Population Genetic Structure and Habitat Connectivity for Jaguar (Panthera onca) Conservation in Belize

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Abstract

Background: Effective connectivity between jaguar (Panthera onca) populations across the American continent will ensure the natural gene flow and the long-term survival of the species throughout its range. Jaguar conservation efforts have focused primarily on connecting suitable habitat in a broad-scale. However, accelerated habitat reduction, limited funding, and the complexity of jaguar behaviour have proven challenging to maintain connectivity between populations effectively. Here we used individual-based genetic analysis in synthesis with landscape permeability models to assess levels of current genetic connectivity and identify alternative corridors for jaguar movement between two core areas in central and southern Belize. Results: We use 12 highly polymorphic microsatellite loci to identify 50 distinct individual jaguars, including 41 males, 3 females and 6 undetermined animals, from scat samples collected in The Cockscomb Basin Wildlife Sanctuary and The Central Belize Corridor. Using Bayesian and multivariate models of genetic structure, we identified one single group across two sampling sites with low genetic differentiation between them. We used fine-scale data on biodiversity features as vegetation types to predict the most probable corridors using least-cost path analysis and circuit theory. Conclusions: The results of our study highlight the importance of expanding the boundaries of the Central Belize Corridor to effectively cover areas that would more easily facilitate jaguar movement between locations.

Background

The jaguar (Panthera onca) is under threat from loss of prey, poaching, habitat destruction, and climate change. Dwindling numbers have put the species on the IUCN Red List as ‘Near-Threatened’ (IUCN 2015). This ever-elusive cat has been an emblem of power to the Maya and a flagship species for the conservation of Mesoamerican forests. Illegal
hunting for its spotted skin and conflict with local ranchers as a potential threat to their cattle caused its inclusion on CITES appendix I (1). A species that was once considered sacred needs comprehensive studies that effectively link fragmented habitat, so that genetic communication can occur between populations (2,3).

Despite its endangered status, efforts to resolve conservation issues have been limited by the difficulty to study this species in the wild. Jaguars are elusive top predators of Neotropical forests that occur at low densities, have large home ranges and require vast areas to harbour viable populations. After a century of being persecuted and suffering habitat loss, jaguars are now restricted to the less than 30% remaining intact patches of forest in Central and South America (4–6). To further reduce the risk of extinction, adequate management of jaguar populations requires connecting them through habitat corridors that allow individuals to disperse and exchange genes (7–9). Habitat deterioration, human-wildlife conflict and the complex dynamics of jaguar populations are rapidly shaping the patterns of genetic diversity we see today (2,10,11). Knowledge of the demographic history, movement patterns, behaviour and genetic diversity of a species are critical components to make adequate conservations decisions (8,12). Furthermore, detailed information at the subpopulation level is valuable for determining geographical conservation priorities (13). The acquisition of data to study elusive predators can prove challenging, but new technologies such as non-invasive genetic monitoring and remote sensing have proven advantageous for identifying genetic discontinuities associated to habitat structure (7,8,14,15).

Genetic information derived from non-invasive sampling can be combined with landscape ecology to improve our understanding of the effect of environmental variables on genetic patterns and evolutionary processes (16,17). The integration of population genetics with landscape ecology and spatial statistics provides a toolbox to study genetic connectivity
by correlating the spatial heterogeneity of landscapes with estimates of gene flow (18). This approach provides important opportunities to identify areas of conservation priority and provide critical knowledge on habitat fragmentation, dispersal ecology, and functional connectivity in complex landscapes for effective conservation planning and management actions (19).

