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Genetic variability and structure of jaguar (*Panthera onca*) in Mexican zoos

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Abstract Genealogical records of animals (studbook) are created to avoid reproduction between closely related individuals, which could cause inbreeding, particularly for such endangered species as the *Panthera onca* (Linnaeus, 1758). Jaguar is the largest felid in the Americas and is considered an important ecological key species. In Mexico, wild jaguar populations have been significantly reduced in recent decades, and population decline typically accompany decreases in genetic variation. There is no current census of captive jaguars in Mexico, and zoos do not follow a standardized protocol in breeding programs based on genetic studies. Here, we emphasise the importance of maintaining an adequate level of genetic variation and propose the implementation of standardised studbooks for jaguars in Mexico, mainly to avoid inbreeding. In addition, achieving the aims of studbook registration would provide a population genetic characterisation that could serve as a basis for ex situ conservation programmes.

Keywords *Panthera onca* · Studbook · Genetic variability · Microsatellite · Population genetics

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Introduction

The jaguar (*Panthera onca*), the largest felid in the Americas, is considered an important and emblematic species for many pre-Columbian cultures (Campos Fernández-Fígares 2002). As most large carnivores, this felid is currently cited in the Red List (IUCN 2013) as Near Threatened (Caso et al. 2008), in CITES Appendix I (2015) and as Endangered in Mexican laws (SEMARNAT 2010). It is estimated that there are approximately 4000 jaguars in the wild throughout the country (Fig. 1), approximately 70 % less than in the 1960s (Chávez and Zarza 2009; Ceballos et al. 2011a, b). The decline in jaguar population is mainly due to habitat loss and fragmentation, and fur trade is still a threat to wild populations (Quigley and Crawshaw 1992; Eizirik et al. 2001; Silver et al. 2004; O'Brien and Johnson 2005: Ruiz-García et al. 2006; Eizirik et al. 2008; Ruiz-García 2013; Roques et al. 2014); it is estimated that only 16 % of the current territory in Mexico is suitable for the increasingly diminished and isolated jaguar populations (Rodríguez-Soto et al. 2011).

Genetic diversity is progressively lost by the action of drift in small populations (O'Brien 1994), and this effect is

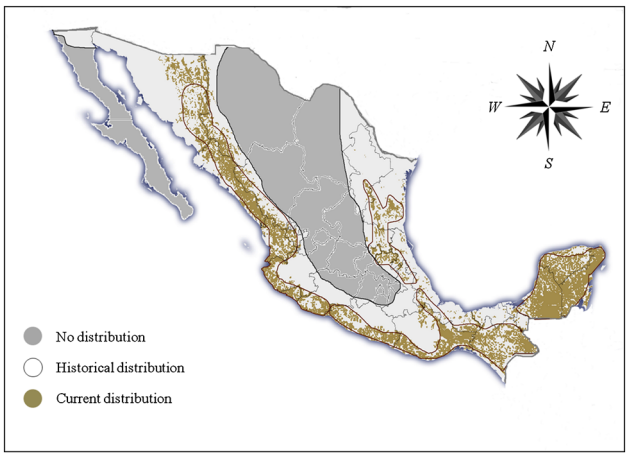

Fig. 1 Historical and current distribution of the jaguar in México (data based on surveys carried out annually since 2008, modified from Ceballos et al. 2011a, b)

further intensified by the reduction of gene flow when these populations become effectively isolated (Briscoe et al. 1992; Frankham 1995; Hedrick and Kalinowski 2000; Keller and Waller 2002; Martínez-Cruz et al. 2004), and this can seriously compromises the survival of a species already at risk of extinction (Hedrick 2001). Previous characterization of genetic variation in jaguar populations revealed moderate to high global genetic diversity in the wild, with some variation among populations and studies (Table 1). Most recent studies have reported a reduced genetic diversity at the periphery of the species range as a consequence of isolation by distance and recent isolation due to fragmentation (Haag et al. 2010; Roques et al. 2014). Mexican jaguar populations in particular show a reduced genetic diversity and a high differentiation to those in Brazil (Roques et al. 2014).

The role of zoos in recent decades as conservation centers has been key to the successful development of conservation programmes for several endangered species: Speke's gazelle (*Gazella spekei*), California condor (*Gymnogyps californianus*), bighorn sheep (*Ovis Canadensis*), Florida panther (*Puma concolor coryi*), European bison (*Bison bonasus*), Mexican grey wolf (*Canis lupus baileyi*) are noticeable examples (Kitchener 1997; Willis and Wiese 1997; Templeton and Read 1998; Meretsky et al. 2000; Whittaker et al. 2004; Hedrick 1994; Olech and Perzanowski 2002; Fredrickson and Hedrick 2002; Fredrickson et al. 2007; Hedrick and Fredrickson 2008; 2010). Captive populations of endangered species can be used as a genetic reservoir and as a source of individuals for reintroductions and for the demographic and genetic reinforcement of extant populations (Woodworth et al. 2002; Frankham 2015). However, captive populations often begins with a small number of individuals distributed in several breeding centers, partly due to spatial constraints (Laikre 1999; Boakes et al. 2007), and if left unmanaged genetic drift and non-random matings results in a rapid loss of genetic diversity and accumulation of inbreeding (Jiménez et al. 1994; Laikre 1999; Keller and Waller 2002; Charlesworth and Willis 2009), with likely reduction of fitness and adaptive potential that can seriously limit their conservation value (Frankham 2015). The erosion of genetic diversity in captive population can however be minimized with the implementation of proper genetic management program that establish the breeding priority and the optimal breeding schemes within and among centers. Such a program is often based on the minimization of average kinship and thus requires exhaustive and reliable genealogical records and/or molecular marker data for kinship estimation.

