NONINVASIVE TRACKING OF JAGUARS (*PANTHERA ONCA*) AND CO-OCCURRING NEOTROPICAL FELIDS IN BELIZE, CENTRAL AMERICA BY GENOTYPING FECES AND REMOTE CAMERA TRAPPING

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ABSTRACT

The elusive jaguar (*Panthera onca*) is extremely difficult to study due to its wide-ranging behavior, crepuscular activity peaks and its occurrence in low population densities in often dense forest habitats. Jaguars are also a species of concern, but our ability to provide for their survival is hampered by our inability to obtain reliable information on the status of their wild populations.

This study combines innovative noninvasive research techniques such as scat detector dogs and molecular scatology to conduct the first genetic study on wild populations of Neotropical felids coexisting across fragmented forest habitats in Belize, Central America. Specifically, we analyzed multi-locus data in jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) collected from 1053 scat samples across their range in the country. First, we optimized 14 polymorphic microsatellite loci for jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*), and assessed their utility for cross-species amplification. Additionally, we tested their reliability for species and individual identification using fecal DNA as he primary DNA source. All microsatellite loci examined successfully cross-amplified in the three target species, and were polymorphic. Second, to maximize PCR amplification success and genotyping accuracy rates, and to minimize genotyping error rates for fecal DNA samples, we evaluated the performance of two fecal DNA storage techniques (dimethyl sulfoxide saline solution/DET buffer, 95% EtOH) suitable for long-term preservation at remote tropical sites. Additionally, we tested fecal DNA samples collected from four different scat locations (top, side, bottom, inside). DET buffer was the superior fecal DNA preservation method and collecting fecal DNA from side and top locations of the scat resulted in the highest PCR success rates.

For the main genetic study, we assessed the genetic conservation status of all three target species across the country of Belize. We examined levels of genetic diversity within different sites, (2) defined potential genetic clusters/populations, (3) and examined levels of gene flow and population structure for all three target species on a countrywide scale. Furthermore, we compared genetic diversity and gene flow levels among the three target species. Wild felids in Belize showed moderate levels of heterozygosity (H_E = 0.60 - 0.70) with jaguars having the lowest genetic diversity with average expected heterozygosities of H_E = 0.60 ± 0.05 and allelic richness (A_R) of 4.94 ± 0.44 followed by pumas with H_E = 0.65 ± 0.06 and A_R of 7.52 ± 0.86 and ocelots with H_E = 0.70 ± 0.05 and A_R of 3.89 ± 0.23. We observed low to moderate levels of differentiation (F_{ST} = 0.00 - 0.15) and weak population structure using spatial Bayesian clustering techniques for all three target species. Although levels of genetic diversity and gene flow across the country are still fairly high, we did detect evidence of fragmentation indicating the risk of further habitat loss and fragmentation for wild felids.

Felids were simultaneously monitored across all study sites by remote sensing camera traps, which allows for a comparison of density estimates obtained from two different noninvasive survey approaches. Furthermore, analytical methods for density estimation are advancing rapidly, making it difficult to choose the optimal technique. Thus, we compared a variety of capture-mark-recapture (CMR) density estimators including the conventional approach of estimating abundance ($\hat{N}$) in programs CAPTURE and MARK and dividing abundance by the effective trapping area (*ETA*), the recently developed spatially explicit capture-recapture (SECR) models, both the likelihood-based approach (ML-SECR) in program DENSITY and the Bayesian approach (B-SECR) in program SPACECAP, and finally the genetic-based mark-recapture one sampling occasion estimator in program CAPWIRE. Although different survey methods using various density estimators produced similar density estimates, confidence levels and coefficients of variation varied, with SECR methods resulting in the least precise estimates. Detection probabilities were generally higher for noninvasive genetic sampling than for camera trapping. Both techniques were shown to be reliable and highly efficient survey methods for density estimation of low-density Neotropical felids living in challenging environments such as the tropics. While less precise, SECR

CMR models are probably a more realistic reflection of our uncertainty. They hold great promise for density estimation studies for wide-ranging and territorial carnivore species, especially if precision can be improved through study design or analysis advancements in the future.

In conclusion, our results demonstrated that noninvasive sampling techniques such as molecular scatology and remote camera trapping are efficient research approaches to study multiple Neotropical felids in a multifaceted way and on a countrywide scale. We believe that the techniques and analyses developed in this study are widely applicable and relevant to the conservation and management of other elusive and difficult to study wild felids worldwide.

To my family

"There are some who can live without wild things

and some who cannot."

Aldo Leopold

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CHAPTER 1

Conservation of jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*)

A General Introduction

Species of interest

Jaguar

The jaguar (*Panthera onca*, Linnaeus 1785; Spanish: tigre; Maya: bolom, barum; Belize: tiger) (Linnaeus 1785) (Fig. 1A) is the largest Neotropical felid, and the only member of the *Panthera* genus in the Americas (Nowell and Jackson 1996, Reid 2009, Macdonald and Loveridge 2010) (Fig. 2). Jaguars are distributed from the southern United States through Central and South America to northern Argentina and can currently be found in at least 18 countries (Swank and Teer 1989, Sanderson et al. 2002, McCain and Childs 2008). Nonetheless, over the last 100 years in the Americas, jaguars have been extirpated from over 54% of their historic range (Nowell and Jackson 1996, Sanderson et al. 2002) and are regionally extinct in El Salvador and Uruguay (Caso et al. 2008). The situation is worse in Mexico and Central America where jaguar populations exist in only 33% of their former range and 75% of populations that do exist are reduced in number (Swank and Teer 1989). Despite its internationally protected status (listed as 'Near Threatened' under IUCN, the International Union for Conservation of Nature, and as an *Appendix I* species under CITES, the Convention on International Trade of Endangered Species of Wild Fauna and Flora), the jaguar's range continues to decrease mainly due to severe deforestation, fragmentation of their preferred habitat, direct persecution, and poaching of

prey (e.g. Hoogenstein and Mondolfi 1992, Nowell and Jackson 1996, Caso et al. 2008).

Although jaguars are considered as generalist species, which occur in a variety of habitat types, they prefer areas with tree cover (Crawshaw and Quigley 1991), close to water bodies (Swank and Teer 1989), far from human settlements and transport corridors. Due to their large home-range sizes which range from 28 – 40 km^2 (Rabinowitz and Nottingham 1986) for adult male jaguars in Belize to 142.1 ± 25 km^2 across the grasslands and forests in the Pantanal of Brazil (Crawshaw and Quigley 1991), jaguars depend on large, well-connected forest areas which also have the potential to support healthy populations of prey species (e.g. Ortega-Huerta and Medley 1999, Scognamillo et al. 2003). Conservation efforts to assess connectivity of jaguars across the landscape on a fine and range-wide scale are still rare, but more are currently underway (e.g. Wultsch et al. in review, Eizirik et al. 2001, Ruiz-Garcia et al. 2006, Haag et al. 2010, Rabinowitz and Zeller 2010, Colchero et al. 2011).

Former research on jaguars focused primarily on food habits and foraging ecology (e.g. Chinchilla 1997, Farrell et al. 2000b, Nunez et al. 2000, Garla et al. 2001, Gonzalez and Miller 2002, Novack et al. 2005, Weckel et al. 2006a, b, de Azevedo and Murray 2007), coexistence of jaguars and pumas (e.g. Scognamillo et al. 2003, Harmsen et al. 2009), human-wildlife conflict (e.g. Polisar et al. 2003, Zimmermann et al. 2005, Michalski et al. 2006) and demographic monitoring estimating jaguar densities (e.g. Maffei et al. 2004, Silver et al. 2004, Soisalo and Cavalcanti 2006, Salom-Perez et al. 2007, Kelly et al. 2008, Sollmann et al. 2011, Noss et al. 2012).

Genetic studies on jaguars are still rare, but focused on primarily on phylogenetics (e.g. Johnson and Obrien 1997, Johnson et al. 2006, Kim et al. 2006, Davis et al. 2010)

and genetic diversity and genetic structure (e.g. Johnson et al. 1999, Eizirik et al. 2001, Moreno et al. 2006b, Ruiz-Garcia et al. 2006). Only a handful of studies were based on wild populations (e.g. Miotto et al. 2007, Haag et al. 2010). Eizirik et al. (2001) conducted the so far only range-wide genetic study on jaguars, which was mostly based on DNA samples (blood, hair, tissue) obtained from museums and captive facilities. They did not detect any major phylogeographic partitions of jaguars across their entire range and did not support traditional classification of jaguars into several subspecies. Historically, up to eight subspecies of jaguars were identified based on geographic origins of specimen and morphological skull characteristics (Nelson and Goldman 1933, Pocock 1939, Seymour 1989, Larson 1997). Eizirik et al. (2001) rather detected no evidence for the existence of major geographical partitions.

Past studies on jaguars in Belize mainly focused on basic ecology, behavior and habitat use (Rabinowitz and Nottingham 1986, Harmsen et al. 2009, Foster et al. 2010a, Harmsen et al. 2010, Harmsen et al. 2011b), diet (Weckel et al. 2006a, Foster et al. 2010b), parasites (Patton et al. 1986, Watt 1987) and abundance/density estimates using remote camera trapping data for mark-recapture analysis (Kelly 2003, Silver et al. 2004, Davis et al. 2011, Harmsen et al. 2011a). Jaguar predation on domestic livestock in Belize was also the focus of research (Rabinowitz 1986).

Puma

The puma (*Puma concolor*, Linnaeus 1771; Spanish: puma; Maya: cha-barum; Belize: red tiger) (Fig. 1B) has the largest geographical range amongst all mammals in the New World (Sunquist and Sunquist 2002b) with a distribution from southern Canada and the

United States through Mexico and Central America to southern Argentina and Chile (Reid 2009, Macdonald and Loveridge 2010). Pumas are also considered as the second largest carnivore species in the Neotropics. Listed as "Least Concern" species under IUCN and in CITES Appendix I (eastern and Central American subspecies) and II (remaining subspecies), since it is considered as a widespread species, it still has disappeared from large portions of his historic range, especially in the Eastern United States (Nowell and Jackson 1996). The only region in the eastern United States, where pumas have survived extinction, is located the Everglade swamp forest in Florida, which is home to a single population of Florida panthers. Florida panthers experienced a severe bottleneck, and currently have a small population size (~ 100 animals) (e.g. Culver et al. 2008, Macdonald and Loveridge 2010).

Generally, pumas are known for long distance dispersal (Ruth et al. 1998, Stoner et al. 2008) up to 1341 km. Pumas have been extensively studied in the United States (e.g. Beier et al. 1995, Sweanor et al. 2000, McRae et al. 2005, Holbrook et al. 2012), but little is known about this species in the Neotropics. Pumas are found in wide variety of habitat types, but generally prefer forested areas with little human disturbance, but movement of pumas through disturbed and human-developed areas has been recorded (e.g. Larue and Nielsen 2008, Macdonald and Loveridge 2010). Abundance and population density estimates for pumas vary greatly across different areas (e.g. Nowell and Jackson 1996, Franklin et al. 1999, Miotto et al. 2007, Kelly et al. 2008, Negroes et al. 2010, Russell et al. 2012).

Additional research on pumas focused on basic ecology (e.g. Spreadbury et al. 1996, Franklin et al. 1999, Pierce et al. 1999, Sweanor et al. 2000, Logan and Sweanor

2001, Grigione et al. 2002), diet and predation (e.g. Iriarte et al. 1991, Cunningham et al. 1999, Hayes et al. 2000, Rau and Jimenez 2002, Hernandez-Guzman et al. 2011), human-wildlife conflicts (e.g. Zarco-Gonzalez et al. 2012), coexistence with other carnivore species (e.g. Di Bitetti et al. 2010, Romero-Munoz et al. 2010, Sollmann et al. 2012a), and movement and dispersal patterns (e.g. Cramer and Portier 2001, Gloyne and Clevenger 2001, Maehr et al. 2002, Clevenger and Waltho 2005, Thompson and Jenks 2005, Larue and Nielsen 2008, Stoner et al. 2008, Elbroch et al. 2009, Elbroch et al. 2010, Kertson et al. 2011).

Former conservation genetic studies addressed a wide range of questions including genetic diversity, gene flow and population structure (e.g. Ernest et al. 2000, Walker et al. 2000a, Ernest et al. 2003, Anderson et al. 2004, McRae et al. 2005, Kurushima et al. 2006, Moreno et al. 2006b, Ruiz-Garcia et al. 2009, Loxterman 2011, Miotto et al. 2011, Andreasen et al. 2012, Castilho et al. 2012, Holbrook et al. 2012, Wheeler and Waller 2012). A range-wide study on pumas and their genomic ancestry detected 6 phylogeographic groupings (or subspecies), with North American pumas being a homogenous group (*Puma concolor cougar*) (Culver et al. 2000). The remaining subspecies were located in Central America (*Puma concolor costaricensis*), eastern South America (*Puma concolor capricornensis*), northern South America (*Puma concolor concolor*), central South America (*Puma concolor cabrerae*) and southern South America (*Puma concolor puma*) (Fig. 3). Additional phylogenetic studies, which included pumas were conducted by Johnson and O'Brien (1997), and Mattern and McLennan (2000).

Former studies on pumas in Belize focused on basic ecology, coexistence, behavior and habitat use (Harmsen et al. 2009, Foster et al. 2010a, Harmsen et al. 2010,

Davis et al. 2011), food habits (Foster et al. 2010b), and abundance and density estimation based on remote-camera data (Kelly et al. 2008).

Ocelot

The ocelot (*Leopardus pardalis*, Linnaeus 1758; Spanish: ocelote; Maya: ek-sush; Belize: tiger cat) (Fig. 1C) is the only medium-sized felid found in the Neotropics (Nowell and Jackson 1996, Reid 2009, Macdonald and Loveridge 2010). Ocelots have a wide geographic distribution ranging from the southern United States (Texas) through Mexico and Central America to northern Argentina (Caso et al. 2008). Historically, this species had a larger distribution in the United States and could be also found in Arkansas and Arizona (Sunquist and Sunquist 2002b). Ocelots are listed as 'Least Concern' species under IUCN and included on CITES Appendix I, however a few populations across its range are threatened with extinction (e.g. southern Texas) (Caso et al. 2008). The main threats ocelots are facing are habitat loss and fragmentation, and illegal skin trade (Macdonald and Loveridge 2010). Generally, this species inhabits a wide variety of habitats and can be considered as generalist species, which is less sensitive to human disturbance than other co-occurring felids (e.g. Caso et al. 2008, Macdonald and Loveridge 2010).

Former studies on ocelots primarily focused on basic ecology, coexistence and habitat use (e.g. Harveson et al. 2004, Pina et al. 2004, Jackson et al. 2005, Aliaga-Rossel et al. 2006, Moreno et al. 2006a, Horne et al. 2009, Di Bitetti et al. 2010), diet (e.g. Meza et al. 2002, Wang 2002, Bianchi and Mendes 2007, Abreu et al. 2008, Martins et al. 2008, Bianchi et al. 2010, Silva-Pereira et al. 2011) and abundance and density estimates

(Trolle and Kery 2005, Di Bitetti et al. 2006, Dillon and Kelly 2007, Di Bitetti et al. 2008, Maffei and Noss 2008, Fusco-Costa et al. 2010, Kolowski and Alonso 2010, Davis et al. 2011, Gonzalez-Maya and Cardenal-Porras 2011, Noss et al. 2012). Genetic studies investigated phylogenetic and phylogeographic relationships (Eizirik et al. 1998, Janecka et al. 2007) and genetic diversity (Grisolia et al. 2007, Janecka et al. 2008b, Janecka et al. 2011b).

Former studies on ocelots in Belize focused on basic ecology and coexistence, and abundance and density estimation (Konecny 1989, Kelly 2003, Dillon and Kelly 2007, 2008, Davis et al. 2011).

The challenges to study elusive forest felids in the tropics

Studying tropical felids often requires physical capture, handling and extensive subsequent monitoring of the animals, an approach which is intrusive, expensive, time-consuming, often dangerous, and considering its often low sample sizes, not efficient enough to gather sufficient information on wild cat populations (Mills et al. 2000a, Gompper et al. 2006). Noninvasive monitoring techniques such as remote camera trapping (Karanth and Nichols 1998) have been developed in response to these inadequacies. Remote camera-trapping, where wild cats are "captured" on film and identified by their distinct coat patterns, has provided the first repeatable estimates of densities and sex-ratios for jaguars (Kelly 2003, Wallace et al. 2003, Maffei et al. 2004, Silver et al. 2004). During the last decade, remote-camera trapping has become to one of the most abundant survey techniques used for Neotropical felids including pumas and ocelots (e.g. Soisalo and Cavalcanti 2006, Dillon and Kelly 2007, Salom-Perez et al.

2007, Kelly et al. 2008, McCain and Childs 2008, Foster et al. 2010a, Mazzolli 2010, Arzate et al. 2011, Davis et al. 2011, Harmsen et al. 2011a, Nunez-Perez 2011, Sollmann et al. 2011, Hernandez-Diaz et al. 2012, Noss et al. 2012). The application of remote camera data is varied. Besides the most common abundance/density estimation studies, remote-camera data also provide information on other co-occurring species including competitors, prey species, human activities, which can be directly related to target species by using trap success rates. Analytically, population size and density estimation uses mark-recapture analysis and a variety of different statistical models, including classic closed capture-recapture models with multiple discrete encounter occasions (Otis et al. 1978, White et al. 1982, Rexstadt and Burnham 1991), and the more recent spatially-explicit mark-recapture (SECR) models applied in a maximum likelihood (ML-SECR) (Efford 2004, Borchers and Efford 2008, Efford et al. 2009, Efford 2011) or a Bayesian (B-SECR) framework (Borchers and Efford 2008, Royle and Young 2008, Royle et al. 2009, Gopalaswamy et al. 2012, Thompson et al. 2012). Choosing the optimal analytical approach is challenging, and has been discussed heavily in recent years (e.g. Efford 2011, Foster and Harmsen 2012, Kelly et al. 2012). When long-term data are available, estimation of survival and recruitment can be furthermore conducted (e.g. Long et al. 2008, O'Connell et al. 2011, Kelly et al. 2012).

Recently, non-invasive genetic monitoring of mammal species has been an approach of increasing importance in the field of wildlife management and conservation (Morin and Woodruff 1996, Waits and Paetkau 2005, Kelly et al. 2012), and has been added to the suite of noninvasive monitoring techniques for wild felids. This approach, where individuals are “genetically tagged” through highly variable microsatellite primers,

is applicable for identification of species, gender and individuals, thus can be also used for population size estimation and monitoring, genetic diversity and genetic structure studies (e.g. Kohn et al. 1999, Eggert et al. 2003, Frantz et al. 2003, Wilson et al. 2003, Waits and Paetkau 2005, Perez et al. 2006). Without ever physically capturing or disturbing the animals of interest, it is a powerful approach to study and/or monitor wide-ranging carnivore species, which often occur at low densities and show cryptic and elusive behavior (Taberlet and Luikart 1999, Long et al. 2008). DNA extracted from biological material from outside the skin such as hair, shed skin, and cells carried from inside the animal to the outside such as in feces, urine, saliva, semen, and regurgitates is collected non-invasively. For carnivores such as wild felids, hair, urine, and feces samples seem to be most the suitable DNA sources. DNA obtained from hair samples has been used to monitor the Andean mountain cat (Johnson et al. 1998), Canadian and Eurasian lynx, bobcat, puma (Turbak 1998, Mills et al. 2000b, Schmidt and Kowalczyk 2006), and ocelots (Weaver et al. 2003). The success rates for felid hair sampling have been very low or close to zero (Downey et al. 2007) plus these techniques are usually biased towards gender due to the use of specific scents or lures. Genotyping individuals from urine samples has been conducted for wolverines (Hedmark et al. 2004) and wolves (Hausknecht et al. 2007) and is considered as a helpful tool as the urine DNA is more homogenous compared to fecal DNA which contains also dietary DNA. The collection of urine DNA is unfortunately strongly dependent on the presence of snow for detection and sampling (Hausknecht et al. 2007), which makes the technique not applicable for tropical environments. Molecular scatology, where individuals are genotyped from sloughed intestinal epithelial cells found in field-collected fecal samples (Hoss et al. 1992, Foran et

al. 1997, Kohn and Wayne 1997), is an especially suitable survey approach for wild felids, which often deposit scat at prominent sites for intra- and interspecific communication (Sunquist and Sunquist 2002b). This survey approach has been used for a variety of wild felids including the Andean cat (Cossios and Angers 2006), pumas (e.g. Ernest et al. 2000, Ernest et al. 2003, Miotto et al. 2007), bobcats (Long et al. 2007a), leopards (Perez et al. 2006), snow leopards (e.g. Janecka et al. 2008a, Karmacharya et al. 2011) and tigers (e.g. Wan et al. 2003, Bhagavatula and Singh 2006, Reddy et al. 2011, Sugimoto et al. 2012). In recent years, molecular scatology studies increased scat-collection rates successfully in the field by using professionally trained scat detector dogs (e.g. Smith et al. 2001, Wasser et al. 2004, Harrison 2006, Long et al. 2007b, Dematteo et al. 2009, Vynne et al. 2011b, de Oliveira et al. 2012) (Fig. 4). Low DNA quantity and quality are major concerns for molecular scatology studies, especially when conducted in the tropics (e.g. Michalski et al. 2011, Vynne et al. 2012). Degraded DNA decreases PCR amplification success and cause genotyping errors (Taberlet et al. 1996), which has been addressed by several approaches.

Understanding current patterns of genetic diversity within populations, and assessing levels of genetic connectivity among populations is crucial for developing conservation and management strategies of potentially fragmented wildlife populations (e.g. Allendorf et al. 2013). Overall, molecular population genetics approaches on jaguars and other Neotropical felids are still rare (e.g. Eizirik et al. 2001, Ruiz-Garcia et al. 2006), and noninvasive fecal genotyping of wild populations has only been conducted on a relatively small scale in different countries across their range (e.g. Zuercher et al. 2003,

Moreno et al. 2006b, Miotto et al. 2007, Haag et al. 2009, Haag et al. 2010, Castilho et al. 2011, Michalski et al. 2011, Roques et al. 2011, Vynne et al. 2011b).

Study objectives

This is the first countrywide study of three Neotropical felids, jaguars, pumas, and ocelots in Belize, Central America using two different noninvasive survey techniques, remote-camera trapping and molecular scatology. To gather knowledge on a countrywide scale and efficiently obtain multi-faceted information on three wild felid species existing across fragmented forest habitats, we defined the following research objectives for this project:

(1) ***Objective 1*** - Develop standardized field and laboratory protocols for a molecular scatology study of three Neotropical felids

   a) Conduct non-invasive Individual and Species Identification of Jaguars (*Panthera onca*), Pumas (*Puma concolor*) and Ocelots (*Leopardus pardalis*) in Belize, Central America using Cross-Species Microsatellites and Fecal DNA (***Chapter 2***)

   b) Assess effects of storage methods and scat sample location on PCR amplification success, genotyping accuracy, and genotyping error rates using fecal samples of jaguars (*Panthera onca*) and co-occurring Neotropical felids from two tropical habitats in Belize, Central America (***Chapter 3***)

(2) ***Objective 2*** – Assess conservation genetic status of three Neotropical felids in Belize, Central America

a) Determine genetic diversity, genetic structure and gene flow of three Neotropical felids across five study sites in Belize, Central America (***Chapter 4***)

(3) ***Objective 3*** - Comparison of two noninvasive survey techniques in wild felid conservation and management: genotyping feces versus remote camera trapping

a) Compare of Population Density Estimation Methods for Sympatric Jaguars (*Panthera onca*) and Pumas (*Puma concolor*) in Belize, Central America using Non-invasive Genetic-Sampling, Remote-Camera Trapping and Capture-Recapture Models (***Chapter 5***)

(4) ***Objective 4*** – Comparison of different density estimators in wild felid conservation and management using remote-camera trapping and molecular scatology

a) Comparison of Population Density Estimation Methods for Sympatric Jaguars (*Panthera onca*) and Pumas (*Puma concolor*) in Belize, Central America using Non-invasive Genetic-Sampling, Remote-Camera Trapping and Capture-Recapture Models (***Chapter 5***)

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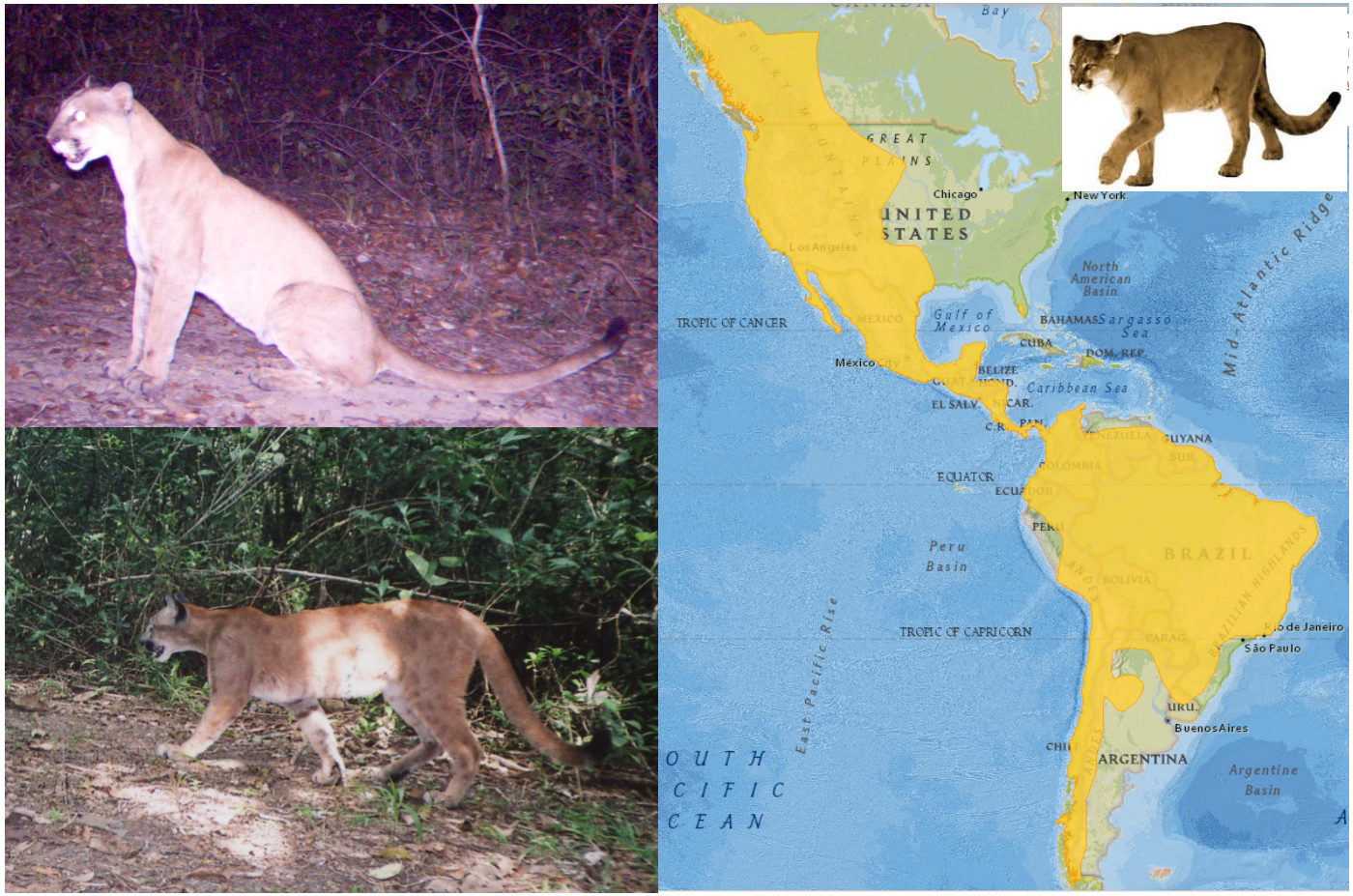
Fig. 1 Geographic distribution of (A) jaguars (*Panthera onca*), (B) pumas (*Puma concolor*), and (C) ocelots (*Leopardus pardalis*) (IUCN 2012). Photographs of jaguars, pumas, and ocelots taken by remote camera traps in Belize, Central America. Current distribution is marked in yellow.

(A) Jaguar

(B) Puma


Chicago
New York
UNITED STATES
Los Angeles
North American Basin
Gulf of Mexico
TROPIC OF CANCER
BAHAMAS
Sargasso Sea
CUBA
México
BELIZE
Caribbean Sea
EL SALV.
EQUATOR
GUYANA
Peru Basin
BRAZIL
BOLIVIA
TROPIC OF CAPRICORN
São Paulo
Buenos Aires
ARGENTINA
Argentine Basin
Mid-Atlantic Ridge
East Pacific Rise

(C) Ocelot


UNITED STATES
Los Angeles
North American Basin
MEXICO
Gulf of Mexico
BAHAMAS
Sargasso Sea
CUBA
México City
DOM. REP.
BELIZE
GUA
Caribbean Sea
EL SALV.
CAR.
C.R.
PAN.
VENEZUELA
GUYANA
SUR.
COLOMBIA
EQUATOR
ECUADOR
PERU
Peru Basin
BRAZIL
BOLIVIA
PARAG.
São Paulo
TROPIC OF CAPRICORN
East Pacific Rise
URU.
Buenos Aires
CHILE
ANDES
ARGENTINA
Argentine Basin

Fig. 2 Phylogenetic relationships among felid species including jaguars (*Panthera onca*), puma (*Puma concolor*), and ocelot (*Leopardus pardalis*) (Johnson et al. 2006).

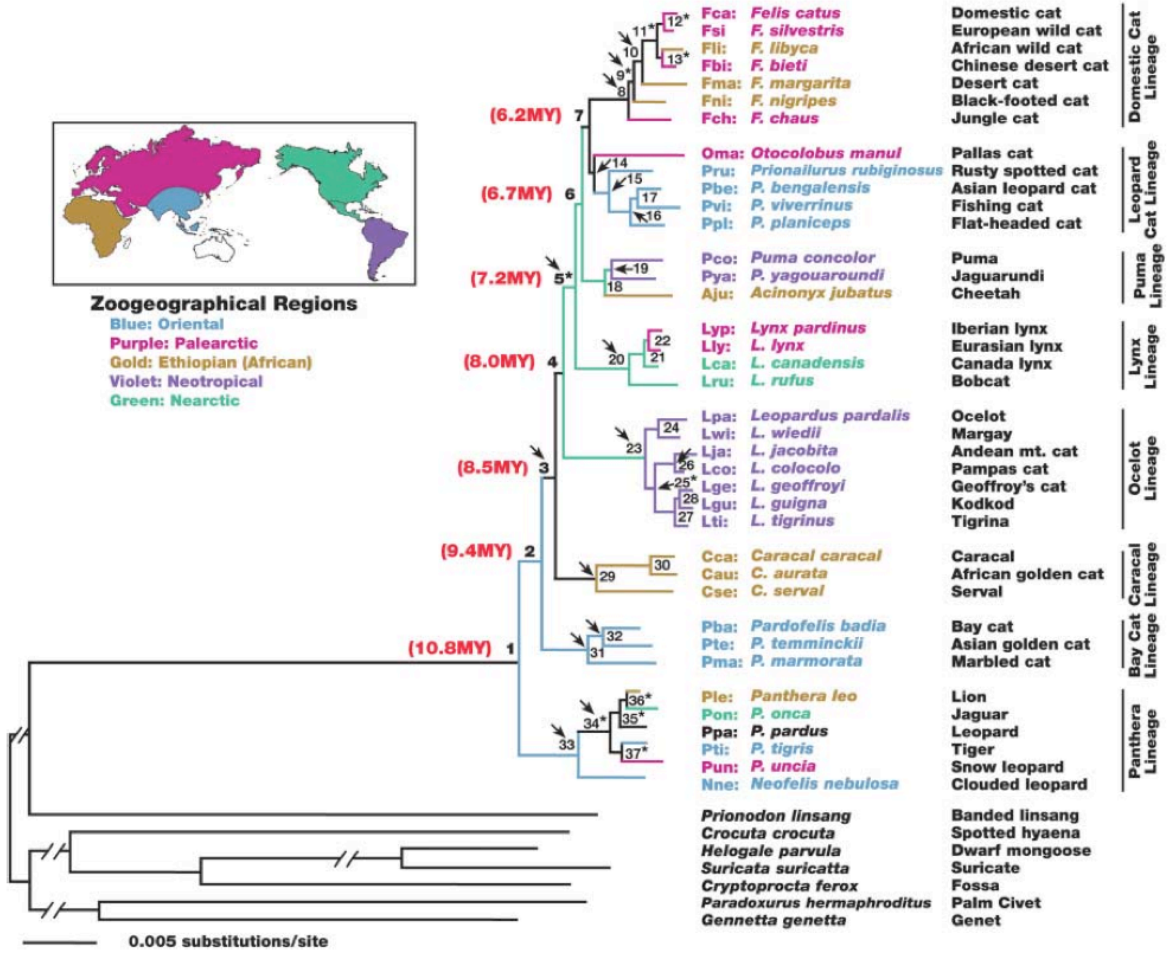


Fig. 3 Geographic ranges of six subspecies of pumas as defined by Culver et al. (2000) with North American pumas being homogenous group (*Puma concolor cougar)*. The remaining subspecies are located in Central America (*Puma concolor costaricensis*), eastern South America (*Puma concolor capricornensis*), northern South America (*Puma concolor concolor*), central South America (*Puma concolor cabrerae*) and southern South America (*Puma concolor puma*).

Fig. 4 Scat detector dog work in Belize, Central America. PI Wultsch with scat detector dog Bruiser detecting wild felid scat in Fireburn Nature Reserve in northern Belize. Wild jaguar scat pictured below – also detected at Fireburn Nature Reserve, Belize in 2010.

CHAPTER 2

Non-invasive Individual and Species Identification of Jaguars (*Panthera onca*), Pumas (*Puma concolor*) and Ocelots (*Leopardus pardalis*) in Belize, Central America using Cross-Species Microsatellites and Fecal DNA

Abstract

There is a great need to develop efficient, noninvasive genetic sampling methods to study wild populations of multiple, co-occurring, threatened felids. This is especially important for molecular scatology studies occurring in challenging tropical environments where DNA degrades quickly and the quality of fecal samples varies greatly. We optimized 14 polymorphic microsatellite loci for jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*), and assessed their utility for cross-species amplification. Additionally, we tested their reliability for species and individual identification using DNA from feces of wild felids detected by a scat detector dog across Belize in Central America. All microsatellite loci were successfully amplified in the three target species, were polymorphic with average expected heterozygosities of $H_E = 0.60 \pm 0.18$ for jaguars, $H_E = 0.65 \pm 0.20$ for pumas, and $H_E = 0.72 \pm 0.12$ for ocelots, and had an overall PCR amplification success of 61%. We used this nuclear DNA primer set to successfully identify species and individuals from 50% of 1053 field-collected scat samples. This set of optimized microsatellite multiplexes represents a powerful tool for future efforts to conduct noninvasive studies on multiple, wild Neotropical felids.

Keywords: *Panthera onca*, *Puma concolor*, *Leopardus pardalis*, non-invasive genetic sampling, individual identification, cross-species microsatellites, molecular scatology, Bayesian clustering

Introduction

Jaguars (*Panthera onca*) and co-occurring Neotropical felids, such as pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*), are extremely difficult to study in the wild due to their elusive nature. As landscapes change and human impacts increase, felid populations become increasingly fragmented and ecological processes such as dispersal, intra-guild competition, and top-down trophic impacts (Linnell and Strand 2000) are affected, but remain understudied. Consequently, there is a great need for efficient survey methods that can be applied to multiple co-occurring species instead of focusing research efforts on a single species. Non-invasive genetic monitoring has been of increasing importance in the field of wildlife management and conservation in recent years (e.g. Morin and Woodruff 1996, Waits and Paetkau 2005), providing the potential to gain valuable information on multiple species existing across a fragmented landscape. Molecular scatology, in which individuals are genotyped from sloughed intestinal epithelial cells in field-collected fecal samples (Hoss et al. 1992, Kohn and Wayne 1997), is especially suitable for wild felids, which often deposit scat at prominent sites for intra- and interspecific communication (Sunquist and Sunquist 2002a). Additionally, DNA extracted from feces can be obtained without physically capturing or disturbing animals of interest (Taberlet et al. 1999). Noninvasive genetic sampling also holds great promise for providing large sample sizes for multiple species simultaneously and identification of species, gender and individuals, which is beneficial for a wide array of analyses (e.g. Waits and Paetkau 2005, Kelly et al. 2012).

In order to efficiently monitor threatened species, robust identification of individuals is crucial for various types of studies (e.g. capture-recapture analysis).

However, individual-based molecular scatology studies of single and especially of multiple wild felids are rare, particularly in tropical regions. Multi-species studies often do not go beyond the species level (e.g. Zuercher et al. 2003, Cossios and Angers 2006, Haag et al. 2009, Michalski et al. 2011, Roques et al. 2011), with a few exceptions (e.g. Singh et al. 2004, Trigo et al. 2008, Mondol et al. 2012). The lack of studies of individual-based genetic population monitoring may be explained by the challenges in accurately identifying individuals from fecal nuclear DNA collected in tropical environments. The extent of DNA degradation is affected by various environmental factors (high temperatures, precipitation and UV radiation) (e.g. Brinkman et al. 2010, Vynne et al. 2012), which are particularly concerning in tropical climates where excessive heat and humidity cause DNA to deteriorate rapidly. Generally, low DNA quantity and quality are major concerns for molecular scatology studies in the tropics (e.g. Michalski et al. 2011, Vynne et al. 2012), since they decrease PCR amplification success and cause genotyping errors (Taberlet et al. 1996). Genotyping errors increase the chance of misidentification of individuals and potentially bias population estimates (Taberlet and Luikart 1999, Taberlet et al. 1999, Mills et al. 2000a, Waits and Leberg 2000).

To conduct a noninvasive genetic study of multiple Neotropical felid species simultaneously, a set of highly polymorphic microsatellite markers (SSRs, simple sequence repeats in the nuclear genome) is needed that amplifies multiple target species and has the potential to identify species and individuals from fecal DNA. Cross-species amplification of microsatellites depends on the conservation of primer sequences, which has been described for several mammalian taxa (e.g. Moore et al. 1991). Cross-species

microsatellites are transferrable between closely related species, which makes them a cost-effective and efficient approach for conservation genetic studies of multiple target species. Menotti-Raymond and O'Brien (1995) and Menotti-Raymond et al. (1999) characterized microsatellite loci for the domestic cat (*Felis catus*) and described their utility for cross-species amplification due to conserved flanking primer sequences across Family Felidae. Cross-species microsatellites subsequently were used for several felids (Menotti-Raymond and O'Brien 1995, Shankaranarayanan et al. 1997, Johnson et al. 1999, Carmichael et al. 2000, Williamson et al. 2002, Singh et al. 2004, Buckley-Beason et al. 2006, Moreno et al. 2006b, Grisolia et al. 2007, Trigo et al. 2008, Hertwig et al. 2009). Their application is diverse and has helped facilitate comparisons among closely related feline species for assessing levels of genetic diversity and phylogeogaphic patterns (Johnson et al. 1999, Moreno et al. 2006b, Grisolia et al. 2007), to recognize new feline species (Wilting et al. 2007), to assess hybridization between wild and domestic cats (e.g. Wiseman et al. 2000, Randi et al. 2001, Trigo et al. 2008), and to detect illegal hunting and trafficking of threatened felids (Maudet et al. 2004, Singh et al. 2004). Nevertheless, cross-species amplification of microsatellites has not often been used for demographic and genetic monitoring of multiple felids in the wild, which could ultimately improve conservation and management activities for these elusive species. Besides the comparison of population genetic parameters among multiple closely-related species, cross-species microsatellites can also be applied for species identification based on species-specific allele sizes at multiple loci, a technique which has not often been used for noninvasive studies of wild carnivores (e.g. Pilot et al. 2007, Wilting et al. 2007).

We conducted a 4-year noninvasive genetic study of three co-occurring felids (jaguar, puma, and ocelot) across several study sites in tropical Belize, Central America. We initially screened 20 microsatellite loci developed in earlier feline studies (Menotti-Raymond et al. 1999), and identified 14 microsatellite markers for a molecular scatology study of three neotropical felids suitable for answering a variety of questions relevant to conservation and management. Specifically, our objective was to define a set of highly polymorphic microsatellite loci applicable for reliable and cost-effective species and individual identification across three target species. Furthermore, we evaluated the reliability of genotypes from fecal samples of highly variable DNA quality and quantity as a primary DNA source by quantifying PCR amplification success, genotyping accuracy and error rates.

Methods

Study Area

We conducted 2- to 3-month long scat surveys across 5 main study sites (Mountain Pine Ridge Forest Reserve – MPR, Rio Bravo Conservation and Management Area – RB, Cockscomb Basin Wildlife Sanctuary – CBWS, Chiquibul Forest Reserve and National Park – CFRNP, Fireburn/Balam Na Nature Reserve – FB) and 2- to 10-day surveys at several other sites (Big Falls – BF, Bladen Nature Reserve - BNR, Boden Creek Ecological Preserve - BC, Bull Run Farm – BRF, Golden Stream Corridor Preserve - GS, Hidden Valley Reserve – HVR, Machaca Hills – MH, Manatee Forest Reserve – MFR, Sarstoon-Temash National Park - STNP, Shipstern Nature Preserve – SNP, Tiger Sandy Bay - TSB) from 2007- 2010 across Belize, Central America (17°15’ N, 88°45’ W; Fig.

1). All sites except BF, BRF, MH and TSB are part of the national system of Protected Areas in Belize. The study sites also fall within the forests of La Selva Maya (The Mayan Forest), a recognized biodiversity hotspot that forms part of the northern section of the Mesoamerican Biological Corridor. Across study sites, elevation ranges from 0 to 1120 m. Mean annual rainfall varies from 1524 mm in the north to 4064 mm in the south, with a pronounced wet season from June to December. Average annual temperatures fluctuate between 17.7 and 31.3 °C. A high diversity of native habitat types is represented within the study sites, including lowland and submontane broad-leaved moist and wet forests, lowland and submontane pine forests, mangrove and littoral forests, lowland savannah, shrub land, and wetland.

Sample Detection

Fecal samples were detected using a professionally trained scat detector dog (PackLeader LLC, Gig Harbor, WA, USA). Opportunistic searches were conducted within study sites (Wasser et al. 2004) to detect scat samples along roads, trails, game trails, off-trail, and across various landscape features (e.g. streams) and habitat types. The scat detector dog was trained to locate scat samples of all five native feline species (jaguars, pumas, ocelots, margays, and jaguarundis). All scat samples located by the scat detector dog, regardless of their appearance or suspected age, were collected and genotyped.

Fecal DNA Storage and Extraction

From each scat, one ~ 0.5 mL sample was collected and stored at room temperature in sterile 2 mL screw-top tubes filled with dimethyl sulfoxide saline solution (DET buffer,

Seutin et al. 1991) at 1: ≥ 4 volume scat-to-solution ratio. Scat samples were collected using disposable gloves and wooden sampling sticks. Fecal DNA extractions were conducted in a separate room at the Laboratory for Ecological, Evolutionary and Conservation Genetics (LEECG) at the University of Idaho (Moscow, ID, US), dedicated to noninvasive genetic studies, in order to avoid contamination while working with low-concentration DNA samples. The QIAamp DNA Stool Mini Kit protocol (Qiagen, Inc.) was used to extract DNA from all fecal samples. An extraction negative was added to each extraction run to control for contamination.

Microsatellite screening and selection

A total of 20 microsatellite loci (F53, F85, F98, F124, FCA008, FCA032, FCA043, FCA090, FCA096, FCA100, FCA124, FCA126, FCA132, FCA212, FCA225, FCA229, FCA275, FCA391, FCA441, FCA741) originally identified for the domestic cat (Menotti-Raymond et al. 1999) was tested on fecal samples from wild neotropical felids (19 jaguars, 18 pumas and 12 ocelots) collected in the first two study sites (MPR, RB). Microsatellite performance was evaluated by assessing genetic variation, probabilities of identity for unrelated individuals and siblings (Waits et al. 2001), polymerase chain reaction (PCR) amplification success, genotyping accuracy and genotyping error rates.

Microsatellite amplification and genotyping

After testing, 14 highly polymorphic loci were selected and arranged in three PCR multiplex reactions (multiplex 1 - F124, FCA391, FCA043, FCA275, FCA096, FCA126, FCA090; multiplex 2 - F85, F98, FCA741, FCA225, FCA008, and multiplex 3 - F53 and

FCA441) in order to increase performance and efficiency. The three multiplexes each contained 7 µL PCR mixture. Multiplex 1 consisted of 3.5 µL 1 x concentrated Qiagen Master Mix (Qiagen, Inc.), 0.88 µL of primers (0.10µM for F124, 0.34µM for FCA391, 0.07µM for FCA043, 0.13µM for FCA275, 0.21µM for FCA096, 0.20µM for FCA126, 0.20µM for FCA090), 0.7 µL of 0.5 x concentrated Qiagen Q solution (Qiagen, Inc.), 0.02 µL H_2O, and 1.8 µL DNA extract. Multiplex 2 consisted of 1 x concentrated Qiagen 3.5 µL Master Mix, 0.65 µL of primers (0.20µM for F85, 0.09µM for F98, 0.11µM for FCA741, 0.43µM for FCA225, 0.10µM for FCA008), 0.7 µL of 0.5 x concentrated Qiagen Q solution, 0.15 µL H_2O, and 1.8 µL DNA extract. Multiplex 3 only differed in the amount of water (0.76 µL) and primers (0.24 µL; 0.20µM for F53, 0.14µM for FCA441) added. Microsatellite PCR amplifications were conducted using a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad Laboratories, Inc.) starting with an initial denaturation step of 15 min at 95 °C; followed by 13 cycles of 30 s at 94 °C for denaturation, 1.5 min at 60 °C with a decrease in annealing temperature of 0.8 °C in each cycle, and 1 min elongation at 72 °C; followed by 30 cycles of 30 s at 94 °C for denaturation, 1.5 min at 50 °C for annealing, and 1 min elongation at 72 °C; and 30 min at 60 °C for final elongation. A PCR negative was included in each group of PCR reactions to control for contamination. We visualized PCR products using an ABI PRISM® 377 automated DNA sequencer (Applied Biosystems™), and genotypes were identified using the software GENEMAPPER, version 3.7 (Applied Biosystems™).

Initially, we performed a minimum number of 2 PCRs for all scat samples using the screening multiplex 1 (7 loci). Depending on the number of successfully amplified loci within multiplex 1, we placed the scat samples in three categories describing

amplification success: (1) bad (0-2 loci amplified), (2) medium (3-5 loci amplified), and (3) good (6-7 loci amplified). We conducted 1- 4 additional PCR runs for scat samples placed into the medium and good quality categories. Scat samples with ≤ 2 loci amplified were discarded from the study. The total number of PCRs ranged from 2 to 6 replicates per locus. In order to finalize the consensus genotypes, a multi-tube approach was used where at least three identical homozygote PCR results were required for homozygote genotypes, and each allele had to be observed in two independent PCRs in order to record a heterozygous genotype.

Species and individual identification

Scat samples were assigned to feline species based on species-specific alleles and allelic ranges initially identified across several loci from known fecal DNA samples of 12 jaguars, 4 pumas, 12 ocelots, 30 margays and 4 jaguarundis obtained from captive facilities (Belize Zoo, Belize; Feline Conservation Center, CA, US; Naples Zoo, FL, US, Profelis, Costa Rica).

For individual identification, we estimated probabilities of identity (per locus and cumulative) for unrelated individuals ($P_{(ID)}$) and siblings ($P_{(ID)sib}$) per species as described by Waits et al. (2001) using GIMLET, version 1.3.3. (Valiere 2002). Cumulative $P_{(ID)}$ and $P_{(ID)sib}$ values were calculated per species for screening multiplex 1, and the minimum number of loci required for individual identification was estimated following a criterion of $P_{(ID)sib} < 0.01$ as suggested by Mills et al. (2000a) and Waits et al. (2001).

Once consensus genotypes were finalized for multiplex 1, we used GenAlEx, version 6.41 (Peakall and Smouse 2006) to determine the minimum number of

individuals per species by checking for repeated matching genotypes, which if detected, were classified as recaptures of individual felids. Near-matching genotypes, which differed at only 1 or 2 loci, were genotyped two more times and rematched. We selected the scat sample with the highest PCR product quality for each detected individual and screened these using Multiplex 2 (5 loci) and 3 (2 loci). We obtained consensus genotypes for the final two multiplexes after performing an additional 2 to 6 PCR replicates per locus. Genotypes, which were only detected once, were further examined using the software RELIOTYPE (Miller et al. 2002) to assess their reliability. If the accuracy of multi-locus scores was below 95%, additional PCR replicates were conducted.

To verify species assignment of all unique jaguar, puma, and ocelot individuals, we conducted assignment tests with the Bayesian clustering software STRUCTURE, version 2.3.3 (Pritchard et al. 2000). Species assignment for all individual genotypes detected was verified by analyzing clustering patterns based on distinctive allele frequencies and the most likely number of genetic clusters (*K*). The admixture model was used (predefined $K = 1$ to 5 corresponding to the five native feline species; 10 runs per *K* value; length of burn-in period: 10^5 iterations; number of MCMC iterations after-burn-in: 10^6). The optimal K value was chosen by calculating the posterior probability for each *K* value, which is based on estimated maximum log-likelihood values. After finding the optimal *K*, individuals were assigned to distinct genetic clusters using the % of the genotype's ancestry (*Q*) attributed to each genetic cluster. For successful species identification, *Q* values had to be $\geq 95\%$.

Data analysis

GenAlEx, version 6.41 (Peakall and Smouse 2006) was used to assess genetic variation per species at single loci, and across all loci by calculating the number of alleles (N_A), and estimating observed (H_O) and expected heterozygosities (H_E). Additionally, we determined allelic richness (A_R) using the rarefaction method with HP-RARE, version 1.0 (Kalinowski 2005) and polymorphic information content (*PIC*) with CERVUS, version 3.0 (Kalinowski et al. 2007).

To calculate PCR amplification success, genotyping accuracy and genotyping error rates, we selected the last 2 PCR runs for all loci across all scat samples. PCR amplification success rates were assessed by calculating the percentage of successful PCRs across all samples tested and for all samples with finalized species identification. Genotyping accuracy rates were estimated by calculating the percentage of successful PCRs, which matched the finalized consensus genotype. Genotyping error was quantified by calculating the rate of false alleles (FA) and allelic dropouts (ADO) following the protocols of Broquet and Petit (2004). False allele rates were calculated for all consensus genotypes and allelic dropout rates were estimated only for heterozygous genotypes.

Hardy-Weinberg equilibrium and linkage disequilibrium (LD) were tested for all three species with GENEPOP, version 4.1 (Raymond and Rousset 1995) using default settings for Markov chain parameters. The presence of null alleles was examined with MICRO-CHECKER (Van Oosterhout et al. 2004). Data was tested for normality and homoscedasticity. Statistical differences between groups were evaluated using non-parametric Kruskal-Wallis and post-hoc Wilcoxon rank-sum tests in program R, version

2.15 (R Development Core Team 2009). All results for multiple significance tests were adjusted by applying sequential Bonferroni correction (Rice 1989).

Results

Sample detection

The total number of fecal DNA samples located by the scat detector dog across all study sites was 1053, with 110 collected in MPR, 203 in RB, 223 in CBWS, 111 in CFRNP, 217 in FB, and 189 samples during several short-term surveys across various sites (BF, BNR, BC, BRF, GS, HVR, MH, MFR, STNP, SNP, and TSB) in Belize.

Microsatellite selection

Microsatellite DNA from fecal samples of wild jaguars, pumas and ocelots collected in MPR and RB amplified across all 20 initially selected 20 loci, confirming their cross-species utility. Nonetheless, six microsatellite loci (FCA032, FCA100, FCA124, FCA132, FCA212, FCA229) were dropped from the analysis due to low levels of genetic variation ($N_A \leq 2$ and $H_E \leq 0.5$ for FCA100, FCA132, and FCA212 in jaguars; $N_A = 1$ and $H_E = 0$ for FCA032, and FCA212 in pumas; $H_E \leq 0.5$ for FCA132 in ocelots), and overlapping allelic size ranges (FCA124 with FCA043, FCA229 with FCA275). Based on $P_{(ID)sib}$ estimates, loci FCA132 for jaguars, FCA032 and FCA212 for pumas, and FCA132 for ocelots were the least powerful primers for individual identification among those tested. We also assessed primer performance across 74 fecal samples collected in MPR and RB in terms of PCR amplification success and genotyping error rates. Loci FCA032 and FCA100 had generally low PCR amplification success rates. Loci FCA100

and FCA132 showed high rates of allelic dropout and loci FCA100 and FCA229 produced high rates of false alleles.

Species and individual identification

After screening, 14 highly polymorphic microsatellite loci were selected, and used for species and individual identification. Species-specific alleles and allelic ranges were described for all target species using fecal samples obtained from captive facilities and from the wild in Belize. Across all finalized loci, for ocelots 71.3% of the alleles described were species-specific followed by 68.8% for pumas, and 62.5% for jaguars. The most powerful loci for species assignment (100% of alleles were species-specific) identified were F124 and FCA126 for jaguars, F124, FCA096, and F98 for pumas and F124 and F85 for ocelots.

Species identification was confirmed by Bayesian assignment methods, which estimated the number of *K* clusters with an increase of likelihood values, peaking at $K = 3$. At $K = 3$, all individuals tested were successfully assigned to one species cluster with an average proportion of membership or *Q* of 0.997 ± 0.004 for jaguars, 0.995 ± 0.007 for pumas, and 0.997 ± 0.001 for ocelots (Fig. 2).

For individual identification, cumulative $P_{(ID)}$ and $P_{(ID)sib}$ values were calculated per species for the screening multiplex 1 (7 loci), which helped to assess the minimum number of loci required for individual identification. With the criterion of $P_{(ID)sib} < 0.010$, a minimum of 6 finalized loci for jaguars and 5 loci for pumas and ocelots were needed to identify individuals with high statistical confidence (Fig. 3). The cumulative $P_{(ID)}$ and $P_{(ID)sib}$ values for 7 loci of multiplex 1 were 3.8E-06 and 0.005 for jaguars, 4.1E-08 and

0.002 for pumas, and 2.1E-07 and 0.002 for ocelots. For all 14 loci, cumulative $P_{(ID)}$ and $P_{(ID)sib}$ estimates were 9.1E-11 and 5.3E-05 for jaguars, 2.1E-13 and 1.5E-05 for pumas, and 8.5E-14 and 6.8E-06 for ocelots (Table 1).

In summary, of 1053 scat samples collected across all study sites, 530 (50%) were successfully identified to the species and individual level. In total, we detected 162 individual felids (74 jaguars, 54 pumas and 32 ocelots). Jaguars were genetically "captured" 307 times, pumas 161 times, and ocelots 62 times (Table 2).

Characterization of cross-species microsatellites

For the finalized set of microsatellite loci, the number of alleles per locus ranged from 3-8 (5.14 ± 1.66) for jaguars, 3-15 (8.00 ± 3.50) for pumas and 5-10 (7.21 ± 1.53) for ocelots. Mean allelic richness was highest for pumas (6.91 ± 2.78), followed by ocelots (6.35 ± 1.42) and jaguars (4.59 ± 1.64). The average expected and observed heterozygosities for all loci were H_E = 0.60 ± 0.18 and H_0 = 0.57 ± 0.21 for jaguars, H_E = 0.65 ± 0.20 and H_0 = 0.61 ± 0.21 for pumas, H_E = 0.72 ± 0.12 and H_0 = 0.59 ± 0.18 for ocelots. Based on mean PIC values, another measure of polymorphism for marker loci, ocelots showed the highest diversity (0.69 ± 0.12) followed by pumas (0.62 ± 0.20) and jaguars (0.55 ± 0.17) (Table 3).

No loci deviated significantly from HWE for jaguars. Loci FCA043 ($P <$ 0.000), F85 (P = 0.008) and F98 (P = 0.011) revealed significant deviations from HWE for pumas after using sequential Bonferroni correction ($P \leq$ 0.015). After performing HWE tests for northern and southern pumas separately, a deviation from HWE was detected only for locus F85 at both locations, which most likely was caused by null

alleles. For ocelots, loci FCA391 ($P < 0.000$), FCA275 ($P < 0.000$), FCA096 ($P = 0.003$), and FCA741 ($P < 0.000$) deviated significantly from HWE. Linkage disequilibrium was detected in four cases (F124/F53, FCA096/FCA441 for jaguars; 124/FCA275, FCA096/F98 for pumas).