The establishment of corridors to improve population connectivity, particularly in Mesoamerica, has been among some of the most important efforts to prevent the loss of biodiversity in the world’s biologically richest regions (20). One of the most important jaguar populations in Mesoamerica can be found in the forests of Belize, a core and critical area for the species throughout its range (21). According to the Belize National Protected Areas System Plan (22), 36% of Belize land territory is under conservation management. In particular, with its 390 square kilometres of protected forests, The Cockscomb Basin Wildlife Sanctuary harbours one of the largest concentrations of jaguars in the country (21,23). This area is connected to other Natural Protected Areas via the Central Belize Corridor, which extends over 750 square kilometres and is considered the most critical and important corridor of the Belize National Protected Areas Systems (24). Range-wide corridors have been established as a major tool to improve population connectivity and thus aid the subsistence of jaguars (5). However, to build a well-connected protection network, considerations must be taken on the spatial scale at which conservation strategies are implemented.

Broad-scale conservation efforts will benefit from information gained at a finer scale, especially in heterogeneous or fragmented areas (3,9,25). The effective collaboration among scientists, practitioners, non-governmental organisations and politicians will tap the full potential of reservoir projects and conservation actions across the jaguar’s range. Here, we present a comprehensive study on genetic structuring and patterns of landscape
heterogeneity to identify alternative habitat corridors for gene flow between populations within two principal locations within a jaguar stronghold in Belize. Using jaguar scat samples, we investigate population genetic structure, levels of inbreeding and gene flow. Additionally, we correlate landscape features and patterns of gene flow to examine landscape permeability for jaguars between The Cockscomb Basin Wildlife Sanctuary and The Central Belize Corridor.

Results

Genetic variation

A total of 536 scat samples collected across the two sampling areas were positively matched to *P. onca*. Other identified species included *Puma concolor, Leopardus wiedii*, *Leopardus pardalis, Herpailurus yaguarondi,* and *Canis familiaris*. Genotyping revealed a total of 50 unique multilocus genotypes (37 from the Cockscomb reserve and 13 from the Central Corridor); these included 41 prospective males, 3 prospective females and 6 unidentified genders (Additional file 3). MICROCHEKER detected three loci (FCA 212, 229, and 075), showing signs of a null allele, but did not find evidence of scoring mistakes or large allele dropout (Additional file 1).

Twelve microsatellite loci were successfully amplified with a mean expected heterozygosity $H_E = 0.61$ (SD = 0.042), and a mean observed heterozygosity $H_O = 0.55$ (SD = 0.05). The mean number of alleles per locus (NA = 9.33) ranged from 3–10 (Table1). The mean polymorphic information content $PIC = 0.642$. The genotyping results allowed the identification of 50 distinct individual jaguars, 37 corresponding to Cockscomb Reserve and 13 to Central Corridor, the geographical coordinates assigned to each individual was determined by averaging the coordinates of all the samples corresponding to that particular individual. Tests for departure from Hardy-Weinberg Equilibrium were
variable for each locus, with four loci deviating from HWE (Table 1). This deviation could be explained by a deficit of heterozygotes within the population potentially caused by inbreeding or by the presence of null alleles ($F_{IS} = 0.22$, $p$-value = 0.001). Furthermore, this test showed evidence of low genetic differentiation ($F_{ST} = 0.021$, $p$-value = 0.007; $F_{IT} = 0.237$, $p$-value = 0.001); linkage disequilibrium was not significant for any pair of loci. The PCA of genetic diversity shows overlapping of the two sites, indicating overlapping of allele frequencies and little differentiation between groups (Figure 1D). The AMOVA analysis revealed that less than 2% of genetic variance occurred among individuals in the Cockscomb Reserve and individuals in the Central Corridor; and showed low levels of genetic differentiation between groups ($F_{ST} = 0.015$, $p$-value = 0.026). Results from the Mantel test showed significant evidence of isolation by distance ($R_{xy} = 0.167$, $p$-value = 0.01; Figure 1C).

Population Structure and relatedness

Data analysis using STRUCTURE revealed that $k = 1$ had the highest mean probability of density value, and $k = 4$ had the highest delta-K value (Figure 1B). This was consistent with the results from TESS where $k = 1$ also had the highest probability ($\Delta K = 10.15$), in both cases no clear pattern of genetic structure can be observed when rendering the assignment probability in bar plots (Figure 1B). Results from GENELAND revealed that $k = 1$ also had the highest probability in 8 of 10 runs, and the final map does not show a clear population boundary between sampling sites (Figure 1E). The DAPC analysis showed that the lowest BIC value (68.42) corresponded to $K = 2$ and is represented in a single discriminant function; however, the BIC difference between $K = 2$ and $K = 1$ is negligible (~1).

