Using Medium and Large-Sized Mammals as Indicator Species to Measure Connectivity and Large Infrastructure Impacts in Costa Rica

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Roberto Salom Perez

Major Professors: Lisette Waits, Ph.D.; Bryan Finegan, Ph.D.
Committee Members: Daniel Thornton, Ph.D.; Ryan Long, Ph.D.;
Fernando Casanoves, Ph.D.
Department Administrator: Lisette Waits, Ph.D.

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Authorization to Submit Dissertation

This dissertation of Roberto Salom Perez, submitted for the degree of Degree of Doctor of Philosophy with a Major in Natural Resources and titled "**Using Medium and Large-Sized Mammals as Indicator Species to Measure Connectivity and Large Infrastructure Impacts in Costa Rica**," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professors: _____ Date: _____
Lisette Waits, Ph.D.

_____ Date: _____
Bryan Finegan, Ph.D.

Committee Members: _____ Date: _____
Daniel Thornton, Ph.D.

_____ Date: _____
Ryan Long, Ph.D.

_____ Date: _____
Fernando Casanoves, Ph.D.

Department
Administrator: _____ Date: _____
Lisette Waits, Ph.D.

Abstract

Conservation of biodiversity in all its forms, genetics, species and ecosystems, is essential to maintain the resilience of ecosystems and the stability of their functions and services. Over the past two centuries ecosystems are being changed, mainly associated to human-related impacts, global forest cover is being lost at a rate of 0.6% per year and extinction of species are occurring at a much higher rate than in most previous periods. Additionally, many protected areas are not enough to guarantee the long-term survival of species that require large extents to sustain viable populations. Thus, connectivity between protected or natural areas through dispersal or biological corridors is crucial for the conservation of wide-ranging species and biodiversity in general.

Moreover, low genetic diversity and reduced gene flow may decrease the ability of a species to survive by reducing its ability to adapt to environmental changes or human-related threats, decreasing fitness and increasing genetic drift. Therefore, measuring genetic diversity and population structure is vital to quantify biodiversity at an evolutionary significant unit (i.e. populations with high genetic or ecological distinction) level.

Medium and large-sized mammals play an important role in the ecosystems where they occur, but are also subject to several threats. Given, their high habitat requirements in terms of food and extent of area some medium and large-sized mammals are at higher risk of extinction than other species. This makes them especially vulnerable to habitat alterations and human related pressures. Consequently, medium and large-sized mammals can be monitored and used as indicator species to test for connectivity between protected or core areas, and assess the impacts of human activities (e.g. large infrastructure projects) on wildlife.

This research intended to use medium and large-sized mammals to evaluate the condition of a critical link of the Mesoamerican Biological Corridor (MBC) in Costa Rica. Also, we intended to measure the impacts of a hydroelectric reservoir on the mammal community in this corridor. Rather than using single species analyses, we used hierarchical community occupancy models in a Bayesian framework. This, not only allowed for the use of data of rare species, but we believed helped produce stronger inferences on the status of the

landscape in terms of biodiversity. Our results suggest that the status of this critical link with respect to medium and large-sized mammals is precarious.

Furthermore, we were able to measure the impacts of the reservoir on medium and large mammals' occupancy by including a dynamic component and comparing results in three time periods: before the flooding of the reservoir, immediately after the completion of the flooding of the reservoir, and approximately one year after the flooding of the reservoir. We found considerable changes in medium and large-sized mammals' occupancy before and after the filling of the reservoir.

In this work we also evaluated genetic diversity and population structure of ocelots (*Leopardus pardalis*) Costa Rica, and compared the genetic diversity of Costa Rican ocelots with that of jaguars (*Panthera onca*) and pumas (*Puma concolor*) in the country, and with ocelots in Belize. We found relatively high levels of genetic diversity for ocelots in Costa Rica and no patterns of genetic substructure, suggesting high levels of gene flow throughout the country and no strong barriers to movement. Additionally, genetic diversity of Costa Rican ocelots was higher than that of jaguars and pumas, and levels of genetic diversity were slightly higher in Costa Rican ocelots when compared to their counterparts in Belize, confirming the south to north decrease in genetic diversity reported in other studies for the species.

We believe the community approaches implemented here can be used to monitor biodiversity and measure the effects of infrastructure in general on wildlife, help avoid impacts, and plan proper mitigation actions. Our study also provides critical baseline information to understand what is the status of the ocelot populations in the country.

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Dedication

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Introduction

Biodiversity

Biodiversity is essential to maintain the resilience of ecosystems and the stability of their functions (1,2). It is also critical for maintaining productivity of several agricultural systems, reducing the risk of spread of infectious diseases, and supporting a range of ecosystem services (1,3).

Ecosystems are being changed and extinctions are occurring much faster than in the past due to human intervention, especially after the 1800s (3–5). The current rate of biodiversity loss may surpass the ability of the system to recuperate and guarantee its functionality (6). In the light of this problematic, several initiatives directed to the preservation of biodiversity have led to the establishment of international conservation agreements and committees (i.e. Convention on International Trade of Endangered Species of Flora and Fauna, Convention on Biological Diversity, Commission on Sustainable Development, Aichi Targets).

Still, it is estimated that the global forest cover is being lost at a rate of 0.6% per year; where conversion to agro-industrial land uses is the main cause of forest cover loss in tropical areas (7). These shrinking natural areas might not be enough, by themselves, to guarantee the long-term survival of species that require large extents to sustain viable populations (8,9). Thus, connectivity between these forested areas through dispersal or biological corridors is crucial for the conservation of wide-ranging species.

Biological corridors

According to Rosenberg et al. (10), a corridor is a linear landscape element that provides for movement between habitat patches or core areas. The concept behind biological corridors comes from the Island Biogeography Theory that states that islands with smaller size and at a greater distance from the mainland will have fewer species (11). If we consider the patches of forest as islands in a matrix of different land uses, we expect that the biggest and most connected patches will not only hold higher biodiversity but will also be able to hold a greater

number of individuals. Likewise, they will provide greater stability to the different species by avoiding the effects of environmental stochasticity, inbreeding depression and genetic drift associated with small and isolated populations (12,13).

Some authors have argued that the island biogeographic model is over-simplistic and may not accurately depict the complexity of the environment and how species interact with it; advocating for a landscape approach where the area is composed of a gradient of different cover types (14). Nevertheless, in general terms, the role of corridors as areas allowing for dispersal of individuals at a small scale, and gene flow between wildlife populations through several generations at a larger scale, is recognized as being of great importance for the long-term survival of species.

In Mesoamerica, Paseo Panthera came out in 1990 as a plan to connect protected areas from southeastern Mexico to Panama, and increase cooperation between the countries in the isthmus (15). In 1996 this evolved into the Mesoamerican Biological Corridor (MBC), a project that was signed by the Ministers of environmental agencies of each country in the region, under the Central American Commission of Environment and Development.

As a result, Costa Rica created a permanent Biological Corridor Program within the Ministry of Environment and Energy. To date there are 44 official biological corridors active in the country covering 32% of the national territory (16). At a size of just over 51,100 km^2 Costa Rica is said to hold 5% of the world´s biodiversity in 0.3% of the land mass, including 249 species of mammals (17). Additionally, Costa Rica has been recognized for its efforts on conservation, having approximately 25% of the country under protected areas and supporting several sustainable and environmental policies. This country, along with the rest of the Central American isthmus, is a critical link for wildlife between North and South America.

Mammals as indicator species

Indicator species are those where their change in status (e.g. presence, absence or abundance) may reveal a certain condition such as: defining a certain habitat type, evaluating the integrity of an area, detecting an impact in the ecosystem or can sometimes act as surrogates for other species (18–20).

Many mammals are charismatic species that help draw attention to biodiversity loss and the impacts to the ecosystem (21). Alongside birds, mammals are the focus of conservation initiatives and fund allocation even when they are not necessarily the most threatened species in the system (22).

However, medium and large-sized mammals play an important role in the ecosystems where they occur, frequently representing the majority of the wildlife biomass in an area (23), being the top predators and executing a top-down control over other vertebrates (24) or playing an important role as seed consumers (25–27) or dispersers (26,28,29). These species are also among the most hunted animals, and may comprise the main source of protein for some humans (23). Moreover, the large body size and endothermy of large mammals translates into a high metabolic rate. Having a high metabolic rate implies that these species require a high intake of food, which usually means that they need large spatial areas to survive and maintain viable populations. Consequently, many medium and large-sized mammals are at higher risk of extinction than other vertebrates, are especially vulnerable to habitat alterations and human related pressures, and thus, are good sentinel species of these events (5,30–33). Therefore, there are several conservation initiatives that have used mammals as indicator species (8,9,34,35). Still, monitoring of other species (i.e. invertebrates, amphibians, fish, plants) may be necessary to assess the full scope of the impacts on biodiversity and the ecosystem (18,20).

Infrastructure and wildlife

Roads, railways, hydroelectric dams, canals, wind parks, powerlines, pipelines, oil wells, urbanization and agro-industrial constructions are just a few of the increasing infrastructure development taking place, especially after the industrial era (32,36). In fact, infrastructure development is seen as a growth indicator of country economies and is an important component of every political agenda (37).

However, human infrastructure can cause several impacts on wildlife, such as habitat loss and fragmentation, hinder local movements (barrier effect), impede dispersal and gene flow between populations, create and edge effect over natural areas, direct mortality, and decrease species abundances (32,36,38–40). Few studies have performed Before-After-Control-Impact

(BACI) sampling designs or have information about the initial status of the area prior to the construction of infrastructure (32). On top of that, some institutions in charge of these projects are not required to carry on a thorough scientific research of its impacts, and usually base their mitigation actions, if any, on limited information (39,41).

The importance of genetics for biodiversity and non-invasive genetic sampling for wild cats

Genetics is one of the main recognized forms of biodiversity, alongside ecosystems and species (42). If populations are isolated and genetic diversity is decreased, species become at higher risk, because they are less able to adapt to environmental changes or human-related threats, suffer a decrease in fitness (e.g. through inbreeding and loss of genetic diversity), and an in increase in genetic drift (13,43,44). With genetics, one can monitor the status of species or distinctive populations, also known as evolutionarily significant units, that may require different conservation considerations (13).

Given their elusive nature, low densities, and generally nocturnal habits, wild cats have always been hard to study and most research was initially based on indirect methods (e.g. tracks, scats, and pelts). The development of the Polymerase Chain Reaction (PCR) technique (45,46) to amplify DNA provided the opportunity to use lower quality samples in genetic monitoring, and opened the door for non-invasive genetic sampling (NGS) of vertebrate species by collecting feces, hair, or other materials without having to capture or handle the animals.

It was not until the year 2000 that the first studies using NGS for wild cats were published (47–49). Since then, the use of NGS has increased significantly with studies on at least 19 different species of wild cats, and the largest number on the tiger (*Panthera tigris*) (49).

The main advantages of NGS over the more traditional methods of studying rare or elusive animals (e.g. telemetry, camera traps, tracks) are that there is no need to handle or capture the animal, the amount of information obtained is significantly increased, fewer permits are required compared to invasive methods, elusive and rare species can be sampled, the number of samples may be greater, the training of field personnel to gather samples is relatively easy, and the cost, in some cases, may be lower (50–53). Yet, there are some drawbacks to using

this sampling technique for genetic studies, such as: low quality and quantity of DNA is obtained which can cause genotyping errors or low amplification success, expenses may be higher (depending on the number of replicates and the markers utilized), and there could be a potential sex bias in sampling depending on the species behavior and sampling design (50,53,54).

Sources for obtaining DNA through NGS are varied, but the most common ones used for wild cats are feces and hair (50,55). To increase the number of samples encountered some researchers have used trained scat detecting dogs (52). This also helps to decrease the collection of samples from non-target species (56).

Genetic markers most commonly used in wild cat genetic studies are DNA sequences from short (usually 400-600bp) regions of the mitochondrial DNA (mtDNA), and nuclear DNA microsatellites (microsats) (49). Microsats are used to identify individuals, calculate genetic diversity, and evaluate population structure, relatedness, parentage, and gender (49,55,57).

The types of information that can be gathered from genetic monitoring have been increasing over the years. Specifically for NGS of wild cats, researchers have assessed: species ID, individual ID, sex ID, diet analysis, habitat modelling (e.g. species occupancy, habitat use), genetic diversity, phylogeography, gene flow, forensics (e.g. source of a hunted animal), hybridization, and mating systems (49,52,53,58–64). Individual identification also allows estimating population size, movements, and assignment tests; method that assigns individuals to the population they most likely came from. Studies on other groups of wildlife have also been able to gather information on demographics, relatedness, parentage analysis, monitoring of individuals released into the wild, attacks on domestic and wild prey, and pathogens (51,55).

Genetic monitoring with NGS has also been used in wild cats to compare results with other methodologies (e.g. camera traps, tracks) (52,57,65,66). Results vary with each study, but NGS with fecal samples has the disadvantage that feces are usually collected opportunistically and are found in clumps, underestimating the sampling area and overestimating population size (49). With a proper methodological design and as costs of genetic analysis keep decreasing, NGS will continue to be important complements or even a substitute of more traditional methods.

General description of this dissertation

In Chapter 1: "Evaluating the status of medium and large-sized mammals through a community occupancy model in a critical link of the Mesoamerican Biological Corridor", I used a multi-level community occupancy model in a Bayesian framework to evaluate the status of medium and large-sized mammals in the Barbilla-Destierro Biological Sub-Corridor (Corridor) and two jaguar core areas, the Central Volcanic Cordillera and Talamanca in Costa Rica. I did this by surveying an area of ~963 km^2 with camera-traps between 2013 and 2017, having 16,904 trap nights across 209 stations; becoming one of the most intensive surveys of its kind in Costa Rica.

In Chapter 2: "Using a dynamic community model to measure the effects of a large hydroelectric project on medium and large-sized mammals in a critical Mesoamerican Biological Corridor", I used a hierarchical dynamic community model in a Bayesian framework to measure the effects of the flooding of a hydroelectric reservoir on medium and large-sized mammals in the Corridor. I surveyed the central portion of the Corridor, covering an area of 416 km^2, with camera traps in three different time periods between 2013-2018; before the flooding of the reservoir, immediately after the completion of the flooding of the reservoir, and approximately one year after the flooding of the reservoir.

Finally, in Chapter 3 "Evaluating genetic diversity and structure for ocelots (*Leopardus pardalis*) in Costa Rica" I evaluated genetic diversity and population structure of ocelots using 15 microsatellite loci in 31 successfully genotyped samples gathered throughout the country. I also compared the genetic diversity of Costa Rican ocelots with that of jaguars (*Panthera onca*) and pumas (*Puma concolor*) in the country, and with ocelots in Belize.

I believe the studies presented here provide transcendental information on how medium and large-sized mammals can be monitored and used as indicator species to test for connectivity between protected areas, and assess the impacts of large infrastructure projects on wildlife. Additionally, I executed one of the first conservation genetics studies of a wild cat species across the country, and show how this type of research can offer important data on connectivity of a carnivore species. This can be used to properly inform management decisions and guarantee long-term survival of these and other species, preserve biodiversity and improve the resilience of ecosystems in general.

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Chapter 1: Evaluating the status of medium and large-sized mammals through a community occupancy model in a critical link of the Mesoamerican Biological Corridor

Abstract

Connectivity of protected and other natural areas through biological corridors is essential to the conservation of biodiversity and to maintain ecosystem resilience. These corridors should be monitored to evaluate their status and to determine if they are being effective. Herein we used a multi-level community occupancy model in a Bayesian framework to evaluate the status of medium and large size mammals in a critical link of the Mesoamerican Biological Corridor (MBC) in Costa Rica. We used camera traps to detect medium (between 1-15 kg) and large (>15 kg) mammal species in the Barbilla-Destierro Biological Sub-Corridor (Corridor) and two jaguar core areas; Central Volcanic Cordillera and Talamanca Cordillera in Costa Rica, between 2013 and 2017. Camera traps operated for 16,904 trap nights across 209 stations, covering an area of ~963 km^2. We found that the most important covariate for the community of medium and large-sized mammals was forest cover (+); although responses to covariates per species were highly variable. As expected, medium and large mammal species richness and puma habitat use probability were lower in the Corridor area than in the two jaguar core areas. Jaguar habitat use probability was highest in the Talamanca Cordillera and in the east side of the Corridor. Low habitat use probability values for jaguar in the Central Volcanic Cordillera suggest that this is not the jaguar stronghold previously assumed. Roads in the area may be having a barrier effect especially for large prey species, jaguars and pumas. Pumas and jaguars were strongly correlated with large prey, and to a lesser degree, with medium prey species. Urgent actions are needed to guarantee connectivity of mammal populations in this critical link of the MBC. Conservation actions in the area should focus on restoring areas in the central-west portion of the Corridor, implement measures to decrease the barrier effect related to the two main roads and increase habitat quality, especially for large species.

Key words: Mesoamerica, jaguar, puma, hierarchical model, multi-species

Introduction

Biodiversity is essential to maintain the resilience of ecosystems and the stability of their functions (1,2). It is also critical for maintaining productivity of several agricultural systems, reducing the risk of spread of infectious diseases, and supporting a range of ecosystem services (1,3). Forests are one of the most biodiverse areas, nonetheless, they are under great threat. It is estimated that the global forest cover is being lost at a rate of 0.6% per year, and conversion to agro-industrial land uses is the main cause of forest cover loss in tropical areas (4). Furthermore, these natural areas might not be enough, by themselves, to guarantee the long-term survival of species that require large spatial extents of habitat to sustain viable populations (5,6). Thus, connectivity between forested areas through dispersal or biological corridors is crucial for the conservation of wide-ranging species that depend on this type of habitat.

With only 0.50% of the world's land mass, the Mesoamerica region holds ~7% of the planet's biodiversity (7). Because of its relatively small size and its geographic position between North and South America, this isthmus has functioned for millennia as a natural funnel for wildlife, becoming arguably the most critical region for connectivity in the Americas. Consequently, in 1996 the governments in the region created the Mesoamerican Biological Corridor (MBC), an initiative that aims to preserve biodiversity and connect protected and other natural areas from southern Mexico to Panama. Nevertheless, the region holds a very high deforestation rate and some researchers have already highlighted possible areas where connectivity might be broken or close to break (8–12).

Medium and large-sized mammals play an important role in the ecosystems where they occur, frequently representing the majority of the wildlife biomass in an area (13), being the top predators and executing a top-down control over other vertebrates (14) or playing an important role as seed consumers (15–17) or dispersers (16,18,19). A large number of carnivore, omnivore and scavenger animals, and decomposer organisms, with an indirect effect on soil and nutrient cycling, depend on medium and large mammal species for survival (20). Medium and large-sized mammals are also among the most hunted animals, and may

comprise the main source of protein for some humans (13). Moreover, the large body size and endothermy of large mammals translates into a high metabolic rate. Having a high metabolic rate implies that these species require a high intake of food, which usually means that they need large spatial areas to survive and maintain viable populations. Consequently, many medium and large-sized mammals are at higher risk of extinction, and are especially vulnerable to habitat alterations and human related pressures. These factors makes them good sentinel species of these events, by decreasing their abundance or disappearing from areas where these occur (21–24). Additionally, several mammal species have been proposed as umbrella species; those whose conservation is supposed to provide protection to other sympatric species. Yet, some investigators suggest that the use of a single species as an "umbrella" to preserve biodiversity has not been tested appropriately (25–27; but see: 20,28–31). Moreover, researchers that work with one species are frequently faced with small sample numbers that limit the type of analyses and conclusions they can make (32–34). Accordingly, the use of a multiple species vs. a single-species approach to assess biodiversity in important areas, evaluate the effects of impacts or management actions, and test connectivity, has proven to be a useful alternative (12,35–37; Petracca et al. in press, Salom-Pérez et al. in prep.).

Herein we used a multi-level or hierarchical community occupancy model in a Bayesian framework developed by Dorazio and Royle (38) and modified by Zipkin et al. (35) and Zipkin et al. (36). This model accounts for species-level variation as well as for community combined responses to measured covariates (35,36,38,39), and allows the generation of occupancy estimates for species that have low abundance and/or low detection probabilities (35,36). Additionally, this model considers variation in surveys (e.g. associated with site/year) through random effects.

With this model, our goal was to establish baseline information on the status of medium and large-sized mammals in a critical wildlife corridor and the core areas it connects in Costa Rica, surveyed over a period of four years. Our study area is the Barbilla-Destierro Biological Sub-Corridor (hereon referred to as Corridor) and a portion of two presumed jaguar core areas. This area is a crucial corridor for the MBC and the Jaguar Corridor Initiative (JCI), the largest-scale carnivore conservation effort existing to date (8,10,40). The JCI aims to preserve jaguar (*Panthera onca*; IUCN: near threatened; (41)) populations and range-wide habitat connectivity, from Mexico to northern Argentina, by identifying and securing dispersal

corridors between core populations (8). The MBC and the JCI highly overlap in Mesoamerica and have been seen as complementary initiatives. This area may be important as habitat for certain species that can tolerate certain alteration, and as a dispersal area for other, perhaps more forest dependent species, to maintain regional population connectivity at a long-term over several generations.

Our specific objectives were to: 1) determine the main environmental and human-related factors driving the presence of 25 medium and large mammal species in the study area, 2) estimate species richness of medium and large mammal species, 3) estimate habitat use probability for jaguars and pumas (*Puma concolor*; IUCN: least concern; (42)), and 4) evaluate if there is a relationship between jaguar and puma habitat use and prey richness. We expected that medium and large mammal species richness would be lower in the Corridor with respect to the core areas, given that the latter areas have greatest forest cover and are classified as protected areas or indigenous territories. Finally, we hypothesized that habitat use probability of jaguars and pumas would be related to large prey species richness.

Materials and methods

Study area

The study area comprises the Barbilla-Destierro Biological Sub-Corridor (Corridor), and a portion of two Jaguar Conservation Units (JCUs), the Central Volcanic Cordillera (CVC) JCU and the Talamanca-Cordillera Central (TC) JCU (Figure 1.1). The JCUs are expert-defined areas that support, or could support, at least 50 adult jaguars, adequate prey and good habitat quality (6,43).

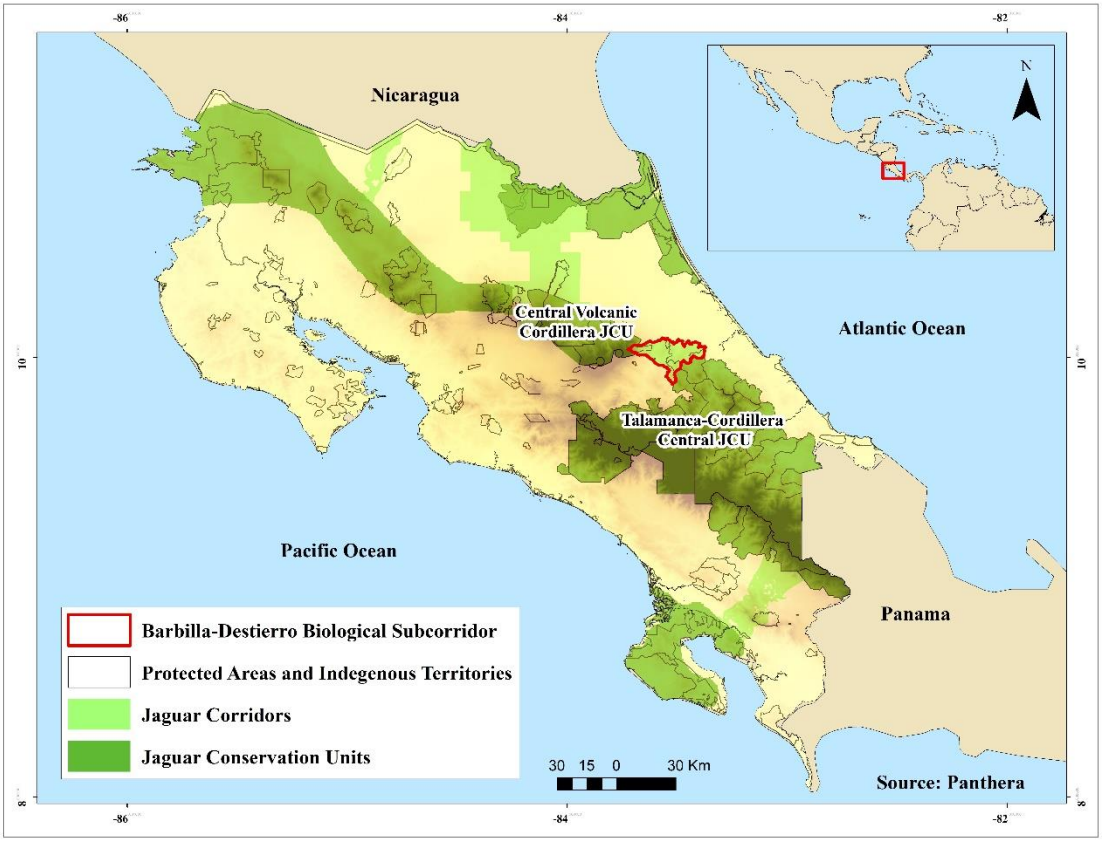

Figure 1.1. Jaguar Conservation Units (dark green), jaguar corridors (light green), and protected areas in Costa Rica. The red polygon indicates the jaguar corridor that is part of our study area.

The first JCU, CVC JCU, comprises a continuous block of protected areas in Costa Rica (IUCN categories II and VI; ~1,153 km^2). Approximately 75% of this JCU is covered by primary and secondary forest. The CVC JCU is an area of great ecological importance, as several major rivers originate in these mountains. The second JCU, TC JCU, is shared between Costa Rica and Panama. The TC JCU connects to the eastern side of the Corridor and encompasses a continuous group of protected areas (IUCN categories II and VI) and indigenous territories (~7,002 km^2) on the Costa Rican side. Primary and secondary forests represent 79% of the total area in Costa Rica.

The Corridor is approximately 362 km^2, of which 58% is protected (IUCN category VI; Forest Reserves and a Protected Zone) or indigenous territory. No other suitable connections for jaguars have been identified between the Central Volcanic and Talamanca JCUs, and more broadly between Nicaragua and Panama (10). Almost all the area is privately owned, mostly composed of small farms. About 64% of Corridor is covered by primary and secondary forest

(2015 Aster Image). The rest of the area is dominated by pasture lands for livestock (20%) and agriculture (14%). The Corridor also includes two two-lane paved roads that connect the towns of Turrialba and Siquirres.

Study design and data collection

We conducted camera trap surveys to assess the presence of medium (between 1-15 kg) and large (>15 kg) mammal species over the study area. We created a grid system of 16 km^2 cells over three sites: the entire Corridor and portions of the CVC and TC JCUs (Figure 1.2). Cell size represented approximate home range size of jaguars in Central America, one of the target species in our analyses, and presumably the species with the largest extent (44–46).