PCR amplification success across all samples was 62% ranging from 47 to 89% per locus. PCR amplification success for samples with finalized species ID differed significantly among species ($H = 17.53$, $P < 0.000$; Kruskal-Wallis rank sum test) with highest mean rates for jaguars (90%), followed by pumas (88%) and ocelots (73%). Pair-wise comparisons between species using post-hoc Wilcoxon rank-sum tests revealed that PCR amplification success rates for ocelots were significantly lower compared to jaguars ($W = 183$, $P < 0.000$, $r = -1.14$) and pumas ($W = 102$, $P = 0.001$, $r = -0.88$). Genotyping accuracy across all samples was 90%, which also differed significantly among species ($H = 29.51$, $P < 0.000$; Kruskal-Wallis rank-sum test) with highest mean rates for jaguars (93%) followed by ocelots (86%) and pumas (75%). Post-hoc Wilcoxon rank-sum tests revealed significant differences between all species pairs tested. Genotyping error was estimated by calculating mean allelic dropout and false allele rates for all samples collected (ADO, 15% ± 2.8; FA, 2% ± 1.0). Allelic dropout rates for samples with finalized species ID varied significantly among species ($H = 9.39$, $P = 0.009$; Kruskal-Wallis rank-sum test) with mean rates of 13% for jaguars, 12% for pumas and 17% for ocelots. Wilcoxon rank-sum tests showed that allelic dropout rates were significantly higher for ocelots than for jaguars ($W = 42$, $P = 0.012$, $r = -0.67$) and pumas ($W = 39$, $P = 0.007$, $r = -0.72$; Table 5 and Fig. 4). Feline species did not differ significantly ($H = 0.68$,

P = 0.711; Kruskal-Wallis rank-sum test) in mean rates for FA of jaguars (1% ± 1.0), pumas (1% ± 1.2), and ocelots (2% ± 1.3) (Table 4, 5 and Fig. 4).

Discussion

Selection and performance of cross-species microsatellite loci

Microsatellites represent a powerful type of neutral genetic marker commonly used to answer a variety of population genetic and ecological questions (e.g. Sunnucks 2000, Selkoe and Toonen 2006, Wang 2011, Allendorf et al. 2013). Due to many conservation challenges, species of concern often are studied in a comparative way and cross-species microsatellites have been identified for several different taxa (e.g. Barbara et al. 2007). We successfully cross-amplified 20 published microsatellite loci developed for the domestic cat in three Neotropical feline species. We conducted thorough screening of all loci tested and selected 14 polymorphic markers, which efficiently and reliably identified species and individuals from field-collected, fecal DNA samples of three feline species with highly variable DNA quality and quantity. Proper selection of genetic markers is crucial since it impacts all subsequent population genetic analyses (e.g. Taberlet and Luikart 1999). In order to increase the cost-efficiency of our study, we arranged the 14 loci in three multiplexes and configured multiplex 1 with 7 highly polymorphic loci, which were used for sample screening, and species and individual identification. Multiplexes 2 and 3 were developed to add additional loci for fine-scale genetic structure or parentage analyses not reported here and were only run for one sample per individual.

The genotyping performance of the microsatellite loci was tested based on several parameters including genetic variation, PCR amplification success, genotyping accuracy

and genotyping error rates. Results of the current study suggest that the cross-species microsatellite set optimized here is an efficient and powerful tool for conservation genetic studies of multiple Neotropical felids. The high variability indicates the potential usefulness for examining genetic structure of the target species. Although we conducted this study in tropical Belize and included all fecal samples detected by the scat detector dog (n = 1053), many of which were highly degraded, our analysis showed medium to high mean PCR amplification success rates using nuclear loci (61% ± 12.4) compared to other studies of tropical felids with scat detector dogs (jaguars, 26 - 41%: Michalski et al. 2011) or without detector dogs (pumas, 5 - 45%, Miotto et al. 2007). Other molecular scatology studies focusing on leopards or tigers in tropical environments (e.g. Bhagavatula and Singh 2006, Mondol et al. 2009a, Borthakur et al. 2011, Dutta et al. 2012) reported success rates similar to, or higher than, our study (> 60%). Mean genotyping error rates differ widely among noninvasive felid studies focusing on tigers (ADO = 0 - 3.5%, FA = 0, Mondol et al. 2009a; ADO = 0 - 64.8%, FA = 0 - 9.0%, Bhagavatula and Singh 2006), leopards (ADO = 0 - 45.4%, FA = 0 - 7.6%, Dutta et al. 2012; ADO = 0 - 7.4%, FA = 0 - 1.5%; Mondol et al. 2009b) and pumas (ADO = 10.6%, Miotto et al. 2007). Mean genotyping error rates for this study (ADO = 15% ± 2.6; FA = 2% ± 1.0) were relatively low considering that low-quality fecal samples detected by our scat detector dog were included to the analysis.

PCR amplification success and genotyping error rates for fecal DNA samples often vary greatly among studies depending on primer selection and design (e.g. Housley et al. 2006), and due to various other factors including fecal DNA quality and quantity, scat sample origin (e.g. species, environmental factors), and the choice of field and

laboratory techniques (e.g. Wasser et al. 1997, Piggott 2004, Soto-Calderon et al. 2009, Stenglein et al. 2010). The detection method used for scat sampling (humans and/or scat detector dogs) also affects success and error rates since studies using scat detector dogs have the potential to locate deteriorated scat samples not easily detectable by humans (e.g. Long et al. 2007b). We examined additional factors affecting success and error rates (e.g. storage and collection methods, scat sample condition, and the natural environment surrounding the sample), and provide further recommendations to increase reliability of field and laboratory techniques in another study (Wultsch et al. In prep.).

Species and individual identification

Recent studies of Neotropical felids suggested that techniques for individual identification of fecal samples need further improvement in field and laboratory techniques (e.g. Michalski et al. 2011). We addressed this by optimizing a powerful set of cross-species microsatellite loci, which can be used reliably for species and individual identification of co-occurring jaguars, pumas and ocelots based on field-collected fecal samples. In total, we collected 1053 scat samples from the wild and successfully genotyped 50.33% of the scats to the species and individual level. We used probability of identity estimates to assess statistical confidence for individual identification with $P_{(ID)sib} < 0.010$ as the deciding criterion (Mills et al. 2000a, Waits et al. 2001). The discriminatory power of the probability of identity estimates were high, which indicates a strong resolving power between individuals, also when related. These estimates indicate strong resolving power for this primer set in individual identification of multiple species even when close relatedness among study animals may be at issue.

To increase the efficiency of this study, we used the same nuclear markers to identify species by examining species-specific alleles and applying Bayesian clustering analysis to verify species assignment. The application of species-specific alleles for species identification of fecal samples collected during field samples has been used mainly used in wildlife forensics to differentiate between several big cats (e.g. Singh et al. 2004) or to detect hybridization between two wild feline species (bobcat and lynx; Schwartz et al. 2004). Here, we demonstrated the potential of this approach for individual-based monitoring of multiple species in the wild. Besides species identification, cross-species microsatellite loci have wide application and are especially useful when several closely related species at risk need to be managed and conserved simultaneously.

In conclusion, we strongly encourage the development of rigorous field and laboratory protocols especially for noninvasive genetic studies conducted in tropical environments hostile to DNA samples. The combination of the most informative markers and the assessment of locus-specific success and error rates both within and among three target species helped to optimize a set of polymorphic nuclear primers which improved our ability to efficiently and accurately identify genotypes (at the species and individual level) from often highly-degraded scat samples. Additionally, using a microsatellite marker set that amplifies across species represents an efficient and powerful way to study multiple co-occurring species on both the individual, and a population level, and simultaneously to evaluate the conservation status (demographics, genetic diversity, and connectivity) of potentially threatened species.

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Table 1 Summary of probability of identity estimates ($P_{(ID)}$ and $P_{(ID)sib}$; per locus and cumulative) obtained from fecal samples of jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*) collected across several study sites in Belize.

	Panthera onca (*n* = 73)				***Puma concolor*** (*n* = 54)				***Leopardus pardalis*** (*n* = 31)			
	Per locus		*Cumulative*		*Per locus*		*Cumulative*		*Per locus*		*Cumulative*	
Locus												
Multiplex 1	**$P_{(ID)}$**	**$P_{(ID)sib}$**	**$P_{(ID)}$**	**$P_{(ID)sib}$**	**$P_{(ID)}$**	**$P_{(ID)sib}$**	**$P_{(ID)}$**	**$P_{(ID)sib}$**	**$P_{(ID)}$**	**$P_{(ID)sib}$**	**$P_{(ID)}$**	**$P_{(ID)sib}$**
F124	0.171	0.470	0.171	0.470	0.032	0.325	0.032	0.325	0.077	0.382	0.077	0.382
FCA391	0.135	0.431	0.023	0.203	0.163	0.466	0.005	0.152	0.200	0.480	0.015	0.183
FCA043	0.218	0.496	0.005	0.100	0.084	0.383	4.4E-04	0.058	0.227	0.526	0.003	0.097
FCA275	0.200	0.477	0.001	0.048	0.122	0.431	5.4E-05	0.025	0.064	0.366	2.2E-04	0.035
FCA096	0.113	0.409	1.1E-04	0.020	0.081	0.388	4.4E-06	0.010	0.129	0.426	2.9E-05	0.015
FCA126	0.161	0.470	1.8E-05	0.009	0.177	0.484	7.7E-07	0.005	0.066	0.368	1.9E-06	0.006
FCA090	0.210	0.506	3.8E-06	0.005	0.053	0.351	4.1E-08	0.002	0.110	0.405	2.1E-07	0.002
Multiplex 2												
F85	0.295	0.548	1.1E-06	2.6E-03	0.038	0.333	1.5E-09	5.5E-04	0.095	0.393	2.0E-08	8.8E-04
F98	0.259	0.537	2.9E-07	1.4E-03	0.567	0.760	8.7E-10	4.2E-04	0.117	0.415	2.3E-09	3.7E-04
FCA741	0.900	0.949	2.6E-07	1.3E-03	0.123	0.417	1.1E-10	1.7E-04	0.084	0.380	1.9E-10	1.4E-04
FCA225	0.183	0.483	4.8E-08	6.3E-04	0.567	0.762	6.1E-11	1.3E-04	0.041	0.338	7.9E-12	4.7E-05
FCA008	0.320	0.592	1.5E-08	3.7E-04	0.470	0.702	2.9E-11	9.3E-05	0.117	0.418	9.3E-13	2.0E-05
Multiplex 3												
F53	0.077	0.385	1.2E-09	1.4E-04	0.044	0.343	1.3E-12	3.2E-05	0.181	0.482	1.7E-13	9.5E-06
FCA441	0.077	0.374	9.1E-11	5.3E-05	0.170	0.458	2.1E-13	1.5E-05	0.505	0.718	8.5E-14	6.8E-06
Total	0.237	0.509			0.192	0.472			0.144	0.436		

Table 2 Number (*n*) of individual jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*) and number of captures per species across study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary; CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize.

	Panthera onca		***Puma concolor***		***Leopardus pardalis***	
Study Sites	***N***	***No. Captures***	***n***	***No. Captures***	***n***	***No. Captures***
MPR	10	81	2	6	1	1
RB	9	30	16	36	11	28
CBWS	16	74	7	36	7	10
CFRNP	8	49	6	9	6	10
FB	8	24	11	55	3	8
Others	23	49	14	19	4	5
Total	74	307	56	161	32	62

Table 3 Characterization of 14 microsatellite loci for jaguars, pumas, and ocelots in Belize, including number of alleles (N_A), allelic richness (A_R) using the rarefaction method (Kalinowski 2005), observed (H_O) and expected heterozygosities (H_E), polymorphic information content (*PIC*), *P*-value for the HWE test (P_{HW}), and frequency of null alleles (F_{Null}).

	Panthera onca (*n* =73)							***Puma concolor*** (*n* = 54)						***Leopardus pardalis*** (*n* = 31)					
Locus	N_A	A_R	H_O	H_E	*PIC*	P_{HW}	F_{Null}	N_A	A_R	H_O	H_E	*PIC*	P_{HW}	F_{Null}	N_A	A_R	H_O	H_E	*PIC*	P_{HW}	F_{Null}
F124	6	5.69	0.60	0.62	0.58	0.35	0.03	15	13.23	0.81	0.87	0.85	0.41	0.03	8	6.04	0.72	0.77	0.75	0.60	0.04
FCA391	6	5.99	0.62	0.71	0.66	0.24	0.07	7	6.88	0.52	0.65	0.61	0.11	0.10	5	3.63	0.43	0.64	0.57	0.00	0.14
FCA043	3	3.00	0.52	0.61	0.54	0.39	0.07	9	8.17	0.74	0.78	0.74	0.00	0.01	5	4.13	0.61	0.56	0.53	0.81	-0.04
FCA275	3	3.00	0.56	0.65	0.58	0.26	0.07	7	6.61	0.69	0.70	0.67	0.72	0.01	8	6.36	0.46	0.80	0.78	0.00	0.21
FCA096	7	6.87	0.77	0.74	0.69	0.87	-0.02	7	7.00	0.75	0.77	0.74	0.19	-0.01	6	4.59	0.58	0.71	0.67	0.05	0.08
FCA126	6	5.86	0.76	0.65	0.62	0.38	-0.11	6	5.96	0.60	0.62	0.59	0.88	0.02	8	6.12	0.73	0.80	0.77	0.64	0.04
FCA090	5	5.00	0.58	0.59	0.55	0.57	-0.01	9	8.34	0.79	0.83	0.80	0.07	0.03	5	4.24	0.76	0.74	0.70	0.98	-0.01
F85	4	3.92	0.41	0.55	0.46	0.06	0.13	10	9.85	0.71	0.85	0.84	0.02	0.09	9	5.35	0.83	0.76	0.72	0.47	-0.05
F98	4	3.91	0.54	0.58	0.51	0.53	0.03	3	2.94	0.22	0.26	0.24	0.01	0.07	6	4.66	0.70	0.73	0.69	0.79	0.02
FCA741	3	3.00	0.05	0.05	0.05	1.00	-0.02	10	8.47	0.71	0.73	0.68	0.07	0.01	5	5.00	0.25	0.78	0.75	0.01	0.33
FCA225	7	6.84	0.58	0.61	0.58	0.41	0.05	4	3.86	0.25	0.26	0.24	0.69	0.00	10	7.41	0.87	0.84	0.83	0.56	-0.02
FCA008	4	3.88	0.41	0.47	0.43	0.21	0.07	5	4.35	0.31	0.33	0.31	0.17	0.05	7	4.95	0.70	0.72	0.68	0.82	0.02
F53	8	7.98	0.76	0.76	0.73	0.72	-0.01	14	14.00	0.80	0.84	0.82	0.39	0.03	5	4.95	0.62	0.63	0.59	0.53	0.00
FCA441	6	6.00	0.90	0.79	0.76	0.11	-0.07	6	5.63	0.67	0.67	0.61	0.93	0.00	3	2.36	0.38	0.32	0.28	1.00	-0.21

Significance tests for HWE were adjusted by applying sequential Bonferroni correction ($P < 0.015$).

Table 4 Summary of PCR amplification success, genotyping accuracy and genotyping error rates for 14 microsatellite loci for all samples and all jaguar, puma, and ocelot samples with finalized species ID detected across 5 study sites surveyed in Belize. *PCR*, % polymerase chain reaction amplification success; *GA*, % genotyping accuracy; *ADO*, % allelic dropout; *FA*, % false alleles.

	All Samples *n* = 1053*				***Panthera onca*** *n* = 307*				***Puma concolor*** *n* = 161*				***Leopardus pardalis*** *n* = 62*			
Primer ID ***Multiplex 1***	***PCR***	***GA***	***ADO***	***FA***	***PCR***	***GA***	***ADO***	***FA***	***PCR***	***GA***	***ADO***	***FA***	***PCR***	***GA***	***ADO***	***FA***
F124	56.22	89.49	13.45	0.69	93.08	92.21	10.51	0.42	89.23	68.42	9.30	1.28	81.67	85.71	16.67	1.02
FCA391	46.73	89.55	13.75	1.79	83.02	90.51	11.94	1.22	86.61	77.54	8.85	1.75	59.17	88.24	16.67	0.00
FCA043	54.24	89.28	14.65	1.53	83.04	93.97	8.20	0.75	76.43	69.01	15.85	0.51	81.90	88.37	13.11	2.33
FCA275	57.75	90.65	14.87	0.74	97.76	94.63	8.93	0.58	93.84	68.70	10.61	0.37	62.50	79.49	29.27	2.56
FCA096	52.99	86.86	18.02	2.00	89.18	88.69	17.72	1.33	86.82	60.00	13.27	1.73	77.50	88.10	21.88	3.57
FCA126	56.24	85.57	16.84	1.41	94.22	88.69	13.33	1.21	92.59	65.32	11.48	0.38	88.33	85.19	14.63	1.85
FCA090	59.76	88.96	15.21	1.32	92.75	90.27	14.58	0.63	91.14	70.97	10.19	0.39	82.50	89.80	13.11	2.04
Multiplex 2																
F85	77.81	89.63	14.18	2.94	86.75	94.66	8.16	2.29	87.03	77.04	12.04	1.97	74.55	84.93	15.79	2.74
F98	80.77	91.24	17.62	1.39	88.55	93.23	12.12	1.59	89.02	92.92	16.33	0.00	83.93	87.50	16.36	0.00
FCA741	66.93	93.78	15.67	0.50	88.18	96.60	26.92	0.00	79.10	73.23	9.71	1.50	15.74	87.50	0.00	0.00
FCA225	76.17	86.19	20.45	2.53	85.24	89.31	15.48	1.53	86.77	81.30	16.87	2.56	74.11	81.69	19.23	2.82
FCA008	88.39	92.81	12.19	0.51	94.34	95.68	7.01	0.36	95.29	84.33	14.77	0.58	87.72	85.42	16.05	1.04
Multiplex 3																
F53	73.47	88.41	13.46	3.77	83.95	91.72	8.51	4.14	81.60	70.93	10.00	3.23	72.00	82.61	23.26	2.90
FCA441	66.33	90.00	9.22	1.82	91.56	90.36	8.12	1.20	86.79	77.78	8.91	4.13	71.43	89.80	15.38	0.00
Mean	61.17	89.22	14.95	1.51	89.40	92.18	12.25	1.23	87.30	74.11	12.01	1.46	72.36	86.02	16.53	1.63
SD	*12.37*	*2.30*	*2.75*	*0.95*	*4.68*	*2.64*	*5.32*	*1.03*	*5.37*	*8.45*	*2.88*	*1.22*	*18.43*	*3.08*	*6.48*	*1.27*

All samples were analyzed with multiplex 1. Multiplexes 2 and 3 were only used for samples with finalized individual ID.

Table 5 PCR amplification success (*PCR*), genotyping accuracy (*GA*) and genotyping error (allelic dropout, *ADO*; false allele, *FA*) rates calculated across 14 microsatellite loci for Neotropical felids in Belize. Rates are compared across three feline species using Kruskal-Wallis rank-sum tests (KW) and pairwise Wilcoxon rank-sum tests* (*P*-value < 0.05).

Response Variable	Species	Observed Difference (%)	*P* - Value*
PCR	Jaguar - Puma	2.10	0.427
KW, $P < 0.000$	Jaguar - Ocelot	17.04	1.86E-05
	Puma - Ocelot	14.94	0.001
GA	Jaguar - Puma	18.07	3.91E-05
KW, $P < 0.000$	Jaguar - Ocelot	6.16	2.60E-05
	Puma - Ocelot	11.91	3.00E-04
ADO	Jaguar - Puma	0.24	0.541
KW, $P = 0.009$	Jaguar - Ocelot	4.28	0.012
	Puma - Ocelot	4.52	0.007
FA	Jaguar - Puma	0.23	0.696
KW, $P = 0.711$	Jaguar - Ocelot	0.40	0.420
	Puma - Ocelot	0.17	0.712

Fig. 1 Locations of short and long-term survey sites across Belize, Central America, including Mountain Pine Ridge Forest Reserve (MPR), Rio Bravo Conservation and Management Area (RB), Cockscomb Basin Wildlife Sanctuary (CBWS), Chiquibul Forest Reserve and National Park (CFRNP), Fireburn/Balam Na Nature Reserve (FB) and survey sites Big Falls (BF), Bladen Nature Reserve (BNR), Boden Creek Ecological Preserve (BC), Bull Run Farm (BRF), Golden Stream Corridor Preserve (GS), Hidden Valley Reserve (HVR), Machaca Hills (MH), Manatee Forest Reserve (MFR), Sarstoon-Temash National Park (STNP), Shipstern Nature Preserve (SNP), and Tiger Sandy Bay (TSB).

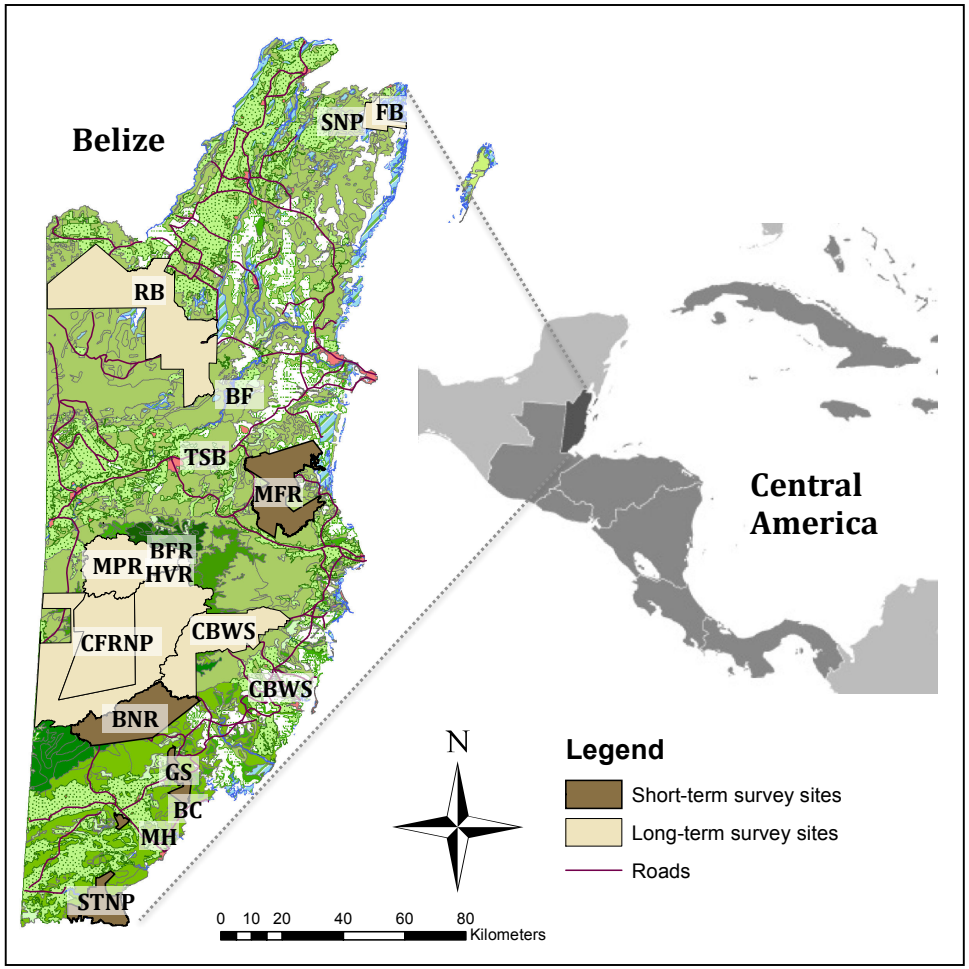


Fig. 2 Species assignment using Bayesian clustering with STRUCTURE, version 2.3 (Pritchard et al. 2000). Bar plot represents the assignment of individuals to Neotropical feline species (jaguar, puma and ocelot) in Belize. Each vertical bar represents one individual. Each genetic cluster (colored in a different grey shade) represents one species.

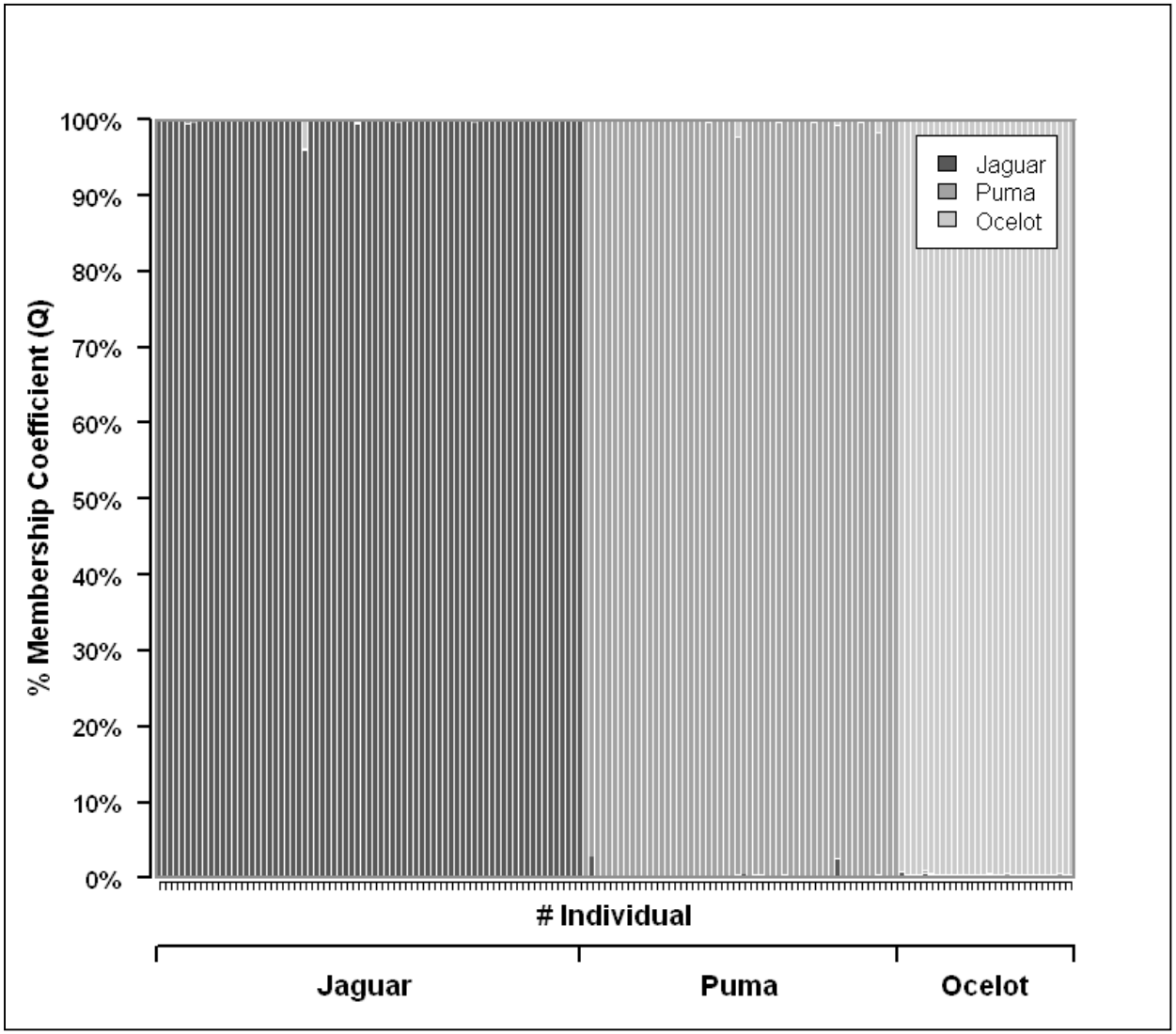


Fig. 3 Relationship between the probability of identity (cumulative) for unrelated individuals ($P_{(ID)}$) and siblings ($P_{(ID)sib}$) for loci within multiplex 1 (7 loci; used for screening, individual and species identification) in jaguars (n = 65), pumas (n = 54) and ocelots (n = 31). DNA was isolated from fecal samples collected across several study sites in Belize. $P_{(ID)sib} < 0.010$ was used as the criterion for individual identification.

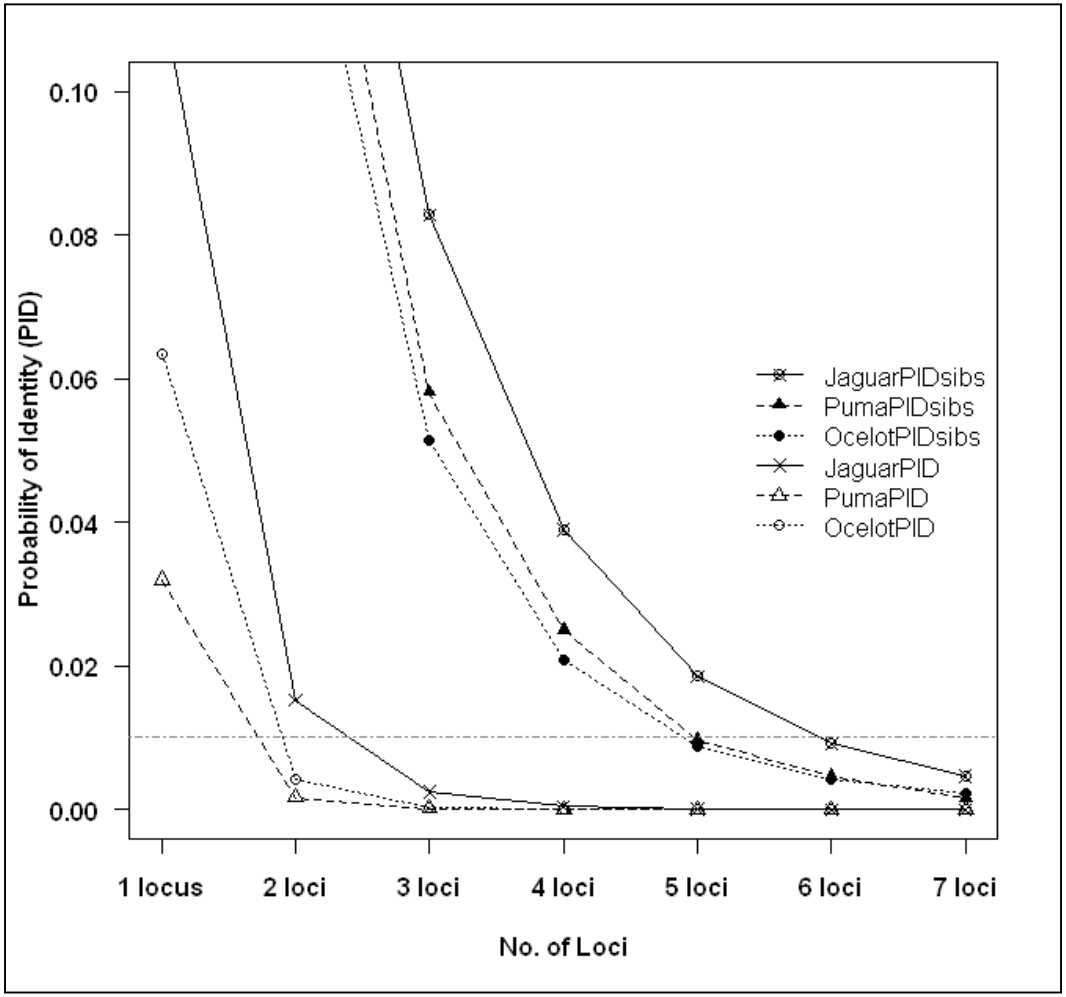
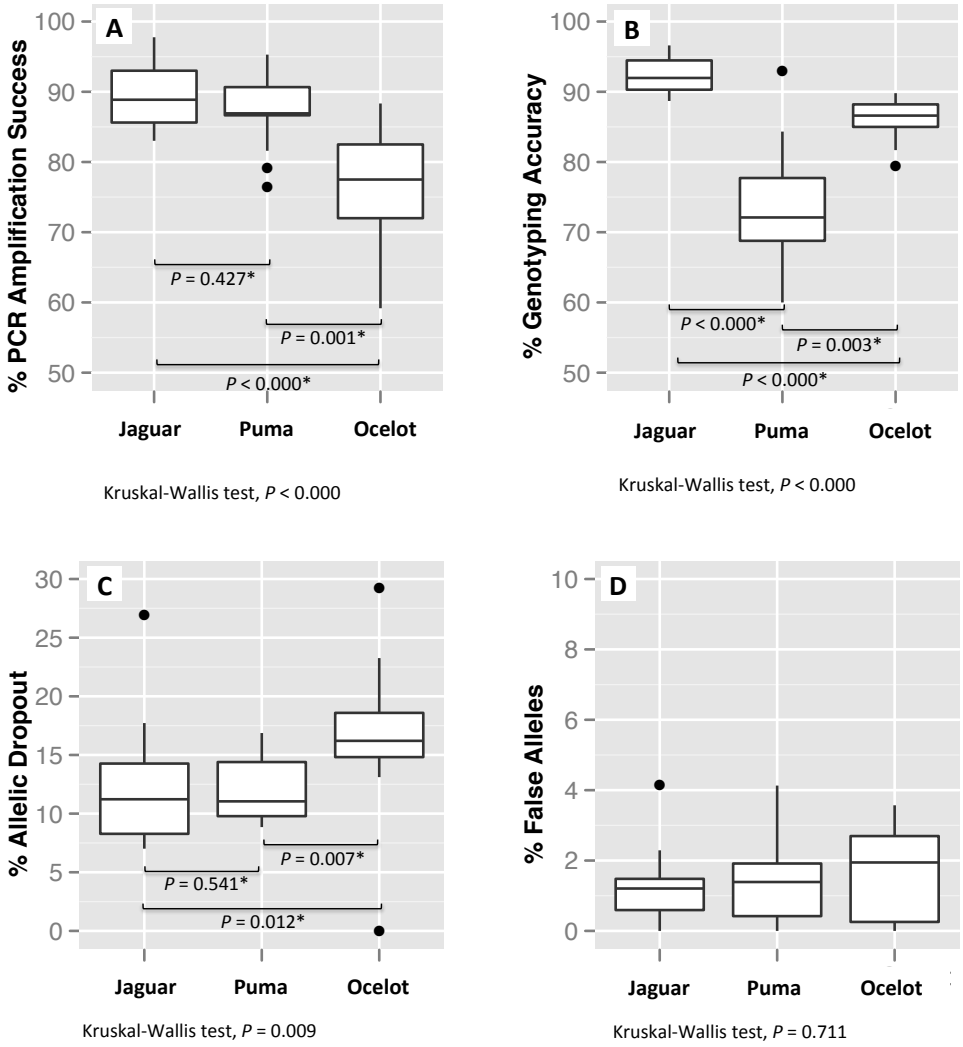


Fig. 4 PCR amplification success (A), genotyping accuracy (B), and genotyping error rates (C, allelic dropout; D, false alleles) for 14 microsatellite loci for all jaguar, puma, and ocelot scat samples located across 5 study sites surveyed in Belize. In the box-and whisker plot, the central value represents the median and the central box represents the value from the 25th to the 75th percentile. *Post-hoc Wilcoxon rank-sum tests were conducted for pairwise comparisons of success and error rates between species.



CHAPTER 3

Effects of storage methods and scat sample location on PCR amplification success, genotyping accuracy, and genotyping error rates using fecal samples for jaguars (*Panthera onca*) and co-occurring Neotropical felids in two tropical habitats in Belize, Central America

Abstract

Molecular scatology studies of elusive species such as wild felids have potential for obtaining large amounts of data for species conservation. However, methods must be optimized, especially when working in challenging tropical environments where rates of DNA degradation are elevated due to hot and humid weather conditions. To maximize PCR amplification success and genotyping accuracy rates, and to minimize genotyping error rates for fecal DNA samples of jaguars (*Panthera onca*) and co-occurring Neotropical felids collected in Belize, Central America, we evaluated the performance of two fecal DNA storage techniques (dimethyl sulfoxide saline solution/DET buffer, 95% EtOH) suitable for long-term preservation at remote tropical sites. Additionally, we tested fecal samples collected from four different scat locations (top, side, bottom, inside) and from two different tropical habitats (tropical broadleaf versus tropical pine forest). DET buffer was the superior fecal DNA preservation method, which increased PCR amplification success rates by 44% ($P = 0.006$) and increased genotyping accuracy by 17% ($P = 0.024$) compared to 95% EtOH-stored samples. PCR amplification success of fecal DNA collected at the more open pine-forest habitat differed significantly ($P = 0.048$) across locations on the scat with highest mean success rates obtained from the top (85% ± 6.5) followed by the side (79% ± 9.4), bottom (76% ± 11.9) and inside (69% ± 10.3) of scat samples. Scat samples collected at the more closed-canopy broadleaf site did not show any significant differences in amplification success calculated across locations on each scat. Based on these results, we recommend optimizing field-sampling methods by conducting a pilot study prior to any long-term and large-scale molecular scatology studies.

Key words: Panthera onca, *Puma concolor*, *Leopardus pardalis*, molecular scatology, genotyping error, fecal DNA storage, noninvasive genetic sampling, tropics

Introduction

Molecular scatology is a continually advancing non-invasive genetic monitoring approach in which individuals are genotyped using DNA from exfoliated intestinal epithelial cells found in their feces (scat) (Albaugh et al. 1992, Hoss et al. 1992, Constable et al. 1995, Kohn and Wayne 1997). This technique is especially suitable for elusive, rare or difficult-to-study species such as wild felids since scat is a prominent sign that the animals leave behind for intra-specific communication and territorial marking (e.g. Sunquist and Sunquist 2002a). Without ever capturing wild felids and by simply collecting and analyzing their scat samples, researchers can determine species, gender, and individual, and hence molecular scatology can be used for ecological, demographic, and population genetic monitoring (e.g. Kohn and Wayne 1997, Reed et al. 1997, Kohn et al. 1999, Ernest et al. 2000, Waits and Paetkau 2005, Kelly et al. 2012).

Although molecular scatology studies have been applied extensively in the Northern Hemisphere, there is a great need to standardize this noninvasive genetic sampling technique for wild felid studies in warmer and more humid climates such as the tropics, which have higher feline diversity than any other geographical zone (Macdonald and Loveridge 2010). Despite the potential power of this approach, several problems and limitations have been documented when applying noninvasive genetic sampling, including low quantity and quality of the host DNA due to enzymatic and bacteria-mediated DNA degradation, the presence of polymerase chain reaction (PCR) inhibitors (e.g. digestive enzymes, microorganisms, bile salts, bilirubin), and contamination with

non-target DNA (bacteria, diet, etc.) (Handt et al. 1994, Kohn and Wayne 1997, Frantzen et al. 1998, Taberlet et al. 1999, Murphy et al. 2003, Broquet et al. 2007). These factors may cause PCR failure or genotyping errors, leading to erroneous microsatellite genotypes (e.g. Taberlet et al. 1996). These genotyping errors may result in either allelic dropout (ADO) due to non-amplification of one allele in a heterozygous genotype, or it may result in false alleles (FA) caused by PCR slippage errors or contamination with non-target DNA (Gagneux et al. 1997, Taberlet et al. 1999, Broquet and Petit 2004).

In the tropics, fecal samples are exposed to high temperatures, elevated levels of humidity and UV-light, and a high diversity of microorganisms, which can accelerate rates of DNA degradation (Lindahl 1993, Farrell et al. 2000a, Piggott 2004, Murphy et al. 2007, Vynne et al. 2012). The extent of DNA degradation of fecal DNA prior to molecular analysis also is influenced by the target species itself and its diet (e.g. Murphy et al. 2003), scat sample condition (e.g. Piggott 2004, Murphy et al. 2007, Santini et al. 2007) and various field and laboratory techniques, including the choice of fecal DNA collection and storage methods (e.g. Wasser et al. 1997, Murphy et al. 2002, Piggott and Taylor 2003, Soto-Calderon et al. 2009, Stenglein et al. 2010).

Generally, DNA storage techniques aim to minimize DNA degradation prior to laboratory analysis. DNA degradation in feces is caused by enzymes (endogenous endo- and exo-nucleases), which cleave DNA strands. DNA storage techniques inactivate these enzymes by removing water through drying or drying agents (e.g. silica, ethanol), by eliminating cations through chelators (e.g. ethylenediaminetetraacetic acid) or by storing samples at low temperatures (Beja-Pereira et al. 2009). A wide variety of these storage methods has been applied for preservation of DNA in felid scats, including freezing (e.g.

Foran et al. 1997, Ernest et al. 2002, Leberg et al. 2004, Sugimoto et al. 2006), air drying (e.g. Farrell et al. 2000a, Novack et al. 2005, Weckel et al. 2006a), silica desiccation (e.g. Palomares et al. 2002, Russello et al. 2004, Haag et al. 2009, Janecka et al. 2011a), or liquid storage using buffer solutions (e.g. 20% dimethyl sulfoxide buffer, Vynne et al. 2012), or ethanol (EtOH) (e.g. Onorato et al. 2006, Mukherjee et al. 2007, Pandey et al. 2007, Mondol et al. 2009a, Rozhnov et al. 2009, Michalski et al. 2011, Dutta et al. 2012). A two-step storage method (24 h storage in EtOH followed by desiccation with silica) has been also applied in a few felid studies (Fernandes et al. 2008, Roques et al. 2011, Reddy et al. 2012b) (Table 1).

This abundance of different preservation techniques for noninvasive genetic sampling makes it challenging for new studies to select the most efficient storage method (e.g. Beja-Pereira et al. 2009), which choice depends on a variety of factors including the target species, its life history (e.g. carnivorous vs. herbivorous diet), environmental conditions, study logistics, DNA storage time and DNA type (mitochondrial vs. nuclear) (e.g. Frantzen et al. 1998, Piggott and Taylor 2003, Soto-Calderon et al. 2009). Comparative fecal DNA preservation studies comparing the effectiveness of different techniques for carnivores are rare. Out of 59 reviewed molecular scatology studies of wild felids from 1997 to present (Table 1), only two studies of tigers (Bhagavatula and Singh 2006, Reddy et al. 2012a) included an empirical evaluation of fecal DNA storage techniques. Generally, silica desiccation (30%) was the most common fecal DNA storage technique followed by EtOH (30%), freezing (14%), air-or sun drying (14%), the two-step method (5%), and buffer (2%). In tropical-subtropical zones, felid studies used a variety of preservation methods, including, for example, freezing (e.g. Miotto et al.

2007), silica desiccation (e.g. Reddy et al. 2011) and EtOH storage (e.g. Mukherjee et al. 2010, Tende et al. 2010).

Since fecal DNA is not uniformly distributed along the length of scat samples (Johnson et al 2005), it is also important to consider the sampling location on the scat. Direct contact of fecal samples with soil and its decomposers (e.g. bacteria, fungi) increases rates of DNA degradation (e.g. Nsubuga et al. 2004, Hajkova et al. 2006, Santini et al. 2007), and direct exposure to UV-light leads to DNA damage (Santini et al. 2007). Consequently, a wide variety of collection protocols for fecal DNA sampling has been used to maximize the success of noninvasive genetic studies of different species. Techniques for fecal DNA collection include collecting fecal material from the scat surface by swabbing (e.g. Frantz et al. 2003, Lampa et al. 2008), scraping (e.g. Nagata et al. 2005, Perez et al. 2006, Stenglein et al. 2010), washing (e.g. Palomares et al. 2002) or homogenizing fragment or entire scat samples prior to DNA extraction (e.g. Wasser et al. 1997, Frantzen et al. 1998). A few carnivore studies have empirically tested for differences in DNA quality using different sampling locations within a scat sample and uniformly recommended fecal DNA sampling from the outside/surface of the scat sample for higher PCR DNA amplification success (e.g. Piggott and Taylor 2003, Pires and Fernandes 2003, Stenglein et al. 2010). Out of the 59 wild felid molecular scatology studies reviewed (Table 1), only a small portion (17%) reported specific scat location collection protocols (e.g. Pires and Fernandes 2003, Nagata et al. 2005, Bidlack et al. 2007, Michalski et al. 2011, Vynne et al. 2012). An empirical evaluation of scat locations for felid DNA studies has not yet been conducted.

Molecular scatology studies of wild felids in tropical regions have been increasing in numbers, but PCR amplification success and genotyping error rates vary greatly particularly when nuclear primers are used for individual-based monitoring. We conducted a noninvasive genetic study of three co-occurring felids (jaguar, *Panthera onca*; puma, *Puma concolor*; ocelot, *Leopardus pardalis*) using a scat detector dog, a set of highly polymorphic microsatellite loci and fecal DNA samples collected across several study sites in Belize, Central America. Our main objective was to identify the most efficient protocol for fecal DNA collection and storage. Specifically, we aimed to examine the effects of: (1) two different liquid storage methods (dimethyl sulfoxide saline solution/DET buffer versus 95% ethanol), and (2) four sampling locations within the scat sample (top, side, bottom, inside) on PCR amplification success, genotyping accuracy and microsatellite genotyping error rates when using neotropical felid scats of varying quality collected across two study sites with differing environmental conditions in Belize. We emphasize the importance of optimizing field-sampling protocols to increase the efficiency and reliability of noninvasive genetic monitoring techniques.

Methods

Study Area

We conducted 2-3-month scat surveys at two initial study sites (Mountain Pine Ridge Forest Reserve – MPR and the Rio Bravo Conservation and Management Area – RB) from 2007- 2008 in Belize, Central America (Fig. 1). We collected scat samples from May to August 2007 at the MPR site and from January to April 2008 at the RB site. Both sites surveyed are part of the national system of Protected Areas in Belize and fall within

the forests of La Selva Maya (The Mayan Forest), a recognized biodiversity hotspot that forms part of the northern section of the Mesoamerican Biological Corridor. The MPR site, located in central-west Belize (16°57' N, 88°54' W), occupies ~ 430 km^2 of predominantly pine forest with some broadleaf moist forest interspersed. The canopy is sparse and dominant vegetation includes Caribbean pine (*Pinus caribaea*), Honduran pine (*Pinus oocarpa*), oaks (*Quercus spp.*), palmetto palm (*Acoelorrhaphe wrightii*) and tiger fern (*Dicranopteris pectinata*), growing on a granite massif with primarily acidic soils (Dubbin et al. 2006). Elevation ranges from 120 to 1,017 m and annual rainfall averages from 1,550 to 2,108 mm with a wet season between June and December. Average temperatures fluctuate between 17 and 29 °C, with January as the coolest month. During our field survey at MPR from May to August 2007, we recorded an average minimum temperature of 22.8 °C, an average maximum temperature of 30.7 °C, and a daily rainfall average of 9.7 mm.

The RB site, which is located in northwestern Belize (17°42' N, 88°54' W), is the largest protected area (934.3 km^2) within the country, and has a diversity of natural forest (broadleaf, pine, and mangrove forest), lowland savanna and marsh habitat (Bridgewater et al. 2002). Elevation ranges from 4 – 241 m and average annual rainfall ranges from 1,549 – 1,600 mm with a dry season between February and May. Average temperatures fluctuate between 26 and 32°C, with January as the coolest month. During our field survey at RB from January to April 2008, we recorded an average minimum temperature of 20.7 °C, an average maximum temperature of 25.7 °C, and a daily rainfall average of 2 mm.

Fecal Sample Detection, Collection and Storage

Fecal samples were detected using a professionally trained scat detector dog (PackLeader LLC, Gig Harbor, WA, USA). We conducted opportunistic searches within the study sites to find scat samples along roads, trails, game-trails, off-trail, and across various landscape features (e.g. ridge lines, forest edges, streams) and across different habitat types. The scat detector dog was trained to locate scat samples of all five native feline species (jaguars, pumas, ocelots, margays and jaguarundis). All scat samples located by the scat detector dog (independent of their age and appearance) were collected and genotyped.

For each fecal sample, ~ 0.5 mL fecal material was collected and stored at ambient temperature in two sterile 2 mL screw-top tubes filled with either dimethyl sulfoxide saline solution (20% DMSO, 0.25M EDTA, 100mM Tris, pH 7.5, and NaCl to saturation; Seutin et al. 1991) or 95% EtOH at 1: ≥ 4 volume scat-to-solution ratio. Vials were prefilled with storage liquids prior to fieldwork. Scat samples were collected using disposable gloves and wooden sampling sticks. After the placement of the fecal fragment in the storage vial, wooden sticks were further used to break scat fragments apart and immerse them fully into the storage liquid. Additionally, for each intact scat located, ~ 0.5 mL of fecal material was collected from 4 different locations (top, side, bottom, inside) of the scat. Scat vials were stored for up to eight months under room temperature until extraction.

Upon detection of each scat in the field, we categorized all samples based on overall appearance, color, moisture level, odor strength, and presence of mold. We used a hand-held Global Positioning System (GPS) unit to record the geographic location.

Rainfall (in mm) and temperature (minimum and maximum in °C) data were recorded on a daily basis.

Fecal DNA Extraction

Fecal DNA extractions were conducted in a separate room at the Laboratory for Ecological, Evolutionary and Conservation Genetics (LEECG) at the University of Idaho (Moscow, ID, USA), dedicated to noninvasive genetic studies to avoid contamination while working with low concentration DNA samples. The QIAamp DNA Stool Mini Kit protocol (Qiagen, Inc.) was used to extract DNA from all fecal samples. An extraction negative was added to each extraction run to control for contamination.

PCR amplification and microsatellite genotyping

Carnivore-specific mitochondrial cytochrome b primers (146 bp) (Farrell et al. 2000a) were used initially to verify the feline origin of the scat samples. The 15 µL PCR mix contained 3 µL $MgCl_2$, 1.5 µL buffer, 1.2 µL dNTPs, 6.32 µL H_2O, 0.41 µL Farrell forward primer, 0.41 µL Farrell reverse primer, 0.3 mg BSA, 0.07 µL Taq polymerase, and 1.8 µL DNA extract. The PCR reaction was performed starting with a 10 min denaturation step at 95 °C, followed by 45 cycles (92 °C for 30 s, 50 °C for 45 s, 72 °C for 40 s), and two concluding steps, 72 °C for 2 min and 4 °C for 30 min. Amplicon sequences were analyzed with the program SEQUENCHER 3.0 (Gene Codes Corporation, Inc., Ann Harbor, MI) and compared to reference sequences listed in GenBank (National Center for Biotechnology Information, 2009).

A set of ten highly polymorphic microsatellite primers (FCA032, FCA096, FCA100, FCA124, FCA126, FCA132, FCA212, FCA225, FCA229, FCA275), which were originally developed, based on the domestic cat (Menotti-Raymond and O'Brien 1995, Menotti-Raymond et al. 1999) were initially identified for the collection and storage method studies. Microsatellite amplifications were conducted in three multiplexes (multiplex 1, FCA032, FCA100, FCA124; multiplex 2, FCA126, FCA212, FCA229; multiplex 3, FCA096, FCA225, FCA132, FCA275) each containing 7 µL PCR mixture. Multiplexes 1 and 2 consisted of 3.5 µL 1 x concentrated Qiagen Master Mix (Qiagen, Inc.), 1.54 µL of primers (0.4µM for FCA032 F and R, 0.6µM for FCA100 F and R, 0.1µM for FCA124 F and R), 0.7 µL of 0.5 x concentrated Qiagen Q solution (Qiagen, Inc.), 0.26 µL H_2O and 1.0 µL DNA extract. Multiplex 3 consisted of 1 x concentrated Qiagen 3.5 µL Master Mix, 1.96 µL of primers (0.2µM for each F and R primer of multiplex 3), 0.7 µL of 0.5 x concentrated Qiagen Q solution, and 1.0 µL DNA extract. Microsatellite PCR amplifications were conducted using a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad Laboratories Inc.). For multiplexes 1 and 2, we started with an initial denaturation step of 15 min at 95 °C, followed by 13 cycles of 30 s at 94 °C for denaturation, 1.5 min at 62.4 °C with a decrease in annealing temperature of 0.3 °C in each cycle, and 1 min elongation at 72 °C, followed by 32 cycles of 30 s at 94 °C for denaturation, 1.5 min at 60 °C for annealing, and 1 min elongation at 72 °C, and 30 min at 60 °C for final elongation. For multiplex 3, we changed the annealing temperature to 57 °C. A PCR negative was added in each group of PCR reactions to control for contamination. We visualized PCR products using an ABI PRISM® 377 automated DNA

sequencer (Applied Biosystems™) and genotypes were identified using the software GENEMAPPER, version 3.7 (Applied Biosystems™).

Data analysis

PCR amplification success, genotyping accuracy and genotyping error rates

To calculate PCR amplification success, genotyping accuracy and genotyping error rates (allelic dropout - ADO, false alleles – FA), we selected the last 2 PCR runs for all loci across all scat samples. Rates were calculated across all loci and were assessed by calculating the percentage of successful PCRs across all samples tested and all samples with finalized species identification. Genotyping accuracy rates were estimated by calculating the percentage of successful PCRs, which match the finalized consensus genotype. Genotyping error was quantified by calculating the rate of allelic dropout and false alleles following the protocols of Broquet and Petit (2004).

Comparison of two fecal DNA storage methods across scat samples of varying quality

A subset of 30 scats (15 high/fresh- and 15 low/degraded-quality samples) was stored in two liquid storage techniques (DET buffer, 95% EtOH). The scat samples were categorized in low- and high-quality samples based on physical appearance, odor strength, color and presence/absence of mold prior to collection. PCR amplification success, genotyping accuracy and genotyping error rates were calculated across all loci and compared across fecal samples stored with two storage techniques. Statistical differences between groups were evaluated using non-parametric Wilcoxon signed-rank tests in program R, version 2.15 (R Development Core Team 2009).

Effect of four sampling locations on fecal DNA across two distinct tropical habitat types

For a subset of 40 intact scats, fecal DNA samples were collected from four different sample locations (top, bottom, side and inside) within each scat sample. The fecal DNA samples were collected at two sites (20 at MPR, 20 at RB), which differ in habitat type and environmental conditions. Statistical differences between groups were evaluated using non-parametric Kruskal-Wallis, Wilcoxon rank-sum and Wilcoxon signed-rank tests in program R, version 2.15 (R Development Core Team 2009).

Results

Comparison of two fecal DNA storage methods using scat samples of varying quality

The PCR amplification success rate for all scat samples tested (n = 30) was significantly higher (Wilcoxon signed-rank test, V = 53, P = 0.006, r = -0.87) when stored in DET buffer (62% ± 19.9) than when stored in 95% EtOH storage (43% ± 14.9). Likewise, genotyping accuracy rate for DET samples (88% ± 8.2) was significantly higher (Wilcoxon signed-rank test, V = 42, P = 0.024, r = -0.71) than for those stored in 95% EtOH-stored samples (75% ± 15.2). Genotyping error rates, (ADO, FA) were both higher when using EtOH (ADO, 32% ± 17.6; FA, 7% ± 7.5) than when using DET storage (ADO, 22% ± 28.9; FA, 3% ± 4.2), but variances were high and differences detected were not significant (Wilcoxon signed-rank test; ADO, V = 10, P = 0.155, r = -0.45; FA, V = 8, P = 0.183, r = -0.42) (Table 2A, Fig. 2, Fig. 3).

For high-quality fecal samples (n = 15), the mean PCR amplification success rate was significantly higher (Wilcoxon signed-rank test, V = 48, P = 0.041, r = -0.65) when stored in DET buffer (67% ± 22.3) then when stored in EtOH (50% ± 21.7). Genotyping accuracy was higher for fecal DNA stored in DET (88% ± 11.5) than in 95% EtOH (75%

± 18.5), but results were not significantly different (Wilcoxon signed-rank test, $V = 36$, $P = 0.124$, $r = -0.49$). ADO and FA were both higher when using EtOH (ADO, 37% ± 23.7; FA, 5% ± 6.1) than when using DET storage (ADO, 21% ± 31.2; FA, 3% ± 4.2), but differences were not significant (Wilcoxon signed-rank test; ADO, $V = 12$, $P = 0.25$, $r = -0.36$; FA, $V = 9$, $P = 0.447$, r = -0.24) (Table 2B, Fig. 2, Fig. 3).

For low-quality samples, the mean PCR amplification success rate was significantly higher (Wilcoxon signed-rank test, $V = 54$, $P = 0.004$, $r = -0.91$) when stored in DET buffer (57% ± 18.5) than when stored in EtOH (35% ± 10.2). Genotyping accuracy rate for DET-stored samples (88% ± 12.4) was higher than for 95% EtOH-stored samples (75% ± 18.8), but the difference was not significant (Wilcoxon signed-rank test, $V = 37$, $P = 0.097$, $r = -0.52$). ADO and FA were both higher when using EtOH (ADO, 26% ± 17.2; FA, 10% ± 10.9) than when using DET (ADO, 20% ± 18.8; FA, 3% ± 5.0), but the differences were not significant (Wilcoxon signed-rank test, ADO, $V = 9$, $P = 0.450$, $r = -0.24$; FA, $V = 6$, $P = 0.205$, $r = -0.40$) (Table 2C, Fig. 2, Fig. 3).

Effect of four sampling locations on fecal DNA amplification success across two distinct tropical habitat types

Microsatellite PCR amplification success rates were higher across all scat sampling locations (top, side, bottom, inside) for samples collected at the MPR site ($n = 20$) in comparison to the RB site ($n = 20$), but differences between sites were not significant (Table 3 and 4). The same trend was observed for genotyping accuracy rates across scat sampling locations between the sites. Genotyping accuracy rates for the bottom of the scat were significantly higher at MPR compared to RB samples (Wilcoxon rank-sum test,

$W = 77$, $P = 0.045$, $r = -0.45$) (Table 3 and 4). Allelic dropout rates were higher across all sample locations for RB compared to MPR. Allelic dropout rates for the bottom location of RB samples were more than twice than the MPR samples, and the difference was significant (Wilcoxon rank-sum test, $W = 13$, $P = 0.010$, $r = -0.58$) (Table 3 and 4). False allele rates were higher for all scat sampling locations from scats at the RB site, with an exception of the side location of scats. However, differences in false allele rates were only significant for top location of scats (Wilcoxon rank-sum test, $W = 19$, $P = 0.020$, $r = -0.52$) (Table 3 and 4).