The genetic status of captive jaguars is mostly unknown. Moreno et al. (2006) assessed genetic variability of jaguars in Brazilian zoos and reported moderate to high levels of polymorphism (suited genetic variability in this species), but genetic data on Mexican captive jaguars is currently lacking. Some Mexican zoos do not exhaustively record births, deaths, or translocations and do not carry a standardized studbook, this difficult an appropriate genetic management in these populations (Ralls et al. 1988; Laikre 1999). Considering these circumstances, we carried out a research in order to: (1) To evaluate some parameters of genetic variation on 56 captive jaguars, (2) To assign individuals to genetic clusters approximating the breeding processes carried out in zoos, and (3) To establish a

Table 1 Levels of genetic variability with samples from free-living, captivity and museums jaguars

Author(s)	Year	Site	Number of samples	Parameter
Eizirik et al.	2001	Americas	44	H = 0.622–0.724
Moreno et al.	2006	Brazilian zoos	39	PIC = 0.69
Ruiz-García et al.	2006	Colombia	62	H = 0.846
Haag et al.	2010	Brazil	59	H = ~0.73
Roques et al.	2014	Brazil	90	H = 0.67–0.7
Roques et al.	2015	Mexico and Brazil	102	H = 0.6 (Mexico), 0.73–0.84 (Brazil)

PIC polymorphism information content, *H* heterozygosity

baseline information useful to implement measures that unify and standardize a prospective studbook in Mexico based on genetic and genealogical information of jaguars.

Materials and methods

Sampling

Fourteen Mexican zoos provided fresh blood samples of 56 jaguars with unknown origin and practically no information on their pedigree. The handling of all individuals was carried out according to the protocols established in each zoo, based on NOM-126-SEMARNAT-2000 and collecting permit No. SGPA/DGVS/01685/11. Blood was collected in tubes with EDTA and stored at 4 °C until processing.

Molecular methods

Total genomic DNA was extracted from blood samples according to the protocol of Sambrook and Russell (2001). DNA samples were visualized by agarose gel electrophoresis. A set of 11 labeled microsatellite markers developed by Menotti-Raymond et al. (1999) and optimized by Roques et al. (2014) was used for individual genotyping (Table 2). PCR conditions were: a first denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C, lasting 30 s, annealing at 57–60 °C, lasting 90 s and extension at 72 °C, lasting 30 s, and a final extension step of 30 min at 72 °C. PCR reactions consisted of 3 µl of DNA extract (15 ng/µl) in a final volume of 20 µl containing Type-it Multiplex PCR Master Mix 1X (QIAGEN®), BSA 0.01 %, and 0.2 µM of each primer (Sigma-Aldrich®) and 0.4 U of Taq polymerase (Bioline®). Allele sizes were determined using the ABI 3130xl Genetic Analyzer System (Applied Biosystems), based on the size standard GS-600 LIZ (Life Technologies Inc.) using GENEMAPPER software v4.0 (Applied Biosystems®) and manually checked to assure reproducibility and correct misreading.

Genotyping

After assigning consensus genotypes to jaguar individuals we tested for genotyping errors with the software MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004), with a 95 % confidence interval and 1000 repetitions; this program can help identify null alleles and accordingly adjust genotype frequencies. FCA115, FCA547 and FCA566 showed a heterozygote deficit consistent with the presence of null alleles. Genotypes were also evaluated with the software FREENA (Chapuis and Estoup 2007), a computer program that allows several statistical treatments on microsatellite datasets with null alleles. Based on INA-corrected allele frequencies we checked whether there are significant differences between values of Fst due to the presence of null alleles (Chapuis and Estoup 2007), using a t-test implemented in GRAPHPAD QUICK CALCS to assess the statistical significance of differences.

Genetic structure

With STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009) we assessed a Bayesian cluster analysis for inferring the probability of individual assignment to a varying number of distinct populations. The procedure uses a Markov Chain Monte Carlo (MCMC) approach to estimate the data fit to each range of potential K clusters. The simulations was performed using 1,000,000 burn in periods and 1,000,000 MCMC iterations, with correlated allele frequencies and an admixture model without prior information on population origin. We selected the most likely number of clusters based on the maximum value of ΔK, following the Evanno method as implemented in STRUCTURE HARVESTER 0.6.92 (Evanno et al. 2005; Earl and von Holdt 2012). We calculated the distribution of the genetic variance between and within clusters and individuals using an analysis of molecular variance (AMOVA) based on Fst as implemented by GENALEX 6.5. (Peakall and Smouse 2006). A phylogenetic tree was constructed from the genetic distances in accordance with the stepwise mutation model (SMM): Nei's genetic distance (Nei 1972) with all individuals using POPULATIONS 1.2.30 (Langella 2002). These distances were used to construct a NJ tree to cluster individuals by genetic similarity with FIGTREE 1.4.2. (Rambaut and Drummond 2010). Finally in GENETIC STUDIO (Dyer 2009) we estimated Nei's genetic distance (D_{Nei}) between clusters.

Genetic variability

Allelic frequencies were used to calculate some genetic parameters including the mean number of alleles per locus (A), the mean number of effective alleles per locus (Ae), observed and expected heterozygosities (Ho, He), and the allelic fixation index (Fst), using GENALEX 6.5. The data set were evaluated to detect deviations from the Hardy–Weinberg Equilibrium (HWE) in the global captive population and in each specific population with ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) and GENEPOP (Raymond and Rousset 1995) using 100,000 Markov chain steps and 100,000 dememorisation steps. The significant values inferred to LD or HWE deviations were corrected for multiple comparisons with a False Discovery Rate (FDR) approach according to Benjamini and Hochberg (1995) as implemented by the QVALUE software (Storey 2002) for R (version 3.0.1; R Development Core Team 2012).

Table 2 Microsatellite loci evaluated in *Panthera onca* by 11 fluorescent primers (Chirhart et al. 2000)

Literature markers name	Markers name in this study	Primers sequences (5′–3′)	Dye	Allele size (in bp)	Annealing to (in °C)	Repeat unit
F115	FCA115	F:CTCACACAAGTAACTCTTTG R:CCTTCCAGATTAAGATGAG	6-FAM	185–225	57	Tetranucleotide
F90a	FCA90	F:ATCAAAAGTCTTGAAGAGCATGG TGTTAGCTCATGTTCATGTGTCC	6-FAM	100–120	62	Dinucleotide
FCA26	FCA26	F:GGAGCCCTTAGAGTCATGCA TGTACACGCACCAAAAACAA	6-FAM	126–160	60	Dinucleotide
N82b	FCA82	F:TCACCGCTTAAGAAGAGGCTA R:GTGAAGCTTCCGAAATGAGG	VIC	190–210	57	Dinucleotide
F176	FCA176	F:GGAAACTTGGAAAGCAAAACC R:TCCACAGTTGGAGTTCTTAAGG	PET	213–233	57	Dinucleotide
V547b	FCA547	F:GGTGACAAAACAAAACAAAGCA R:GGAGCCTGCATAGGATTCAC	VIC	215–235	60	Dinucleotide
FCA77	FCA77	F:GGCACCTATAACTACCAGTGTGA R:ATCTCTGGGGAAATAAATTTTGG	NED	110–154	57	Dinucleotide
N43a	FCA43	F:GAGCCACCCTAGCACATATACC R:AGACGGGATTGCATGAAAAG	NED	100–130	60	Dinucleotide
N566b	FCA566	F:TGCTCAAACAGATAAGGCTGAA R:CCCACTCATGCTGTCTCTCA	NED	155–175	57	Dinucleotide
FCA24	FCA24	F:CCCAGCTTTGTCTCTTACTGTG R:CATCCTCCCCTAATGCCC	PET	210–235	60	Dinucleotide
FCA126	FCA126	F:GCCCCTGATACCCTGAATG R:CTATCCTTGCTGGCTGAAGG	PET	150–170	57	Dinucleotide