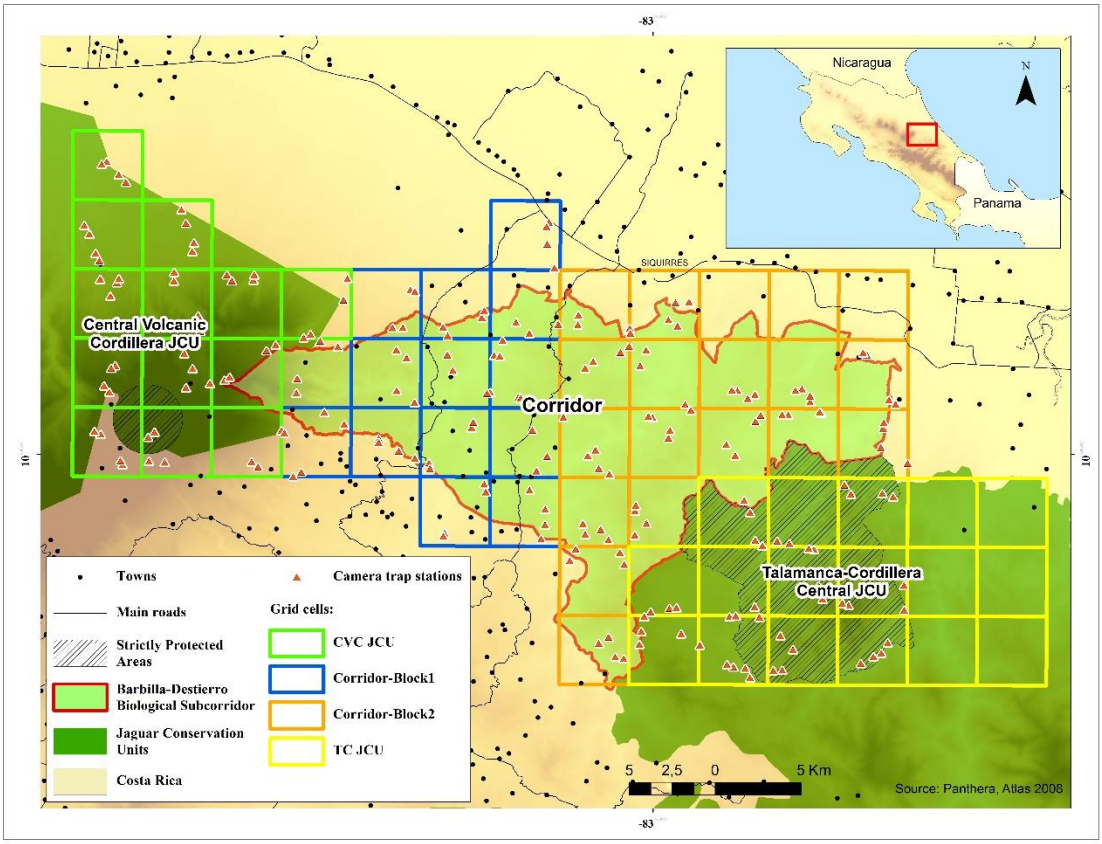

Figure 1.2. Study area with a grid of 16 km^2 cells in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

The 16 km^2 grid-cells were later subdivided into four sub-cells of 4 km^2 each. We sampled two sub-cells per grid-cell with two stations (one camera trap per station) in each sub-cell for a period of approximately three months. Selection of these sub-cells was random, but had to be adjusted when there was no forest, permits were not given or access was very complicated (e.g. very high slopes). This allowed for a more widespread distribution of the camera stations in the cells, while decreasing the logistical difficulties in surveying every sub-cell. The study area was divided in four different blocks and surveyed at different times to allow for a higher density of cameras per area, and due to limitations in the number of cameras available and logistical considerations. The Corridor site was surveyed in two phases (Corridor-Block 1: Oct 2013 - January 2014; Corridor-Block 2: January 2014 - May 2014). The CVC JCU site was surveyed from August 2014 to April 2015, and the TC JCU site was surveyed between September 2016 and April 2017.

We placed motion sensitive camera traps (Panthera® V3, V4, V5 and V6) in forested areas, strapped to trees at approximately 0.4-0.5 m above ground, a height intended to detect medium and large size mammals. Cameras were set to function 24 hours and take three shots in every event during the day and one shot during the night. One camera of each 4 km^2 surveyed sub-cell was placed off a trail and the other one was placed on a human-made trail (when available), in an attempt to detect species that may avoid human trails (Figure 1.3).

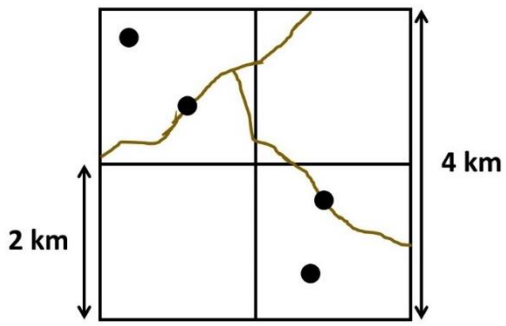

Figure 1.3. Graphic representation of a 16 km^2 cell, 4 km^2 sub-cells and the planned distribution of the camera stations in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017. Black dots represent camera stations and brown lines represent human-made trails.

Camera location was recorded using a GPS device (Garmin®). Cameras were checked approximately every 6 weeks to download pictures and perform camera maintenance. Data

were processed on Panthera IDS (Integrated Data Systems; version 1.13.786), where the species, date, time and number of individuals in each photograph were recorded.

For all the occupancy-based analyses, we collated data from all stations in each grid-cell to make detection histories ("1" = detected, "0" = not detected, "NA" = inactive camera) for each of the 25 medium and large mammal species; including domestic pig. We included domestic pig as part of jaguar and puma prey species, given that they could represent an important prey item for jaguars and pumas in the study area (personal observation). Species included in the analysis were: agouti (*Dasyprocta punctata*), armadillo nine-banded (*Dasypus novemcinctus*), armadillo northern-naked-tailed (*Cabassous centralis*), coati (*Nasua narica*), coyote (*Canis latrans*), deer red-brocket (*Mazama temama*), deer white-tailed (*Odocoileus virginianus*), fox (*Urocyon cinereoargenteus*), grison (*Gallictis vittata*), jaguar, jaguarundi (*puma yagouaroundi*), margay (*Leopardus wiedii*), ocelot (*L. pardalis*), oncilla (*L. tigrinus*), opossum (*Didelphis marsupialis*), paca, (*Cuniculus paca*), peccary collared (*Pecari tajacu*), domestic pig (*Sus scrofa*), puma, rabbit (*Sylvilagus brasiliensis*), raccoon (*Procyon lotor*), skunk (*Conepatus semistriatus*), tamandua (*Tamandua mexicana*), tapir (*Tapirus bairdii*), and tayra (*Eira barbara*). Each sampling occasion (*k*) was one week.

We selected site covariates *a priori* as being thought to be the main drivers affecting habitat use of medium and large sized-mammals in the study area (8,10,11,43,47,48) (see Appendix 1, Table S1.1). Site covariates expected to have a positive relationship to most medium and large mammal habitat use, were: enhanced vegetation index (EVI) (49), forest (forest v1: global data base, (50); forest v2: product specific to Costa Rica, (48)), distance from major settlements, and distance from major, medium or minor settlements (48). On the other hand, site covariates expected to have a negative relationship were: elevation (51), ruggedness (52), distance to strictly-protected areas (48), distance to JCUs (Panthera unpub. data), settlement density (48) and human presence (this study; see Appendix 1). To calculate effort, a covariate on detection, I calculated and standardized the sum of all trap nights on each occasion for every grid-cell.

Multi-level community occupancy model

Given that our survey occurred over a long period of time (~3.5 years), the closure assumption (i.e., there are no changes in occupancy during the survey period) is likely

violated. Thus, in this investigation Ψ can be interpreted as estimating habitat use probability (53). In order to calculate jaguar and puma habitat use probability and obtain medium and large-sized mammal species richness, we used a multi-level (or hierarchical) community occupancy model in a Bayesian framework. Occupancy models account for imperfect detection of species by incorporating probability of detection, based on the capture history of species (34,54). Thus, species estimates derived from such models are more precise and have less bias than raw species counts or traditional species richness estimators (39). The multi-level community approach allows the generation of more precise species-specific occupancy estimates by drawing such estimates from the community collective data across the landscape (35,36,38,39; Petracca et al. in press). This is especially important when there are rare or elusive species present in an area. An added advantage of this model is that no previous assumptions on the community composition are required (35,36). Furthermore, unlike traditional occupancy models, the Bayesian framework can account for unobserved heterogeneity across species, space or time (10). This type of heterogeneity could be expected in the current investigation given the relatively large area it covered and the fact that the blocks were surveyed in different time periods.

To determine the site covariates included in the global community occupancy model, we first ran single-species occupancy models in a maximum-likelihood framework using R Presence (R Core Team 2015®; version 3.4.3), for each medium and large mammal species separately. This assumed independence between species observations (54).

For each species, we ran all possible combinations of site covariates, with and without the human presence covariate, that were not highly correlated with each other ($r < 0.6$; (55); Appendix 1, Table S1.2); assuming no interactions. We included standardized effort as a covariate on detection in all models; offset variable to account for differences in effort across grid-cells. In total, there were 79 models for each species (Appendix 1, Table S1.3).

Then, we calculated the Akaike Information Criterion with correction for small samples (AICc) for all 79 models across species (56). The AIC across species (community value) was calculated for each model by adding the negative 2 * natural log of the likelihood ($-2 \ln(\hat{L})$) across species.

We calculated AICc by applying the following equation:

$$\text{AICc} = (2c - \text{AIC}) + ((2c^2 + 2c)/(n - c - 1));$$

where *c* is the number of estimated parameters and the sample size *n* was the total number of species (56; Petracca et al. in press). The best-performing community model for all species was determined based on the lowest AICc.

To build the model we defined true occurrence $z(i,j)$ as a binary variable in which $z(i,j)$ = 1 if species *i* occurred in the grid-cell *j* (and = 0 otherwise). We modeled occurrence from a Bernoulli random variable, where $z(i,j) \sim \text{Bern}(\Psi_{i,j})$, where $\Psi_{i,j}$ is the probability that species *i* occurs at grid-cell *j*. To account for imperfect detection, we modeled a probability of detection, where observed data, denoted by $y(i,j,k) \sim \text{Bern}(p_{i,j,k} * z(i,j))$, where $p_{i,j,k}$ is the detection probability of species *i* in grid-cell *j* in the survey occasion (week) *k*.

The multi-level community model we utilized to calculate habitat use probability is represented by:

$$\text{Logit}(\Psi_{i,j}) = \xi_{il} + \alpha_i D_j;$$

where $\xi_{il} \sim \text{Normal}(\mu_\xi, \tau_\xi)$ is the random intercept for each species *i* at each block *l*, μ_ξ is the community-level or hyper parameter mean for Ψ intercept and τ_ξ is its precision. This is used to account for potential heterogeneity due to differences between species and in space and/or time (35,36; Petracca et al. in press). α_i are estimated beta coefficients for habitat use for species *i*, where $\alpha_i \sim \text{Normal}(\mu\alpha, \tau\alpha)$, $\mu\alpha$ is the community-level or hyper parameter mean for each site covariate and $\tau\alpha$ is its precision; and D_j are the values for the covariates at grid-cell *j*. The logit transformation for detection probability was modelled through this equation:

$$\text{Logit}(p_{i,j,k}) = v_{il} + \beta_i effort_{j,k};$$

where $v_{il} \sim \text{Normal}(\mu_v, \tau_v)$ is the random intercept for each species *i* at each block *l*, μ_v is the community-level mean for *p* intercept and τ_v is its precision. β_i is the estimated beta coefficient for effort for species *i*, where $\beta_i \sim \text{Normal}(\mu_\beta, \tau_\beta)$, μ_β is the community-level mean for effort and τ_β is its precision; and *effort(j,k)* are the values for the covariate on detection at grid-cell *j* on occasion *k*.

Additionally, we tested and modeled the correlation between habitat use and detection probability with species correlation parameter rho (ρ) to account for the effect of species abundance on detection probabilities (37). These analyses were done in R (R Core Team

2015®; version 3.4.3) using the package jagsUI (59), running three MCMC chains with 30,000 iterations, 5,000 burn-in, and a thinning of three.

We estimated jaguar and puma habitat use probability using the community model selected. We obtained species richness per grid-cell as a derived parameter from the summation of $z(i,j)$ values, which averages the number of occurring species across iterations. We calculated species richness for: 1- all medium and large mammal species (without domestic pig), 2- jaguar and puma large prey species (including domestic pig-see justification in Results), 3- jaguar medium prey, and 4- puma medium prey. In order to select the medium and large prey species for jaguars and pumas, we conducted a literature review of publications on jaguar and puma diet and predation reports from Mexico to Panama (Appendix 1, Table S1.4).

Finally, we compared jaguar and puma habitat use probabilities with medium and large prey species richness through independent Pearson´s correlations. We did this to evaluate if areas with higher probability of jaguar or puma habitat use had higher number of large or medium prey.

Results

Camera traps operated for 16,904 trap nights in total across 209 stations. Fifty-five out of 63 total cells were surveyed, covering 87.30% of the study area (minimum convex polygon= 962.57 km^2). We registered 2,946 independent records of 24 medium and large wild terrestrial mammal species and domestic pig (n = 25) (Appendix 1, Table S1.5).

The five most widespread species across the study area, derived from the Bayesian analysis, were: nine-banded armadillo (occurring in an estimated 89.32% of the area), coati (86.87%), ocelot (85.94%), tayra (82.35%) and jaguarundi (70.53%).

Two community models for medium and large size mammals had a ΔAICc <2. The best model, had the following covariates (relation to both jaguar and puma habitat use shown in parenthesis): EVI (jaguar +, puma -), forest v2 (+), ruggedness (+), distance to strictly-protected area (-) and Human presence (+) (Figure 1.4, Table 1.1). The second-best model (ΔAICc=0,64) shared the last three covariates, but had elevation instead of EVI (correlated at -0.80 with elevation) and forest v1 instead of forest v2 (correlated at 0.91 with forest v1). Thus, we decided to keep only the first model in the final community model. Effort, measured

as the sum of all trap nights on each occasion, was also part of the top model as a covariate on detection. There was a positive correlation between occupancy and detection (ρ=0.26; 95% CI=-0.04 to 0.91).

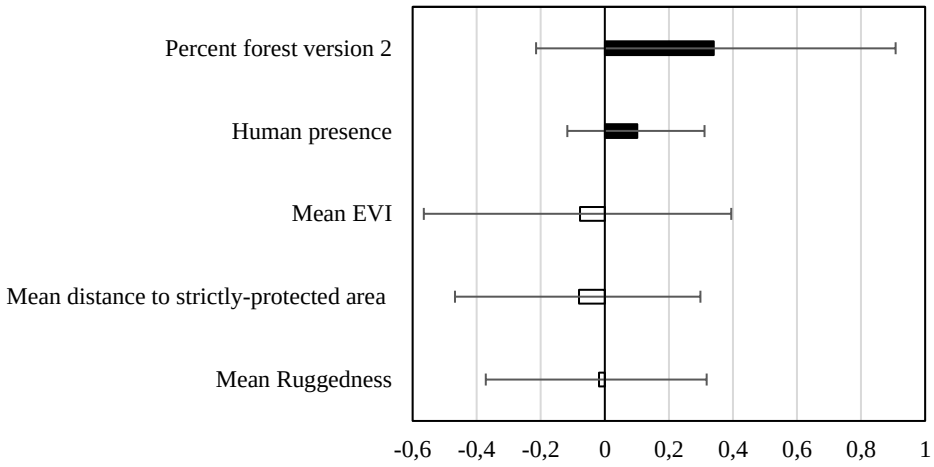

Figure 1.4. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for occupancy (Ψ) of medium and large wild mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017. Estimates with a negative influence are shown in white, and the ones with a positive influence are shown in black.

Table 1.1. Community-level summary of hyperparameters for the covariates on detection and occupancy in the top model, driving occurrence of medium and large wild mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor, and portions of the Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017. Posterior mean with standard deviation and 95% credible intervals, and an indicator of convergence ($\hat{R}$), for which values <1.1 indicate convergence.

Model parameter	Covariates	Beta (SD)	95% credible interval	$\hat{R}$
Occupancy (Ψ)	Percent forest version 2	0.34 (0.28)	-0.21, 0.91	1.000
	Human presence (human detections per 1,000 trap nights)	0.10 (0.11)	-0.12, 0.31	1.002
	Mean Enhanced Vegetation Index	-0.08 (0.24)	-0.57, 0.39	1.000
	Mean distance to strictly-protected area	-0.08 (0.19)	-0.47, 0.30	1.001
	Mean Ruggedness	-0.02 (0.18)	-0.37, 0.32	1.000
Detection (p)	Effort (sum of all trap nights on each occasion)	0.39 (0.05)	0.30, 0.49	1.000

Variables informing occupancy had a large 95% CI, and all of them overlapped zero, which suggests a high variability of their effect on medium and large mammal species responses (Figure 1.4, 1.5; Appendix 1, Figures S.1-4) (37). Percent forest had the greatest influence on species richness, an effect size more than three times that of any other variable (Figure 1.4). This effect varied among species, with a strong positive effect (95% CI not overlapping zero) for collared peccary, jaguar, domestic pig, paca, puma, agouti and ocelot, and strong negative effects on coyote, armadillo nine-banded and raccoon (Figure 1.5).

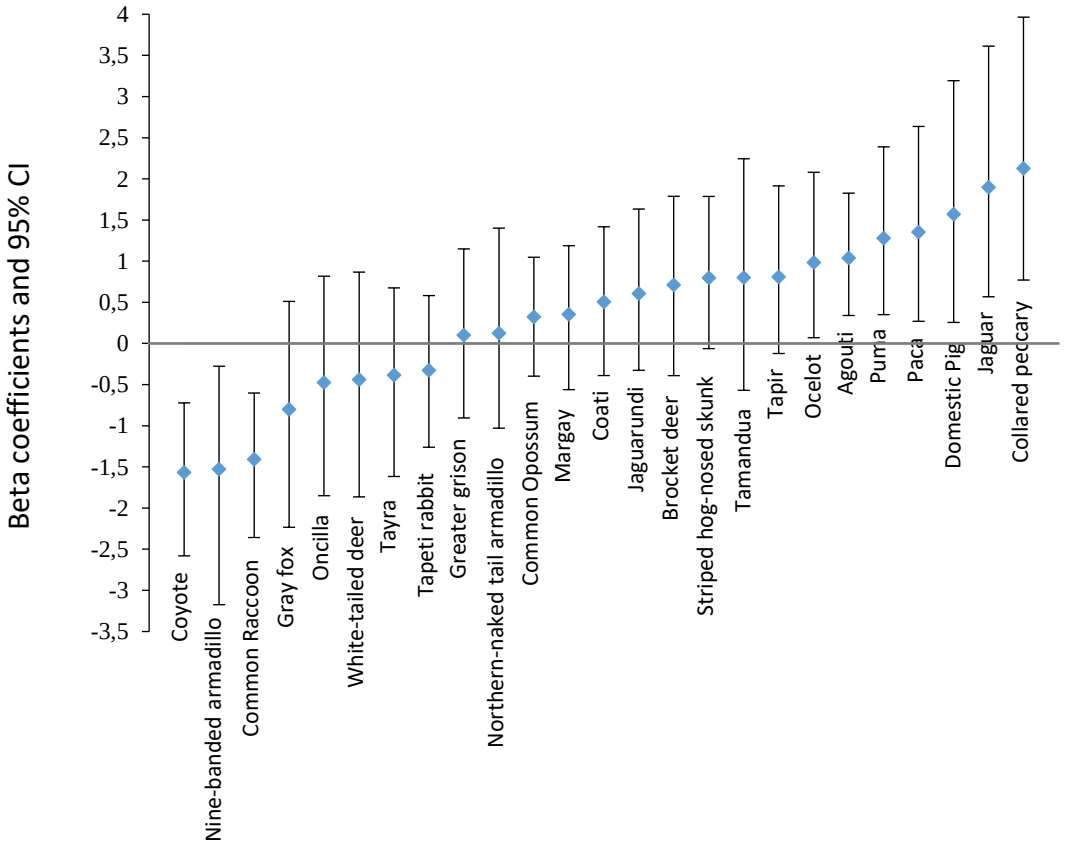

Figure 1.5. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for the influence of Percent forest on occupancy (Ψ) of medium and large mammals and domestic pig (*n* = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

Medium and large mammal species richness estimates per grid-cell ranged from 6.02 to 14.54 (95% CI 5-16; $\bar{x}$ = 10.60±1.90), with TC JCU having the highest species richness estimate overall ($\bar{x}$ = 11.86±1.15), followed by CVC JCU ($\bar{x}$ = 11.03±1.79), and Corridor ($\bar{x}$ = 9.78±1.84) (Figure 1.6, C).

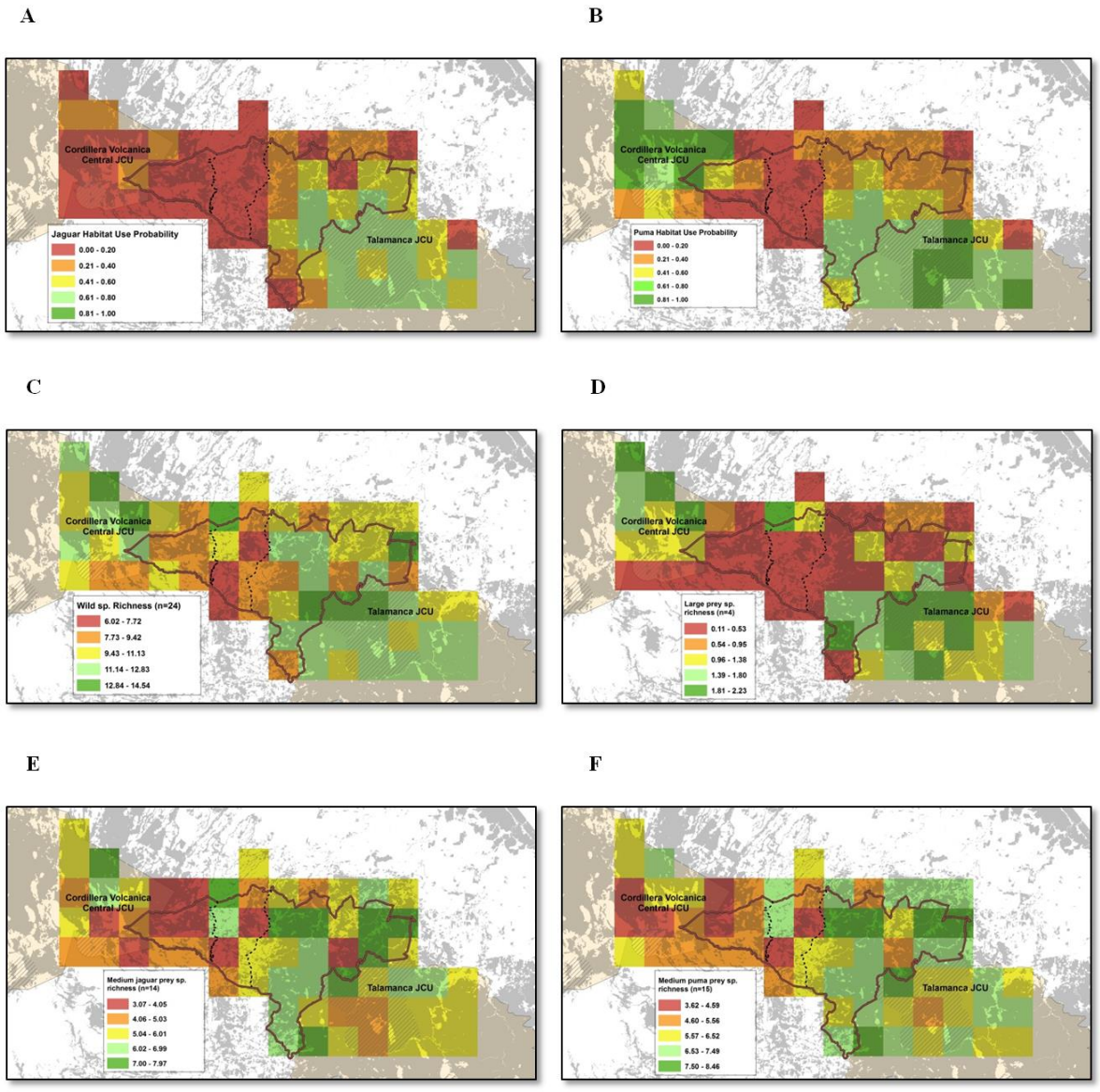

Figure 1.6. Habitat use probability for jaguar (A) and puma (B), and richness of medium and large-sized mammals (C), large prey (D), medium jaguar prey (E), and medium puma prey (F) in Barbilla-Destierro Biological Sub-Corridor (Corridor, red polygon), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units (both in faded pink color), 2013-2017. Forest is shown in gray; the two main roads are shown in dotted lines; and the strictly protected areas are shown as striped polygons.

We determined there were four large mammal prey species for both jaguar and puma in the area (Appendix 1, Table S1.4). Also, there were ten medium prey species for jaguars and 11 medium prey species for pumas. Jaguar and puma medium prey species richness was higher in the eastern side of the Corridor in comparison to the rest of the Corridor and the two JCUs (Figure 1.6, E,F). On the other hand, large prey species richness was distinctly higher in the TC JCU, followed by CVC JCU and lowest in the Corridor area; especially in the west-central

section (Figure 1.6, D). Jaguars are less distributed in the study area (estimated to occur in 29.74% of the study area), than pumas (49.68%). Jaguar habitat use probability was higher on the east side of the study area, towards the TC JCU, while puma had a higher habitat use probability in the CVC JCU, followed by the TC JCU and the eastern section of the Corridor (Figure 1.6, A,B). Jaguar and puma estimates of habitat use were correlated to a greater degree with large prey species richness (jaguar, $r = 0.59$, 95% CI = 0.40 to 0.73, $t = 5.71$, $df = 61$, $p<0.001$; puma, $r = 0.72$, 95% CI = 0.57 to 0.82, $t = 7.99$, $df = 61$, $p<0.001$) than with medium prey species richness (jaguar, $r = 0.36$, 95% CI = 0.12 to 0.56, $t = 3.06$, $df = 61$, $p = 0.003$; puma, $r = 0.23$, 95% CI = -0.01 to 0.46, $t = 1.89$, $df = 61$, $p = 0.064$).

Discussion

To our knowledge this is the most intensive camera-trap study on a continuous area in Costa Rica (60), and the first to take place in this critical link between CVC JCU and TC JCU. Here, we found that the most important covariate (direction of the sign shown in parentheses) for medium and large-sized mammals habitat use was forest cover (+), followed by human presence (+), calculated as the number of human detections per 1,000 trap nights per stations, EVI (-), distance to strictly protected areas (-) and ruggedness (-). Elevation also had some importance as it appeared in the second-best model according to the AICc values. In contrast, distance to JCU, and other measures related to human disturbance (i.e. distance to primary roads, distance to settlements or settlement density) had little explanatory value.