The performance of the seven relatedness estimators was analysed to provide information on the degree of resolution expected in our dataset. Mean relatedness amongst
individuals from the Central Corridor was \(-0.046 \pm 0.068\) (SE = 0.008) and from Cockscomb Reserve \(-0.15 \pm 0.086\) (SE = 0.003). Amongst all individuals mean relatedness was \(-0.01 \pm SD 0.08\) (SE = 0.002). Overall, individuals from the Central Corridor were more closely related to each other than to those in the Cockscomb Reserve.

**Landscape permeability**

The least-cost path analysis inferred the best route from the most northern point in the Central Corridor to the most southern point in the Cockscomb Reserve with a total length from the origin to the destination of 152,502.7m, and a straightness index of 1.4255 with 106,979.2 m (Figure 1A, see Additional file 4). Alternative paths connecting both areas and considering all individuals were detected using CIRCUITSCAPE (Figure 1A, see Additional file 4). Both analyses coincide that an important path connecting both areas falls outside any Natural Protected Areas or the Biological Corridor in its most western point at approximately Latitude 17.1963 Longitude -88.6791 covering an area of approximately 117 square kilometres. Also noteworthy was the break that occurs in Hummingbird Highway between Cayo District and Stann Creek District (Figure 1A, see Additional file 4).

**Discussion**

**Population genetics**

This study presents estimates of genetic variation for individuals sampled within two core areas in Central and Southern Belize. Twelve polymorphic microsatellite loci were useful to successfully identify 50 distinct individuals that correspond to 41 males, 3 females and 6 undetermined sexes. The relatively low number of females could be explained by the sampling methodology rather than reflecting the proportion of sexes in the area. Sampling was conducted close to paths or dirt roads, and closer to human settlements; because
females could be more elusive, have smaller home ranges, hide their scats and avoid crossing open spaces and wide paths (3,12,26) this method could favour the sampling of male scats and therefore bias the analysis towards the more frequently observed sex.

Studies on dispersal in large felines show that males are the dispersing sex, while females tend to be more philopatric (9,11,27,28); other measurements for genetic differentiation between sexes and more female scat samples are necessary to confirm sex-biased dispersal in this area.

Data derived from microsatellite analysis was also useful to estimate the genetic diversity, gene flow and population structure within and between the Cockscomb Reserve and the Central Corridor. Overall, our results show evidence of gene flow between sites with low population differentiation (FST = 0.021, between groups component of AMOVA = 0.015) and a lack of heterozygosis within certain loci (Table1). Several methodological approaches were useful to analyse this genetic differentiation and to evaluate if subtle structuring of the jaguar populations occurs. The results from the Bayesian clustering analyses, assigned all 50 individuals to one single cluster and does not show clear patterns of population structure (Figure 1B). Bayesian clustering methods rely on genetic information to ascertain population membership and operate by minimising Hardy-Weinberg and linkage disequilibria (29). Additionally, properties of our data as sample size, number of loci, polymorphisms, and null alleles could have also influenced their performance (30,31). Their power to detect population structure has been shown to decrease in accuracy at very low levels of population differentiation (FST<0.05) (29) as the number of estimated populations can be affected by a violation of model assumptions and cryptic relatedness (32). Furthermore, GENELAND and DAPC also indicated a single cluster as the most probable number of populations. GENELAND assigned individuals to each population considering the sampling locations and measurements of genetic
differentiation; as this method considered spatial autocorrelation and is more able to detect low levels of genetic differentiation, it more accurately reflects the true k (33). Congruent results from our genetic clustering analyses (k = 1) suggest there is no population structuring between individuals in the Central corridor and individuals in the Cockscomb Reserve. The very low levels of genetic differentiation found in this study could be the result of limited dispersal between sampling localities caused by behavioural characteristics of the species, but more likely caused by the presence of significant isolation by distance and a sampling gap between the two regions. Studies conducted with radio telemetry show that jaguars depend on large patches of habitat and can have home ranges that surpass 100 km² (3,21,34) however, females have smaller home ranges and tend to avoid roads and human-dominated landscapes at a higher degree, showing preference for intact forests (3,35). Although having large home ranges and the ability to move considerable distances, jaguars tend to avoid human-dominated areas and show gender-specific differences (3,35). Genetic subdivision across the country has been discussed by Wultsch et al. (2016); suggesting that habitat fragmentation and human disturbance are limiting gene flow within jaguar populations. Other studies have also demonstrated that jaguars are highly sensitive to habitat fragmentation in human-dominated landscapes and that although dispersal capabilities of the species may slow the effect of drift, the effect of large-scale habitat loss and fragmentation may contribute to genetic differentiation within a short period of time (2,8).