Allele size and annealing temperatures conditions are indicated. 6-FAM, NED, PET, VIC: fluorophore acronyms

Effective population size, genetic bottlenecks, and relatedness

Effective population size was estimated with the software LDNE (Waples 2006; Waples and Do 2008), based on the linkage disequilibrium method (Hill 1981). We used the software BOTTLENECK 5.1.26 (Cornuet and Luikart 1996; Piry et al. 1999) to test for a trace of genetic bottlenecks events. We estimated the observed and expected heterozygosity under the infinite allele model (IAM), SMM and the two-phase model (TPM), with settings at 90 % SMM, 10 % IAM, and 10 % variance and default values (70 % SMM, 30 % IAM, and 10 % variance). Both settings were calculated with 10,000 replicates and excess of heterozygosity was tested with Wilcoxon test. We evaluated relatedness among individuals with the program ML-RELATE (Kalinowski et al. 2006), which takes into account null alleles and is based on maximum likelihood tests, Also we presented a summary of the of relationships assigned with the highest likelihood, consistent with the genetic data at the 0.05 level of significance; a graphical comparison of the number of pairs of individuals probably related (ML RELATE) and the number of pairs of individuals having a relationship according to the information provided by the veterinarians of each zoo.

Results

Population sampling and potential scoring errors

Genotypes from fifty-six blood samples from Mexican zoos were obtained by microsatellite analysis with 11 loci (Roques et al. 2014). The analysis of genotypes with MICRO-CHECKER showed three loci with potential null alleles: FC115, FC547 and FC566. However, since the adjusted Fst values did not significantly differ from the unadjusted values (Fst-INA = 0.096, Fst = 0.095; t = 0.015, $P = 0.99$, gl = 20) we kept all loci for further analyses.

Genetic structure

Three genetic clusters were obtained with STRUCTURE software (LnPr ($k = 3$) = −1716.35 Fig. 2), according to the Evanno method (based on the maximum likelihood of

k clusters; Fig. A1). At least 85 % of jaguars were assigned with a probability of 95 % to the clusters obtained (Table 3), several individuals were assigned partially to one, two or all three clusters (Fig. 2). Results of AMOVA showed that most of the genetic variation resided within individuals (76 %; $P = 0.001$), then between individuals within clusters (16 %; $P = 0.001$) and finally between clusters (8 %; $P = 0.001$, Table 4). The Fst and Nei's genetic distances were slightly moderate among Clusters, moderate between Cluster 1 to Cluster 2 (Fst = 0.051 Nei's = 0.2142), moderate between Cluster 1 to Cluster 3 (Fst = 0.113 Nei's = 0.4857), and high between Cluster 2 to Cluster 3 (Fst = 0.151 Nei's = 0.5425). In agreement with AMOVA, the NJ tree showed the Clusters 1 and 2 are more similar between them, instead the Cluster 3 is the most different from all (Fig. A2). Also the results of the NJ tree and STRUCTURE showed signs of admixture (Fig. A2 and Fig. 2).

Table 3 Number of individuals that were assigned to a cluster at a 95 % probability in structure

P	Number of assigned jaguars	(%)
>90 %	30	53.57
>80 %	9	17.86
>70 %	8	14.29
>60 %	2	3.57
45–60 %	7	12.50
Total	56	100

Genetic variability

After FDR correction, no significant overall linkage disequilibrium was detected in any Cluster, so we considered the eleven loci as independent markers. We measured Ho, He, A, Ae, Shannon's Information Index (I) and Fst values per locus for each of the three inferred clusters and for the overall captive jaguar population (CJP), see Table 4. In the genetic Cluster 1, the following values were obtained: A ($\bar{x} = 7$), Ae ($\bar{x} = 4.4$), Ho and Ho and He were the same ($\bar{x} = 0.72$) and, Fst ($\bar{x} = -0.01$); Cluster 2: A ($\bar{x} = 4.4$), Ae ($\bar{x} = 3.13$), Ho ($\bar{x} = 0.53$) He ($\bar{x} = 0.62$) and, Fst ($\bar{x} = 0.12$) and Cluster 3: A ($\bar{x} = 3.63$), Ae ($\bar{x} = 2.8$), Ho ($\bar{x} = 0.7$) He ($\bar{x} = 0.59$) and, Fst ($\bar{x} = -0.2$).

In summary, levels of genetic diversity were moderate to high (0.53–0.72) with an overall mean of 0.65 (Table 4). Ho was higher in genetic Cluster 1 (0.72), but considering that genetic Cluster 3 contained only eight individuals, it would be proportionately higher (0.71), while the Cluster 2 has a moderate heterozygosity (0.53). In addition, the values of the inbreeding coefficient were low to moderate: in the captive jaguar population CJP (Fis; from −0.12 to 0.21), the Cluster 1 (Fis; from −0.12 to 0.14), the Cluster 2 (Fis; from −0.13 to 0.35), and Cluster 3 (Fis; from −0.49 to 0.25; Table A2).