Forest cover had an effect that was at least three times higher than any other covariate, and seemed to be especially important for species like collared peccary, jaguar, domestic pig, puma, agouti and ocelot. These species, except for the domestic pig, are known to depend, or at least be associated with vegetation cover (41,42,61–63). Meyer et al. (11), found that forest cover was positively associated with occupancy for seven medium and large-sized mammals in Panama; but contrary to our results, had a negative relationship with puma and jaguar occupancy. Almost all domestic pigs encountered were in the eastern side of the corridor, specifically in or near indigenous territories with high forest cover. These animals belong to the indigenous people, roam freely in the forest and are practically feral. On the other hand, coyote, nine-banded armadillo and raccoon seem to avoid areas with high forest cover in the

study area. This was not surprising as these are adaptable species that can be found in open and/or disturbed areas (61,64–66).

Contrary to our predictions, human presence and EVI were positively and negatively associated with overall species habitat use respectively. But, both effects were subtle (Beta community values: human presence = 0.10 ±0.11; EVI = -0.08 ±0.24) and the 95% CI highly overlapped zero. This suggests varied species responses to these covariates and not a generalized preference for areas with human presence, or avoidance of areas with higher EVI. Measuring human presence as every adult human detected by the cameras, independent of their background or possible reason for being in the forest, could be the reason for the relationship we found. Indigenous people, farmers, tourists, and some hunters use the sites we surveyed. Evidently, not all of these have a strong negative effect on mammals (e.g. tourists), and some may actually be actively looking for these animals and selectively walking on the same trails or areas where they occur (e.g. hunters, indigenous).

With respect to EVI, it was surprising to see that its values across the study area were not correlated with forest cover. EVI has been shown to be correlated to gross primary production (GPP; a measure of productivity closely related to vegetation cover), and to perform better in dense vegetation than the normalized difference vegetation index (67,68). On the other hand, Sims et al. (69), found poor correlation between EVI and GPP in some evergreen sites in North America. More research on the association between EVI, GPP and forest cover in the study area may be needed to understand these results. Responses to the distance to strictly protected areas and ruggedness were also highly variable among species, but overall were negative as expected.

As we anticipated, medium and large mammal species richness was lower in the Corridor area than the two JCUs. The lowest values for richness of all species, richness of jaguar and puma prey, and jaguar and puma habitat use probabilities, were towards the central-west section of the Corridor, with low jaguar habitat use probability values extending inside the CVC JCU. These low-value grid-cells in the central-west section of the Corridor coincide with areas with less forest cover, higher settlement density and lie between the two main roads. Although these aforementioned anthropogenic covariates did not have an explanatory value in our model, they are known to have a negative effect on certain mammal species (see below). We believe that the two main roads, and the associated increased human presence and

pressures, may be having a barrier effect especially on large prey species, jaguars and pumas. Roads can affect a wide number of species through direct mortality (i.e. roadkills), by limiting home ranges, decreasing abundance, hindering dispersal and isolating populations, changing the characteristics of the habitat, and increasing human access (23,60,70–73). Rytwinski and Fahrig (74), found that road density and quantity were negatively related with reproductive rate and body size for mammals in Canada, suggesting a major effect of roads on large mammals with low reproductive rate (i.e. number of offspring and litters per year). Also, road avoidance has been documented for jaguars in Mexico and Belize, with stronger effects on females (75,76). A similar trend was found for pumas in Florida (77). We detected at least two jaguars, one male and one of unidentified sex, less than 2 km from the most-western main road (Route 10), but none on the other side. In fact, previous camera trap monitoring surveys in the study area since 2011 have only documented one male jaguar to the east of Route 10 (78; Salom-Pérez et al. unpub. data). Detections of puma and other large mammals have also been scarce between these two main roads (this study, Salom-Pérez et al. unpub. data). Thus, we believe that large prey species, jaguars and pumas may be able to get close to the road, if other conditions exist (e.g. forest cover, low human pressures), but will seldom cross and establish in the area between them. Specific studies on the effect of these roads on large mammal species should be made to recommend and implement proper management actions. Moreover, future studies should not only focus on using distance to roads as the road-related covariate; as this will probably not reflect the barrier effect it may pose on certain species, like in our case. We suggest complementing with studies on roadkills, crossing of individuals and gene flow with samples from both sides of the road.

The highest habitat use probability for jaguars was located inside or near a strictly protected area (i.e. Barbilla National Park, IUCN category II) in the TC JCU. It was surprising to see that jaguar habitat use probability in CVC JCU was very low, considering the area has high forest cover, seems to have enough prey and is part of a block of protected areas of ~1,153 km^2, an area previously assumed to be a core area for jaguars (40,48). Even so, most of the area surveyed in this JCU is not strictly protected (IUCN category VI), the terrain is very rugged and some areas have high elevation (i.e. above 2,000 and up to 3,300 m.a.s.l.). These conditions do not favor jaguar presence (41,79). In contrast, pumas have high habitat use probability in both JCUs. Pumas are known to occur more frequently in higher elevations than

jaguars, and could be benefitting from the apparent absence of a direct competitor in the CVC JCU (42,79,80). A recent investigation further west into the CVC JCU found no sign of jaguar, adding extra support to the hypothesis that this area is not the jaguar stronghold previously assumed (Velado et al. unpub. data). It remains to be established whether low jaguar presence in this JCU is explained by the site conditions mentioned above or if it responds to more historical pressures (e.g. hunting, isolation) or other variables.

Given their large home range sizes, wide distribution and relative sensitivity to human disturbance, it has been argued that conservation of important areas for large carnivores may help protect other wildlife (i.e. that these may serve as umbrella species) (81,82). Consequently, a large-scale conservation initiative has been implemented based on jaguar core (JCUs) and connectivity areas (6,83). Nonetheless, some authors question the seemingly careless use of the umbrella concept, given that there are few studies that have empirically tested this assumption (25–27,84). Thorne et al. (85), found that puma-based priority areas proportionally contained most broad-scale biodiversity elements analyzed in the central coast of California, but did not do well in representing some endangered terrestrial vertebrates. On the other hand, the Jaguar Corridor Initiative (JCUs and the corridor network that connects them) was found to be effective in protecting high-quality habitat for other mammal species, especially for larger ones, in Latin America (30). Similarly, Figel et al. (31), showed evidence that this jaguar conservation network included the majority of endemic herpetofauna species distribution in Central America. In our study, we found that puma and jaguar habitat use probabilities were strongly correlated with large prey species richness and, to a lesser degree, with medium prey species. This indicates that conservation actions directed to increase presence of these two carnivores should focus on improving the habitat conditions for prey species. Moreover, this relation seems to support the concept of jaguars and pumas as umbrella species for most large and medium-sized mammals in the area. However, we recommend additional studies that would include other taxa to test the umbrella value of these large carnivores over a broader number of species. When possible, the use of a group of species with different habitat requirements to plan a conservation strategy and monitoring programs should be favored (35–37,86,87). Yet, when resources are scarce, as it frequently occurs in the conservation world, the use of one or few verified umbrella species may be of great value (20,30,31,88).

Conclusions

We believe that the model presented here is an important tool for monitoring the status of a community of species, leading to better-informed management actions. The hierarchical community approach in a Bayesian framework allows for the use of data of rare species, that would otherwise be discarded or used improperly. Additionally, it helps produce stronger inferences on the status of a landscape in terms of biodiversity. The model is quite versatile as it can account for methodological differences (e.g. data taken over several sites or different years) through random effects.

Forest cover was the most important factor evaluated for the community of medium and large-sized mammals. Our study suggests that people who use forest resources in some way (e.g. hunters, indigenous people, tourists) can be positively related with forest animals. Low values found especially in the western side of the Corridor of almost all occupancy-based parameters measured here, suggest that the status of this critical link with respect to medium and large-sized mammals is precarious. Urgent actions are needed to guarantee connectivity of mammal populations elsewhere in Costa Rica and in the MBC. Pumas and jaguars were correlated with other large mammal species. Therefore, conservation actions in the area should focus on restoring areas in the central-west portion of the Corridor, between the two main roads, and increasing habitat quality for these prey species.

Further research should be made on the effect of roads on jaguars, pumas and large prey species as they might be an important barrier for regular movements and dispersal. Proper mitigation actions (e.g. underpasses, signage, arboreal crossings) should be implemented after identifying the most likely crossing points.

The Central Volcanic Cordillera JCU does not seem to be the jaguar stronghold previously assumed. More research may be needed to corroborate this hypothesis and find out why it is not favorable for jaguars.

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Chapter 2: Using a dynamic community model to measure the effects of a large hydroelectric project on medium and large-sized mammals in a critical Mesoamerican Biological Corridor

Abstract

Hydroelectric projects are one of the most important sources of renewable energy worldwide. Until now, very few studies have been able to obtain information before the flooding phase to evaluate the effects of these projects and their associated reservoirs on wildlife. Additionally, most investigations have focused on measuring the differences in species richness between reservoir-created islands and mainland areas. In this research we used a hierarchical dynamic community model in a Bayesian framework to measure the effects of the flooding of a hydroelectric reservoir on medium and large-sized mammals in a critical Mesoamerican corridor in Costa Rica. We found there was considerable increase in mean species occupancy in more than 65% of the study area from the pre-flooding period to the early-flooding (~1-2 months after the filling of the reservoir), resulting in a ~17% increase in occupancy overall. This increase could reflect species trying to escape from the reservoir and looking for refuge areas, thus increasing species occupancy. In contrast, mean species occupancy only decreased ~3% between the early-flooding and the post-flooding (~12 months after the filling of the reservoir) periods. There was no difference in how mean species occupancy changed between areas close (< 4 km) vs. areas far away (> 4km) from the reservoir among time periods, suggesting that the effect could be more widespread than expected. We found that mean occupancy changes among time periods varied by species. The majority of species showing a change, increase or decrease, among time periods had lower mean occupancy values when compared with species that remained relatively constant. Changes among time periods did not seem to be related to how dependent species are on forest areas or their tolerance to disturbance. We believe that dynamic community models can help reach more accurate inferences by basing conclusions on group responses rather than on individual species. The approach used here can be used to measure the effects of infrastructure in general on wildlife, help avoid impacts and plan proper mitigation actions.

Key words: occupancy, reservoir, infrastructure, impacts, Bayesian

Introduction

In the era of climate change, hydroelectric power and other renewable energy sources (e.g. solar, wind) are seen as important elements to eliminate the use of carbon-based fuels (1). While direct and indirect benefits of hydroelectric projects are palpable (e.g. energy production, employment, water for irrigation, flooding control), some have questioned how "green" they really are (2). Potential impacts related to these projects include alteration of biological and physical characteristics of rivers and aquatic systems downstream, disruption of connectivity of river systems and fauna, erosion, sedimentation, fragmentation, edge effects, increased access to previously remote undisturbed areas, release of greenhouse gases from reservoirs, methylmercury bioaccumulation, and social-related impacts (e.g. displacement, changes in economic activities, changes in access and use of water and land) (3–8). Consequently, some countries have started to remove dams as a river restoration tool (9–11). Nonetheless, between 1960 and 2005 the quantity of water in reservoirs related to dams grew four times, holding six times as much as natural rivers (12). The number of dams keeps rising, with 59,000 large dams ($\geq$ 15 m wall height) currently established worldwide, of which approximately 25% are being used to generate hydropower (13,14).

Negative effects of dams on aquatic fauna are arguably the most evident; blocking or restricting their migration, preventing their reproduction, degrading their habitat, isolating populations, and even generating local extinctions (2,3,6,15–17). Other studies have examined the potential impact of dams and their associated reservoirs on non-aquatic species by mainly measuring the differences between reservoir-created islands and mainland areas used as control sites (8,18). In general, these investigations found a decrease in species richness in reservoir islands with respect to mainland areas in all taxonomic groups studied, suggesting that lentic systems can become barriers to species movement. Most of these studies were made after the flooding phase and therefore were not able to compare the "before and after" species scenarios. Santos et al. (19), measured pre and post dam effects on four threatened carnivore species in Portugal, and found that they moved to refuge or "escape" areas with favorable habitat and enough prey, and in general, suffered a reduction in range. Changes in species

status (e.g. occupancy, range), resulting from disturbance or fragmentation, can lead to overcrowding of some areas, increase competition for resources like food, shelter and reproduction, and eventually affect fitness and survival of individuals (20,21). This can cause a loss of species, and consequently in biodiversity, even several years after the event; a process known as extinction debt (18,21,22).

In this investigation we used a hierarchical dynamic community model in a Bayesian framework to measure the short-term effects of the flooding of a hydroelectric reservoir on medium and large-sized mammals in a critical Mesoamerican corridor in Costa Rica. Gross or naïve estimates of species presence or traditional richness evaluations used to compare sites or changes in time fail to account for imperfect detection of species (23). In contrast, occupancy models consider detection probabilities of species and therefore produce unbiased estimates of species occupancy (23). In a dynamic framework, occupancy is dependent on the occupancy status in the previous time period, improving estimates of change (24). Additionally, the hierarchical structure has been shown to improve predictions in comparison to models where parameters were estimated independently (25). Our model adapted the hierarchical dynamic model presented by Miller & Grant (25) to incorporate multiple species. By using a multiple-species or community model, one can generate occupancy estimates for rarer species by drawing such estimates from community-level hyperparameters (23–26; Petracca et al. in press). The community model also allows for stronger inferences when compared to traditional single-species models that could be biased or influenced by other processes occurring in the area (25,28,29). Lastly, unlike traditional occupancy models, the Bayesian framework can account for unobserved heterogeneity (e.g. across species, time or sites) (30).

The objective of this investigation was to evaluate short-term effects of a flooding, related to a hydroelectric project, on medium and large-sized mammals in a critical corridor in Mesoamerica. Specifically, we evaluated: 1) the effects on the overall occupancy of medium and large mammal species, and 2) changes in occupancy for each species across the study area among three time periods (pre-flooding, early flooding and post-flooding). We expected that mean occupancy of medium and large-sized mammals would increase in the early flooding period with respect to the pre-flooding period, in the areas closer to the reservoir that would function as refuge areas, while other areas would remain relatively unaffected.

Materials and methods

Study area

Our study area is a portion of the Barbilla-Destierro Biological Sub-Corridor (hereon referred to as the Corridor) and surrounding areas close to the hydroelectric reservoir area, covering an area of 416 km^2. The Corridor is located in central-eastern Costa Rica, and connects two blocks of protected areas in the Central Volcanic Cordillera (CVC) and Talamanca (Figure 2.1).

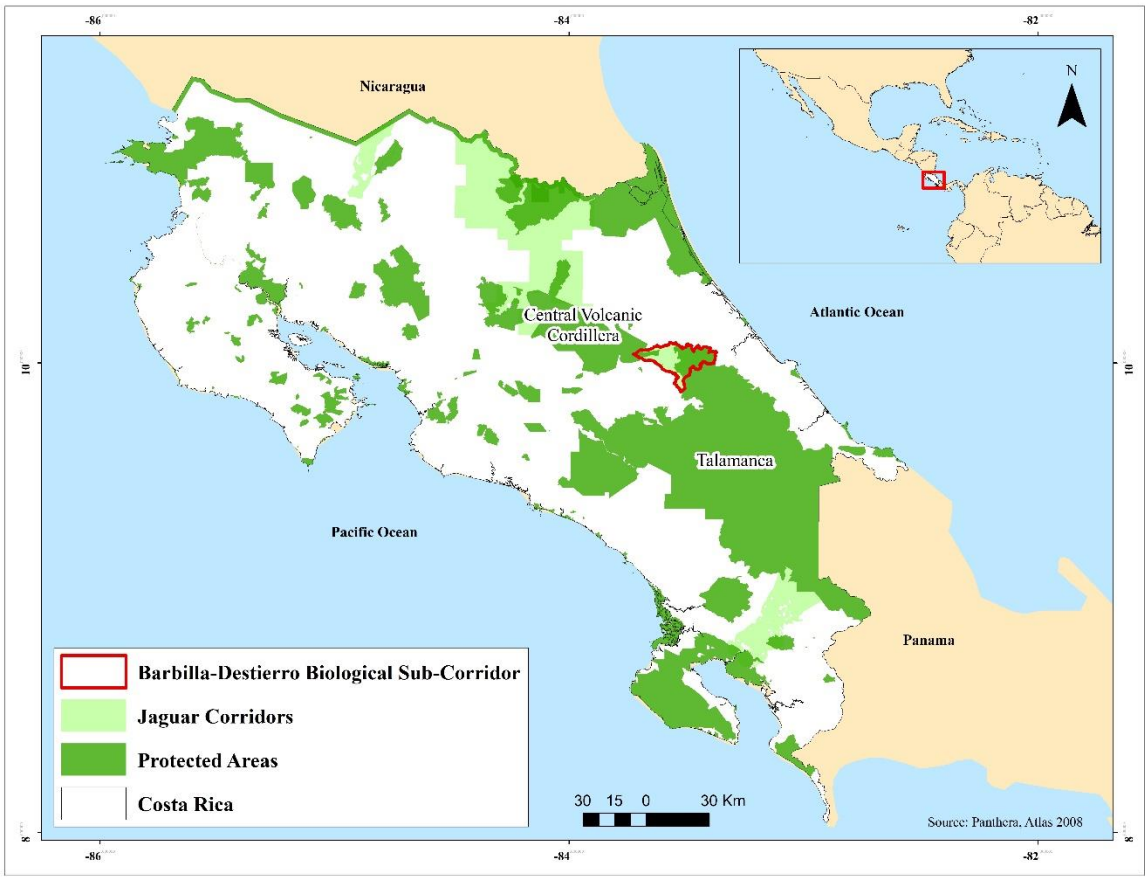

Figure 2.1. Protected areas (dark green) and jaguar corridors (light green) in Costa Rica. Red polygon indicates the Corridor area.

The Corridor is one of the most important links for jaguar (*Panthera onca)* connectivity at a local and regional level (30,31). No other suitable connections have been identified between

the Central Volcanic and Talamanca mountain ranges in Costa Rica, and more broadly between Nicaragua and Panama.

The local Costa Rican Electricity Institute (ICE-for its Spanish acronym) started constructing in 2010 the largest hydroelectric project, in terms of energy production (installed capacity: 305 megawatts), in Central America. ICE started flooding, in late November 2015, a ~7 km^2 area to create a reservoir in the center-west section of the Corridor; a critical area of connectivity between relatively continuous forest patches that originate from the protected areas to the east and west (Figure 2.2).

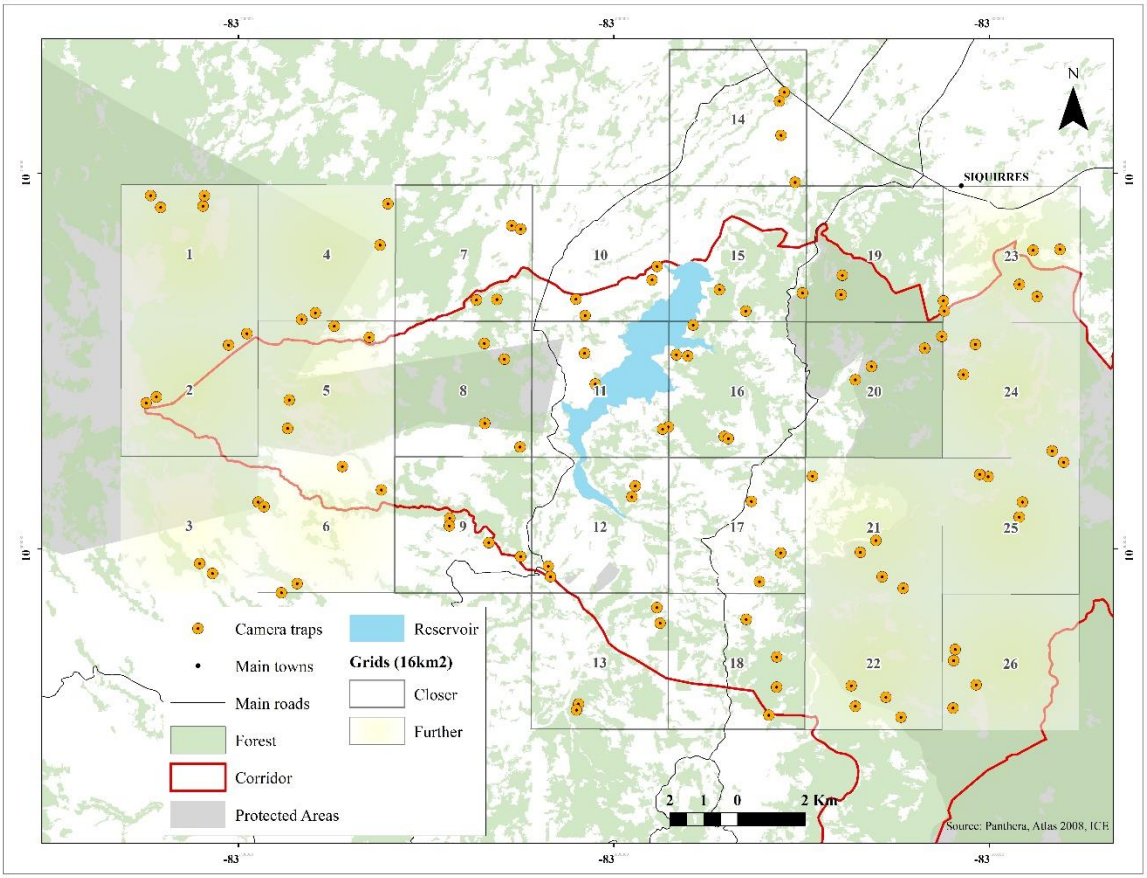

Figure 2.2. Location of camera trap stations within the 16 km^2 grid cells in the Barbilla-Destierro Biological Sub-Corridor (Corridor), Costa Rica, 2013-2018.

Study design and data collection

Camera traps were used to capture detections of medium (between 1-15 kg) and large (>15 kg) mammal species within the study area. We created a grid system of 26 cells (16 km^2 each) over the study area. Cell size represented approximate home range size of jaguars in Central America (32–34), as this investigation is part of a larger long-term monitoring project for the species. The 16 km^2 cells were later subdivided into four sub-cells of 4 km^2 each. We sampled two sub-cells per cell with two stations (one camera trap per station) in each sub-cell. This allowed for a more widespread distribution of camera stations in the cells, while decreasing the logistical difficulties in surveying each sub-cell. We placed one camera of each 4 km^2 surveyed sub-cell off a trail and the other one was placed on a human-made trail (when available), in an attempt to detect species that may avoid human trails (Figure 2.3).

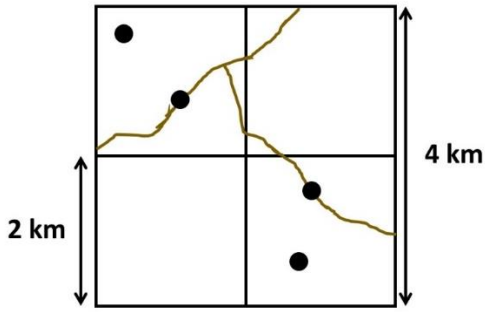

Figure 2.3. Graphic representation of a 16 km^2 cell, 4 km^2 sub-cells and the planned distribution of the camera stations in Barbilla-Destierro Biological Sub-Corridor (Corridor), 2013-2018. Black dots represent camera stations and brown lines represent human-made trails.

We placed motion-sensitive camera traps (Panthera® V3, V4, V5 and V6) in forested areas, strapped to trees at approximately 0.4-0.5 m above ground, a height intended to detect medium and large-sized mammals. We set the cameras to function 24 hours daily and to take three shots in every event during the day and one shot during the night. We recorded camera location using a GPS device (Garmin®), and checked them approximately every six weeks to download pictures and perform camera maintenance. We processed data on Panthera IDS (Panthera Integrated Data Systems; version 1.13.786), where the species, date, time and number of individuals in each photograph were recorded.

We surveyed the study area in three different time periods: T1 (pre-flooding), before the flooding of the reservoir: October 2013 – April 2015; T2 (early flooding), immediately after the completion of the flooding of the reservoir: March 2016 – August 2016; and T3 (post-flooding), approximately one year after the flooding of the reservoir: August 2017 – January 2018 (Figure 2.4).

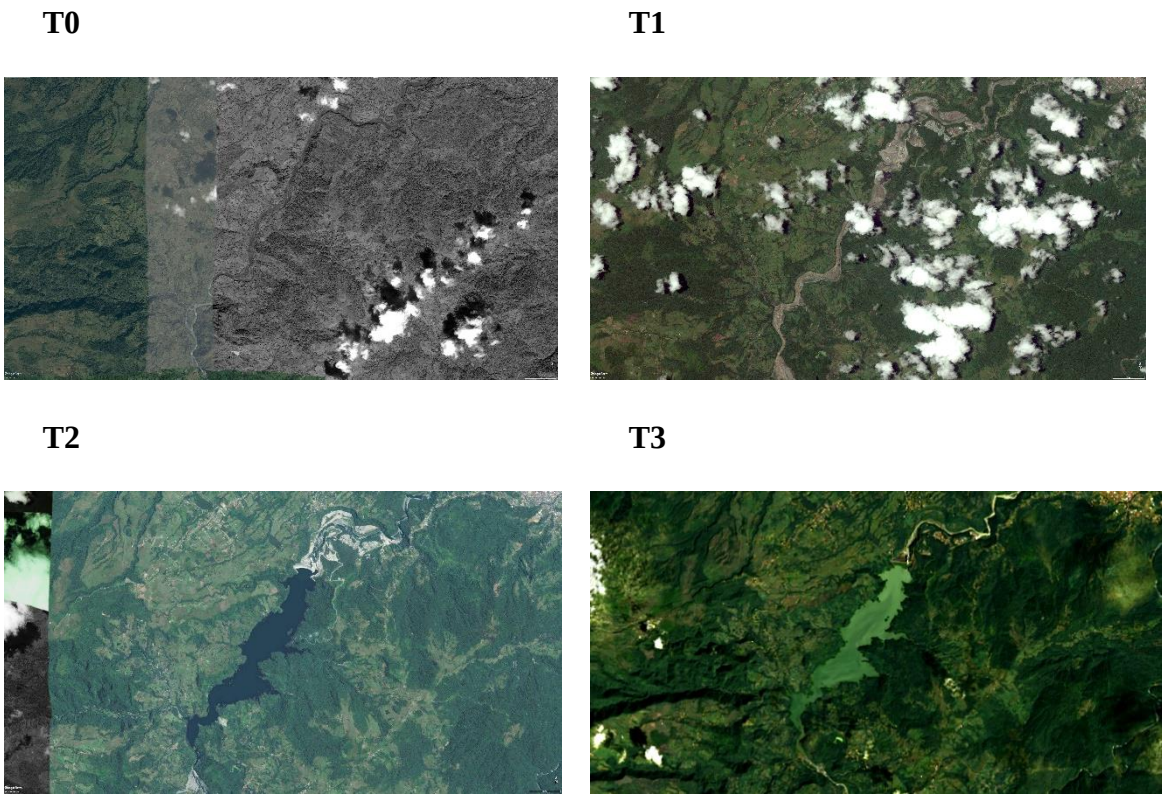

Figure 2.4. Satellite images of the reservoir area location from different time periods in Barbilla-Destierro Biological Sub-Corridor (Corridor), 2013-2018. T0: November, 2008; before any work related to the hydroelectric project. T1: May, 2014; impact of construction work near the dam site on the northern end and on the riverbed is evident, flooding has not started. T2: March, 2016; flooded area is at ~71% of its maximum level and the hydroelectric project is operational. T3: December, 2017; flooded area is ~95% of its maximum level. Google Earth Images Landsat/Copernicus, © 2019 Maxar Technologies and Landsat 8.

