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**Article** *in* European Journal of Wildlife Research · May 2016

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**European Journal of Wildlife Research**

ISSN 1612-4642

Eur J Wildl Res
DOI 10.1007/s10344-016-1019-8


Springer

Eur J Wildl Res
DOI 10.1007/s10344-016-1019-8

ORIGINAL ARTICLE

# Gene flow and genetic structure of the puma and jaguar in Mexico

**Marina Zanin[1] · Begoña Adrados[2] · Noa González[2] · Severine Roques[2] · Daniel Brito[3] · Cuauhtemoc Chávez[4] · Yamel Rubio[5] · Francisco Palomares[2]**

Received: 22 January 2016 / Revised: 19 April 2016 / Accepted: 26 April 2016


**Abstract** Gene flow among populations and subpopulations homogenizes allele frequencies. This mechanism is strongly influenced by species dispersal ability, frequently correlating genetic variation with distance among individuals, which is also known as an isolation-by-distance pattern. Species with high dispersal abilities are expected to show a limited isolation-by-distance pattern compared to those with reduced dispersal. Here, we use non-invasive genetic sampling of faeces to evaluate how isolation-by-distance is differentially structured in jaguar and puma populations in Mexico. We have optimized and validated a reliable and standardized non-invasive genetic sampling protocol to monitor pumas based on 12 microsatellite markers, as well as applied a previously published and consistent protocol for jaguars. We found that jaguars and pumas were not uniform and panmictic populations. Spatial trends in allele frequencies for both species generated clinal patterns. However, pumas showed a stronger isolation-by-distance pattern than jaguars, which was expected since pumas seem to have a lower dispersal ability than jaguars. The genetic structures of both species differed at the level of subpopulations. These results reinforce the differences in intensity of isolation-by-distance between two generalist species with high dispersal ability.

**Keywords** Faeces · Genetic clusters · Microsatellite markers · Spatial principal component analysis

## Introduction

Dispersal is a mechanism that ensures gene flow among populations and homogenizes allele frequencies (Slatkin 1985). It is strongly influenced by species dispersal ability, as individual movements permit gene transfer among populations and subpopulations (Lenormand 2002; François and Durand 2010). The relationship between gene flow and species dispersal abilities is explained by isolation-by-distance patterns and its variations, such as 'isolation-by-effective distance' or 'resistance' (Sexton et al. 2014). The principle is simple; neighbours are genetically more similar than distant individuals, but the genetic variation among distant individuals decreases for species with greater dispersal abilities (Wright 1943).

Studies of gene flow are difficult for rare and low density species because genetic samples traditionally involved animal captures. To circumvent this difficulty, non-invasive genetic sampling of faeces has emerged as a reliable means of sourcing biological material from elusive species (Palomares et al. 2002; Wei et al. 2012; Ramón-Laca et al. 2015). This method has proven particularly successful for elusive felids

**Electronic supplementary material** The online version of this article (doi:10.1007/s10344-016-1019-8) contains supplementary material, which is available to authorized users.

✉ Marina Zanin
marinazaning@gmail.com

[1] Departamento de Biologia, Universidade Federal do Espírito Santo, Centro de Ciências Humanas e Naturais, Vitória, Espírito Santo, Brazil

[2] Departamento de Biología de la Conservación, Estación Biológica de Doñana - CSIC, Sevilla, Spain

[3] Departamento de Ecologia, Universidade Federal de Goiás, Goiânia, Goiás, Brazil

[4] Universidad Autónoma Metropolitana-Unidad Lerma, Lerma, Estado de México, México

[5] Universidad Autónoma de Sinaloa, Escuela de Biología, Culiacán Rosales, Sinaloa, México

Published online: 03 May 2016

(Bhagavatula and Singh 2006; Perez et al. 2006; Mondol et al. 2009; Roques et al. 2014), which commonly use faeces to mark territories, depositing it in visible locations (Davison et al. 2002).

Puma (*Puma concolor*, Linnaeus, 1771) and jaguar (*Panthera onca*, Linnaeus 1758) are elusive felids for which investigations of gene flow can be important due to their considerable environmental plasticity and dispersal abilities. Currently, pumas and jaguars are not listed as threatened according to the IUCN Red List (IUCN 2015), mainly due to their wide continental distributions, which gives more options for conservation. However, localized studies have diagnosed habitat loss and fragmentation as significant threats for both species, which generate isolated (Balkenhol et al. 2009; Haag et al. 2010; Andreasen et al. 2012) and unviable populations (Reed 2004; Hostetler et al. 2012; Galetti et al. 2013). These effects seem to be more severe for the jaguar than for the puma (IUCN 2016) because jaguars are less tolerant to anthropogenic alterations (De Angelo et al. 2011). Overall, both species are displaying a general trend of population decline (IUCN 2015), and it seems that their conservation status will probably worsen in the near future (Zanin et al. 2015).