Effective population size, genetic bottlenecks, and relatedness

The effective population size was estimated in 17.3 (95 % CI 13.4–22.7), with a harmonic mean sample size of 51.9 (Table 5). Evidence of a recent genetic bottleneck associated with a heterozygote excess (BOTTLENECK results) was observed for all captive jaguar population CJP with a variance of 30 % and a probability of 70 %, under the IAM and TPM models ($P = 0$), also with a variance of 10 % and a probability of 90 %, under the IAM model ($P = 0$). When analyzed independently each cluster the same phenomenon was observed, all Clusters showed possible

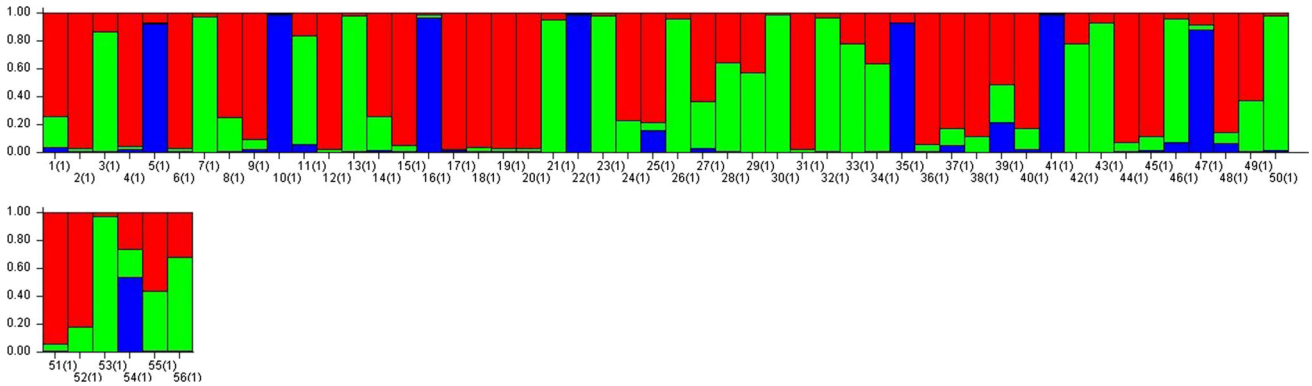

Fig. 2 Estimation of genetic ancestry in each of the three inferred clusters for the 56 captive jaguars using STRUCTURE 2.3.4. Each individual is represented by a single vertical line divided into *k-coloured* ensemble. *Colour length* in *vertical lines* represents the proportion of ancestry from each of the three inferred clusters in each individual. *Each number* corresponds to an individual and the *number inside the brackets* represents the captive population

Table 4 Analysis of molecular variance (AMOVA) based on Fst values

Source of variation	D.F.	Sum of squares	Variance components	Percentage of variation
Between clusters	1	33.778	0.365 Va	8
Between individuals	53	248.981	0.702 Va	16
Within individuals	56	184.5	3.295 Vb	76
Total	111	629.141	4.361	

Fixation index Fst: 0.084; $P = 0.001$

Table 5 Measures of diversity at 11 microsatellites in the three jaguar clusters obtained from 56 captive individuals

Genetic cluster	Locus	A	Ae	I	Ho	He	Fst
Cluster 1	FCA24	5	2.62	1.18	0.57	0.62	0.08
	FCA26	6	2.99	1.41	0.72	0.67	−0.09
	FCA43	3	2.62	1.02	0.61	0.62	0.02
	FCA77	7	4.03	1.60	0.83	0.75	−0.11
	FCA82	6	3.07	1.35	0.77	0.67	−0.14
	FCA90	5	3.38	1.31	0.74	0.70	−0.05
	FCA115	17	12.05	2.65	0.85	0.92	0.07
	FCA126	6	2.50	1.20	0.62	0.60	−0.04
	FCA176	6	4.10	1.53	0.73	0.76	0.04
	FCA547	8	5.26	1.83	0.79	0.81	0.03
	FCA566	8	5.41	1.81	0.71	0.81	0.12
Mean		7	4.37	1.54	0.72	0.72	−0.01
Cluster 2	FCA24	4	2.99	1.19	0.72	0.66	−0.09
	FCA26	3	2.21	0.92	0.63	0.55	−0.15
	FCA43	3	2.52	0.99	0.53	0.60	0.13
	FCA77	5	2.50	1.17	0.50	0.60	0.17
	FCA82	3	1.21	0.37	0.19	0.17	−0.08
	FCA90	4	2.96	1.16	0.61	0.66	0.08
	FCA115	9	7.11	2.08	0.62	0.86	0.27
	FCA126	5	2.91	1.23	0.50	0.66	0.24
	FCA176	3	2.97	1.09	0.53	0.66	0.21
	FCA547	5	3.79	1.42	0.50	0.74	0.32
	FCA566	5	3.34	1.4	0.50	0.70	0.29
Mean		4.455	3.136	1.185	0.530	0.624	0.125
Cluster 3	FCA24	4	2.61	1.16	0.50	0.62	0.19
	FCA26	3	2.33	0.95	0.87	0.57	−0.53
	FCA43	2	1.44	0.48	0.37	0.30	−0.23
	FCA77	3	1.85	0.78	0.62	0.46	−0.36
	FCA82	5	3.92	1.47	0.86	0.74	−0.15
	FCA90	3	2.67	1.04	0.62	0.62	0.00
	FCA115	5	4.08	1.49	0.98	0.75	−0.32
	FCA126	2	2	0.69	0.50	0.50	0.00
	FCA176	3	2.84	1.07	0.98	0.65	−0.54
	FCA547	4	1.97	0.95	0.62	0.49	−0.27
	FCA566	6	4.67	1.67	0.86	0.79	−0.09
Mean		3.636	2.762	1.068	0.713	0.591	−0.210
Overall mean		5.03	3.42	1.26	0.65	0.65	−0.03

A, number of different alleles; Ae, number of effective alleles; I, Shannon's Information Index; Ho, observed Heterozygosity; He, expected heterozygosity; Fst, fixation index

bottlenecks under the two models used for this analysis (Table A3).

The relatedness analysis based on the maximum likelihood (ML) estimator, according to the amount of shared alleles, in Cluster 1 revealed 8 full siblings (FS), 44 half siblings (HS) and 9 parent/offspring (PO) relationships; we also evaluated the differences between the Ln Likelihood (LnL) for the other relationships categories (Table 7). There were significant differences between FS and PO comparisons, however in HS relationships there were no differences compared with unrelated (U) and FS. In Cluster 2 revealed 7 FS, 22 HS and 7 PO relationships; there were significant differences between all the relationships categories compared but in FS with PO. In Cluster 3 revealed 6 FS, 17 HS and 5 PO relationships assigned by ML; in this cluster all comparisons with other categories of relationships categories had significant differences (Table 7).