For the occupancy-based analyses, we collated data from all the stations in each grid cell to make detection histories ("1" = detected, "0" = not detected, "NA" = inactive camera) for each of the 24 medium and large mammal species detected (Table S1). Each sampling occasion (*k*) was one week.

We calculated effort, a covariate on detection, as a standardized value of the sum of all trap nights within each sampling occasion (*k*) across the grid cells.

Dynamic community model

In order to estimate occupancy of medium and large mammals among time periods T1, T2 and T3, all related to flooding of the hydroelectric reservoir, we used a dynamic community occupancy model in a Bayesian framework.

Our model is based on the basic dynamic model presented by MacKenzie et al. (24) and the hierarchical model by Miller & Grant (25), with the latter model altered to incorporate multiple species rather than sampling locations. Here, occupancy in a site on the first period was calculated directly, but occupancy on subsequent periods was dependent on the occupancy in the previous period. True occurrence *z* for species *i* in site (grid-cell) *j* in time period 1 was modeled from a Bernoulli distribution, where $z_{i,j,1} \sim \text{Bern}(\Psi_{i,j,1})$ and $\Psi_{i,j,1}$ is the probability that species *i* occurs in grid cell *j* in time period 1. For successive time periods, true occurrence *z* was modeled as $z_{i,j,t} \sim \text{Bern}(muZ_{i,j,t})$, where $muZ_{i,j,t}$ is dependent on (1) the probability of extinction (ε) at a previously occupied site (i.e. in time *t*-1), and (2) the probability of colonization (γ) of a previously unoccupied site. Thus, probability of occupancy at subsequent time periods *t* can be modeled as:

$$muZ_{i,j,t} = z_{i,j,t-1} * \left(1 - \varepsilon_{i,j,t-1}\right) + (1 - z_{i,j,t-1}) * \gamma_{i,j,t-1};$$

where ε and γ are calculated through the logit functions:

$$\text{Logit}\left(\varepsilon_{i,j,t}\right) = \alpha + \xi_t + \varsigma_i;$$

$$\text{Logit}\left(\gamma_{i,j,t}\right) = \eta + \theta_t + \pi_i;$$

where α and η are the intercepts, ξ_t and θ_t are random effects of time period, and ς_i and π_i are random effects of species. ξ_t and θ_t are drawn from normally-distributed hyperparameters with mean μ_ξ and μ_θ, and precision τ_ξ and τ_θ, respectively; and ς_i and π_i are drawn from normally-distributed hyperparameters with mean μ_ς and μ_π, and precision τ_ς and τ_π, respectively.

The logit transformation for detection probability was modelled through this equation:

$$\text{Logit}\left(p_{i,j,k,t}\right) = \omega_{i,t} + \beta_1 * effort_{j,k,t};$$

where $\omega_{i,t} \sim \text{Normal}(\mu_\omega, \sigma_\omega)$ is on a logit scale and is the random intercept for detection probability of each species *i* in time period *t;* and is drawn from a normally-distributed hyperparameter with mean μ_ω and precision σ_ω. β_1 is a fixed effect and is the estimated beta

coefficient for effort, where $\beta \sim$ Normal (0,0.01), and $effort_{j,k,t}$ is the covariate value on detection at site *j* on occasion *k* in time period *t*.

We ran these analyses in R (R Core Team 2015®; version 3.4.3) using the package jagsUI (35), running three MCMC chains with 10,000 iterations, 2,500 burn-in, and a thinning rate of three.

We obtained the following derived parameters using estimates of true occurrence, $z_{i,j,t}$: mean probability of occupancy for all species at each site (calculated as a mean of $z_{i,j,t}$ for each site and time period), and mean probability of occupancy for each species across the study area by time period (calculated as a mean of $z_{i,j,t}$ for each species and time period).

We estimated average percent of change in mean occupancy values of areas close to the reservoir (edge of grid cell closer than 4 km from any point of the reservoir) and areas further away (edge of grid cell further than 4 km from any point of the reservoir) (Figure 2.2).

Results

The number of weeks (*k*) and trap nights (*tn*) for each time period were: T1, *k*=35, *tn*=7,780; T2, *k*=19, *tn*=9,669; T3, *k*=24, *tn*=9,041; Total *tn*= 26,490 across 104 camera trap stations. The greatest increase in mean species occupancy occurred between the time period before the flooding and the early flooding period, where mean occupancy in T2 ($\bar{x}$=0.50, SD=0.08) across the whole study area was on average 17.11% greater than in T1 ($\bar{x}$= 0.43, SD= 0.08). Twelve grid cells had their mean species occupancy augmented in more than 10% in T2, and five were augmented more than 30%. The rest where within 10% of their original value in T1 (Figures 2.5, 2.6*a*).

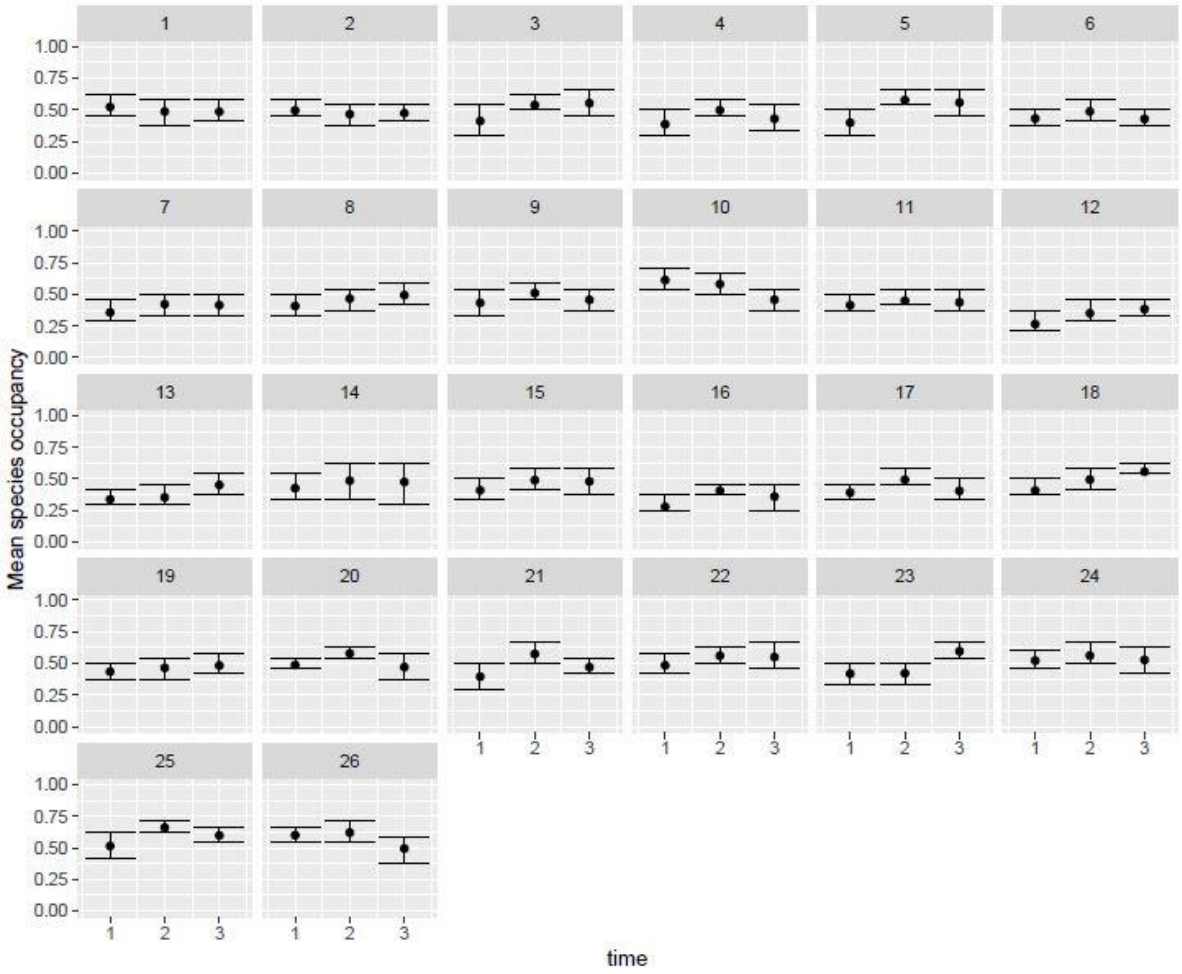

Figure 2.5. Overall mean occupancy values and 95% CI for the 24 medium and large mammal species for each of the 26 grid cells in T1: before the flooding of the hydroelectric reservoir, T2: immediately after the flooding, and T3: ~ 1 year after the flooding in Barbilla-Destierro Biological Sub-Corridor (Corridor), 2013-2018.

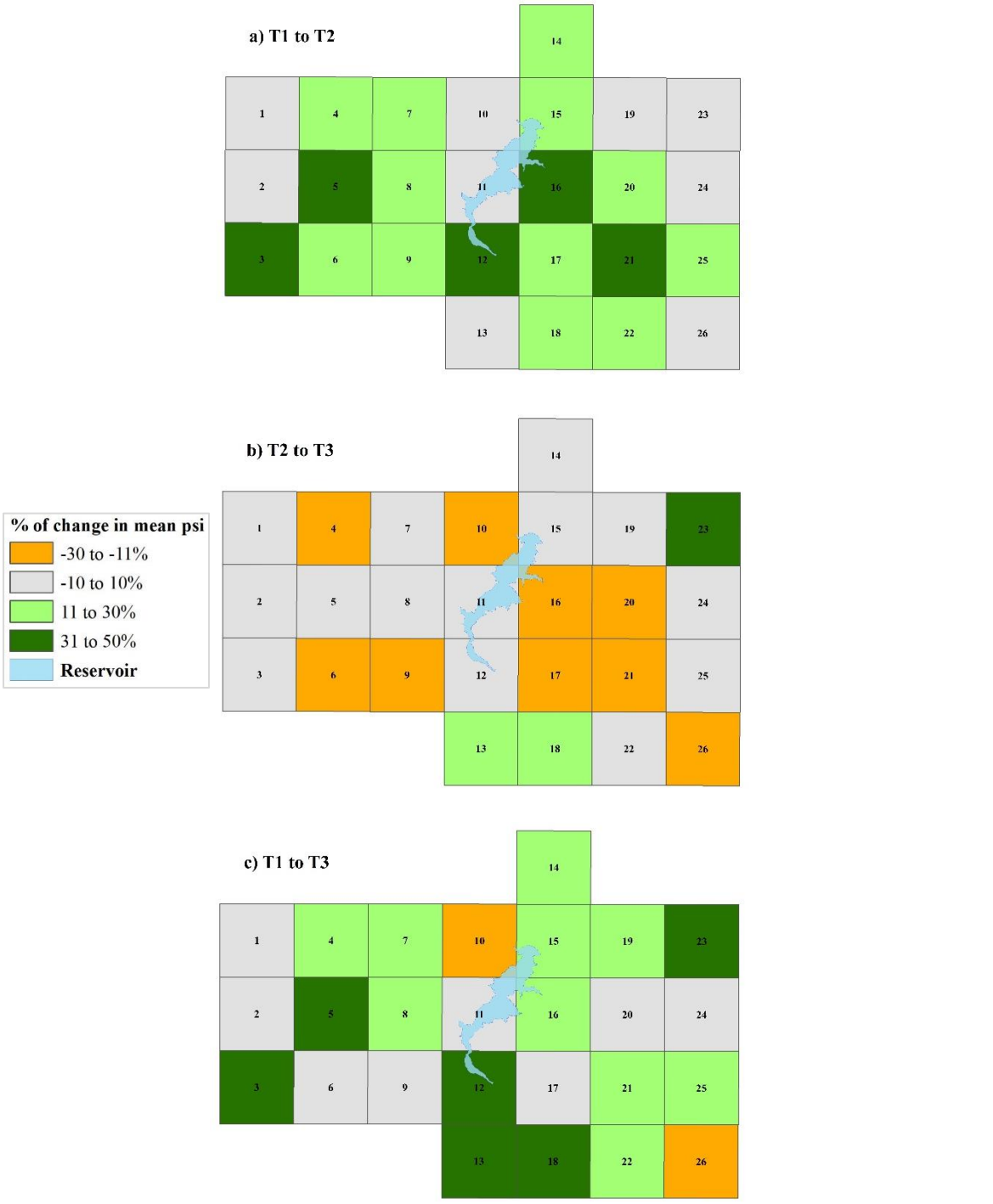

Figure 2.6. Percent of change in mean occupancy values for the 24 medium and large mammal species across the 26 grid cells between a) T1, before the flooding of the hydroelectric reservoir and T2, immediately after the flooding; between b) T2 and T3, ~ 1 year after the flooding; and between c) T1 and T3, in Barbilla-Destierro Biological Sub-Corridor (Corridor), 2013-2018.

Mean species occupancy overall between T2 and T3 ($\bar{x}$= 0.48, SD= 0.06) was similar; it dropped on average only 2.71%. However, nine grid cells had a drop of more than 10% in occupancy, 14 were more or less stable (between -10% and 10%), two increased more than 10%, and only one had an increase of over 30% (Figures 2.5, 2.6*b*). As a result, T3 still had 13.39% higher mean occupancy overall than T1 (Figures 2.5, 2.6*c*).

When comparing the percent of change in mean species occupancy for areas close to the reservoir area vs. areas further away, the values were very similar among time periods (Table 2.1).

Table 2.1. Average percent of change and standard deviation in mean occupancy values for the 24 medium and large mammal species comparing areas near the reservoir vs. areas further away between a) T1, before the flooding of the hydroelectric reservoir and T2, immediately after the flooding; between b) T2 and T3, ~ 1 year after the flooding; and between c) T1 and T3, in Barbilla-Destierro Biological Sub-Corridor (Corridor), 2013-2018.

	T1 to T2	T2 to T3	T1 to T3
Close to reservoir (edge of grid cell closer than 4 km from any point of the reservoir)	17.09 (±12.34)	-2.15 (±13.60)	14.41 (±17.99)
Further away from reservoir (edge of grid cell further than 4 km from any point of the reservoir)	17.13 (±18.48)	-3.38 (±16.02)	12.20 (±19.26)

When looking at estimates per species, mammals that showed considerable change (more than 10% increase or decrease) from T1 to T2 had lower mean occupancy across the study site than species that were relatively constant (species with change, $\bar{x}$=0.30, SD=0.25; species constant, $\bar{x}$=0.59, SD=0.28). The same relationship held true from T2 to T3 (species with change, $\bar{x}$=0.41, SD=0.28; species constant, $\bar{x}$=0.67, SD=0.29). Between T1 and T2, only white-tailed deer (*Odocoileus virginianus*, decrease in ~42%) and jaguarundi (*Puma yagouaroundi*, decrease in ~25%) showed a considerable decrease in mean probability of occupancy across the study area. On the other hand, 11 species had an increase in occupancy of more than 10%, including some that doubled or more than doubled their state in T1 (i.e. northern naked-tail armadillo, *Cabassous centralis*, increase in ~106%; greater grison, *Galictis vittata*, increase in ~393%; margay, *Leopardus wiedii*, increase in ~99%; and collared peccary, *Pecari tajacu*, increase in ~106%). Eleven species remained relatively stable (between -10 and 10% change) (Figure 2.7).

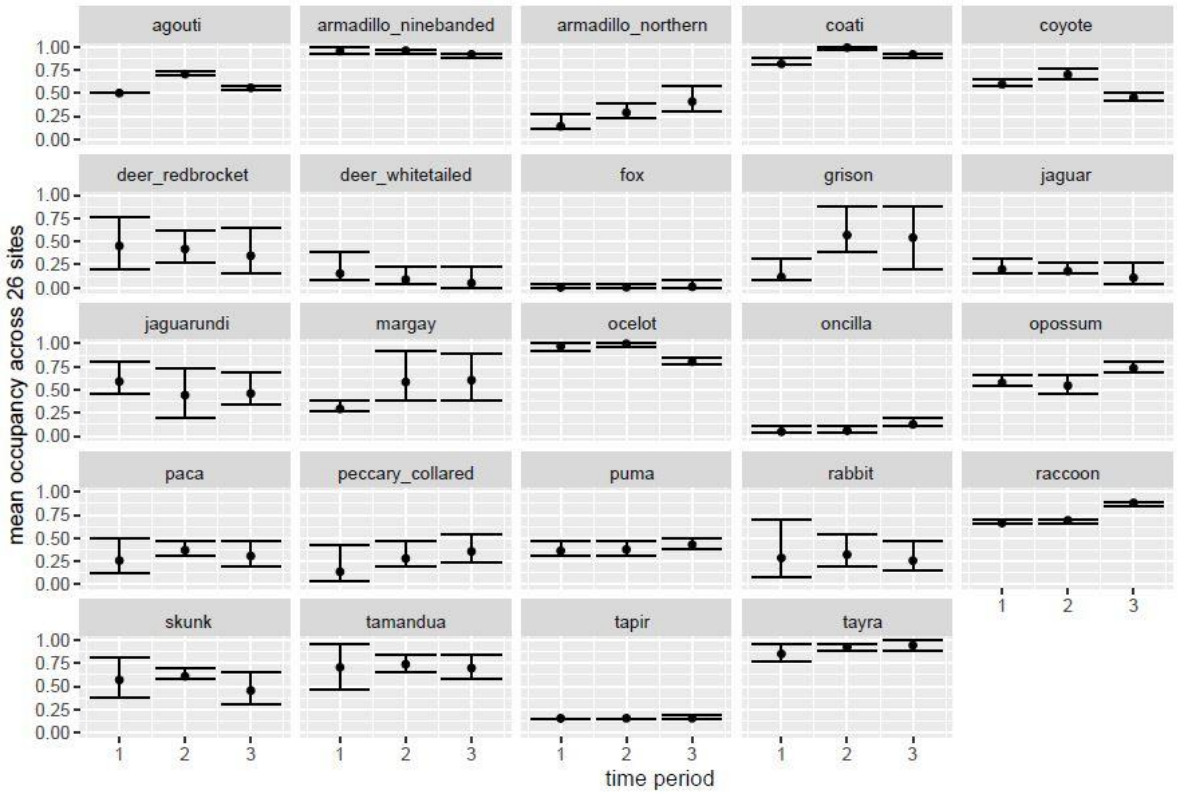

Figure 2.7. Mean occupancy values and 95% CI for each of the 24 medium and large mammal species across the study area in T1: before the flooding of the hydroelectric reservoir, T2: immediately after the flooding, and T3: ~ 1 year after the flooding in Barbilla-Destierro Biological Sub-Corridor (Corridor), 2013-2018.

We found that nine species decreased in mean occupancy more than 10% between T2 and T3; with jaguar, white-tailed deer and coyote (*Canis latrans*) decreasing more than 35%. Whereas seven species increased more than 10%; with northern naked tail armadillo, fox (*Urocyon cinereoargenteus*), oncilla (*L. tigrinus*) and opossum (*Didelphis marsupialis*) augmenting more than 34%. The rest of the species (*n*=8) remained relatively stable (between -10 and 10%) in this time period (Figure 2.7).

Discussion

Human infrastructure can cause several impacts over wildlife, such as habitat loss and fragmentation, the creation of barriers to movement, impede dispersal and gene flow between populations, create an edge effect over natural areas, cause changes in behavior, reduce fitness,

cause direct mortality, and decrease species abundances (36–39). Yet, few studies have information about the initial status of landscapes prior to the construction of infrastructure, including dams and hydroelectric projects (39). In Costa Rica, 98% of the energy has come from renewable sources in the last four years, where hydroelectric energy accounts for more than 70% (40). Thus, knowing the effects of these projects, in Costa Rica and elsewhere, is of utmost importance to avoid their impacts or know how to mitigate them.

Herein we monitored a corridor area impacted by a hydroelectric dam before and after the flooding, and used a dynamic community model to measure the potential effects on medium and large-sized mammals. Our study focused on the single most drastic alteration of the landscape associated with the hydroelectric scheme, the filling of the 7 km^2 reservoir.

Overview

We found there was considerable increase in mean species occupancy in more than 65% of the study area from the pre-flooding period-T1 to the early-flooding-T2; resulting in a ~17% increase overall. In contrast, only about 11% of the study area showed an increase and 35% showed a decrease between the early-flooding and the post-flooding-T3 phases; overall decrease was small across the study area (less than 3%). In other words, changes in occupancy in medium and large-sized mammals, potentially attributed to the flooding, were still evident at least one year after the hydroelectric project became operational. In other studies, negative effects on species richness on reservoir islands has been documented for several decades after isolation (18,41). It remains to be seen if mean species occupancy around the studied reservoir will keep decreasing, when will it stabilize, and if it will stabilize below or above its pre-flooding value.

While the increase in species occupancy detected here may be seen as positive, this change could reflect species trying to escape from the impact and look for refuge or escape areas (19). Individuals moving to areas that might already be occupied and limited in resources, may increase competition and potentially have negative effects on their fitness and survival. Nevertheless, we recommend caution when looking at these results as confidence intervals of overall species occupancy across the study area and individual species occupancy by site highly overlapped between time periods.

The effect of flooding of the reservoir

We didn´t find a clear spatial pattern in how mean species occupancy changed between areas close vs. areas far away from the reservoir among time periods; even when loss of forest was three times greater in the area close to the reservoir. One possibility is that the area impacted is bigger than what we expected, causing species to move to areas that were more than 10 km away from the reservoir looking for refuge areas with suitable habitat. Benitez-Lopez et al. (39), performed a meta-analysis on 49 studies including data on 243 mammal species, and found that population densities declined with proximity to infrastructure. They reported that the declines were usually evident up to ~5 km from infrastructure, but could extend up to 17 km for certain taxa and depending on habitat type. Additionally, they found that the effect extended over larger distances in non-forested areas when compared to forests (39). This could be a factor explaining the detection of changes in areas far apart from the reservoir, as the area closer to it is highly fragmented by agriculture and urbanization. Also, species might be moving to areas already occupied by conspecifics or other guild competitors, and would be forced to keep moving if resources or mates are not sufficient (42,43).

An alternative explanation is that the changes detected here have little or no relation with the flooding. Nevertheless, we consider this unlikely as there was no other major event or pressure (e.g. major land use change, harsh climatic conditions) occurring in the area during the study period. Additionally, we don´t think these changes are due to natural variation because of two main factors: 1) our pre-flooding period was long enough (1.5 years) to capture the natural variation in species occupancy, and 2) the increase occurred in almost half of the species (see below).

The majority of species showing a change, increase or decrease, among time periods had lower mean occupancy values when compared with species that remained relatively constant. This was expected, as for species with low occupancy even small changes in the number of detections may yield proportionally larger changes in occupancy. Hence, we propose that changes in several species with low occupancies may serve as early indications of disturbance; that might not be evident for species with high occupancy values until disturbance is stronger. Yet, more studies are needed to corroborate this hypothesis. Also, we recommend caution when evaluating one or a few species with low occupancies that could be affected by other factors, and suggest the use of community approaches such as the one presented here.

Consistent with the community values discussed above, 11 out of 24 species increased their mean occupancy across the study area between the pre-flooding and the early flooding period. Benitez-Lopez et al. (39), found that the effect of infrastructure was detected over larger distances for Artiodactyla species in comparison to Rodentia species, and argued that mammals with larger body sizes and larger area requirements are more sensitive to disturbance and habitat fragmentation. Additionally, Irving et al. (8) studied the changes of a reservoir on bird communities on island and mainland sites and found greater effects on disturbance-intolerant species. In our study, only two large-sized mammals (collared peccary-increase and white-tailed deer-decrease) showed considerable changes in their occupancy, while the other four large species remained constant (red brocket deer, jaguar, puma and tapir). Nonetheless, the occupancy of these four species is concentrated in areas further away from the reservoir with highest forest cover (Salom-Perez et al. in prep.), where the reservoir-related effects could have been diluted.

Out of the 24 species, only white-tailed deer and jaguarundi showed a decrease of more than 10% in mean occupancy between the pre-flooding and the early flooding period. Mean occupancy values for white-tailed deer were low (< 0.16) throughout the study period and its detection was concentrated in a small portion of the study area (in three out of 26 grid cells). Thus, changes in occupancy estimates for white-tailed deer in the area could be caused by numerous factors (e.g. hunting, change in home range, change in forage area). On the other hand, jaguarundis can be found in fragmented or disturbed areas with access to forest or vegetation cover (41; Salom-Pérez et al. in prep.). Thus, a decrease in mean occupancy for this species was less expected than for others. Yet, a combination of habitat reduction related to the reservoir and an increase in occupancy of other mesopredators, such as ocelots (*L. pardalis*) and coyotes, in our landscape may have a negative effect on jaguarundis (44,45).

Post-flooding changes

Species that decreased in mean occupancy across the study area between early flooding and post-flooding periods included some that are usually more affected by disturbances (e.g. paca, *Cuniculus paca*; red brocket deer, *Mazama temama;* jaguar), and others known to be relatively tolerant to disturbed areas and/or prefer open areas (e.g. white-tailed deer, ocelot, coyote). The same occurred with species that increased more than 10% in mean occupancy,

with puma (*Puma concolor*) and collared peccary on one hand, and common opossum and common raccoon (*Procyon lotor*) on the other (Figure 2.7). Thus, changes between these two periods did not seem to be related to how dependent species are on forest areas or how much they tolerate disturbance. Species can be affected by changes in their habitat in different ways, depending on aspects like home range size, distribution with respect to the source of change, food availability, movement capabilities, and dependency on certain natural cover or certain habitat types (8,19,22).

Mitigation actions

Since the start of the hydroelectric project, the Costa Rican Electricity Institute has been implementing a number of mitigation actions following environmental safeguards set by the financial institutions that support the project (i.e. Inter-American Development Bank, World Bank), and their own guidelines (46). Some of the main actions designed to avoid biodiversity loss and secure connectivity of animal populations include reforestation projects, protection and restoration of a buffer of at least 50 m around the reservoir, payment for environmental services (an incentive to protect or restore the forest) to landowners, support of sustainable production activities, and environmental education and the setup of their own monitoring program. Alongside, they have implemented a monitoring project on vertebrates since 2014 based, in part, on recommendations derived from the present study (47). It will be very important to evaluate the potential positive effects of these measures on the mammal community and other species in the years to come.

Conclusions

With this research we have demonstrated the value of repeated monitoring and the use of a dynamic model, especially when trying to measure impacts of human-related infrastructure on wildlife. One-time surveys or environmental impact studies, usually performed associated with this type of projects, do little to properly account for impacts and plan appropriate mitigation actions. Additionally, hydroelectric projects should gather baseline information before any disturbance (*e.g.* dam construction, new roads, road paving, extraction of materials from the river, higher human presence) is made.