Here, we used non-invasive genetic samples to examine variations in local and regional genetic structuring of pumas and jaguars in Mexico. We first optimized and validated a reliable, efficient and standardized non-invasive genetic sampling protocol for individual identification of pumas, following the recently published protocol for jaguars (Roques et al. 2014). Since dispersal capacity normally correlates positively with body size (Whitmee and Orme 2013), we expected greater dispersal ability for jaguars compared to pumas. Thus, we predicted a smoother isolation-by-distance signal for jaguars than pumas throughout the study area. Populations or subpopulations should also be differentially structured in both species because these structures are mediated by dispersal ability (Waples and Gaggiotti 2006), so we also expected lower genetic structuring (i.e. fewer clusters) for jaguars compared to pumas due to enhanced genetic transfer in the former.

### Study area

Our study sites were located in Mexico, between longitudes 107° 52′ 12″ W and 86° 31′ 48″ W and latitudes 24° 27′ N and 14° 28′ 48″ N (Fig. 1). This region still contains a large proportion of pristine habitat, composed of grasslands, scrublands, temperate forests, and lowland, medium and montane forests (Bontemps et al. 2011). The study area also contains anthropogenic landcover, especially agricultural and urban areas (Bontemps et al. 2011).

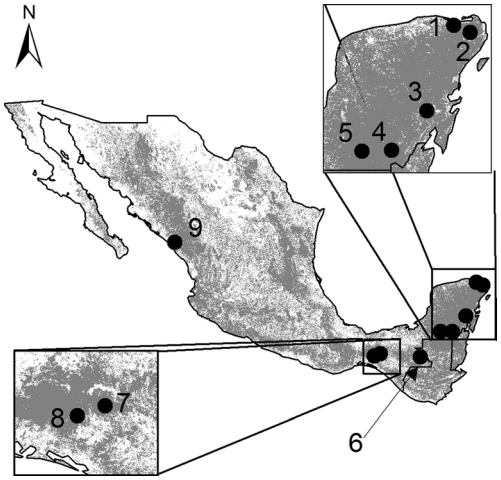


**Fig. 1** Location of sampling sites in Mexico: (*1*) El Edén; (*2*) El Zapotal; (*3*) Petcacab; (*4*) Ejido Caoba; (*5*) Calakmul; (*6*) La Cojolita; (*7*) Selva El Ocote; (*8*) Los Ocotones; and (*9*) El Carmen. *Grey colour* represents native vegetation cover

## Methods

### Sample collection and preservation

Faecal samples were collected from nine locations within the study area (Fig. 1). Distances among sampling locations varied from 60 to 1900 km. Samples were sought between 2005 and 2012 by actively searching along dirt roads and trails by experienced people. Samples were stored in 100 ml plastic containers with silica gel and the locations were georeferenced using a GPS.

### DNA extraction for species and individual identification

DNA extraction of faecal samples was conducted according to GuSCN/silica protocols (Boom et al. 1990; Höss and Svante 1993; Frantz et al. 2003). Each batch of extractions ($n = 12$–15) included one PCR negative extraction control to monitor contamination by exogenous DNA. DNA extractions of faecal samples were performed in a UV-sterilized laminar flow hood in an isolated laboratory specially designated for the manipulation of non-invasively sourced biological material. Faecal samples were screened for species identity using species-specific primers (Roques et al. 2011). DNA extraction, species identification and marker amplifications are described in the Supplementary Material 1.