Discussion

Genetic structure and genetic variability

Although captive jaguars are not a natural population, genetic analysis could be useful for conservation on this endangered species. The analysis of fifty-six blood samples from captive animals showed three genetic clusters based on the Δk estimated by STRUCTURE (Fig. 2). Cluster 1 (red) with 29 individuals, Cluster 2 (green) with 19 individuals and Cluster 3 (blue) only 8 individuals. This software infers population structure, assigning individuals to the closest population, identifying migrants and admixed individuals. We start from the premise that captive jaguar population have some degree of structure because many animals from highly differentiated populations have been reproduced each other (Brazilian, Venezuelans with individuals from Mexican origin), as mentioned by zookeepers. More than 85 % of the jaguars were assigned to one of three clusters ($P = 95$ %), in several cases it was observed that some individuals were partially assigned to more than one cluster (Table 3), probably these characteristics of variable proportions of membership to the clusters within individuals was due to the mate of jaguars from very different populations, a common situation in the sampled animals.

AMOVA results revealed that there was moderate genetic differentiation between the three clusters formed according to allelic frequencies; it may result from different founder individuals (presumably from differentiated populations), in turn this difference is probably to be a result of the diverse geographical origin of jaguars and the effects of different selective breeding. We attribute these results to the variation within individuals (76 %) and are concordant with the NJ tree: Clusters 1 and 2 were more similar instead cluster 3 was the most distant. This assignment probably was originated from the selective breeding, chiefly South American individuals mated with Mexicans, for example reproduction of melanistic jaguars have been increased because they are very attractive to the visitors (pers. obs); in Mexico there is no evidence of black jaguars in wild, the northernmost record being from Costa Rica (Meyer 1994; Eizirik et al. 2003; Cartín-Núñez and Carrillo 2009), and few sightings are reported in Belize without further solid evidence. Both Fst analysis and AMOVA showed significant genetic differentiation among clusters, although the Fst value between Clusters 1 and 2 was small, it is statistically significant (0.051). The values of Nei's distances were also significant to consider the clusters with a degree of differentiation.

Despite limitations in space and number of individuals, in this research we found moderate to high levels of heterozygosity (0.53–0.72; Table 5). Because this captive population is probably formed by individuals of several origins, levels of heterozygosity could be raised as a result of the mixture of individuals from populations with some degree of differentiation (Luo et al. 2008). The highest record of heterozygosity in jaguars reported by Ruiz-García et al. 2006 for Colombian jaguars ($H = 0.84$), is perhaps derived from the population subdivision that they assessed (Boecklen 1986; Swindell and Bouzat 2006). Other research about genetic diversity in jaguar was developed by Haag et al. (2010), the average $Ho = 0.73$ was high, nonetheless they reported allelic loss, differentiation between populations isolated and low effective sizes, this processes possibly caused by recent genetic drift. In other large felids genetic diversity has been reported low levels, cheetah (*Acinonyx jubatus*) $He = 0.64$–0.70 in Namibia (Marker et al. 2008; Luo et al. 2004) obtained a mean observed heterozygosity in several samples of tiger subspecies (*P. tigris*) ranged from 0.40 (Ho minimum in (*P. t. altaica*) to 0.66 (Ho maximum in *P. t. corbetti*). In contrast, Indian leopard (*P. pardus*) showed high levels of $Ho = 0.74$ (Dutta et al. 2013).

Effective population size, genetic bottleneck and relatedness

There is no a regular update of the census of jaguars in Mexican zoos, the most recent data is from 2003, published in the workshop "*El papel de los zoológicos de Mesoamérica y el Caribe en la conservación de los jaguares*" (The role of zoos in Mesoamerica and the Caribbean in the conservation of jaguars) developed by the Conservation Breeding Specialist Group (CBSG). They estimated 135 jaguars (62 males, 67 females and 7 unknown), however, it has not been updated a census to verify whether this

Table 6 Estimating of effective population size based on LD method

	LDNe		
	Harmonic mean sample size	Ne	Confidence interval (95 %)
Captive jaguars	51.9	17.3	13.4–22.7

number has increased or decreased. In several zoos where we collected blood samples, veterinarians mentioned that they try to avoid the breeding between close-related individuals, however, in the absence of an updated and standardized jaguar studbook, consanguineous matings cannot be completely avoided and an effective genetic management cannot be implemented, what will adversely affect genetic variation (Witzenberger and Hochkirch 2011). On the contrary, breeding programs that are genetically managed based on mean kinship, retained genetic diversity and delayed the effects of inbreeding, even when there is scarce information about the pedigree of individuals, these programs can still be implemented by using molecular marker data to estimate kinship among individuals. The data generated by this study provides thus a starting point for the implementation of genetic management program of captive jaguars in Mexico, since in more than half of the relationships assigned, it was verified that there was a high probability of consistency with the information provided by zoo keepers. Also, it could be detected that some families are formed due to the limited interchange that has occurred in four zoos having proximity (data not shown). Accordingly, this may also be reflected in the small effective population size obtained (Ne = 17) considering there have been no reproductions guided in a program to maintain genetic variation, for example, random mating that includes the greatest number of jaguars (Table 6). In the three clusters a recent genetic bottleneck was detected (Table A3), it is possible that this result indicated a restricted reproduction between few individuals (founder members) and furthermore, the captive jaguar population has recently been formed (it is estimated that in the 1980s).

Table 7 Relationships assigned with the maximum likelihood according to genetic data at the 0.05 level of significance

Number of pairs	R[a]	LnL(R)	LnL(U)	LnL(HS)	LnL(FS)	LnL(PO)
Cluster 1						
8	FS	−51.82 to (−46.44)	−54.49 to (−48.31)	−52.88 − 47.11	–	−10,050.82 to (−10,045.44)
			0.032*	0.03*		0.003*
44	HS	−57.98 to (−49.96)	−58.2 to (−50.77)	–	−61.8 to (−51.36)	−10,056.98 to (−10,048.96)
			0.9		0.2	0.0001*
9	PO	−14.08 to (−15.9)	−15.13 to (−16.28)	−14.54 to (−15.98)	−14.73 to (−16.38)	–
			0.005*	0.008*	0.001*	
Cluster 2						
7	FS	−26.15 to (−23.48)	−27.09 to (−28.42)	−26.85 to (−25.53)	–	−10,025.15 to (−23.51)
			0.017*	0.027*		0.078
22	HS	−39.31 to (−41.37)	−39.65 to (−41.73)	–	−40.65 to (−43.56)	−10,038.31 to (−10,040.37)
			0.0001*		0.0001*	0.0001*
7	PO	−39.95 to (−41.61)	−40.5 to (−43.26)	−40.03 to (−41.65)	−42.25 to (−41.95)	–
			0.002*	0.014*	0.007*	
Cluster 3						
6	FS	−72.18 to (−69.44)	−96.77 to (−76.96)	−80.52 to (−69.5)	–	−75.47 to (−10,068.44)
			0.001*	0.007*		0.0001*
17	HS	−84.21 to (−57.14)	−92.41 to (−60.95)	–	−87.3 to (−57.51)	−10,083.21 to (−10,056.14)
			0.0001*		0.0001*	0.0001*
5	PO	−78.15 to (−52.94)	−98.24 to (−68)	−82.55 to (−55.87)	−80.48 to (−56.32)	–
			0.001*	0.001*	0.019*	