We detected changes in mean occupancy for mammal species across the study area, suggesting a possible impact related to the reservoir extending for more than 10 km. For future studies measuring the impact of infrastructure on medium and large-sized mammals, it would be worthwhile to use control areas that have similar conditions to the area under study, but that are at least 20 km away.

Considerable changes in medium and large-sized mammals' occupancy were still evident at least one year after the flooding. Thus, we recommend repeating the survey in subsequent years at least until the values measured stabilize. Afterwards, surveys can be spread by 5-10 years to continue monitoring potential changes related to the impact or to the mitigation measures that could be applied.

We found that mean occupancy changes among time periods varied by species, indicating that one could reach entirely different conclusions depending on the species studied. This underscores the value of using community models that can help reach more accurate inferences by basing conclusions on group responses rather than on individual species. Additionally, future studies should consider other groups of wildlife (e.g. other mammals, amphibians, reptiles, birds) that may respond differently to impacts.

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Chapter 3: Evaluating genetic diversity and structure for ocelots (Leopardus pardalis) in Costa Rica

Abstract

Ocelots (*Leopardus pardalis*) have a very large range, occurring from southern United States to northern Argentina. They occupy a wide variety of habitats but they are associated with areas with well-structured vegetation cover. Additionally, they usually are the most abundant wild cat in the areas where they occur and are listed as least concern by IUCN. Yet, all but one, of the genetic studies of the species that include individuals from Central America are based on very small sample sizes. To our knowledge, this is the first conservation genetic study on ocelots in Costa Rica and the second one in Mesoamerica to measure genetic diversity and population structure of the species at a countrywide level. We evaluated genetic diversity and population structure of ocelots using 15 microsatellite loci in 31 successfully genotyped samples gathered throughout the country. We also compared the genetic diversity of Costa Rican ocelots with that of jaguars (*Panthera onca*) and pumas (*Puma concolor*) in the country, and with ocelots in Belize. Genetic diversity of ocelots in Costa Rica was relatively high (N_A = 6.87 ± 1.71; rarified A_R = 5.50 ± 1.36; H_O = 0.73 ± 0.12; H_E = 0.79 ± 0.08). We didn´t find patterns of genetic substructure, suggesting high levels of gene flow throughout the country and no strong barriers to movement. As expected, genetic diversity of Costa Rican ocelots was higher than that of jaguars and pumas using seven shared loci. Additionally, levels of genetic diversity were slightly higher in Costa Rican ocelots when compared to their counterparts in Belize, confirming the south to north decrease in genetic diversity reported in other studies for the species. Our study provides critical baseline information to understand what is the status of the ocelot populations in the country. Future studies in this and other threatened or keystone species should incorporate conservation genetics to properly inform management decisions and guarantee their long-term survival and improve the resilience of ecosystems in general.

Key words: conservation genetics, gene flow, microsatellites, non-invasive genetic sampling

Introduction

Conservation efforts should consider the three main recognized forms of biodiversity: genetics, species and ecosystems (1). While genetic diversity of wild populations is increasingly being studied by the scientific community, it is still largely ignored in many global conservation strategies (e.g. Aichi Targets 2011-2020), where prioritization has been given to the wild relatives of domesticated species of direct economic (2,3). This approach overlooks the importance of evaluating the genetic diversity and population structure of wild species that may have an important role in maintaining the resistance and resilience of ecosystems, or that may be threatened with extinction (4). A reduction in genetic diversity and the isolation of populations may decrease the ability of a species to survive by reducing its ability to adapt to environmental changes or human-related threats, decreasing fitness (e.g. through inbreeding and loss of genetic diversity), and increasing genetic drift (5–7).

Wild cats in general have a higher extinction risk than most other groups of vertebrates, because of their life history traits, habitat requirements, and the pressures they face (8). They generally have low population densities and slow growth rates which translates into slow recovery rates (8,9). Additionally, they are usually located in high trophic levels depending almost entirely on animal prey, and have relatively large and high-quality area requirements (9–11). This makes them especially vulnerable to habitat degradation and fragmentation. Furthermore, wild cats have been historically persecuted by humans for a number of reasons; for their pelts, because they are seen as competitors or threats, or to protect domestic animals (9,11,12). Consequently, negative fitness effects related to the loss of genetic variation have already been documented for multiple wild cat species (5,13–15).

The number of investigations on genetic diversity and population structure on the ten species of wild cats in the Neotropical realm is growing (*Leopardus colocolo*(16–18)*; L. geoffroyi:* (16,18,19)*; L. guigna*: (16,20); *L. jacobita:* (17,21)*; L. pardalis*: (22–31); *L. wiedii*: (22,23); *L. tigrinus*: (16,18,19,23,24); *Panthera onca*: (24,30,31,33–40); *Puma concolor*: (24,30,31,35,39,41–43); *P. yagouaroundi*: (35,44,45)). While ocelots (*L. pardalis*) have been the focus of more genetic studies than other Neotropical cats, investigations that include

samples from Central America have been based on small sample sizes with the exception of studies of Wultsch et al. (30,31) in Belize.

The ocelot is found from southern United States (Arizona and Texas) to northern Argentina, and is usually the most abundant wild cat in the areas where it occurs (46). The major threats to this wild cat across its range are: loss and fragmentation of their habitat, retaliatory killing, and illegal trade of their pelts (46–48). Their association with well-structured vegetation cover, high abundance, relatively large home ranges and almost ubiquitous presence (46,49,50), make the ocelot a valuable species to study the potential effects of extensive fragmentation and habitat alteration, especially in cases where low densities and lack of data make it difficult to study other species of conservation concern such as jaguar and puma.

Genetic diversity in ocelots is generally high in South America, moderate in Central America and low in the northern-most populations in Tamaulipas and Texas (21–23,25,26,28–31). In the most comprehensive study of this species in Central America, Wultsch et al. (31) found that in Belize ocelots had higher genetic diversity than pumas and jaguars, and genetic differentiation in ocelot populations was either low or non-existent throughout the country. In Costa Rica, the only genetic research on wild cats evaluated jaguar and puma genetic diversity and population structure using nuclear DNA microsatellite loci (39). She found moderate levels of genetic diversity for jaguars and pumas, and indications of genetic subdivision for both species in certain parts of the country.

The goal of the present study was to evaluate genetic diversity and population structure of ocelots in Costa Rica. Specifically, we assessed if ocelots in Costa Rica are a single panmictic population or if there is any evidence for population substructure. We also compared the genetic diversity of Costa Rican ocelots with that of jaguars and pumas in the country, and with ocelots in Belize. We expected that ocelots would be a single panmictic population, and that gene flow has not been strongly affected by human or natural barriers. We also believed that ocelots would have a higher genetic diversity compared to jaguars and pumas in Costa Rica, due to the ocelot´s ability to use a wider array of habitats and move through them, and because they are presumed to have larger effective population sizes. We also hypothesized that genetic diversity of Costa Rican ocelots would be lower than that in Belize populations, given that connectivity between populations and effective population size in Belize, whose

populations are presumably connected with those in Guatemala and Mexico, are expected to be higher than Costa Rica. This study will help establish important baseline information on the population status of ocelots in Costa Rica, evaluate how it compares with other larger wild cats and with ocelots in Belize, and determine if ocelots are being affected by human-related or natural barriers to connectivity.

Materials and methods

Study area

This study was executed throughout Costa Rica. At a size of just over 51,100 km^2 Costa Rica is said to hold 5% of the world´s biodiversity in 0,3% of the land mass, including 249 species of mammals (51). This country, along with the rest of the Central American isthmus, is a critical link for wildlife between North and South America. Consequently, Costa Rica has been recognized for its efforts on conservation, having approximately 25% of the country under protected areas.

Elevation in the country goes from 0 to 3,820 meters, and it has two main seasons: Rainy (May-November) and Dry (December-April). A variety of Life Zones are present in Costa Rica, from Tropical Dry Forest, to Rain Forest and Paramo (high altitude non-forest vegetation) (52).

There are six species of wild cats recognized in the country: jaguar (*P. onca*), puma (*P. concolor*), ocelot, jaguarundi (*P. yagouaroundi*), margay (*L. wiedii*), and oncilla (*L. tigrinus*). The ocelot is presumed to be the most common and least threatened of the felid species (46), while the jaguar is considered Near Threatened, and the puma is listed as Least Concern by the IUCN (53,54). Ocelots are the largest of the small spotted cats in the Americas, and while they can occupy a wide variety of habitats, they are usually associated with areas with significant forest cover (46). In Costa Rica they can be found throughout the country (55).

Field sampling and DNA sample storage

We collected 68 biological samples from throughout Costa Rica for genetic analysis (Figure 3.1). We gathered ocelot samples either by sampling from museums or private collections, road kills, or the wild. We collected some fecal samples in the wild using a scat detector dog across several field sites. Other fecal samples were provided by collaborators that found them opportunistically. Genetic samples used for this study included tissue, feces, hair, blood, teeth and bone (see Results for details on sample sizes). After collection, we kept samples in a dry place and took them to the lab as fast as possible (usually within 72 hours). Once they came from the field, we kept samples in a freezer at -20 °C until DNA extraction.

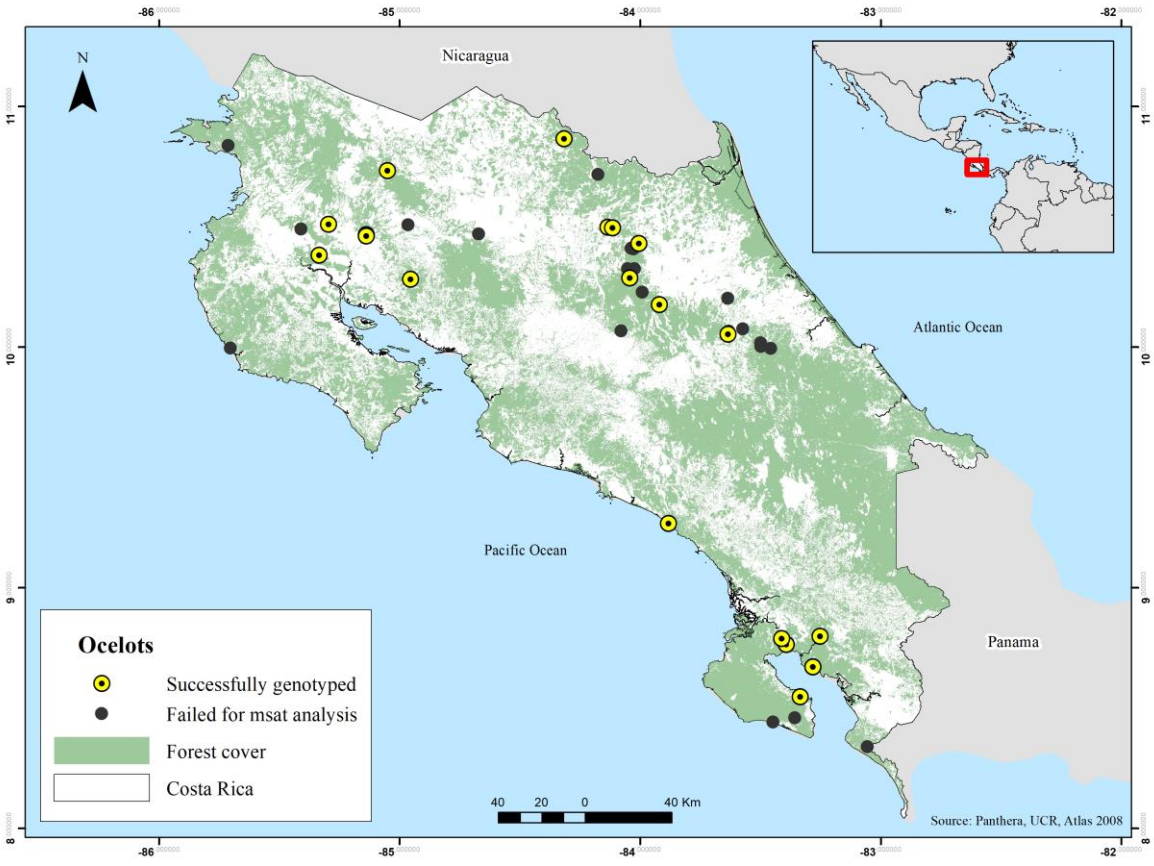

Figure 3.1. Location of the samples successfully identified as ocelots (Leopardus pardalis; n = 68), using DNA sequencing of regions of four mitochondrial genes and successfully genotyped using 15 microsatellite loci (n = 31) in Costa Rica. Thirty-seven samples failed microsatellite analysis.

DNA extraction

We extracted DNA from samples at the Genetics Conservation Laboratory at the School of Biology, University of Costa Rica, using different protocols according to the type of material (see Supplementary Data SD1). For fecal samples, we used the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA, USA) protocol for isolation from stool for human DNA analysis with modifications based on Chaves et al. (56). For samples obtained from museum specimens (teeth, hair, bones, tissue), we used the QIA amp DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) with modifications based on (43). Finally, for fresh samples (blood and tissue), we used the QIA amp DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) following standard protocols. We stored all purified DNA samples at -20 °C until the remaining analyses.

Species identification with mitochondrial DNA

We conducted species identification at the Global Felid Genetics Program at the Sackler Institute for Comparative Genomics at the American Museum of National History (AMNH), New York, USA. We screened all samples for species identification using species-specific primers amplifying regions of four mitochondrial genes: cytochrome b (H15149, (57,58)), 12S (L1085, H1259, (59)), 16S (L2513, H2714, (59)), 16Scp (16Scp-F, 16Scp-F, (59)) and adenosine triphosphate-6 (ATP6-DF3, ATP6- DR2, (60)). We edited sequences using Sequencher, version 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA) and Geneious Pro, version 5.6.5 (Auckland, New Zealand), and aligned them to an in-house reference database compiled for carnivore species. We assessed the relationships and sequence similarity among species by constructing a phylogenetic tree using the neighbor-joining method to infer the origin of the samples.

Microsatellite amplification and genotyping

We conducted all microsatellite genotyping at the University of Idaho Laboratory for Evolutionary, Ecological and Conservation Genetics. We tested 21 polymorphic microsatellite loci, most of which were used by Soto (39) and/or Wultsch et al. (30) (F53, F98,

F124, FCA008, FCA043, FCA045, FCA075, FCA090, FCA096, FCA100, FCA117, FCA124, FCA126, FCA132, FCA208, FCA225, FCA229, FCA275, FCA391, FCA506, FCA559) (61,62). We also tested two sex markers: AMEL and ZN fingers (63,64). Due to poor performance (insufficient amplification or messy unscorable patterns), we excluded F53, FCA559, FCA506, FCA100, FCA 208, FCA225, and AMEL. We identified the gender of the ocelot samples by using ZF-1F/ZFX-1R and ZFY-2F/ZF-2R primer sets developed for tigers (*P. tigris*) (65).

We arranged the rest of the loci (n =15 plus the sex marker) in two PCR multiplex reactions using a total volume of 10 µL each. Multiplex 1 consisted of 5µl 2 x concentrated Qiagen Master Mix (Qiagen, Inc., Valencia, CA, USA), 1.46 µl of primers (0.07 µM for F98, 0.06 µM for F124, 0.12 µM for FCA008, 0.30 µM for FCA043, 0.07 µM for FCA117, 0.09 µM for FCA126, 0.20 µM for FCA132, 0.25 µM for FCA275, 0.30 µM for FCA391), 1.0 µl of 5 x concentrated Qiagen Q solution (Qiagen, Inc., Valencia, CA, USA), 0.54 µl H_2O, and 2.0 µl DNA extract. Multiplex 2 consisted of 10 µl 2 x concentrated Qiagen Master Mix (Qiagen, Inc.), 0.79 µl of primers (0.12 µM for FCA045, 0.03 µM for FCA075, 0.20 µM for FCA090), 0.11 µM for FCA124, 0.08 µM for FCA229, 0.25 µM for Zn fingers), 1.0 µl of 5 x concentrated Qiagen Q solution (Qiagen, Inc.), 1.21 µl H_2O, and 2.0 µl DNA extract. We ran FCA096 was run in singleplex with 10 µl 2 x concentrated Qiagen Master Mix (Qiagen, Inc., Valenica, CA, USA), 0.07 µM for FCA096 1.0 µl of 5 x concentrated Qiagen Q solution (Qiagen, Inc.), 1.93 µl H_2O, and 2.0 µl DNA extract.

We conducted microsatellite PCR amplifications starting with an initial denaturation step of 15 min at 94 °C; followed by 13 cycles of 30 s at 94 °C for denaturation, 1.5 min at 62 °C with a decrease in annealing temperature of 0.4 °C in each cycle, and 1 min elongation at 72 °C; followed by 42 cycles of 30 s at 94 °C for denaturation, 1.5 min at 57 °C for annealing, and 1 min elongation at 72 °C; and 30 min at 60 °C for final elongation, followed by 10 min at 4 °C for cooldown. We included a PCR negative in each group of PCR reactions to control for contamination. We also included a PCR positive control in each reaction.

Primers were fluorescently labeled and we visualized PCR products using an ABI 3130xl DNA analyzer (Applied Biosystems™, Carlsbad, CA, USA). Genotypes were identified using the software GENEMAPPER, version 5.0 (Applied Biosystems™, Carlsbad, CA, USA). To

finalize consensus genotypes and to minimize genotyping error, we used a multi-tube approach (66) with a minimum of 4 repetitions for each microsatellite multiplex and ocelot sample. Two PCRs were required to confirm a heterozygote and three to confirm a homozygote. To confirm the individual identification and assess the resolving power of the 15 microsatellite loci, we used GenAlEx, version 6.503 (67) to calculate the probabilities of identity, $P_{(ID)}$, the probability of identity and $P_{(ID)sibs}$, the probability of identity between siblings.

Data analysis

We used program GenAlEx, version 6.503 (67) to assess genetic variation at single loci and across all loci by calculating the number of alleles (N_A), estimate observed (H_O) and expected heterozygosities (H_E), and fixation/inbreeding index (F). Additionally, we determined allelic richness (A_R) using the rarefaction method with HP-RARE, version 1.1 (68). We tested linkage disequilibrium and Hardy-Weinberg equilibrium using Genepop, version 4.2 and applied the Holm-Bonferroni correction (69–71).

To investigate genetic structure and visualize similarities and dissimilarities of the genotype data, we calculated pairwise genetic differences between individuals in GenAlEx, version 6.503 (67), and later used this information in a principal coordinate analysis (PCoA). To complement this information we used Program STRUCTURE, version 2.3.4 to implement a non-spatial clustering analysis (72). We also performed additional analyses in STRUCTURE to test specific hypotheses about potential barriers: 1- main mountain ranges, and 2- the Great Metropolitan Area (GMA), the highest human population density area (see below). These factors, alongside roads, are some of the few variables that we hypothesized may have an effect on ocelot dispersal given their high habitat plasticity. We grouped samples *a priori* in four groups based on their location with respect to the highest mountains in the country (>1,200 masl) (NW: Northwest, NE: Northeast, SW: Southwest, U: Unknown location) (Figure 3.2). Figures with STRUCTURE results were made using Pophelper version 1.0.10.

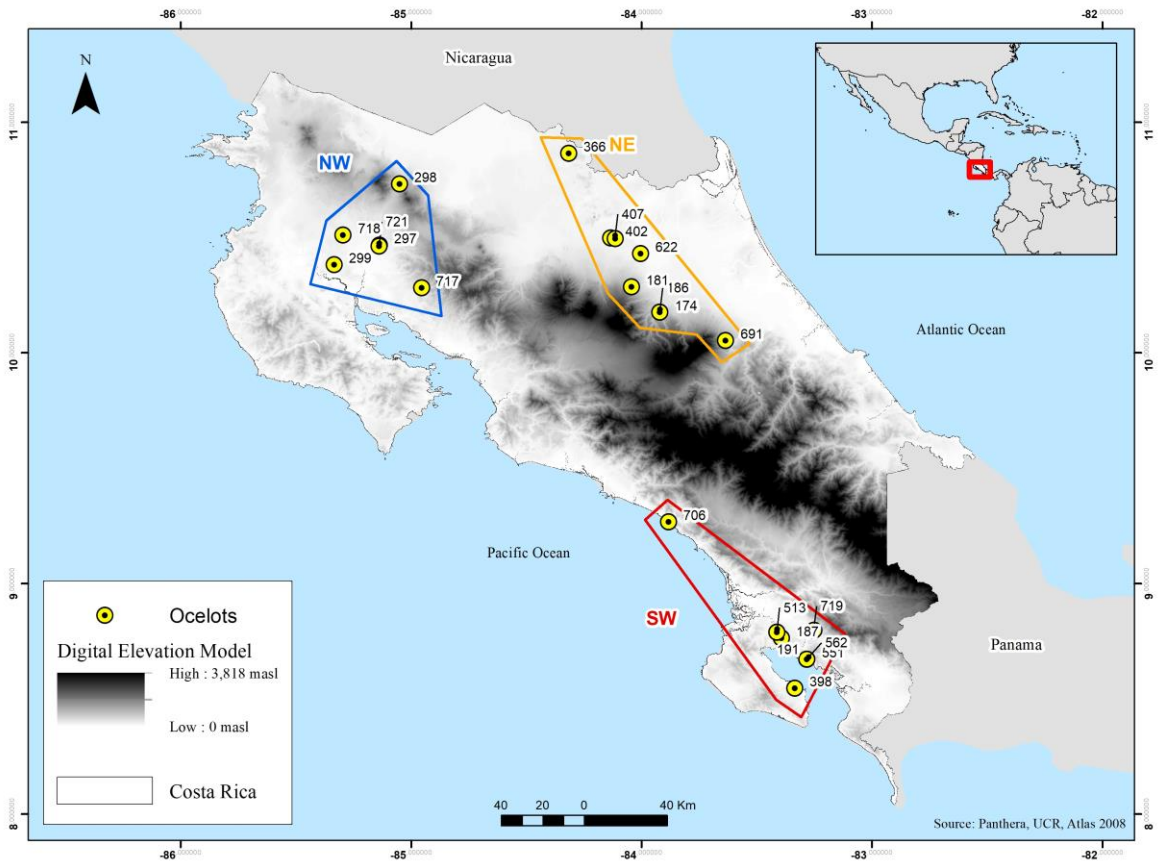

Figure 3.2. Groups of ocelot (*Leopardus pardalis*) samples established *a priori* based on their location with respect to the highest mountains in the country (>1,200 masl) (NE: Northeast, *n* = 8; NW: Northwest, *n* = 6; SW: Southwest, *n* = 9; U: Unknown location, *n* = 7) to test for population structure in Costa Rica. Two individuals were recaptured in the NE group, so for analysis purposes n = 6. Sample reference number is shown next to each location.

The same was done by separating samples north (N) and south (S) of the GMA, that concentrates 2.8 million inhabitants (~60% of the Costa Rican population) in an area of 2,044 km² (including the main cities of San Jose, Alajuela, Heredia and Cartago) (Figure 3.3). Only one sample per individual was used in these analyses (n =28). A similar analysis using main roads as a potential barrier was considered but could not be conducted due to low sample size in the groups.

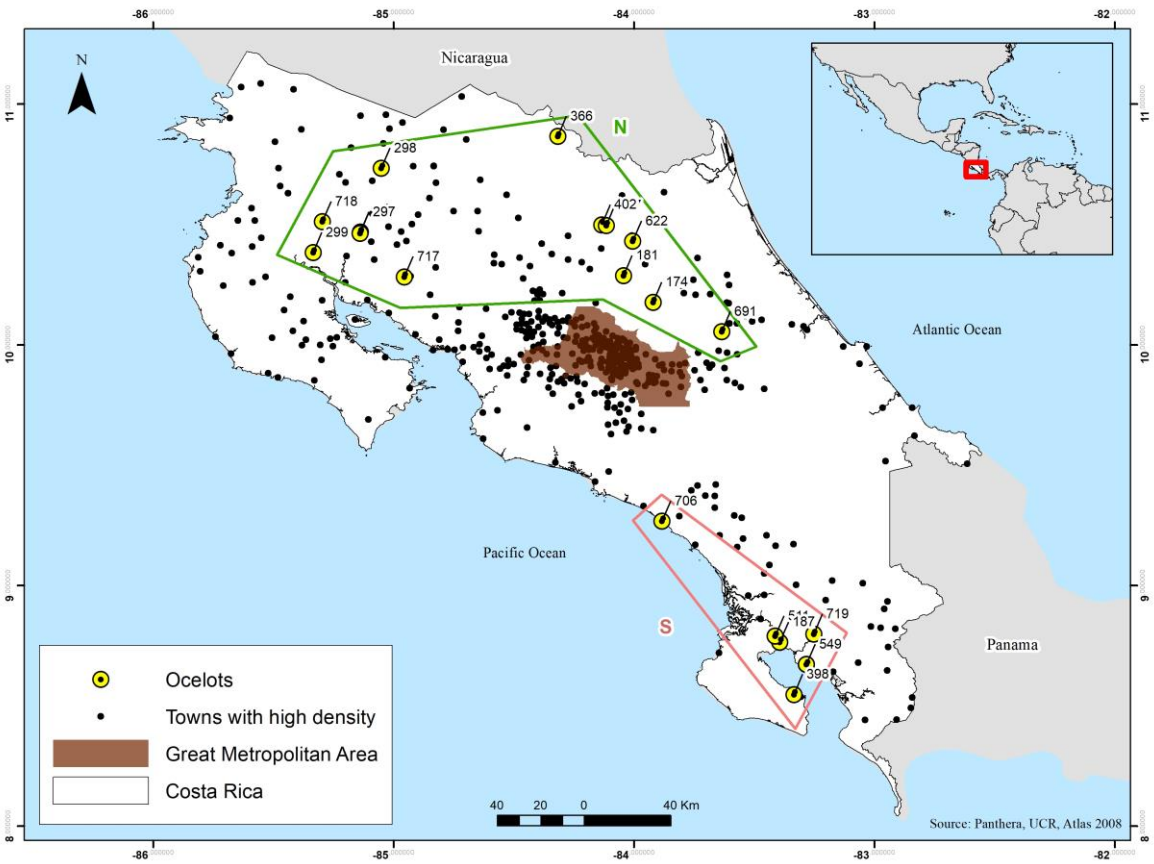

Figure 3.3. Groups of ocelot (*Leopardus pardalis*) samples established *a priori* based on their location with respect to the Great Metropolitan Area (N: North, *n* = 14; South, *n* = 9; U: Unknown location, *n* = 7) to test for population structure in Costa Rica. Two individuals were recaptured in the N group, so for analysis purposes *n* = 12. Sample number is shown next to each location.

Additionally, we calculated estimates of relatedness and defined relationships between pairs of individuals using maximum likelihood, as an alternative measure of gene flow using program ML-RELATE, version April, 2008 (73). Relationship estimates included the following classes: unrelated (U), half-sibling (HS), full- sibling (FS), and parent-offspring (PO).

Isolation by distance (IBD) was examined to determine whether a significant correlation exists between pairwise genetic (codominant genotypic distance calculated in GenAlEx, version 6.503 (67) and geographic distances, and the log of genetic distance versus geographic distances, by applying a simple Mantel test with 10,000 permutations.