For overall comparison of four different scat DNA sampling locations (top, side, bottom, inside) within study sites, Kruskal-Wallis rank-sum tests showed that PCR amplification success across sample locations from scats at the MPR site differed significantly ($H = 12.00$, $P = 0.007$), with highest success rates at the top (85% ± 6.5) followed by the side (79% ± 9.4), bottom (76% ± 11.9) and inside (69% ± 10.3) of scat samples. Pairwise comparisons using Wilcoxon signed-rank tests detected significant differences between the top and the side ($V = 51$, $P = 0.014$, $r = -0.55$), the top and the bottom ($V = 51$, $P = 0.019$, $r = -0.52$), the top and the inside ($V = 55$, $P = 0.006$, $r = -0.62$), the side and the inside ($V = 55$, $P = 0.002$, $r = -0.69$), and the bottom and the inside ($V = 49$, $P = 0.027$, $r = -0.49$). Genotyping accuracy rates also showed significant differences (Kruskal-Wallis rank-sum test, $H = 7.98$, $P = 0.046$) with highest accuracy rates for the bottom (90% ± 5.2) followed by the top (87% ± 8.7), inside (85% ± 7.8) and side (80% ± 10.0) location. Pairwise comparisons using Wilcoxon rank-sum tests detected significant differences between the top and the side ($V = 43$, $P = 0.018$, $r = -0.53$), the side and the bottom ($V = 0$, $P = 0.009$, $r = -0.58$), and the side and the inside (V

= 0, P = 0.009, r = -0.58). Allelic dropout (H = 6.17, P = 0.104) and false allele (H = 6.12, P = 0.106) rates were not significantly affected by the four different locations for sampling of a scat sample (Table 5, Fig. 4 and 5).

For RB samples, Kruskal-Wallis rank-sum tests showed that PCR amplification success (H = 2.64, P = 0.451), genotyping accuracy (H = 2.01, P = 0.571), allelic dropout (H = 3.13, P = 0.579) and false allele (H = 0.77, P = 0.856) rates were not significantly impacted by the four different sampling locations of a scat sample (Table 5, Fig. 4 and 5).

Discussion

Fecal DNA storage methods for scat samples of varying quality at remote tropical field sites

Despite the many advantages that noninvasive genetic sampling offers to wild felid studies, the quality and quantity of DNA obtained from scat samples is often low (e.g. Taberlet et al. 1999, Waits and Paetkau 2005), thus optimization of field and laboratory protocols is critical. This is particularly important when scats are collected in tropical environments where DNA degradation rates accelerate due to extreme weather conditions (e.g. Lindahl 1993, Nsubuga et al. 2004, Vynne et al. 2012). This study is the first to evaluate fecal DNA storage methods and collection protocols for wild Neotropical felids. Our results demonstrate the importance of selecting the most efficient and practical field sampling techniques prior to molecular analysis in the laboratory.

Fecal DNA sample preservation is a crucial component of every molecular scatology study. Yet, only a handful of molecular scatology studies have examined different preservation methods in tropical environments (e.g. Frantzen et al. 1998, Soto-

Calderon et al. 2009), and only two have focused on felids. For tigers (*Panthera tigris*) in India, PCR amplification success rates did not significantly differ between EtOH or silica preservation (Bhagavatula and Singh 2006). Reddy et al. (2012a) evaluated three storage methods (silica desiccation, EtOH, and the two-step storage method) for captive tigers and found that the two-step method yielded two to three times more DNA than storage with silica and EtOH. Comparative fecal DNA preservation studies of other carnivore species assessing PCR amplification success or genotyping error rates across different storage methods found significant differences between storage approaches and overall recommended DET buffer, EtOH and silica desiccant for carnivore studies (e.g. Wasser et al. 1997, Frantzen et al. 1998, Murphy et al. 2000, Murphy et al. 2002, Frantz et al. 2003, Piggott and Taylor 2003, Soto-Calderon et al. 2009, Bubb et al. 2011, Panasci et al. 2011).

Due to the remoteness of most field sites and limited access to cooling and freezing facilities, a long-term preservation method, which allows reliable storage of fecal DNA samples under room temperature for several months, is most practical. We found that the choice of a fecal DNA preservation method had a significant impact on PCR amplification success and genotyping accuracy rates and that DET buffer was the superior fecal DNA preservation technique compared to 95% EtOH (Table 2). PCR amplification success and genotyping accuracy rates were significantly higher by 44% and 17%, while genotyping error rates for allelic dropout and false alleles were lower for samples stored in DET buffer. PCR amplification success rates for low- and high-quality scat samples showed similar trends. PCR amplification success rates were higher for both low- and high-quality samples stored in DET buffer, but the impact was more

pronounced for low-quality samples, suggesting that the correct choice of a fecal DNA preservation method is even more crucial when low quality and degraded scat samples are prevalent.

Genotyping error rates overall also decreased by storing samples in DET buffer (Table 2) compared to EtOH preservation. Our findings were consistent with several other studies examining the same preservation techniques. Seutin et al. (1991) recommended the use of DET buffer to preserve DNA in avian tissue at ambient temperatures for extended times and suggested that DNA storage with 70% EtOH was less successful in comparison. Frantzen et al. (1998) found that DET buffer was the most effective technique for nuclear DNA preservation of baboon scats in a tropical environment. In contrast, Frantz et al. (2003) did not find differences in amplification success between DET buffer and 70% EtOH for fecal DNA samples of European badgers. Panasci et al. (2011) detected equal success for coyote fecal DNA storage in DET buffer and 95% EtOH when diet of coyotes was not considered. However, when effects of feeding habits were included, DET buffer preservation was most efficient method for animals with a plant-based diet and 95% EtOH preservation was better for obligate and facultative meat-eaters. This is contrary to our findings where DET buffer had significantly higher success rates than 95% EtOH for carnivorous felids. Differences in findings between studies can be explained by multiple factors, including the target species, DNA sample type and condition, storage time, environmental influences, and the choice of field and laboratory techniques (e.g. Beja-Pereira et al. 2009, Panasci et al. 2011).

Besides efficiency in producing reliable genotypes, storage time for fecal DNA samples is often a deciding factor when choosing a storage technique for field studies. Generally, concentrated EtOH (> 70%) reduces water content from the scat, which decreases DNA degradation caused by bacteria, where DET buffer uses high concentration of salts to inactivate the enzymes causing DNA degradation (Seutin et al. 1991, Kilpatrick 2002). Over time, EtOH does not efficiently preserve supporting components of tissues (e.g. proteins) and their DNA becomes acidified (Jackson et al. 2012). According to Kilpatrick (2002), DET buffer provided the best protection from DNA degradation of high molecular weight DNA in tissues stored under room temperatures for up to 2 years, whereas DNA yield using EtOH storage was relatively low. Soto-Calderon (2009) reported that microsatellite amplification success rates for tropical ungulate scats stored in EtOH significantly declined over time after three months of storage relative to success rates measured one week or one month after collection.

Non-invasive genetic studies at remote field sites need simple fecal DNA preservation techniques, which require minimum storage space, easy portability in the field and transportation by air (if needed). We used sterile 2 mL screw-top tubes and showed that preservation of small fecal fragments (~ 0.5 mL) is sufficient for microsatellite genotyping. Fecal DNA storage vials/containers of most other felid genetic studies using liquid storage techniques ranged from 10 – 50 mL (Table 1, e.g. Bhagavatula and Singh 2006, Michalski et al. 2011, Vynne et al. 2011b). Storage in 2 mL vials requires minimal space, which is important when a large number of scat samples is handled. Additionally, no further treatment is necessary after fecal samples are placed in vials with storage liquids. Generally, EtOH does not require any special precautions

while it is handled in the field, but sometimes leakage occurs when used with plastic vials. Thus, frequent checks of EtOH levels in storage vials plus the use of alcohol-resistant markers for labeling are recommended. EtOH is available at most field sites, but transportation by air is regulated since it is flammable and classified as dangerous goods/hazardous materials. The International Air Transport Association allows transportation of limited quantities (5 L by passenger aircraft) (e.g. Kilpatrick 2002). In contrast, DET buffer is a non-explosive substance, but it includes DMSO, which must be handled with care (with gloved hands preferably) and skin contact should be avoided (e.g. David 1972, Kilpatrick 2002). Seutin et al. (1991) described DET as a safe substance when handled with precautions. DET buffer must be prepared in a laboratory facility prior to fieldwork, but is not classified as dangerous goods/hazardous materials, thus no special regulations to transport DET buffer by air apply.

Alternative fecal DNA storage techniques such as freezing and silica desiccation were not considered for this study for several reasons. Freezing is one of the most conventional preservation techniques for DNA, but was not possible in our study since we had no access to reliable freezers. Preserving fecal DNA using silica desiccation, which is a common method, was not used since it was not considered practical for long-term storage of a large amount of fecal samples in our study. Preservation with silica often requires additional drying of moist (fresh or rain-soaked) fecal samples using an oven, air or sun prior to the actual preservation (Murphy et al. 2000). Further handling of fecal samples is time-consuming, increases the risk of contamination particularly when a large amount of samples is handled simultaneously, and requires designated areas for drying. In summary, we recommend the use of DET buffer to preserve fecal DNA

samples of Neotropical felids for long-term field studies, which require storage under room temperature and handling a large number of fecal samples.

Effect of four sampling locations within scat sample on fecal DNA

We found that specific sampling locations on or in a scat sample significantly affect PCR amplification success rates. Fecal DNA collected from the surface of the scat is usually last in contact with the intestinal lining and the first to desiccate, thus collection of fecal DNA from the surface is most promising, as suggested by former studies (Stenglein et al. 2010). Fecal DNA collected from the top of a scat may be negatively affected by direct UV-light and rainfall (e.g. Brinkman et al. 2010). The side location affected is also by these factors, but their impact may not be as direct as the top. Soil decomposers may have a greater impact on fecal DNA samples collected from the bottom and inside locations. Santini et al. (2007) suggested that direct contact with the soil keeps the fecal sample moist, which accelerates the invasion with decomposer organisms.

Scat samples collected at the more open, submontane MPR (pine forest) site, showed overall higher PCR amplification success and genotyping accuracy rates and lower genotyping error rates across all scat sample locations compared to samples collected at the lowland, closed canopy RB site. However, differences were only significant for genotyping accuracy and allelic dropout rates for the bottom location of scats, and for false allele rates of the top location of scats. Despite lower temperatures at the RB site and thicker canopy cover, false allele rates for fecal samples collected from the top of scats were significantly higher compared to MPR samples. Genotyping accuracy rates for the bottom location at MPR were higher and may be explained by the

high level of dryness for fecal samples detected at this site. Allelic dropout rates for the bottom location were also significantly higher for RB samples, which suggests that contact with moist soil/ground of broadleaf forest habitat at RB may increase the rate of DNA degradation by decomposers.

We found scat sampling location within scats had a significant impact on PCR amplification success rates for fecal DNA collected at the MPR site. Generally, we detected that PCR success rates were significantly higher when fecal DNA was collected from the outside (top, side, bottom) of the scat compared to the inside, similar to past studies (Stenglein et al. 2010). Success rates were highest for samples collected from the top of the scat, followed by the side, bottom and inside location of scats. This may be explained by the degree of desiccation, which varies across locations within freshly deposited samples (< 2 days), but potentially also across older scats depending on the habitat. Desiccation by air in more open and dry ecosystems naturally preserves fecal DNA (e.g. Murphy et al. 2007). Top of scats are most likely to dry first in open environments due to exposure to UV-light and wind, followed by the sides, bottom and inside of scats. PCR amplification success rates did not significantly differ for scat locations from RB samples, where scats overall had higher levels of moisture and were predominately detected in broadleaf forest habitat. In summary, we recommend thorough inspection of fecal samples upon detection in the field, to select the most promising location for the fecal DNA sample. In our study, the top and side locations, which were usually the driest, resulted in highest PCR amplification success rates, and thus can be considered the preferred sampling location for fecal DNA.

In conclusion, we recommend optimizing field-sampling methods used for molecular scatology studies prior to molecular analysis. A study investigating effects of various factors (scat sample condition, microhabitat, etc.) on PCR amplification success, genotyping accuracy and genotyping error rates is currently in progress, which will help explain our findings in great detail and provide more suggestions for future molecular scatology studies. Overall, we showed that selection of an appropriate fecal DNA preservation method and of a fecal DNA collection location protocol significantly increased the success of our molecular scatology study by 44%. We predict that our results will be consistent with other felids in similar tropical environments, and suggest that other researchers conduct a pilot study to verify performance of field sampling protocols chosen for a particular species and region.

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Table 1 Molecular scatology studies of wild felids across temperate, desert-steppe, tropical-subtropical and highland climate zones, including description of fecal DNA storage method (preservation type, scat amount, storage vial, fecal DNA sample location within scat sample). *EtOH, ethanol; NA, not available; RT, room temperature.*

Climate zone	Felid Species	Study Location	Fecal DNA Storage Method	Storage Temp.	Scat Amount	Storage Vial/Container	Scat Location	Genetic Analysis	Reference
Temperate	Iberian lynx	Spain	Stored with silica gel	NA	3-5 cm fragment	Paper envelope and moisture indicator	NA	mtDNA sequencing	Palomares et al. (2000)
	Iberian lynx	Portugal	Stored with silica beads separated with porous tissue	-20 °C	NA	NA	Tip	mtDNA sequencing	Pires and Fernandes (2003)
	Amur tiger	Russia	Stored with silica gel	RT	25 g (silica) and 1-2 g (scat)	50 ml	NA	mtDNA sequencing	Russello et al. (2002)
	Leopard cat, domestic cat	Japan	Stored in absolute EtOH	4 °C	NA	NA	NA	mtDNA sequencing	Kurose et al. (2003)
	Tiger, leopard	Russia	Stored frozen at -20 °C	-20 °C	1 x 2cm^2 fragment	NA	Scraped fecal surface (50 mg)	mtDNA sequencing	Nagata et al. (2004)
	Tiger, leopard	Russia	Stored frozen at -20 °C	-20 °C	NA	NA	NA	mtDNA sequencing	Sugimoto et al. (2006)
	Puma, bobcat	MI, US	Wrapped in tissue, stored in plastic bags in cool environment	-20 °C	NA	Plastic bags	Yes, not specified	mtDNA sequencing	Swanson and Rusz (2005)
	Iberian lynx, wildcat	Portugal	Stored with silica after soaking with EtOH upon collection	NA	NA	NA	NA	mtDNA sequencing	Fernandes et al. (2005)
	Tiger	Russia	Stored in 96% EtOH	NA	NA	NA	NA	mtDNA sequencing, microsatellites	Rozhnov et al. (2009)
Desert-steppe	Puma	CA, US	Stored frozen at -20 °C	-20 °C	NA	NA	NA	microsatellites	Ernest et al. (2000)
	Puma	CA, US	Stored frozen at -20 °C	-20 °C	NA	NA	NA	microsatellites	Ernest et al. (2002)
	Puma	CA & NV, US	Stored frozen at -20 °C	-20 °C	NA	NA	NA	microsatellites	Ernest et al. (2003)

	Puma	LA, US	Stored frozen	NA	NA	Plastic container	NA	mtDNA sequencing	Leberg et al. (2004)
	Puma	ID, US	Stored in absolute EtOH	-80 °C	NA	NA	NA	mtDNA sequencing	Onorato et al. (2006)
	Leopard	Israel	Stored in paper bags, dried at 60 °C if necessary	RT	NA	Paper bags	NA	mtDNA sequencing, microsatellites	Perez et al. (2006)
	Puma, bobcat	CA, US	Stored with clay desiccant bag	-80 °C	NA	Plastic or paper bags	Outside or end	mtDNA sequencing	Bidlack et al. (2007)
	Bobcat, mountain lion, domestic cat	CA, US	Stored with silica gel beads	NA	5 (silica): 1 (scat) ratio	NA	NA	mtDNA sequencing, microsatellites	Ruell and Crooks (2007)
	Cheetah, leopard	Algeria	Not specified	NA	NA	NA	NA	mtDNA sequencing, Microsatellites	Busby et al. (2009)
	Lion	Sudan	Stored in 99% EtOH	-50 °C	NA	NA	NA	mtDNA sequencing, microsatellites	Tende et al. (2010)
	Puma	TX, US	Not specified	NA	NA	NA	External portion (for extract.)	mtDNA sequencing, microsatellites	Gilad et al. (2011)
	Puma, bobcat	AZ, US	Stored with silica desiccant	-20 °C	NA	Paper bag, sealed plastic container	Scraped fecal surface in lab	mtDNA sequencing, microsatellites	Naidu et al. (2011)
Tropical-subtropical	Jaguar, puma, ocelot	Venezuela	Air-dried on sterile paper, bile powder stored in zip-lock bag in dark and dry location	NA	NA	Zip-lock bag	NA	mtDNA sequencing	Farrell et al. (2000a)
	Leopard	India	Preservation-free	NA	NA	NA	NA	mtDNA sequencing	Verma et al. (2003)
	Jaguar, puma, jaguarundi, ocelot, oncilla, Geoffroy's cat	Paraguay	Stored in WhirlPak collection bag	-20 °C	NA	WhirlPak bag	NA	mtDNA sequencing	Zuercher et al. (2003)
	Tiger	China	Dried over flame of hot coal, stored with silica	NA	NA	Plastic bag	NA	Southern blot analysis	Wan et al. (2003)

Jaguar, puma	Guatemala	Sun-dried on raised platform, stored in envelopes temporarily, dried 2-4 days at 48 °C	NA	NA	Envelope, water-resistant container (scat); polyurethane bottle (pile powder)	NA	mtDNA sequencing	Novack et al. (2005)
Jaguar	Belize	Air-dried, treated with pyrethrum-based insecticide (up to 6 months)	-20 °C	NA	Paper bags	NA	mtDNA sequencing	Weckel et al. (2006a)
Puma	Brazil	Preservation-free	-22 °C	NA	Sterile tubes	NA	mtDNA sequencing, microsatellites	Miotto et al. (2007)
Tiger	India	Stored in absolute EtOH	NA	NA	Sterile plastic vials (30 mL)	NA	mtDNA sequencing	Mukherjee et al. (2007)
Leopard	India	Stored in 70% EtOH	-20 °C	NA	NA	NA	mtDNA sequencing	Pandey et al. (2007)
Tiger	India	Stored in EtOH, silica	RT	4 (EtOH): 1 (scat)	50 ml screw cap tubes	NA	mtDNA sequencing, microsatellites	Bhagavatula and Singh (2006)
Jaguar, puma	Brazil	Stored with silica gel	-20 °C	4 g silica/feces ratio	15 mL	NA	mtDNA sequencing	Haag et al. (2009)
Leopard	India	Stored in 90% EtOH	RT	NA	NA	NA	mtDNA sequencing, microsatellites	Mondol et al. (2009b)
Tiger	India	Stored in 90% EtOH	RT	NA	NA	Outside	mtDNA sequencing, microsatellites	Mondol et al. (2009a)
Tiger	India	Stored in EtOH or with silica gel	NA	5 mL EtOH/g scat, 4 g silica/g scat	25 mL	NA	mtDNA sequencing	Sharma et al. (2009)
Jaguar, puma	Belize	Stored with silica gel	RT	NA	NA	External layer (for extract.)	mtDNA sequencing	Foster et al. (2010b)
Jaguar, puma	Belize	Stored with silica gel	RT	NA	NA	NA	mtDNA sequencing	Harmsen et al. (2010)
Jungle cat, leopard cat	India	Stored in 90-100% EtOH	NA	NA	NA	NA	mtDNA sequencing	Mukherjee et al. (2010)
Jungle cat, leopard cat,	India	Not specified	NA	NA	NA	NA	mtDNA sequencing	Mukherjee et al. (2010a)

	Asiatic wildcat, rusty-spotted cat, snow leopard								
	Tiger	India	Not specified	NA	NA	NA	NA	mtDNA sequencing	Pokorny et al. (2010)
	Tiger	India	Stored with silica gel	-20 °C	20 g scat	Plastic vial	NA	mtDNA sequencing, microsatellites	Borthakur et al. (2011)
	Jaguar, puma, ocelot, jaguarundi	Brazil	Stored in 96% EtOH	3 °C (field), -20 °C (lab)	6 g scat	50 mL	Outside	mtDNA sequencing, microsatellites	Michalski et al. (2011)
	Puma	Brazil	Preservation-free	-22 °C	NA	Sterile tubes	NA	mtDNA sequencing, microsatellites	Miotto et al. (2007)
	Tiger	India	Stored with silica	-20 °C	NA	Zip-lock bag	NA	mtDNA sequencing, microsatellites	Reddy et al. (2011)
	Jaguar, puma, ocelot, jaguarundi, margay	Brazil, Mexico	Stored in absolute EtOH, later with silica pellets	RT	NA	30 mL(EtOH), 100 mL(silica)	NA	RCP-PCR, mtDNA sequencing	Roques et al. (2011)
	Tiger	Nepal	Stored in EtOH or with silica gel	NA	5 mL EtOH/g scat, 4 g silica/g scat	25 mL	NA	mtDNA sequencing	Sharma et al. (2011)
	Jaguar, puma	Brazil	Stored in 20% DMSO buffer	Frozen	NA	vial (40 mL), buffer (25 mL)	NA	mtDNA sequencing	Vynne et al. (2012)
	Puma, oncilla, domestic cat	Brazil	Stored with silica beads or 94-100% EtOH	-20 °C	NA	50 mL, 15 mL	Outside	mtDNA sequencing	Chaves et al. (2012)
	Leopard	India	Stored in absolute EtOH	NA	NA	NA	NA	mtDNA sequencing, microsatellites	Dutta et al. (2012)
	Tiger	India	Preservation-free	-20 °C	NA	Zip-lock bag	NA	qPCR, microsatellites	Reddy et al. (2012b)
	Tiger	India	Stored w. silica beads or two-step method (24 h EtOH storage, followed by silica)	-20 °C	NA	Zip-lock bag	NA	mtDNA sequencing, microsatellites	Reddy et al. (2012a)

Highland	Andean cat, Pampas cat, domestic cat, Geoffroy's cat	Peru, Argentina	Not specified	NA	NA	NA	NA	ribosomal DNA	Cossios and Anger (2006)
	Snow leopard	China, India, Mongolia	Stored with silica desiccant, separated with Kimwipe tissue	NA	NA	Vial(15 mL, silica(12 mL)	NA	mtDNA sequencing, microsatellites	Janecka et al. (2008a)
	Snow leopard	China, Kyrgyzstan	Stored in 90% EtOH	NA	4 mL (EtOH): 1 mL (scat)	Transport tubes	NA	mtDNA sequencing, microsatellites	McCarthy et al. (2008)
	Snow leopard	Pakistan	Stored with silica desiccant, separated with Kimwipe tissue	NA	NA	15mL	NA	mtDNA sequencing	Anwar et al. (2011)
	Snow leopard	Mongolia	Stored with silica desiccant	NA	1 cm^2 of each scat	15mL	NA	mtDNA sequencing, microsatellites	Janecka et al. (2011a)
	Snow leopard	Nepal	Stored with silica	RT	NA	NA	NA	mtDNA sequencing, microsatellites	Karmacharya et al. (2011)
	Snow leopard	Mongolia	Stored with silica gel (~6 ml)	NA	NA	10 mL	NA	mtDNA sequencing, microsatellites	Shezad et al. (2012)
	Snow leopard	Nepal	Stored in 70% EtOH or sun-dried	NA	NA	NA	NA	mtDNA sequencing, microsatellites	Wegge et al. (2012)

Table 2 PCR amplification success (PCR), genotyping accuracy (GA) and genotyping error (ADO, FA) rates calculated across 10 microsatellite loci for Neotropical felids. Rates were calculated across: (A) all, (B) high/fresh-quality, and (C) low/degraded-quality scat samples collected at the Mountain Pine Ridge Forest Reserve (MPR) in Belize, Central America.

(A) All Samples (*n* = 30)

Locus	PCR (%)		GA (%)		ADO (%)		FA (%)	
	DET	ETOH	DET	ETOH	DET	ETOH	DET	ETOH
FCA032	35.96	35.16	72.41	83.33	23.53	17.65	13.79	6.67
FCA096	71.91	32.97	91.53	72.41	8.57	30.00	3.39	6.90
FCA100	19.10	14.29	100.00	100.00	0.00	0.00	0.00	0.00
FCA124	55.06	57.14	86.96	69.39	12.50	37.84	2.17	2.04
FCA126	76.40	58.24	81.54	66.67	23.53	53.57	6.15	3.92
FCA132	72.53	49.44	85.00	93.02	100.00	25.00	0.00	2.33
FCA212	73.63	49.44	95.24	77.27	9.09	41.67	0.00	0.00
FCA225	61.54	30.34	93.88	76.92	8.00	21.43	2.04	11.54
FCA229	75.82	38.20	80.70	47.06	30.30	60.00	1.75	17.65
FCA275	78.02	60.67	91.80	62.75	5.88	27.59	1.64	21.57
Mean	62.00	42.59	87.91	74.88	22.14	31.47	3.09	7.26

(B) High Quality Samples (*n* = 15)

Locus	PCR (%)		GA (%)		ADO (%)		FA (%)	
	DET	ETOH	DET	ETOH	DET	ETOH	DET	ETOH
FCA032	35.56	31.11	64.29	76.92	30.00	18.18	14.29	7.69
FCA096	77.78	31.11	87.50	92.31	17.65	11.11	3.13	0.00
FCA100	22.22	15.56	100.00	100.00	NA	NA	0.00	0.00
FCA124	53.33	75.56	95.83	81.25	0.00	25.00	4.17	3.13
FCA126	86.67	66.67	94.44	56.67	5.00	64.71	2.78	6.67
FCA132	75.56	66.67	71.88	93.10	100.00	25.00	0.00	0.00
FCA212	80.00	60.00	97.22	66.67	4.76	50.00	0.00	0.00

FCA225	75.56	35.56	93.33	81.25	5.56	22.22	3.33	6.25
FCA229	84.44	42.22	85.29	42.11	19.05	81.82	2.94	10.53
FCA275	80.00	77.78	88.24	59.38	4.55	35.00	2.94	18.75
Mean	67.11	50.22	87.80	74.96	20.73	37.00	3.36	5.30

(C) Low Quality Samples (*n* = 15)

Locus	**PCR** (%)		**GA** (%)		**ADO** (%)		**FA** (%)	
	DET	**ETOH**	**DET**	**ETOH**	**DET**	**ETOH**	**DET**	**ETOH**
FCA032	36.36	39.13	80.00	88.24	14.29	16.67	13.33	5.88
FCA096	65.91	34.78	96.30	56.25	0.00	45.45	3.70	12.50
FCA100	15.91	13.04	100.00	100.00	0.00	0.00	0.00	0.00
FCA124	56.82	39.13	77.27	47.06	22.73	52.94	0.00	0.00
FCA126	65.91	50.00	65.52	80.95	50.00	36.36	10.34	0.00
FCA132	69.57	31.82	100.00	92.86	NA	NA	0.00	7.14
FCA212	67.39	38.64	92.59	94.12	16.67	16.67	0.00	0.00
FCA225	47.83	25.00	94.74	70.00	14.29	20.00	0.00	20.00
FCA229	67.39	34.09	73.91	53.33	50.00	33.33	0.00	26.67
FCA275	76.09	43.18	96.30	68.42	8.33	11.11	0.00	26.32
Mean	56.92	34.88	87.66	75.12	19.59	25.84	2.74	9.85

Table 3 PCR amplification success (PCR), genotyping accuracy (GA) and genotyping error (ADO, FA) rates calculated across 10 microsatellite loci for fecal DNA samples of Neotropical felids collected from four locations (1 top, 2 side, 3 bottom, 4 inside) within scats at two sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area) in Belize, Central America.

MPR Fecal Samples (*n* = 20)

	PCR				GA				ADO				FA			
Locus	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
FCA032	83.33	74.29	79.49	72.50	100.00	84.62	87.10	89.66	0.00	23.08	13.33	13.33	0.00	3.85	6.45	3.45
FCA096	83.33	77.14	71.79	65.00	78.57	85.19	92.86	92.31	15.38	7.69	15.38	9.09	0.00	11.11	0.00	3.85
FCA100	75.00	60.00	56.41	50.00	72.00	65.00	85.00	73.68	21.43	30.00	7.69	20.00	16.00	20.00	10.00	15.79
FCA124	88.89	88.57	89.74	72.50	87.10	77.42	84.85	86.21	11.54	17.86	14.29	12.50	3.23	6.45	3.03	3.45
FCA126	83.33	82.86	87.18	75.00	82.76	79.31	81.25	80.00	29.41	37.50	27.78	31.25	0.00	0.00	3.13	3.33
FCA132	83.33	85.71	74.36	75.00	100.00	100.00	100.00	100.00	NA	NA	NA	NA	0.00	0.00	0.00	0.00
FCA212	91.67	91.43	82.05	82.50	87.50	77.42	90.63	84.38	15.79	31.58	14.29	25.00	3.13	3.23	0.00	0.00
FCA225	75.00	71.43	56.41	57.50	88.46	79.17	90.91	81.82	17.65	17.65	6.67	17.65	0.00	8.33	4.55	4.55
FCA229	88.89	74.29	74.36	62.50	83.33	65.38	89.29	76.00	15.00	17.65	5.26	21.05	6.67	19.23	7.14	8.00
FCA275	94.44	82.86	86.84	80.49	90.63	82.76	90.63	87.50	14.29	11.11	9.09	13.64	0.00	10.34	3.13	3.13
Mean	84.72	78.86	75.86	69.30	87.03	79.63	89.25	85.15	15.61	21.57	12.64	18.17	2.90	8.25	3.74	4.55

RB Fecal Samples (*n* = 20)

	PCR				GA				ADO				FA			
Locus	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
FCA032	70.00	87.50	73.68	75.00	82.14	76.47	88.89	83.33	26.67	43.75	36.36	28.57	3.57	2.94	0.00	0.00
FCA096	60.00	62.50	67.57	67.50	77.27	77.27	76.00	69.23	20.00	18.75	29.41	21.05	9.09	4.55	4.17	15.38
FCA100	67.50	80.00	66.67	80.00	76.00	70.00	78.26	70.00	14.29	16.67	25.00	24.00	4.00	10.00	8.33	0.00
FCA124	92.50	82.50	84.62	87.50	81.08	78.79	71.88	85.71	11.76	13.33	25.93	6.67	2.70	9.09	10.00	8.57
FCA126	87.50	87.50	87.50	90.00	70.59	71.43	60.00	69.44	19.35	3.45	27.59	10.00	5.88	22.86	14.29	16.67
FCA132	65.00	70.00	64.10	67.50	92.31	77.78	91.67	88.46	0.00	55.56	40.00	28.57	7.69	3.70	0.00	3.85
FCA212	70.00	70.00	50.00	62.50	92.59	88.46	95.00	91.67	4.76	14.29	5.88	5.00	3.70	0.00	0.00	4.17
FCA225	35.00	37.50	33.33	42.50	64.29	61.54	81.82	76.92	66.67	71.43	50.00	42.86	7.14	0.00	0.00	0.00
FCA229	77.50	82.50	67.50	75.00	80.65	75.00	62.96	72.41	10.71	17.86	26.09	16.00	6.45	9.38	3.70	10.34
FCA275	70.00	75.00	64.10	80.00	71.43	68.97	84.00	84.38	41.18	47.37	20.00	15.00	3.57	0.00	4.00	6.25
Mean	69.50	73.50	65.91	72.75	78.83	74.57	79.05	79.16	21.54	30.24	28.63	19.77	5.379	6.25	4.45	6.52

Table 4 PCR amplification success (PCR), genotyping accuracy (GA) and genotyping error (allelic dropout, ADO; false allele, FA) rates calculated across 10 microsatellite loci for Neotropical felids in Belize, Central America. Rates are compared across four scat locations (1 top, 2 side, 3 bottom, 4 inside) between two sites, the Mountain Pine Ridge Forest Reserve (MPR, $n = 20$) and Rio Bravo Conservation Management Area (RB, $n = 20$) using pairwise Wilcoxon rank sum tests (P-value < 0.05).

Response Variable	Site/Scat Location	Observed Difference (%)	*P* - Value
PCR	MPR/Top - RB/Top	15.22	0.063
	MPR/Side - RB/Side	5.36	0.384
	MPR/Bottom - RB/Bottom	9.95	0.121
	MPR/Inside - RB/Inside	3.45	0.447
GA	MPR/Top - RB/Top	8.20	0.121
	MPR/Side - RB/Side	5.06	0.121
	MPR/Bottom - RB/Bottom	10.20	0.045
	MPR/Inside - RB/Inside	5.99	0.151
ADO	MPR/Top - RB/Top	5.93	0.902
	MPR/Side - RB/Side	8.67	0.624
	MPR/Bottom - RB/Bottom	15.99	0.010
	MPR/Inside - RB/Inside	1.60	0.870
FA	MPR/Top - RB/Top	2.48	0.020
	MPR/Side - RB/Side	2.00	0.470
	MPR/Bottom - RB/Bottom	0.71	0.877
	MPR/Inside - RB/Inside	1.97	0.402

Table 5 PCR amplification success (PCR), genotyping accuracy (GA) and genotyping error (allelic dropout, ADO; false allele, FA) rates calculated across 10 microsatellite loci for Neotropical felids in Belize, Central America. Rates are compared across four scat locations (1 top, 2 side, 3 bottom, 4 inside) at two different sites, the Mountain Pine Ridge Forest Reserve (MPR, n = 20) and Rio Bravo Conservation Management Area (RB, n = 20) using Kruskal-Wallis rank-sum tests (KW) and pairwise Wilcoxon signed-rank tests* (P-value < 0.05).

MPR Site	Scat Locations	Observed Difference (%)	*P*-Value*	RB Site	Scat Locations	Observed Difference (%)	*P*-Value*
PCR	Top - Side	5.60	0.014	**PCR**	Top - Side	4.30	0.105
KW, P = 0.007	Top - Bottom	7.90	0.019	KW, P = 0.451	Top - Bottom	3.60	0.155
	Top - Inside	14.50	0.006		Top - Inside	2.70	0.166
	Side - Bottom	2.30	0.375		Side - Bottom	7.90	0.033
	Side - Inside	8.90	0.002		Side - Inside	1.60	0.765
	Bottom - Inside	6.60	0.027		Bottom - Inside	6.30	0.004
GA	Top - Side	8.80	0.018	**GA**	Top - Side	5.85	0.013
KW, P = 0.046	Top - Bottom	5.20	0.234	KW, P = 0.057	Top - Bottom	0.65	0.922
	Top - Inside	1.80	0.193		Top - Inside	0.20	1.000
	Side - Bottom	14.00	0.009		Side - Bottom	6.50	0.232
	Side - Inside	7.00	0.009		Side - Inside	5.65	0.124
	Bottom - Inside	7.00	0.076		Bottom - Inside	0.85	1.000
ADO	Top - Side	5.83	0.107	**ADO**	Top - Side	19.00	0.064
KW, P = 0.104	Top - Bottom	6.22	0.294	KW, P = 0.372	Top - Bottom	16.90	0.322
	Top - Inside	1.94	0.294		Top - Inside	22.90	1.000
	Side - Bottom	12.06	0.020		Side - Bottom	2.10	0.846
	Side - Inside	3.89	0.107		Side - Inside	3.90	0.084
	Bottom - Inside	8.17	0.080		Bottom - Inside	6.00	0.002
FA	Top - Side	12.35	0.014	**FA**	Top - Side	0.35	1.000
KW, P = 0.106	Top - Bottom	4.20	0.439	KW, P = 0.856	Top - Bottom	3.05	0.770
	Top - Inside	7.05	0.050		Top - Inside	1.40	0.625
	Side - Bottom	8.15	0.024		Side - Bottom	2.70	0.183
	Side - Inside	5.30	0.033		Side - Inside	1.75	0.813
	Bottom - Inside	2.85	0.208		Bottom - Inside	4.45	0.183

Fig. 1 Location of two study sites: the Mountain Pine Ridge Forest Reserve (MPR), and the Rio Bravo Conservation and Management Area (RB) in Belize, Central America.

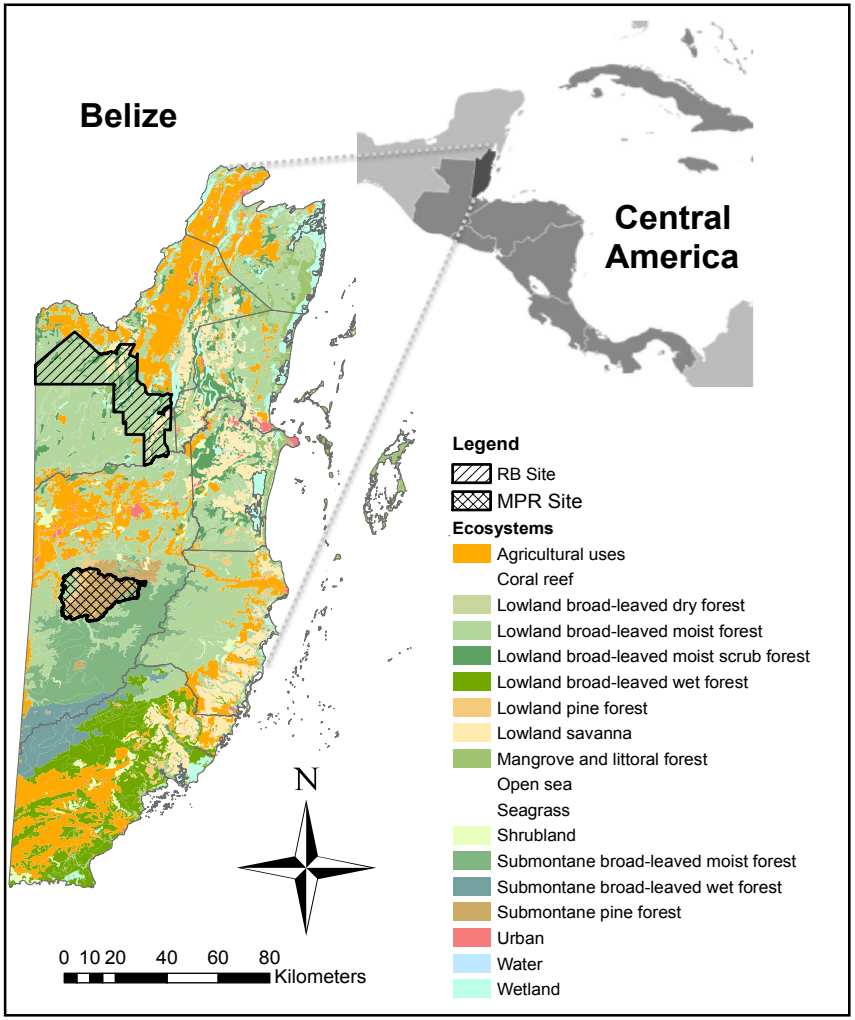


Fig. 2 PCR amplification success (PCR), and genotyping accuracy (GA) rates calculated across 10 microsatellite loci for Neotropical felids in Belize, Central America. Rates were assessed across all samples (n = 30), high/fresh-quality ones (n = 15), and low/degraded-quality scat samples (n = 15) collected at the Mountain Pine Ridge Forest Reserve in Belize, Central America. Statistical significance of differences was examined using pairwise Wilcoxon signed-rank tests (P - value < 0.05). In the box-and whisker plot, the central value represents the median and the central box represents the value from the 25th to the 75th percentile.

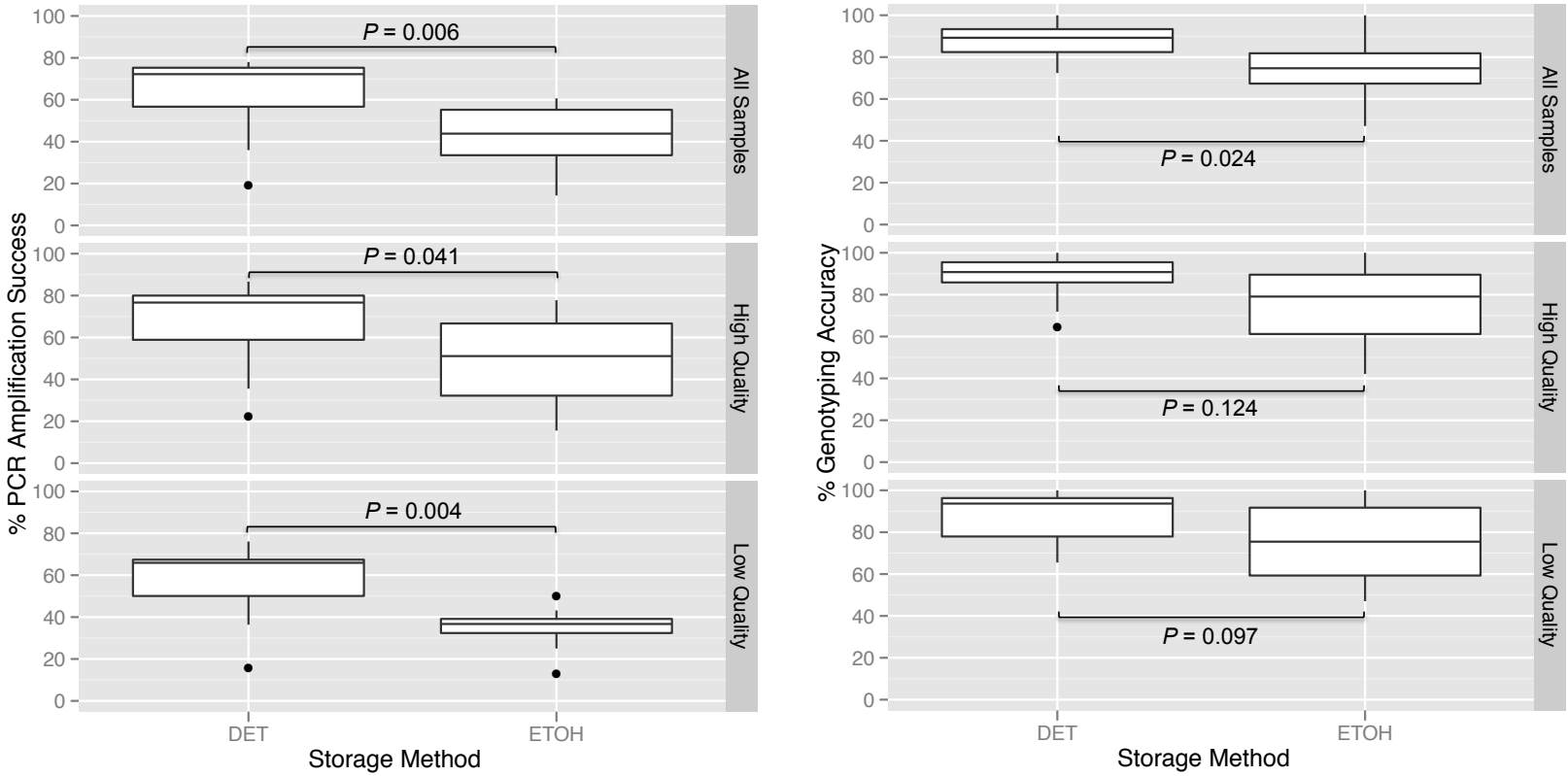


Fig. 3 Allelic dropout (ADO), and false allele (FA) rates calculated across 10 microsatellite loci for Neotropical felids in Belize, Central America. Rates were assessed across all samples (n = 30), high/fresh-quality ones (n = 15), and low/degraded-quality scat samples (n = 15) collected at the Mountain Pine Ridge Forest Reserve in Belize, Central America. Statistical significance of differences was examined using pairwise Wilcoxon signed-rank tests (P - value < 0.05). In the box-and whisker plot, the central value represents the median and the central box represents the value from the 25th to the 75th percentile.

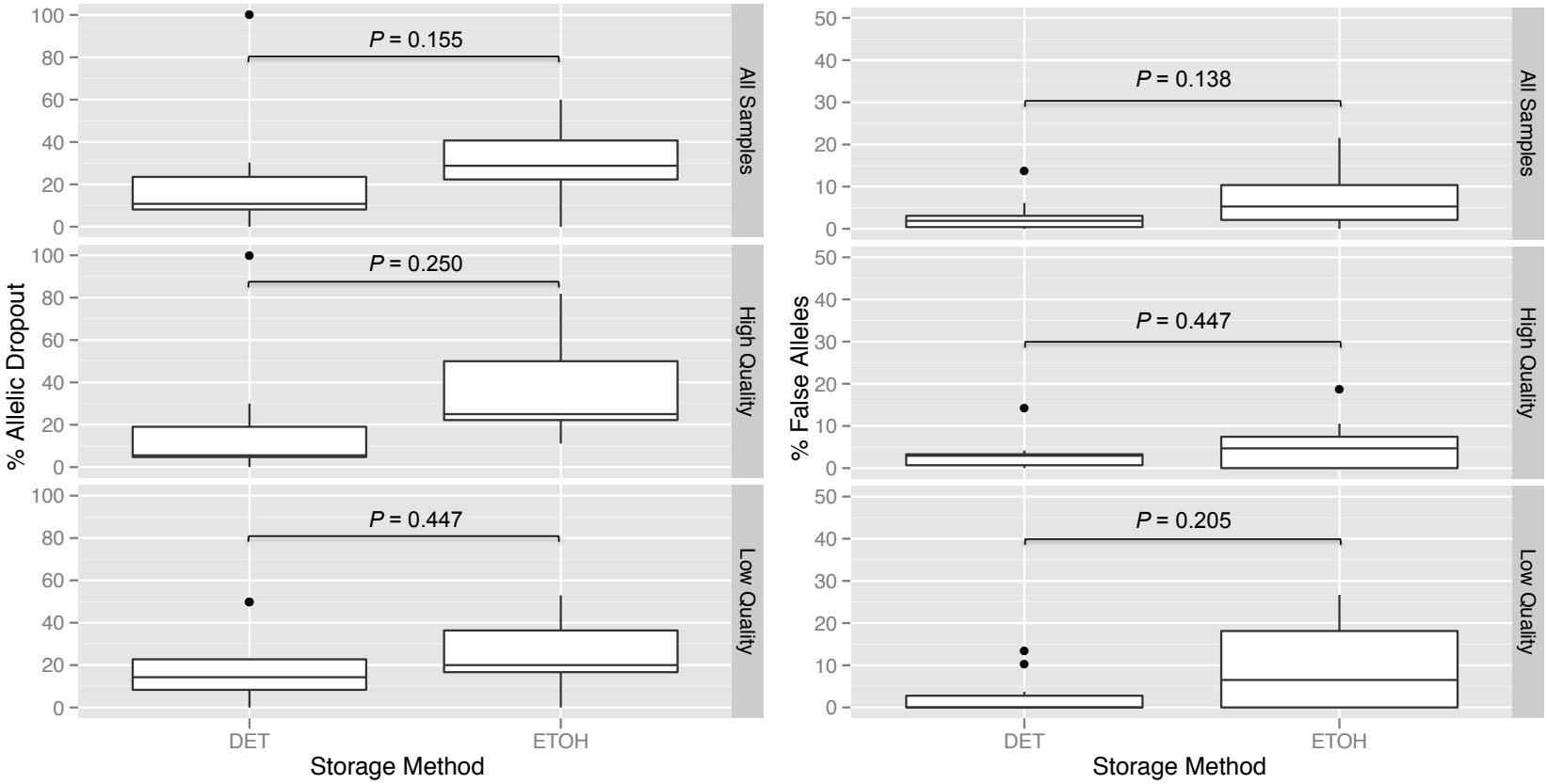


Fig. 4 PCR amplification success (PCR) and genotyping accuracy (GA) rates calculated across 10 microsatellite loci for Neotropical felids in Belize, Central America. Rates are compared across four scat locations (1 top, 2 side, 3 bottom, 4 inside) at two sites, the Mountain Pine Ridge Forest Reserve (MPR, n = 20) and Rio Bravo Conservation Management Area (RB, n = 20). Kruskal-Wallis rank-sum tests and pairwise Wilcoxon signed-rank tests were used for statistical testing (P - value < 0.05). In the box-and whisker plot, the central value represents the median and the central box represents the value from the 25th to the 75th percentile.

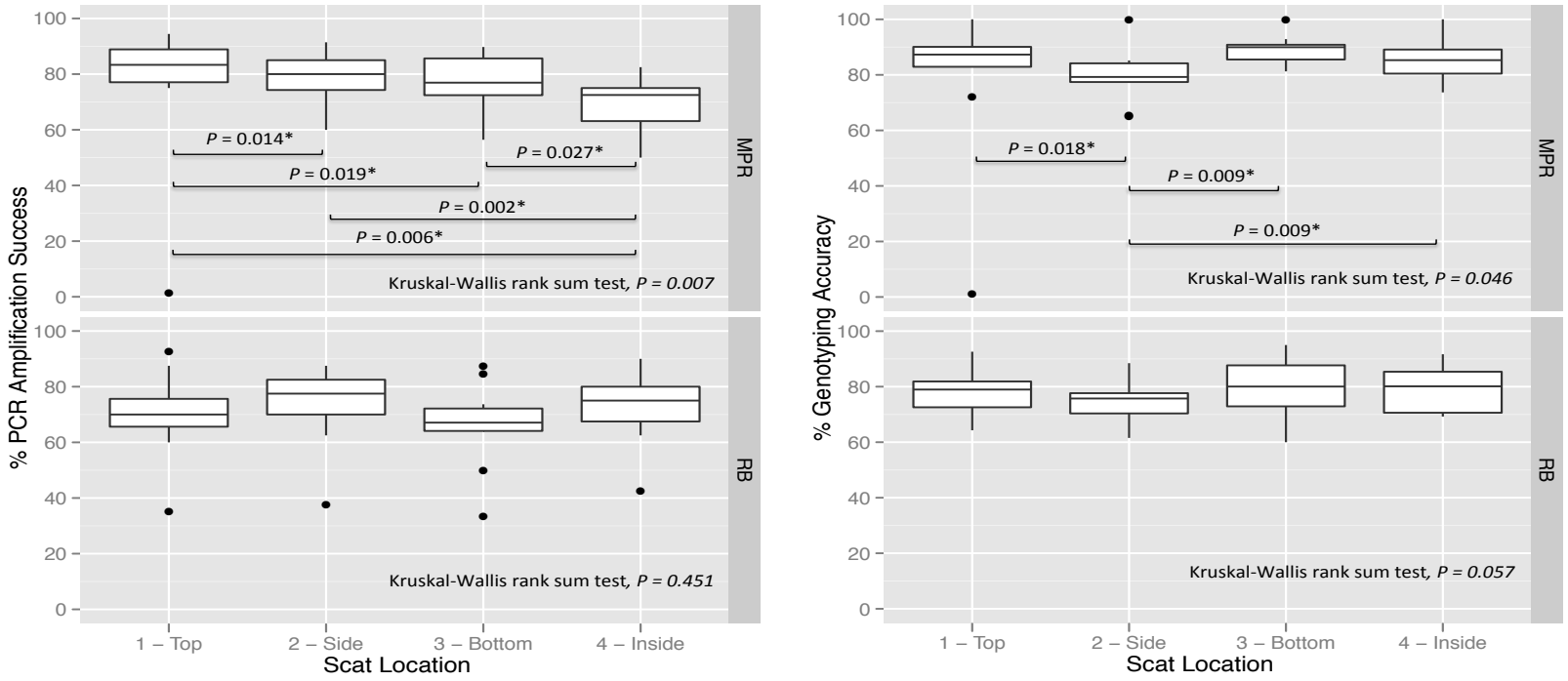
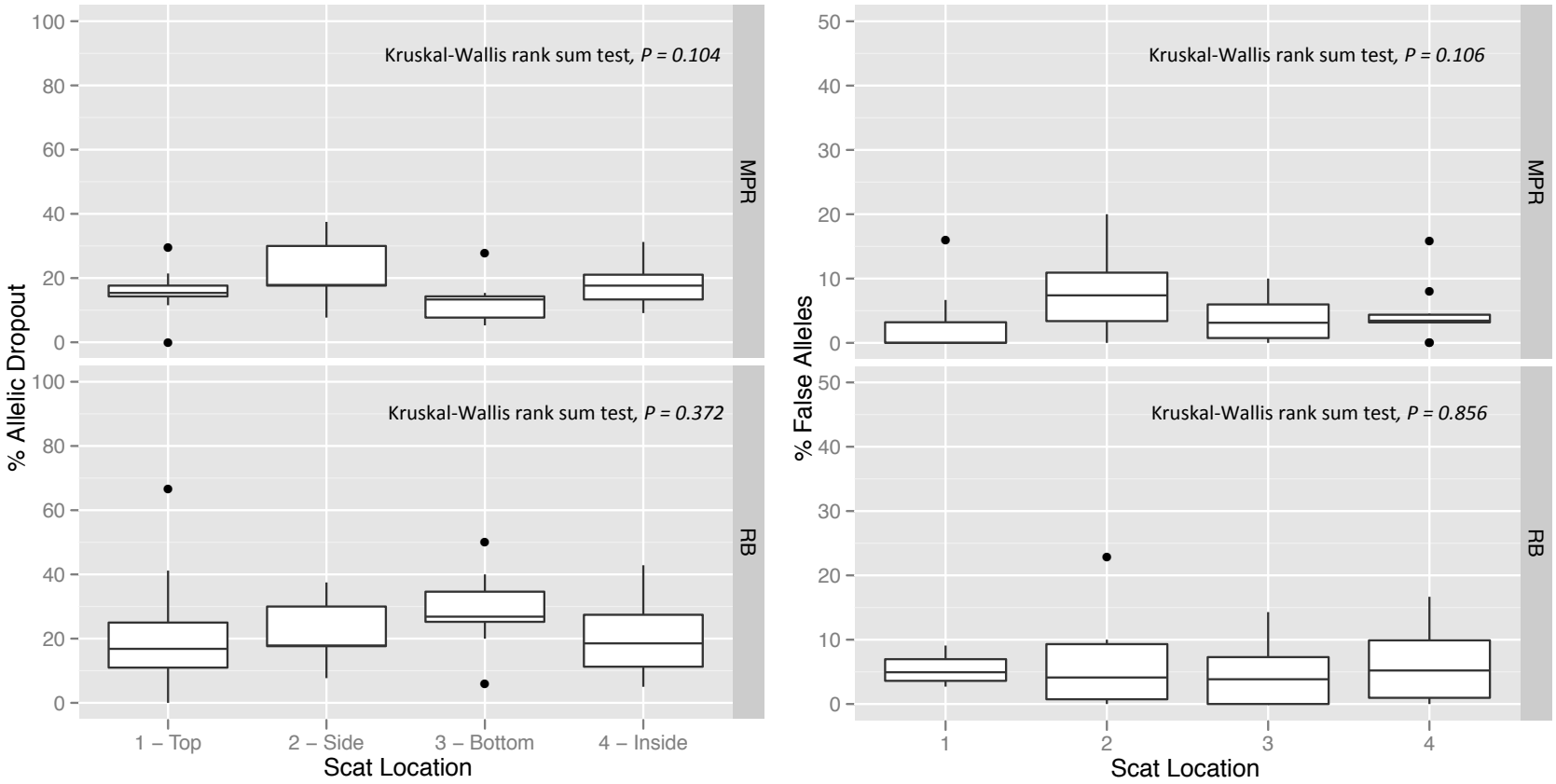


Fig. 5 Allelic dropout and false allele rates calculated across 10 microsatellite loci for Neotropical felids in Belize, Central America. Rates are compared across four scat locations (1 top, 2 side, 3 bottom, 4 inside) at two sites, the Mountain Pine Ridge Forest Reserve (MPR, n = 20) and Rio Bravo Conservation Management Area (RB, *n* = 20). Kruskal-Wallis rank sum tests and pairwise Wilcoxon signed-rank tests were used for statistical testing (*P* - value < 0.05). In the box-and whisker plot, the central value represents the median and the central box represents the value from the 25th to the 75th percentile.



CHAPTER 4

Genetic Diversity, Genetic Structure and Gene Flow of Jaguars (*Panthera onca*), Pumas (*Puma concolor*) and Ocelots (*Leopardus pardalis*) in Belize, Central America

Abstract

Wild felids are greatly impacted by habitat loss and fragmentation. Genetic monitoring of species can quantify changes in genetic diversity and gene flow in response to these major threats. This study combined innovative noninvasive research techniques, such as scat detector dogs and molecular scatology to conduct the first genetic study on wild populations of Neotropical felids coexisting across fragmented forest habitats in Belize, Central America. We analyzed data from 14 highly polymorphic microsatellite loci in 1053 scat samples from jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) collected across their range in the country. We assessed levels of genetic diversity within geographic sites, defined potential genetic clusters/populations, and examined levels of gene flow and population structure for the three target species on a countrywide scale. We compared genetic diversity and gene flow levels among the three target species. Wild felids in Belize showed moderate levels of heterozygosity (H_E = 0.60 - 0.70), with jaguars having the lowest genetic diversity average expected heterozygosities of H_E = 0.60 ± 0.05 and allelic richness (A_R) of 4.94 ± 0.44, followed by pumas with H_E = 0.65 ± 0.06 and A_R of 7.52 ± 0.86, and ocelots with H_E = 0.70 ± 0.05 and A_R of 3.89 ± 0.23. We observed low to moderate levels of genetic differentiation (F_{ST} = 0.00 - 0.15) and weak population structure using spatial Bayesian clustering techniques for all three target species. Although levels of genetic diversity and gene flow across the country are still fairly high, we did detect evidence of fragmentation indicating the risk of further habitat loss and fragmentation for wild felids. Our results demonstrate that fecal DNA analysis is an effective approach for monitoring genetic diversity and structure of multiple Neotropical felids on a countrywide scale.