R relatedness, *LnL* Ln of the Likelihood, *U* unrelated, *HS* half siblings, *FS* full siblings, *PO* parent/offspring

* Significant difference

[a] Probable relationship assigned by Maximum Likelihood in ML Relate (Kalinowski et al. 2006)

To avoid the loss of genetic diversity caused by of the lack of accurate planning in the genetic management of captive jaguar populations, this research would contribute to be the starting point for further establishment of appropriate genetic management for Mexican zoos.

Although we had a moderate number of loci, the relatedness analysis was mostly consistent with the other results. Cluster 1 showed the largest number of possible relationships (Total = 61), of which 2 were known (1 parent/offspring and 1 full siblings). In Cluster 3 it was found only one parent/offspring relationship, however this two melanistic jaguars formed one of the confirmed relationships. The relationship with the largest possibilities in the three clusters was HS (Table 7), it is expected to be ambiguous because of the challenge of calculating and assigning individuals that share only 25 % of the genetic material and may actually be categorized in other relationships such as cousins, uncles/nephews, grandparents/grandchildren. Hence, we emphasize the necessity for further analysis of parentage based on genetic information, preferably using a high number of molecular markers to improve the precision of the estimates. In three of the four known relationships we observed consistency on the assignment of the individuals into the same cluster, one of full siblings (Fig. 2, see bars 14 and 38) and two of parent/offspring (Fig. 2, see bars 15 and 17; 16 and 22). The fourth relationship of parent/offspring was not very clear (bars 56 and 39 of the Fig. 2), there is the possibility that parents and offspring were assigned in different clusters, the most likely cause that we consider is because these parents have different origins, and each parent could be assigned to a different cluster meanwhile offspring are assigned to the proportion inherited from each parent. In general, it is likely that our results show a medium level of relatedness among individuals; which suggests that litters have been distributed in zoos that have maintained a limited exchange of individuals. Following the protocol of reproduction based on the mean kinship (MK) as suggested by Willoughby et al. (2015) is strongly recommended to avoid the loss of genetic variation, if there is the purpose to establish a studbook for captive jaguars, it is important to have an intensive relatedness analysis prior to start a breeding program.

Conclusions

Captive breeding has become an important tool in animal conservation, but the lack of a well-executed management program aimed at minimizing the impact of drift and inbreeding can reduce their usefulness for species conservation. Wild Mexican jaguars have declined and show signs of genetic erosion, a situation that could eventually demand supplementation from a healthy captive genetic stock, there was no information about genetic status of captive jaguars, and our research is the first to provide basic information on genetic variation of captive jaguars in Mexico. They need to be genetically managed based on genealogical and molecular information to guarantee the appropriate management to retain genetic diversity for jaguar conservation programmes. This paper is the first in genetically evaluate captive populations of jaguars in Mexico and provides the basis to designing breeding programs that conserve the genetic diversity of these populations.

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References

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B Met 57(1):289–300

Boakes EH, Wang J, Amos W (2007) An investigation of inbreeding depression and purging in captive pedigreed populations. Heredity 98:172–182. doi:10.1038/sj.hdy.6800923

Boecklen WJ (1986) Optimal design of nature reserves: consequences of genetic drift. Biol Conserv 38(4):323–338. doi:10.1016/0006-3207(86)90058-3

Briscoe DA, Malpica JM, Robertson A, Smith GJ, Frankham R, Banks RG, Barker JSF (1992) Rapid loss of genetic variation in large captive populations of Drosophila flies: implications for the genetic management of captive populations. Conserv Biol 6(3):416–425. doi:10.1046/j.1523-1739.1992.06030416.x

Campos Fernández-Fígares MM (2002) El caballo y el jaguar: sobre la historia verdadera de la conquista de la Nueva España. Comares Editores, España

Cartín-Núñez M, Carrillo E (2009) New record of black jaguar, *Panthera onca* (Carnivora: Felidae) in Costa Rica. Brenesia 71–72:67–68

Caso A, Lopez-Gonzalez C, Payan E, Eizirik E, de Oliveira T, Leite-Pitman R, Kelly M, Valderrama C (2008) *Panthera onca*-The IUCN Red List of Threatened Species. Version 2014.3. www.iucnredlist.org. Downloaded on 29 April 2015

Ceballos G, Chávez C, Zarza H (2011a) El Jaguar en México. Alianza WWF/Telcel CONANP, UNAM. Servicios Ecológicos y Científicos, México, Distrito Federal

Ceballos G, Chávez C, List R, Zarza H, Medellín RA (2011b) Jaguar Conservation and Management in Mexico: case studies and perspectives, Alianza WWF/Telcel CONANP, UNAM. Servicios Ecológicos y Científicos, México, Distrito Federal

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24(3):621–631. doi:10.1093/molbev/msl191

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Nat Rev Genet 10:783–796. doi:10.1038/nrg2664

Chávez C, Zarza H (2009) Distribución potencial del hábitat del jaguar y áreas de conflicto humano-jaguar en la península de Yucatán. Rev Mex Mastozool 13:46–62

Chirhart SE, Honeycutt RL, Greenbaum IF (2000) Microsatellite markers for the deer mouse *P. maniculatus*. Mol Ecol 9:1669–1672

CITES (2015) Convention on international trade in endangered species of wild fauna and flora appendices I, II and III. http://www.cites.org/eng/app/appendices.php. Accessed 10 April 2015