Local-scale connectivity

The relatively high levels of gene flow and low genetic differentiation found in our study attest to the success of the corridor established to connect these two areas of Belize, which were a continuum of jaguar habitat in the distant past. These results are especially
informative to aid conservation efforts in other areas of the species range, such as those of the Atlantic Forest of South America, where there is a lack of genetic connectivity among isolated remnant jaguar populations (36). However, anthropogenic barriers (such as Hummingbird highway) could be altering gene flow between core jaguar areas and should alert conservation managers to improve connectivity in future conservation actions and corridor management. The negative impact of roads on jaguar populations should be especially taken into consideration to improve existing corridors or design new ones; road construction and/or expansion within protected areas increases the accessibility of hunters to jaguars and their natural prey, and leads reduction of the potential of using these lands to sustain viable populations of top predators (37). Rabinowitz & Zeller (2010) conducted a range-wide model of landscape connectivity to identify potential corridors that connect jaguar populations across the Americas. Their study provided critical information for conservation actions such as corridor design across the range of the species. However, even if extremely useful for large-scale planning, the model proves more challenging for local or regional corridor design and zoning. The model depends on a least-cost path analysis that relies on coarse-grain environmental data to determine habitat connectivity and could ignore factors that affect how animals utilise the landscape (38). This range-wide corridor could be improved with fine-scale studies that advise targeted-conservation actions (12,38).

Our least-cost path analysis predicted from the most southern point in the Cockscomb Reserve to the most northern point in the Central Corridor falls out of the areas designated as protected or biologically important. Furthermore, using circuit theory, we incorporated landscape features and information derived from all our identified individuals to predict other suitable pathways for gene flow between the two areas. The resistance map of movement probability shows a clear break between the Cockscomb Reserve and
the Central Corridor. This break coincides with ‘Hummingbird Highway’, a clear anthropogenic boundary between the two sites (Figure 1A). Currently, jaguars seem to move across the two sites, but this highway, other roads and urbanisation, in general, could be shaping population structure by presenting physical barriers to gene flow. To improve connectivity between these sites, the coverage of the Central Corridor needs to expand so that its boundaries cover the areas that would more easily facilitate jaguar movement as evidenced in this study. Conservation efforts should focus on habitat restoration of corridor networks that increase the resistance surface linking Cockscomb Reserve and the Central Corridor to secure movement between and across jaguar core areas could include building wildlife crossings where the resistance surface for movement breaks (e.g. highway junctions in Cayo District and Stann Creek District). The uncertainty over the dispersal ability of jaguars and the extent of use of corridors highlight the importance of incorporating data at a regional scale to better delineate corridors that facilitate gene flow (9,39).