Individual genotyping for jaguars was conducted using an optimized set of 11 microsatellite markers (Palomares et al. 2012; Roques et al. 2014). For individual identifications of puma, we first tested 18 microsatellite loci: 8 microsatellite loci were originally developed for *Felis catus* (Fca043, Fca045, Fca077, Fca82b, Fca090, Fca126, Fca547b and Fca566b; Menotti-Raymond et al. 1999) and the other 10 markers were originally described for *Puma concolor*

(PcoA208w, PcoA216w, PcoC217w, PcoB316w, PcoA339w, PcoB003w, PcoB010w, PcoB210w, PcoC108w and PcoC112w; Kurushima et al. 2006). From these 18 initial markers, we selected the 12 that worked best on faecal samples based on their genetic variability indices (polymorphism, probability of identity, allelic diversity, observed and expected heterozygosity), PCR amplification success and error rates (allelic dropout and false alleles; Table S1). For full details of puma microsatellite marker selection, see Supplementary Material 1.

After establishing the microsatellite markers for both species, samples were genotyped four times and a consensus genotype was constructed for each sample. To consider a locus as being homozygous, the same allele had to be observed in at least three replicates, without observing a different allele in the fourth. Heterozygous loci were those with two different alleles in at least two replicates.

### Isolation-by-distance among individuals

We used Spatial Principal Component Analyses (sPCA) to summarize both the genetic diversity and to reveal any possible isolation-by-distance pattern. As for classical Principal Component Analysis (PCA), sPCA is an ordination method to consolidate variables, but it has the advantage of optimizing the data variance for principal component scores as well as encompassing spatial structure (Jombart et al. 2008). Therefore, when applied to allele frequency data, the genetic variability among individuals is expressed into a few uncorrelated components, which maximize genotypic variance while taking spatial information into account (Jombart et al. 2008). Moreover, the use of sPCA to explore genetic data does not require populations to be in Hardy-Weinberg equilibrium or linkage equilibrium, since it is not based on a genetic model (Jombart et al. 2008).

This approach requires the generation of a connection network to define the neighbouring sites, transforming the PCA into a spatially explicit method. We used a distance-based neighbourhood graph with 100 km as a threshold of maximal distance between connected individuals as this is a reasonable average of species home-ranges (Cullen Jr. 2006; Cavalcanti and Gese 2009). This type of connection network is recommended for data with an aggregated distribution (Jombart et al. 2008), such as those used in this study. Spatial structure was examined by conducting Moran's I test (Moran 1948; Moran 1950), which may assume positive or negative values. Therefore, the sPCA eigenvalues can reveal two types of spatial pattern: positive Moran's I (global structure) and negative Moran's I (local structure) (*sensu* Thioulouse et al. 1995). A pattern of global spatial structure occurs when the allelic frequencies among neighbours are more similar than those of a random distribution, whereas a local spatial structure occurs when the allelic frequencies among neighbours are more dissimilar than those of a random distribution (Jombart et al. 2008).

Selection of principal components was conducted graphically, balancing the genetic variability and spatial structure expressed in the principal components. The criterion of percentage variability explained by eigenvalues (common in a classical PCA) cannot be applied in a sPCA because the principal components also express the product of spatial autocorrelation. Therefore, we considered the abrupt decrease in information contained in the eigenvalues as a threshold to select principal components (Legendre and Legendre 1998). Due to the somewhat subjectivity of our criterion, global and local tests (Jombart et al. 2008) were applied to confirm the presence of a spatial pattern in the axes. These analyses were done in R software (R Core Team 2013) using the *adegenet* package (Jombart and Ahmed 2011).

### Genetic clustering analysis

Bayesian cluster analyses were performed to investigate population structure in the dataset, i.e. to assign individuals into clusters. It has been recommended to confirm genetic structure patterns using both non-spatial and spatial approaches (Chen et al. 2007; Frantz et al. 2009), so we employed the STRUCTURE (non-spatial—Pritchard et al. 2000; Falush et al. 2003) and TESS (spatial—Chen et al. 2007; Durand et al. 2009) software packages to identify populations. Both of these software packages use Markov Chain Monte Carlo algorithms to identify $k$ populations without a priori group definition. They also have the option of applying an admixture model, which permits efficient classification of individuals into a population even if the source population has not been sampled (Durand et al. 2009). The main difference between the two algorithms applied by these packages is that TESS assumes geographical continuity of allele frequencies, which infers that neighbouring sites are more similar than distant sites (François et al. 2006). This feature allows TESS to detect clines and/or clusters, making it the most efficient Bayes algorithm applied to data on the effects of isolation-by-distance (François and Durand 2010).