Keywords: Panthera onca, *Puma concolor*, *Leopardus pardalis*, genetic diversity, gene flow, Bayesian clustering, molecular scatology, genetic structure, Belize, dispersal

Introduction

Habitat loss and fragmentation are among the largest threats to wild felids (e.g. Nowell and Jackson 1996, Weber and Rabinowitz 1996, Macdonald and Loveridge 2010), including tigers (*Panthera tigris*) (e.g. Mondal and Nagendra 2011, Tian et al. 2011, Harihar and Pandav 2012, Rathore et al. 2012, Reddy et al. 2012c, Sunarto et al. 2012), leopards (*Panthera pardus*) (e.g. Johnsingh and Negi 2003), snow leopards (*Panthera uncia*) (e.g. Forrest et al. 2012), and jaguars (*Panthera onca*) (e.g. Eizirik et al. 2001, McCain and Childs 2008, Conde et al. 2010, Haag et al. 2010, De Angelo et al. 2011, Vynne et al. 2011a). Landscape changes due to forest loss, degradation, and anthropogenic developments and disturbances can severely impact the degree of genetic connectivity and dispersal (e.g. Crooks 2002, Frankham 2006, Urquiza-Haas et al. 2009). By altering potential dispersal corridors and negatively impacting genetic connectivity across the landscape, levels of reproductive fitness and adaptive potential may decrease. Genetic differentiation and the risk for inbreeding may rise (e.g. Peacock and Smith 1997, Gaggiotti 2003, Aurambout et al. 2005, Frankham 2006).

Wild felids, such as the large-bodied jaguar, have large space requirements, occur at low densities, and depend on forest habitat, and thus are especially affected by changes of habitat connectivity and heterogeneity (e.g. Nowell and Jackson 1996, Sunquist and Sunquist 2002b, Haag et al. 2010, Macdonald and Loveridge 2010, Vynne et al. 2011a). Over the last 100 years in the Americas, the impact of land conversion on wide-ranging species such as jaguars has severely increased leading to a listing as near threatened under IUCN. Jaguars have been extirpated from over 54% of their historic range (Nowell and Jackson 1996, Sanderson et al. 2002). The situation is worse in Mexico and Central

America, where jaguar populations exist in only 33% of their former range, and 75% of populations that do exist, are reduced in number (Swank and Teer 1989). Despite its internationally protected status (as an *Appendix I* species under CITES, the Convention on International Trade of Endangered Species of Wild Fauna and Flora), the jaguar's range continues to decrease, mainly due to severe deforestation and fragmentation of their preferred habitat (e.g. Hoogenstein and Mondolfi 1992, Nowell and Jackson 1996, IUCN 2012). Increased human presence and anthropogenic development have resulted in the conversion of continuous forest habitat to small, potentially isolated patches, causing loss of biodiversity (e.g. Turner 1996, Bender et al. 1998). Jaguars now exist in fragmented forest areas across their range. This is especially concerning in Belize, which has been experiencing widespread land conversion and development, as well as privatization of public lands. According to Cherrington et al. (2010), Belize has lost about 17% of its forest cover over the last 30 years, which has mainly affected forested areas outside of protected areas. Local conservation efforts for jaguars are currently underway in central Belize, but more research is needed to scientifically define and prioritize future conservation and management efforts for wild felids on a countrywide scale.

Although the jaguar is the largest felid in the Americas (Kitchener 1991, Nowell and Jackson 1996), it is still considered the least-studied of all great cats. Conservation genetic studies of wild jaguars and other elusive Neotropical felids are particularly rare (e.g. Miotto et al. 2007, Haag et al. 2010, Michalski et al. 2011) due to the difficulties in obtaining sufficient DNA sample sizes from wild felid populations in tropical environments. Previous genetic studies on jaguars and other Neotropical felids often were based on invasive samples (e.g. blood, tissue) obtained from captured and captive

animals or from museum specimens (e.g. Eizirik et al. 2001, Ruiz-Garcia et al. 2006). Recent advances in noninvasive sampling, including the use of molecular scatology and scat detector dogs (e.g. Wultsch et al. in press, Waits and Paetkau 2005, Long et al. 2007b, Stenglein et al. 2010, Vynne et al. 2011b, Kelly et al. 2012), make this approach a powerful and more feasible way to genetically study multiple elusive forest carnivores in tropical environments. The use of noninvasive genetic samples (e.g. scats) and highly polymorphic genetic markers such as microsatellites make it possible to analyze genetic variation and population structure efficiently (e.g. Balloux and Lugon-Moulin 2002, Waits and Paetkau 2005, Allendorf et al. 2013). Additionally, statistical methods such as model-based Bayesian clustering, which uses multilocus data to assess genetic structure, has the potential to detect genetic discontinuities caused by limited gene flow due to dispersal barriers, habitat loss and fragmentation (e.g. Pritchard et al. 2000, Chen et al. 2007, Guillot 2009, Francois and Durand 2010). All these developments have expanded the possibilities genetic monitoring has to offer tremendously over the last years.

We conducted a four-year (2007 – 2010), noninvasive genetic study on jaguars and two co-occurring felids, pumas and ocelots, using a scat detector dog, a set of microsatellite loci and fecal DNA samples collected across several study sites in Belize, Central America. We hypothesize that felids living in the most northern and southern sites of Belize experience lower levels of genetic connectivity due to isolation of protected areas in these parts of the country and to a higher degree of human disturbance. Furthermore, we hypothesized that jaguars would show more genetic structure than pumas, since pumas reportedly are long-distance dispersers and are more likely to move through disturbed and fragmented areas than jaguars (e.g. Larue and Nielsen 2008, Stoner

et al. 2008). In order to test these hypotheses and assess the conservation status of wild felids in Belize, we specifically aimed to: (1) estimate levels of genetic diversity within different regions of the country, (2) define any genetic clusters/populations, (3) and examine levels of gene flow and dispersal patterns for all three target species. Understanding levels of genetic diversity and the spatial genetic structure of wild felid populations in Belize is vital for planning and prioritizing future management and conservation efforts for wild felids.

Methods

Study area

We conducted 2- to 3-month long scat surveys across 5 study sites (Mountain Pine Ridge Forest Reserve – MPR, Rio Bravo Conservation and Management Area – RB, Cockscomb Basin Wildlife Sanctuary – CBWS, Chiquibul Forest Reserve and National Park – CFRNP, Fireburn/Balam Na Nature Reserve – FB) and 2- to 10-day surveys at several other sites (Big Falls – BF, Bladen Nature Reserve - BNR, Boden Creek Ecological Preserve - BC, Bull Run Farm – BRF, Golden Stream Corridor Preserve - GS, Hidden Valley Reserve – HVR, Machaca Hills – MH, Manatee Forest Reserve – MFR, Sarstoon-Temash National Park - STNP, Shipstern Nature Preserve – SNP, Tiger Sandy Bay - TSB) from 2007- 2010 across Belize, Central America (17°15’ N, 88°45’ W; Fig. 1). All sites except BF, BRF, MH and TSB are part of the national system of Protected Areas in Belize. For the genetic diversity and structure study, we predefined groups of individuals based on geographical regions. For jaguars and pumas, we evaluated five geographical regions in Belize, which included the following study sites: north (FB,

SNP), north-central (RB, BF, MFR, TSB), central (MPR, CFRNP, BFR, HVR), south-central (CBWS), and south (BNR, BC, GS, MS, STNP) (Fig. 2A). For ocelots, we evaluated samples from the north (FB, SNP, RB, BF, MFR, TSB), and south (MPR, CFRNP, BFR, HVR, BNR, BC, GS, MS, STNP) (Fig. 2B). Overall, the study sites also fall within La Selva Maya (the Mayan Forest), a recognized biodiversity hotspot that forms part of the northern section of the Mesoamerican Biological Corridor. Across study sites, elevation ranges from 0 to 1120 m, and mean annual rainfall varies from 1524 mm in the north to 4064 mm in the south with a pronounced wet season from June to December. Average annual temperatures fluctuate between 17.7 and 31.3 °C. A high diversity of native habitat types occurs within the study sites, including lowland and submontane broad-leaved moist and wet forests, lowland and submontane pine forests, mangrove and littoral forests, lowland savannah, shrub land, and wetland.

Fecal sampling and DNA extraction

Fecal samples were detected opportunistically in the field by using a professionally trained scat detector dog (PackLeader LLC, Gig Harbor, WA, USA) following the study design described in detail by Wultsch et al. (in review). From each scat, one ~ 0.5 mL sample was collected and stored at ambient temperature in sterile 2 mL screw-top tubes filled with dimethyl sulfoxide saline solution (DET buffer, Seutin et al. 1991) at 1: ≥ 4 ratio by volume. Scat samples were collected using disposable gloves and wooden sampling sticks. Fecal DNA extractions were conducted in a separate room at the Laboratory for Ecological, Evolutionary and Conservation Genetics (LEECG) at the University of Idaho (Moscow, ID, US), dedicated to noninvasive genetic studies, in order

to avoid contamination while working with low-concentration DNA samples. The QIAamp DNA Stool Mini Kit protocol (Qiagen, Inc.) was used to extract DNA from all fecal samples. An extraction negative was added to each extraction group to monitor for contamination.

Microsatellite genotyping

We PCR amplified 14 highly polymorphic microsatellite loci, which were arranged in three PCR multiplex reactions (multiplex 1 - F124, FCA391, FCA043, FCA275, FCA096, FCA126, FCA090; multiplex 2 - F85, F98, FCA741, FCA225, FCA008, and multiplex 3 - F53 and FCA441) (Wultsch et al. in review). Gender was determined by DNA markers associated with the Y sex chromosome carried by males (Zn, Zn-finger; Amel, Amelogenin), but not by females (Pilgrim et al. 2005). The three multiplexes each contained a 7 μL PCR mixture. Multiplex 1 consisted of 3.5 μL 1 x concentrated Qiagen Master Mix (Qiagen, Inc.), 0.98 μL of primers (0.10μM for F124, 0.34μM for FCA391, 0.07μM for FCA043, 0.13μM for FCA275, 0.21μM for FCA096, 0.20μM for FCA126, 0.20μM for FCA090, and 0.14μM for Zn), 0.7 μL of 0.5 x concentrated Qiagen Q solution (Qiagen, Inc.), 0.02 μL H_2O and 1.8 μL DNA extract. Multiplex 2 consisted of 1 x concentrated Qiagen 3.5 μL Master Mix, 0.85 μL of primers (0.20μM for F85, 0.09μM for F98, 0.11μM for FCA741, 0.43μM for FCA225, 0.10μM for FCA008, and 0.29μM Amel), 0.7 μL of 0.5 x concentrated Qiagen Q solution, 0.15 μL H_2O and 1.8μL DNA extract. Multiplex 3 only differed in the amount of water (0.76 μL) and primers (0.24 μL; 0.20μM for F53, and 0.14μM for FCA441) added. Microsatellite PCR amplifications were conducted using a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad

Laboratories, Inc.) starting with an initial denaturation step of 15 min at 95 °C, followed by 13 cycles of 30 s at 94 °C for denaturation, 1.5 min at 60 °C with a decrease in annealing temperature of 0.8 °C in each cycle, and 1 min elongation at 72 °C, followed by 30 cycles of 30 s at 94 °C for denaturation, 1.5 min at 50 °C for annealing, and 1 min elongation at 72 °C, and 30 min at 60 °C for final elongation. A PCR negative was added in each group of PCR reactions to control for contamination. We visualized PCR products using an ABI PRISM 377® automated DNA sequencer (Applied Biosystems™), and genotypes were identified using the software GENEMAPPER, version 3.7 (Applied Biosystems™).

To finalize the consensus genotypes and to minimize genotyping error, a multi-tube approach (Taberlet et al. 1996) was used in which at least three identical homozygote PCR results were required to identify homozygous genotypes and each allele had to be observed in two independent PCRs to record a heterozygous genotype. Hence, the total number of PCRs ranged from 2 to 6 replicates per locus. Once consensus genotypes were finalized for multiplex 1, we used GenAlEx, version 6.41 (Peakall and Smouse 2006) to determine the minimum number of individuals per species by checking for repeated matching genotypes which, when detected, were interpreted as recaptures of individual felids. Genotypes which were only detected once were further examined using the software RELIOTYPE (Miller et al. 2002) to assess their reliability. If their accuracy of multi-locus scores was below 95%, additional PCR replicates were conducted. For individual identification, we estimated probabilities of identity (per locus and cumulative) for unrelated individuals ($P_{(ID)}$) and siblings ($P_{(ID)sib}$) per species using GIMLET, version 1.3.3. (Valiere 2002). Scat samples were assigned to feline species based on species-

specific alleles and allelic size ranges described for jaguars, pumas and ocelots (Wultsch et al. in review).

Data analysis

Genetic diversity

The program GenAlEx, version 6.41 (Peakall and Smouse 2006) was used to assess genetic variation per species and geographical region (north, north-central, central, south-central, and south for jaguars and pumas; north and south for ocelots) at single loci, and across all loci by calculating the number of alleles (N_A), the number of private alleles (A_P) and estimating observed (H_O) and expected heterozygosities (H_E). Additionally, we determined allelic richness (A_R) using the rarefaction method with HP-RARE, version 1.0 (Kalinowski 2005).

We assessed for Hardy-Weinberg equilibrium (*HW*) and linkage disequilibrium (*LD*) for all three species using GENEPOP, version 4.1 (Raymond and Rousset 1995) with default settings for Markov chain parameters. The presence of null alleles was examined with MICRO-CHECKER (Van Oosterhout et al. 2004).

Statistical differences between groups were evaluated using non-parametric Kruskal-Wallis and post-hoc Wilcoxon rank-sum tests in program R, version 2.15 (R Development Core Team 2009).

Genetic structure and gene flow

To determine whether jaguars or co-occurring felids in Belize represent a single panmictic population or multiple subdivided populations and whether populations at certain sites experience less gene flow than others, we used various approaches to assess

genetic structure and measured levels of gene flow in both direct and indirect ways. Direct measures of gene flow helped to gain inferences on genetic structure and gene flow based on direct (or recent) events relative to the rate of genetic changes. Direct methods give insight into patterns of contemporary gene flow, which seems to be an appropriate way to study long-lived carnivore species with extended generation times such as jaguars, pumas and ocelots. In contrast, indirect methods are based on the divergence of allele frequencies (Wright 1931, Wright 1951) caused by evolutionary forces such as gene flow and genetic drift, and hence provide inferences on population genetic processes occurring over many generations.

Direct methods

SPATIAL AND NON-SPATIAL ASSIGNMENT TESTS

In order to assess gene flow directly and gain insight into contemporary rates of genetic change, we conducted assignment tests, which assign multi-locus genotypes to the population in which that genotype most likely originated (e.g. Paetkau et al. 2004, Allendorf et al. 2013). Assignment tests may directly identify dispersers/genetic migrants, which are sampled in one population, but originated in another one. We applied several different types of assignment tests and models using aspatial and spatial Bayesian clustering techniques (e.g. Pritchard et al. 2000, Guillot et al. 2005, Chen et al. 2007, Francois and Durand 2010).

We applied the non-spatial Bayesian clustering software STRUCTURE, version 2.3.3 (Pritchard et al. 2000), which uses multi-locus genotype data to examine genetic structure and describe the number of genetic clusters. The admixture model was used

with the number of putative genetic groups/clusters (*K)* ranging from 1 to 10 for jaguars and pumas and from 1 to 5 for ocelots; 10 runs per *K* value; length of burn-in period: 200,000 iterations; number of Markov Chain Monte Carlo (MCMC) iterations afterburning: 2,000,000]. The optimal *K* value was chosen by calculating the posterior probability for each *K* value, which is based on estimated maximum log-likelihood values (Pritchard et al. 2000). After finding the optimal *K*, individuals were assigned to distinct genetic clusters using the percentage of genotypes' ancestry (*Q*) attributed to each genetic cluster. We additionally estimated delta *K* values as suggested by Evanno et al. (2005) using program STRUCTURE-HARVESTER (Earl and Vonholdt 2012).

We also used a spatial Bayesian clustering method, which includes geographic coordinates and assumes spatial separation of genetically distinct groups. A spatial prior and the techniques of triangulation and tessellation are used to test this assumption.

We implemented the spatial Bayesian clustering method in two programs, including programs GENELAND, version 4.0.3 (Guillot et al. 2005), and TESS, version 2.3 (Chen et al. 2007). We used the spatially explicit model in GENELAND, version 4.0.3 (100000 iterations, thinning = 1), and set the number of potential populations to *K* equals 1 to 10. The uncorrelated frequency model, which uses the Dichirilet distribution (Pritchard et al. 2000) was used to model allele frequencies as recommended by Guillot et al. (2005).

We implemented these models also in program TESS, version 2.3 (Chen et al. 2007). We estimated the number of genetic clusters (K_{max}) by using models with and without admixture with 100 independent simulations with 20,000 burn-in period for 100,000 MCMC iterations. In the admixture model, we estimated the fraction of individual genomes, which originate from different parental populations (Durand et al.

2009). In the model without admixture, we assessed the number of true genetic clusters from which individuals originated. To identify the maximum population number (K_{max}), we used the degree of stabilization of individual posterior probabilities. Additionally, the Deviance Information Criterion (DIC, Spiegelhalter et al. 2002) was applied for model selection. We calculated the mean DIC value for each K_{max} using 10% of the runs with the lowest DIC values.

IDENTIFICATION OF FIRST-GENERATION MIGRANTS

Additional assignment tests were conducted in program GENECLASS, version 2.0 (Paetkau et al. 2004, Piry et al. 2004) to identify potential first-generation migrants of all three target species between different geographical regions. We used two different approaches, including the likelihood-based method and *Lhome*:*Lmax* ratio and *Lhome* values (Paetkau et al. 2004), and also the Bayesian method using MCMC resampling (Rannala and Mountain 1997, Paetkau et al. 2004).

DISPERSAL FROM RELATEDNESS

We used maximum likelihood estimates of relatedness and defined relationships between pairs of individuals, which included the classes unrelated (*U*), half-sibling (*HS*), full-sibling (*FS*), and parent-offspring (*PO*), in program ML-RELATE (Kalinowski et al. 2006). By assessing the degree of relatedness for pairs of felids detected at different sites, we directly assessed contemporary gene flow.

Indirect methods

Indirect methods assess the degree of divergence of predefined subpopulations (Wright 1951, Excoffier et al. 1992). We used *F*-statistics to examine the degree of genetic differentiation by calculating pairwise F_{ST} values (Weir and Cockerham 1984) between predefined groups of felids detected across different geographical areas (north, north-central, central, south-central, and south) using ARLEQUIN, version 3.5 (Excoffier and Lischer 2010). Statistical significance for F_{ST} was tested using 10,000 permutations and Bonferroni correction (Rice 1989). Furthermore, analysis of molecular variance (AMOVA) was conducted to examine partitioning of genetic variation among and within groups, using 10,000 permutations (Excoffier et al. 1992).

Additionally, we performed Mantel tests using pairwise (individual-based) genetic and geographic distances calculated in program GenAlEx, version 6.41 (Mantel 1967, Peakall and Smouse 2006) to test whether isolation by distance (*IBD*) is present for our study systems (within species) in Belize.

Results

Scat sample detection and number of felid captures

The total number of fecal DNA samples located with the scat detector dog across all study sites was 1053, with 110 collected in MPR, 203 in RB, 223 in CBWS, 111 in CFRNP, 217 in FB, and 189 samples during several short-term surveys across various sites (BF, BNR, BC, BRF, GS, HVR, MH, MFR, STNP, SNP, and TSB) in Belize, 2007 – 2010.

Of 1053 scat samples collected, 530 (50%) were successfully identified to species and individual level. In total, jaguars were genetically "captured" 307 times, pumas 161 times, and ocelots 62 times. At the individual level, we detected 65 jaguars (57 males, 8 females), 54 pumas (32 males, 22 females) and 30 ocelots (15 males, 15 females) (Table 1). For individual identification, cumulative $P_{(ID)}$ and $P_{(ID)sib}$ values were calculated per species for the screening multiplex 1 (7 loci), which helped to assess the minimum number of loci required for individual identification. With the criterion of $P_{(ID)sib} < 0.010$, a minimum of 6 finalized loci for jaguars and 5 loci for pumas and ocelots were needed to identify individuals with statistical confidence. The cumulative $P_{(ID)}$ and $P_{(ID)sib}$ values for 7 loci of multiplex 1 was 3.8E-06 and 0.005 for jaguars, 4.1E-08 and 0.002 for pumas, and 2.1E-07 and 0.002 for ocelots. For all 14 loci, cumulative $P_{(ID)}$ and $P_{(ID)sib}$ estimates were 9.1E-11 and 5.3E-05 for jaguars, 2.1E-13 and 1.5E-05 for pumas, and 8.5E-14 and 6.8E-06 for ocelots, indicating that the probability that two individuals sampled from the same population have matching genotypes was low, even when animals are related.

Genetic diversity

The number of alleles per locus (N_A) ranged from 3 - 8 (5.00 ± 0.44) for jaguars, 3 - 15 (8.00 ± 0.93) for pumas and 3 - 10 (6.43 ± 0.52) for ocelots. Mean allelic richness (A_R) using rarefaction was highest for pumas (7.52 ± 0.86), followed by jaguars (4.95 ± 0.44) and ocelots (3.89 ± 0.23). The average expected and observed heterozygosities for all loci were H_E = 0.60 ± 0.05 and H_0 = 0.57 ± 0.05 for jaguars, H_E = 0.65 ± 0.06 and H_0 = 0.61 ± 0.06 for pumas, H_E = 0.70 ± 0.04 and H_0 = 0.62 ± 0.05 for ocelots. Estimates of average expected heterozygosity calculated across all loci did not differ significantly between

species (Kruskal – Wallis rank-sum test, H = 4.29, P = 0.117) (Table 2). Diversity estimates also did not differ significantly across regions for jaguars (Kruskal – Wallis rank-sum tests, A_R, H = 0.72, P = 0.95; H_e, H = 1.15, P = 0.886) and ocelots (Wilcoxon rank-sum tests, A_R, W = 45, P = 0.670; H_e, W = 43, P = 0.889). Pumas differed significantly in allelic richness estimates between the south and most other regions (A_R, Kruskal – Wallis rank-sum test, H = 14.11, P = 0.007), but not in expected heterozygosity estimates (H_e, Kruskal – Wallis rank-sum test, H = 8.07, P = 0.09) (Table 3 – 5). Jaguars and pumas differed marginally in numbers of private alleles (A_P) (Wilcoxon rank-sum test, W = 0, P = 0.054). A_P ranged from 0 to 4 private alleles for jaguars, with lowest values for the north (A_P = 0) and the south (A_P = 2), and highest for central (A_P = 4) and south-central (A_P = 4) regions. For pumas, we detected 4 to 10 private alleles across sites, with lowest numbers for the north (A_P = 4) and south (A_P = 4), and highest numbers for the north-central region (A_P = 10). Ocelots had a higher number of private alleles in the south (A_P = 19) compared to the north (A_P = 16). Sample sizes for ocelots in general and jaguars and pumas in the north and south were considerably lower, so diversity estimates based on alleles should be treated with caution (also when rarefaction was applied) (Table 6).

No loci deviated significantly from *HWE* for jaguars. Loci FCA043 (P < 0.000), and F98 (P = 0.011) for pumas and FCA391 (P < 0.000), FCA275 (P < 0.000), and FCA741 (P = 0.011) for ocelots revealed significant deviations from HWE after using sequential Bonferroni correction ($P \leq 0.015$) (Table 2). Linkage disequilibrium was detected in a few cases (F124/F53, FCA096/FCA441 for jaguars; 124/FCA275, FCA096/F98 for pumas), which were not however consistent across geographic regions.

Genetic structure and gene flow

Non-spatial Bayesian clustering analyses in program STRUCTURE showed that the mean log likelihood of the data was highest at $K = 1$ for jaguars (mean *LnP(K)* = -2008.50), pumas (mean *LnP(K)* = -1977.14), and ocelots (mean *LnP(K)* = -1087.10), indicating that there is little evidence for population subdivision for all three target species in Belize. *LnP(K)* values for jaguars decreased continuously with increasing values for *K*. For pumas, we also detected a distinct peak of *LnP(K)* at $K = 5$ (Fig. 3 and 4).

Spatial Bayesian clustering analysis in program GENELAND detected no distinct genetic differentiation for jaguars across Belize ($K = 1$). For pumas, GENELAND identified two distinct clusters ($K = 2$) corresponding to the most northern study site (Fireburn/Balam Na Nature Reserve), whereas all other pumas from central and southern Belize were clustered together. For ocelots, we also detected two distinct genetic clusters ($K = 2$), where ocelots in north-central and central Belize (Rio Bravo Conservation Management Area, Big Falls Area) form a distinct cluster. Interestingly, two ocelots detected in south-central Belize also got assigned to this cluster. The remaining ocelots were grouped into a second cluster, which spans from southern Belize all the way up to the most northern site within the country (Fig. 6).

Spatial Bayesian clustering analysis using program TESS revealed higher estimates for *K* than did program STRUCTURE with a $K_{max} = 7$ (No admixture model) and 7 (admixture model) for jaguars, a $K_{max} = 5$ (No admixture model) and 7 (admixture model) for pumas, and a $K_{max} = 6$ (No admixture model) and 6 (admixture model) for

ocelots (Fig. 3 and 4). The admixture model estimates fractions of individuals' genome which belong to different parental populations ('ancestry groups'). The no-admixture model aims to detect true genetic clusters from which individuals originate. In comparison to the other clustering methods, TESS detected the largest amount of clusters. We also observed that the percentage of genotype's ancestry (*Q*) attributed to some clusters was distinctively low, indicating that the number of *K* may be overestimated as reported before (Guillot 2009).

Using GENECLASS, we identified three first-generation migrants for jaguars. The three animals were detected and came from: (1) the central and south, (2) the central-south and south, and (3) the south and north sites. For pumas, we detected a total of four first-generation migrants. The animals were located and originally came from: (1) north and north-central, (2) north-central and north, (3) central and north, and (4) south and south-central (Table 9). For ocelots, we identified one first-generation migrant, which was detected in the north (Rio Bravo Conservation Management Area) and originally came from the south. Our results using two-ratio and *Lhome*:*Lmax* values (Paetkau et al. 2004), and the Bayesian method using MCMC resampling (Rannala and Mountain 1997) were consistent.

The percentage of unrelated jaguars per site was highest in the south (82%), followed by central (79%), south-central (70%), north (68%) and north-central (67%), which indicates that individuals particularly at northern sites were more highly related. The northern site has the highest percentage of closely related animals (7% FS, 7% PO). At the north-central site we detected the highest percentage for half-siblings (28%). When estimating pairwise relationships values among individuals living at different

geographical sites, we observed a general trend that percentage of related individuals among sites decreases with geographical distance, with an exception for the southern site (Table 10). The percentage of unrelated pumas per site was highest in central Belize (84%), followed by south-central (81%), north-central (79%), north (67%) and south (60%), which indicates that individuals at northern and southern sites were relatively highly related. When estimating pairwise relationships values among individuals living at different geographical sites, we also observed a general trend that % of related individuals among sites decreased with geographical distance, with an exception between the north-central, central and south sites (Table 10). Ocelots had a higher percentage for unrelated individuals within the southern site (84%) than in the north (76%), indicating that ocelots detected in the north are generally more closely related than southern ocelots. Comparison of relationships among pairs of individuals between sites revealed that most were unrelated (91%), 8% of them were half-siblings and 1% full-siblings (Table 10).

Pairwise F_{ST} values between most geographical regions indicated little to moderate genetic differentiation for jaguars ($F_{ST} < 0.050$). However, levels for genetic differentiation were moderate (F_{ST} between 0.050 and 0.150) between the north and the south-central ($F_{ST} = 0.094$) and the north-central and south-central ($F_{ST} = 0.093$) regions for jaguars. Both F_{ST} estimates were significantly different from zero ($P = 0.021$ and $P = 0.002$). For pumas, little genetic differentiation was detected among most regions. Moderate F_{ST} values (> 0.050) were observed between the north and central, and north-central and south areas. We detected highest genetic differentiation for pumas between the north and south ($F_{ST} = 0.148$) and south-central and southern areas ($F_{ST} = 0.131$). F_{ST} estimates for pumas were not statistically significant ($P < 0.05$). For ocelots, a significant

F_{ST} value of 0.100 was detected between northern and southern ocelots ($P < 0.000$) (Table 7, Fig. 6).

Mantel tests showed no correlation (r = 0.002, $P = 0.350$) between genetic and geographical distances for jaguars, verifying that geographical distance is not the main factor impacting the observed genetic differentiation. A weak positive relationship between genetic and geographic distance was detected for pumas ($r = 0.017$, $P = 0.010$) and ocelots ($r = 0.014$, $P = 0.010$) indicating that isolation by distance has a small influence on both species (Fig. 7).

Analysis of molecular variance revealed that genetic variance among populations was substantially lower (3.93% for jaguars, 4.60% for pumas, and 9.95% for ocelots) than within populations. For all three target species, most of genetic variation (> 90%) was within populations/geographical sites.

Discussion

Genetic diversity

Wild felids are negatively affected by habitat loss and fragmentation (e.g. Nowell and Jackson 1996, Crooks 2002, Sunquist and Sunquist 2002b, Macdonald and Loveridge 2010). Monitoring of genetic diversity and understanding patterns of genetic structure and gene flow are vital in order to maintain long-term genetic diversity of species (e.g. Schwartz et al. 2007, Allendorf et al. 2013). Over the last 10 years, only a few conservation genetic studies have been conducted on wild Neotropical felids (e.g. Miotto et al. 2007, Haag et al. 2010, Michalski et al. 2011). This is the first study assessing levels of genetic diversity and differentiation of jaguars, pumas and ocelots in Central

America. The analysis revealed that genetic diversity estimates across five sites in Belize were lowest for jaguars, followed by pumas and ocelots (Table 2). Eizirik et al. (1998) stated that larger Neotropical felids (e.g. jaguars) have a three-to-ten-times lower genetic diversity than to smaller felids (e.g. ocelots), which we did not observe in Belize. Ruiz-Garcia et al. (2006) compared levels of genetic diversity of Colombian jaguars to past puma studies (e.g. Culver et al. 2000, Walker et al. 2000b) and stated that jaguars have significantly higher levels of genetic diversity, which we did not detect for Belizean jaguars and pumas. Diversity estimates calculated for all three target species across several sites in Belize did not show significance differences among regions, but levels were lowest in the very north (H_E = 0.58) and south (H_E = 0.44) for pumas, in northern Belize for ocelots (H_E = 0.61), and the very north of the country for jaguars (H_E = 0.54). In comparison to jaguar studies in other countries, levels of genetic diversity of Belizean jaguars were relatively low. For example, Brazilian (H_E = 0.73) (Haag et al. 2010) and Colombian jaguars (H_E = 0.85) (Ruiz-Garcia et al. 2006) were genetically more diverse. Nonetheless, it a strict direct comparison of diversity measures between studies cannot be made since different microsatellite loci were used by most studies. In 2001, a genetic study looking at jaguars across their entire range (Eizirik et al. 2001) resulted in similar genetic diversity estimates for Central America compared to what we found in Belize. Genetic diversity estimates observed for pumas resulted in similar estimates compared to past studies, which revealed an H_0 of 0.63 (± 0.11) for Central American pumas and generally higher diversity levels for South American and lower ones for North American pumas (Culver et al. 2000). A study on pumas in southern Brazil, though, showed lower levels of genetic diversity (H_E = 0.61) and the authors explained that pumas suffered from

a bottleneck in the past and consequently lost their genetic diversity (Castilho et al. 2011, Castilho et al. 2012). Belizean ocelots had lower levels of genetic diversity compared to South American specimens (e.g. Grisolia et al. 2007), but higher ones than ocelots studied in northeastern Mexico and southern parts of the United States (Janecka et al. 2011b).

Genetic structure and gene flow

Despite severe habitat loss and fragmentation and extirpation from more than 50% of their historical distribution, jaguars and other Neotropical felids have maintained moderate to high levels of genetic diversity and little genetic differentiation has been detected across their range (e.g. Eizirik et al. 2001, Ruiz-Garcia et al. 2006). Moreno et al. (2006b) used four *Felis catus* microsatellite loci to estimate levels of genetic variability in jaguars and pumas in Brazil and found a similar or greater amount of genetic variability in wild felids compared to the domestic cat. Moreno et al. (2006b) concluded that although jaguars and pumas are considered to be threatened species, they still have moderate to high levels of genetic diversity. Jaguars generally do not show strong genetic structure for most areas across their range (Eizirik et al. 2001). Former delineation into eight subspecies for jaguars (Pocock 1939, Seymour 1989) was not confirmed by Eizirik et al. (2001). Instead, Eizirik et al. (2001) found no evidence for the existence of major geographical partitions. On a smaller geographic scale, Ruiz-Garcia et al. (2006) found incomplete isolation of jaguar populations in Colombia caused by the Andes mountains. Haag et al. (2010) examined levels of genetic diversity and genetic structure of jaguars among different local areas in the Atlantic forest region in Brazil, and

found that jaguars exist in four distinct genetic clusters and not in a single panmictic population.

Conservation genetic studies on pumas have been conducted primarily in the United States (e.g. Ernest et al. 2000, Ernest et al. 2003, McRae et al. 2005, Loxterman 2011, Holbrook et al. 2012, Wheeler and Waller 2012), and relatively little is known on the genetic status of this species in Latin America (e.g. Miotto et al. 2007, Castilho et al. 2011, Michalski et al. 2011, Castilho et al. 2012). A range-wide study on pumas and their genomic ancestry detected 6 phylogeographic groupings (or subspecies), with North American pumas being a homogenous group (*Puma concolor cougar*) (Culver et al. 2000). The remaining subspecies were located in Central America (*Puma concolor costaricensis*), eastern South America (*Puma concolor capricornensis*), northern South America (*Puma concolor concolor*), central South America (*Puma concolor cabrerae*) and southern South America (*Puma concolor puma*). Conservation genetic studies on pumas in the United States detected genetic structure on a fine landscape scale on several occasions. Overall, previous studies concluded that levels of genetic differentiation for pumas are low when habitat is relatively continuous (e.g. Culver et al. 2000), but increase when habitat becomes fragmented and disturbed (e.g. Ernest et al. 2003). Holbrook et al. (2012) described partitioning of pumas into three genetically differentiated groups in New Mexico and Texas, with pumas in southern Texas being the more isolated population. Walker et al. (2000b) also reported that puma populations in south and west Texas were genetically somewhat differentiated compared to pumas across their range. McRae et al. (2005) detected a strong north-south division for pumas in the southwestern United States including animals for the study from Arizona, Colorado, New Mexico and

Utah. Ernest et al. (2003) revealed distinct genetic subdivision between coastal and inland pumas in California caused by geographic barriers and isolation by distance. Loxterman's (2011) study showed that puma subpopulations in Idaho and surrounding areas did not act as a large panmictic population; the study area was rather separated by the Snake River floodplain, which acted as a barrier to movement.

Considering the dispersal capabilities of these large cats (relatively unknown for Neotropical jaguars and pumas, and assumed to be similar to e.g. North American pumas, Anderson et al. 1992, Sweanor et al. 2000, Stoner et al. 2008) and the presence of still-large tracts of forests, we did not expect to see strong genetic differentiation within Belize, which has only a total area of ~ 22,966 km^2 (Fig. 1). With the genetic structure study we mainly aimed to assess levels of contemporary gene flow between different sites across the country, and in doing so help prioritize future conservation and management efforts for wild felids in Belize. We used a variety of direct and indirect approaches and found evidence for weak genetic structure for all three targets species in Belize, with jaguars showing the lowest levels of genetic differentiation across the country, followed by ocelots and pumas. Non-spatial Bayesian clustering results obtained from program STRUCTURE showed that jaguars, pumas and ocelots do not exist in genetically distinct populations, but rather live in panmixia in Belize (Fig. 3 and 4). However, a spatial clustering approach conducted in program GENELAND indicated that both pumas and ocelot populations are separated in two distinct genetic clusters within the country of Belize. For pumas, we detected that the most northern site (Fireburn/Balam Na Nature Reserve) was genetically the most distinct one, and different from all other sites. Fireburn Nature Reserve is one of the more geographically isolated

protected areas we studied, but additionally this part of the country has been experiencing severe land conversion and forest loss in recent years. For ocelots, we detected that the north-central and central areas of the country are genetically differentiated and form their own distinct cluster. These forest habitats are in general surrounded by more open habitat and a higher degree of human developments and disturbance, thus movement from central to northern or southern site may be restricted (Fig. 6).

The other Bayesian clustering method we used in program TESS indicated low levels of genetic differentiation, which were more obvious when the admixture model was used. The admixture model in program TESS generally estimates fractions of individuals' genome, which originate from different parental populations (Chen et al. 2007). Although this approach does not detect "true" genetic clusters/populations, it gives insight into the spatial distribution of genomic ancestry ('ancestry groups'). In order to interpret results from the Bayesian clustering analysis, we mapped individuals based on the most likely number of *K* to see if genetic patterns match with distinct landscape features. Most of the differentiation we could observe was distributed between the northern (north and north-central) and southern (central, south-central, south) sites (Fig. 3 and 4), which are separated by a wide stretch of human-dominated landscape and the Belize Western highway in central Belize. Bayesian clustering analysis using the admixture model in TESS, showed that fractions of individuals' genome differed particularly between northern and southern sites (Fig. 4), indicating that the more-developed and human-dominated areas in central Belize affect movements of wild felids to some degree. We agree that program TESS overestimates ("oversplits") the number of K as reported earlier in the literature (e.g. Guillot 2009), nonetheless, we believe that the

distinct change in individual's composition of ancestry groups between certain areas (e.g. between north and south) gives indication for population structure on a fine landscape scale, which needs to be interpreted carefully.

Generally, when using different clustering approaches to infer population structure, it is strongly recommended to evaluate and consider estimates of *K*. A few studies reported that TESS has a tendency to report larger numbers of *K* than estimates obtained from other non-spatial Bayesian clustering methods (e.g. Guillot 2009), whereas program STRUCTURE also produces results which need to be interpreted with care. Pritchard et al. 2000 indicated that mean *LnP(K)* values in STRUCTURE only may be an indicator for the true number of genetic clusters and recommended to decide for the smallest value of *K* that captures the major structure in the data. Additionally, sampling design needs to be taken into consideration, since opportunistic sampling as well as sampling density may cause inconsistencies in clustering analysis (e.g. McRae et al. 2005), which may be the case in our study. False genetic structure can be caused by clustered sampling under the effect of isolation by distance and also by sampling of family groups (e.g. Anderson and Dunham 2008, Frantz et al. 2009, Schwartz and McKelvey 2009).

Contemporary gene flow analysis found evidence for recent dispersal events among our study sites. An estimation of first-generation migrants among study sites inferred three migrants for jaguars, four migrants for pumas and one for ocelots. Two of the jaguars were detected in central and south-central Belize, but originated from southern Belize. One male jaguar was detected in southern Belize (Golden Stream Corridor Preserve), and was genetically assigned to the most northern site, which is

located approximately 200 - 250 km away. To the best of our knowledge, this may be the first detection of a long-distance dispersal event for a jaguar in Central America based on a noninvasive study. For pumas, we detected two dispersers between north-central and the most northern site. One animal was detected in the Mountain Pine Forest Reserve in central Belize, but also originated from the most northern site. Finally, one puma was detected in the Golden Stream Corridor Preserve in southern Belize, and originated from the neighboring Cockscomb Wildlife Basin Sanctuary in south-central Belize. Generally, we could observe that most dispersers detected moved away from either the most northern or the most southern sites. Past research has suggested that one migrant per generation is sufficient to maintain sufficient levels of gene flow (e.g. Kimura and Ohta 1971). Others have suggested that 10 or more migrants per generation would be more appropriate (e.g. Vucetich and Waite 2000, Couvet 2002, Efremov 2006). Based on the results of our migration study, where we detected several dispersal events among different areas in Belize in the short-time frame of our study (relative to the long generation time of our target species), we believe that gene flow levels are still relatively high in most areas. Most dispersal events though were directional since animals moved away from most northern and southern sites of the country.

We also applied conventional *F*-statistics (Wright 1931) to infer population structure, and could observe low-to-moderate levels of differentiation (F_{ST} = 0.000 - 0.150). Moderate levels of genetic differentiation, which were significant, were observed for jaguars (F_{ST} = 0.093 - 0.094) and ocelots (F_{ST} = 0.100) between northern and southern sites (Fig. 6). Recent habitat loss and fragmentation in the country may have not yet produced effects measurable as genetic differentiation.

By using different approaches to examine genetic structure and assess gene flow levels, we observed that gene flow levels estimated for felids differed to some degree across the landscape of Belize. We do not include ocelots into the discussion here due to the limited sample size we detected for this species. For both large felids, we detected indication for weak genetic differentiation especially between northern and southern sites, which are separated by a ~ 50 km wide stretch of open and human-dominated landscape located in central Belize and a ~ 60 km long area in the northern part of the country, which has been heavily farmed and developed. Compared to jaguars, pumas additionally showed lower levels of genetic diversity between the most northern and southern sites and an overall higher number of private alleles. We observed higher percentages of related individuals within the most northern and southern sites and a generally lower occurrence of pairwise relationships estimated among all sites compared to jaguars. Jaguars showed the lowest genetic diversity levels at the northern and south-central sites and percentage of related individuals were also highest at northern sites. Overall, we could observe regional differences in genetic connectivity levels for both species, and a tendency for pumas to be less connected than jaguars. Generally, pumas are known to use a wider variety of habitats than jaguars (Sunquist and Sunquist 2002b), including more open and drier areas (e.g. Nunez et al. 2000), and are often considered opportunistic regarding their habitat use. Movement of pumas through disturbed and human-developed areas has been reported (e.g. Scognamillo et al. 2003, Larue and Nielsen 2008, Macdonald and Loveridge 2010). In contrast, jaguars have a tendency to avoid open areas and prefer dense forest habitats (e.g. Crawshaw and Quigley 1991). A previous study on habitat use of jaguars and pumas in Belize (Foster et al. 2010a)

concluded that pumas were less likely to be found outside of protected areas than to jaguars, which was opposite to findings of Vynne et al. (2011a) and Silviera (2004). Foster et al. (2010a) reasoned that differential tolerance to human disturbance and resource limitation may be responsible, and furthermore stated that pumas are not affected by direct persecution outside of protected areas. Davis et al. (2011) also reported that pumas, and jaguars to a lesser extent, were sensitive to human disturbance, even within protected areas in Belize. Several studies investigated effects of intra-guild competition and space partitioning between sympatric jaguars and pumas and concluded that overall habitat use by both species is comparable (e.g. Scognamillo et al. 2003, Harmsen et al. 2009, Foster et al. 2010a). Conservation genetic studies, which detected genetic structure for pumas on a regional scale, concluded that limited gene flow may be caused by several factors. McRae et al. (2005) explained that habitat barriers, such as lowland deserts, grasslands, agricultural and human developments, decrease levels of gene flow in the southwestern United States. Holbrook et al. (2012) detected genetic differentiation for pumas in Texas and New Mexico, but also showed long-distance dispersal events for this species. They concluded that mortality rates for dispersers are high due to human-wildlife conflicts in this area. Former fine-scale genetic structure studies on jaguars, which are still rare, detected remarkably high genetic differentiation for populations living at close geographical proximity (~ 69 to 500 km), but separated by a highly disturbed and human-dominated landscape (Haag et al. 2010). In conclusion, despite the dispersal capabilities of these large felids, they can be affected by habitat loss, fragmentation and increase of human developments on a fine landscape scale, suggesting that further anthropogenic changes will increase levels of genetic differentiation in

Belize. Northern and central Belize have been experiencing widespread land conversions over recent years, mainly caused by local communities, which have been expanding agricultural areas tremendously. Additionally, wildlife-human conflicts have been occurring in these areas, resulting in direct persecution of the two larger felids (particularly jaguars). In southern Belize, forest habitat has beendisappearing at an alarming rate. Local communities clear forest habitat to create pasture and croplands (local name *milpa*). Furthermore, development of infrastructure (e.g. roads) across the country has facilitated habitat loss and fragmentation (Chomitz and Gray 1996).

Conservation and management implications for wild felids in Belize

Interpreting the results from all analyses, we conclude that genetic diversity for wild felids in Belize is moderate to high and that levels of genetic differentiation are still relatively low. Although Belize has a high proportion of forest cover (~ 62.7%) and protected areas (~ 36%) compared to other Central American countries, we believe that levels of genetic differentiation are likely to increase if habitat loss and fragmentation continues at the current rate. We recommend prioritizing countrywide conservation and management efforts for wild felids based on multi-faceted research including results from conservation genetics studies. With Central America being one of the areas with the highest deforestation rates (> 2% yearly; Brooks et al. 2002) in the world, and a lack of genetic studies on wild felids in general, we recognize the need to conduct further genetic-based research of wild felids in these areas. Our study demonstrated that fecal DNA analysis is an efficient research approach to study and monitor levels of genetic diversity and structure of multiple Neotropical felids on a countrywide scale.

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Table 1 Total number of individual jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*) (including their sex) detected based on DNA markers in fecal samples detected across five geographical regions in Belize, Central America, 2007 - 2010. Geographical regions include: north (FB, Fireburn/Balam Na Nature Reserve; SNP, Shipstern Nature Reserve), north-central (RB, Rio Bravo Conservation and Management Area; BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay), central (MPR, Mountain Pine Ridge Forest Reserve; CFRNP, Chiquibul Forest Reserve and National Park; BFR, Bull Run Farm; HVR, Hidden Valley Reserve), south-central (CBWS, Cockscomb Basin Wildlife Sanctuary), south (BNR, Bladen Nature Reserve; BC, Boden Creek Ecological Preserve; GS, Golden Stream Corridor Preserve; MS, Machaca Hills; STNP, Sarstoon-Temash National Park). BCC, Belize Central Corridor (includes BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay). # Ind., number of individual jaguars, pumas, or ocelots; M, males; F, females.

		Jaguar			Puma			Ocelot		
Region	**Site**	**#Ind.**	**Sex**		**#Ind.**	**Sex**		**#Ind.**	**Sex**	
			M	**F**		**M**	**F**		**M**	**F**
North	FB + SNP	8	8	0	13	8	5	3	1	2
North-Central	RB	9	6	3	16	6	10	11	8	3
	BCC	7	7	0	3	2	1	1	0	1
Central	MPR + BFR	10	7	3	4	3	1	1	0	1
	CFRNP+HVR	8	7	1	6	3	3	6	0	6
South-Central	CBWS	15	15	0	7	6	1	7	6	1
South	GS + BC + BNR	5	5	0	1	1	0	1	0	1
	MH + STNP	3	2	1	4	3	1	0	0	0
Total		65	57	8	54	32	22	30	15	15

Table 2 Genetic diversity estimates across 14 microsatellite loci for jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*) across five geographical regions in Belize, Central America. N_A, number of alleles; A_R, allelic richness using the rarefaction method; H_O, observed heterozygosity; H_E, expected heterozygosity; F, fixation index; P_{HW}, P values for exact tests of Hardy-Weinberg equilibrium (level of significance, $\alpha = 0.05$); n = sample size.

	***Panthera onca* (n = 65)**						***Puma concolor* (n = 54)**						***Leopardus pardalis* (n = 30)**					
Locus	N_A	A_R	H_O	H_E	F	P_{HW}	N_A	A_R	H_O	H_E	F	P_{HW}	N_A	A_R	H_O	H_E	F	P_{HW}
F124	6	5.78	0.63	0.64	0.03	0.31	15	13.23	0.81	0.87	0.06	0.41	8	4.45	0.72	0.77	0.06	0.60
FCA391	6	6.00	0.62	0.71	0.13	0.25	7	6.88	0.52	0.65	0.20	0.11	5	3.04	0.43	0.64	0.32	0.00
FCA043	3	3.00	0.52	0.62	0.15	0.38	9	8.17	0.74	0.78	0.04	0.00	5	3.14	0.59	0.56	-0.06	0.81
FCA275	3	3.00	0.54	0.65	0.17	0.21	7	6.61	0.69	0.70	0.02	0.72	8	4.67	0.46	0.80	0.42	0.00
FCA096	7	6.92	0.74	0.74	-0.01	0.79	7	7.00	0.75	0.77	0.01	0.19	6	3.67	0.58	0.71	0.18	0.05
FCA126	6	5.90	0.76	0.64	-0.19	0.48	6	5.96	0.60	0.62	0.04	0.88	8	4.58	0.73	0.80	0.08	0.64
FCA090	5	5.00	0.59	0.59	0.01	0.75	9	8.34	0.79	0.83	0.04	0.07	5	3.72	0.76	0.74	-0.02	0.98
F85	4	3.95	0.40	0.55	0.28	0.04	10	9.85	0.71	0.85	0.17	0.02	9	4.08	0.83	0.76	-0.09	0.47
F98	3	3.00	0.50	0.56	0.10	0.36	3	2.94	0.22	0.26	0.18	0.01	6	3.76	0.70	0.73	0.04	0.79
FCA741	3	3.00	0.05	0.05	-0.02	1.00	10	8.47	0.71	0.73	0.02	0.07	5	5.00	0.25	0.78	0.68	0.01
FCA225	6	5.90	0.58	0.63	0.07	0.27	4	3.86	0.25	0.26	0.03	0.69	10	5.23	0.87	0.84	-0.03	0.56
FCA008	4	3.90	0.41	0.48	0.13	0.23	5	4.35	0.31	0.33	0.07	0.17	7	3.83	0.70	0.72	0.03	0.82
F53	8	8.00	0.75	0.77	0.02	0.40	14	14.00	0.80	0.84	0.04	0.39	5	3.34	0.62	0.63	0.02	0.53
FCA441	6	6.00	0.89	0.79	-0.13	0.09	6	5.63	0.67	0.67	-0.01	0.93	3	1.99	0.38	0.32	-0.20	1.00
Mean	5.00	4.95	0.57	0.60	0.05		8.00	7.52	0.61	0.65	0.07		6.43	3.89	0.62	0.70	0.10	
SE	0.44	0.44	0.05	0.05	0.03		0.93	0.86	0.06	0.06	0.02		0.52	0.23	0.05	0.04	0.06	

Table 3 Genetic diversity estimates across 14 microsatellite loci for jaguars (*Panthera onca*) from five geographical regions in Belize, Central America. Geographical regions include: north (FB, Fireburn/Balam Na Nature Reserve; SNP, Shipstern Nature Reserve), north-central (RB, Rio Bravo Conservation and Management Area; BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay), central (MPR, Mountain Pine Ridge Forest Reserve; CFRNP, Chiquibul Forest Reserve and National Park; BFR, Bull Run Farm; HVR, Hidden Valley Reserve), south-central (CBWS, Cockscomb Basin Wildlife Sanctuary), south (BNR, Bladen Nature Reserve; BC, Boden Creek Ecological Preserve; GS, Golden Stream Corridor Preserve; MS, Machaca Hills; STNP, Sarstoon-Temash National Park). N_A, number of alleles; A_R, allelic richness using the rarefaction method; H_O, observed heterozygosity; H_E, expected heterozygosity; n = sample size.

Jaguars	**North (n = 8)**				**North-Central (n = 16)**				**Central (n = 18)**				**South-Central (n = 15)**				**South (n = 8)**			
Locus	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E
F124	3	3.00	0.50	0.66	5	4.09	0.75	0.69	4	3.40	0.53	0.56	4	3.51	0.67	0.58	5	4.44	0.63	0.63
FCA391	3	3.00	0.57	0.64	4	3.75	0.73	0.68	4	3.90	0.63	0.74	5	3.93	0.53	0.66	3	2.86	0.57	0.56
FCA043	3	2.95	0.38	0.53	3	2.62	0.56	0.56	3	2.97	0.69	0.62	3	2.96	0.62	0.60	3	3.00	0.13	0.65
FCA275	3	2.70	0.38	0.32	3	2.99	0.56	0.66	3	2.98	0.72	0.65	3	2.99	0.47	0.64	3	2.95	0.38	0.60
FCA096	5	4.85	1.00	0.77	7	5.30	0.67	0.80	4	3.71	0.65	0.70	4	3.34	0.67	0.63	4	3.99	1.00	0.73
FCA126	4	3.98	0.86	0.66	4	3.26	0.69	0.55	5	3.84	0.59	0.57	5	4.15	1.00	0.71	5	4.44	0.75	0.63
FCA090	3	2.70	0.38	0.32	5	3.81	0.69	0.59	5	3.57	0.50	0.56	4	3.36	0.67	0.64	3	2.99	0.63	0.63
F85	3	2.99	0.57	0.52	3	2.38	0.33	0.41	3	2.62	0.44	0.55	4	2.80	0.40	0.53	3	2.86	0.29	0.56
F98	3	3.00	0.63	0.63	3	2.89	0.53	0.60	3	2.73	0.41	0.48	2	1.98	0.29	0.34	3	2.99	0.88	0.62
FCA741	1	1.00	0.00	0.00	1	1.00	0.00	0.00	2	1.40	0.07	0.06	3	2.00	0.17	0.16	1	1.00	0.00	0.00
FCA225	3	2.95	0.38	0.57	4	3.54	0.67	0.66	6	3.87	0.56	0.59	4	3.32	0.57	0.50	4	3.98	0.71	0.66
FCA008	2	2.00	0.29	0.41	3	2.36	0.38	0.42	4	3.20	0.41	0.53	3	2.74	0.47	0.42	3	2.95	0.50	0.53
F53	6	6.00	0.83	0.79	5	4.57	0.80	0.76	7	4.89	0.76	0.71	7	4.70	0.64	0.61	6	5.20	0.75	0.73
FCA441	4	3.99	1.00	0.74	5	4.65	0.79	0.76	5	4.75	0.88	0.79	5	4.65	0.91	0.75	5	4.85	1.00	0.76
Mean	3.29	3.22	0.55	0.54	3.93	3.37	0.58	0.58	4.14	3.42	0.56	0.58	4.00	3.32	0.58	0.55	3.64	3.46	0.59	0.59
SE	0.32	0.32	0.08	0.06	0.38	0.30	0.06	0.05	0.36	0.24	0.05	0.05	0.33	0.22	0.06	0.04	0.34	0.29	0.08	0.05

Table 4 Genetic diversity estimates across 14 microsatellite loci for pumas (*Puma concolor*) from five geographical regions in Belize, Central America. Geographical regions include: north (FB, Fireburn/Balam Na Nature Reserve; SNP, Shipstern Nature Reserve), north-central (RB, Rio Bravo Conservation and Management Area; BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay), central (MPR, Mountain Pine Ridge Forest Reserve; CFRNP, Chiquibul Forest Reserve and National Park; BFR, Bull Run Farm; HVR, Hidden Valley Reserve), south-central (CBWS, Cockscomb Basin Wildlife Sanctuary), south (BNR, Bladen Nature Reserve; BC, Boden Creek Ecological Preserve; GS, Golden Stream Corridor Preserve; MS, Machaca Hills; STNP, Sarstoon-Temash National Park). N_A, number of alleles; A_R, allelic richness using the rarefaction method; H_O, observed heterozygosity; H_E, expected heterozygosity; n = sample size.

Pumas	**North (n = 8)**				**North-Central (n = 16)**				**Central (n = 18)**				**South-Central (n = 15)**				**South (n = 8)**			
Locus	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E
F124	7	6.70	0.67	0.76	9	6.59	0.89	0.79	7	6.85	0.70	0.79	6	6.00	1.00	0.81	4	4.00	1.00	0.75
FCA391	5	4.58	0.67	0.56	5	4.11	0.59	0.65	5	4.90	0.40	0.71	3	3.00	0.17	0.29	2	2.00	1.00	0.50
FCA043	4	4.00	0.69	0.73	7	5.95	0.76	0.77	4	3.95	0.60	0.62	6	6.00	1.00	0.79	2	2.00	1.00	0.50
FCA275	6	5.57	0.77	0.63	6	5.22	0.63	0.65	5	4.95	0.80	0.77	3	3.00	0.29	0.58	4	4.00	1.00	0.70
FCA096	7	6.13	0.85	0.77	7	5.64	0.74	0.67	5	5.00	0.80	0.78	5	5.00	0.86	0.74	3	3.00	0.25	0.53
FCA126	4	3.93	0.67	0.61	6	4.70	0.53	0.58	4	4.00	0.70	0.64	5	5.00	0.43	0.55	3	3.00	0.75	0.53
FCA090	5	4.38	0.46	0.44	8	6.57	0.94	0.79	7	7.00	1.00	0.85	6	6.00	0.83	0.78	5	5.00	0.60	0.72
F85	7	6.85	0.80	0.79	6	5.49	0.67	0.77	6	6.00	0.67	0.77	6	6.00	0.80	0.82	3	3.00	0.50	0.63
F98	3	2.85	0.09	0.24	2	2.00	0.41	0.39	2	2.00	0.11	0.10	1	1.00	0.00	0.00	2	2.00	0.50	0.38
FCA741	5	4.67	0.77	0.72	4	3.53	0.61	0.68	5	5.00	0.67	0.75	6	6.00	1.00	0.78	2	2.00	0.50	0.38
FCA225	3	2.76	0.08	0.23	3	2.52	0.39	0.32	2	2.00	0.20	0.18	3	3.00	0.33	0.29	1	1.00	0.00	0.00
FCA008	3	2.76	0.25	0.23	3	2.75	0.37	0.38	5	4.90	0.50	0.54	2	2.00	0.17	0.15	1	1.00	0.00	0.00
F53	7	6.51	0.67	0.75	11	10.16	0.91	0.87	6	6.00	0.78	0.72	4	4.00	1.00	0.72	0	NA	0.00	0.00
FCA441	4	3.85	0.55	0.61	5	4.50	0.76	0.70	4	4.00	0.67	0.67	3	3.00	0.60	0.46	2	2.00	1.00	0.50
Mean	5.00	4.68	0.57	0.58	5.86	4.98	0.66	0.64	4.79	4.75	0.61	0.63	4.21	4.21	0.61	0.55	2.43	2.62	0.58	0.44
SE	0.42	0.39	0.07	0.06	0.66	0.56	0.05	0.04	0.41	0.40	0.06	0.06	0.46	0.46	0.10	0.07	0.36	0.33	0.11	0.07

Table 5 Genetic diversity estimates across 14 microsatellite loci for ocelots (*Leopardus pardalis*) from two geographical regions in Belize, Central America. Geographical regions include: north (FB, Fireburn/Balam Na Nature Reserve; SNP, Shipstern Nature Reserve; RB, Rio Bravo Conservation and Management Area; BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay), and south (MPR, Mountain Pine Ridge Forest Reserve; CFRNP, Chiquibul Forest Reserve and National Park; BFR, Bull Run Farm; HVR, Hidden Valley Reserve; CBWS, Cockscomb Basin Wildlife Sanctuary; BNR, Bladen Nature Reserve; BC, Boden Creek Ecological Preserve; GS, Golden Stream Corridor Preserve; MS, Machaca Hills; STNP, Sarstoon-Temash National Park). N_A, number of alleles; A_R, allelic richness using the rarefaction method; H_O, observed heterozygosity; H_E, expected heterozygosity; n = sample size.