Conclusion

Our results provide a screenshot of genetic patterns of animals whose scats were sampled during 2008–2010 and represent an early warning sign of demographic consequences of population subdivision. Strong signals of genetic differentiation or inbreeding might not be so evident before a population experiences an irreversible bottleneck. The results provided here present additional information on potential corridors to maintain gene flow and mitigate the effect of habitat fragmentation and anthropogenic infrastructures that could increase genetic differentiation and potentially subdivide the jaguar population inhabiting two core areas in Belize: The Cockscomb Reserve and the Central Corridor. The results of this research provide a scientific basis for conservation decisions concerning jaguar populations in Belize and land management projects planned for the country in the
future. This research provides critical information that must be taken into consideration in coordinated conservation actions that promote genetic connectivity among isolated remnant jaguar populations across its range.

Methods

Sampling

Scat collection was conducted between 2008–2010 within two areas in Central Belize (Figure 1A) as part of the Global Felid Genetics Program: The Central Belize Corridor and Cockscomb Basin Wildlife Sanctuary. The Central Belize Corridor (17.349140° N, 88.455310° W, 50m elevation) has been identified as an important link between jaguar populations in northern and southern Belize (24). Cockscomb Basin Wildlife Sanctuary (16.7162° N, 88.6608° W, 500 m elevation) houses the highest density of jaguars in Mesoamerica (21,23). These two areas play a crucial role in the maintenance of the Mesoamerican Biological Corridor, comprised of a network of protected areas stretching from Mexico to Panama (21). A total of 852 scats were collected opportunistically along paths, trails, roads, and transects. Samples were georeferenced at the time of collection and stored individually at room temperature using silica gel beads until DNA extractions. All faecal material has been deposited in the Sackler Institute for Comparative Genomics at the American Museum of Natural History.

DNA extraction and species identification

About 200mg of the dry sample was shaved from each scat and used to extract genomic DNA using a QIAamp DNA extraction Stool Mini Kit (Qiagen Inc., Valencia, California, USA). Samples were screened to identify species via PCR using three mitochondrial gene regions including cytochrome b (H15149)(40,41), 12S rDNA (L1085 and H1259)(42) 16S rDNA (L2513 and H2714)(42) and 16Scp (16S cp-F 16S cp-R)(42). PCR amplifications were done
using G&E Ready-to-go PCR Beads (GE Healthcare) in 25ul reaction volumes containing 0.2uM of each primer, 0.3uL BSA and 1ul of DNA. PCR profiles for each reaction are available as supplementary material (see Additional file 2). All amplifications included negative controls. Cycle-sequencing was performed with the BidDye® Terminator v. 1.1 cycle Sequencing kit (Applied Biosystems, Lennik, Belgium) using the PCR primers. Amplified products were visualised and scored in an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA). Sequences were aligned and manually corrected using GENEIOUS v.6.5 (Biomatters Ltd., Auckland, New Zealand). Species identification was achieved by comparing consensus sequences to known felid references and by constructing a phylogenetic tree with 93% similarity using the Jukes-Cantor neighbourhood-joining model (43). Species ID was validated if at least three gene fragments successfully identified the same species.

**Individual and sex identification**

Samples identified as *P. onca* were genotyped for twelve polymorphic microsatellite loci (FCA 32, 75, 96, 107, 126, 100, 124, 132, 212, 229, 275, and 225)(44). Each sample was screened 4–6 times to have enough replicates to assign the alleles correctly. The genotypes were validated if they successfully scored at least three times. Samples that failed to amplify at least six loci were not considered in our analysis. We amplified sets of 3 primers in multiplex PCR reactions and FCA225 by itself. Amplification was performed using the Qiagen Multiplex Master Mix ®, with a final volume of 20ul following manufacturers recommendations.