Comparison with other studies

We compared ocelot (*n* =28) diversity and structure estimates to Costa Rican jaguars (*n* =39) and pumas (*n* =48) previously studied (39) by subsampling the dataset to contain only the loci used in both studies (number of shared microsatellite loci=7). We used the same approach and also compared our estimates to Belizean ocelots (*n* =30) (30,31) based on 9 shared microsatellite loci. To compare genetic diversity estimates, we calculated unbiased expected heterozygosity (uH_E) and H_O with GenAlEx, version 6.503 (67), and rarified A_R with HP-RARE, version 1.1 (68). We performed pairwise t tests by locus in R (R Core Team 2015®; version 3.4.3) between Costa Rican ocelots with jaguars and then with pumas to test for significance. We also ran similar tests between Costa Rican and Belizean ocelots. GenAlEx was also used to calculate G_{ST} and $G``_{ST}$ (given small sample size) to assess the level of genetic differentiation between Costa Rica and Belize ocelots (74–76). Genotypes for both populations were called in the same laboratory and allele sizes were standardized.

Results

Genotyping and equilibrium summary statistics

Out of the 68 Costa Rican ocelot samples, only 31 were successfully genotyped to individual level (Table 3.1). Successfully genotyped samples came from the following sources (ordered by success rate): blood, tissue, scat and hair. The majority of the successfully genotyped samples (~77%) came from wild individuals, followed by captive animals (~19%), and museum specimens (~3%). Only 24 samples had an exact (n = 15), or approximate geographic location (n = 9) (Figure 3.1).

Table 3.1. Information on the microsatellite analysis success (y= successful, n = not successful) and sample type of the ocelot (Leopardus pardalis) samples (n = 68) using 15 microsatellite loci in Costa Rica.

	Successfully genotyped (n/y)							
	Museum (*n* =5)		Captive (*n* =12)		Wild (*n* =51)			
Sample Type	n	y	n	y	n	y	*% successful*	Total
Blood	---	---	---	4	---	---	*100%*	4
Tissue	2	---	---	---	1	9	*75%*	12
Scat	---	---	5	2	26	15	*35%*	48
Hair	2	1	1	---	---	---	*25%*	4
Total	4	1	6	6	27	24	*46%*	68

Three individuals sampled in wild were recaptured; two of them were recaptured at the same site and the samples of the third individual were separated by 2.13 km. We identified gender in fourteen ocelot individuals (five females, nine males and 14 sex unknown). Cumulative $P_{(ID)sibs}$ estimates for all 15 loci was 6.7E-07, and the most informative loci were 4.9E-03 (5 loci) and 7.5E-03 (6 loci), indicating a high power to differentiate between individuals. Cumulative $P_{(ID)sibs}$ for the three individuals recaptured were 1.6E-05 (12 loci) for one and 6.0E-05 (10 loci) for the other two.

Departure from Hardy-Weinberg equilibrium was significant in five out of 15 tests at the 0.05 significance level and in one out of the 15 loci (FCA126) using the Bonferroni corrected p value (0.0031). Deviations from linkage equilibrium were detected in five out of 105 tests at the 0.05 significance level and 0 out of five tests at the Bonferroni corrected p value (0.008). All loci were retained in the rest of the analyses.

Genetic diversity

Genetic diversity of ocelots in Costa Rica was relatively high. The average number of alleles (N_A) was 6.87 (±1.71, SD), rarified A_R= 5.50 (±1.36, SD), H_O= 0.73 (±0.12, SD), and H_E= 0.79 (±0.08, SD). The fixation index (F) was 0.05 (±0.15, SD), suggesting that there is almost no inbreeding or excess of heterozygosity.

Genetic structure

The non-spatial analysis using STRUCTURE supports a panmictic population of ocelots in Costa Rica. The highest value in the likelihood curve is at K=1 (Figure 3.4) and the ancestry

plot for *K*=2 shows evidence of oversplitting; thus, supporting *K*=1 (Figure 3.5). Also, the two hypothesis-based STRUCTURE analyses with *a priori* clustering of individuals gave *K*=1 as the most supported number of genetic groups.

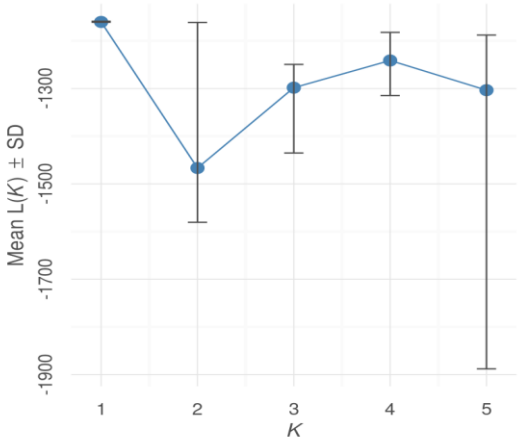

Figure 3.4. Inference of number of genetic clusters (*K*) for ocelots (*Leopardus pardalis*) based on the mean log likelihood L(*K*), using the admixture model with correlated allele frequencies without prior sampling location, obtained in STRUCTURE version 2.3.4 for ocelots (*n* = 28) in Costa Rica.

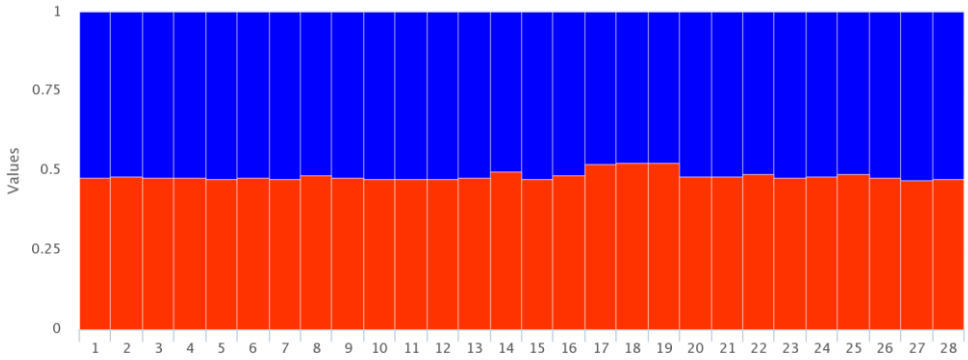

Figure 3.5. Ancestry barplots of ocelot (*Leopardus pardalis*) samples with *K*=2, based on the mean log likelihood L(*K*), using the admixture model with correlated allele frequencies without prior sampling location, obtained in STRUCTURE version 2.3.4 for ocelots (*n* = 28) in Costa Rica. Each bar represents one individual ocelot and colors within each bar represent the rate of membership (value) for each genetic cluster.

Consistent with STRUCTURE results, the Principal Coordinate Analysis showed no patterns of genetic substructure, further supporting the hypothesis of a panmictic population of Costa Rican ocelots. There is no clear geographic clustering of samples based on their location with respect to the highest mountains in the country or with respect to the Great

Metropolitan Area (Figures 3.6, 3.7). The first axis of the PCoA explains 14.22% of the variation, and the second axis explains 9.18%.

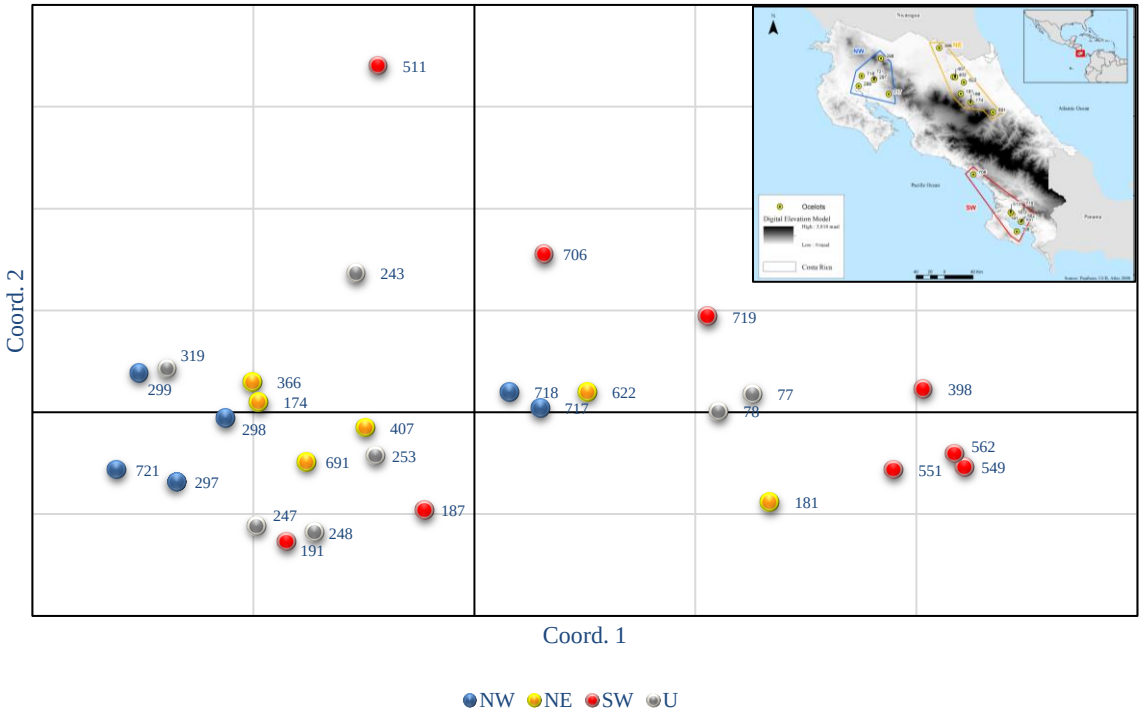

Figure 3.6. Principal Coordinate Analysis calculated with GenAlEx, version 6.503 for the first two axis for the groups of ocelot (*Leopardus pardalis*) samples established *a priori* based on their location with respect to the highest mountains in the country (>1,200 masl) (NE: Northeast, *n* =6; NW: Northwest, *n* =6; SW: Southwest, *n* =9; U: Unknown location, *n* =7) to test for population structure in Costa Rica. Insert shows Figure 3.2 with sample location; see Materials and Methods for more information.

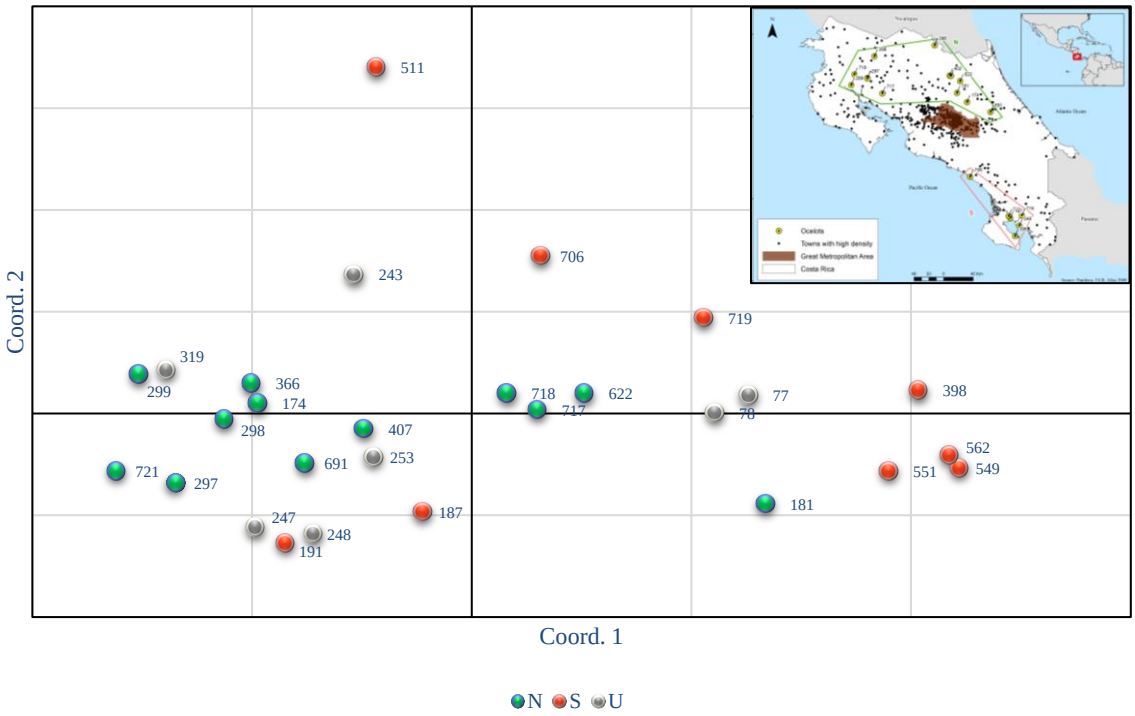

Figure 3.7. Principal Coordinate Analysis calculated with GenAlEx, version 6.503 for the first two axis for the groups of ocelot (*Leopardus pardalis*) samples established *a priori* based on their location with respect to the Great Metropolitan Area (N: North, *n* =12; South, *n* =9; U: Unknown location, *n* =7) to test for population structure in Costa Rica. Insert shows Figure 3.3 with sample location; see Materials and Methods for more information.

Examination of isolation by distance revealed a weak positive relationship between geographic distance (km) vs. genetic distance (n = 21, R^2= 0.04, p= 0.007, Figure 3.8), and between geographic distance vs. the log of genetic distance (n =21, R^2= 0.05, p= 0.009, Figure 3.9).

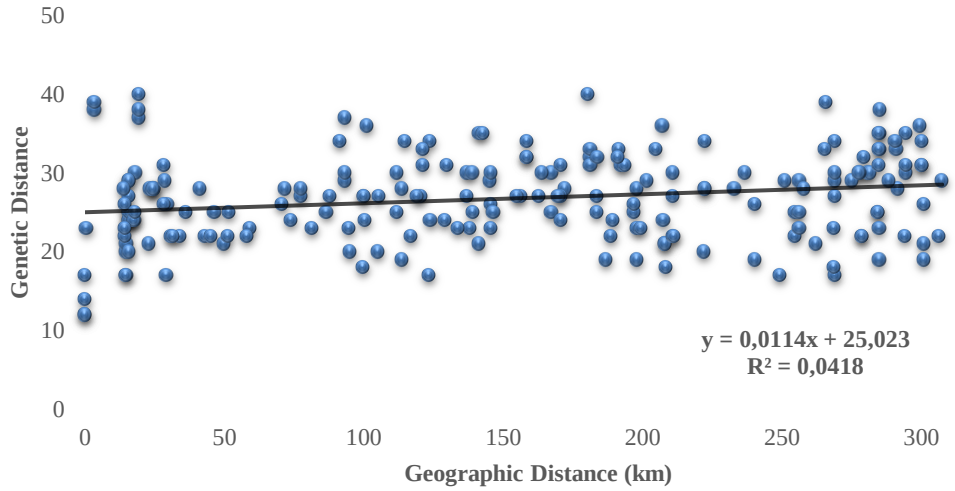

Figure 3.8. Isolation by distance for ocelots (*Leopardus pardalis*) in Costa Rica assessed by plotting pairwise codominant genotypic distance calculated in GenAlEx, version 6.503 versus pairwise geographic distances (km) using a simple Mantel test.

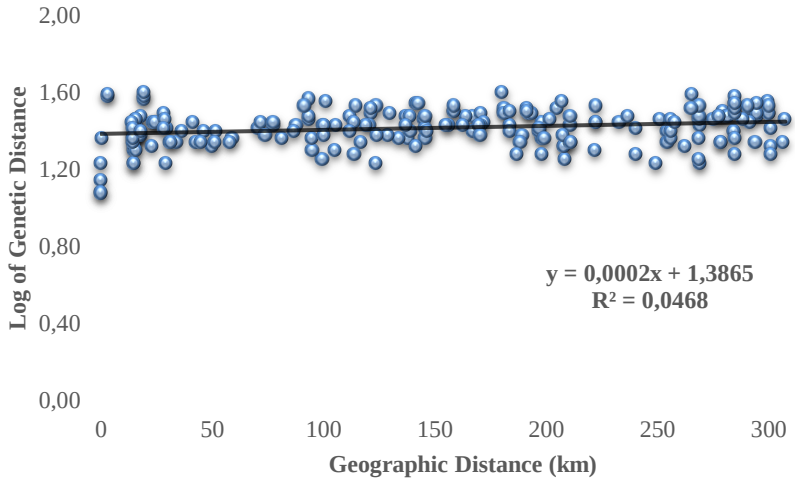

Figure 3.9. Isolation by distance for ocelots (*Leopardus pardalis*) in Costa Rica assessed by plotting the log of the pairwise codominant genotypic distance calculated in GenAlEx, version 6.503 versus pairwise geographic distances (km) based on 15 microsatellite loci using a simple Mantel test.

Out of the 756 possible pairwise combinations of individuals, 20 had a high relatedness (equal or higher than 0.5). Seven of the relationships were classified as full siblings and six as parent-offspring (Table 3.2, Figure 3.10). The distances between these highly-related individuals range from 0 to 195 km in a straight line (avoiding large bodies of water when present).

Table 3.2. Relatedness (maximum likelihood estimates) and Ln (*Likelihood*) for each of the four following relationships: U= Unrelated, HS= Half Siblings, FS= Full Siblings, PO= Parent-Offspring, between ocelot (*Leopardus pardalis*) samples in Costa Rica calculated by ML-RELATE version April, 2008, based on 15 nuclear DNA microsatellite loci. Only PO and FS classified relationships are shown.

					Delta Ln(*L*)[c]					
Ind1	**Ind2**	**Relatedness**	***R*[a]**	**LnL(*R*)[b]**	**U**	**HS**	**FS**	**PO**	**Location**	**Approximate distance (km)**
551	549	0.87	FS	-19.39	6.93	3.69	-	1.84	Osa-Osa	0
191	187	0.82	FS	-28.9	7.75	3.95	-	1.58	Osa-Osa	Not available
562	551	0.75	FS	-25.6	6.92	2.75	-	0.58	Osa-Osa	Not available
562	549	0.71	FS	-32.42	6.35	3.23	-	9999	Osa-Osa	0
253	181	0.50	PO	-31.76	2.04	0.81	1.56	-	*Captivity*-Sarapiqui	Not available
622	187	0.50	PO	-26.63	1.46	0.38	1.93	-	Sarapiqui-Osa	195*
717	622	0.50	PO	-28.61	0.96	0.37	1.95	-	Abangares-Sarapiqui	105*
719	398	0.50	PO	-16.73	1.85	0.68	1.28	-	Osa-Osa	52*
77	366	0.50	PO	-27.46	1.45	0.5	0.78	-	*Captivity* -Crucitas	Not available
78	398	0.50	PO	-21.68	1.16	0.5	1.47	-	*Captivity* -Osa	Not available
706	511	0.39	FS	-74.26	2.68	1.05	-	9999	Barú-Osa	74
77	719	0.32	FS	-19.36	1.23	0.33	-	9999	*Captivity* -Osa	Not available
298	297	0.31	FS	-56.73	2.77	0.83	-	9999	Tenorio-Cañas	31*

[a]*R*: relationship with the highest likelihood.[b] LnL(*R*): log likelihood of R. [c]Delta Ln(*L*): how much lower the log likelihoods are for the other relationships. A Delta Ln(*L*) of '9999' indicates that the relationship is not possible. *Indicates distance is approximate.

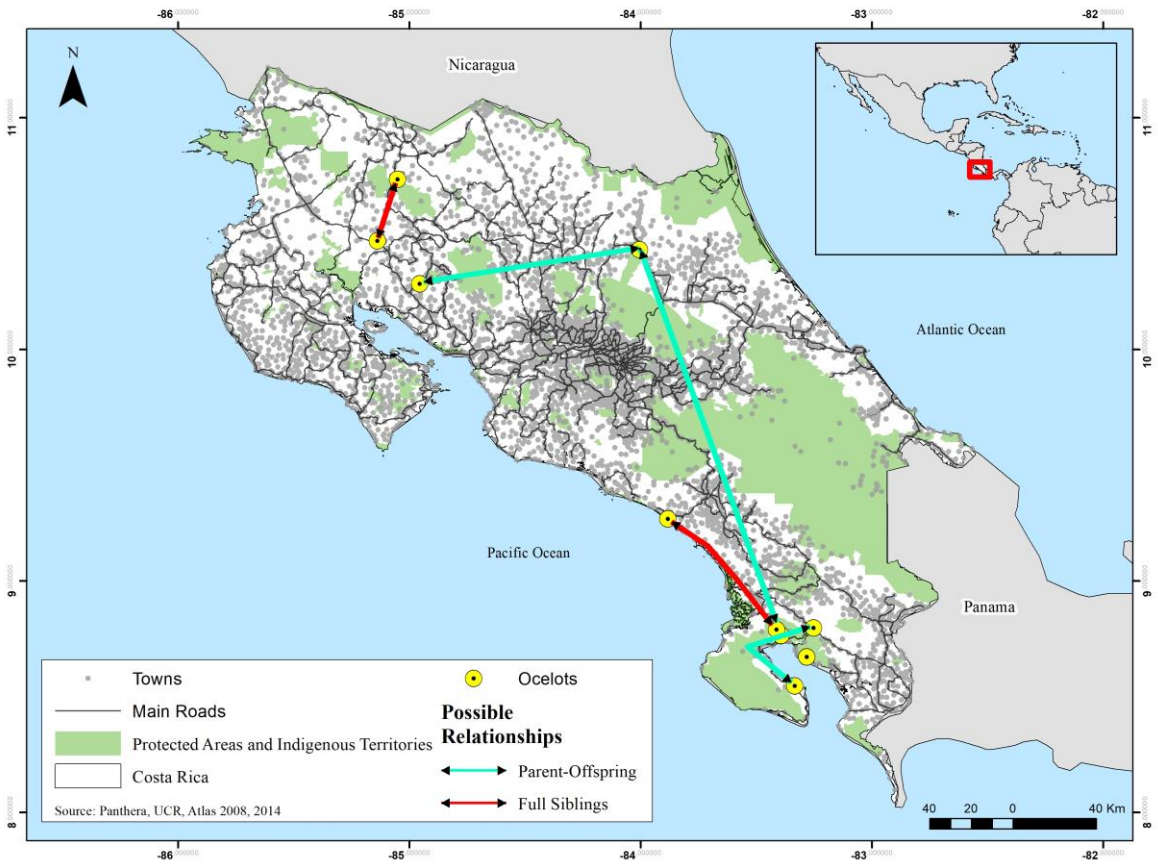

Figure 3.10. Possible close relationships (Full Siblings or Parent-Offspring) between ocelot (*Leopardus pardalis*) samples of known location in Costa Rica calculated by ML-RELATE based on 15 nuclear DNA microsatellite loci.

Comparison of genetic diversity in ocelots with other Neotropical felids and sites

In Costa Rica, the genetic diversity of ocelots is higher than that of jaguars, and pumas using seven shared loci (Table 3.3).

Table 3.3. Summary statistics of genetic diversity for *Leopardus pardalis*, *Puma concolor*, and *Panthera onca* in Costa Rica, using 7 shared microsatellite loci.

Species	*n*	H_O	uH_E	A_R	Source
Leopardus pardalis	28	0.71 (±0.14)	0.80 (±0.09)	4.38 (±0.70)	This study
Puma concolor	49	0.53 (±0.11)*	0.79 (±0.03)	3.99 (±0.22)	(39)
Panthera onca	38	0.52 (±0.09)*	0.63 (±0.11)*	3.09 (±0.57)*	(39)

n, number of individuals; H_O, mean (±SD) for observed heterozygosity; uH_E, mean (±SD) for unbiased expected heterozygosity; A_R, mean (±SD) for allelic richness using the rarefaction method; * indicates relationship is significant ($p < 0.05$) compared to *L.pardalis*

Contrary to our prediction, genetic diversity of ocelots is slightly higher in Costa Rica than in Belize based on nine shared loci, but only expected heterozygosity estimates were significantly different (Table 3.4). F-statistics for Costa Rica and Belize ocelots across all shared loci were G_{ST}= 0.069 (SE= 0.024, p= 0.001) and $G``_{ST}$ = 0.559 (SE= 0.162, p= 0.001).

Table 3.4. Summary statistics of genetic diversity for *Leopardus pardalis* in Costa Rica and Belize.

Country	*n*	H_O	uH_E	A_R	Source
Costa Rica	28	0.72 (±0.09)	0.80 (±0.09)	5.66 (±1.67)	This study
Belize	30	0.63 (±0.12)	0.73 (±0.08)*	4.87 (±0.94)	(31)

n, number of individuals; H_O, mean (±SD) for observed heterozygosity; uH_E, mean (±SD) for unbiased expected heterozygosity; A_R, mean (±SD) for allelic richness using the rarefaction method; * indicates significant relationship (p< 0.05) compared to *L. pardalis* in Costa Rica

Two genetic groups are supported based on the STRUCTURE analyses including Costa Rica and Belize ocelots (Figure 3.11). There is very little shared ancestry between groups and no evidence of migration or gene flow between these countries based on the current samples (Figure 3.12).

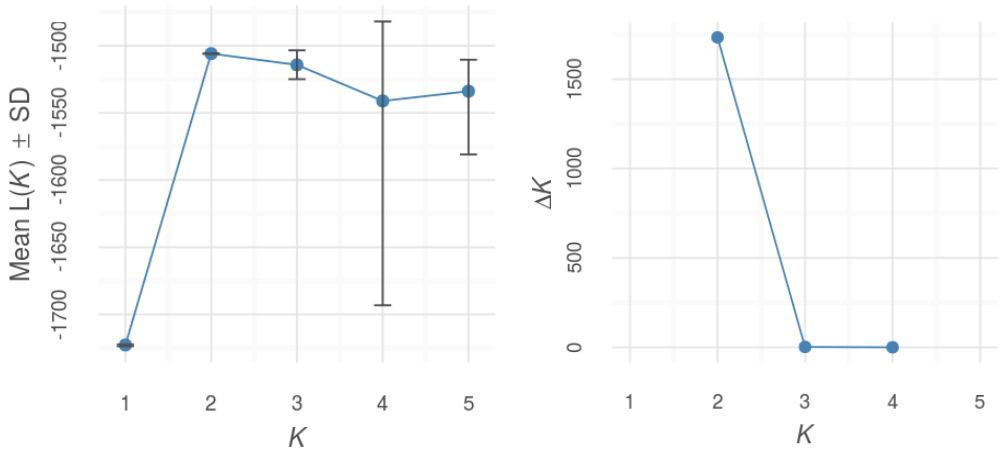

Figure 3.11. Inference of number of genetic clusters (*K*) based on (a) the mean log likelihood L(*K*) and (b) delta K (*ΔK*), using the admixture model with correlated allele frequencies without prior sampling location, obtained in STRUCTURE version 2.3.4 for Costa Rican (*n* =28) and Belizean (*n* =30) ocelots (*Leopardus pardalis*); suggesting *K* = 2.