We ran admixture models in STRUCTURE and TESS, using 10,000 iterations after a burn-in period of 100,000 iterations, for $k=1$–9 for STRUCTURE and $k=2$–9 for TESS, with 10 independent runs for each value of $k$. For the non-spatial model, the logarithms of the probability of the data (LnP(D); Pritchard et al. 2000) and $\Delta k$ (Evanno et al. 2005) were plotted against $k$ to identify the plateau of the curve and, consequently, to estimate the number of clusters (François and Durand 2010); for the spatial model, this relationship was determined using the deviance information criterion (DIC; Spiegelhalter et al. 2002). The CLUMPP software (Jakobsson and Rosenberg 2007) was used to average the

admixture proportions of individuals over the 10 replicates of the most likely *k*.

These Bayesian methods are efficient for assigning individuals into populations, but they may fail to identify subdivisions within populations (Frantz et al. 2009; Jombart et al. 2010). Thus, a classical statistical analysis may be appropriate to detect fine-scale changes in the genetic configuration because it is not based on models of population genetics. Thus, we performed a Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010). DAPC uses PCA as a prior step to Discriminant Analysis (DA), reducing the dimensionality of genetic data and making the dataset fulfil DA requirements, i.e. have a higher number of sampling units than variables (Legendre and Legendre 1998; Jombart et al. 2010). Then, individuals are assigned to pre-defined clusters by way of increasing between-group variability while reducing within-group variation (Jombart et al. 2010). We used the sampling areas as a priori individual clusters and made a graphical interpretation of individuals' ordination and assignment to define the final demes. For this analysis, we only considered those locations where three or more individuals had been sampled. DAPC was performed using the *adegenet* package (Jombart and Ahmed 2011) in R software (R Core Team 2013).

### Descriptive statistics for genetic clusters and global data

To measure the genetic diversity of the identified groups, we calculated allele richness and rarefied allele richness (using the Hp-Rare software; Kalinowski 2005), and the observed ($H_o$) and expected heterozygosity ($H_e$) under Hardy-Weinberg assumptions (using the FSTAT software; Goudet 2002). Significance of deviations from Hardy-Weinberg equilibrium was evaluated through a Bonferroni correction of *p* values (Rice 1989). We estimated inbreeding for each subpopulation to measure the degree of substructure by calculating $F_{IS}$ over subpopulations and loci using the FSTAT software (Goudet 2002) with 10,000 permutations. The degree of population differentiation was measured by $F_{ST}$ using the SPAGeDi software (Hardy and Vekemans 2013).

## Results

### Samples and individual identification

We collected a total of 205 faecal samples from jaguars and 205 from pumas, ranging between 0–66 and 1–57 samples per study site for each species, respectively (Table 1). Twelve microsatellite markers were selected to genotype pumas from faecal samples (Supplementary Material 1, Table S1). This set of microsatellite markers could be efficiently used to genotype pumas because the probability of two full siblings differing by at least two loci was high (*P* mismatch = 0.007; see Fig. S1 for complete mismatch probability distribution).

We genotyped a total of 185 and 151 faecal samples for pumas and jaguars, respectively. Both amplification and extraction were efficient, as indicated by the Quality Index and number of complete loci (Table 1). The number of alleles per locus ranged between 4 and 12 for pumas and 2 and 16 for jaguars (Table 1). The differences between observed and expected heterozygosity did not suggest deviation from Hardy-Weinberg equilibrium (Table S2 and S3). We identified 67 unique genotypes (individuals) for pumas and 34 for jaguar (Table 1).

### Clinal patterns

We selected the first principal component from each sPCA because the eigenvalues decreased strongly thereafter (Fig. S2). The selected principal components summarized a significant amount of the genetic variance (Variance ≈ 0.3 for both species) and captured the spatial structure (Fig. S2). A global test confirmed the global spatial pattern indicated by a positive eigenvalue score (global test; puma = 0.06, $p < 0.01$; jaguar = 0.09, $p < 0.01$), and there was no evidence for a local spatial pattern for either species (local test; puma = 0.03, $p = 0.18$; jaguar = 0.05, $p = 0.62$). This result suggests a gradual change in genetic configuration (i.e. an isolation-by-distance pattern) for both species. However, the transition is more gradual for jaguars than pumas because their higher scores suggest more similar allelic frequencies in the global data.

### Population structure

We observed differing numbers of clusters in each species according to the plateauing curve of the Bayesian methods used. STRUCTURE inferred two populations for pumas and eight for jaguars according to the $\Delta k$ scores (Fig. 2a, b), while LnP(D) scores suggested five populations for pumas (Fig. 2c), and the results were inconclusive for jaguars (Fig. 2d). The DIC score estimated by TESS identified two as the most likely *k* for both species (Fig. 2e, f).