PCR reaction conditions were optimised in the annealing temperature for each set of primers as follows: 95°C for 15min, followed by 13 cycles of 94 °C for 30s, 57.4–62.4 °C for 90s, 72°C for 60s, followed by 32 cycles of 94°C for 30s, 55–60 °C for 90 s, 72°C for 60s and 60 °C for 30min (see Additional file 2). Amplified products were visualised and
scored in an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA). PCR products were analysed using GeneMapper v4.0 following the quality index measure to validate genotypes (Miquel et al. 2006). In order to further validate the allele calling, we used FRAGMAN v.1.0.8 package in R (45). Identification of unique multilocus genotypes was achieved using the ALLELEMATCH package in R v.2.5 (46), which accommodates for genotyping error and missing data by making pairwise comparisons and finding similarity scores for each pair of profiles and clustering similar ones. To determine if genotypes were not different individuals matched by chance, the cumulative probability of identity for unrelated individuals (PID) and siblings (Psib) was calculated. Loci with Psib < 0.010 were positively identified as single individuals (15).

Gender was determined following the PCR-CTPP method for sex identification developed by Wei et al. (2008) based on zinc finger alleles (ZFX/ZFY). The two forward primers (ZF-1F and ZFY-2F) were fluorescently labelled with FAM and MAX, respectively. Amplification was performed using G&E Ready-to-go PCR Beads (GE Healthcare) in a final volume of 25ul containing 2mM of each primer and 5ul of DNA. The PCR conditions were as follows: 95°C for 5min, followed by 13 cycles of 95 °C for 45s, 63 °C for 60s, 72°C for 60s, followed by 35 cycles of 95°C for 45s, 53 °C for 45s, 72°C for 60s and 72 °C for 10min (see Additional file 2). DNA amplifications were visualised in a 5% agarose gel to confirm the molecular sexing. Amplified products were visualised and scored in an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA). PCR products were analysed using GeneMapper v4.0.

Population genetics analyses

MICROCHECKER v 2.2.3 (47) was used to screen null alleles in each locus. The rarefaction procedure implemented in HIERFSTAT v 2.9.3.250 (48) was used to estimate the expected number of alleles and to compare allelic richness (r). Measures of genetic diversity as average number of alleles per locus (A), allelic richness (AR), observed (Ho) and...
expected heterozygosity \((He)\) deviations from Hardy-Weinberg equilibrium \((HWE)\), inbreeding coefficient \((FIS)\), and multivariate analyses were performed using the ADEGENET package in R v.2.0.1 (49). Genetic diversity among sampled individuals was summarised in a Principal Component Analysis (PCA) based on the allele frequencies. Linkage disequilibrium \((LD)\) between pairs of loci was performed in GENEPOP v. 4.2 (50) with default settings. We used GenAlex v6.0 (51) to explore multivariate patterns of molecular diversity relative to populations via Principal Coordinate Analysis \((PCoA)\) and Mantel tests of matrix correspondence to test for Isolation by Distance \((IBD)\); we assessed the partitioning of genetic variation between sampling localities with Analysis of Molecular Variance \((AMOVA)\).

**Population Structure**

We estimated population genetic structure using Bayesian assignment methods with STRUCTURE v2.3.4 (52), which assigns individuals to a number \(K\) of genetically homogeneous groups, based on the Bayesian estimate in accordance to the expected Hardy-Weinberg equilibrium and absence of linkage disequilibrium between loci. We ran STRUCTURE with the LOCPRIOR option to allow sampling location to assist in the clustering, and we performed 20 independent runs for \(k = 1-10\). We set a burn-in period of 100,000 and 1,000,000 MCMC iterations and assumed an admixture model with correlated allele frequencies. To determine the optimal number of clusters and render bar plots, we implemented the Evanno method (30) using POPHELPER package in R v.1.2.1 (53). Furthermore, we inferred spatial genetic structure with TESS 2.3.1 (54). This program assumes that population memberships follow a hidden Markov random field model where the log-probability of an individual belonging to a particular population, given the population membership of its closest neighbours, is equal to the number of neighbours belonging to this population (55). We tested the CAR, and BYM models with linear trend
surface to define the spatial prior for admixture (31); we set a burn-in period of 100,000 and 1,000,000 sweeps through 10 independent runs testing the maximal number of clusters from 1–10. To decide the optimal $K$, we plotted the deviance information criterion (DIC) against $K_{max}$. We used GENELAND v4.0.6 (55) as an additional method to infer the number of populations and the spatial location of genetic discontinuities. This program allows using georeferenced individual multilocus genotypes to infer the number of populations and uses the spatial location of genetic discontinuities between those populations. We determined $K$ across 20 independent runs with 1,000,000 MCMC iterations. Thinning was set at 100, allowing $K$ to vary from 1 to 10. We used the correlated allele model and set the maximum rate of the Poisson process at 50 (the number of individuals), the maximum number of nuclei in the Poisson-Voronoi tessellation at 150 (three times the number of individuals), and the uncertainty of spatial coordinates of the collection at 25 meters. We re-ran the analysis ten times to check for consistency across runs.