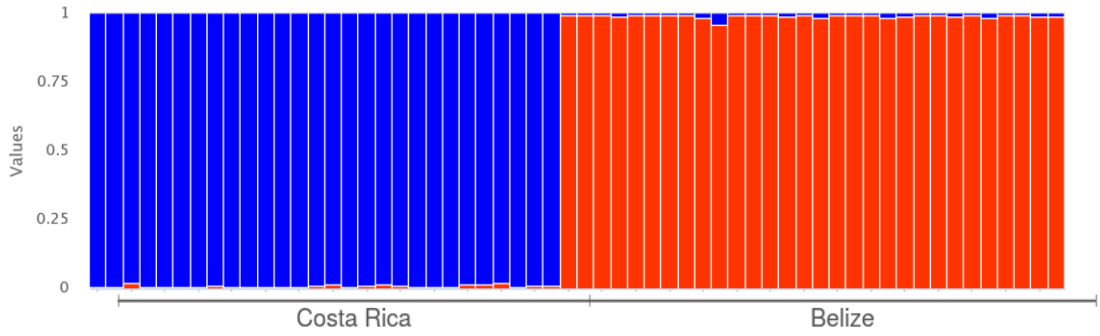

Figure 3.12. Ancestry barplots of ocelot samples with *K*=2, based on the mean log likelihood L(*K*), using the admixture model with correlated allele frequencies without prior sampling location, obtained in STRUCTURE version 2.3.4 for Costa Rican (*n* = 28) and Belizean ocelots (*n* =30) (*Leopardus pardalis*). Each bar represents one individual ocelot and colors within each bar represent the rate of membership (value) for each genetic cluster.

Discussion

Conservation genetics is an essential field of investigation for understanding genetic processes and genetic health of populations to assist managers in the preservation of biodiversity. This field of research provides invaluable information on topics like the status of species and populations, genetic diversity, population structure, connectivity, effects of habitat loss and fragmentation, fitness, adaptation, and restoration of populations (7). This information is critical to minimize genetic diversity loss, giving species more opportunity to withstand impacts or disturbances (5–7). To our knowledge, this is the first conservation genetic study on ocelots in Costa Rica and the second one in Mesoamerica to measure genetic diversity and population structure of ocelots at a countrywide level.

Sampling at this countrywide scale was made possible by the use of non-invasive genetic sampling (NGS) of scat and hair which composed 70% of our samples. The main advantages of NGS over the more traditional methods of studying rare or elusive animals (e.g. telemetry, camera traps, tracks) are that there is no need to handle or capture the animal, the amount of information obtained is significantly increased, fewer permits are required (e.g., compared to telemetry), elusive and rare species can be sampled, the number of samples may be greater, the training of field personnel to gather samples is relatively easy, and the cost in some cases may be lower (77–80). Yet, there are some drawbacks to using this sampling technique for genetic studies, such as: low quality and quantity of DNA is obtained which can cause

genotyping errors or low amplification success, expenses may be higher (depending on the number of replicates and the markers utilized), and there could be a potential sex bias in sampling depending on the species behavior and sampling design (77,80,81). We obtained high microsatellite analysis success rates for blood and tissue samples and considerably lower for scat and hair, resulting in less than half of the original samples successfully genotyped. Wultsch et al. (80) found that sample storage technique, type of habitat where it is collected, and the part of scat (e.g. top, bottom, side, inside), have significant effects on polymerase chain reaction amplification success and genotyping accuracy rates. Older scats have also been shown to have lower success rates (24). Future studies should take these results into account to increase accuracy and improve the use of resources.

We found genetic diversity of ocelots in Costa Rica to be relatively high. When comparing with other studies using microsatellite loci, Costa Rican ocelots have higher genetic diversity than populations in the northernmost populations in southern USA and northern Mexico (26,27,29) and Belize (31) and similar (24) or lower than populations studied in South America (23,32). This confirms a south to north decrease in genetic diversity for ocelots. However, caution should be taken with these comparisons as the microsatellite markers employed were not the same as the ones in the present study except in Belize.

Our analyses regarding population structure and relatedness indicate a high level of genetic connectivity of ocelots throughout the country. We found no evidence of genetic substructure or landscape barriers. Consequently, neither the highest mountains (> 1,200 masl) nor the Great Metropolitan Area, the highest density urban area in the country, seem to pose a significant barrier to Costa Rican ocelots. Conversely, Soto (39) found indications of genetic subdivision in jaguars and pumas in certain areas of Costa Rica. In Belize, Wultsch et al. (31) reported that ocelots had lower levels of genetic subdivision than pumas, but not jaguars. Additionally they found a high genetic connectivity for ocelots in Belize and detected two male first-generation dispersal events of a minimum of ~60-80 km in a landscape that included agriculture and human settlements (31). In this study, we found closely related individuals separated by up to ~195 km, implying that ocelots have a great movement capacity. Ocelots occur in a wide variety of habitats and have been reported in relatively disturbed areas (e.g. pasture/agriculture land, forests near populated areas, fragmented forests) (9,46,47,50,82,83).

This habitat plasticity may allow ocelots to move through altered areas, border high density urban areas, cross roads and highways and maintain relatively high levels of genetic flow (46,83,84; Araya-Gamboa et al. unpub. data). However, our genetic substructure analyses could have been affected by relatively low samples sizes and number of loci. It is possible that with more comprehensive geographic sampling and more loci we could detect some finer scale genetic structure and evidence of areas that are less permeable to ocelots. Still, we believe our finding of no major barriers in the sample groups analyzed here is likely to be robust with our small sample size.

Multiple studies have found that ocelots need areas with high vegetation cover, are positively correlated with primary productivity and are affected by human-related pressures (e.g. logging, poaching, settlements, roads) (46,49,50). Additionally, roads are not fully permeable to their movement as animals end up injured or killed by cars (84–86). In Costa Rica, the Wildlife Friendly Roads group (VAVS; Spanish) indicates that ocelots are the most frequently roadkilled wild cat (Pomareda et al. unpub. data). Moreover, highly fragmented landscapes with human-related pressures might be too big of a challenge even for the ocelot (29). Therefore, it is possible that ocelots are able to maintain healthy populations in fragmented or altered landscapes when they are combined with significant forest fragments that will sustain enough prey and provide cover, and where logging, poaching and other human-induced threats are controlled or mitigated.

As expected, genetic diversity for ocelots is higher when compared to that of pumas and jaguars in Costa Rica (Table 3.3). This corroborates the thesis that threatened status is inversely correlated with genetic diversity (4). Similar results were found in Belize, where genetic diversity was higher for ocelots followed by pumas and jaguars (31). In general, ocelots can use a greater number of habitat types, can rely on smaller prey, have higher densities and seem to be less affected by disturbances than pumas or jaguars (46,53,54). All these factors contribute to having larger effective population sizes, which could explain the higher genetic diversity found in ocelots.

Genetic diversity of ocelots is higher in Costa Rica than in Belize, opposing our *a priori* prediction. We theorized that effective population size and connectivity of Belizean populations would be greater and that this would yield higher genetic diversity in Belize. Our

hypothesis was based on the facts that Belize has a very high percentage of forest cover (68%), and is well connected to the Selva Maya and other forested areas in Guatemala and Mexico (87). But, as mentioned earlier, higher genetic diversity estimates in southern vs northern latitudes is consistent with what has been reported in other studies (23,26,27,29,32). This has also been found to be true in jaguars in Mesoamerica, where genetic diversity was highest for the southernmost population analyzed in Costa Rica and lowest for the northernmost samples from Mexico (40). These gradients of diversity may be explained by historical events, such as post-glacial recolonization from South America into Central and North America (88).

Conclusions

In this study we were able to determine that ocelots in Costa Rica have a relatively high genetic diversity and are still well connected. Habitat plasticity of ocelots may allow them to disperse through fragmented and altered habitats, although from previous studies we know that the presence of vegetation cover and the reduction and mitigation of threats are essential to guarantee the long-term survival of their populations. As expected, ocelots presented a higher genetic diversity than pumas and jaguars. Yet, in certain cases they may serve as proxy to study the potential effects of human-related threats, especially in cases where lack of data for the other larger carnivores might hinder the use of robust analyses and models. We also corroborated the south to north gradient in genetic diversity reported in other ocelot studies. A continuous monitoring program of threatened and keystone species that includes genetic evaluations is essential to prevent problems related to the loss of genetic diversity and reductions in genetic flow.

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Appendices

Appendix 1: Supporting information for Chapter 1

Supporting information 1.1: Additional information on covariates selected a priori as being thought to have an influence on habitat use probability of medium and large mammals in Barbilla-Destierro Biological Sub-Corridor (Corridor), and portions of the Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

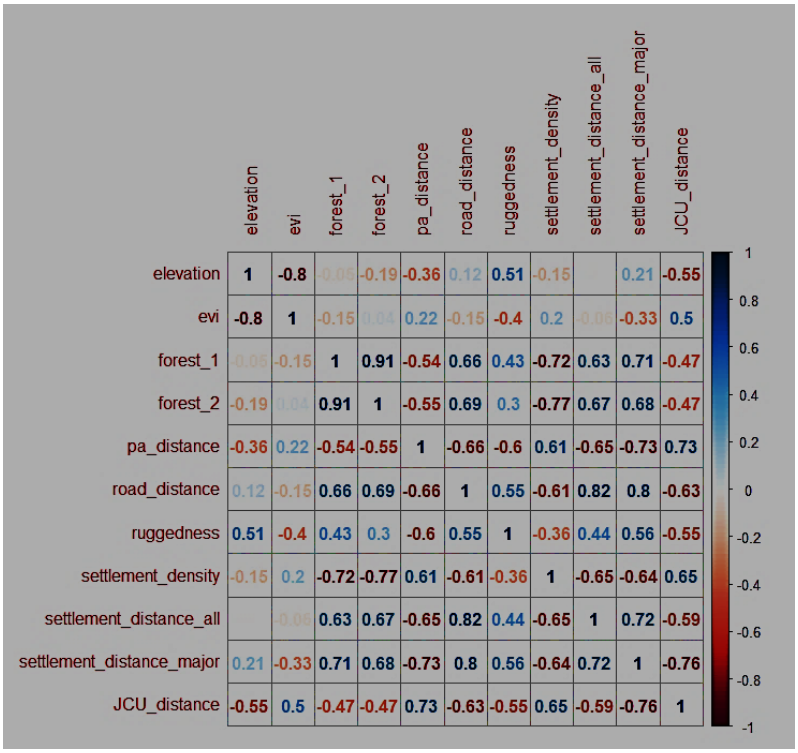
We calculated and standardized (1) site covariates in each 16 km^2 grid cell using Arc Map 10.3.1 (ESRI®), QGIS 3.4.4 (Creative Commons Attribution-ShareAlike 3.0 license) and R (R Core Team 2015®; version 3.4.3) (Table S1.1). Mean EVI (2) and percent forest version 1 (forest v1;(3)) values varied by year. The data for EVI and forest v1 came from the exact dates and as annual product respectively, for each of the blocks surveyed. For forest v1 we used a 30% forest cover threshold. Forest version 2 (forest v2) was a product specific to Costa Rica (4), and JAXA land use classification using RADAR images was used for areas with clouds. We created the distance layers (minimum distance to a primary road, (4); minimum distance to a major settlement, (4); minimum distance to any settlement, (4); minimum distance to a strictly-protected area, (4); minimum distance to a JCU, Panthera unpub. data) by calculating the distance from the center point of each grid cell to the closest point, line or polygon in each of the layers. For the minimum distance to primary road covariate only paved roads of one or more lanes were considered. Mean settlement density was estimated for each grid cell by counting the number of settlements and adjusting for density, multiplying minor settlements (towns) by a factor of one, mid-size settlements (main settlements in Districts -excluding IGN exclusive areas-, suburbs and urbanizations) by a factor of three, and major settlements (main settlements in Provinces, Cantons and Districts -including IGN exclusive areas-) by a factor of five. Finally, human presence in each grid cell was the number of human detections per 1,000 trap nights in the stations. Human events were not considered if they occurred within the same hour in the same station (camera trap). To calculate Effort, a

covariate on detection, I calculated and standardized the sum of all trap nights on each occasion for every grid cell.

Table S1.1. Information of covariates selected a priori as being thought to have an influence on habitat use probability of medium and large mammals in Barbilla-Destierro Biological Sub-Corridor (Corridor), and portions of the Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

Covariate	Source	Resolution	Year	Hypothesized relationship to mammal habitat use
Mean EVI (Enhanced Vegetation Index)	MOD13Q1 – 16 Day	250 m	2013, 2014, 2015, 2016, 2017	+
Percent forest version 1 (forest v1)	Hansen et al. 2013	30 m	CORRIDOR-Block1:2013, CORRIDOR-Block2: 2014, CVC: 2015, Talamanca: 2017	+
Percent forest version 2 (forest v2)	Costa Rican National Forestry Inventory-SIREFOR	30 m	2012	+
Mean Elevation	SRTM DEM	30 m	NA	-
Mean Ruggedness	SRTM DEM	30 m	NA	-
Minimum distance to a primary road	Costa Rican National Geographic Institute (IGN)	scale 1:25,000	2005	+
Minimum distance to a major settlement	IGN and National Institute of Statistics and Census (INEC)	scale 1:25,000	2013	+
Minimum distance to any settlement	IGN and INEC.	scale 1:25,000	2013	+
Mean settlement density	IGN and INEC	scale 1:25,000	2013	+
Minimum distance to a strictly-protected area	CENIGA, IUCN Ia & II categories	scale 1:50,000	2011	-
Minimum distance to JCU	Panthera unpub. data	250 m	2017	-
Human presence	This study	NA	2013-2017	-

Table S1.2. Correlation of site covariates selected a priori as being thought to have an influence on habitat use probability of jaguars and pumas in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.



	elevation	evi	forest_1	forest_2	pa_distance	road_distance	ruggedness	settlement_density	settlement_distance_all	settlement_distance_major	JCU_distance
elevation	1	-0.8	-0.05	-0.19	-0.36	0.12	0.51	-0.15		0.21	-0.55
evi	-0.8	1	-0.15	0.04	0.22	-0.15	-0.4	0.2	-0.06	-0.33	0.5
forest_1	-0.05	-0.15	1	0.91	-0.54	0.66	0.43	-0.72	0.63	0.71	-0.47
forest_2	-0.19	0.04	0.91	1	-0.55	0.69	0.3	-0.77	0.67	0.68	-0.47
pa_distance	-0.36	0.22	-0.54	-0.55	1	-0.66	-0.6	0.61	-0.65	-0.73	0.73
road_distance	0.12	-0.15	0.66	0.69	-0.66	1	0.55	-0.61	0.82	0.8	-0.63
ruggedness	0.51	-0.4	0.43	0.3	-0.6	0.55	1	-0.36	0.44	0.56	-0.55
settlement_density	-0.15	0.2	-0.72	-0.77	0.61	-0.61	-0.36	1	-0.65	-0.64	0.65
settlement_distance_all		-0.06	0.63	0.67	-0.65	0.82	0.44	-0.65	1	0.72	-0.59
settlement_distance_major	0.21	-0.33	0.71	0.68	-0.73	0.8	0.56	-0.64	0.72	1	-0.76
JCU_distance	-0.55	0.5	-0.47	-0.47	0.73	-0.63	-0.55	0.65	-0.59	-0.76	1

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Supporting information 1.2: Individual species results and AICc values for the 79 models evaluated for medium and large wild mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor, and portions of the Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

Table S1.3. Individual species results and selection criteria for the 79 models evaluated for medium and large wild mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor, and portions of the Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

Table provided in an Excel file, and available from the author at robertos@uidaho.edu

Supporting information 1.3: Additional description on literature review conducted to select species included as prey species for jaguars (*Panthera onca*) and pumas (*Puma concolor*) in Barbilla-Destierro Biological Sub-Corridor, and portions of the Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

We found and reviewed 23 researches, between peer-reviewed publications and thesis, describing jaguar and puma prey from Mexico to Panama (Table S1.4). In total, there were four large prey species for both jaguars and pumas, ten medium prey species for jaguars and 11 medium prey species for pumas. We included species as medium or large terrestrial mammal prey if the species or genus were mentioned in more than one document. The ones that were not mentioned or that were mentioned only in one document were left out, as they were considered opportunistic cases, and thus probably not having considerable effect on jaguar or puma habitat use in terms of prey. Thus, species mentioned in one document but not considered for this analysis were fox, coyote, ocelot and tayra for jaguar, and coyote for puma. Species that were not mentioned as prey for jaguar and puma were armadillo northern-naked-tailed, jaguarundi, margay, oncilla, and tapir. Additionally, puma was not reported as prey for jaguars, and grison, jaguar and ocelot were not reported in puma´s diet in any of the documents. We included domestic pig as part of jaguar and puma diet given that they could represent an important prey item for jaguars and pumas in the study area. This is especially

true in or near the indigenous territories present in the east side of the Corridor, where domestic pigs are feral and roam freely, were frequently detected by the camera traps, and were predated by both species on several occasions (before and during this study; personal observation). Other domestic animals or arboreal species were not considered in this analysis because I considered that camera traps (or their location) were not ideal for detecting these species.

Table S1.4. List of species or genus found in the literature review of 23 investigations related to diet of jaguars (Panthera onca) or pumas (Puma concolor) from Mexico to Panama. In gray are species or genus detected in the present study, and in green are species or genus considered for the estimate of prey richness.

Class	Order	Family	Genus	Species	Note	Citations as jaguar diet	Citations as puma diet
Aves	Galliformes	Cracidae	*Crax*	*rubra*		2; 3; 15	21
Aves	Galliformes	Cracidae	*Ortalis*	*poliocephala*			12
Aves	Galliformes	Cracidae	*Ortalis*	*vetula*		21	21
Aves	Galliformes	Phasianidae	*Agriocharis*	*ocellata*		13; 23	
Aves	Galliformes	Phasianidae	*Gallus*	*gallus*	Domestic animal	1	
Aves	NA	NA	NA	NA	Bird	1; 2; 5; 6; 18; 23	5; 6; 8; 10; 18
Malacostraca	Decapoda	NA	NA	NA			14
Mammalia	Artiodactyla	Bovidae	*Bos*	*taurus*	Domestic animal	14	
Mammalia	Artiodactyla	Bovidae	*Capra*	*hircus*	Domestic animal	22	12; 19; 22
Mammalia	Artiodactyla	Bovidae	NA	NA	Domestic animal		12; 18
Mammalia	Artiodactyla	Bovidae	*Ovis*	*aries*	Domestic animal	14	
Mammalia	Artiodactyla	Cervidae	*Mazama*	sp.		6; 13	6; 13
Mammalia	Artiodactyla	Cervidae	*Mazama*	*temama*		1; 3; 7; 14; 18; 21; 23	3; 8; 10; 14; 18; 21
Mammalia	Artiodactyla	Cervidae	NA	NA		2; 6	6
Mammalia	Artiodactyla	Cervidae	*Odocoileus*	*virginianus*		5; 6; 13; 15; 21; 22	4; 5; 6; 12; 13; 14; 15; 18; 21; 22

Table S1.4 Continues

Class	Order	Family	Genus	Species	Note	Citations as jaguar diet	Citations as puma diet
Mammalia	Artiodactyla	Cetacea	NA	NA	Marine dolphin	17	
Mammalia	Artiodactyla	Suidae	*Sus*	*scrofa*	Domestic animal		19
Mammalia	Artiodactyla	Tayassuidae	*Pecari*	*tajacu*		2; 4; 5; 6; 7; 9; 10; 13; 14; 15; 18; 21	4; 5; 6; 8; 10; 13; 14; 15; 18; 19; 21
Mammalia	Artiodactyla	Tayassuidae	*Tayassu*	*pecari*		1; 3; 6; 7; 10; 13; 14; 15; 23	6; 13; 14
Mammalia	Carnivora	Canidae	*Canis*	*familiaris*	Domestic animal	14; 21	
Mammalia	Carnivora	Canidae	*Canis*	*latrans*		15	21
Mammalia	Carnivora	Canidae	*Canis*	sp.	Unclear if domestic or wild	13	
Mammalia	Carnivora	Canidae	*Cerdocyon*	*thous*		10	
Mammalia	Carnivora	Canidae	*Urocyon*	*cinereoargenteus*		14	6; 12; 18; 21
Mammalia	Carnivora	Felidae	*Leopardus*	*pardalis*		3	
Mammalia	Carnivora	Felidae	NA	NA	*Panthera onca or Puma concolor*	23	
Mammalia	Carnivora	Mephitidae	*Conepatus*	*leuconotus*		22	
Mammalia	Carnivora	Mephitidae	*Conepatus*	*mesoleucus*			12
Mammalia	Carnivora	Mephitidae	*Conepatus*	*semistriatus*		13	
Mammalia	Carnivora	Mephitidae	*Conepatus*	sp.		14; 18	21

Table S1.4 Continues

Class	Order	Family	Genus	Species	Note	Citations as jaguar diet	Citations as puma diet
Mammalia	Carnivora	Mephitidae	NA	NA	*Spilogale putorius or Conepatus semistriatus*	1	
Mammalia	Carnivora	Mephitidae	*Spilogale*	*putorius*			12
Mammalia	Carnivora	Mustelidae	*Eira*	*barbara*		13	13; 18
Mammalia	Carnivora	Mustelidae	*Galictis*	*vittata*		14; 18	
Mammalia	Carnivora	Mustelidae	*Mustela*	*frenata*			12
Mammalia	Carnivora	Procyonidae	*Bassariscus*	*astutus*			12
Mammalia	Carnivora	Procyonidae	*Bassariscus*	*sumichrasti*			13
Mammalia	Carnivora	Procyonidae	*Nasua*	*narica*		1; 2; 5; 6; 7; 9; 13; 14; 18; 21; 22	5; 6; 8; 11; 12; 13; 18; 19; 21; 22
Mammalia	Carnivora	Procyonidae	*Potos*	*flavus*		1; 6; 7; 13; 14; 18; 23	6; 13; 14; 18
Mammalia	Carnivora	Procyonidae	*Procyon*	*lotor*		14; 18; 22	12; 21; 22
Mammalia	Cyngulata	Dasypodidae	*Dasypus*	*novemcinctus*		1; 2; 5; 6; 7; 13; 14; 18; 21; 22; 23	4; 5; 6; 8; 10; 12; 13; 14; 15; 18; 22
Mammalia	Didelphimorphia	Didelphidae	*Didelphis*	*marsupialis*		1	8; 15
Mammalia	Didelphimorphia	Didelphidae	*Didelphis*	sp.		13; 14	
Mammalia	Didelphimorphia	Didelphidae	*Didelphis*	*virginiana*		5; 21	5; 12; 15; 22
Mammalia	Didelphimorphia	Didelphidae	*Marmosa*	*canescens*			5

Table S1.4 Continues

Class	Order	Family	Genus	Species	Note	Citations as jaguar diet	Citations as puma diet
Mammalia	Didelphimorphia	Didelphidae	*Philander*	*opossum*		13; 23	8; 13
Mammalia	Lagomorpha	Leporidae	*Sylvilagus*	*brasiliensis=gabbi*		23	
Mammalia	Lagomorpha	Leporidae	*Sylvilagus*	*cuniculaurius*			12; 22
Mammalia	Lagomorpha	Leporidae	*Sylvilagus*	*floridanus*		4; 15; 22	12; 22
Mammalia	Lagomorpha	Leporidae	*Sylvilagus*	sp.		13	13; 21; 22
Mammalia	NA	NA	NA	NA	Small mammal		8
Mammalia	Perissodactyla	Equidae	*Equus*	sp.	Domestic animal	21	
Mammalia	Pilosa	Bradypodidae	*Bradypus*	variegatus		10	8; 10
Mammalia	Pilosa	Bradypodidae	NA	NA		23	
Mammalia	Pilosa	Choloepodidae	*Choloepus*	*hoffmanni*		3	8; 10
Mammalia	Pilosa	Myrmecophagidae	*Tamandua*	*mexicana*		1; 2; 14; 18	8; 10; 13
Mammalia	Primates	Atelidae	*Alouatta*	*palliata*			3; 19
Mammalia	Primates	Atelidae	*Ateles*	*geoffroyi*		13; 15; 23	3
Mammalia	Primates	Cebidae	*Cebus*	*imitator*		3	3; 8
Mammalia	Primates	NA	NA	NA		6; 10	6; 10
Mammalia	Rodentia	Cricetidae	*Ototylomis*	*phyllotis*			13
Mammalia	Rodentia	Cricetidae	*Peromyscus*	*yucatanicus*		13	13
Mammalia	Rodentia	Cuniculidae	*Cuniculus*	*paca*		2; 6; 7; 13; 14; 18; 23	6; 8; 10; 13; 14; 18
Mammalia	Rodentia	Dasyproctidae	*Dasyprocta*	*punctata*		6; 9; 10; 13; 18	3; 6; 8; 10; 13

Table S1.4 Continues

Class	Order	Family	Genus	Species	Note	Citations as jaguar diet	Citations as puma diet
Mammalia	Rodentia	Echimyidae	*Proechimys*	*semispinosus*			3; 8
Mammalia	Rodentia	Erethizontidae	*Sphiggurus*	*mexicanus*			3; 6; 14
Mammalia	Rodentia	Heteromyidae	*Heteromys*	*desmarestianus*			13
Mammalia	Rodentia	Heteromyidae	*Heteromys*	sp.		14	
Mammalia	Rodentia	Heteromyidae	*Lyomis*	sp.			12
Mammalia	Rodentia	NA	NA	NA		1	5; 8
Mammalia	Rodentia	Sciuridae	*Sciurus*	*aureogaster*			12
Mammalia	Rodentia	Sciuridae	*Sciurus*	*granatensis*			8
Reptilia	Crocodilia	Crocodylidae	*Crocodylus*	sp.		13	
Reptilia	NA	NA	NA	NA		1; 10	
Reptilia	Squamata	Iguanidae	*Ctenosaura*	*pectinata*		5; 22	5; 22
Reptilia	Squamata	Iguanidae	*Ctenosaura*	*similis*			15
Reptilia	Squamata	Iguanidae	*Iguana*	*iguana*		3; 14; 18; 23	8; 14
Reptilia	Squamata	NA	NA	NA	Snake	1; 2	5; 8; 14
Reptilia	Squamata	Teiidae	*Cnemidophorus*	sp.			13
Reptilia	Testudines	Cheloniidae	*Chelonia*	*mydas*		20; 23	
Reptilia	Testudines	Cheloniidae	*Lepidochelys*	*olivacea*		3; 15	
Reptilia	Testudines	Geoemydidae	*Rhinoclemmys*	*areolata*		13	
Reptilia	Testudines	Kinosternidae	*Kinosternon*	*integrum*			12

Table S1.4 Continues

Class	Order	Family	Genus	Species	Note	Citations as jaguar diet	Citations as puma diet
Reptilia	Testudines	Kinosternidae	*Staurotypus*	*triporcatus*		15	
Reptilia	Testudines	NA	NA	NA	River turtle	1; 2; 18	

*NA: Not availble

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Supporting information 1.4: Additional information on number of occupied cells, relative abundance and number of independent detections of medium and large mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

Table S1.5. Number of occupied cells, relative abundance and number of independent detections of medium and large mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