Ocelots	**North (n = 15)**				**South (n = 15)**			
Locus	N_A	A_R	H_O	H_E	N_A	A_R	H_O	H_E
F124	7	6.17	0.86	0.82	7	5.21	0.60	0.67
FCA391	4	3.50	0.38	0.59	4	3.62	0.50	0.53
FCA043	5	4.14	0.57	0.61	4	3.42	0.62	0.48
FCA275	8	6.42	0.60	0.81	4	3.92	0.27	0.63
FCA096	5	4.32	0.62	0.71	5	4.50	0.55	0.70
FCA126	7	5.90	0.73	0.73	7	5.69	0.73	0.80
FCA090	4	3.76	0.71	0.71	5	4.37	0.80	0.73
F85	7	4.86	0.87	0.74	7	5.28	0.79	0.72
F98	4	3.66	0.73	0.66	5	4.19	0.67	0.68
FCA741	1	1.00	0.00	0.00	4	4.00	0.33	0.72
FCA225	7	6.27	0.92	0.83	9	7.47	0.82	0.83
FCA008	6	5.34	0.87	0.77	5	3.87	0.53	0.65
F53	4	3.42	0.54	0.48	5	4.42	0.69	0.71
FCA441	2	1.88	0.13	0.12	3	2.53	0.54	0.41
Mean	5.07	4.33	0.61	0.61	5.29	4.46	0.60	0.66
SE	0.55	0.44	0.07	0.07	0.44	0.32	0.04	0.03

Table 6 Summary of genetic diversity estimates for jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) across five geographical regions in Belize, Central America. Geographical regions include the following study sites for jaguars and pumas: north (FB, Fireburn/Balam Na Nature Reserve; SNP, Shipstern Nature Reserve), north-central (RB, Rio Bravo Conservation and Management Area; BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay), central (MPR, Mountain Pine Ridge Forest Reserve; CFRNP, Chiquibul Forest Reserve and National Park; BFR, Bull Run Farm; HVR, Hidden Valley Reserve), south-central (CBWS, Cockscomb Basin Wildlife Sanctuary), south (BNR, Bladen Nature Reserve; BC, Boden Creek Ecological Preserve; GS, Golden Stream Corridor Preserve; MS, Machaca Hills; STNP, Sarstoon-Temash National Park). Geographical regions include the following study sites for ocelots: north (FB, SNP, RB, BF, MFR, TSB), and south (MPR, CFRNP, BFR, HVR, BNR, BC, GS, MS, STNP). N_A, number of alleles; A_R, allelic richness using the rarefaction method; A_P, number of private alleles; H_E, expected heterozygosity.

Region	N_A			A_R			A_P			H_E		
	Jaguar	***Puma***	***Ocelot***	***Jaguar***	***Puma***	***Ocelot***	***Jaguar***	***Puma***	***Ocelot***	***Jaguar***	***Puma***	***Ocelot***
North	3.29	5.00	5.07	3.22	4.68	4.33	0	4	16	0.54	0.58	0.61
North-Central	3.93	5.86	---	3.37	4.98	---	3	10	---	0.58	0.64	---
Central	4.14	4.79	---	3.42	4.75	---	4	6	---	0.58	0.63	---
South-Central	4.00	4.21	---	3.32	4.21	---	4	6	---	0.55	0.55	---
South	3.64	2.43	5.29	3.46	2.62	4.46	2	4	19	0.59	0.44	0.66

Table 7 Pairwise F_{ST} estimates and associated P – values for: (A) jaguars (*Panthera onca*), (B) pumas (*Puma concolor*), and (C) ocelots (*Leopardus pardalis*) among five geographical regions obtained in program ARLEQUIN, version 3.5. (Excoffier and Lischer 2010). Above the diagonal (P – values), below the diagonal (pairwise F_{ST} estimates).

(A) Jaguar

Geograph. Region	1 North	2 North-central	3 Central	4 South-central	5 South
1 North	*	0.265	0.119	0.021	0.278
2 North-central	0.025	*	0.150	0.002	0.100
3 Central	0.050	0.025	*	0.186	0.334
4 South-central	0.094	0.093	0.022	*	0.737
5 South	0.028	0.049	0.012	0.000	*

(B) Puma

Geograph. Region	1 North	2 North-central	3 Central	4 South-central	5 South
1 North	*	0.105	0.084	0.218	0.062
2 North-central	0.038	*	0.146	0.629	0.191
3 Central	0.060	0.044	*	0.255	0.305
4 South-central	0.038	-0.025	0.031	*	0.079
5 South	0.148	0.062	0.031	0.131	*

(C) Ocelot

Geograph. Region	1 North	2 South
1 North	*	< 0.000
2 South	0.100	*

Table 8 Summary results of analysis of molecular variance (AMOVA) for jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) detected across five geographical regions in Belize, Central America and conducted in program ARLEQUIN, version 3.5 (Excoffier and Lischer 2010). *d.f.,* degrees of freedom, *P*, *P*-value for AMOVA.

(A) ***Jaguar***

Source of variation	*d.f.*	Sum of Squares	Variance components	Percentage of variation	*P*
Among Populations	4	9.24	0.06	3.93	0.021
Within Populations	60	91.28	1.52	96.07	
Total	64	100.52	1.58	1.00	

(B) ***Puma***

Source of variation	*d.f.*	Sum of Squares	Variance components	Percentage of variation	*P*
Among Populations	4	5.89	0.05	4.60	0.051
Within Populations	49	48.28	0.99	95.40	
Total	53	54.17	1.03	100.00	

(C) ***Ocelot***

Source of variation	*d.f.*	Sum of Squares	Variance components	Percentage of variation	*P*
Among Populations	1	5.37	0.22	9.95	0.003
Within Populations	28	56.53	2.02	90.05	
Total	29	61.90	2.24	100.00	

Table 9 Analysis for first-generation migrants using program GENECLASS, version 2.0 (Piry et al. 2004) for jaguars (*Panthera onca*), pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) across five regions (1 north, 2 north-central, 3 central, 4 central-south, and 5 south) in Belize, Central America. Bold values indicate fist-generation migrants with a probability value below 0.01. First-generation migrants are marked in bold letters.

(A) ***Jaguars***

Nr	Sample ID	Home	*-LOG(L_home / L_max)*	*P*	1 North *-log(L)*	2 North-central *-log(L)*	3 Central *-log(L)*	4 South-central *-log(L)*	5 South *-log(L)*
1	FBJ1	1	2.571	0.023	10.978	8.407	11.219	12.250	11.153
2	FBJ2	1	3.237	0.025	12.669	9.432	12.312	14.763	13.190
3	FBJ3	1	0.000	0.652	12.236	15.658	14.291	18.305	15.175
4	FBJ4	1	2.378	0.051	14.674	12.295	14.691	14.739	12.942
5	FBJ5	1	0.000	0.638	10.567	14.061	11.695	13.431	13.753
6	FBJ6	1	0.122	0.244	10.694	11.929	10.572	14.916	12.673
7	FBJ7	1	0.000	0.641	6.383	8.822	7.262	7.630	8.887
8	FBJ8	1	1.288	0.121	11.105	9.817	11.553	13.409	10.508
9	RBJ1	2	0.000	0.590	12.237	10.763	13.691	17.066	13.612
10	RBJ2	2	0.000	0.589	10.826	9.734	10.461	12.392	11.608
11	RBJ3	2	1.068	0.071	11.646	11.687	10.619	12.898	12.347
12	RBJ4	2	0.000	0.589	15.658	14.114	14.253	17.012	14.329
13	RBJ5	2	0.000	0.588	14.318	13.261	14.326	15.718	13.331
14	RBJ6	2	0.000	0.593	13.005	10.442	12.865	17.497	14.743
15	RBJ7	2	0.000	0.589	12.104	11.451	14.819	15.161	14.485
16	RBJ8	2	0.000	0.588	14.200	11.985	12.277	12.897	12.734
17	RBJ9	2	0.000	0.637	8.891	7.243	9.155	8.995	8.155
18	BZJ7	2	0.000	0.590	12.626	11.078	13.114	14.517	13.319
19	BZJ8	2	0.000	0.592	13.401	11.818	15.206	17.738	13.862
20	BZJ9	2	0.000	0.590	11.710	11.224	13.340	15.385	13.865

21	BZJ10	2	0.000	0.591	11.890	11.504	12.923	15.778	14.090
22	BZJ12	2	0.000	0.590	15.897	13.190	16.989	16.578	14.284
23	BZJ13	2	0.000	0.589	11.311	10.670	12.155	13.885	11.981
24	BZJ14	2	2.556	0.013	12.432	14.989	13.319	16.986	13.960
25	MPRJ1	3	0.113	0.443	12.197	11.802	9.980	9.867	10.872
26	MPRJ2	3	0.000	0.733	13.705	10.856	10.080	11.261	12.086
27	MPRJ3	3	0.000	0.738	18.015	15.471	13.055	14.083	14.676
28	MPRJ4	3	0.000	0.785	8.436	6.669	5.440	6.589	6.946
29	MPRJ5	3	0.000	0.736	11.817	12.012	9.341	10.583	11.503
30	MPRJ6	3	0.000	0.702	10.200	10.810	9.068	11.602	11.234
31	MPRJ7	3	0.671	0.349	6.435	8.512	7.106	6.455	6.772
32	MPRJ8	3	2.501	0.081	14.156	11.585	14.086	15.730	12.742
33	MPRJ9	3	1.697	0.167	15.382	16.823	13.407	11.710	13.417
34	MPRJ10	3	0.000	0.740	15.384	13.714	11.233	14.014	14.527
35	CBJ1	3	0.000	0.734	15.813	16.447	15.024	16.171	16.386
36	CBJ2	3	0.000	0.731	11.485	12.195	10.106	10.900	11.055
37	CBJ3	3	1.841	0.127	11.009	10.748	12.002	10.161	10.976
38	CBJ4	3	0.000	0.732	13.449	10.445	9.893	10.483	11.467
39	CBJ5	3	0.083	0.452	12.680	16.362	11.724	11.642	13.671
40	CBJ6	3	1.073	0.248	13.162	12.837	13.910	13.586	13.601
41	**CBJ7**	**3**	**4.432**	**0.007**	15.476	12.589	16.648	13.893	**12.216**
42	CBJ8	3	1.361	0.198	15.380	17.786	13.704	12.343	12.695
43	CCJ1	4	0.000	0.678	14.156	15.940	13.277	10.786	11.343
44	CCJ2	4	0.000	0.647	19.871	17.870	13.217	12.831	13.376
45	CCJ3	4	0.000	0.662	11.631	12.171	9.931	8.415	8.616
46	CCJ4	4	0.000	0.674	19.529	17.290	14.991	12.402	13.594
47	CCJ5	4	1.381	0.088	13.856	12.689	10.639	12.020	11.918
48	CCJ6	4	0.000	0.664	13.510	12.333	11.613	10.143	10.687

49	CCJ7	4	0.000	0.674	13.482	15.209	12.401	11.362	12.292
50	CCJ8	4	0.000	0.653	14.823	13.229	10.472	9.990	11.165
51	**CCJ9**	**4**	**4.715**	**0.001**	13.810	11.743	11.165	15.553	**10.838**
52	CCJ10	4	0.076	0.316	16.608	16.623	13.874	12.510	12.435
53	CCJ11	4	0.009	0.332	14.533	12.708	12.404	12.413	12.987
54	CCJ12	4	0.000	0.597	9.445	8.626	7.557	6.411	6.580
55	CCJ13	4	1.627	0.094	14.131	12.846	11.906	13.533	13.745
56	CCJ14	4	0.000	0.612	9.707	7.819	6.795	5.466	6.323
57	CCJ15	4	1.071	0.155	13.872	15.643	9.786	10.857	10.085
58	FBJ9	5	1.776	0.091	13.577	11.465	12.700	12.533	13.240
59	FBJ11	5	2.620	0.044	15.052	15.317	13.200	13.511	15.820
60	BZJ1	5	1.089	0.146	12.665	12.192	9.646	9.657	10.734
61	BZJ2	5	1.559	0.110	13.315	14.064	14.927	14.020	14.875
62	BZJ3	5	0.082	0.290	18.991	14.087	15.942	14.712	14.169
63	BZJ4	5	0.293	0.277	17.851	13.770	13.182	10.837	11.130
64	**BZJ24**	**5**	**5.235**	**0.003**	**8.688**	11.723	10.488	11.871	13.922
65	BZJ25	5	0.000	0.645	11.536	12.368	11.465	11.205	10.692

(B) ***Pumas***

Nr	**Sample ID**	**Home**	**-LOG(L_home / L_max)**	***P***	**1 North -log(L)**	**2 North-central -log(L)**	**3 Central -log(L)**	**4 South-central -log(L)**	**5 South -log(L)**
1	FBC1	1	0.000	0.563	9.556	10.545	15.675	15.702	18.899
2	FBC2	1	0.000	0.552	12.785	14.155	17.990	16.972	21.635
3	**FBC3**	**1**	**6.679**	**0.000**	19.363	**12.685**	16.319	12.800	18.292
4	FBC4	1	3.243	0.013	16.093	12.851	13.305	18.437	19.071
5	FBC5	1	0.000	0.560	11.331	15.828	16.531	19.987	21.834

6	FBC6	1	0.000	0.554	13.352	18.917	19.295	18.448	24.036
7	FBC7	1	0.000	0.561	13.595	15.592	16.357	21.455	23.358
8	FBC8	1	0.000	0.560	9.627	13.172	12.571	15.549	20.068
9	FBC9	1	0.000	0.560	9.635	10.924	9.932	11.619	16.699
10	FBC10	1	0.000	0.558	10.533	16.189	16.871	14.983	21.152
11	FBC11	1	1.730	0.031	15.912	16.078	14.183	15.583	15.183
12	FBC12	1	0.000	0.559	12.468	18.495	19.062	17.639	22.779
13	FBC13	1	0.000	0.560	10.605	12.627	15.419	17.174	21.035
14	RBC1	2	0.000	0.555	16.692	12.671	17.570	16.641	19.500
15	RBC2	2	0.000	0.564	14.408	13.299	16.786	18.373	18.881
16	RBC3	2	0.416	0.081	16.272	16.689	22.965	19.183	16.980
17	RBC4	2	0.000	0.552	18.112	15.224	15.322	18.692	22.747
18	RBC5	2	0.000	0.554	16.044	15.104	16.679	18.013	18.413
19	RBC6	2	0.000	0.553	15.520	12.597	13.713	14.158	20.086
20	RBC7	2	0.875	0.059	17.556	17.666	16.790	19.343	22.475
21	RBC8	2	0.000	0.553	22.587	18.109	21.692	20.690	21.614
22	RBC9	2	0.000	0.566	12.698	10.235	17.144	20.948	19.097
23	RBC10	2	0.000	0.576	15.665	12.585	15.734	19.097	20.364
24	RBC11	2	1.421	0.039	14.418	15.839	14.897	17.538	18.443
25	RBC12	2	0.000	0.557	14.775	10.317	16.832	18.064	19.191
26	RBC13	2	0.000	0.559	16.154	9.507	15.588	17.275	20.980
27	RBC14	2	0.000	0.559	14.072	10.273	16.160	16.673	15.829
28	RBC15	2	0.000	0.557	20.210	13.213	19.649	21.899	24.823
29	RBC16	2	0.000	0.556	16.300	11.104	18.099	14.897	16.315
30	BZC7	2	2.313	0.022	16.198	13.333	11.020	14.771	15.513
31	**BZC8**	**2**	**3.216**	**0.008**	**12.150**	15.366	13.368	12.390	15.167
32	BZC9	2	0.000	0.583	6.104	4.092	7.721	6.422	8.902
33	MPRC1	3	0.000	0.543	12.983	13.575	11.011	12.724	17.239

34	MPRC2	3	0.000	0.527	18.186	16.520	15.475	16.931	19.143
35	CBC1	3	0.000	0.527	16.797	15.903	15.512	17.445	17.802
36	CBC2	3	0.874	0.031	17.882	19.212	16.283	15.410	19.018
37	CBC3	3	0.008	0.054	16.280	15.998	14.944	14.936	17.836
38	CBC4	3	0.000	0.524	22.956	17.127	17.070	19.933	22.103
39	CBC5	3	0.529	0.040	20.462	19.929	15.949	15.421	22.350
40	CBC6	3	0.000	0.533	19.103	15.499	13.200	17.573	20.192
41	**BZC4**	**3**	**2.534**	**0.006**	**12.660**	18.271	15.194	17.308	24.060
42	BZC6	3	0.000	0.531	21.872	18.814	13.412	20.930	21.836
43	CCC1	4	0.000	0.585	14.971	14.310	14.213	13.904	13.945
44	CCC2	4	1.513	0.073	13.539	11.481	11.440	12.953	17.251
45	CCC3	4	0.362	0.138	18.987	17.744	15.299	15.660	19.352
46	CCC4	4	0.635	0.128	17.974	19.033	16.339	16.563	15.928
47	CCC5	4	3.364	0.016	14.524	16.589	12.502	15.866	17.786
48	CCC6	4	0.000	0.592	16.133	16.773	14.659	12.499	17.231
49	CCC7	4	1.915	0.047	11.909	12.329	11.647	10.125	8.210
50	FBC14	5	0.000	0.585	6.433	5.778	6.526	5.897	4.443
51	FBC15	5	0.822	0.166	8.684	4.660	8.210	7.682	5.482
52	BZC1	5	0.990	0.020	15.788	12.632	13.474	11.260	12.250
53	BZC2	5	0.000	0.559	10.759	9.906	10.214	8.631	8.449
54	**BZC3**	**5**	**4.870**	**0.000**	14.417	14.251	12.950	**9.672**	14.542

(C) *Ocelots*

Nr	Sample ID	Home	-LOG(L_home / L_max)	*P*	1 North -log(L)	2 South -log(L)
1	FBO1	1	0.000	0.514	19.819	21.641
2	FBO2	1	0.935	0.011	17.933	16.998
3	FBO3	1	0.000	0.518	12.396	17.606
4	**RBO1**	**1**	**1.727**	**0.005**	17.289	15.562
5	RBO2	1	0.000	0.522	9.292	12.989
6	RBO3	1	0.000	0.512	14.468	21.715
7	RBO4	1	0.000	0.512	13.290	15.357
8	RBO5	1	0.000	0.514	13.556	22.329
9	RBO6	1	0.000	0.514	14.436	19.637
10	RBO7	1	0.000	0.513	19.624	21.686
11	RBO8	1	0.000	0.515	10.446	17.290
12	RBO9	1	0.000	0.514	10.786	16.365
13	RBO10	1	0.000	0.520	10.469	11.835
14	RBO11	1	0.000	0.514	17.056	17.671
15	BZO2	1	0.000	0.513	12.492	14.674
16	MPRO1	2	0.000	0.544	12.204	11.385
17	CBO1	2	0.000	0.535	18.931	16.020
18	CBO2	2	0.000	0.533	14.377	12.851
19	CBO3	2	0.000	0.529	17.468	16.955
20	CBO4	2	0.000	0.529	17.192	15.517
21	CBO5	2	0.000	0.532	15.899	12.802
22	CBO6	2	0.000	0.524	14.943	14.234

23	CCO1	2	0.000	0.538	12.222	10.128
24	CCO2	2	0.000	0.539	14.358	13.663
25	CCO3	2	0.000	0.536	16.934	13.552
26	CCO4	2	3.174	0.001	10.073	13.247
27	CCO5	2	0.146	0.053	19.212	19.358
28	CCO6	2	0.000	0.535	17.685	11.678
29	CCO7	2	0.000	0.534	14.582	14.239
30	BZO1	2	0.091	0.052	15.860	15.951

Table 10 Proportions of unrelated and related (unrelated, half sibling, full sibling, *and* parent-offspring) individuals within and among five regions (1 north, 2 north-central, 3 central, 4 central-south, and 5 south) for jaguars (*Panthera onca*), pumas (*Puma concolor*), and ocelots (*Leopardus pardalis*) in Belize. Estimates were obtained in program ML-RELATE (Kalinowski et al. 2006). *U*, unrelated; *HS*, half-sibling; *FS*, full-sibling, *PO*, parent-offspring.

(A) ***Jaguars***

	1 North				**2 North - Central**				**3 Central**				**4 South - Central**				**5 South**			
	U	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***
1	**0.68**	**0.18**	**0.07**	**0.07**																
2	0.71	0.24	0.02	0.02	**0.67**	**0.28**	**0.04**	**0.02**												
3	0.82	0.15	0.03	0.00	0.85	0.13	0.01	0.01	**0.79**	**0.12**	**0.06**	**0.03**								
4	0.90	0.09	0.00	0.01	0.91	0.09	0.00	0.00	0.79	0.17	0.03	0.02	**0.70**	**0.24**	**0.04**	**0.03**				
5	0.78	0.19	0.02	0.02	0.82	0.16	0.01	0.02	0.84	0.13	0.01	0.01	0.75	0.18	0.03	0.05	**0.82**	**0.14**	**0.00**	**0.04**

(B) ***Pumas***

	1 North				**2 North - Central**				**3 Central**				**4 South - Central**				**5 South**			
	U	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***
1	**0.67**	**0.24**	**0.06**	**0.03**																
2	0.90	0.08	0.02	0.00	**0.79**	**0.13**	**0.04**	**0.05**												
3	0.92	0.08	0.01	0.00	0.94	0.05	0.01	0.01	**0.84**	**0.13**	**0.02**	**0.00**								
4	0.96	0.04	0.00	0.00	0.93	0.06	0.01	0.00	0.80	0.20	0.00	0.00	**0.81**	**0.14**	**0.05**	**0.00**				
5	0.92	0.06	0.02	0.00	0.77	0.15	0.03	0.05	0.88	0.12	0.00	0.00	0.63	0.23	0.03	0.11	**0.60**	**0.20**	**0.10**	**0.10**

(C) ***Ocelots***

	1 North				**2 South**			
	U	***HS***	***FS***	***PO***	***U***	***HS***	***FS***	***PO***
1	**0.76**	**0.18**	**0.01**	**0.05**				
2	0.91	0.08	0.01	0.00	**0.84**	**0.14**	**0.00**	**0.02**

Fig. 1 Map of main ecosystems in Belize, Central America

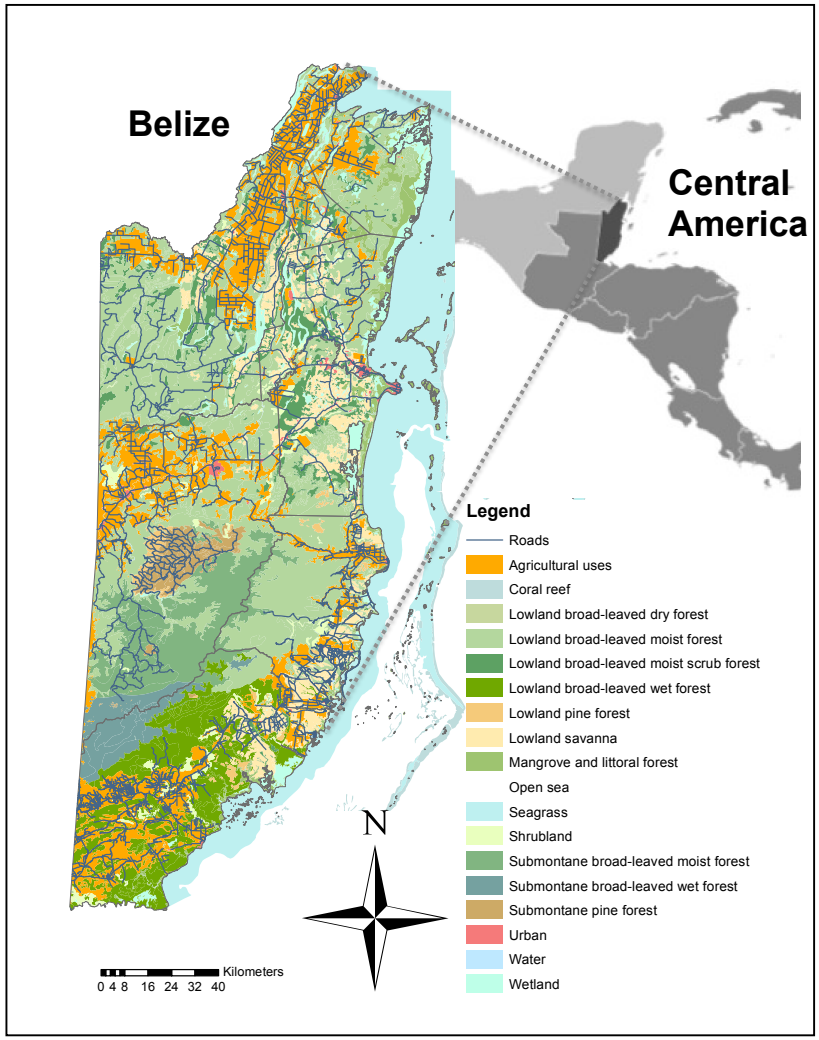


Fig. 2 Map of short- and long-term survey sites for Neotropical felids across various geographical regions in Belize, Central America. Geographical regions include the following study sites for jaguars and pumas: (A) north (FB, Fireburn/Balam Na Nature Reserve; SNP, Shipstern Nature Reserve), north-central (RB, Rio Bravo Conservation and Management Area; BF, Big Falls; MFR, Manatee Forest Reserve; TSB, Tiger Sandy Bay), central (MPR, Mountain Pine Ridge Forest Reserve; CFRNP, Chiquibul Forest Reserve and National Park; BFR, Bull Run Farm; HVR, Hidden Valley Reserve), south-central (CBWS, Cockscomb Basin Wildlife Sanctuary), south (BNR, Bladen Nature Reserve; BC, Boden Creek Ecological Preserve; GS, Golden Stream Corridor Preserve; MS, Machaca Hills; STNP, Sarstoon-Temash National Park). (B) Geographical regions include the following study sites for ocelots: north (FB, SNP, RB, BF, MFR, and TSB), and south (MPR, CFRNP, BFR, HVR, BNR, BC, GS, MS, and STNP).

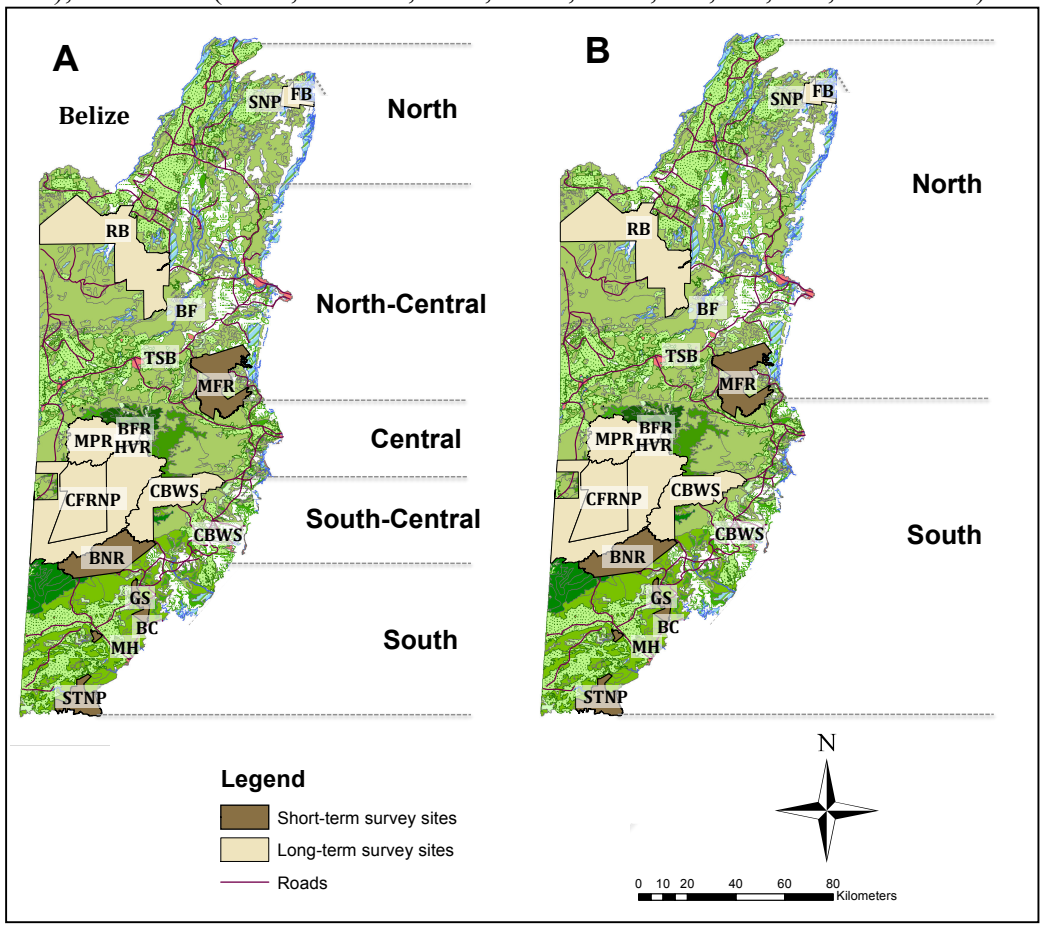
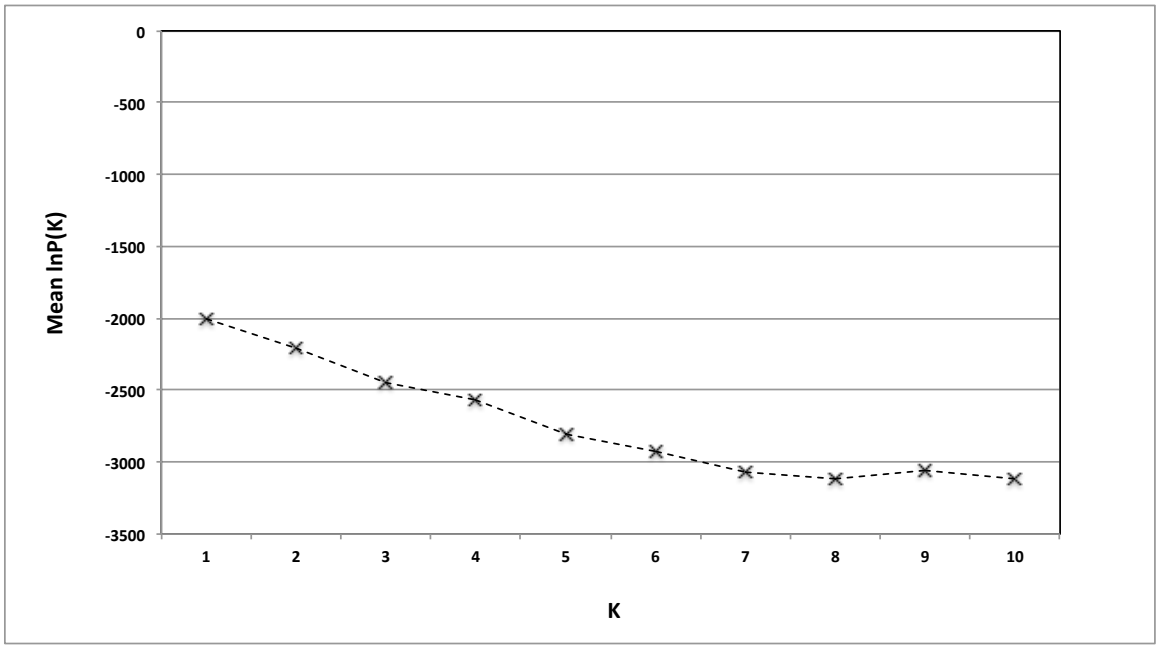
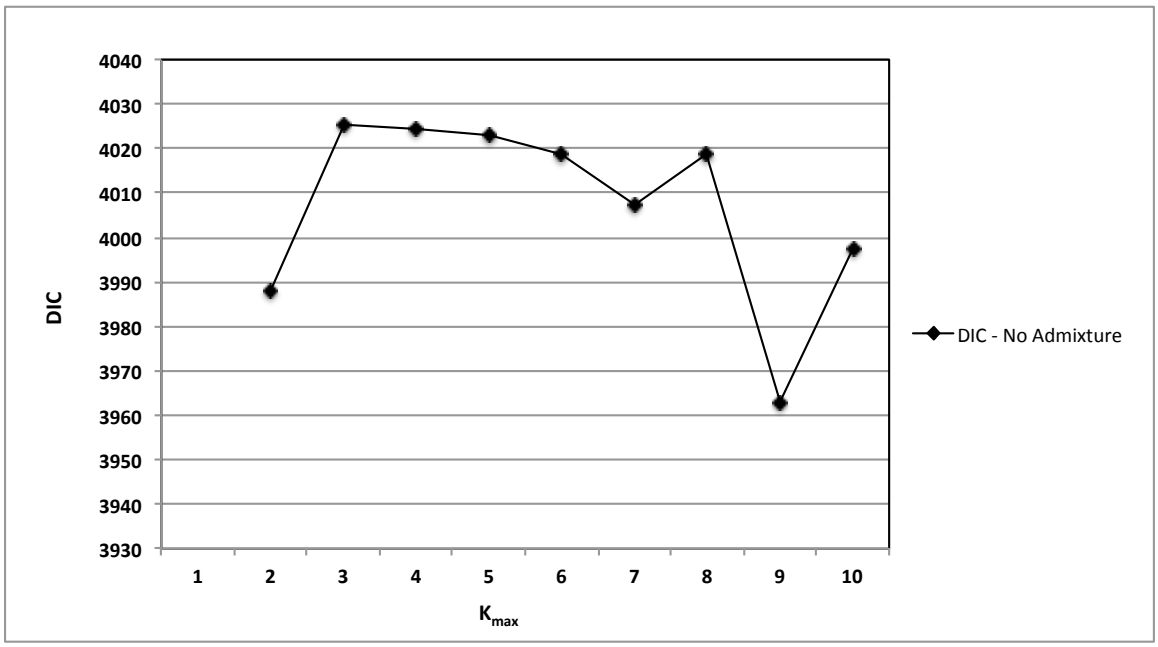


Fig. 3 Inference of the likely number of populations/genetic clusters (*K*) for: (1) jaguars (*Panthera onca*), (2) pumas (*Puma concolor*), and (3) ocelots (*Leopardus pardalis*) across five regions in Belize using two Bayesian clustering methods. Analysis was conducted for $K = 1$ to 10. I. Mean log likelihood (*lnP(K)*) (A) values obtained from program STRUCTURE, version 2.3.3 (Pritchard et al. 2000). II. DIC as function of K_{max} using program TESS, version 2.3 (Chen et al. 2007) and applying the Admixture (B) and No Admixture (C) models.

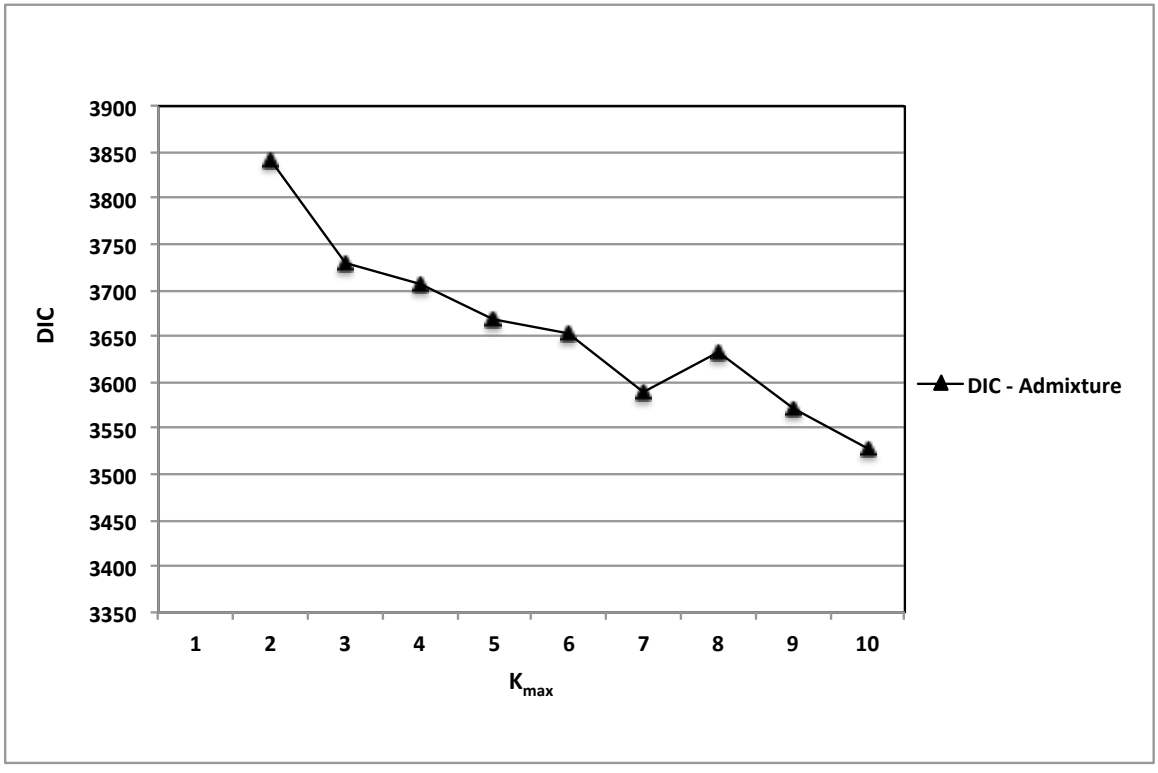
(I.) 1A - *Jaguars - STRUCTURE (Admixture Model)*



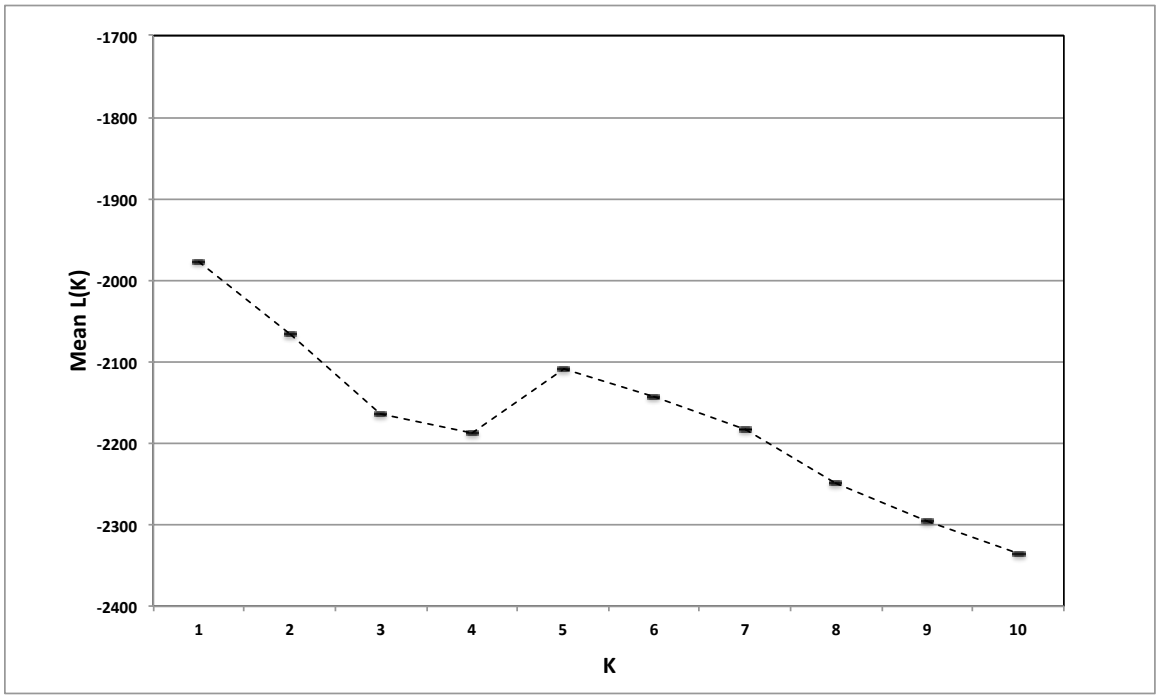
(II.) 1 B - *Jaguars –TESS (No Admixture Model)*



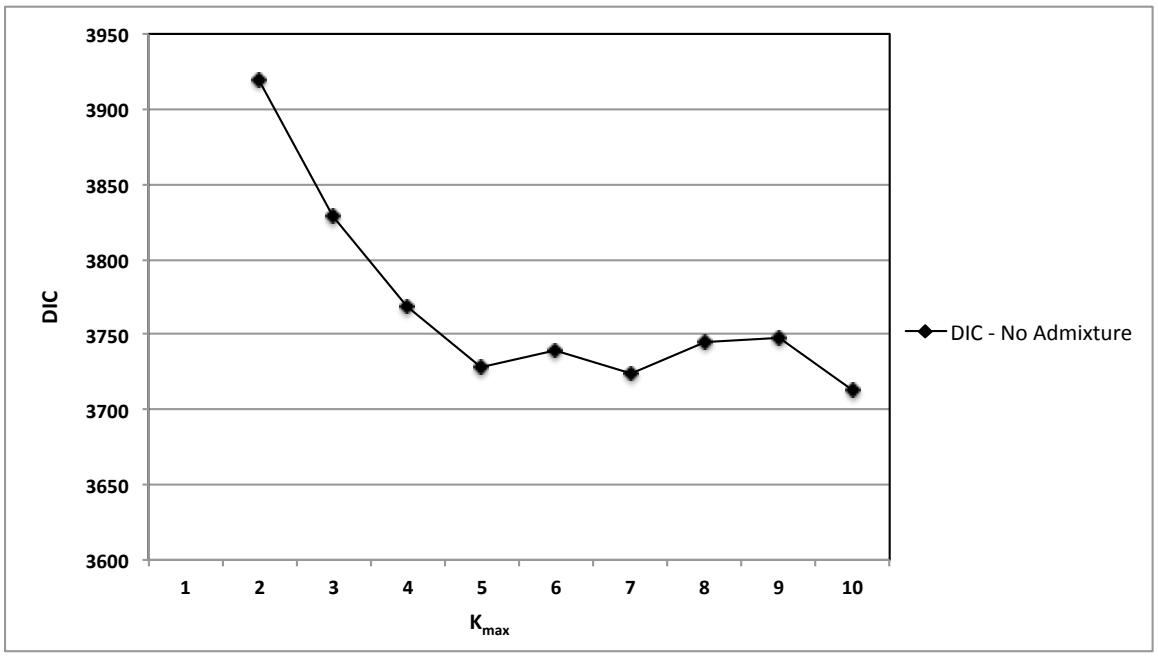
(II.) 1 C - *Jaguars –TESS (Admixture Model)*



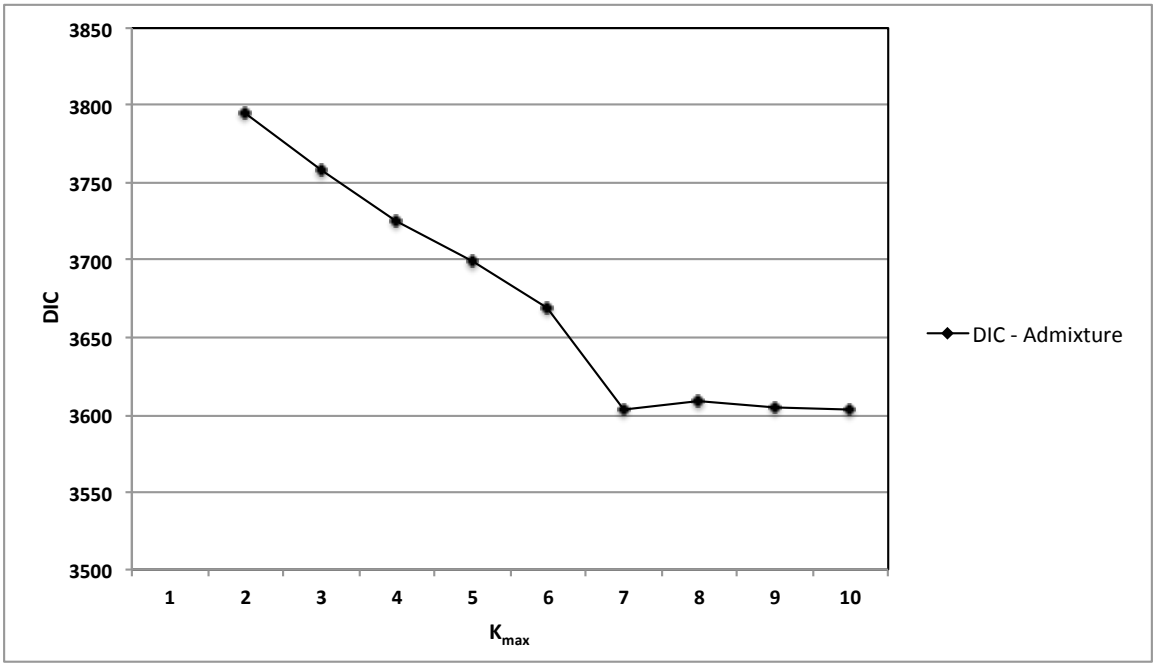
(I.) 2A - *Pumas - STRUCTURE (Admixture Model)*



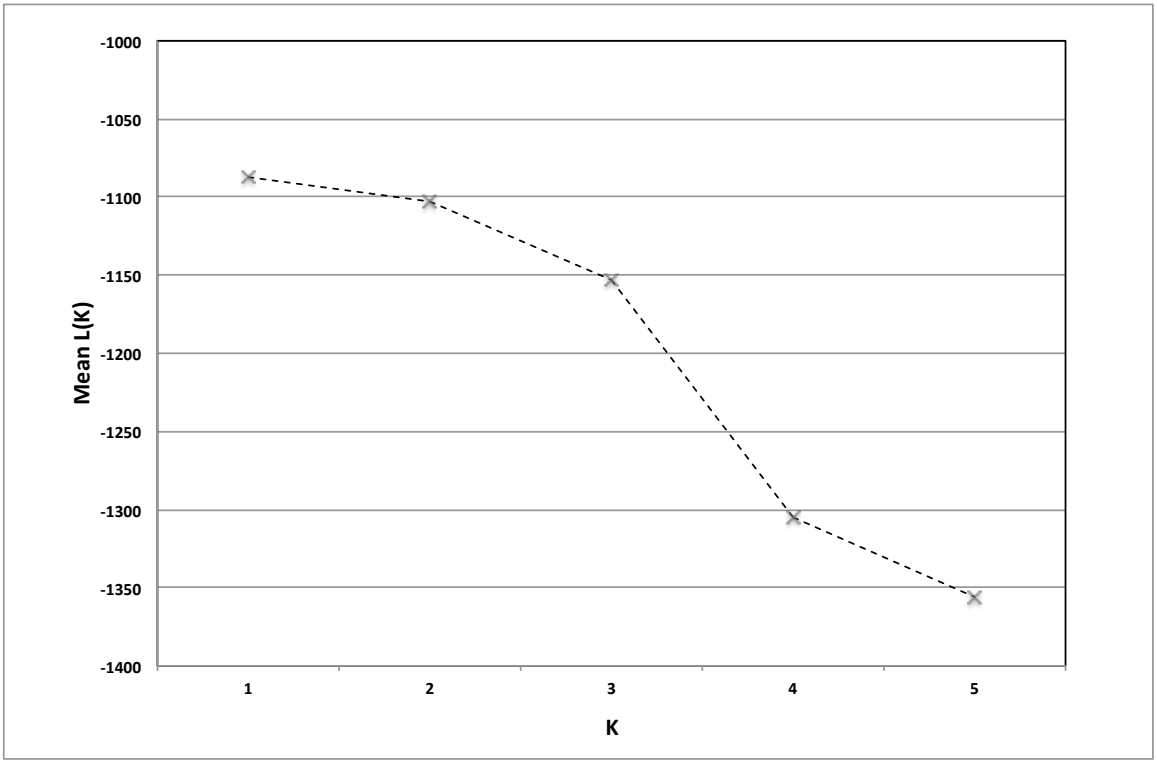
II.) 2B - *Pumas - TESS (No Admixture Model)*



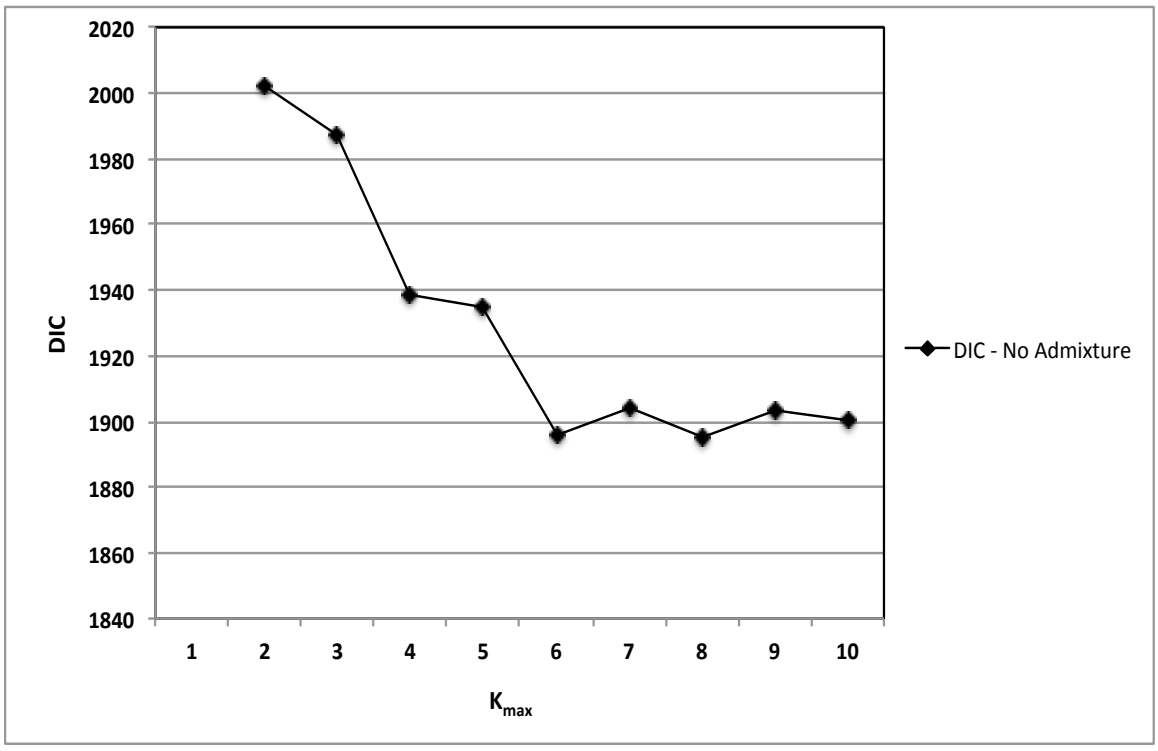
(II.) 2C - *Pumas - TESS (Admixture Model)*



(I.) 3A - *Ocelots - STRUCTURE (Admixture Model)*



(II.) 3B - *Ocelots - TESS (No Admixture Model)*



(II.) 3C - *Ocelots - TESS (Admixture Model)*

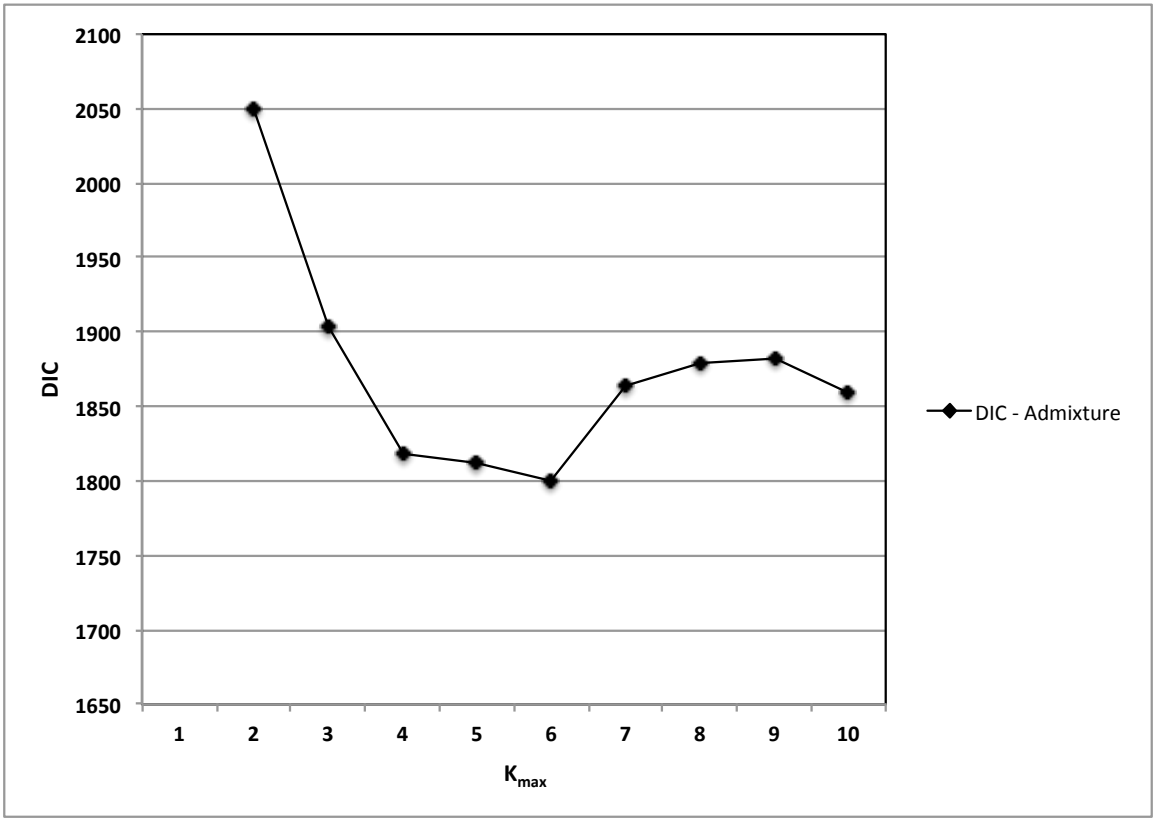
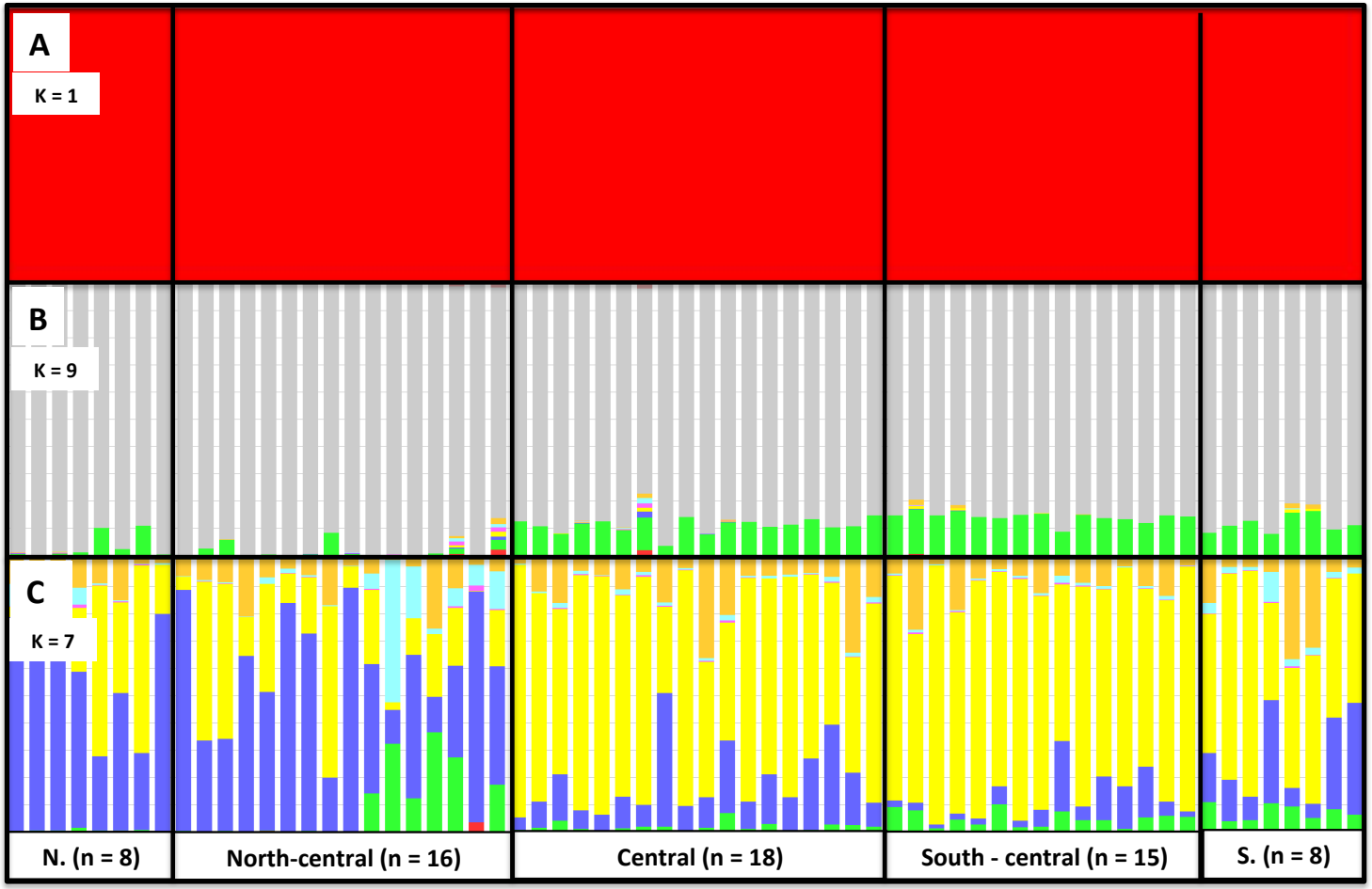
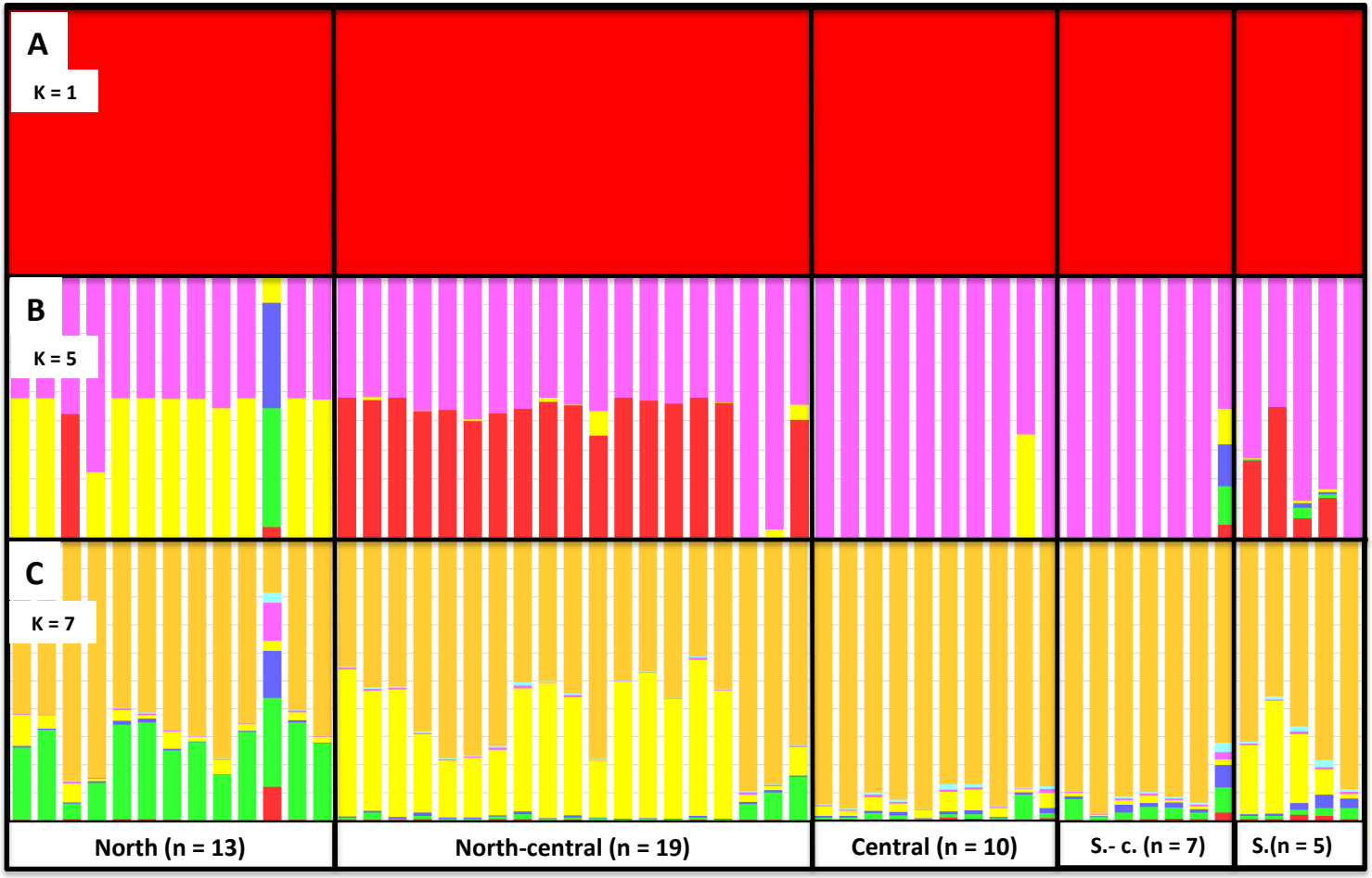


Fig. 4 Barplots results obtained from Bayesian clustering methods for (1) jaguars (*Panthera onca*), (2) pumas (*Puma concolor*), and (3) ocelots (*Leopardus pardalis*) across five regions in Belize, Central America. Inference for number of genetic clusters (*K*) was based on mean log likelihood (*lnP(K)*) (A) obtained from program STRUCTURE, version 2.3.3 (Pritchard et al. 2000). Inference for number of genetic clusters (K_{max}) using program TESS, version 2.3 (Chen et al. 2007) was based on average DIC values derived from the (B) No Admixture and (C) the Admixture models. Each bar represents one individual felid and the color of the bar represents the % of membership (*Q*) the individual belongs to different genetic clusters.