To further explore the genetic diversity and structure among individuals, we reduced the dimensions via a Discriminant Principal Component Analysis (DAPC) without a priori group assignment using the ADEGENT package in R. v2.0.1 (49,56). The tools implemented in DAPC allow solving complex population structures by summarising the genetic differentiation between groups while overlooking within-group variation, therefore achieving the best discrimination of individuals into pre-defined groups (57). This multivariate method is useful to identify clusters of genetically related individuals when group priors are lacking. Estimation of clusters is performed by comparing the different clustering solutions using the Bayesian Information Criterion (BIC). We compared the results from the three Bayesian approaches and the DAPC to provide confidence in the spatial designation of genetic groupings.
Relatedness

Levels of genetic relatedness were calculated using seven estimators as implemented in the RELATED package in R v1.0 (58). Pairwise relatedness was calculated using the estimators described by Queller and Goodnight, 1989; Li et al., 1993; Ritland, 1996; Lynch and Ritland, 1999 and Wang, 2002), as well as the dyadic likelihood estimator described in Milligan (2003) and the triadic likelihood estimator from Wang (2007). Genotyping errors and inbreeding estimations were incorporated into the model, and confidence intervals (95%) were obtained through bootstrapping across loci. Allele frequencies were used to simulate pairs of individuals of known relatedness based on Parent-Offspring, Full siblings, Half siblings and Unrelated individuals.

Landscape Connectivity

We predicted the most effective corridor via least-cost path analysis using GDISTANCE package in R v1.1 (66). This approach offers the shortest cost-weighted distance between two sampling points; the program allows calculating grid-based distances and routes and is comparable to ArcGIS Spatial Analyst (67), GRASS GIS (GRASS Development Team 2012), and CIRCUITSCAPE (68). The package implements measures to model dispersal histories and contains specific functionality for geographical genetic analyses (66). The least-cost path analysis was inferred from the most northern GPS point in the Central Corridor to the most southern point in the Cockscomb Reserve. Additionally, we used CIRCUITSCAPE v3.5 (68) to model resistance surfaces of the landscape as an alternative to the least-cost path analysis, which assumes that gene flow is associated to total cost along a single optimal path (69). Circuit theory considers all possible paths and is useful to assess different interactions between different landscape features. With these two approaches, we were able to identify the most probable routes for dispersal and gene flow
between localities.

Ecosystem preference costs were based on literature review and expert opinion of habitat use by jaguars (3,5,6,35,70–72). To model each ecosystem as analogous to an electrical circuit, each pixel was assigned a resistance value in a scale of 0—9 based upon land cover (Table 2). The resistance value represents the relative effort required to move from one point to another, and the map of resistance values is used to derive all possible pathways for jaguar movement. Spatial data were obtained from the Biodiversity and Environmental Resource Data System of Belize (BERDS)(73).

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

AM and NR analysed, interpreted the genetic data and wrote the manuscript. JF performed the GIS simulations. IDF performed part of the laboratory analyses, BH and RF conducted the sample collection. SR and GA read and approved the final manuscript.

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Additional Files (not included)

Additional file 1: Figure S1. Probability graphs of K as calculated by Evanno et al. 2005.

Figure S2. Box plots, and density plot of relatedness values for simulated pairs of individuals of known relatedness. Table S2. Results from MICROCHECKER for 12 microsatellite loci.