		Occupied cells		Relative abundance (# of independent records/1000 trap nights)				
Common name	**Scientific name**	Mean	SD	Corridor	CVC JCU	TC JCU	Overall	**# of independent detections**
Agouti	*Dasyprocta punctata*	41.28	1.39	32.95	16.02	49.81	33.90	573
Armadillo Nine Banded	*Dasypus novemcinctus*	56.27	1.04	33.62	27.47	3.14	24.31	411
Ocelot	*Leopardus pardalis*	54.14	1.38	24.13	10.01	25.35	21.53	364
Coati White Nosed	*Nasua narica*	54.73	1.96	22.12	11.44	8.53	16.33	276
Coyote	*Canis latrans*	25.12	1.67	16.76	14.31	0.00	11.83	200
Opossum Common	*Didelphis marsupialis*	42.35	2.41	8.60	20.89	6.28	10.53	178
Tayra	*Eira barbara*	51.88	2.48	14.63	2.29	5.16	9.58	162
Raccoon Common	*Procyon lotor*	31.58	1.81	16.42	2.00	1.12	9.41	159

Table S1.5 Continues

Common name	Scientific name	Occupied cells		Relative abundance (# of independent records/1000 trap nights)				# of independent detections
		Mean	SD	Corridor	CVC JCU	TC JCU	Overall	
Paca	*Cuniculus paca*	24.92	2.60	1.45	0.29	14.58	4.67	79
Puma	*Puma concolor*	31.30	2.56	1.34	9.73	7.18	4.61	78
Margay	*Leopardus wiedii*	24.26	2.29	1.23	8.01	5.38	3.73	63
Jaguarundi	*Puma yagouaroundi*	44.44	4.92	3.91	2.58	4.04	3.67	62
Jaguar	*Panthera onca*	18.74	2.70	1.45	0.00	8.53	3.02	51
Pig Domestic	*Sus scrofa*	13.46	2.08	3.02	0.00	4.26	2.72	46
Tapir Baird	*Tapirus bairdii*	9.14	0.97	0.34	8.87	1.79	2.48	42
Rabbit Tapeti	*Sylvilagus brasiliensis*	12.33	1.21	0.11	10.87	0.45	2.43	41
Skunk Striped Hog Nosed	*Conepatus semistriatus*	28.40	4.59	3.24	1.72	0.90	2.31	39
Deer Red Brocket	*Mazama temama*	22.77	3.27	0.56	4.29	2.92	1.95	33
Peccary Collared	*Pecari tajacu*	18.62	3.89	0.22	2.29	4.49	1.77	30
Tamandua Northern	*Tamandua mexicana*	39.12	7.87	1.90	0.57	0.22	1.18	20
Armadillo Nothern Naked Tailed	*Cabassous centralis*	7.06	2.64	1.34	0.00	0.45	0.83	14
Grison Greater	*Gallictis vittata*	13.38	4.98	0.89	0.57	0.22	0.65	11
Oncilla	*Leopardus tigrinus*	7.01	2.53	0.00	2.00	0.00	0.41	7
Deer White Tailed	*Odocoileus virginianus*	6.95	3.83	0.34	0.29	0.00	0.24	4
Fox Grey	*Urocyon cinereoargenteus*	1.88	1.39	0.00	0.86	0.00	0.18	3
							TOTAL	**2,946**

Supporting information 1.5: Community-level hyperparameter estimates for the influence of covariates on occupancy (Ψ) and detection (p) of in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

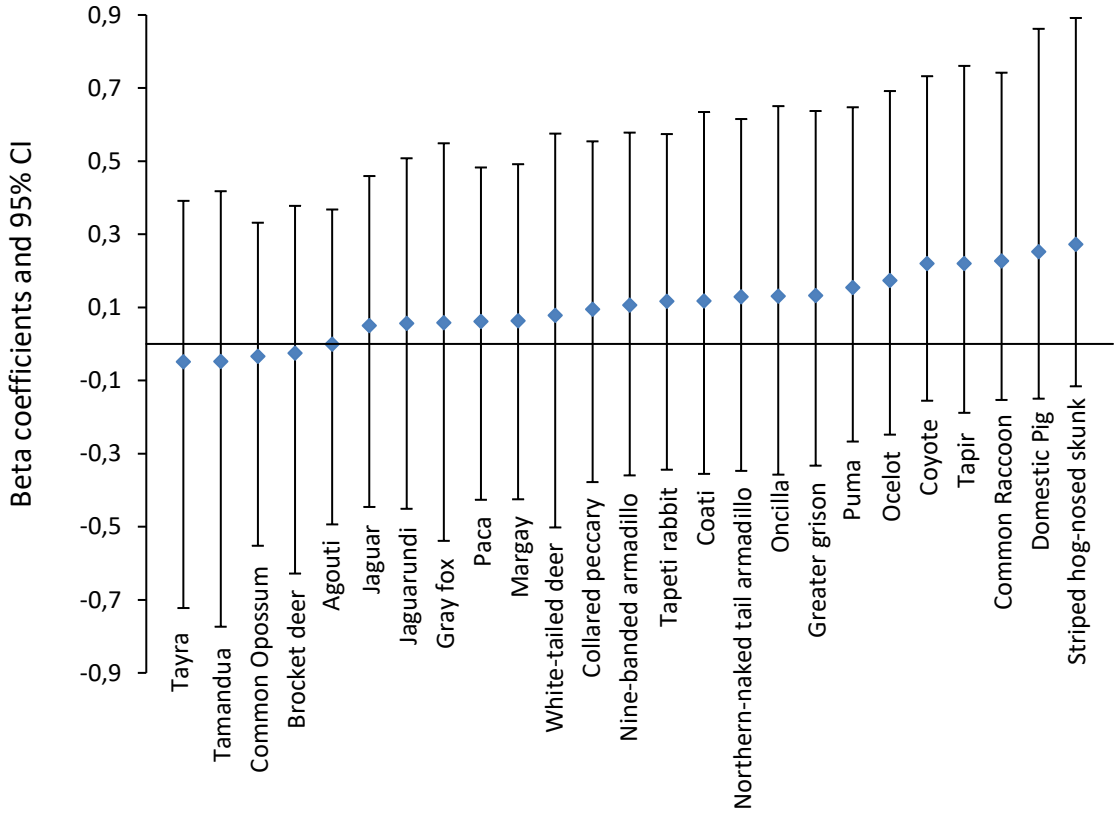

Figure S1.1. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for the influence of Human presence on occupancy (Ψ) of medium and large mammals and domestic pig (n = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

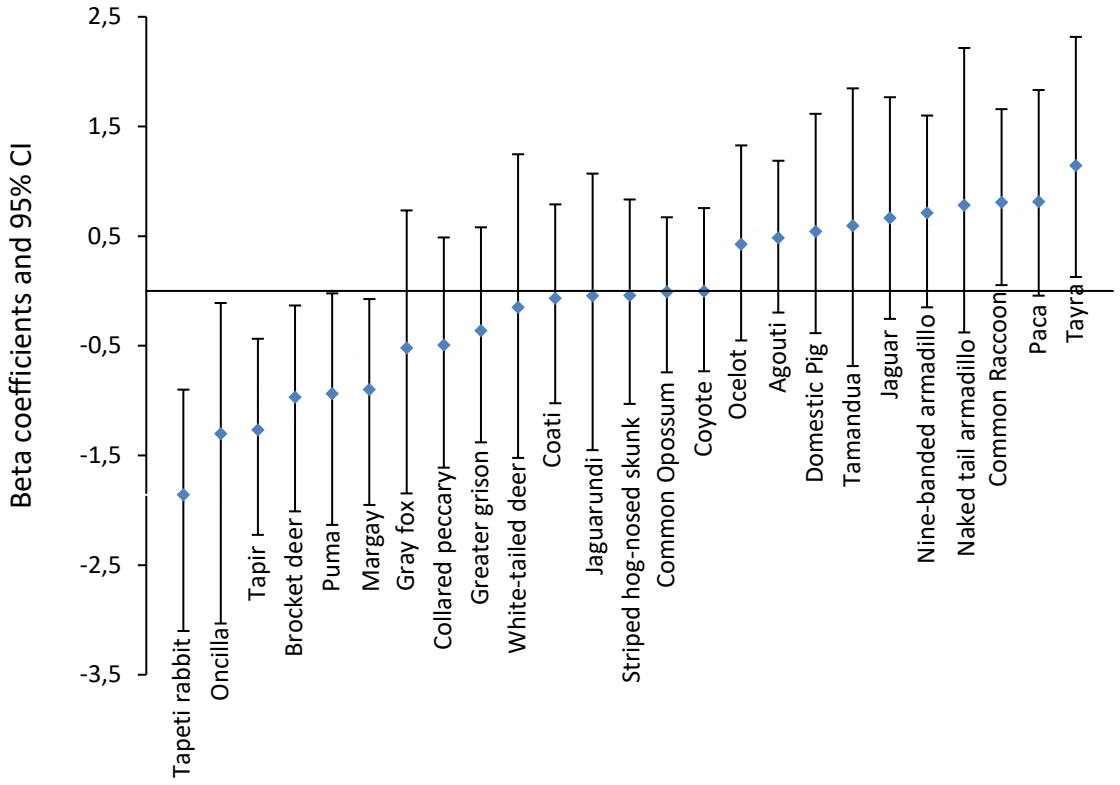

Figure S1.2. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for the influence of Mean EVI on occupancy (Ψ) of medium and large mammals and domestic pig (*n* = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

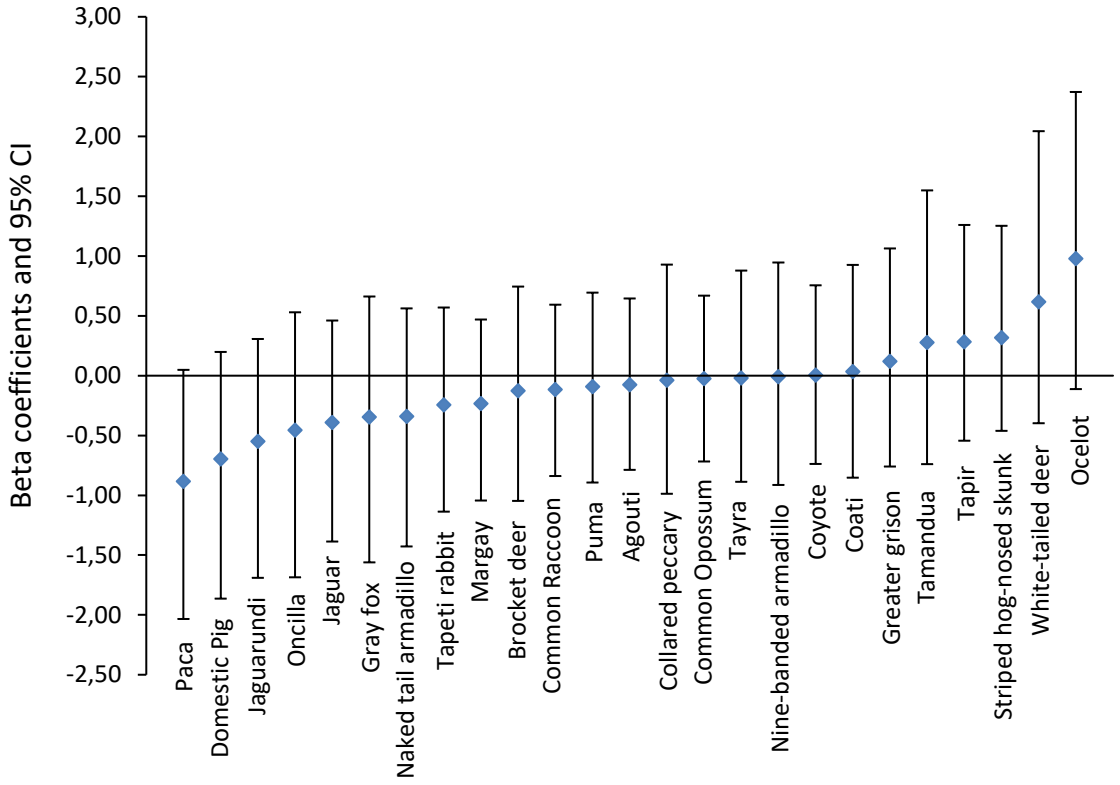

Figure S1.3. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for the influence of Mean distance to strictly-protected area on occupancy (Ψ) of medium and large mammals and domestic pig (*n* = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

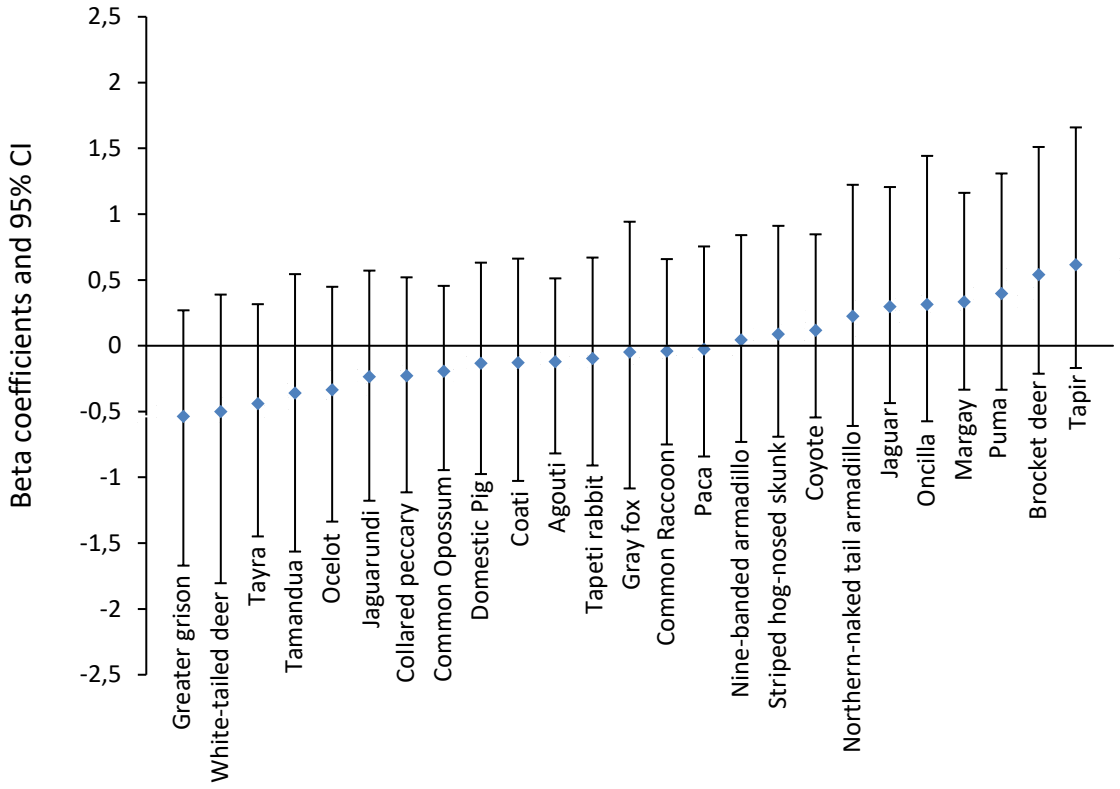

Figure S1.4. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for the influence of Mean ruggedness on occupancy (Ψ) of medium and large mammals and domestic pig (*n* = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

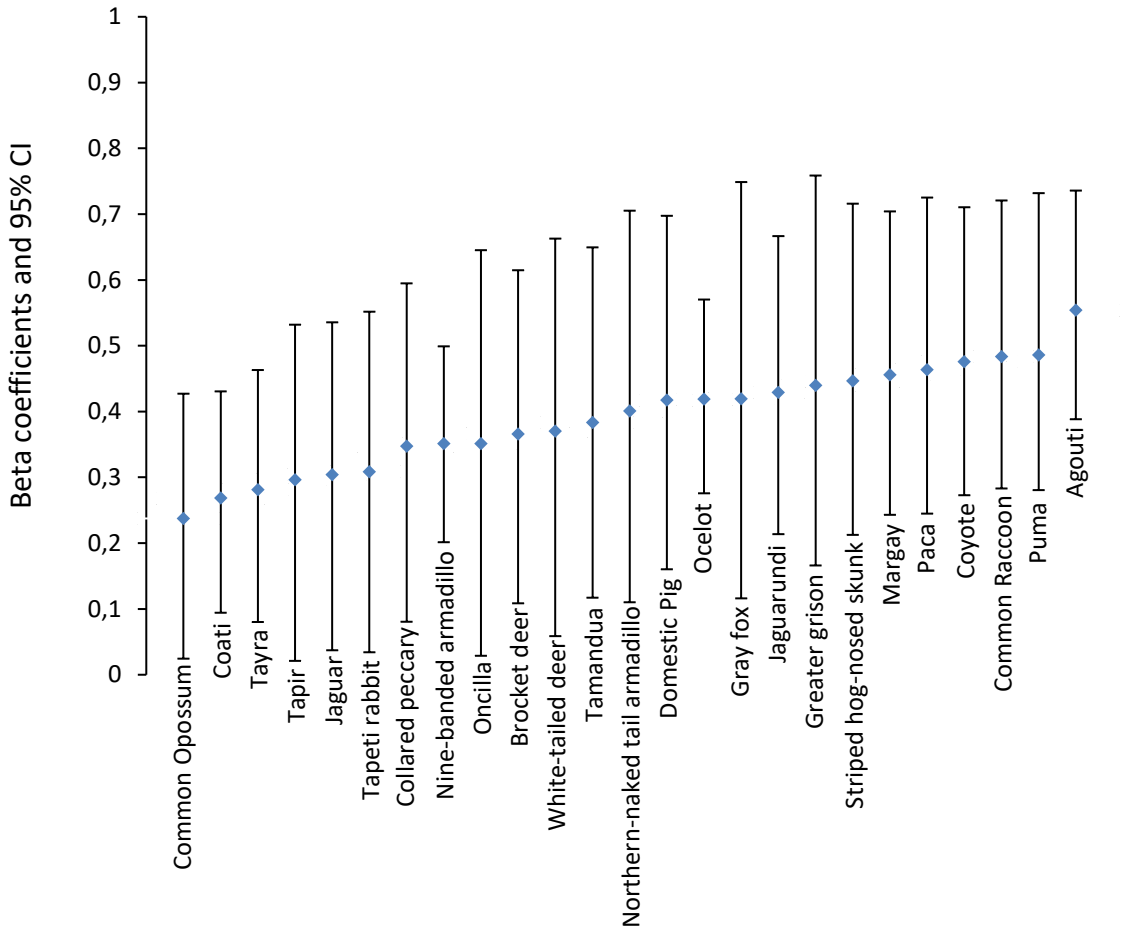

Figure S1.5. Community-level hyperparameter estimates (with 95% Bayesian Credible Intervals) for the influence of Effort (sum of all trap nights on each occasion) on detection (p) of medium and large mammals and domestic pig (*n* = 25) in Barbilla-Destierro Biological Sub-Corridor (Corridor), and a segment of Central Volcanic Cordillera and Talamanca-Cordillera Central Jaguar Conservation Units, 2013-2017.

Appendix 2: Supporting information for Chapter 2

Supporting information 2.1: List of the 24 medium and large mammal species detected, mean probability of occupancy per survey period in Barbilla-Destierro Sub-Corridor (Corridor), Costa Rica, 2013-2018.

Table S2.1. List of the 24 medium and large mammal species detected, mean probability of occupancy per survey period in Barbilla-Destierro Sub-Corridor (Corridor), Costa Rica, 2013-2018.

		Mean Ψ ± SD by species		
Common name	**Scientific name**	**Pre-flooding**	**Early flooding**	**Post-flooding**
Agouti	*Dasyprocta punctata*	0.50 ± 0.00	0.71 ± 0.02	0.56 ± 0.02
Armadillo Nine Banded	*Dasypus novemcinctus*	0.95 ± 0.03	0.96 ± 0.01	0.92 ± 0.01
Armadillo Nothern Naked Tailed	*Cabassous centralis*	0.14 ± 0.04	0.28 ± 0.04	0.41 ± 0.07
Coati White Nosed	*Nasua narica*	0.82 ± 0.02	0.99 ± 0.02	0.92 ± 0.01
Coyote	*Canis latrans*	0.59 ± 0.03	0.70 ± 0.03	0.45 ± 0.03
Deer Red Brocket	*Mazama temama*	0.45 ± 0.15	0.42 ± 0.09	0.35 ± 0.13
Deer White Tailed	*Odocoileus virginianus*	0.15 ± 0.08	0.09 ± 0.06	0.05 ± 0.07
Fox Grey	*Urocyon cinereoargenteus*	0.00 ± 0.01	0.00 ± 0.02	0.01 ± 0.02
Grison Greater	*Gallictis vittata*	0.12 ± 0.08	0.57 ± 0.14	0.54 ± 0.19
Jaguar	*Panthera onca*	0.20 ± 0.04	0.18 ± 0.03	0.11 ± 0.07
Jaguarundi	*Puma yagouaroundi*	0.59 ± 0.09	0.44 ± 0.14	0.46 ± 0.09
Margay	*Leopardus wiedii*	0.29 ± 0.04	0.58 ± 0.13	0.60 ± 0.13
Ocelot	*Leopardus pardalis*	0.97 ± 0.03	1.00 ± 0.01	0.80 ± 0.02
Oncilla	*Leopardus tigrinus*	0.05 ± 0.02	0.06 ± 0.03	0.13 ± 0.02
Opossum Common	*Didelphis marsupialis*	0.58 ± 0.04	0.54 ± 0.04	0.73 ± 0.03
Paca	*Cuniculus paca*	0.26 ± 0.11	0.37 ± 0.04	0.31 ± 0.08
Peccary Collared	*Pecari tajacu*	0.14 ± 0.12	0.28 ± 0.07	0.36 ± 0.08
Puma	*Puma concolor*	0.36 ± 0.04	0.38 ± 0.04	0.43 ± 0.03
Rabbit Tapeti	*Sylvilagus brasiliensis*	0.28 ± 0.16	0.32 ± 0.09	0.26 ± 0.09
Raccoon Common	*Procyon lotor*	0.66 ± 0.02	0.69 ± 0.01	0.88 ± 0.01
Skunk Striped Hog Nosed	*Conepatus semistriatus*	0.57 ± 0.11	0.61 ± 0.03	0.46 ± 0.09
Tamandua Northern	*Tamandua mexicana*	0.71 ± 0.12	0.74 ± 0.05	0.70 ± 0.08
Tapir Baird	*Tapirus bairdii*	0.15 ± 0.00	0.15 ± 0.01	0.16 ± 0.01
Tayra	*Eira barbara*	0.85 ± 0.06	0.92 ± 0.01	0.94 ± 0.03

Appendix 3: Supporting information for Chapter 3

Supporting Information 3.1: DNA Extraction modifications for fecal and museum samples of ocelots in Costa Rica.

For fecal samples, we used the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA, USA) protocol for isolation from stool for human DNA analysis with the following modifications based on Chaves et al. (1). We shaved thin slices ~200 mg of fecal material from the outside of the scat. If sample was solid, we placed it into a tube and added 1.5 ml of Buffer ASL. If the sample was made mostly from hair we poured ~4 ml of Buffer ASL in a Petri dish with the sample. We soaked the sample and let it dissolve. We then collected the liquid and aliquoted 1.5 ml in each 2 ml tube. Later, we vortexed the tubes with the samples for 15 sec and let them incubate overnight at 55-65 °C on a rotator (~22 rpm). The next day we centrifuged samples during 3 min at 13,300 rpm, and then transferred 1.5 ml of the supernatant to a new 2 ml reaction tube. We added one InhibitEX tablet to the tube and vortexed continuously for 1 min and incubated for suspension at room temperature. Later, we centrifuged for 12 min at 13,300 rpm, and then transferred 600 µl to a 2 ml reaction tube with proteinase K and homogenized by mixing manually. We added 600 µl of AL Buffer and vortexed for 15 sec. We let it incubate for 15 min at 70 °C, and then added 600 µl of 100% Ethanol and vortexed for another 15 sec. Later we transferred 600 µl lysate to a QIA amp spin column in a 2 ml collection tube. We then centrifuged at 13,300 rpm for 1 min and discarded the filtrate after each transfer. We repeated these steps until all lysate was filtered. At that time, we transferred the QIA amp spin column to a new collection tube and added 500 µl of AW1 Buffer and centrifuged at 13,300 rpm for 1 min. We transferred the QIA amp spin column to a new collection tube and added µl of AW2 Buffer and centrifuged at 13,300 rpm for 2 min. We again transferred the QIAam spin column to a new collection tube and centrifuged at 13,300 rpm for 2 min to dry the column. We then transferred the QIA amp spin column to a new Eppendorf tube. We carefully opened the QIA amp spin column and pipetted 60 µl Buffer AE (heated) directly onto the QIA membrane, closed the cap and let it incubate at room temperature for 40 min. After that time, we centrifuged for 3 min at 8,000 rpm. We pipetted more 60 µl Buffer AE in the same tube onto the QIA membrane, closed the cap and let it incubate for 15 min at room temperature. We centrifuged for 3 min at 8,000 rpm. Finally,

we transferred 90 µl of the resulting DNA into two new 1.5 ml Eppendorf tubes and labeled them as “stock” and “back up”. We made extractions in a separate bench and used pipettes specifically for feces to avoid contamination, and included negative controls of DNA extraction to monitor for it.

For samples obtained from museum specimens (teeth, hair, bones, tissue), we used the QIA amp DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) with the following modifications based on Caragiulo et al. (2): prior to extraction, we rinsed the samples with distilled water, later soaked them in phosphate Buffer (PBS) 1X for a week at room temperature, changing the PBS every other day. We placed the removed PBS in a separate sterile 100 µl microcentrifuge tube and used it in a separate DNA extraction. After 7 days, we removed all PBS removed and added 20 µl of proteinase K, 1 µl of 1 M dithiothreitol, and 180 µl of ATL Buffer to a new 1.5 ml microcentrifuge tube with the sample. We checked samples daily and added an additional 10 µl of Proteinase K per day of additional incubation. We kept samples in incubation at 56 °C for 3 days. We followed the rest of the manufacturer´s protocol with the following exceptions: we used cold ethanol, we inverted samples by hand instead of vortexing to prevent shearing of DNA, we heated AE Buffer to 70 °C prior to addition to the spin-column membrane to improve DNA yield as recommended by the manufacturer, we carried on elution in two steps with a 40 and 15 min incubation at room temperature, and total elution volume was 150 µl done in two 75 µl stages. We made extractions in a separate bench and used pipettes specifically for these samples to avoid contamination.

References for Supporting information 3.1:

1. Chaves SL, Dias I, Pomilla C. Extraction of genomic DNA from carnivore fecal samples using QIAamp DNA Stool Mini Kit [Internet]. 2010. Available from: http://research.amnh.org/genomics/Resources/Extraction-genomicDNA
2. Caragiulo A, Dias-Freedman I, Clark JA, Rabinowitz S, Amato G. Mitochondrial DNA sequence variation and phylogeography of Neotropic pumas (*Puma concolor*). Mitochondrial DNA. 2014;25(4):304–12.