(1) ***Jaguars***



(2) ***Pumas***



(3) ***Ocelots***

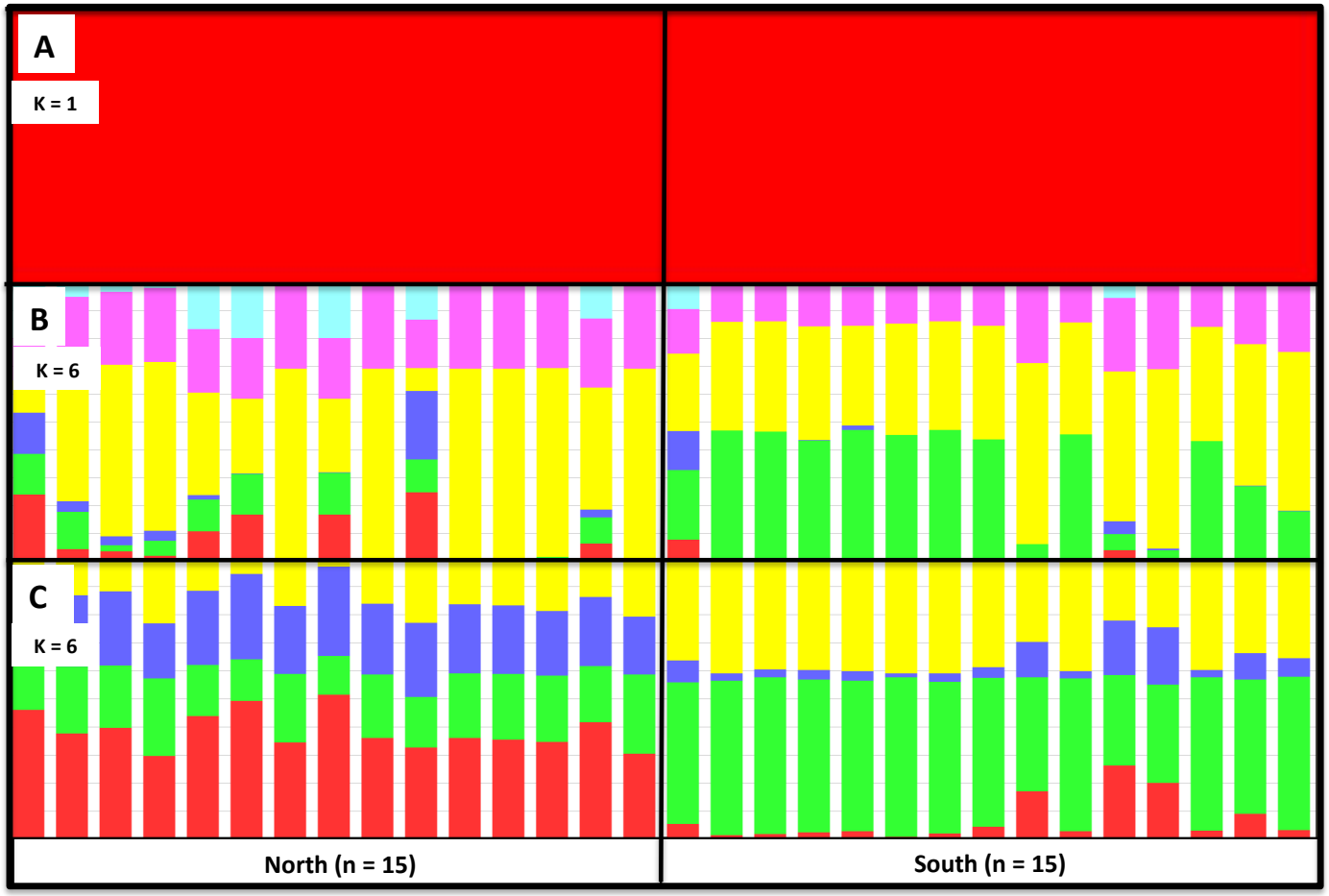
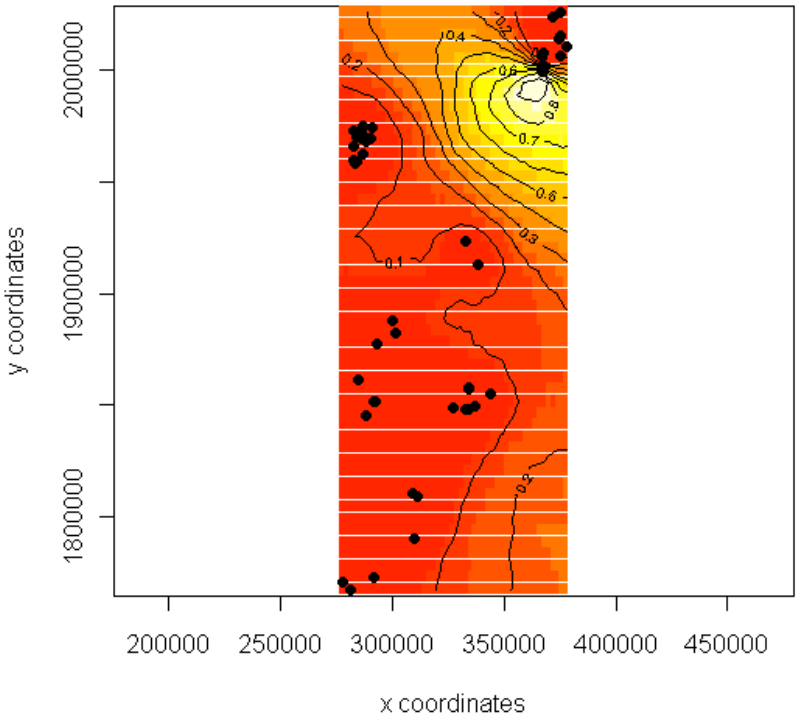


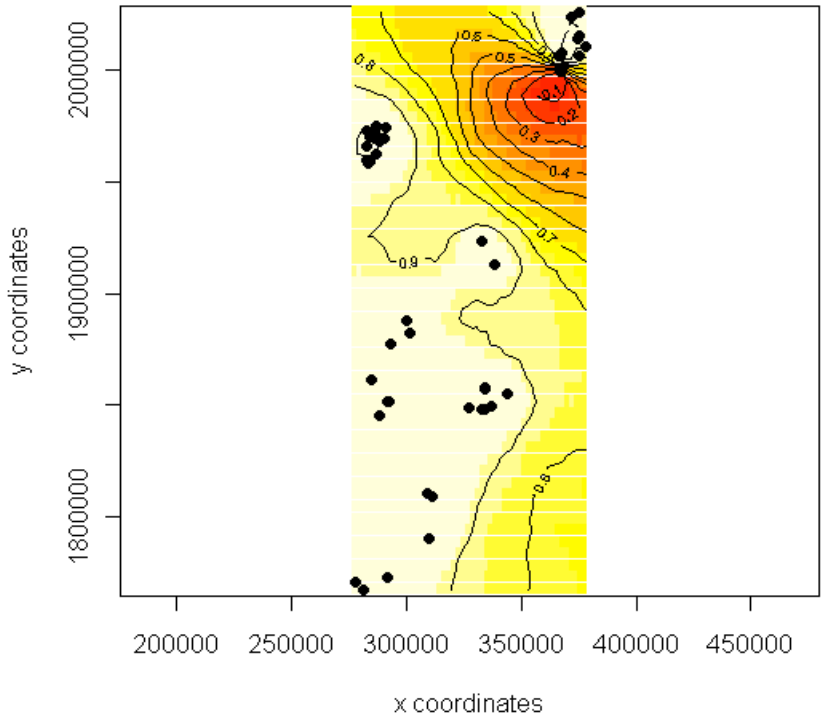
Fig. 5 Genetic assignment and maps of posterior probability belonging to distinct genetic clusters for (A) pumas (*Puma concolor*), and (B) ocelots (*Leopardus pardalis*) distributed across five geographical regions (north, north-central, central, south-central, south for pumas; north and south for ocelots) in Belize, Central America, in program GENELAND, version 4.0.3. (Guillot et al. 2005). *K*, number of distinct genetic clusters.

(A) Pumas (*K* = 2)

Map of posterior probability to belong to cluster 1

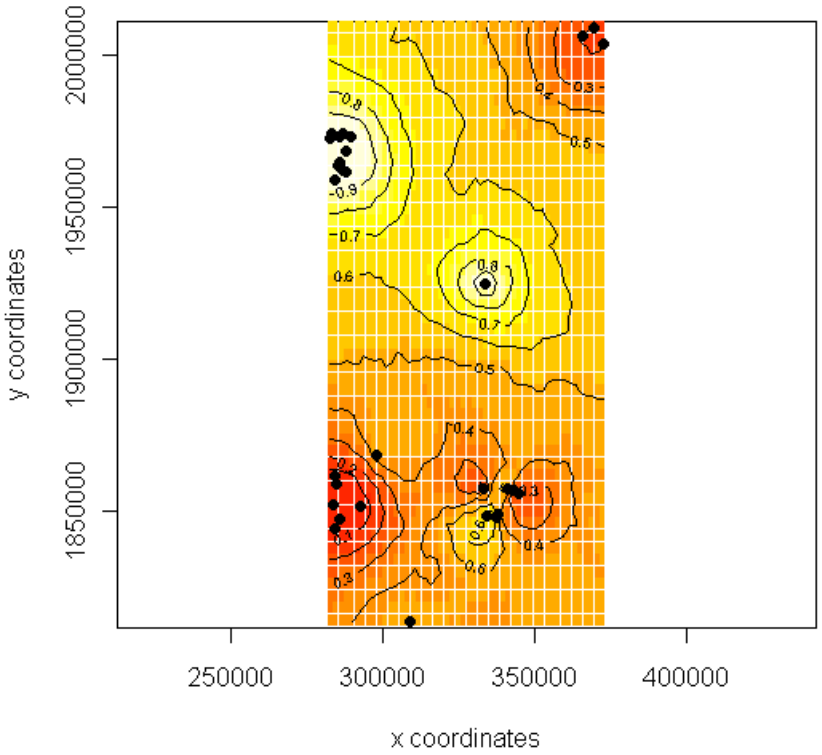


Map of posterior probability to belong to cluster 2



(B) Ocelots (K = 2)

Map of posterior probability to belong to cluster 1



Map of posterior probability to belong to cluster 2

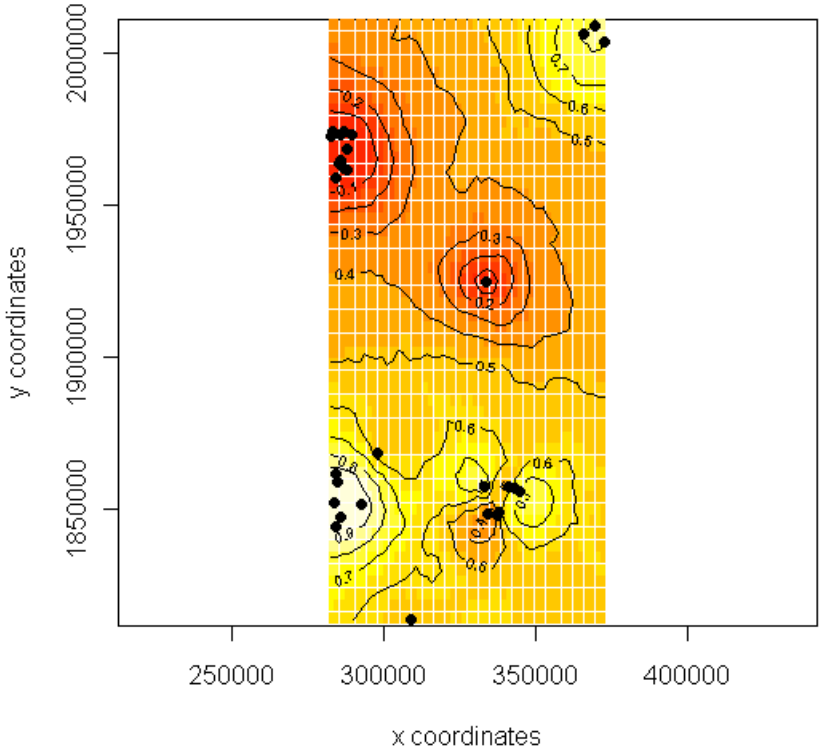


Fig. 6 Pairwise F_{ST} values calculated in program ARLEQUIN, version 3.5 (Excoffier and Lischer 2010) for: (A) jaguars (*Panthera onca*), (B) pumas (*Puma concolor*), and (C) ocelots (*Leopardus pardalis*) between five geographical regions (north, north-central, central, south-central, south for jaguars and pumas; north and south for ocelots) in Belize, Central America. *n*, sample size (number of individuals detected per species and site based on fecal DNA analysis); * indicates F_{ST} value is significant ($P < 0.05$).

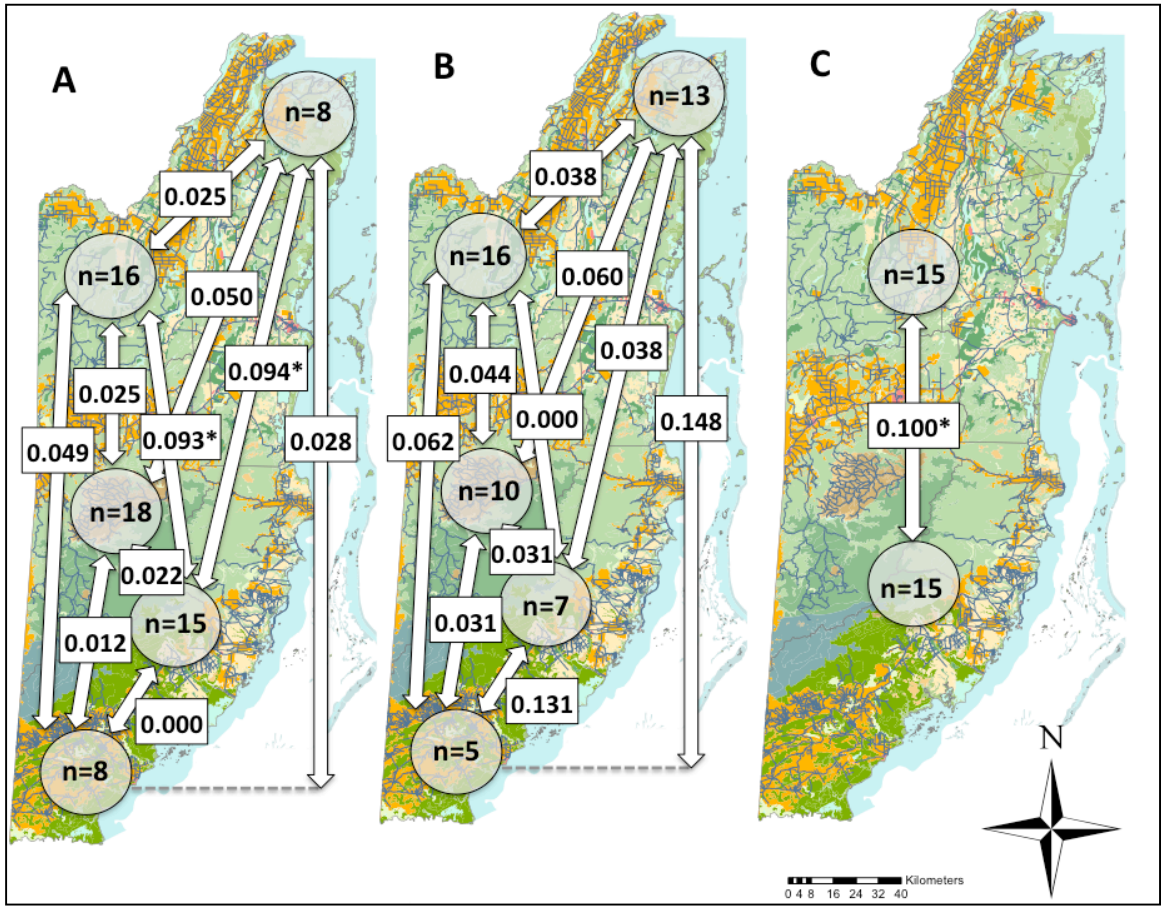
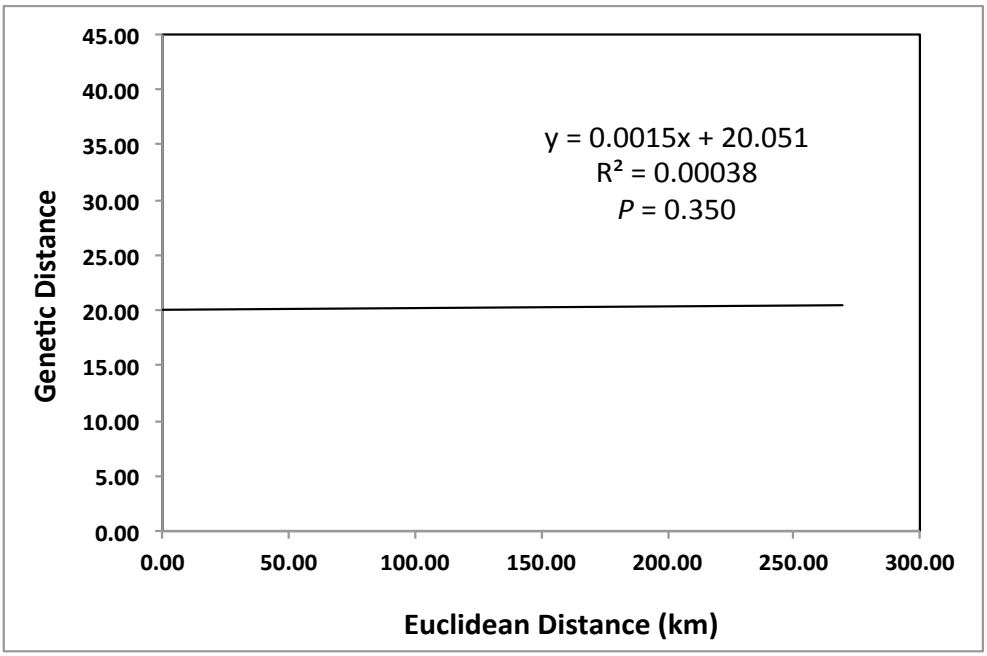
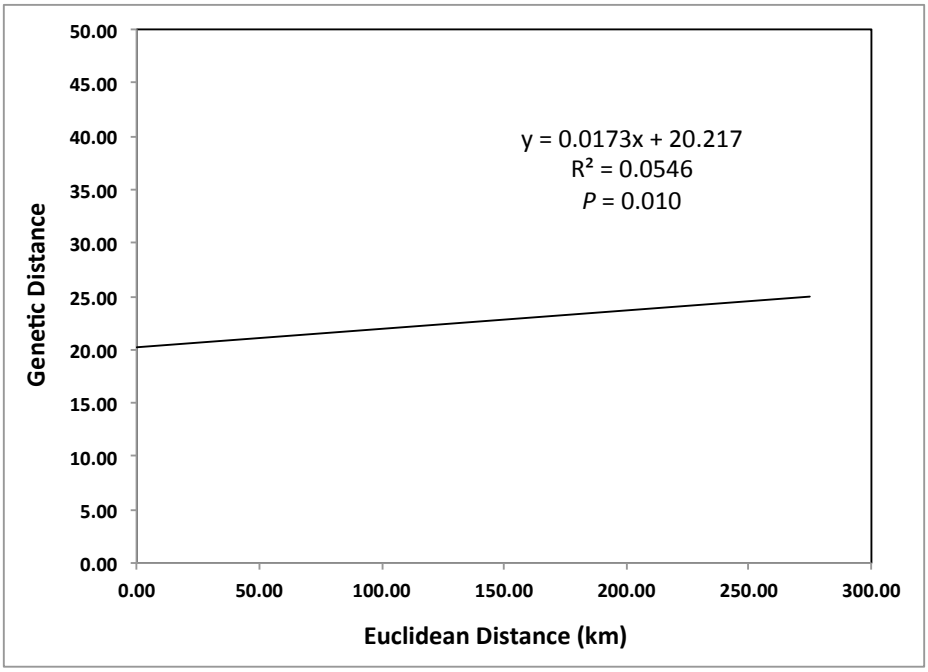


Fig. 7 Relationships between Euclidean distance and genetic distances for jaguars (*Panthera onca*) (Mantel test, $r = 0.002$, $R^2 < 0.000$, $P = 0.350$), pumas (*Puma concolor*) (Mantel test, $r = 0.017$, $R^2 = 0.055$, $P = 0.010$), and ocelots (*Leopardus pardalis*) (Mantel test, $r = 0.014$, $R^2 = 0.023$, $P = 0.010$) distributed across five geographical regions (north, north-central, central, central-south and south) in Belize, Central America.

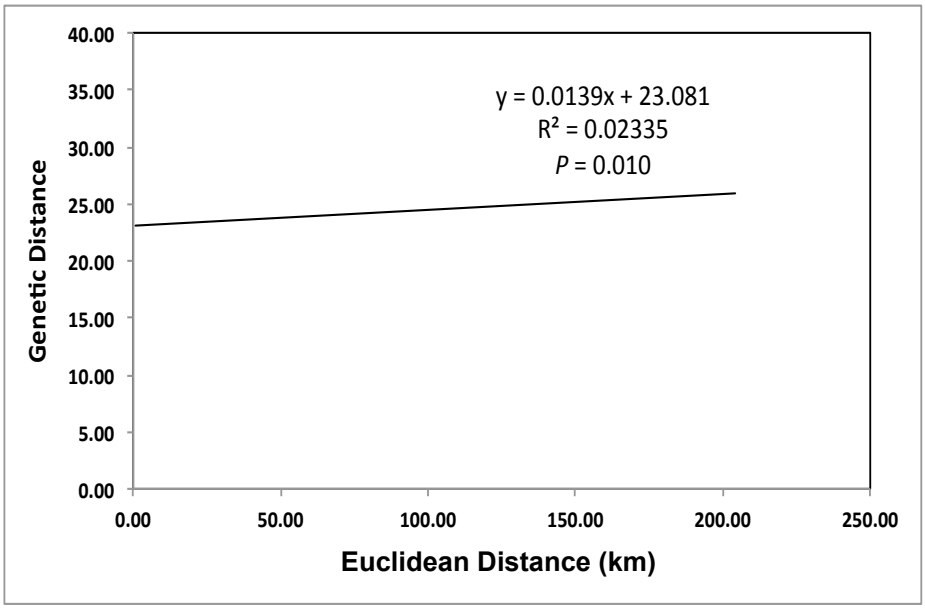
(A) ***Jaguars***



(B) ***Pumas***



(C) **Ocelots**



CHAPTER 5

Comparison of Population Density Estimation Methods for Sympatric Jaguars (*Panthera onca*) and Pumas (*Puma concolor*) in Belize, Central America using Non-invasive Genetic-Sampling, Remote-Camera Trapping and Capture-Mark-Recapture Models

Abstract

Population density is a crucial parameter in the field of wildlife conservation and management. Reliable density estimates for large carnivores, such as jaguars (*Panthera onca*) and pumas (*Puma concolor*), are particularly difficult to obtain due to their wide-ranging and elusive behaviors, occurrence at low density, and heterogeneous detection probabilities. Additionally, field survey techniques and analytical methods are advancing rapidly, making it difficult to choose the optimal survey approach and design for the target species and the environment in which it lives. We simultaneously conducted two noninvasive field surveys (remote camera trapping and molecular scatology with a scat detector dog) to study jaguars and pumas across five study sites in Belize, Central America from 2007 to 2009. We surveyed 5 sites and compared a variety of capture-mark-recapture (CMR) density estimators, including the conventional approach of estimating abundance ($\hat{N}$) in programs CAPTURE and MARK and dividing abundance by the effective trapping area (*ETA*), the recently developed spatially explicit capture-recapture (SECR) models, both the likelihood-based approach (ML-SECR) in program DENSITY and the Bayesian approach (B-SECR) in program SPACECAP, and finally the genetic-based mark-recapture one-sampling occasion estimator in program CAPWIRE. Although different survey methods using various density estimators produced similar density estimates, confidence levels and coefficients of variation varied, with SECR methods resulting in the least precise estimates. Detection probabilities were generally higher for noninvasive genetic sampling than for camera trapping. Both techniques were shown to be reliable and highly efficient survey methods for density estimation of low-density Neotropical felids living in challenging environments such as the tropics. While less precise, SECR CMR models are probably a more realistic reflection of our

uncertainty. We conclude that they hold great promise for density estimation studies for wide-ranging and territorial carnivore species, especially if precision can be improved through study design or analytic advancements in the future.

Keywords: Jaguar, puma, *Panthera onca*, *Puma concolor*, density estimation, capture-mark-recapture, molecular scatology, microsatellites, scat detector dog, remote-camera trapping, SECR, MMDM, Belize

Introduction

The jaguar (*Panthera onca*) and puma (*Puma concolor*) are the two largest felids in the Americas, and are generally extremely difficult to study due to their wide-ranging movements, crepuscular activity peaks, and occurrence at low population densities in dense forest habitats (Nowell and Jackson 1996). Listed as near-threatened (jaguars) and as least-concern species (pumas) on the IUCN (International Union for the Conservation of Nature and Natural Resources) Red List, some populations face local extirpation due to habitat loss, fragmentation and direct persecution in Central and South America (Caso et al. 2008). Our ability to promote for survival of these wild felids is hampered by our inability to obtain reliable information on their ecology (e.g. Nowell and Jackson 1996, Ruiz-Garcia et al. 2006, Macdonald and Loveridge 2010). Studying tropical felids often requires physical capture, handling and extensive subsequent monitoring, an approach, which is intrusive, expensive, time-consuming, and often dangerous. Additionally, physical capture usually results in low sample size that is not effective for inferring information relevant to conservation, such as estimates for population size and density. Noninvasive monitoring techniques, including remote camera trapping, have been developed in response to these inadequacies (Karanth 1995, Karanth and Nichols 1998). Camera-trapping, where wild cats are captured on film and identified by their distinct

coat patterns, has provided the first repeatable estimates of densities and sex-ratios for jaguars (e.g. Kelly 2003, Wallace et al. 2003, Maffei et al. 2004, Silver et al. 2004, Soisalo and Cavalcanti 2006, Salom-Perez et al. 2007, Kelly et al. 2008, Harmsen et al. 2009, Davis et al. 2011).

In recent years, molecular scatology studies, where individuals are genotyped from intestinal epithelial cells found in their feces (scat) (Hoss et al. 1992, Kohn and Wayne 1997, Kohn et al. 1999), have been added to the suite of noninvasive monitoring techniques for wild felids. This approach, where individuals are "genetically tagged" through highly variable microsatellite DNA markers, is applicable for identification of species, gender, and individuals, thus also can be used for population size estimation and monitoring (e.g. Kohn et al. 1999, Eggert et al. 2003, Frantz et al. 2003, Wilson et al. 2003, Waits and Paetkau 2005, Perez et al. 2006). Recently, molecular scatology studies have benefitted from increased scat-collection rates in field studies by using professionally trained scat detector dogs (e.g. Smith et al. 2001, Wasser et al. 2004, Harrison 2006, Long et al. 2007b, Dematteo et al. 2009, Vynne et al. 2011b, de Oliveira et al. 2012). Nonetheless, noninvasive genetic sampling studies of wild Neotropical felids are still rare (e.g. Haag et al. 2009, Michalski et al. 2011, Roques et al. 2011, Vynne et al. 2011a) and have been applied only to population size estimation on a very few occasions (e.g. Miotto et al. 2007).

Generally, population size and density estimation for carnivores is challenging (e.g. Kelly et al. 2012, Pollock et al. 2012). Choosing the optimal noninvasive survey approach for wild felids in challenging tropical environments is critical for providing reliable demographic information. Performance of noninvasive survey methods depends

on a variety of factors, including the target species, its biology and natural environment, survey design, and logistical constraints (e.g. Long et al. 2008, Lindberg 2012, Pollock et al. 2012), all of which need to be considered carefully when designing a new field study. Several recent carnivore studies simultaneously applied two or more noninvasive survey techniques, compared their efficiency, and reviewed advantages and disadvantages (e.g. Gompper et al. 2006, Harrison 2006, Long et al. 2007a, Rosellini et al. 2008, Hajkova et al. 2009, Vine et al. 2009, Janecka et al. 2011a, Galaverni et al. 2012). Most studies focused their evaluation of effectiveness and comparison of survey methods on detection rates only (e.g. Harrison 2006, Long et al. 2007a, Rosellini et al. 2008, Vine et al. 2009), while a few other studies also considered population size and density estimates (Bellemain et al. 2005, Solberg et al. 2006, Hajkova et al. 2009).

Analytically, population size and density estimation based on noninvasive wildlife surveys (e.g. remote camera trapping, noninvasive DNA sampling) and capture-mark-recapture analysis (CMR) use a variety of different statistical models, including classic, closed capture-recapture models with multiple discrete encounter occasions (Otis et al. 1978, White et al. 1982, Rexstadt and Burnham 1991), and the more recent spatially-explicit mark-recapture (SECR) models applied in a maximum likelihood (ML-SECR) (Efford 2004, Borchers and Efford 2008, Efford et al. 2009, Efford 2011) or a Bayesian (B-SECR) framework (Borchers and Efford 2008, Royle and Young 2008, Royle et al. 2009, Gopalaswamy et al. 2012, Thompson et al. 2012). Additionally, CMR models specifically targeting noninvasive genetic studies were develop to address genotyping error or continuous sampling design and single trapping occasions, where animals are randomly drawn from the population as opposed to the traditionally used multiple

discrete capture occasion models (Gazey and Staley 1986, McKelvey and Schwartz 2004, Lukacs and Burnham 2005a, Lukacs and Burnham 2005b, Miller et al. 2005, Petit and Valiere 2006, Knapp et al. 2009, Wright et al. 2009). Accumulation or rarefaction curve analysis also have been used to estimate animal abundance from noninvasive genetic data (e.g. Kohn et al. 1999, Eggert et al. 2003), but CMR techniques are considered superior (Lukacs and Burnham 2005b).

Overall, assumptions of classic CMR models, especially geographic closure, are violated often and thus population size and density estimations suffer from various biases (e.g. Lukacs and Burnham 2005b, Kelly et al. 2012, Pollock et al. 2012). Classic CMR models provide abundance estimates and thus require additional calculation of the effective trapping area (*ETA*) to obtain density estimates. A variety of methods have been used to estimate *ETA,* including the creation of minimum convex polygons around the trapping array or circular buffer areas around trap stations (e.g. Karanth and Nichols 1998, Kelly et al. 2012, Pollock et al. 2012). The size of buffer areas varies greatly based on the choice of buffer distances, which are based often on mean maximum distances moved (MMDM) representing the target species' home-range diameters. Most commonly, ½ MMDM values are applied (e.g. Karanth 1995, Maffei et al. 2004, Silver et al. 2004, Kelly et al. 2008), but full MMDMs also have been used by several studies (e.g. Parmenter et al. 2003, Soisalo and Cavalcanti 2006). Occasionally, density estimates are calculated using both buffer distances, ½ MMDM and MMDM (e.g. Trolle et al. 2007, Gerber et al. 2012). Consequently, the estimation of *ETA* size has been a problematic issue frequently discussed and questioned in the recent literature (e.g. Efford 2004, Dillon and Kelly 2007, Maffei and Noss 2008, Foster and Harmsen 2012, Kelly et al. 2012).

Large carnivores such as jaguars and pumas are wide-ranging, thus potentially move outside the study site and violate the population-closure assumption of traditional closed-population mark-recapture models (e.g. Karanth and Nichols 1998, Gardner et al. 2009). Additionally, the "edge effect" has been described, where animals which live only partially within the survey area get captured and thus inflate population estimates (White et al. 1982). Remote-camera trapping studies of jaguars and other large carnivores are often gender-biased and thus influence detection probabilities across individuals (e.g. Silver et al. 2004, Soisalo and Cavalcanti 2006).

Scat sampling in comparison is potentially also influenced by sex- and age-biased scent-marking behaviors (e.g. Dallas et al. 2003) and environmental factors (e.g. Norris and Michalski 2010, Cristescu et al. 2012), leading to heterogeneous detection probabilities. Noninvasive genetic studies face additional challenges when applied to population size estimation (e.g. Lukacs and Burnham 2005b). Low DNA quantity and quality obtained from noninvasive DNA sources is known to result in genotyping errors (e.g. Taberlet et al. 1996, Pompanon et al. 2005). Genotyping errors increase the chance of erroneous genotypes and misidentification of individuals, which potentially bias population estimates when not addressed appropriately (e.g. Taberlet and Luikart 1999, Taberlet et al. 1999, Mills et al. 2000a, Waits and Leberg 2000, Creel et al. 2003). According to Roon et al. (2005), genotyping errors may cause a bias of up to 200% of the population density estimate. Creel et al. (2003) also showed that genotyping error levels could potentially inflate the population size by five times. Thus, it is highly recommended that noninvasive genetic mark-recapture studies include thorough error-checking protocols and apply genetic markers powerful enough to accurately differentiate

among individuals (e.g. Waits and Leberg 2000, Broquet and Petit 2004, Lukacs and Burnham 2005a, Waits and Paetkau 2005). Additionally, depending on the noninvasive genetic sample type (e.g. scat) and environmental conditions of the study site, the time of scat deposition may be unknown, which may violate the geographical closure assumption (e.g. Lukacs and Burnham 2005b). This potential violation may be of less concern when fecal samples are collected in a tropical environment where degradation rates are usually high and fecal samples disappear quickly.

SECR models use information on capture locations of animals and estimate capture probabilities as a function of animal's home range center to the trap site. Specifically, SECR models combine a state model (distribution of activity centers of animals) and an observation model (spatial detection function) and estimate density by either using the maximum-likelihood (ML-SECR) or the Bayesian (B-SECR) method (e.g. Efford 2004, Borchers and Efford 2008, Royle and Young 2008). SECR models have been used primarily for density estimation using remote-camera trapping data (e.g. Royle et al. 2011, Noss et al. 2012), but recently also have been used for noninvasive genetic studies using hair (Gardner et al. 2009, Tredick and Vaughan 2009, Gardner et al. 2010, Kery et al. 2011) and scat samples (Thompson et al. 2012). Different density estimators have been compared to one other using remote camera trap (e.g. O'Brien and Kinnaird 2011, Sollmann et al. 2011, Gerber et al. 2012, Noss et al. 2012) and noninvasive genetic sampling data (e.g. Robinson et al. 2009, Obbard et al. 2010). Comparative analysis between spatial and non-spatial approaches showed that classic CMR models have a tendency to overestimate population densities compared to the SECR approach (Obbard et al. 2010, Gerber et al. 2012), which is particularly

problematic when the target species is at risk.

During our 3-year noninvasive mark-recapture study focusing on jaguars and pumas, we simultaneously conducted scat surveys (with scat detector dog) and remote-camera trapping across five study sites in tropical Belize, Central America. Specifically, our objectives were: (1) to compare population density estimators for sympatric jaguars and pumas across five study sites using noninvasive genetic sampling combined with traditional non-spatial, spatially explicit and genetic-based mark-recapture models. Additionally, we aim: (2) to compare detection effort, capture rates and population density estimates for jaguars across multiple study sites obtained from two noninvasive survey approaches (remote camera trapping and molecular scatology) and various density estimation techniques, and discuss their application for carnivore studies in challenging and remote environments such as the tropics. This is the first study empirically evaluating the impact of different noninvasive survey methods and analytical approaches used for population density estimation of Neotropical felids. Finally, we conclude with recommendations for future noninvasive studies aiming to demographically monitor secretive and elusive wild felids.

Methods

Study area

We conducted 2- to 3-month long scat and remote camera trapping surveys across five study sites (Mountain Pine Ridge Forest Reserve – MPR, Rio Bravo Conservation and Management Area – RB, Cockscomb Basin Wildlife Sanctuary – CBWS, Chiquibul Forest Reserve and National Park – CFRNP, Fireburn/Balam Na Nature Reserve – FB)

from 2007- 2009 across Belize, Central America (17°15' N, 88°45' W; Fig. 1, Table 1). All sites are part of the national system of Protected Areas in Belize. The study sites also fall within the forests of La Selva Maya (The Mayan Forest), a recognized biodiversity hotspot that forms part of the northern section of the Mesoamerican Biological Corridor. Across study sites, elevation ranges from 0 to 1120 m, and mean annual rainfall varies from 1524 mm in the north to 4064 mm in the south, with a pronounced wet season from June to December. Average annual temperatures fluctuate between 17.7 and 31.3 °C. A high diversity of native habitat types is represented within the study sites, including lowland and submontane broad-leaved moist and wet forest, lowland and submontane pine forest, mangrove and littoral forest, lowland savannah, shrub land, and wetland.

Noninvasive field surveys

Remote camera trapping

At each study site, simultaneous with the scat survey, we established a systematic grid of 23 to 47 passive-infrared camera stations, which were active between 63 to 102 days (Table 3, Fig. 2). The camera-trapping grid was systematically spaced at 3-km (± 200 m) intervals for jaguars. The spacing was based on the smallest home range recorded in Belize for one female radio-collared jaguar of 10 km^2 (Rabinowitz and Nottingham 1986). At four of the study sites, we also had a nested grid with spacing of 1.5 km between traps to target ocelots. Two opposing camera traps were placed at each camera station along existing roads, trails, game trails or freshly cut trails ~ 30 cm above on trees ground. The cameras were active 24 hours/day and operated for a minimum of a 60-day survey period per study site. The main camera types used in this study were DeerCam

DC200 and DC300 film cameras (DeerCam, Park Falls, WI, US) and Reconyx digital cameras (Reconyx, Inc., WI, US). We identified and sexed jaguars based on individual spot patterns, scars, and secondary sex characteristics from the photographic data obtained.

Scat detection and survey design

Fecal samples were detected by using a professional scat detector dog (PackLeader LLC, Gig Harbor, WA, USA) following training and handling protocols commonly applied for scent detecting (e.g. narcotics) and search-and-rescue dogs (e.g. Button 1990, Schoon 1996, Smith et al. 2001). The scat detector dog was trained prior to the field study to locate scat samples of all five native feline species (jaguars, pumas, ocelots, margays and jaguarundis). For the initial scent training, we used known fecal samples (from ≤ 10 individuals of both sexes and all target species) obtained from captive facilities (Belize Zoo, Belize; Feline Conservation Center, CA, US; Naples Zoo, FL, US). Opportunistic scat searches were conducted by the dog team (handler and scat detector dog), with the dog working off-leash to detect scat samples along roads, trails, game-trails, off-trail, and across various landscape features (e.g. streams) and habitat types during three main field seasons (June – August 2007, January – August 2008, April – July 2009). In the field, the dog detection team usually consisted of an additional 1-2 other people who help with orienteering (e.g. through rugged terrain), scat collection and carrying water and field gear, as needed.

For the CMR analysis, we superimposed each of the 5 study areas with a scat-sampling grid consisting of 29 - 46 (2 x 2 km) cells. We visited each cell once during

each sampling period and searched it opportunistically for ~ 2 km with the scat detector dog (Fig. 2). Detection effort was kept constant across cells by measuring survey km using a hand-held Global Positioning System unit. Each sampling period lasted 8-10 days and represented one "encounter occasion" in CMR capture histories. Each study site was sampled 4 - 5 times to create 4 - 5 encounter occasions for subsequent CMR analysis (Table 2).

Fecal DNA sampling

From each scat, one ~ 0.5 mL sample was collected and stored at ambient temperature in sterile 2mL screw-top tubes filled with DET buffer (Seutin et al. 1991) at 1: ≥ 4 ratio by volume. Scat samples were collected using disposable gloves and wooden sampling sticks.

Laboratory analysis

Fecal DNA extraction, microsatellite amplification and genotyping

Fecal DNA extractions were conducted in a separate room at the Laboratory for Ecological, Evolutionary and Conservation Genetics (LEECG) at the University of Idaho (Moscow, ID, US). A total of 14 highly polymorphic microsatellite loci (multiplex 1 - F124, FCA391, FCA043, FCA275, FCA096, FCA126, and FCA090; multiplex 2 - F85, F98, FCA741, FCA225, and FCA008, and multiplex 3 - F53 and FCA441), optimized for Neotropical felids in Belize, were used for species and individual identification following laboratory and analytical protocols described in detail by Wultsch et al. (in review).

Additionally, genotyping errors were minimized by applying various error-checking protocols (Wultsch et al. in review). Genotypes, which were only detected once were further examined using the software RELIOTYPE (Miller et al. 2002) by assessing their reliability. If their accuracy in multi-locus scoring was below 95%, additional PCR replicates were conducted. To verify individual identification, we used probability of identity estimates to assess statistical confidence for individual identification with $P_{(ID)sib} < 0.010$ as the deciding criterion (Mills et al. 2000a, Waits et al. 2001).

Data analysis

Comparison of two noninvasive field methods

Performance of the two noninvasive field methods (remote camera trapping vs. molecular scatology) was evaluated by comparing overall detection effort and success. For remote camera trapping, we reported the total number of camera stations, total survey duration in days, total camera trap nights, mean trap nights per camera station, and number of photographs captured per survey day. For capture rates, we calculated the number of photographs, number of jaguar or puma captures, number of individual jaguars captured, average number of observations per individual jaguar, and trap success for jaguars as capture events per 100 trap nights across five study sites. The number of capture events equals the number of identifiable animals in photographs. Animals that were captured within 30 minutes at the same station were considered to be one photographic event (Kelly 2003, Silver et al. 2004).

For the molecular scatology study, we reported the total survey distance in km, survey distance in km to detect one scat sample, total survey duration in days, number of

days to detect on scat sample, number of scat samples detected per survey day, and the number of scat samples it took to capture one jaguar or puma. Additionally, we reported the number of scat samples detected, number of jaguar or puma captures, number of individual jaguars or pumas captured, and the average number of observations per individual jaguar or puma. Statistical differences between groups were evaluated with Student's *t* - tests in program R, version 2.15 (R Development Core Team 2009).

Furthermore, we conducted a demographic study on jaguars and pumas by simultaneously using these two noninvasive field survey methods. We used eight different methods to estimate population densities. Their 95% confidence intervals (*CI*) were used to test for differences in density estimates among methods, study sites and target species. Non-overlapping 95% confidence intervals were interpreted as indicating a substantial difference between estimation techniques. Coefficients of variation (*CV*) were calculated to assess and compare levels of precision among density estimators.

CMR capture histories – single versus multiple session models

For CMR analysis, each photograph from an individual felid or genotype from a scat sample (with successful individual identification using microsatellite primers) represented a single capture. All captures were used to develop capture histories of all individuals for each species within each study site. For classic CMR models, we recorded whether an individual had been detected (e.g. photographs, scats with successful individual ID) or not (1, detection; 0, non-detection) during an encounter occasion. For the camera trapping survey, we increased low detection rates of jaguars by collapsing the initial one day-long encounter occasions of the photographic data into multiple day-long

encounter occasions based on the closure test and the stability of abundance estimates obtained in program CAPTURE (White et al. 1982, Rexstadt and Burnham 1991, Dillon and Kelly 2007). For the scat survey, scats were collected during 4 or 5 predefined encounter occasions (each 8 – 10 days) ("multiple session model"). For the SECR and CAPWIRE models, the number of detections per individual and species was counted for one overall sampling occasion ("single session model").

Abundance and density estimation

Conventional closed CMR models

Abundance estimates were calculated using traditional closed mark-recapture models in program CAPTURE (Otis et al. 1978, White et al. 1982, Rexstadt and Burnham 1991) and MARK, version 6.0 (White and Burnham 1999). Population density estimates ($\hat{D}$) then were calculated by dividing the abundance estimate for each species and study site ($\hat{N}$) by the *ETA*. For the remote camera trapping survey, *ETA* was determined by applying a buffer value equal to ½ and full mean maximum distance moved (½ MMDM and MMDM) among camera locations for all individuals recaptured at least once (Dice 1938, Wilson and Anderson 1985, Karanth and Nichols 1998), which was added around camera stations to calculate the size of the total survey area. For the scat survey, we used the same method to calculate *ETA* by creating buffer values (½ MMDM and MMDM) around the centers of the scat survey grid cells (Fig. 3). *A priori* hypotheses were tested to evaluate the most important factors influencing detection probabilities, including heterogeneity, sex, behavior, and time for both photographic and genetic data collected on jaguars and pumas.

PROGRAM CAPTURE

We ran all available models in Program CAPTURE with our *a priori* reasoning noted below. We ran the discriminant function analysis (*DFA*) within CAPTURE for model selection.

- *M(o)*: null model - detection probabilities remain constant over time, between sexes and among individuals.
- *M(b*): behavior model – detection probability varies caused by "trap" response (e.g. for cameras-trapping – trap-shy or trap-happy due to curiosity or aversion to foreign scent and human activity, presence of camera equipment and camera flash, disturbed vegetation; for genetics – defecation rate or scat location may be influenced by foreign scent/human and or scat detector dog, overall human activity and disturbance).
- *M(t):* time model – detection probability varies over time and across encounter occasions caused by seasonal changes (e.g. presence of jaguars influenced by water level, prey availability, hunting or other anthropogenic impacts; scat detection influenced by weather conditions) and/or varying levels of human activity during survey (e.g. initial setup of study – investigation of study area, trail cutting, deployment of camera equipment, etc.) (e.g. Crawshaw and Quigley 1991).
- *M(h)*: heterogeneity model - detection probabilities are affected by sex, age, and social status, and result in individually unique capture probabilities (e.g. Harmsen et al. 2011a).

- *M(tb):* time and behavior model – capture probability varies across encounter occasions, and recapture probabilities differ from capture probabilities (e.g. trap response and seasonal changes both affect detection probability for jaguars).
- *M(th):* time and heterogeneity model – detection probability varies with each individual and each encounter occasion (e.g. detection probability varies among individuals and in response to temporal changes).

PROGRAM MARK

- We used the closed Huggins Full Closed Capture with Heterogeneity model (Huggins 1991) implemented in Program MARK. Since capture and recapture probabilities may vary among individuals, across time, and due to other factors, we tested various models including heterogeneity (*h*), behavior (*b*), and time (*t*). We additionally included sex as a covariate.
- We constructed models in Program MARK that were equivalent to those in CAPTURE (*M(o)*, *M(b)*, *M(t)*, *M(h)*, *M(tb)*, *M(th)* and *M(tbh)*). Program MARK uses a maximum likelihood framework to estimate parameters of interest and implements Akaike's Information Criterion (AIC; Akaike 1973) for model selection.
- Since MARK has higher flexibility for including other variables in the detection process, additional models were constructed and evaluated with AIC (genetic data included sex as covariate) (examples listed below):

- *p(.) c(sex)* = capture probability is constant, recapture probability is affected by sex (e.g. for camera and genetic surveys females often are detected only once or a very few times during the survey).
- *p(sex) c(sex)* = capture and recapture probabilities differ and are affected by the sex of the animal (e.g. overall detection probabilities for females is often lower, e.g. jaguars).
- *p(h + t) = c(h + t)* = detection probability is affected by heterogeneity and differs across encounter occasions due to, for example, varying levels of human activity (e.g. detection probability decreases during survey due to presence of research team, some individuals are more affected than others). Heterogeneity was incorporated using a two-class mixture. This means that in the mixture, group 1 always has a by the same amount higher detection probability compared to group 2.
- *p(.) c(t + sex)* = capture and recapture probabilities differ and recapture probability changes across encounter occasions and based on the sex of animal (e.g. female jaguars often are detected only once or a very few times during the survey – for genetic data females tend to be detected more likely at the beginning of the survey).

Spatially explicit CMR models

We also estimated densities from photographic and genetic CMR data by using SECR models. The capture probability using the SECR approach can be defined as a function of distance between the unknown home range/activity centers of the animals and the trap

stations (e.g. Efford 2004, Borchers and Efford 2008, Royle and Young 2008, Gardner et al. 2009). The detection probability decreases as distance from the home range centers increases. For the camera survey, trap stations equal the geographical location of the remote camera stations. For the genetic scat survey using a scat detector dog, we used the centers of the scat sampling grid cells as fixed survey points and kept the search intensity across sampling grids equal by recording the sampling effort in survey km. Unlike Thompson et al. (2012), the survey design for this study was spatially structured and we conducted temporal repetition of grid cell surveys.

PROGRAM DENSITY – ML-SECR

ML-SECR methods were developed initially by Efford (2004) and draw elements from both the classic distance sampling and also mark-recapture analysis. This method incorporates a state model for the population density and a distance-dependent detection process, which also can be described as a function of the distance between the animal's home range and the trap location. Capture histories of individuals are a function of both models, which are fitted by using maximum likelihoods. Density estimates obtained from ML-SECR methods are not affected by the choice of trap layout and number of traps, but relative precision was based on the number of recaptures, which were recommended to be > 20 (Efford et al. 2004).

We applied the likelihood-based SECR approach in program DENSITY, version 5.0 (Efford 2004). We selected the *M(h)* Jackknife or *M(h)* Chao models

and ran the analysis using three different detection functions (negative exponential, hazard rate, half-normal).

Detection function

- Hazard-rate detection functions assume that animals move around the activity centers, are territorial and have clearly defined home ranges.
- Negative exponential detection functions assume that animals mainly move around their activity center, but also venture out occasionally.
- Half-normal detection functions assume that animals have established activity centers, but home ranges around them are not defined as clearly compared with the other two detection functions.

Detector type

The setting "proximity" was selected to define the detector type since it allows for multiple captures on the same day and detectors act independently of each other, which was most fitting for our study. Other possible detector types available are for example 'single-catch', and 'multi-catch' types, which are used when true traps are in use.

Buffer

Buffer distance for the habitat mask (or state-space) depends on the scale of movement of target species and the detection functions chosen for the analysis (e.g. hazard and lognormal require large buffers for stable density estimates). We tested the effect that different buffer sizes would have on density estimates and

decided to base buffer distances on the root-pooled spatial variance (RPSV), which is a simple measure of the home range size. We tested four different buffer distances (>RPSV, 2xRPSV, 3xRPSV, 5xRPSV) for density estimation based on the genetic data of MPR jaguars. Density estimates around five times of RPSV (~ 15 km) were most stable (Table 7) thus, a buffer distance of 15 km was chosen for the ML-SECR analysis.

Factors impacting g0 and s

We tested effects of behavior (*b*, response to capture) and individual heterogeneity (*h2*, 2-class finite mixture) on the intrinsic trappability (*g0*) and the spatial scalar (*s* o*r* σ) based on the following models:

- *g0*[.] = detection probabilities at the home range center vary due to spatial scale of activity space,
- *g0*[*b*] = detection probabilities vary due to trap response (e.g. trap-happy individuals = learnt response affect *g0*),
- *g0*[*h2*] = detection probabilities vary due to intrinsic differences in the 2-class finite mixture (e.g. males and females – varying levels of activity, varying preference or avoidance of area types within home ranges – e.g. open areas, roads and trails),
- *s*[.] = distance from activity center that affects detection probability (= detection scale) is constant among animals,
- *s*[*b*] = detection scale changes a function of trapping due to trap response. For example, after first capture, animals used either more or less space.