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144(4):2001–2014

Dutta T, Sharma S, Maldonado JE, Wood TC, Panwar HS, Seidensticker J (2013) Fine-scale population genetic structure in a wide-ranging carnivore, the leopard (Panthera pardus fusca) in central India. Divers Distrib 19(7):760–771. doi:10.1111/ddi.12024

Dyer RJ (2009) Genetic studio: a suit of programs from spatial analysis of genetic-marker data. Mol Ecol Resour 9:110–113

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361. doi:10.1007/s12686-011-9548-7

Eizirik E, Heup KJ, Menotti-Raymond M, Crawshaw PG, O'Brien SJ, Johnson WE (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Mol Ecol 10:65–79. doi:10.1046/j.1365-294X.2001.01144.x

Eizirik E, Yuhki N, Johnson WE, Menotti-Raymond M, Hannah SS, O'Brien SJ (2003) Molecular genetics and evolution of melanism in the cat family. Curr Biol 13:448–453. doi:10.1016/S0960-9822(03)00128-3

Eizirik E, Haag T, Santos AS, Salzano FM, Silveira L, Azevedo FCC, Furtado MM (2008) Jaguar Conserv Genet. CAT News 4:31–34

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Excoffier L, Lischer HEL (2010) ARLEQUIN suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567. doi:10.1111/j.1755-0998.2010.02847.x

Falush D, Stephens M, Pritchard J (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164(4):1567–1587

Frankham R (1995) Conservation genetics. Annu Rev Genet 29:305–327. doi:10.1146/annurev.ge.29.120195.001513

Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol Ecol Early View. doi:10.1111/mec.13139

Fredrickson R, Hedrick P (2002) Body sizes in endangered Mexican wolves: effects of inbreeding and cross-lineage matings. Anim Conserv 5:39–43. doi:10.1017/S1367943002001051

Fredrickson RJ, Siminski P, Woolf M, Hedrick PW (2007) Genetic rescue and inbreeding depression in Mexican wolves. Proc R Soc Lond B Bio 274:2365–2371. doi:10.1098/rspb.2007.0785

Haag T, Santos AS, Sana DA, Morato RG, Cullen L Jr, Crawshaw PG, De Angelo C, Di Bitetti MS, Salzano FM, Eizirik E (2010) The effect of habitat fragmentation on the genetic structure of a top predator: loss of diversity and high differentiation among remnant populations of Atlantic Forest jaguars (*Panthera onca*). Mol Ecol 19:4906–4921. doi:10.1111/j.1365-294X.2010.04856.x

Hedrick PW (1994) Purging inbreeding depression and the probability of extinction: full-sib mating. Heredity 73:363–372. doi:10.1038/hdy.1994.183

Hedrick PW (2001) Conservation genetics: where are we now? Trends Ecol Evol 16:629–636. doi:10.1016/S0169-5347(01)02282-0

Hedrick PW, Fredrickson R (2008) Captive breeding and the reintroduction of Mexican and red wolves. Mol Ecol 17:344–350. doi:10.1111/j.1365-294X.2007.03400.x

Hedrick PW, Fredrickson R (2010) Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conserv Genet 11:615–626. doi:10.1007/s10592-009-9999-5

Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. Annu Rev Ecol Syst 31:139–162

Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. Genet Res 38(03):209–216. doi:10.1017/S0016672300020553

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9(5):1322–1332. doi:10.1111/j.1755-0998.2009.02591.x

Jiménez JA, Hughes KA, Alaks G, Graham L, Lacy RC (1994) An experimental study of inbreeding depression in a natural habitat. Science 266(5183):271–273. doi:10.1126/science.7939661

Kalinowski ST, Wagner AP, Taper ML (2006) ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes 6(2):576–579. doi:10.1111/j.1471-8286.2006.01256.x

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17(5):230–241. doi:10.1016/S0169-5347(02)02489-8

Kitchener AC (1997) The role of museums and zoos in conservation biology. Int Zoo Yb 35:325–336. doi:10.1111/j.1748-1090.1997.tb01228.x

Laikre L (1999) Hereditary defects and conservation genetic management of captive populations. Zoo Biol 18:81–99. doi:10.1002/(SICI)1098-2361(1999)18:2<81:AID-ZOO1>3.0.CO;2-2

Langella O (2002) Populations 1230 Copyright (C) 1999 Olivier Langella CNRS-UPR9034. http://bioinformatics.org/~tryphon/populations/

Luo SJ, Kim JH, Johnson WE, van der Walt J, Martenson J, Yuhki N, Miquelle DG, Uphyrkina O, Goodrich JM, Quigley HB, Tilson R, Brady G, Martelli P, Subramaniam V, McDougal C, Hean S, Huang SQ, Pan W, Karanth UK, Sunquist M, Smith JLD, O'Brien SJ (2004) Phylogeography and genetic ancestry of tigers (*Panthera tigris*). PLoS Biol 2(12):2275–2293. doi:10.1371/journal.pbio.0020442

Luo SJ, Johnson WE, Martenson J, Antunes A, Martelli P, Uphyrkina O, Traylor-Holzer K, Smith JLD, O'Brien SJ (2008) Subspecies genetic assignments of worldwide captive tigers increase conservation value of captive populations. Curr Biol 18(8):592–596. doi:10.1016/j.cub.2008.03.053

Marker LL, Wilkerson AJP, Sarno RJ, Martenson J, Breitenmoser-Würsten C, O'Brien SJ, Johnson WE (2008) Molecular genetic insights on cheetah (*Acinonyx jubatus*) ecology and conservation in Namibia. J Hered 99(1):2–13. doi:10.1093/jhered/esm081

Martínez-Cruz B, Godoy JA, Negro JJ (2004) Population genetics after fragmentation: the case of the endangered Spanish imperial eagle (*Aquila adalberti*). Mol Ecol 13(8):2243–2255. doi:10.1111/j.1365-294X.2004.02220.x

Menotti-Raymond M, David VA, Lyons LA, Schäffer AA, Tomlin JF, Hutton MK, O'Brien SJ (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 57(1):9–23. doi:10.1006/geno.1999.5743

Meretsky VJ, Snyder NF, Beissinger SR, Clendenen DA, Wiley JW (2000) Demography of the California Condor: implications for reestablishment. Conserv Biol 14:957–967. doi:10.1046/j.1523-1739.2000.99113.x

Meyer JR (1994) Black jaguars in Belize? A survey of melanism in the jaguar, *Panthera onca*. http://biological-diversity.info/Black_Jaguar.htm