STRUCTURE and TESS had similar assignment proportions for puma at $k = 2$, for which we identified a clear discontinuity in assignment proportions in the region of El Carmen (location 9), suggesting that pumas from that locality are a different population from the pumas of our eastern study sites (Fig. 3). A similar pattern was observed for jaguars analysed by TESS (Fig. 3). The other assignment proportions generated by the Structure software (puma $k = 5$; jaguar $k = 2$ and 8; Fig. 2) showed a continuous and unbroken allele frequency distribution, making it impossible to define clusters and suggesting

**Table 1** General results for each sampling location and faecal DNA amplification success for pumas and jaguars. *NFS* number of faecal samples, *GEN* number of genotypes identified, *QI* mean quality index (the mean efficiency of amplification in four Polymerase Chain Reaction - PCR), *SD* standard deviation, *NL* mean number of loci, and *IN* number of individuals (males/females indicated in parenthesis)

| Sampling location | Puma | | | | | Jaguar | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NFS | GEN | QI (SD) | NL (SD) | IN (M/F) | NFS | GEN | QI (SD) | NL (SD) | IN (M/F) |
| El Edén | 36 | 32 | 0.66 (0.28) | 8.50 (3.53) | 13 (6:7) | 62 | 37 | 0.65 (0.27) | 7.70 (3.19) | 6 (6:0) |
| El Zapotal | 57 | 53 | 0.72 (0.27) | 9.08 (3.30) | 13 (5:8) | 66 | 51 | 0.77 (0.26) | 7.41 (3.74) | 7 (7:0) |
| Petcacab | 14 | 14 | 0.62 (0.23) | 7.93 (2.95) | 7 (2:5) | 25 | 18 | 0.67 (0.34) | 7.64 (3.63) | 5 (4:1) |
| Ejido Caoba | 13 | 13 | 0.84 (0.17) | 10.23 (1.92) | 4 (1:3) | 16 | 14 | 0.81 (0.11) | 10.00 (2.00) | 4 (3:1) |
| Calakmul | 27 | 23 | 0.72 (0.27) | 8.74 (3.29) | 9 (3:6) | 23 | 20 | 0.75 (0.19) | 8.75 (2.02) | 7 (6:1) |
| La Cojolita | 3 | 3 | 0.59 (0.51) | 7.00 (6.08) | 2 (1:1) | 0 | – | – | – | – |
| Selva El Ocote | 1 | 1 | 1 | 12.00 (0.00) | 1 (0:1) | 0 | – | – | – | – |
| Los Ocotones | 23 | 21 | 0.85 (0.23) | 10.29 (2.78) | 3 (2:1) | 2 | 1 | 0.73 | 9.00 (0.00) | 1 (1:0) |
| El Carmen | 31 | 25 | 0.76 (0.13) | 9.56 (1.47) | 14 (7:7) | 11 | 10 | 0.85 (0.19) | 9.00 (2.34) | 4 (2:2) |
| Overall | 205 | 185 | 0.73 (0.25) | 9.11 (3.07) | 66 (25:39) | 205 | 151 | 0.74 (0.25) | 8.00 (3.17) | 34 (29:5) |

that these $k$ numbers were overestimated (Fig. 3). The fixation index confirmed genetic differentiation among puma populations ($F_{ST} = 0.08$, $p < 0.01$) and jaguar populations ($F_{ST} = 0.15$, $p < 0.01$).