- $s[h2]$ = detection scale differs between different groups (e.g. some animals use more space than others, have larger home ranges, etc.).

PROGRAM SPACECAP – B-SECR

B-SECR can be considered as a Bayesian extension of spatially-explicit mark-recapture models first adopted by Efford (2004). These models can be generally classified as generalized linear models (GLMs). According to Royle et al. (2009), analysis of these models is challenging due to several unknown variables (random effects) (e.g. activity centers of individuals, also the number of activity centers). To make interference more efficient, Bayesian analysis based on data augmentation was applied (Royle et al. 2007). B-SECR was initially developed for remote camera trapping studies and considers an array of traps in which each trap functions independently and animals can be photographed at any trap, multiple times, and during any capture occasion. A hierarchical model is used for the observed spatial capture history, which is based on two processes: (1) distribution of animals across the landscape, and (2) an encounter process which describes whether animals are encountered by traps as a function of their location (Royle and Young 2008, Royle et al. 2009).

To implement the B-SECR method, we ran the R package SPACECAP (Royle et al. 2009). For the state-space, we created a grid of cells (1 x 1 km) to superimpose over the survey areas and included a buffer area of 15 km for jaguars and pumas following the protocol described by Noss et al. (2012). We tested the following models: trap response absent or present, spatial-capture recapture, half-

normal detection function and Bernoulli's encounter model. The B-SECR models closely resemble the time and heterogeneity model, M(th), which allows detection probabilities to vary with each individual and each encounter occasion. Behavior effects (trap response absent or present) can be added, which creates a M(tbh) model. We conducted the analysis using 60,000 iterations, with a 10,000 burn-in value, a thinning rate of 1, and data augmentation of 5 – 10 times the number of animals detected for each species at each site. Convergence of Marcov chains was examined by using the Geweke statistic (Geweke 1992).

Genetic CMR models

For the genetic data, we additionally estimated densities by using the single-session models in CAPWIRE (Miller et al. 2005, Petit and Valiere 2006), which accommodate multiple captures of single individuals during the sampling session (i.e. number of sampling occasions equals the maximum number of captures per individual). We conducted a likelihood ratio test (with $P < 0.1$) to determine the presence of capture heterogeneity and choose between two implemented models. One model assumes constant capture rate among individuals (ECM, even capturability model), and the other one accounts for heterogeneous capture probabilities among individuals (TIRM, two innate rate model). Both models were used to estimate population sizes with 95% confidence intervals.

CMR ASSUMPTIONS

Generally, for the CMR analysis, we made the following assumptions based on our field sampling and laboratory protocols: (1) populations were demographically (i.e. no births, deaths, immigration or emigration) and geographically (i.e. old scat samples are not detected due to high degradation rates or genotyping of old and degraded DNA fails) closed for the short duration of the scat surveys, (2) and elaborate laboratory and analytical protocols effectively controlled for genotyping errors and misidentification of individual genotypes.

MODEL SELECTION AND AVERAGING

The Akaike Information Criterion (sample-size adjusted AICc, Akaike 1973) was used to compare the set of candidate models and model weights (ω_i) derived from ΔAIC_c values in order to select the appropriate, most parsimonious model describing the variation in the data (Burnham and Anderson 2002). All models with $\Delta AIC_c < 2$ were considered as competing models.

Results

Comparison of two noninvasive survey methods – molecular scatology vs. remote camera trapping

Photographic and genetic capture rates and effort

We captured jaguars 465 times across all sites (15 – 223 capture events per site) using remote camera traps in a total of 451 survey days (8,129 total photos over 10,433 camera-trap nights). For the molecular scatology survey, we captured jaguars 252 times (24 – 79

times per site) over 217 survey days (1,008 total scat samples over 1,788.05 km survey with scat detector dog). We photographically identified a total number of 63 individual jaguars (8 – 21 individual jaguars per site) and genetically a total of 49 individual jaguars (8 – 14 individual jaguars per site) across all sites. The overall detection of individuals between survey methods did not differ significantly (Student's t – test, t = 1.01, P = 0.36). Additionally, the molecular scatology survey identified 41 individual pumas (2 – 16 individual pumas per site). We did not attempt to identify pumas in photographs by their subtle marks, but will attempt that in future studies.

The average number of captures per individual jaguar by site ranged from 1.67 to 24.78 (8.74 ± 9.57) for remote cameras and from 3.00 to 7.90 (5.07 ± 2.03) for molecular scatology and was not significantly different between methods (Student's t – test, t = 0.68, P = 0.53). Average number of captures per individual puma and site using molecular scatology ranged from 1.67 to 4.27 (2.94 ± 1.02) (Table 4A and B).

Trap success rates ranged from 1.14 to 7.39 jaguars per 100 trap-nights (4.07 ± 2.55) across sites and from 0.59 to 3.97 pumas per 100 trap-nights (2.26 ± 1.37) when using remote camera traps. Not including blank photographs with no animals, an average number of 17.5 photos (± 13.61) of actual animals were collected to capture one jaguar and 34.4 photos (± 99.41) of actual animals were collected to capture one puma. For the molecular scatology study (using a scat detector dog), we covered an average of 2.03 survey km (± 0.93) to detect one scat sample. On average, we located 4.57 scat samples (± 1.27) per day using a scat detector dog at a field site. An average of 5.28 scat samples (± 3.74) had to be collected to capture one jaguar and 11.60 scat samples (± 6.07) to capture one puma.

It took 0.32 to 6.60 days (across different study sites) (0.97 ± 2.48) to photographically capture one jaguar and 0.49 to 2.17 days (across different study sites) (1.11 ± 0.71) to genetically capture one jaguar. The time to capture animals using either method did not differ significantly (Student's *t*-test, $t = 1.21$, $p = 0.28$). To capture pumas, it took between 1.27 to 9 days (1.91 ± 3.28) using remote camera traps and 1.11 and 6.50 days (across different study sites) (2.93 ± 2.25) using noninvasive genetic sampling (Table 2 and 4C). The difference in time among survey methods to capture one animal did not differ significantly for pumas (Student's *t*-test, $t = 0.35$, $p = 0.74$).

Factors influencing detection probabilities for jaguars and pumas in genetic mark-recapture study

For non-spatial CMR analysis in program CAPTURE, *M(o)* was most commonly selected as the top model for abundance estimation of jaguars and pumas based on genetic data. The time and behavior model *M(th)* outranked other models for pumas at CBWS. The time and heterogeneity model *M(bh)* performed best for jaguars and pumas at FB. Generally, *M(h)* and *M(bh)* ranked second- or third-best among models with an exception at CBWS (for jaguars and pumas) and FB (for pumas), where *M(t)* was ranked higher based on the *DFA* scores (Table 5).

Results from program MARK indicated that heterogeneity and sex (including additive and interactive models) were the most important factors influencing detection probabilities (especially recapture probabilities) for jaguars and pumas across most sites. At two study sites (CBWS for jaguars, FB for pumas), additive models including the factors time, behavior, heterogeneity and sex were listed as top candidate models

affecting detection probabilities. For the remaining study sites, behavior and time were listed as less important factors affecting detection probabilities, with AIC_c model weights (ω_i) below 0.06 (Table 6). Models with unreasonable abundance estimates or high standard errors were disregarded.

Spatial CMR analysis in program DENSITY ranked models using negative exponential and half-normal detection functions as best. Detection probabilities varied mainly due to spatial scale of activity space (*g0*[.]), intrinsic differences (heterogeneity) in two classes (e.g. difference in spatial use of two classes) (*g0*[*h2*]), and distance from activity center (*s*[.]). Results for jaguars at CBWS detected by remote cameras were an exception, since hazard detection functions were ranked highest and indicated that intrinsic trappability varied mainly due to trap response (*g0*[*b*]) (Table 8). Models with unreasonable density estimates or high standard errors were disregarded.

B-SECR analysis using SPACECAP ranked models incorporating trap response at the MPR and FB sites, and no trap response at the RB site consistently as highest (for both species and survey techniques). A trap response is defined as the behavioral reaction of animals (e.g. return to trap station – "trap-happy") after being captured initially. Molecular scatology data on jaguars at CFRNP and on jaguars and pumas at CBWS selected for no trap response models, whereas puma genetic data at CFRNP and jaguar camera data at CBWS indicated that detection probabilities were influenced by a trap response (Table 9).

Comparison of density estimators – non-spatial, spatial and genetic-based approaches - with noninvasive genetic and photographic survey data

We used eight methods of density estimation to analyze the genetic data sets for jaguars and pumas across five sites, including: (1) CAPTURE – ½ MMDM, $\hat{N}$ /ETA with half MMDM as buffer distance (Table 5), (2) CAPTURE - MMDM, $\hat{N}$ /ETA with MMDM as buffer distance (Table 5), (3) MARK – ½ MMDM, $\hat{N}$ /ETA with half MMDM as buffer distance (Table 6 and 7), (4) MARK – MMDM, $\hat{N}$ /ETA with MMDM as buffer distance (Table 6), (5) ML – SECR, maximum likelihood-based spatially explicit mark-recapture in program DENSITY (Table 7 and 8), (6) B – SECR, Bayesian spatially explicit mark-recapture in program SPACECAP (Table 9), (7) CAPWIRE – ½ MMDM, genetic-based $\hat{N}$ /ETA with half MMDM as buffer distance (Table 10), and (8) CAPWIRE – MMDM, genetic-based $\hat{N}$ /ETA with MMDM as buffer distance (Table 10). Genetic and photographic CMR analysis for jaguars was compared using six density estimators including CAPTURE and MARK – ½ MMDM, $\hat{N}$ /ETA with half and full MMDM as buffer distance (Table 5), ML – SECR, maximum likelihood based spatially explicit mark-recapture analysis in program DENSITY, and B - SECR , Bayesian-based spatially explicit CMR in program SPACECAP, and (Table 11).

For the genetic data, density estimates in CAPTURE, MARK and CAPWIRE using ½ MMDM as buffer values resulted in substantially higher density estimates for jaguars and pumas across most study sites (Table 11, Fig. 4) compared to density estimates derived using MMDM values. For example, density estimates obtained in program CAPTURE approximately doubled when ½ MMDM were used as buffer distances compared to MMDM values (Table 11, Fig. 5). Comparison of additional

density estimators ($\hat{N}$ /ETA with MMDM, spatially explicit and genetic-based methods) indicated little difference in their estimates (Fig. 4 and 5).

Comparing the two different survey approaches (remote camera vs. genetics), we found that conventional CMR techniques using programs CAPTURE and MARK resulted in differences in jaguar density estimates, with higher estimates for genetic sampling at MPR and higher estimates for remote camera trapping at RB and CBWS sites. Spatially explicit derived density estimates (ML–SECR and B–SECR) for jaguars using the molecular scatology and remote camera trapping approach did not differ significantly across all sites (Table 11, Fig. 6).

Levels of precision varied depending on the choice of density estimator. ML-SECR estimators resulted in the least precise density estimates, with *CV*s ranging from 0.01 - 0.46 for genetic study on jaguars and 0.34 - 0.69 for pumas (Table 11). Capture probabilities were highest for traditional $\hat{N}$ /ETA methods, ranging from 0.12 to 0.18 for camera studies of jaguars, 0.44 to 0.58 for genetic studies of jaguars, and 0.28 to 0.30 for genetic studies of pumas (Fig. 6 and 7). Capture probabilities were much lower for SECR methods, with mean capture probabilities of 0.05 (ML-SECR) and 0.03 (B-SECR) for camera studies on jaguars, and 0.09 (ML-SECR) and 0.03 (B-SECR) for genetics on jaguars. The average capture probabilities derived from SECR methods also were low for pumas (ML –SECR: p = 0.08; B-SERC: p = 0.02). CAPWIRE also had relatively low capture probabilities, ranging from 0.06 to 0.18 for jaguars and 0.04 to 0.07 for pumas (Fig. 6 and 7).

Comparison of density estimates for jaguars and pumas across sites

Density estimates differed when various density estimators were applied, but we could also see a variation in density estimates across study sites. Density estimates (number of individuals per 100 km^2) per study sites varied considerably for jaguars with highest estimates at the CBWS site ranging from 3.23 to 6.22 (using genetics) and 1.99 to 8.3 (using cameras), followed by the RB site with 1.64 to 4.84 (using genetics) and 2.62 to 5.08 (using cameras), the MPR site with 1.41 to 3.31 (using genetics) and 1.41 to 2.05 (using cameras), the CFRNP site with 0.62 to 2.06 (using genetics) and 1.01 to 2.51 (using cameras), and the FB site with 0.82 to 2.20 (using genetics) and 0.40 to 1.99 (using cameras) (Table 11).

For pumas differences in density estimates (number of individuals per 100 km^2) obtained from noninvasive genetic sampling only, were less varied, but more dramatic with highest density estimates at the RB site ranging from 5.12 to 12.02, followed by the FB site with 1.36 to 4.21, and fairly similar estimates for the CBWS site with 1.09 to 2.90 and the CFRNP site with 1.26 to 2.72 (Table 11).

Discussion

The application of noninvasive field methods (e.g. remote camera trapping, molecular scatology) for demographic monitoring has become increasingly popular for wide-ranging and cryptic carnivores which would otherwise be difficult to study (e.g. Waits and Paetkau 2005, Long et al. 2008, Kelly et al. 2012). Yet, to date, no study has been designed specifically to compare results of standardized remote camera trapping protocols to genetic mark-recapture following repeated surveys at a landscape scale. This

is the first study empirically evaluating the effectiveness of these different noninvasive survey methods and the impact on the analytical approaches used for population density estimation of Neotropical felids.

Comparison of different density estimators

To obtain reliable and efficient abundance and density estimates, it is crucial to select the most appropriate analytical approach to minimize violations of density estimator assumptions and optimize the study design to obtain detection probabilities above the recommended $P > 0.1$ or 0.2 (Otis et al. 1978). Generally, conventional grid-based $\hat{N}$ /*ETA* density estimation techniques face multiple challenges, including the choice of trap layout, trap spacing, trapping grid size and the calculation method for the effective trapping area. All of these factors have been extensively discussed in the literature and have made the use of the traditional MMDM estimation techniques questionable (e.g. Parmenter et al. 2003, Efford 2004, Dillon and Kelly 2007, Maffei and Noss 2008, Gardner et al. 2009, Foster and Harmsen 2012, Kelly et al. 2012). According to Wilson and Anderson (1985), using ½ MMDM values as *ETA* buffer distance results in more precise and less biased density estimates, but is overall believed to underestimate the effective trapping area, especially when the *ETA* value is relatively small compared to the animal's home range size (e.g. White et al. 1982, Parmenter et al. 2003, Dillon and Kelly 2008).

To address these issues, SECR density estimators (B – SECR and ML – SECR) have been developed (e.g. Efford et al. 2004, Borchers and Efford 2008, Royle and Young 2008, Gardner et al. 2009, Efford 2011, Borchers 2012). Past studies (e.g. Obbard

et al. 2010, Sollmann et al. 2011, Gerber et al. 2012, Noss et al. 2012) have compared conventional CMR methods to newer spatially explicit CMR models, and have concluded that SECR methods are the more appropriate density estimators for wide-ranging carnivores. These recent comparisons resulted in significantly lower density estimates for SECR models versus non-spatial closed population models (in MARK and CAPTURE), which does not match with our findings. Generally, our $\hat{N}$ /ETA density estimates (using ½ MMDM values) were higher than most SECR estimates, but due to low levels of precision in SECR models, CIs overlapped for all estimates but jaguars at the CFRNP site (with genetic data). $\hat{N}$ /ETA density estimates (using full MMDMs) also did not differ from SECR estimates, which is unlike what former studies have shown (e.g. Obbard et al. 2010, Gerber et al. 2012). Density estimates for the two SECR approaches (ML – SECR and B – SECR) did not differ for either target species (using both survey techniques) across all study sites.

The relatively low precision of our SECR density estimates (for both survey techniques) likely resulted from the small samples sizes obtained at most of our study sites (especially for pumas, with *CV*s ranging from 0.34 - 0.69). Although the average sampling area size was about 200+ km^2 per site, larger sampling areas or higher sampling intensity, if feasible in tropical environments may be beneficial to increase levels of precision for density estimates.

For the molecular scatology study, for example, we recommend using two or three scat detector dogs to survey the same-sized of study area and to survey sampling grids more evenly. Larger sampling intensity also will increase the chance to collect greater numbers and less-degraded scat samples. SECR density estimators are less

sensitive to the grid size and layout (e.g. Efford 2004, Borchers and Efford 2008), but the use of larger sampling grid sizes (several times the size of average home-range size; > 200 km^2 for jaguars in South America) and a sufficient number of trapping devices to increase detection probabilities of target species is strongly recommend (Noss et al. 2012). Sollmann et al. (2012b) also confirmed that SECR methods are less sensitive to trap array size and spacing, but emphasized that reliability and precision of density estimates depends particularly on the amount of data collected and how widely recaptures are spread spatially. Capture probabilities were highest for traditional $\hat{N}$ /ETA methods for genetic studies of jaguars, followed by lower capture probabilities for CAPWIRE and SECR methods (Fig. 6 and 7).

Few studies have compared conventional CMR techniques to genetics-based density estimators (e.g. Robinson et al. 2009, Coster et al. 2011). Past CMR models specifically developed for noninvasive genetic sampling are quite different from conventional CMR techniques (e.g. Lukacs and Burnham 2005b, Robinson et al. 2009) since they are based on single trapping sessions where all captures of individuals (potentially multiple) are included in the analysis and capture information is not lost (e.g. Miller et al. 2005, Petit and Valiere 2006). Traditionally, genetics-based CMR methods assumed constant detection, and only recently have added models including heterogeneity, which still makes multi-occasion models superior, since they use capture histories through time to test for various additional variables (e.g. time, behavior) impacting detection. Robinson et al. (2009) compared estimates obtained from conventional multi-occasion CMR models to estimates from single-session CAPWIRE models and concluded that abundance estimates were similar, but confidence intervals

varied widely, and that conventional CMR methods overall performed best. We included the single-session, genetics-based CAPWIRE abundance estimator (Miller et al. 2005) in our comparison of density estimators (using ½ and full MMDM) since it also accounts for capture heterogeneity, which may be caused by sex- and age-biased scent-marking behaviors (e.g. Dallas et al. 2003), which we believe plays an important role in molecular scatology-based CMR studies. Nonetheless, the models do not test for potential effects of time and behavior on detection probabilities, which both are considered to be important factors.

CAPWIRE is known to perform better when used for smaller populations (< 100 animals), but tends to overestimate population densities when sample sizes are low (Miller et al. 2005). Additionally, an average of 2.5 to 3 observations per individual is recommended to increase the precision of abundance estimates obtained from CAPWIRE (Miller et al. 2005). The mean number of observations per individual for our study across different sites in Belize ranged from 3.33 to 7.90 for jaguars and 1.67 to 4.27 for pumas. In our study, abundances derived from program CAPWIRE did not appear different from those derived from the classic closed CMR estimators using either ½ MMDM or full MMDM buffer distances. CAPWIRE estimates using MMDM buffer distances also did not differ significantly from SECR density estimates, but ½ MMDM- based CAPWIRE estimates appeared higher than the B – SECR estimates (in 6 out of 10 comparisons) and ML – SECR estimates (in 2 out of 10 comparisons). Additional CMR models developed for abundance estimation, which correct for genotyping error (e.g. Lukacs and Burnham 2005a, Knapp et al. 2009) were not applied in this study, since fecal DNA genotypes

were screened extensively for genotyping errors and misidentification of individual genotypes.

Comparison of two noninvasive survey methods – molecular scatology vs. remote camera trapping

Noninvasive survey methods have become increasingly popular and have been widely used, especially for carnivore species (e.g. Long et al. 2008, Kelly et al. 2012). In recent years, several carnivore studies simultaneously applied two or more noninvasive survey techniques to directly compare performance of different methods (e.g. Gompper et al. 2006, Harrison 2006, Long et al. 2007a, Rosellini et al. 2008, Hajkova et al. 2009, Vine et al. 2009, Janecka et al. 2011a, Galaverni et al. 2012), and overall concluded that techniques should be carefully chosen based on the target species, its environment and overall research objectives. According to Long et al. (2007a) and Harrison et al. (2006), noninvasive genetic sampling using scat detector dogs had the highest detection rates compared to other noninvasive techniques. Galaverni et al. (2012) used remote camera traps and noninvasive genetic sampling to monitor wolves and concluded that both techniques were consistent in their performance, and suggested that simultaneous use would be beneficial. Janecka et al. (2011a) found that density estimates obtained from noninvasive genetic sampling were higher than those for remote camera trapping estimates, due to potential collection of scat samples from subadults, which were not included in the remote camera trapping analysis.

Our results showed that remote-camera trapping and the molecular scatology approach were overall equally effective in monitoring jaguars and pumas across five sites

in Belize. We did not detect significant differences in the number of individual jaguars, the number of observations per individual jaguar, or the number of days required to capture animals photographically vs. genetically. Survey methods showed variation in density estimates, mainly based on the density estimator utilized. Detection probabilities were generally higher when genetic sampling was used. Density estimates based on SECR analysis (both ML–SECR and B–SECR) did not appear to differ between techniques and across study sites, but levels of precisions were generally low. Traditional CMR techniques revealed differences in density estimates for the genetic and remote camera study across three of the five study sites. The drier MPR study site resulted in higher density estimates for the genetic study. Two of the broadleaf sites (RB and CBWS) showed higher density estimates when remote cameras were used.

Remote-camera trapping is an effective survey method, which has been widely used for abundance and density estimation of distinctively marked felids, including ocelots (e.g. Dillon and Kelly 2008, Davis et al. 2011), jaguars (e.g. Silver et al. 2004, Soisalo and Cavalcanti 2006), leopards (e.g. Kauffman et al. 2007, Balme et al. 2009), and tigers (e.g. Karanth and Nichols 1998, Sunarto et al. 2012). The technique can be also used for subtly marked (e.g. Kelly et al. 2008) or uniquely tagged animals (e.g. Bridges et al. 2004). Remote-camera data also provide information on other co-occurring species including competitors, prey species, and human activities, which can be directly related to target species by using trap success rates, but also estimation of survival and recruitment when long-term data are available (e.g. Kelly et al. 2012). Remote camera surveys face challenges such as durability and reliability of equipment (especially in hot, humid and cold climates) and the choice of an appropriate survey design and camera

setup, especially when used for demographic monitoring (e.g. Dillon and Kelly 2007, Long et al. 2008, Maffei and Noss 2008, Kelly et al. 2012).

Non-invasive genetic sampling faces different challenges. High temperatures, precipitation and UV radiation have the potential to reduce DNA quality and quantity, which is particularly concerning when noninvasive genetic sampling is conducted in tropical climates (e.g. Brinkman et al. 2010, Vynne et al. 2012). Generally, low DNA quantity and quality are major concerns for molecular scatology studies in the tropics (e.g. Michalski et al. 2011, Vynne et al. 2012), since they decrease PCR amplification success and cause genotyping errors (Taberlet et al. 1996). In our study, RB and CBWS had lower PCR amplification success rates than other sites, and the MPR site the highest (> 80% successful for species and individual identification) (Wultsch et al. in review). We implemented a pilot study at the beginning of this study and optimized 14 highly polymorphic microsatellite loci for five Neotropical felids (jaguars, pumas, ocelots, margays and jaguarundis) to increase reliability and power of individual identification (Wultsch et al. in review). Defecation rate also may vary due to sex and age of animals (e.g. Dallas et al. 2003, Marucco et al. 2009), which has the potential to introduce additional bias to demographic monitoring. Genetic mark-recapture studies have been in general described as having a "complex multi-step process" (Gervasi et al. 2010), which ideally involves optimization of field sampling and laboratory analysis protocols (e.g. Roon et al. 2005, Waits and Paetkau 2005, Boulanger et al. 2006), and applies the most appropriate design for field sampling and method for demographic analysis (e.g. Boulanger et al. 2002). Besides these challenges, genetic-based mark-recapture studies have the potential to increase detection probabilities (especially when scat detector dogs

are used), and to obtain population density estimates also for externally "unmarked" or "untagged" animals, since "genetic tagging" through polymorphic multi– locus data is applied. Simultaneous studies of multiple carnivore species are also feasible (e.g. Long et al. 2007b, Vynne et al. 2011b). Genetic CMR studies also provide additional information on genetic diversity, relatedness, gene flow and population structure, which all are relevant for conservation and management, especially when the target species is poorly studied or threatened (e.g. Waits and Paetkau 2005, Allendorf et al. 2013). Further, scat samples can be used for additional ecological studies focusing on diet and endocrine/hormone physiology (e.g. Long et al. 2008, Kelly et al. 2012).

Financial costs for both field methods, which is the major concern for most field projects, depend on various factors including available resources (e.g. genetic markers for target species etc.), amount and type of equipment used (e.g. size of study area and potential survey of multiple sites; camera brands vary widely in price; use of scat detector dog – potential renting of scat dog and dog handler training or hiring of entire scat dog team) and type of analysis planned (e.g. costs for genetic analysis - species versus individual identification). However, both methods are highly efficient techniques to demographically monitor wide-ranging and elusive tropical forest carnivores such as jaguars and pumas. Their advantages and disadvantages should be considered carefully before starting a research project. Simultaneous use of both techniques may also be beneficial, especially when the target species is extremely difficult to detect and only sparse data are available (e.g. Galaverni et al. 2012, Gopalaswamy et al. 2012).

Management implications and recommendations for future CMR studies

Our study demonstrated that the choice of the field survey method (noninvasive genetic sampling versus remote camera trapping) did not affect density estimates extensively. We recommend choosing the survey methods based on additional short- and long-term research goals, logistics, available resources and the target species and its environment. We believe that careful planning of the sampling area (size, trap layout etc.), the inclusion of thorough error-checking protocols (e.g. genotyping errors for noninvasive genetic data), and the choice of a reliable estimation method are crucial for precise and efficient demographic monitoring of potentially difficult-to-study or threatened species.

For density estimation, we ultimately recommend applying spatially explicit SECR models for future demographic studies, especially when focusing on cryptic, difficult-to-study and wide-ranging wildlife species such as large carnivores. While less precise, SECR CMR models are probably a more realistic reflection of uncertainty. They hold great promise for density estimation studies for wide-ranging and territorial carnivore species, especially if precision can be improved through study design or future analytic advancements.

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Table 1 Description and characteristics of five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, Central America. IUCN (International Union for the Conservation of Nature) protected area categories (Ia – Strict Nature Reserve, Ib Wilderness Area, II National Park, III Natural Monument or Feature, IV Habitat/Species Management Area, V Protected Landscape/Seascape, VI Protected Area with sustainable use of natural resources).

Site	Protected Area Type	IUCN	Size (km^2)	Elevation (m)	Main Ecotypes	Annual rainfall average (mm)
MPR	Forest reserve	VI	513.2	120 - 1016	Lowland and submontane pine forest Lowland and submontane broadleaf most forest Shrubland	1550 - 2018
RB	Private reserve	IV	1050	40 - 160	Subtropical moist broadleaf Lowland savanna Mangrove and littoral forest Shrubland and wetland	1550 - 1600
CBWS	Wildlife sanctuary	IV	437	50 - 1120	Lowland and submontane broadleaf wet forest Lowland and submontane broadleaf moist forest Shrubland	2700
CFRNP	Forest reserve & National Park	VI & II	1775	500	Lowland and submontane broadleaf moist forest Submontane and montane broadleaf wet forest Submontane pine forest Shrubland	1500
FB	Private reserve	IV	7.35	0 - 20	Lowland broadleaf moist forest Mangrove and littoral forest Wetland	1524

Table 2 Description of scat detector dog surveys for jaguars (*Panthera onca*) and pumas (*Puma concolor*) across five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2009. CMR, capture-recapture; # scats, number of scat samples collected in the wild; # SO, number of sampling occasions for capture-recapture study; # grid cells, number of scat survey grid cells (2 x 2 km); total survey duration in days, survey distance in km (not including backtracking); total survey distance in km (including backtracking); scat detection effort (distance in km to detect one scat sample, number of scats detected per day).

CMR Scat Survey Summary						**Scat Dog Survey**				**Scat Detection Effort**	
Site	**Dates**	**# Scats**	**# SO**	**# Grid Cells**	**Total Survey Duration (d)**	**Scat Dog**	**Survey Length (km)**	**Total Distance (km)**	**Average Moving Speed (km/h)**	**Distance (km)/ scat**	**Scats/ Day**
MPR	6/15/07 – 8/14/07	124	4	46	39	Billy	377.71	435.20	NA	3.05	3.18
RB	1/28/08 – 3/31/08	251	5	29	45	Bruiser	388.79	509.75	3.95	1.55	5.58
CBWS	4/3/08 – 7/15/08	265	4	33	44	Bruiser	327.98	413.99	4.25	1.24	6.02
CFRNP	7/2/08 – 8/25/08	126	5	34	37	Bruiser	380.87	532.87	4.77	3.02	3.41
FB	3/19/09 – 6/10/09	242	5	31	52	Bruiser	312.70	407.79	4.47	1.29	4.65
Total		**1008**		**173**	**217**		**1788.05**	**2299.60**	**4.36**	**2.03**	**4.57**

Table 3 Description of remote-camera trapping surveys for jaguars (*Panthera onca*) across five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2009. # camera stations, number of remote camera stations (each station was set up with two remote-camera traps).

Site	Survey Name	Date	Survey length (d)	# Camera stations	Average Trap nights/camera
MPR	5MPR	06/06/07 - 08/17/07	72	47	61.57
RB	1RB	01/12/08 - 04/09/08	88	33	47.76
CBWS	1CC	04/05/08 - 07/16/08	102	48	55.69
CFRNP	3PBL	6/22/08 - 08/24/08	63	29	22.00
FB	1FB	03/13/09 - 07/17/09	126	23	72.22
Total			**451**	**180**	**51.85**

Table 4 Summary of molecular scatology and remote-camera trapping surveys across five study sites in Belize. (A) Description of survey, (B) number of captures, number of ind. and average number of observations per ind., (C) trap success and effort. Cap, capture (RC – photographic capture event, MS – scat sample successfully genotyped; d, number of survey days; HA, human-related photos, P, number of photos (minus blanks); S, number of scats; TS, trap success (capture events per 100 trap nights).

(A) Site	Remote camera survey (RC)				Molecular scatology survey (MS)		
	# Total Photos	#Total Photos (-HA)	# Days	Trap nights	# Scats	# Days	# km
MPR	4386	4168	72	2894	124	39	377.71
RB	5418	1284	88	2579	251	45	388.79
CBWS	3431	1264	102	2673	265	44	327.98
CFRNP	864	585	63	626	126	37	380.87
FB	1875	828	126	1661	242	52	312.70
Total	15974	8129	451	10433	1008	217	1788.05

(B) Site	# Captures Jaguars		# Ind. Jaguars		Avg. # Obs./ Ind. Jaguar		# Captures Pumas		# Ind. Pumas		Avg. # Obs./ Ind. Puma	
	RC	MS	RC	MS	RC	MS	RC	MS	RC	MS	RC	MS
MPR	223	79	9	10	24.78	7.90	17	6	---	2	---	3.00
RB	67	30	16	9	4.19	3.33	66	36	---	16	---	2.25
CBWS	144	70	21	14	6.86	5.00	80	21	---	6	---	3.50
CFRNP	15	49	9	8	1.67	6.13	7	10	---	6	---	1.67
FB	21	24	8	8	2.63	3.00	66	47	---	11	---	4.27
Total	465	252	63	49	7.38	5.07	236	120	---	41	---	2.94

(C) Site	RC		MS		MS		RC		MS	
	Jaguars	Pumas	Jaguars	Pumas	Jaguars	Pumas	Jaguars	Pumas	Jaguars	Pumas
	TS	TS	#P/cap	#P/cap	#S/cap	#S/cap	#d/cap	#d/cap	#d/cap	#d/cap
MPR	7.39	0.59	18.69	245.18	1.57	20.67	0.32	4.24	0.49	6.50
RB	2.52	2.56	19.16	19.45	8.37	6.97	1.31	1.33	1.50	1.25
CBWS	5.95	2.99	8.78	15.80	3.79	12.62	0.71	1.27	0.63	2.10
CFRNP	3.35	1.19	39.00	83.57	2.57	12.60	4.20	9.00	0.76	3.70
FB	1.14	3.97	39.43	12.54	10.08	5.15	6.00	1.91	2.17	1.11
Total	4.07	2.26	17.48	34.44	5.28	11.60	0.97	1.91	1.11	2.93

Table 5 Program CAPTURE - Summary for abundance estimates for jaguars (*Panthera onca*) and pumas (*Puma concolor*) across 5 sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) using the program CAPTURE (Otis et al. 1978, White et al. 1982, Rexstadt and Burnham 1991). P, capture probability; c, recapture probability; Top four models were presented based on *DFS* (discriminant function) scores. Final models selected are marked in bold. Selection was additionally based on standard errors and reasonable abundance estimates.

Study Site	Species	Survey	Closure Test		Model Selection		*p*	*c*	*N*	*SE*
			***Z* Score**	***P* - value**	**Model**	***DFA Score***				
MPR	Jaguar	Camera	-0.590	0.278	M(bh)	1.00	NA	NA	NA	NA
					M(h)	0.86	0.34	NA	12	2.01
					M(b)	***0.85***	***0.24***	***0.47***	***10***	***0.36***
					M(o)	0.67	0.41	NA	10	0.03
	Jaguar	Genetics	-1.300	0.097	***M(o)***	***0.98***	***0.55***	***NA***	***10***	***0.74***
					M(bh)	0.88	NA	NA	13	5.99
					M(h)	0.87	0.34	NA	16	3.57
					M(b)	0.62	0.28	0.71	13	5.99
RB	Jaguar	Camera	-2.255	0.012	M(h)	1.00	0.16	NA	17	3.15
					M(o)	***0.99***	***0.18***	***NA***	***15***	***0.95***
					M(bh)	0.54	NA	NA	16	1.72
					M(b)	0.36	0.16	0.19	16	1.72
	Jaguar	Genetics	-1.132	0.129	***M(o)***	***1.00***	***0.44***	***NA***	***9***	***0.80***
					M(h)	0.81	0.21	NA	19	5.56
					M(bh)	0.57	NA	NA	9	0.99
					M(th)	0.36	NA	NA	17	7.54
	Puma	Genetics	-0.447	0.327	***M(o)***	***1.00***	***0.28***	***NA***	***19***	***2.97***
					M(h)	0.85	0.25	NA	22	4.35

					M(bh)	0.59	NA	NA	18	3.53
					M(th)	0.37	NA	NA	26	9.15
CBWS	Jaguar	Camera	-0.769	0.221	***M(b)***	***1.00***	***0.13***	***0.30***	***27***	***7.73***
					M(bh)	0.84	NA	NA	27	7.73
					M(h)	0.68	0.17	NA	32	6.64
					M(o)	0.68	0.25	NA	22	1.23
	Jaguar	Genetics	-1.674	0.047	M(th)	1.00	NA	NA	21	5.01
					M(o)	***0.68***	***0.58***	***NA***	***15***	***0.75***
					M(t)	0.48	NA	NA	15	0.01
					M(bh)	0.47	NA	NA	15	0.45
	Puma	Genetics	-0.758	0.224	***M(th)***	***1.00***	***NA***	***NA***	***8***	***2.10***
					M(t)	0.73	NA	NA	7	0.00
					M(o)	0.39	0.54	NA	7	0.66
					M(bh)	0.29	NA	NA	7	0.43
CFRNP	Jaguar	Camera	-1.510	0.066	M(o)	1.00	0.14	NA	12	3.26
					M(h)	1.00	0.06	NA	31	9.75
					M(bh)	***0.61***	***NA***	***NA***	***9***	***0.99***
					M(b)	0.55	0.31	0.12	9	0.99
	Jaguar	Genetics	-0.588	0.278	***M(o)***	***1.00***	***0.52***	***NA***	***8***	***0.48***
					M(h)	0.83	0.42	NA	10	1.75
					M(bh)	0.60	NA	NA	8	0.40
					M(th)	0.38	NA	NA	9	1.93
	Puma	Genetics	1.430	0.924	***M(o)***	***1.00***	***0.30***	***NA***	***7***	***1.55***
					M(h)	0.81	0.33	NA	6	2.15
					M(bh)	0.59	NA	NA	6	1.68
					M(th)	0.37	NA	NA	8	3.59
FB	Jaguar	Camera	-0.826	0.204	***M(o)***	***1.00***	***0.12***	***NA***	***9***	***2.75***
					M(h)	0.89	0.09	NA	12	4.39

				M(bh)	0.67	NA	NA	7	1.12
				M(th)	0.50	NA	NA	19	13.44
Jaguar	Genetics	-1.118	0.132	M(o)	1.00	0.32	NA	9	1.63
				M(h)	0.99	0.31	NA	9	2.56
				M(bh)	***0.90***	***NA***	***NA***	***8***	***0.40***
				M(b)	0.72	0.57	0.23	8	0.40
Puma	Genetics	-0.542	0.294	***M(th)***	***1.00***	***NA***	***NA***	***15***	***5.09***
				M(bh)	0.35	NA	NA	11	0.45
				M(t)	0.28	NA	NA	11	0.71
				M(o)	0.18	0.35	NA	12	1.52

Table 6 Program MARK - Capture-recapture models estimating abundance of jaguars (*Panthera onca*) and pumas (*Puma concolor*) using molecular scatology, remote camera trapping and program MARK (White and Burnham 1999) across five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2010. AIC_c = Akaike's Information Criterion, ΔAIC_c = differences in AIC, *Log L* = maximized log-likelihood, *K* = number of parameters, ω_i = Akaike weights, *N* = abundance estimate, and *SE* = standard error. Final models selected are marked in bold. Selection was additionally based on standard errors and reasonable abundance estimates.

Site	Species	Survey	Model Definition	AIC_c	ΔAIC_c	ω_i	*Log L*	*K*	*N*	*SE*	LCI, 95%	UCI, 95%
MPR	**Jaguar**	**Genetic**	{p(.) c(sex)}	41.122	0.000	0.462	1.000	3	21.045	27.021	10.718	179.795
			{p(.) c(het+sex)}	41.954	0.832	0.304	0.660	4	21.045	27.021	10.718	179.794
			{p(het) c(sex)}	43.599	2.476	0.134	0.290	4	21.044	27.003	10.719	179.663
			{p(.) c(het*sex)}	44.576	3.454	0.082	0.178	5	21.044	27.019	10.718	179.784
			{p(het) = c(het)}	47.909	6.787	0.016	0.034	2	20.487	16.033	11.219	100.182
			{p(.) c(.)}	52.326	11.204	0.002	0.004	2	21.045	27.021	10.718	179.794
			{p(het) c(.)}	54.669	13.546	0.001	0.001	3	21.045	27.015	10.719	179.753
			{p(.)=c(.)}	***56.210***	***15.088***	***0.000***	***0.001***	***1***	***10.555***	***0.879***	***10.062***	***14.989***
			{p(t) = c(t)}	58.054	16.932	0.000	0.000	4	10.367	0.700	10.032	14.159
			{p(het+t) = c(het+t)}	59.327	18.205	0.000	0.000	9	15.904	8.967	10.692	60.355
			{p(.) c(sex+t)}	60.398	19.275	0.000	0.000	10	21.046	27.028	10.718	179.848
			{p(t*het) = c(t*het)}	60.695	19.573	0.000	0.000	6	10.614	0.989	10.067	15.633
			{p(.) c(t)}	63.770	22.648	0.000	0.000	9	21.045	27.021	10.718	179.795
			{p(t) c(.)}	70.194	29.072	0.000	0.000	9	12.335	0.000	12.335	12.335
			{p(h+sex+t) c(h+sex+t)}	100.470	59.348	0.000	0.000	19	13.087	0.000	13.087	13.087
RB	**Jaguar**	**Genetic**	***{p(.) c(het+sex)}***	***60.086***	***0.000***	***0.251***	***1.000***	***4***	***10.274***	***2.257***	***9.123***	***22.170***
			{p(.) c(het)}	60.466	0.380	0.208	0.827	3	10.274	2.257	9.123	22.170
			{p(het) = c(het)}	60.826	0.740	0.174	0.691	2	12.191	3.425	9.574	26.749
			{p(het) c(het)}	61.579	1.493	0.119	0.474	4	11.046	3.521	9.205	29.397

			{p(.) c(het*sex)}	62.625	2.539	0.071	0.281	5	10.274	2.257	9.123	22.170
			{p(.) = c(.)}	62.802	2.715	0.065	0.257	1	9.670	0.988	9.082	14.509
			{p(.) c(sex)}	63.235	3.149	0.052	0.207	3	10.274	2.257	9.123	22.170
			{p(.) c(t+b+het+sex)}	64.680	4.594	0.025	0.101	7	10.274	2.257	9.123	22.172
			{p(.) c(.)}	64.734	4.648	0.025	0.098	2	10.274	2.257	9.123	22.172
			{p(.) c(t+b+het)}	66.879	6.793	0.008	0.034	8	10.274	2.257	9.123	22.172
			{p(.) c(t+b)}	69.834	9.748	0.002	0.008	5	10.274	2.257	9.123	22.172
RB	**Puma**	**Genetic**	***{p(sex) = c(sex)}***	***97.392***	***0.000***	***0.162***	***1.000***	***2***	***23.064***	***5.778***	***17.737***	***44.727***
			{p(.) c(het+sex)}	97.834	0.442	0.130	0.802	4	20.857	6.418	16.677	50.826
			{p(het) c(het)}	98.526	1.134	0.092	0.567	4	22.496	9.070	16.846	65.879
			{p(.) = c(.)}	98.674	1.282	0.085	0.527	1	20.362	3.305	17.165	32.333
			{p(.) c(het*sex)}	99.079	1.686	0.070	0.430	5	20.857	6.418	16.677	50.826
			{p(het) = c(het)}	99.308	1.916	0.062	0.384	2	25.701	11.166	17.603	74.703
			{p(b+sex) = c(b+sex)}	99.537	2.145	0.055	0.342	3	24.025	10.671	17.110	73.996
			{p(.) c(sex)}	99.903	2.511	0.046	0.285	3	20.857	6.418	16.677	50.830
			{p(.) c(het)}	99.930	2.538	0.045	0.281	4	20.857	6.418	16.677	50.826
			{p(het) c(het+sex)}	99.981	2.589	0.044	0.274	5	22.682	13.035	16.573	93.872
			{p(het) c(het)}	100.096	2.704	0.042	0.259	4	21.858	7.066	16.914	53.534
			{p(.) c(.)}	100.769	3.377	0.030	0.185	2	20.857	6.418	16.677	50.826
			{p(.) c(t+b+het+sex)}	101.289	3.897	0.023	0.143	7	20.857	6.418	16.677	50.830
			{p(sex) c(sex)}	101.721	4.329	0.019	0.115	4	22.356	10.712	16.654	77.743
			{p(b*sex) = c(b*sex)}	101.721	4.329	0.019	0.115	4	22.356	10.712	16.654	77.742
			{p(het) c(sex)}	102.121	4.729	0.015	0.094	4	20.857	6.418	16.677	50.826
			{p(sex) c(.)}	102.529	5.137	0.012	0.077	3	22.356	10.712	16.654	77.745
			{p(sex) c(t+b+het)}	102.767	5.375	0.011	0.068	7	22.356	10.711	16.654	77.739
			{p(het) c(.)}	102.929	5.537	0.010	0.063	3	20.857	6.525	16.664	51.547
			{p(.) c(t+b+het)}	103.167	5.775	0.009	0.056	7	20.857	6.418	16.677	50.826
			{p(het) c(t+b+sex+het)}	103.631	6.238	0.007	0.044	8	22.265	12.041	16.547	87.684

			{p(het+sex) c(.)}	104.747	7.355	0.004	0.025	4	22.356	10.712	16.654	77.745
			{p(b*het*sex) c(b*het*sex)}	105.676	8.284	0.003	0.016	8	33.564	38.141	17.320	249.611
			{p(het*sex) c(.)}	107.024	9.632	0.001	0.008	5	22.356	10.712	16.654	77.743
CC	**Jaguar**	**Genetic**	{p(het) c(t+b+het+sex)}	77.485	0.000	0.230	1.000	7	15.634	1.262	15.053	22.578
			{p(.) c(t+b+het+sex)}	***77.758***	***0.273***	***0.201***	***0.872***	***6***	***15.212***	***0.549***	***15.013***	***18.488***
			{p(sex) c(t+b+het)}	77.758	0.273	0.201	0.872	6	15.212	0.549	15.013	18.490
			{p(.) c(t+b+het)}	77.758	0.273	0.201	0.872	6	15.212	0.549	15.013	18.488
			{p(het) = c(het)}	80.788	3.303	0.044	0.192	2	22.125	21.335	15.365	154.153
			{p(t*het) = c(t*het)}	82.080	4.595	0.023	0.101	8	17.141	2.459	15.355	27.919
			{p(.)=c(.)}	82.559	5.074	0.018	0.079	1	15.574	0.861	15.068	19.821
			{p(.) c(het)}	82.875	5.390	0.016	0.068	3	15.212	0.549	15.013	18.490
			{p(h+sex) = c(h+sex)}	83.006	5.521	0.015	0.063	3	22.125	21.338	15.365	154.177
			{p(.) c(t+b)}	83.645	6.161	0.011	0.046	5	15.212	0.549	15.013	18.490
			{p(.) c(.)}	83.949	6.464	0.009	0.040	2	15.212	0.549	15.013	18.490
			{p(sex)=c(sex)}	84.700	7.216	0.006	0.027	2	15.574	0.861	15.068	19.821
			{p(.) c(het+sex)}	85.174	7.689	0.005	0.021	4	15.212	0.549	15.013	18.490
			{p(het*sex) =c(het*sex)}	85.305	7.820	0.005	0.020	4	22.125	21.335	15.365	154.153
			{p(.) c(t)}	85.773	8.288	0.004	0.016	9	15.212	0.549	15.013	18.488
			{p(b+sex) = c(b+sex)}	86.167	8.682	0.003	0.013	3	15.212	0.549	15.013	18.488
			{p(.) c(sex)}	86.167	8.682	0.003	0.013	3	15.212	0.549	15.013	18.490
			{p(sex) c(.)}	86.167	8.682	0.003	0.013	3	15.212	0.549	15.013	18.490
CC	**Puma**	**Genetic**	***{p(.)=c(.)}***	***34.378***	***0.000***	***0.317***	***1.000***	***1***	***6.230***	***0.545***	***6.016***	***9.401***
			{p(.) c(.)}	35.699	1.321	0.164	0.517	2	6.028	0.181	6.001	7.252
			{p(het) = c(het)}	36.369	1.991	0.117	0.370	2	6.357	0.749	6.028	10.552
			{p(sex)=c(sex)}	36.767	2.390	0.096	0.303	2	6.230	0.545	6.016	9.401
			{p(.) c(het)}	38.327	3.950	0.044	0.139	3	6.028	0.181	6.001	7.252
			{p(b+sex) = c(b+sex)}	38.327	3.950	0.044	0.139	3	6.028	0.181	6.001	7.252
			{p(.) c(sex)}	38.327	3.950	0.044	0.139	3	6.028	0.181	6.001	7.252

			{p(sex) c(.)}	38.327	3.950	0.044	0.139	3	6.028	0.181	6.001	7.252
			{p(h+sex) = c(h+sex)}	38.997	4.620	0.032	0.099	3	6.357	0.749	6.028	10.552
			{p(.) c(t+b)}	39.421	5.043	0.026	0.080	5	6.028	0.181	6.001	7.252
			{p(sex) c(sex)}	41.233	6.855	0.010	0.033	4	6.028	0.181	6.001	7.252
			{p(b*sex) =c(b*sex)}	41.233	6.855	0.010	0.033	4	6.028	0.181	6.001	7.252
			{p(.) c(het+sex)}	41.233	6.855	0.010	0.033	4	6.028	0.181	6.001	7.252
			{p(.) c(t+b+het+sex)}	41.338	6.961	0.010	0.031	6	6.028	0.181	6.001	7.252
			{p(sex) c(t+b+het)}	41.338	6.961	0.010	0.031	6	6.028	0.181	6.001	7.252
			{p(.) c(t+b+het)}	41.338	6.961	0.010	0.031	6	6.028	0.181	6.001	7.252
			{p(het*sex) =c(het*sex)}	41.903	7.525	0.007	0.023	4	6.357	0.749	6.028	10.552
CB	**Jaguar**	**Genetic**	***{p(.) c(sex)}***	***55.278***	***0.000***	***0.229***	***1.000***	***3***	***8.171***	***0.516***	***8.009***	***11.367***
			{p(sex) c(sex)}	56.485	1.207	0.125	0.547	4	8.245	0.668	8.014	12.285
			{p(b*sex) = c(b*sex)}	56.485	1.207	0.125	0.547	4	8.245	0.668	8.014	12.285
			{p(.) c(het+sex)}	56.990	1.711	0.097	0.425	4	8.171	0.515	8.009	11.366
			{p(.) = c(.)}	57.035	1.757	0.095	0.416	1	8.232	0.530	8.016	11.285
			{p(het) c(sex)}	57.199	1.920	0.088	0.383	4	8.688	2.092	8.035	21.678
			{p(het) = c(het)}	58.400	3.122	0.048	0.210	2	8.504	0.885	8.049	13.151
			{p(.) c(het)}	59.151	3.872	0.033	0.144	3	8.171	0.515	8.009	11.366
			{p(.) c(.)}	59.212	3.934	0.032	0.140	2	8.171	0.515	8.009	11.366
			{p(het) c(het+sex)}	59.392	4.114	0.029	0.128	5	8.246	0.675	8.014	12.339
			{p(.) c(het*sex)}	59.611	4.333	0.026	0.115	5	8.171	0.515	8.009	11.366
			{p(sex) c(.)}	60.286	5.008	0.019	0.082	3	8.245	0.668	8.014	12.284
			{p(het) c(.)}	60.999	5.721	0.013	0.057	3	8.688	2.093	8.035	21.682
			{p(b*het+sex) c(b*het+sex)}	61.088	5.810	0.013	0.055	6	8.273	0.791	8.015	13.133
			{p(het) c(het)}	61.698	6.419	0.009	0.040	4	8.143	0.456	8.007	11.000
			{p(het+sex) c(.)}	62.451	7.173	0.006	0.028	4	8.688	2.092	8.035	21.681
			{p(.) c(t+b+het+sex)}	62.788	7.509	0.005	0.023	7	8.171	0.516	8.009	11.367
			{p(het*sex) c(.)}	65.073	9.794	0.002	0.008	5	8.688	2.092	8.035	21.680

CB	**Puma**	**Genetic**	***{p(.) = c(.)}***	***38.118***	***0.000***	***0.410***	***1.000***	***1***	***7.730***	***2.118***	***6.265***	***17.290***
			{p(.) c(.)}	40.174	2.056	0.147	0.358	2	10.530	12.020	6.267	82.754
			{p(sex) = c(sex)}	40.419	2.302	0.130	0.316	2	7.730	2.118	6.265	17.290
			{p(het) = c(het)}	40.419	2.302	0.130	0.316	2	7.730	2.118	6.265	17.290
			{p(.) c(sex)}	42.537	4.419	0.045	0.110	3	10.530	12.020	6.267	82.754
			{p(b+sex) = c(b+sex)}	42.628	4.510	0.043	0.105	3	11.249	15.850	6.266	109.517
			{p(.) c(het)}	42.653	4.535	0.042	0.104	3	10.530	12.020	6.267	82.754
			{p(het+sex) = c(het+sex)}	42.898	4.780	0.038	0.092	3	7.730	2.118	6.265	17.290
			{p(.) c(het+sex)}	45.213	7.096	0.012	0.029	4	10.530	12.020	6.267	82.754
			{p(.) c(het*sex)}	48.113	9.996	0.003	0.007	5	10.530	12.020	6.267	82.754
FB	**Jaguar**	**Genetic**	***{p(.) c(.)}***	***51.259***	***0.000***	***0.189***	***1.000***	***2***	***8.171***	***0.515***	***8.009***	***11.366***
			{p(.) = c(.)}	51.388	0.128	0.178	0.938	1	9.850	2.053	8.320	18.707
			{p(het) c(het)}	52.044	0.785	0.128	0.675	4	8.431	0.713	8.045	12.091
			{p(b+sex) = c(b+sex)}	53.602	2.342	0.059	0.310	3	8.171	0.516	8.009	11.367
			{p(sex) c(.)}	53.602	2.342	0.059	0.310	3	8.171	0.515	8.009	11.366
			{p(.) c(het)}	53.602	2.342	0.059	0.310	3	8.171	0.516	8.009	11.367
			{p(.) c(sex)}	53.602	2.342	0.059	0.310	3	8.171	0.515	8.009	11.366
			{p(het) = c(het)}	53.606	2.347	0.059	0.309	2	9.900	3.890	8.154	31.504
			{p(sex) = c(sex)}	53.607	2.347	0.059	0.309	2	9.850	2.053	8.320	18.707
			{p(het*t) c(.)}	54.588	3.329	0.036	0.189	7	8.000	0.002	8.000	8.004
			{p(.) c(t+b)}	54.752	3.493	0.033	0.174	6	8.171	0.515	8.009	11.366
			{p(sex) c(sex)}	56.078	4.819	0.017	0.090	4	8.171	0.515	8.009	11.366
			{p(b*sex) = c(b*sex)}	56.078	4.819	0.017	0.090	4	8.171	0.516	8.009	11.367
			{p(.) c(het+sex)}	56.078	4.819	0.017	0.090	4	8.171	0.515	8.009	11.366
			{p(sex) c(t+b+het)}	57.707	6.448	0.008	0.040	7	8.171	0.515	8.009	11.366
			{p(.) c(t+b+het+sex)}	57.707	6.448	0.008	0.040	7	8.171	0.515	8.009	11.366
			{p(.) c(t+b+het)}	57.707	6.448	0.008	0.040	7	8.171	0.515	8.009	11.366
			{p(.) c(het*sex)}	58.700	7.440	0.005	0.024	5	8.171	0.515	8.009	11.366

			{p(t*het) = c(t*het)}	59.286	8.027	0.003	0.018	10	8.000	0.000	8.000	8.000
			{p(t) = c(t)}	61.005	9.746	0.001	0.008	10	9.616	2.186	8.219	19.926
FB	**Puma**	**Genetic**	***{p(.) c(t+b+het+sex)}***	***69.196***	***0.170***	***0.105***	***0.919***	***7***	***11.210***	***0.563***	***11.012***	***14.601***
			{p(.) c(het)}	69.551	0.525	0.088	0.769	3	11.210	0.563	11.012	14.600
			{p(sex) c(t+b+het)}	69.767	0.741	0.079	0.691	7	11.306	0.812	11.018	16.186
			{p(.) c(t+b+het)}	70.211	1.185	0.063	0.553	7	11.210	0.563	11.012	14.601
			{p(b+sex) = c(b+sex)}	70.625	1.599	0.052	0.450	3	11.778	1.661	11.060	21.136
			{p(.) c(sex)}	70.628	1.602	0.051	0.449	3	11.210	0.563	11.012	14.600
			{p(het) c(t+b+sex+het)}	70.730	1.704	0.049	0.427	8	11.271	0.673	11.017	15.239
			{p(sex) = c(sex)}	70.759	1.733	0.048	0.420	2	16.393	7.098	11.756	49.482
			{p(.) c(het+sex)}	70.999	1.973	0.043	0.373	4	11.210	0.563	11.012	14.601
			{p(het*sex) = c(het*sex)}	71.355	2.329	0.036	0.312	4	17.040	7.211	11.955	49.216
			{p(het) c(het+sex)}	71.607	2.581	0.032	0.275	5	11.360	0.830	11.025	16.151
			{p(.) c(het*sex)}	71.814	2.788	0.028	0.248	5	11.210	0.563	11.012	14.601
			{p(het) c(het)}	72.250	3.224	0.023	0.200	4	13.829	7.494	11.167	58.833
			{p(.) c(.)}	72.290	3.264	0.022	0.196	2	11.210	0.563	11.012	14.600
			{p(sex) c(sex)}	72.513	3.487	0.020	0.175	4	11.306	0.812	11.018	16.186
			{p(b*sex) = c(b*sex)}	72.513	3.487	0.020	0.175	4	11.306	0.812	11.018	16.185
			{p(.) = c(.)}	72.681	3.655	0.018	0.161	1	12.711	1.791	11.317	20.235
			{p(het) c(sex)}	72.958	3.932	0.016	0.140	4	11.210	0.563	11.012	14.600
			{p(het) c(het)}	73.325	4.299	0.013	0.117	4	11.332	0.695	11.026	15.222
			{p(b*het+sex) c(b*het+sex)}	73.347	4.321	0.013	0.115	6	11.993	2.941	11.052	30.149
			{p(het) = c(het)}	73.679	4.653	0.011	0.098	2	14.956	5.289	11.544	39.777
			{p(sex) c(.)}	74.085	5.059	0.009	0.080	3	11.306	0.812	11.018	16.186
			{p(t) = c(t)}	74.320	5.294	0.008	0.071	10	14.385	3.537	11.629	29.230
			{p(het) c(.)}	74.530	5.504	0.007	0.064	3	11.210	0.563	11.012	14.600
			{p(t+sex) = c(t+sex)}	74.548	5.522	0.007	0.063	11	18.092	8.558	12.106	56.465
			{p(.) c(t+b)}	74.625	5.599	0.007	0.061	6	11.210	0.563	11.012	14.600

	{p(het+sex) c(.)}	76.415	7.389	0.003	0.025	4	11.306	0.812	11.018	16.186
	{p(t*het) = c(t*het)}	76.723	7.697	0.002	0.021	10	11.000	0.000	11.000	11.000
	{p(t+b+het) c(t+b+het)}	77.042	8.016	0.002	0.018	11	11.000	0.000	11.000	11.000
	{p(b+sex+t) = c(b+sex+t)}	77.646	8.620	0.002	0.013	12	16.469	12.587	11.383	89.087
	{p(het*sex) c(.)}	78.029	9.003	0.001	0.011	5	11.710	1.873	11.042	22.949
	{p(b*het*sex) c(b*het*sex)}	78.292	9.266	0.001	0.010	8	11.334	0.840	11.021	16.308
	{p(.) c(t+sex)}	78.433	9.407	0.001	0.009	12	11.210	0.563	11.012	14.601
	{p(.) c(t)}	78.828	9.802	0.001	0.007	10	11.210	0.563	11.012	14.601

Table 7 Program DENSITY - Spatially explicit mark-recapture models estimating densities of jaguars (*Panthera onca*) at Mountain Pine Ridge Forest Reserve, Belize, 2007, using program DENSITY (Efford et al. 2004) and varying buffer sizes (3500, 6000, 9000, and 15000 m). Model (period, no variation, h2, heterogeneity with 2 classes, b, behavioral response); detection function (*Negexp* = negative exponential curve, *hazard* = hazard rate curve, *halfnorm* = half-normal curve); *K*, number of parameters; *Log L*, maximized log-likelihood; AIC_c, Akaike's Information Criterion; ΔAIC_c, differences in AIC; ω_i, Akaike weights, *D*, density estimate (individual jaguars per $100km^2$); *SE*, standard error; *g0*, detection probability at theoretical activity center; *s,* spatial scalar. Models listed had $\Delta AIC_c \leq 20$.