Additional file 2: Table S1. PCR profiles for sex identification, microsatellite multiplexes and sex identification.

Additional file 3: Table S3. List of genotypes for 50 individual jaguars identified in the Cockscomb Reserve and the Central Corridor.

Additional file 4: GIS map projections for least-cost path and resistance surface of habitat suitable for jaguar movement in Belize.

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Tables

Table 1. The number of alleles (NA), allelic richness (AR), observed (HO) and expected heterozygosity (HE), fixation index (FIS), its standard error (SE), and P-value for the test of Hardy–Weinberg equilibrium for 12 microsatellite loci amplified for 50 jaguars. Bold values indicate loci not in Hardy–Weinberg equilibrium following Bonferroni correction.

| Locus   | NA | AR  | HO   | HE   | Fis  | SE  | P-value |
|---------|----|-----|------|------|------|-----|---------|
| FCA032  | 5  | 3.523 | 0.540 | 0.629 | 0.167 | 0.023 | 0.219   |
| FCA100  | 5  | 3.687 | 0.520 | 0.597 | 0.147 | 0.056 | 0.085   |
| FCA124  | 4  | 2.989 | 0.571 | 0.653 | 0.119 | 0.178 | 0.507   |
| FCA126  | 6  | 4.454 | 0.766 | 0.709 | -0.103 | 0.035 | 0.475   |
| FCA212  | 2  | 1.989 | 0.163 | 0.273 | 0.437 | 0.355 | 0.015   |
| FCA229  | 6  | 4.172 | 0.596 | 0.729 | 0.240 | 0.313 | <0.0001 |
| FCA096  | 6  | 4.565 | 0.778 | 0.729 | -0.051 | 0.139 | 0.584   |
| FCA132  | 2  | 1.572 | 0.085 | 0.081 | 0.342 | 0.005 | 1.000   |
| FCA275  | 3  | 2.995 | 0.532 | 0.643 | 0.217 | 0.111 | 0.010   |
| FCA075  | 10 | 6.852 | 0.612 | 0.844 | 0.261 | 0.051 | 0.001   |
| FCA208  | 7  | 5.533 | 0.833 | 0.767 | -0.028 | 0.042 | 0.926   |
| FCA225  | 4  | 3.691 | 0.524 | 0.585 | 0.238 | 0.024 | 0.144   |

Table 2. Ecosystem types and cost values for jaguar movement based on expert knowledge. Values range from 0 (highly costly) to 9 (no cost for movement).
| Class                                      | Cost |
|-------------------------------------------|------|
| Submontane broad-leaved moist forest      | 9    |
| Submontane broad-leaved wet forest        | 9    |
| Submontane pine forest                    | 9    |
| Lowland broad-leaved dry forest           | 8    |
| Lowland broad-leaved moist forest         | 8    |
| Lowland moist scrub forest                | 8    |
| Lowland pine forest                       | 7    |
| Lowland savanna                           | 7    |
| Shrubland                                 | 6    |
| Mangrove and littoral forest              | 5    |
| Wetland                                   | 4    |
| Urban                                     | 3    |
| Agricultural                              | 2    |
| Water                                     | 1    |
| Seagrass                                  | 0    |
| Open sea                                  | 0    |
| Coral reef                                | 0    |

**Figures**
Figure 1

Population Structure of Jaguars in Belize. A) Map of Belize Natural Protected Areas, Jaguar Corridor, Least-cost path, resistance surface as predicted by CIRCUITSCAPE, roads and sampling localities; B) Assignment probabilities of population structure as shown in STRUCTURE for K = 1 and K = 4, each bar represents a single individual; C) Mantel's test for correlation between genetic distance and geographic distance (km) showed low correlation, $R_{xy} = 0.167$, $P = 0.01$ from 10,000 randomizations; D) Scatter plot of first two principal components (PCs) of genetic diversity, dots represent individual genotypes at two
sampling locations. Bar plot displays the eigenvalues associated with the components; E) GENELAND map of population membership probability for the most likely $K = 1$. 