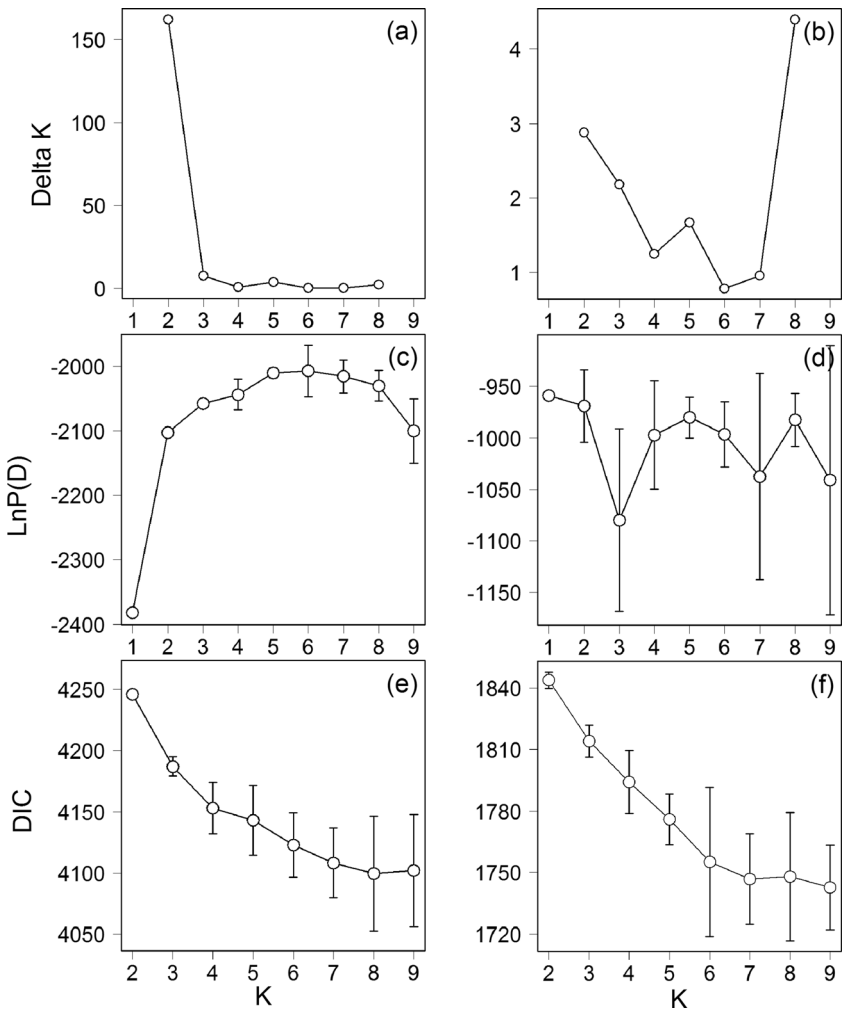


**Fig. 2** Number of genetic clusters ($k$) estimated by STRUCTURE (**a**–**d**) and TESS (**e**, **f**) for pumas (*left panels*) and jaguars (*right panels*). $\Delta k$ results show $k = 2$ for pumas and $k = 8$ for jaguar (**a** and **b**, respectively) while average log likelihood LnP(D) suggest $k = 5$ for pumas and was inconclusive for jaguars (**c** and **d**). The deviance information criterion (DIC) scores computed by the TESS admixture model indicated $k = 2$ for both species. The intervals represented in figures **c**–**f** are the standard deviations

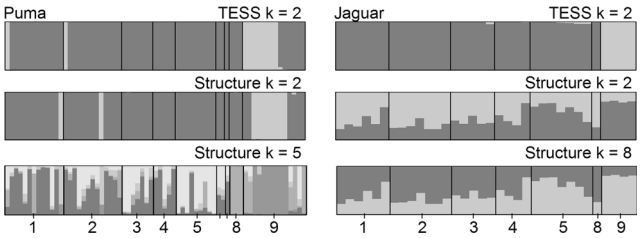


**Fig. 3** Bar plots showing the assignment proportions of individuals from STRUCTURE and TESS cluster analyses. *Left-hand-side and right-hand-side panels* are assignments for different *k* groups for pumas and jaguars, respectively. *Numbers along the bottom panels* indicate the sampling location of individuals (see Fig. 1)