Moreno VR, Grisolia AB, Campagnari F, Milazzotto M, Adania CH, Garcia JF, Souza EBD (2006) Genetic variability of *Herpailurus yagouaroundi*, *Puma concolor* and *Panthera onca* (Mammalia, Felidae) studied using *Felis catus* microsatellites. Genet Mol Biol 29(2):290–293. doi:10.1590/S1415-47572006000200017

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

O'Brien SJ (1994) Genetic and phylogenetic analyses of endangered species. Annu Rev Genet 28(1):467–489. doi:10.1146/annurev.ge.28.120194.002343

O'Brien SJ, Johnson WE (2005) Big Cat Genomics. Annu Rev Genom Hum Genet 6:407–429. doi:10.1146/annurev.genom.6.080604.162151

Olech W, Perzanowski K (2002) A genetic background for reintroduction program of the European bison (*Bison bonasus*) in the Carpathians. Biol Conserv 108:221–228. doi:10.1016/S0006-3207(02)00108-8

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel population genetic software for teaching and research. Mol Ecol Notes 6:288–295. doi:10.1111/j.1471-8286.2005.01155.x

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Quigley HB, Crawshaw PG (1992) A conservation plan for the jaguar *Panthera onca* in the Pantanal region of Brazil. Biol Conserv 61(3):149–157. doi:10.1016/0006-3207(92)91111-5

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Ralls K, Ballou JD, Templeton A (1988) Estimates of lethal equivalents and the cost of inbreeding in mammals. Conserv Biol 2(2):185–193. doi:10.1111/j.1523-1739.1988.tb00169.x

Rambaut A, Drummond A (2010) FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, UK. http://tree.bio.ed.ac.uk/software/figtree/. Accessed 2013 Apr 25

Raymond M, Rousset F (1995) GENEPOP v 12: population genetics software for exact test and ecumenicism. J Hered 86:248–249

Rodríguez-Soto C, Monroy-Vilchis O, Maiorano L, Boitani L, Faller JC, Briones MA, Núñez R, Rosas-Rosas O, Ceballos G, Falcucci A (2011) Predicting potential distribution of the jaguar (*Panthera onca*) in Mexico: identification of priority areas for conservation. Divers Distrib 17:350–361. doi:10.1111/j.1472-4642.2010.00740.x

Roques S, Furtado M, Jácomo AT, Silveira L, Sollmann R, Tôrres NM, Godoy JA, Palomares F (2014) Monitoring jaguar populations *Panthera onca* with non-invasive genetics: a pilot study in Brazilian ecosystems. Oryx 48(03):361–369. doi:10.1017/S0030605312001640

Roques S, Sollman R, Jácomo A, Tôrres N, Silveira L, Chávez C, Keller C, Mello do Prado D, Carignano Torres P, Jorge dos Santos C, Bernardes Garcia da Luz X, Magnusson WE, Godoy JA, Ceballos G, Palomares F (2015) Effects of habitat deterioration on the population genetics and conservation of the jaguar. Conserv Genet. doi:10.1007/s10592-015-0766-5

Ruiz-García M (2013) Jaguar, genes y conservación: La historia poblacional del gran félido americano revelada por el ADN. Investigación y ciencia 441:24–31

Ruiz-García M, Payán E, Murillo A, Álvarez D (2006) DNA microsatellite characterization of the jaguar (*Panthera onca*) in Colombia. Genes Genet Syst 81:115–127. doi:10.1266/ggs.81.115

Sambrook J, Russell WD (2001) Molecular cloning: a laboratory manual, Vol 2. Cold Spring HarborLaboratory Press. Cold Spring Harbor, New York, pp 47–53

SEMARNAT-Secretaría de Medio Ambiente y Recursos Naturales (2010) Norma Oficial Mexicana NOM-059-ECOL-2010, Protección ambiental de especies nativas de México de flora y fauna silvestres en categorías de riesgo y especificaciones para su inclusión, exclusión o cambio en la lista de especies en riesgo. Diario Oficial de la Federación 1–56

Silver SC, Ostro LET, Marsh LK, Maffei L, Noss AJ, Kelly MJ, Wallace RB, Gómez H, Ayala G (2004) The use of camera traps for estimating jaguar *Panthera onca* abundance and density using capture/recapture analysis. Oryx 38(02):148–154. doi:10.1017/S0030605304000286

Storey JD (2002) A direct approach to false discovery rates. J R Stat Soc B 64(3):479–498. doi:10.1111/1467-9868.00346

Swindell WR, Bouzat JL (2006) Selection and inbreeding depression: effects of inbreeding rate and inbreeding environment. Evolution 60(5):1014–1022. doi:10.1111/j.0014-3820.2006.tb01179.x

Templeton AR, Read B (1998) Elimination of inbreeding depression from a captive population of Speke's gazelle: validity of the original statistical analysis and confirmation by permutation testing. Zoo Biol 17:77–94. doi:10.1002/(SICI)1098-2361(1998)17:2<77:AID-ZOO4>3.0.CO;2-6

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. doi:10.1111/j.1471-8286.2004.00684.x

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7:167–184. doi:10.1007/s10592-005-9100-y

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8(4):753–756. doi:10.1111/j.1755-0998.2007.02061.x

Whittaker DG, Ostermann SD, Boyce WM (2004) Genetic variability of reintroduced California bighorn sheep in Oregon. J Wildl Manag 68:850–859. doi:10.2193/0022-541X(2004)068[0850:GVORCB]2.0.CO;2

Willis K, Wiese RJ (1997) Elimination of inbreeding depression from captive populations: Speke's gazelle revisited. Zoo Biol 16:9–16. doi:10.1002/(SICI)1098-2361(1997)16:1<9:AID-ZOO3>3.0.CO;2-E

Willoughby JR, Fernandez NB, Lamb MC, Ivy JA, Lacy RC, DeWoody JA (2015) The impacts of inbreeding, drift and selection on genetic diversity in captive breeding populations. Mol Ecol 24(1):98–110. doi:10.1111/mec.13020

Witzenberger KA, Hochkirch A (2011) Ex situ conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. Biodivers Conserv 20(9):1843–1861. doi:10.1007/s10531-011-0074-4

Woodworth LM, Montgomery ME, Briscoe DA, Frankham R (2002) Rapid genetic deterioration in captive populations: causes and conservation implications. Conserv Genet 3(3):277–288. doi:10.1023/A:1019954801089