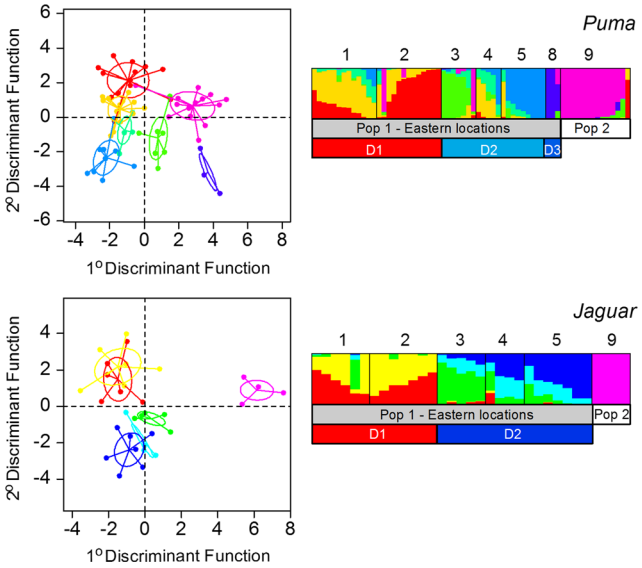


**Fig. 4** Summary of Discriminant Analysis of Principal Component results. Genotype ordination for pumas and jaguars are given in the *left-hand-side panels*, showing differentiation into groups (points and circle of the same colour) and between-group variation (distances between group centroids). The *right-hand-side panels* show the assignment proportions of individuals for each sampling location (numbers above panels, see Fig. 1 legend) and the clusters adopted in this work (populations, and demes—*D*)

## Subpopulation structure

For the DAPC clustering method, we selected the principal components accumulating 80 % of the total variation, which consisted of 6 eigenvalues for puma and 5 eigenvalues for jaguar. The final ordination was conducted with the first two discriminant functions because we observed a strong reduction in explanatory power after the second function. The average assignment probability of DAPC was higher for pumas than for jaguars (puma = 0.85 and jaguar = 0.67). Therefore, we did an additional analysis to evaluate if the higher efficiency for pumas could reveal a biological pattern worthy of investigation, or if it was only a mathematical artefact due to having sampled a higher number of individuals and, thus, disproportionately having greater statistical power. We evaluated the average assignment probability of DAPC by means of 10,000 random subsamples of 33 pumas, i.e. the same sample size for jaguars used in our DAPC analysis. Average assignment probabilities were not different for puma and jaguar considering a dataset of the same size (average assignment probability among 10,000 random puma data set = 0.80; proportion of simulations for pumas with assignment probability lower than that observed for jaguar = 0.07).

DAPC analysis indicates that there is a general genetic gradient for both species. Even within a gradual transition of genetic variation, it was possible to identify breaks in ordination and assignment probabilities suggesting that pumas and jaguars are structured into demes. El Carmen (location 9; population 2) was considered a different cluster for both species, which is expected since it was also identified as a different population by TESS, but jaguars from El Carmen were more isolated than pumas (Fig. 4). We observed a pattern of gradual transition of assignment probabilities between individuals from El Eden and Zapotal (locations 1 and 2—deme 1) for both species, and also among individuals of Petcacab, Ejido Caoba, Calakmul and La Cojolita (locations 3 to 6—deme 2; Fig. 4). Pairwise $F_{ST}$ values confirm the existence of these demes for both species ($F_{ST}$ between demes 1 and 2; puma = 0.03 and jaguar = 0.04). Pumas from Los Ocotones also constituted a different cluster according to DAPC analysis (deme 3; Fig. 4) and differed from demes 1 and 2 (pairwise $F_{ST}$ for pumas: demes 1 and 3 = 0.17; demes 2 and 3 = 0.06). Therefore, we reveal population subdivision from eastern locations (population 1), which could be considered subpopulation genetic structuring for both species. Overall then, we identified two populations for both species from our dataset, one of which could be divided into three subpopulations for pumas and two subpopulations for jaguars (Fig. 4).

We adopted these DAPC clusters to carry out diversity estimates. All loci were polymorphic for both species; the number of alleles across loci in the subpopulations ranged from 1 to 14 for pumas and from 1 to 12 for jaguars (sees Tables S2 and S3 for estimates by locus). Following a rarefaction procedure, the average allele richness over loci ranged from 1.67 to 4.00 for pumas and from 2.57 to 2.79 for jaguars (Table 2). No pair of loci was at linkage disequilibrium after Bonferroni correction for multiple comparisons. In addition, following Bonferroni correction, there was no evidence of inbreeding in subpopulations for either species (Table 1). Pumas exhibited higher variation in genetic diversity than jaguar, for both expected and observed heterozygosities (Table 2).

## Discussion

The efficiency of our genetic profiling protocol for pumas was confirmed through the low probability of mismatch

**Table 2** Genetic diversity estimates for puma (12 microsatellite markers) and jaguar (11 microsatellite markers) subpopulations. AR—rarefied allelic richness averaged over loci; $H_o$—observed heterozygosity; $H_e$—expected heterozygosity; SD—standard deviation; $F_{IS}$—inbreeding (for subpopulations)

| Demes | AR | $H_o$ (SD) | $H_e$ (SD) | $F_{IS}$ |
|---|---|---|---|---|
| *Puma concolor* | | | | |
| El Edén and Zapotal | 3.22 | 0.61 (0.08) | 0.65 (0.11) | 0.12 |
| Petcacab, Cojolita, and Calakmul | 3.29 | 0.65 (0.11) | 0.66 (0.11) | 0.06 |
| Ocotones | 1.67 | 0.69 (0.33) | 0.55 (0.24) | −0.22 |
| El Carmen | 4.00 | 0.71 (0.11) | 0.76 (0.11) | 0.12 |
| *Panthera onca* | | | | |
| El Edén and Zapotal | 2.79 | 0.62 (0.26) | 0.58 (0.23) | −0.06 |
| Petcacab, Caoba, Calakmul and Ocotones | 3.24 | 0.70 (0.19) | 0.66 (0.17) | −0.03 |
| El Carmen | 2.57 | 0.64 (0.20) | 0.59 (0.16) | −0.03 |

and average of the quality index, which was similar to that generated for jaguar and higher than obtained in other studies (Miquel et al. 2006; Haag et al. 2010). The puma and jaguar individual identification protocols enhance our ability to study these elusive felids, even in tropical environments where faecal samples and DNA often degrade quickly due to the high humidity (e.g. Farrell et al. 2000; Brinkman and Hundertmark 2009). Therefore, our results reinforce the huge potential of faecal samples to provide reliable information for genetic and ecological studies.

Overall, the genetic variation revealed that, in our study area, there is not a uniform and panmictic population for either species. The gradual transition across all sampled locations suggests an isolation-by-distance pattern of genetic variation. As we predicted, this gradual change was smoother for jaguars than for pumas, in agreement with the expected higher dispersal ability of the former due to their larger body size. Our species comparison reveals that even two large-bodied felid species, both capable of long-distance dispersal, can differ in the intensity of genetic structuring mediated by isolation-by-distance.

However, isolation-by-distance did not preclude classification of individuals into more than one cluster. The two populations identified for both species suggested a genetic discontinuity between Los Ocotones and El Carmen, perhaps located in the intervening Madre del Sur Mountains, which reach 2500 m above sea level and might work as a biogeographical barrier. Moreover, habitat fragmentation is higher between these two areas, which could exacerbate the barrier effect between localities. As demonstrated by the $F_{ST}$ index, pumas from El Carmen are genetically more similar to eastern individuals than jaguars are. Despite the expectation of a lower dispersal ability for pumas due to their lower body size compared to jaguars, pumas can also be expected to be better dispersers in anthropogenic habitats due to their higher tolerance to anthropogenic cover (Di Bitetti et al. 2010). Therefore, for anthropogenic landscapes, we could observe the opposite pattern than that predicted in our study, which might also explain the lower isolation among puma populations we observed, considering that the habitat of the transition zone is markedly anthropogenic.

Focussed analysis of the eastern populations, which is composed mainly by native vegetation, both species revealed that gene flow is not homogeneous within populations, with groups of individuals organized into subpopulations. In accordance with our predictions, pumas exhibited a higher number of clusters, which we attribute to the lower dispersal ability of the species (Whitmee and Orme 2013). Similar results were found for tigers and leopards in Indian landscapes (Dutta et al. 2013; Sharma et al. 2013), reinforcing that even felines, which exhibit large body size and large dispersal distances, can exhibit genetic structuring at the regional to local scale.

In a conservation context, we did not observe signals of inbreeding depression for any subpopulation or species, and the genetic diversity indices are similar to those of other feline populations (Loxterman 2011; Miotto et al. 2011; Andreasen et al. 2012; Dutta et al. 2013). However, habitat fragmentation between these populations might represent a potential risk for both species through loss of genetic diversity due to a reduction in gene flow and population viability (Flather and Bevers 2002; Ovaskainen and Hanski 2003; Cushman et al. 2006). Species conservation not only relates to maintenance of genetic diversity and avoidance of inbreeding depression, it is also necessary to secure evolutionary and demographic processes. Therefore, the current scenario of a lack of signal for inbreeding depression should not be viewed as a reason to not carry out management strategies. Instead, it should be seen as an opportune time to evaluate the future consequences of recent human-induced changes and to design appropriate conservation plans to avoid further genetic erosion.

**Acknowledgments** This study was supported by Fundación BBVA (project BIOCON 05–100/06) and the Spanish Ministry of Science and Innovation (project CGL2010-16902) for the funds of this work.

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