Buffer (m)	Model	Detection Function	*K*	*Log L*	AIC_c	ΔAIC_c	ω_i	*D* #/100km²	*SE*	*g0*	*SE*	*s*	*SE*
3500	g0[.]s[h2]	Halfnorm	5	-295.72	616.45	0.00	0.61	20.3	0.26	0.06	0.01	290.57	142.68
	g0[.]s[h2]	Negexp	5	-296.62	618.24	1.79	0.25	171.42	1.75	0.15	0.04	63.26	25.56
	g0[h2]s[.]	Halfnorm	5	-297.69	620.39	3.94	0.08	8.31	0.10	0.00	0.00	2300.96	201.38
	g0[.]s[.]	Negexp	3	-306.38	622.75	6.30	0.03	2.98	0.01	0.13	0.04	1530.75	183.52
	g0[h2]s[.]	Negexp	5	-298.89	622.78	6.33	0.03	8.45	0.09	0.01	0.01	1520.27	199.44
	g0[b]s[.]	Negexp	4	-306.07	628.13	11.68	1.77E-03	3.09	0.01	0.10	0.05	1544.62	188.05
	g0[b]s[.]	Halfnorm	4	-306.28	628.56	12.11	1.43E-03	3.14	0.01	0.03	0.02	2398.82	205.96
	g0[.]s[b]	Negexp	4	-306.31	628.63	12.18	1.38E-03	2.94	0.01	0.14	0.04	1590.24	257.78
	g0[.]s[.]	Hazard	4	-306.50	629.00	12.55	1.14E-03	2.96	0.01	0.06	0.02	2222.67	522.17
	g0[.]s[b]	Halfnorm	4	-306.51	629.01	12.56	1.14E-03	2.91	0.01	0.05	0.01	2611.11	332.85
	g0[b]s[b]	Halfnorm	5	-303.41	631.82	15.37	2.79E-04	3.03	0.01	0.02	0.01	3668.05	808.59
	g0[.]s[h2]	Hazard	6	-296.15	632.29	15.84	2.21E-04	25.29	0.21	0.04	0.01	409.76	124.55
	g0[b]s[b]	Negexp	5	-304.26	633.52	17.07	1.19E-04	3.01	0.01	0.03	0.03	2627.87	1113.99
6000	g0[.]s[.]	Negexp	3	-303.11	616.21	0.00	0.35	2.25	0.01	0.14	0.03	1604.89	194.47
	g0[.]s[h2]	Halfnorm	5	-295.74	616.48	0.27	0.30	13.14	0.16	0.06	0.01	337.10	157.05
	g0[.]s[h2]	Negexp	5	-296.55	618.09	1.88	0.13	111.22	1.15	0.15	0.04	72.42	25.81

	g0[.]s[.]	Halfnorm	3	-304.78	619.55	3.34	0.07	2.32	0.01	0.05	0.01	2500.96	229.16
	g0[h2]s[.]	Halfnorm	5	-297.82	620.64	4.43	0.04	6.97	0.09	0.00	0.00	2288.65	197.05
	g0[.]s[.]	Hazard	4	-302.51	621.01	4.80	0.03	2.22	0.01	0.06	0.02	2303.81	514.79
	g0[.]s[b]	Negexp	4	-302.72	621.44	5.23	0.03	2.09	0.01	0.14	0.04	1775.13	298.74
	g0[b]s[.]	Negexp	4	-303.01	622.02	5.81	0.02	2.34	0.01	0.11	0.06	1609.23	196.31
	g0[.]s[b]	Halfnorm	4	-303.39	622.78	6.57	0.01	2.01	0.01	0.06	0.01	2971.35	392.01
	g0[.]s[b]	Halfnorm	4	-303.39	622.78	6.57	0.01	2.01	0.01	0.06	0.01	2970.92	392.22
	g0[h2]s[.]	Negexp	5	-299.52	624.03	7.82	0.01	4.38	0.04	0.01	0.01	1513.78	195.44
	g0[b]s[.]	Halfnorm	4	-304.47	624.93	8.72	4.41E-03	2.50	0.01	0.04	0.02	2491.15	228.86
	g0[b]s[b]	Negexp	5	-300.7	626.39	10.18	2.13E-03	2.07	0.01	0.04	0.03	2845.35	1116.95
	g0[.]s[b]	Hazard	5	-302.38	629.76	13.55	3.94E-04	2.13	0.01	0.06	0.02	2609.32	827.86
	g0[.]s[h2]	Hazard	6	-296.16	632.32	16.11	1.10E-04	15.89	0.14	0.04	0.01	486.66	145.65
9000	g0[.]s[.]	Negexp	3	-302.34	614.68	0.00	0.52	2.03	0.01	0.13	0.03	1651.86	209.59
	g0[.]s[h2]	Halfnorm	5	-295.72	616.43	1.75	0.22	10.64	0.11	0.06	0.01	375.64	158.26
	g0[.]s[.]	Hazard	4	-301.28	618.56	3.88	0.07	1.88	0.01	0.07	0.02	2311.97	512.65
	g0[.]s[.]	Halfnorm	3	-304.59	619.18	4.50	0.05	2.23	0.01	0.05	0.01	2528.18	242.90
	g0[.]s[b]	Negexp	4	-301.85	619.70	5.02	0.04	1.76	0.01	0.14	0.04	1858.73	329.66
	g0[b]s[.]	Negexp	4	-302.25	620.50	5.82	0.03	2.12	0.01	0.11	0.06	1655.68	210.83
	g0[h2]s[.]	Halfnorm	5	-297.81	620.61	5.93	0.03	6.82	0.09	0.00	0.00	2288.72	197.15
	g0[.]s[b]	Halfnorm	4	-302.99	621.98	7.30	0.01	1.77	0.01	0.06	0.01	3087.72	444.63
	g0[b]s[b]	Halfnorm	5	-299.22	623.44	8.76	0.01	1.71	0.01	0.01	0.01	4379.81	934.44
	g0[b]s[b]	Halfnorm	5	-299.22	623.44	8.76	0.01	1.71	0.01	0.01	0.01	4372.58	931.75
	g0[b]s[b]	Negexp	5	-299.76	624.51	9.83	3.81E-03	1.63	0.01	0.04	0.03	2921.10	1065.64
	g0[b]s[.]	Halfnorm	4	-304.29	624.57	9.89	3.70E-03	2.42	0.01	0.04	0.02	2516.38	241.35
	g0[.]s[b]	Hazard	5	-301.18	627.36	12.68	9.17E-04	1.77	0.01	0.06	0.02	2599.26	859.25
	g0[b]s[.]	Hazard	5	-301.20	627.40	12.72	8.99E-04	1.97	0.01	0.06	0.03	2302.65	517.15
	g0[h2]s[.]	Negexp	5	-301.30	627.60	12.92	8.13E-04	2.34	NA	0.05	NA	1609.46	110.74

	g0[.]s[h2]	Negexp	5	-302.14	629.28	14.60	3.51E-04	2.07	0.01	0.13	0.04	1366.25	354.11
15000	g0[.]s[.]	Negexp	3	-302.17	614.33	0.00	0.63	1.96	0.01	0.13	0.03	1670.73	218.23
	g0[.]s[.]	Hazard	4	-300.69	617.38	3.05	0.14	1.64	0.01	0.07	0.02	2319.09	521.89
	g0[.]s[.]	Halfnorm	3	-304.58	619.17	4.84	0.06	2.22	0.01	0.05	0.01	2529.79	244.00
	g0[.]s[b]	Negexp	4	-301.65	619.30	4.97	0.05	1.67	0.01	0.14	0.04	1884.39	336.85
	g0[b]s[.]	Negexp	4	-302.08	620.16	5.83	0.03	2.06	0.01	0.11	0.06	1674.58	219.69
	g0[h2]s[.]	Halfnorm	5	-297.81	620.61	6.28	0.03	6.82	0.09	0.00	0.00	2289.25	197.30
	g0[.]s[h2]	Halfnorm	5	-297.96	620.92	6.59	0.02	3.28	0.02	0.06	0.01	592.73	150.92
	g0[.]s[b]	Halfnorm	4	-302.97	621.94	7.61	0.01	1.74	0.01	0.06	0.01	3098.43	450.00
	g0[b]s[b]	Halfnorm	5	-299.14	623.27	8.94	0.01	1.61	0.01	0.01	0.01	4422.76	940.73
	g0[b]s[b]	Negexp	5	-299.56	624.11	9.78	4.76E-03	1.41	0.01	0.03	0.03	3033.67	1036.25
	g0[b]s[.]	Halfnorm	4	-304.28	624.56	10.23	3.80E-03	2.41	0.01	0.04	0.02	2518.01	242.45
	g0[b]s[.]	Hazard	5	-300.56	626.13	11.80	1.73E-03	1.75	0.01	0.05	0.03	2290.76	525.82
	g0[.]s[b]	Hazard	5	-300.65	626.31	11.98	1.59E-03	1.57	0.01	0.06	0.02	2507.93	892.55
	g0[h2]s[.]	Negexp	5	-301.19	627.38	13.05	9.28E-04	2.17	0.01	0.06	0.05	1640.91	227.15
	g0[.]s[h2]	Negexp	5	-301.94	628.87	14.54	4.41E-04	2.00	0.01	0.13	0.03	1790.54	309.86
	g0[h2]s[h2]	Halfnorm	6	-295.41	630.82	16.49	1.66E-04	17.81	0.24	0.04	0.06	325.05	150.33
	g0[.]s[h2]	Hazard	6	-295.92	631.83	17.50	1.00E-04	15.13	0.11	0.04	0.01	448.66	81.87
	g0[h2]s[h2]	Negexp	6	-297.12	634.23	19.90	3.02E-05	14.34	0.17	1.00	NA	115.50	30.91

Table 8 Program DENSITY - Spatially explicit mark-recapture models estimating densities of jaguars (*Panthera onca*) and pumas (*Puma concolor*) at five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2010, using molecular scatology and remote camera trapping and program DENSITY (Efford et al. 2004). Survey (study site, species, data type), model (period, no variation, h2, heterogeneity with 2 classes, b, behavioral response); detection function (*Negexp* = negative exponential curve, *hazard* = hazard rate curve, *halfnorm* = half-normal curve); *K*, number of parameters; *Log L*, maximized log-likelihood; AIC_c, Akaike's Information Criterion; ΔAIC_c, differences in AIC; ω_i, Akaike weights, *D*, density estimate (individuals per 100 km^2); *SE*, standard error; *LCl*, lower confidence limit (95%); *HCl*, upper confidence limit (95%); *g0*, detection probability at theoretical activity center; *s,* spatial scalar. Models listed had $\Delta AIC_c \leq 20$. Final models selected are marked in bold. Selection was additionally based on standard errors and reasonable abundance estimates.

Survey	Model	Detection Function	*K*	*Log L*	AIC_c	ΔAIC_c	ω_i	*D*	*SE*	*LCI*	*HCI*	*g0*	*SE*	*σ*	*SE*
MPR	***g0[.]s[h2]***	***Negexp***	***5***	***-1021.07***	***2072.13***	***0.00***	***0.68***	***1.41***	***0.58***	***0.65***	***3.05***	***0.10***	***0.01***	***1228.50***	***143.42***
Jaguar	g0[.]s[.]	Negexp	3	-1031.80	2074.41	2.28	0.22	1.07	0.39	0.54	2.12	0.10	0.01	2271.81	197.39
Camera	g0[h2]s[.]	Negexp	5	-1023.90	2077.80	5.67	0.04	1.11	0.40	0.56	2.21	0.06	0.01	2302.37	189.50
	g0[.]s[.]	Halfnorm	3	-1034.15	2079.09	6.96	0.02	1.34	0.47	0.68	2.63	0.05	0.00	3383.99	230.03
	g0[b]s[.]	Negexp	4	-1030.73	2079.45	7.32	0.02	1.20	0.44	0.59	2.42	0.07	0.02	2281.88	200.75
	g0[.]s[b]	Negexp	4	-1030.92	2079.85	7.72	0.01	1.34	0.52	0.64	2.80	0.10	0.01	1944.65	250.65
	g0[.]s[h2]	Halfnorm	5	-1025.93	2081.86	9.73	0.01	1.53	0.60	0.74	3.20	0.05	0.00	1854.36	189.79
	g0[b]s[.]	Halfnorm	4	-1033.13	2084.25	12.12	0.00	1.45	0.52	0.73	2.87	0.03	0.01	3388.46	230.52
	g0[h2]s[.]	Halfnorm	5	-1027.55	2085.10	12.97	0.00	1.45	0.51	0.74	2.84	0.02	0.01	3298.57	198.15
	g0[.]s[b]	Halfnorm	4	-1034.06	2086.13	14.00	0.00	1.41	0.52	0.69	2.85	0.05	0.00	3261.24	348.56
	g0[.]s[.]	Hazard	4	-1034.17	2086.34	14.21	0.00	0.98	0.38	0.47	2.02	0.04	0.01	3701.76	533.77
	g0[.]s[b]	Hazard	5	-1032.88	2095.77	23.64	0.00	1.26	0.53	0.57	2.77	0.04	0.01	3138.81	562.71
	g0[b]s[.]	Hazard	5	-1032.91	2095.83	23.70	0.00	1.13	0.44	0.54	2.37	0.03	0.01	3660.63	506.01
	g0[.]s[h2]	Hazard	6	-1024.95	2103.89	31.76	0.00	1.11	0.47	0.50	2.47	0.05	0.01	1664.58	342.08
	g0[h2]s[.]	Hazard	6	-1025.39	2104.77	32.64	0.00	1.11	0.40	0.55	2.22	0.03	0.00	3574.67	342.62
MPR	***g0[.]s[.]***	***Negexp***	***3***	***-302.17***	***614.33***	***0.00***	***0.63***	***1.96***	***0.01***	***1.02***	***3.79***	***0.13***	***0.03***	***1670.73***	***218.23***
Jaguar	g0[.]s[.]	Hazard	4	-300.69	617.38	3.05	0.14	1.64	0.01	0.80	3.37	0.07	0.02	2319.09	521.89

Genetics	g0[.]s[.]	Halfnorm	3	-304.58	619.17	4.84	0.06	2.22	0.01	1.17	4.22	0.05	0.01	2529.79	244.00
	g0[.]s[b]	Negexp	4	-301.65	619.30	4.97	0.05	1.67	0.01	0.80	3.48	0.14	0.04	1884.39	336.85
	g0[b]s[.]	Negexp	4	-302.08	620.16	5.83	0.03	2.06	0.01	1.03	4.14	0.11	0.06	1674.58	219.69
	g0[h2]s[.]	Halfnorm	5	-297.81	620.61	6.28	0.03	6.82	0.09	0.96	48.49	0.00	0.00	2289.25	197.30
	g0[.]s[h2]	Halfnorm	5	-297.96	620.92	6.59	0.02	3.28	0.02	1.24	8.70	0.06	0.01	592.73	150.92
	g0[.]s[b]	Halfnorm	4	-302.97	621.94	7.61	0.01	1.74	0.01	0.87	3.49	0.06	0.01	3098.43	450.00
	g0[b]s[b]	Halfnorm	5	-299.14	623.27	8.94	0.01	1.61	0.01	0.74	3.52	0.01	0.01	4422.76	940.73
	g0[b]s[b]	Negexp	5	-299.56	624.11	9.78	0.00	1.41	0.01	0.60	3.34	0.03	0.03	3033.67	1036.25
	g0[b]s[.]	Halfnorm	4	-304.28	624.56	10.23	0.00	2.41	0.01	1.22	4.77	0.04	0.02	2518.01	242.45
	g0[b]s[.]	Hazard	5	-300.56	626.13	11.80	0.00	1.75	0.01	0.81	3.78	0.05	0.03	2290.76	525.82
	g0[.]s[b]	Hazard	5	-300.65	626.31	11.98	0.00	1.57	0.01	0.72	3.43	0.06	0.02	2507.93	892.55
	g0[h2]s[.]	Negexp	5	-301.19	627.38	13.05	0.00	2.17	0.01	1.02	4.60	0.06	0.05	1640.91	227.15
	g0[.]s[h2]	Negexp	5	-301.94	628.87	14.54	0.00	2.00	0.01	1.02	3.91	0.13	0.03	1790.54	309.86
	g0[h2]s[h2]	Halfnorm	6	-295.41	630.82	16.49	0.00	17.81	0.24	2.44	130.24	0.04	0.06	325.05	150.33
	g0[.]s[h2]	Hazard	6	-295.92	631.83	17.50	0.00	15.13	0.11	4.03	56.81	0.04	0.01	448.66	81.87
	g0[h2]s[h2]	Negexp	6	-297.12	634.23	19.90	0.00	14.34	0.17	2.27	90.79	1.00	NA	115.50	30.91
RB	***g0[.]s[.]***	***Halfnorm***	***3***	***-406.73***	***821.47***	***0.00***	***0.45***	***2.68***	***0.96***	***1.36***	***5.28***	***0.01***	***0.00***	***4208.22***	***703.17***
Jaguar	g0[h2]s[.]	Halfnorm	5	-404.08	824.15	2.68	0.12	3.39	1.19	1.73	6.62	0.01	0.00	3908.73	592.80
Camera	g0[.]s[.]	Negexp	3	-408.11	824.23	2.76	0.11	2.21	0.79	1.12	4.37	0.03	0.01	2866.17	577.82
	g0[.]s[b]	Halfnorm	4	-406.68	824.99	3.52	0.08	2.56	0.99	1.23	5.32	0.01	0.00	4331.77	810.36
	g0[b]s[.]	Halfnorm	4	-406.69	825.02	3.55	0.08	2.60	0.97	1.28	5.29	0.01	0.01	4220.61	707.80
	g0[.]s[.]	Hazard	4	-406.72	825.08	3.61	0.07	3.31	1.26	1.61	6.80	0.01	0.00	6032.55	1120.73
	g0[b]s[.]	Negexp	4	-408.11	827.85	6.38	0.02	2.18	0.82	1.06	4.46	0.03	0.02	2862.85	577.60
	g0[.]s[b]	Negexp	4	-408.11	827.85	6.38	0.02	2.26	0.91	1.06	4.82	0.03	0.01	2827.93	628.58
	g0[h2]s[.]	Hazard	6	-403.80	828.93	7.46	0.01	3.55	0.93	2.15	5.88	0.00	0.00	7605.10	NA
	g0[.]s[b]	Hazard	5	-406.72	829.44	7.97	0.01	3.31	1.32	1.56	7.05	0.01	0.00	6005.13	1491.89
	g0[.]s[h2]	Halfnorm	5	-406.73	829.47	8.00	0.01	2.69	0.96	1.36	5.29	0.01	0.00	4222.07	NA
	g0[.]s[h2]	Negexp	5	-406.74	829.47	8.00	0.01	2.95	1.09	1.47	5.93	0.03	0.01	2256.66	448.28

	g0[h2]s[.]	Negexp	5	-407.01	830.01	8.54	0.01	2.73	1.06	1.31	5.69	0.01	0.01	2751.44	545.47
	g0[b]s[.]	Hazard	5	-407.02	830.04	8.57	0.01	3.54	NA	NA	NA	0.01	0.00	6895.01	607.80
	g0[.]s[h2]	Hazard	6	-406.72	834.78	13.31	0.00	3.31	1.26	1.61	6.79	0.01	0.00	6031.86	NA
RB	***g0[.]s[.]***	***Negexp***	***3***	***-153.87***	***318.55***	***0.00***	***0.64***	***1.79***	***0.81***	***0.77***	***4.18***	***0.06***	***0.02***	***2422.30***	***597.65***
Jaguar	g0[.]s[.]	Halfnorm	3	-154.71	320.23	1.68	0.27	2.05	0.97	0.85	4.94	0.03	0.01	3545.55	844.59
Genetics	g0[.]s[b]	Negexp	4	-153.64	325.27	6.72	0.02	1.48	0.80	0.54	4.01	0.07	0.03	2707.10	806.42
	g0[.]s[.]	Hazard	4	-153.72	325.43	6.88	0.02	1.68	0.78	0.71	3.98	0.03	0.01	3666.58	1469.61
	g0[b]s[.]	Negexp	4	-153.87	325.74	7.19	0.02	1.75	0.90	0.68	4.51	0.06	0.05	2418.47	595.93
	g0[.]s[b]	Halfnorm	4	-153.95	325.91	7.36	0.02	1.43	0.79	0.52	3.95	0.03	0.01	4432.93	1245.46
	g0[b]s[.]	Halfnorm	4	-154.71	327.43	8.88	0.01	2.08	1.19	0.73	5.89	0.02	0.02	3539.78	854.06
	g0[h2]s[.]	Halfnorm	5	-148.98	327.96	9.41	0.01	0.00	NA	NA	NA	NA	NA	NA	15.37
RB	***g0[.]s[.]***	***Hazard***	***4***	***-196.74***	***405.12***	***0.00***	***0.38***	***5.54***	***1.82***	***2.95***	***10.37***	***0.02***	***0.01***	***2436.61***	***699.21***
Puma	g0[.]s[.]	Negexp	3	-198.95	405.89	0.77	0.26	5.16	1.73	2.72	9.78	0.05	0.02	1740.21	364.27
Genetics	g0[.]s[h2]	Negexp	5	-196.14	408.29	3.17	0.08	7.45	2.75	3.69	15.02	0.05	0.02	1133.41	306.41
	g0[b]s[.]	Hazard	5	-196.24	408.49	3.37	0.07	7.12	3.76	2.70	18.83	0.01	0.01	2371.20	718.51
	g0[.]s[h2]	Halfnorm	5	-196.28	408.55	3.43	0.07	7.24	2.33	3.91	13.41	0.02	0.01	2004.37	344.87
	g0[.]s[b]	Negexp	4	-198.49	408.61	3.49	0.07	6.78	3.35	2.71	16.97	0.04	0.02	1515.25	405.23
	g0[b]s[.]	Negexp	4	-198.50	408.63	3.51	0.06	6.72	3.73	2.43	18.55	0.03	0.02	1767.12	375.67
	g0[.]s[h2]	Hazard	6	-196.14	413.61	8.49	0.01	6.62	2.52	3.22	13.61	0.03	0.01	1674.53	775.72
	g0[h2]s[.]	Negexp	5	-198.80	413.61	8.49	0.01	5.36	1.83	2.80	10.28	0.03	0.02	1736.21	363.97
	g0[.]s[.]	Halfnorm	3	-202.83	413.67	8.55	0.01	3.78	1.41	1.86	7.67	0.01	0.00	3854.35	794.56
	g0[h2]s[.]	Hazard	6	-196.27	413.88	8.76	0.00	6.27	2.31	3.12	12.61	0.02	0.01	2450.85	687.65
	g0[.]s[b]	Halfnorm	4	-201.98	415.60	10.48	0.00	6.68	4.94	1.83	24.35	0.01	0.00	2786.90	1033.75
	g0[b]s[.]	Halfnorm	4	-202.50	416.63	11.51	0.00	4.75	2.70	1.69	13.39	0.01	0.01	3858.30	790.95
	g0[h2]s[.]	Halfnorm	5	-202.52	421.04	15.92	0.00	4.16	1.72	1.91	9.05	0.01	0.00	3889.83	819.22
CC	***g0[b]s[.]***	***Hazard***	***5***	***-768.03***	***1550.05***	***0.00***	***0.63***	***2.51***	***0.63***	***1.55***	***4.08***	***0.03***	***0.01***	***0.02***	***618.40***
Jaguar	g0[.]s[h2]	Hazard	6	-767.34	1552.68	2.63	0.17	2.87	0.75	1.74	4.76	0.03	0.01	0.02	401.88
Camera	g0[.]s[.]	Hazard	4	-772.03	1554.56	4.51	0.07	2.14	0.51	1.34	3.40	0.02	0.01	0.01	605.10

	g0[.]s[.]	Hazard	4	-772.03	1554.56	4.51	0.07	2.14	0.51	1.35	3.40	0.02	0.01	0.01	606.27
	g0[.]s[b]	Hazard	5	-770.73	1555.46	5.41	0.04	2.39	0.62	1.45	3.94	0.02	0.01	0.01	845.28
	g0[h2]s[.]	Hazard	6	-769.65	1557.30	7.25	0.02	5.28	2.94	1.91	14.62	0.05	0.03	0.02	978.87
	g0[h2]s[.]	Hazard	6	-770.61	1559.21	9.16	0.01	2.70	2.40	0.60	12.04	0.03	0.02	0.01	602.09
	g0[.]s[h2]	Negexp	5	-774.79	1563.59	13.54	0.00	3.18	0.88	1.87	5.41	0.03	0.01	0.02	154.33
	g0[.]s[.]	Negexp	3	-779.71	1566.84	16.79	0.00	2.36	0.59	1.45	3.83	0.02	0.01	0.01	168.98
	g0[.]s[b]	Negexp	4	-779.45	1569.40	19.35	0.00	2.48	0.67	1.48	4.16	0.02	0.01	0.01	195.72
CC	***g0[.]s[.]***	***Negexp***	***3***	***-322.46***	***653.09***	***0.00***	***0.54***	***4.12***	***1.22***	***2.34***	***7.27***	***0.12***	***0.03***	***1398.58***	***196.92***
Jaguar	g0[.]s[.]	Hazard	4	-321.99	655.98	2.89	0.13	3.17	1.07	1.67	6.04	0.12	0.05	1079.08	328.97
Genetics	g0[.]s[h2]	Negexp	5	-319.75	656.17	3.08	0.11	12.98	18.5	1.64	102.80	0.14	0.03	236.11	155.55
	g0[b]s[.]	Negexp	4	-322.35	656.71	3.62	0.09	4.34	1.41	2.34	8.06	0.10	0.05	1396.54	198.58
	g0[.]s[b]	Negexp	4	-322.43	656.87	3.78	0.08	4.28	1.50	2.19	8.33	0.12	0.03	1358.11	269.40
	g0[h2]s[.]	Negexp	5	-321.24	659.16	6.07	0.03	6.58	4.50	1.95	22.16	0.01	0.02	1311.52	180.97
	g0[.]s[b]	Hazard	5	-321.39	659.45	6.36	0.02	4.04	1.76	1.79	9.13	0.12	0.04	822.47	316.24
	g0[.]s[.]	Halfnorm	3	-327.88	663.94	10.85	0.00	4.40	1.28	2.51	7.70	0.04	0.01	2231.17	283.58
	g0[.]s[h2]	Halfnorm	5	-323.83	664.32	11.23	0.00	16.28	20.5	2.40	110.24	0.05	0.01	357.81	201.84
	g0[h2]s[.]	Hazard	6	-321.85	666.20	13.11	0.00	5.03	4.21	1.20	20.96	0.01	0.02	1115.37	340.72
	g0[.]s[h2]	Hazard	6	-321.98	666.47	13.38	0.00	3.24	1.08	1.71	6.12	0.12	0.04	1197.29	663.24
	g0[b]s[.]	Halfnorm	4	-327.60	667.20	14.11	0.00	4.80	1.57	2.58	8.96	0.03	0.01	2206.72	279.33
	g0[.]s[b]	Halfnorm	4	-327.86	667.72	14.63	0.00	4.55	1.60	2.33	8.87	0.04	0.01	2165.82	442.74
	g0[h2]s[.]	Halfnorm	5	-325.59	667.84	14.75	0.00	6.89	4.14	2.32	20.46	0.01	0.01	2153.22	259.68
CC	***g0[.]s[.]***	***Negexp***	***3***	***-137.18***	***288.35***	***0.00***	***0.92***	***1.74***	***0.79***	***0.74***	***4.07***	***0.19***	***0.07***	***1502.81***	***301.66***
Puma	g0[.]s[.]	Halfnorm	3	-140.07	294.15	5.80	0.05	1.28	0.61	0.53	3.10	0.08	0.03	3232.54	611.70
Genetics	g0[.]s[.]	Hazard	4	-133.76	295.52	7.17	0.03	1.98	0.93	0.83	4.73	0.18	0.08	1109.79	329.02
	g0[.]s[b]	Negexp	4	-136.64	301.28	12.93	0.00	2.44	1.44	0.84	7.11	0.17	0.06	1162.93	395.86
	g0[b]s[.]	Negexp	4	-137.16	302.32	13.97	0.00	1.69	0.83	0.68	4.21	0.21	0.14	1504.68	303.81
	g0[b]s[.]	Halfnorm	4	-139.93	307.86	19.51	0.00	1.19	0.59	0.47	2.99	0.10	0.06	3261.93	624.68
	g0[.]s[b]	Halfnorm	4	-140.01	308.01	19.66	0.00	1.41	0.80	0.50	3.98	0.07	0.03	3016.35	844.75

CB	***g0[.]s[.]***	***Negexp***	***3***	***-108.11***	***227.03***	***0.00***	***0.54***	***1.01***	***0.47***	***0.42***	***2.41***	***0.06***	***0.04***	***4013.47***	***1195.84***
Jaguar	g0[.]s[.]	Halfnorm	3	-108.51	227.82	0.79	0.36	1.02	0.51	0.40	2.56	0.02	0.01	6372.68	1530.48
Camera	g0[.]s[b]	Negexp	4	-107.50	232.99	5.96	0.03	0.81	0.36	0.36	1.85	0.09	0.05	4384.26	1237.81
	g0[.]s[b]	Halfnorm	4	-107.67	233.34	6.31	0.02	0.79	0.34	0.35	1.78	0.03	0.02	7091.93	1447.78
	g0[b]s[.]	Negexp	4	-107.88	233.76	6.73	0.02	0.88	0.41	0.37	2.11	0.11	0.10	3837.00	1058.43
	g0[.]s[.]	Hazard	4	-108.15	234.30	7.27	0.01	1.02	0.49	0.42	2.49	0.02	0.01	7663.26	4266.97
	g0[b]s[.]	Halfnorm	4	-108.38	234.75	7.72	0.01	0.90	0.46	0.35	2.31	0.03	0.03	6337.78	1373.81
	g0[.]s[h2]	Negexp	5	-107.05	244.11	17.08	0.00	3.48	3.39	0.70	17.27	0.07	0.04	1296.92	778.22
	g0[.]s[h2]	Halfnorm	5	-107.26	244.52	17.49	0.00	4.56	3.58	1.18	17.71	0.03	0.01	9254.10	4250.60
	g0[h2]s[.]	Halfnorm	5	-107.52	245.05	18.02	0.00	2.09	3.06	0.26	17.01	0.04	0.02	6502.81	1997.29
	g0[h2]s[.]	Negexp	5	-107.65	245.30	18.27	0.00	1.99	6.71	0.09	44.58	0.01	0.02	4182.84	1525.04
	g0[b]s[.]	Hazard	5	-107.82	245.65	18.62	0.00	0.87	0.40	0.37	2.04	0.04	0.03	7374.80	3568.77
CB	***g0[.]s[.]***	***Negexp***	***3***	***-227.32***	***466.65***	***0.00***	***0.85***	***0.89***	***0.37***	***0.41***	***1.95***	***0.12***	***0.05***	***2804.09***	***508.55***
Jaguar	g0[.]s[.]	Halfnorm	3	-229.59	471.19	4.54	0.09	0.83	0.36	0.37	1.89	0.05	0.01	4998.06	780.67
Genetics	g0[.]s[.]	Hazard	4	-225.36	472.05	5.40	0.06	0.89	0.38	0.40	1.97	0.06	0.02	3900.96	944.11
	g0[.]s[b]	Negexp	4	-227.21	475.74	9.09	0.01	1.00	0.50	0.40	2.51	0.12	0.05	2578.60	622.66
	g0[b]s[.]	Halfnorm	4	-229.54	480.41	13.76	0.00	0.81	0.36	0.35	1.87	0.05	0.02	4994.43	786.82
	g0[.]s[b]	Halfnorm	4	-229.55	480.44	13.79	0.00	0.89	0.45	0.35	2.28	0.05	0.01	4768.87	1062.63
CB	***g0[.]s[.]***	***Negexp***	***3***	***-53.07***	***124.13***	***0.00***	***0.60***	***2.09***	***1.45***	***0.61***	***7.16***	***0.07***	***0.05***	***2088.02***	***929.25***
Puma	g0[.]s[.]	Halfnorm	3	-53.47	124.94	0.81	0.40	2.13	1.49	0.62	7.32	0.02	0.02	3491.16	1383.26
Genetics															
FB	***g0[.]s[.]***	***Halfnorm***	***3***	***-91.33***	***194.65***	***0.00***	***0.50***	***0.40***	***0.36***	***2.06***	***3.32***	***0.03***	***0.02***	***6598.30***	***1485.88***
Jaguar	***g0[.]s[.]***	***Negexp***	***3***	***-91.39***	***194.77***	***0.12***	***0.47***	***0.41***	***0.37***	***2.08***	***7.11***	***0.07***	***0.04***	***4452.79***	***1451.24***
Camera	g0[b]s[.]	Halfnorm	4	-91.01	203.35	8.70	0.01	2.21	0.14	12.65	1.21	0.01	0.02	6746.09	1826.17
	g0[b]s[.]	Negexp	4	-91.09	203.52	8.87	0.01	2.13	0.14	12.15	2.51	0.03	0.05	4826.47	1922.16
	g0[.]s[b]	Halfnorm	4	-91.28	203.90	9.25	0.00	0.41	0.32	2.05	3.50	0.04	0.02	6838.54	1698.44
	g0[.]s[b]	Negexp	4	-91.38	204.10	9.45	0.00	0.50	0.32	2.49	6.97	0.07	0.04	4412.15	1578.82
	g0[.]s[.]	Hazard	4	-91.40	204.13	9.48	0.00	0.39	0.36	2.00	2.43	0.02	0.01	8865.04	4190.70

FB	***g0[.]s[.]***	***Halfnorm***	***3***	***-124.43***	***260.86***	***0.00***	***0.64***	***0.82***	***0.38***	***0.34***	***1.95***	***0.03***	***0.01***	***6367.66***	***1366.03***
Jaguar	g0[.]s[.]	Negexp	3	-125.09	262.17	1.31	0.33	0.78	0.35	0.34	1.79	0.06	0.03	4517.37	1345.62
Genetics	g0[.]s[b]	Halfnorm	4	-124.17	269.67	8.81	0.01	0.71	0.34	0.30	1.71	0.03	0.01	6986.17	1715.91
	g0[b]s[.]	Halfnorm	4	-124.42	270.17	9.31	0.01	0.80	0.40	0.31	2.03	0.03	0.02	6355.05	1349.41
	g0[.]s[.]	Hazard	4	-124.55	270.44	9.58	0.01	0.89	0.48	0.33	2.39	0.02	0.01	9172.31	3022.72
	g0[.]s[.]	Hazard	4	-124.55	270.44	9.58	0.01	0.89	0.48	0.33	2.39	0.02	0.01	9174.75	3023.76
	g0[.]s[b]	Negexp	4	-125.05	271.44	10.58	0.00	0.73	0.37	0.29	1.86	0.06	0.03	4745.39	1727.13
	g0[b]s[.]	Negexp	4	-125.09	271.50	10.64	0.00	0.78	0.39	0.31	1.97	0.06	0.05	4513.24	1354.06
FB	***g0[.]s[.]***	***Halfnorm***	***3***	***-230.01***	***469.45***	***0.00***	***0.52***	***1.36***	***0.53***	***0.65***	***2.84***	***0.03***	***0.01***	***4764.36***	***782.45***
Puma	g0[.]s[.]	Negexp	3	-230.65	470.72	1.27	0.27	1.29	0.50	0.62	2.68	0.07	0.02	3118.56	606.52
Genetics	g0[.]s[b]	Halfnorm	4	-229.06	472.79	3.34	0.10	1.11	0.44	0.52	2.37	0.04	0.01	5406.31	973.77
	g0[b]s[.]	Halfnorm	4	-229.88	474.43	4.98	0.04	1.26	0.52	0.58	2.75	0.04	0.02	4778.29	776.58
	g0[.]s[b]	Negexp	4	-230.39	475.45	6.00	0.03	1.12	0.48	0.50	2.49	0.07	0.03	3443.05	838.52
	g0[b]s[.]	Negexp	4	-230.60	475.86	6.41	0.02	1.22	0.52	0.55	2.71	0.08	0.05	3110.24	600.43
	g0[.]s[.]	Hazard	4	-230.63	475.92	6.47	0.02	1.35	0.53	0.64	2.83	0.02	0.01	5865.22	2357.51
	g0[.]s[h2]	Halfnorm	5	-229.99	481.97	12.52	0.00	1.53	1.02	0.47	5.01	0.03	0.01	3390.78	1572.28
	g0[h2]s[.]	Halfnorm	5	-230.01	482.03	12.58	0.00	1.36	0.53	0.65	2.85	0.03	0.02	4760.46	781.24
	g0[b]s[.]	Hazard	5	-230.56	483.12	13.67	0.00	1.27	0.54	0.58	2.81	0.03	0.02	6067.95	2529.01
	g0[.]s[h2]	Negexp	5	-230.59	483.17	13.72	0.00	1.44	1.62	0.24	8.50	0.07	0.03	2225.79	1325.41
	g0[h2]s[.]	Negexp	5	-230.65	483.29	13.84	0.00	1.29	0.50	0.62	2.68	0.07	0.04	3116.67	605.91
	g0[.]s[b]	Hazard	5	-231.13	484.27	14.82	0.00	0.89	0.27	0.49	1.61	0.01	0.00	13851.84	674.58

Table 9 Program SPACECAP - Spatially explicit mark-recapture models estimating densities of jaguars (*Panthera onca*) and pumas (Puma concolor) at five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2010, using molecular scatology and remote camera trapping and program SPACECAP (Royle et al. 2009). Model (*NTR*, no trap response; *TR*, trap response); N_{super}, population size; *SD*, standard deviation; *D* (density estimate – number of individuals per 100 km^2); *LCl*, low 95% confidence interval; *UCl*, high 95% confidence interval; Bayes *P*; Bayesian *p* – value; sigma, scale parameter of a bivariate normal encounter function; lam0, intercept for encounter frequency; beta, regression coefficient that measures behavior response; psi, ratio of number of animals. Final models selected are marked in bold. Selection was on values for Bayes *P* and Geweke statistics.

Site	Species	Method	Model	N_{super}	*SD*	*D*	*SD*	95% *LCI*	95% *HCI*	Bayes *P*	*Geweke statistics*			
											sigma	lam0	beta	psi
CFRNP	Jaguar	Cameras	***NTR***	***27***	***12.844***	***1.133***	***0.539***	***0.377***	***2.180***	***0.512***	***6.401***	***0.030***	***NA***	***0.174***
			TR	29	14.046	1.212	0.589	0.377	2.348	0.493	6.439	0.024	0.809	0.186
	Jaguar	Genetics	***NTR***	***15***	***5.862***	***0.623***	***0.246***	***0.335***	***1.090***	***0.502***	***0.592***	***0.547***	***-7.004***	***-0.978***
			TR	15	5.730	0.608	0.240	0.335	1.090	0.519	1.081	0.296	-0.125	-2.400
	Puma	Genetics	NTR	31	32.172	1.296	1.349	0.252	4.570	0.661	-2.291	10.113	-12.620	5.819
			TR	***50***	***39.069***	***2.093***	***1.638***	***0.252***	***5.283***	***0.488***	***-2.785***	***3.294***	***3.067***	***1.343***
CBWS	Jaguar	Cameras	NTR	45	9.980	1.973	0.435	1.177	2.789	0.414	-1.586	-2.315	6.498	8.943
			TR	***46***	***10.435***	***1.985***	***0.455***	***1.220***	***2.876***	***0.439***	***1.230***	***-1.176***	***1.360***	***-1.353***
	Jaguar	Genetics	***NTR***	***80***	***19.837***	***3.468***	***0.864***	***1.917***	***5.185***	***0.491***	***2.230***	***-1.869***	***0.502***	***1.566***
			TR	80	21.105	3.477	0.920	1.874	5.360	0.493	5.318	-2.455	5.397	-1.891
	Puma	Genetics	***NTR***	***25***	***12.216***	***1.094***	***0.532***	***0.305***	***2.092***	***0.458***	***-1.900***	***-0.985***	***3.911***	***3.352***
			TR	34	14.582	1.461	0.635	0.436	2.702	0.530	-0.431	-2.166	3.243	3.460
FB	Jaguar	Cameras	NTR	22	10.476	0.929	0.437	0.334	1.794	0.546	7.088	0.034	0.146	22.271
			TR	24	13.720	0.989	0.572	0.334	2.044	0.598	NA	0.029	0.434	0.155
	Jaguar	Genetics	NTR	22	10.289	0.901	0.429	0.334	1.752	0.572	-0.553	-0.330	3.304	1.206
			TR	***21***	***9.982***	***0.891***	***0.416***	***0.334***	***1.669***	***0.496***	***-2.743***	***1.126***	***-1.322***	***-0.475***

	Puma	Genetics	NTR	35	11.947	1.447	0.498	0.626	2.420	0.482	0.155	-1.231	2.398	1.163
			TR	***35***	***12.309***	***1.446***	***0.514***	***0.584***	***2.420***	***0.476***	***0.151***	***-0.233***	***1.006***	***0.950***
MPR	Jaguar	Cameras	NTR	36	9.866	1.582	0.430	0.784	2.397	0.914	0.908	-1.049	-7.239	-0.430
			TR	***34***	***9.464***	***1.496***	***0.412***	***0.784***	***2.309***	***0.760***	***0.315***	***0.070***	***-0.180***	***-0.808***
	Jaguar	Genetics	NTR	55	15.934	2.398	0.694	1.177	3.791	0.743	4.998	-1.663	-9.396	-0.486
			TR	***57***	***16.795***	***2.468***	***0.732***	***1.264***	***4.009***	***0.678***	***0.882***	***-0.552***	***1.829***	***1.233***
RB	Jaguar	Cameras	***NTR***	***64***	***20.565***	***2.767***	***0.896***	***1.133***	***4.488***	***0.652***	***0.098***	***1.292***	***-3.517***	***-1.270***
			TR	65	21.874	2.842	0.953	1.177	4.793	0.619	0.976	-1.553	1.914	0.122
	Jaguar	Genetics	***NTR***	***51***	***21.284***	***2.240***	***0.927***	***0.610***	***4.009***	***0.528***	***1.245***	***1.172***	***-3.311***	***-1.081***
			TR	54	24.578	2.342	1.071	0.610	4.488	0.579	-0.624	0.276	-0.530	-0.559
	Puma	Genetics	***NTR***	***117***	***37.676***	***5.117***	***1.642***	***2.092***	***8.366***	***0.560***	***2.057***	***-1.555***	***-4.768***	***-0.732***
			TR	126	40.945	5.479	1.784	2.571	9.150	0.558	-1.703	2.206	-3.641	-1.466

Table 10 Program CAPWIRE - Genetic mark-recapture models estimating densities of jaguars (*Panthera onca*) and pumas (*Puma concolor*) at five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2009, using molecular scatology and program CAPWIRE (Miller et al. 2005). SSize, sample size; T_{obs}, number of distinct individuals observed per species and study site; $\hat{N}$, population size estimate; *LCl*, lower confidence bound; *UCl*, upper confidence bound; model, CAPWIRE model used to analyze the data (ECM, even capturability model or TIRM, two innate rate model, was selected a priori); $L_{(ECM)}$, best likelihood score under ECM model; $L_{(TIRM)}$, best likelihood score under TIRM model; Lambda, $L_{(TIRM)} - L_{(ECM)}$; *p*(Lambda), proportions of the simulation (of ECM model), where observed ratio is > than the observed value; $\hat{N}_a$, point estimate of the number of type A individuals per site and species; $\hat{N}_b$, point estimate of the number of type B individuals per site and species; $\hat{\alpha}$, point estimate of relative capture probability of type As to type Bs.

Site	Species	SSize	T_{obs}	$\hat{N}$	*LCI*	*UCI*	Model	$L_{(ECM)}$	$L_{(TIRM)}$	Lambda	*p*(Lambda)	$\hat{N}_a$	$\hat{N}_b$	$\hat{\alpha}$	Mean #Obs./Ind.
MPR	Jaguars	79	10	13	10	16	TIRM	-181.90	-137.53	44.38	0.00	4	9	17.75	7.90
RB	Jaguars	30	9	15	9	20	TIRM	-65.92	-54.25	11.66	0.00	4	11	10.00	3.33
RB	Pumas	36	16	18	16	22	ECM	-99.02	-92.90	6.13	0.16	5	17	3.75	2.25
CC	Jaguars	70	14	16	14	19	TIRM	-184.73	-167.79	16.95	0.00	7	9	5.52	5.00
CC	Pumas	21	6	8	6	11	TIRM	-37.63	-34.01	3.62	0.04	4	4	7.13	3.50
CB	Jaguars	49	8	8	8	9	TIRM	-101.89	-88.82	13.07	0.00	3	5	4.62	6.13
CB	Pumas	10	6	8	6	12	ECM	-17.46	-16.21	1.26	0.34	3	7	3.89	1.67
FB	Jaguars	24	8	10	8	16	TIRM	-49.91	-41.66	8.25	0.00	2	8	6.67	3.00
FB	Pumas	47	11	13	11	16	TIRM	-112.70	-93.36	19.34	0.00	3	10	7.07	4.27

Table 11 Summary of mark-recapture models estimating densities for jaguars (*Panthera onca*) and pumas (*Puma concolor*) at five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize, 2007 – 2010, using molecular scatology, remote camera trapping and programs CAPTURE (N/*ETA*), MARK (N/*ETA*), DENSITY (ML – SECR, maximum likelihood based spatially explicit capture-recapture analysis), SPACECAP (B – SECR, Bayesian spatially explicit capture-recapture analysis and CAPWIRE (N/*ETA*). MMDM, mean maximum distance moved; *ETA*, effective trapping area; *N*, population size; *SE*, standard error; *D*, density estimate (number of individuals per 100 km^2); *SD*, standard deviation; *LCI*, low 95% confidence interval; *UCI*, high 95% confidence interval; *CV*, coefficient of variation.

Site	Species	Method	Density Estimator	Buffer type	Buffer distance (m)	*ETA* (km^2)	*N*	*SE*	*D* (#/100km^2)	*SE*	*SD*	*LCI*	*UCI*	*CV*
MPR	***Jaguar***	***Cameras***	N/*ETA* - CAPTURE	½ MMDM	5615	488.61	10.00	0.36	2.05	0.07		0.00	0.00	0.04
			N/*ETA* - CAPTURE	MMDM	11230	1051.15	10.00	0.36	0.95	0.03		0.00	0.00	0.04
			ML - SECR	---	---	---	---	---	1.41	0.58		0.65	3.05	0.41
			B - SECR	---	---	---	---	---	1.50		0.41	0.78	2.31	0.27
	Jaguar	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	3922	393.02	10.00	0.74	2.54	0.19		0.00	0.00	0.07
			N/*ETA* - CAPTURE	MMDM	7845	746.42	10.00	0.74	1.34	0.10		0.00	0.00	0.07
			N/*ETA* - MARK	½ MMDM	3922	393.02	10.56	0.88	2.69	0.22		2.56	3.81	0.08
			N/*ETA* - MARK	MMDM	7845	746.42	10.56	0.88	1.41	0.12		1.35	2.01	0.08
			ML - SECR	---	---	---	---	---	1.96	0.01		1.02	3.79	0.01
			B - SECR	---	---	---	---	---	2.47		0.73	1.26	4.01	0.30
			CAPWIRE	½ MMDM	3922	393.02	13.00		3.31			2.54	4.07	
			CAPWIRE	MMDM	7845	746.42	13.00		1.74			1.34	2.14	
RB	***Jaguar***	***Cameras***	N/*ETA* - CAPTURE	½ MMDM	3477	295.35	15.00	0.95	5.08	0.32		0.00	0.00	0.06
			N/*ETA* - CAPTURE	MMDM	6954	571.66	15.00	0.95	2.62	0.17		0.00	0.00	0.06
			ML - SECR	---	---	---	---	---	2.68	0.96		1.36	5.28	0.36
			B - SECR	---	---	---	---	---	2.77		0.90	1.13	4.49	0.32
	Jaguar	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	3730	309.92	9.00	0.80	2.90	0.26		0.00	0.00	0.09
			N/*ETA* - CAPTURE	MMDM	7460	614.58	9.00	0.80	1.46	0.13		0.00	0.00	0.09

			N/*ETA* - MARK	½ MMDM	3730	309.92	10.27	2.26	3.32	0.73		2.94	7.15	0.22
			N/*ETA* - MARK	MMDM	7460	614.58	10.27	2.26	1.67	0.37		1.48	3.61	0.22
			ML - SECR	---	---	---	---	---	1.79	0.81		0.77	4.18	0.45
			B - SECR	---	---	---	---	---	2.24		0.93	0.61	4.01	0.42
			CAPWIRE	½ MMDM	3730	309.92	15.00		4.84			2.90	6.45	
			CAPWIRE	MMDM	7460	614.58	15.00		2.44			1.46	3.25	
	Puma	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	2060	191.89	19.00	2.97	9.90	1.55		8.86	15.63	0.16
			N/*ETA* - CAPTURE	MMDM	4119	338.17	19.00	2.97	5.62	0.88		5.03	8.87	0.16
			N/*ETA* - MARK	½ MMDM	2060	191.89	23.06	5.78	12.02	3.01		9.24	23.31	0.25
			N/*ETA* - MARK	MMDM	4119	338.17	23.06	5.78	6.82	1.71		5.25	13.23	0.25
			ML - SECR	---	---	---	---	---	5.16	1.73		2.72	9.78	0.34
			B - SECR	---	---	---	---	---	5.12		1.64	2.09	8.37	0.32
			CAPWIRE	½ MMDM	2060	191.89	18.00		9.38			8.34	11.46	
			CAPWIRE	MMDM	4119	338.17	18.00		5.32			4.73	6.51	
CBWS	***Jaguar***	***Cameras***	N/*ETA* - CAPTURE	½ MMDM	364	325.45	27.00	7.73	8.30	2.38		6.76	19.05	0.29
			N/*ETA* - CAPTURE	MMDM	7295	635.00	27.00	7.73	4.25	1.22		3.46	9.76	0.29
			ML - SECR	---	---	---	---	---	2.51	0.63		1.55	4.08	0.25
			B - SECR	---	---	---	---	---	1.99		0.46	1.22	2.88	0.23
	Jaguar	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	2704	257.17	15.00	0.75	5.83	0.29		0.00	0.00	0.05
			N/*ETA* - CAPTURE	MMDM	5408	464.87	15.00	0.75	3.23	0.16		0.00	0.00	0.05
			N/*ETA* - MARK	½ MMDM	2704	257.17	15.21	0.55	5.92	0.21		5.84	7.19	0.04
			N/*ETA* - MARK	MMDM	5408	464.87	15.21	0.55	3.27	0.12		3.23	3.98	0.04
			ML - SECR	---	---	---	---	---	4.12	1.22		2.34	7.27	0.30
			B - SECR	---	---	---	---	---	3.47		0.86	1.92	5.19	0.25
			CAPWIRE	½ MMDM	2704	257.17	16.00		6.22			5.44	7.39	
			CAPWIRE	MMDM	5408	464.87	16.00		3.44			3.01	4.09	
	Puma	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	2972	276.26	8.00	2.10	2.90	0.76		2.90	7.24	0.26
			N/*ETA* - CAPTURE	MMDM	5944	510.68	8.00	2.10	1.57	0.41		1.57	3.92	0.26

			N/*ETA* - MARK	½ MMDM	2972	276.26	6.23	0.55	2.26	0.20		2.18	3.40	0.09
			N/*ETA* - MARK	MMDM	5944	510.68	6.23	0.55	1.22	0.11		1.18	1.84	0.09
			ML - SECR	---	---	---	---	---	1.74	0.79		0.74	4.07	0.45
			B - SECR	---	---	---	---	---	1.09		0.53	0.31	2.09	0.49
			CAPWIRE	½ MMDM	2972	276.26	8.00		2.90			2.17	3.98	
			CAPWIRE	MMDM	5944	510.68	8.00		1.57			1.17	2.15	
CFRNP	***Jaguar***	***Cameras***	N/*ETA* - CAPTURE	½ MMDM	3974	359.09	9.00	0.99	2.51	0.28		0.00	0.00	0.11
			N/*ETA* - CAPTURE	MMDM	7949	723.85	9.00	0.99	1.24	0.14		0.00	0.00	0.11
			ML - SECR	---	---	---	---	---	1.01	0.47		0.42	2.41	0.47
	Jaguar	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	4228	396.00	8.00	0.48	2.02	0.12		2.02	2.27	0.06
			N/*ETA* - CAPTURE	MMDM	8456	802.00	8.00	0.48	1.00	0.06		1.00	1.12	0.06
			N/*ETA* - MARK	½ MMDM	4228	396.00	8.17	0.52	2.06	0.13		2.02	2.87	0.06
			N/*ETA* - MARK	MMDM	8456	802.00	8.17	0.52	1.02	0.06		1.00	1.42	0.06
			ML - SECR	---	---	---	---	---	0.89	0.37		0.41	1.95	0.42
			B - SECR	---	---	---	---	---	0.62		0.25	0.34	1.09	0.40
			CAPWIRE	½ MMDM	4228	396.00	8.00		2.02			2.02	2.27	
			CAPWIRE	MMDM	8456	802.00	8.00		1.00			1.00	1.12	
	Puma	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	2996	294.00	7.00	1.55	2.38	0.53		2.04	4.76	0.22
			N/*ETA* - CAPTURE	MMDM	5992	553.68	7.00	1.55	1.26	0.28		1.08	2.53	0.22
			N/*ETA* - MARK	½ MMDM	2996	294.00	7.73	2.12	2.63	0.72		2.13	5.88	0.27
			N/*ETA* - MARK	MMDM	5992	553.68	7.73	2.12	1.40	0.38		1.13	3.12	0.27
			ML - SECR	---	---	---	---	---	2.09	1.45		0.61	7.16	0.69
			B - SECR	---	---	---	---	---	2.09		1.64	0.25	5.28	0.78
			CAPWIRE	½ MMDM	2996	294.00	8.00		2.72			2.04	4.08	
			CAPWIRE	MMDM	5992	553.68	8.00		1.44			1.08	2.17	
FB	***Jaguar***	***Cameras***	N/*ETA* - CAPTURE	½ MMDM	5392	453.00	9.00	2.75	1.99	0.61		1.77	4.64	0.31
			N/*ETA* - CAPTURE	MMDM	10783	991.00	9.00	2.75	0.91	0.28		0.81	2.12	0.31
			ML - SECR						0.40	0.36		2.06	3.32	0.90

Jaguar	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	5260	455.00	8.00	0.40	1.76	0.09		0.00	0.00	0.05
		N/*ETA* - CAPTURE	MMDM	10520	970.99	8.00	0.40	0.82	0.04		0.00	0.00	0.05
		N/*ETA* - MARK	½ MMDM	5260	455.00	8.17	0.52	1.80	0.11		1.76	2.50	0.06
		N/*ETA* - MARK	MMDM	10520	970.99	8.17	0.52	0.84	0.05		0.82	1.17	0.06
		ML - SECR	---	---	---	---	---	0.82	0.38		0.34	1.95	0.46
		B - SECR	---	---	---	---	---	0.89		0.42	0.33	1.67	0.47
		CAPWIRE	½ MMDM	5260	455.00	10.00		2.20			1.76	3.52	
		CAPWIRE	MMDM	10520	970.99	10.00		1.03			0.82	1.65	
Puma	***Genetics***	N/*ETA* - CAPTURE	½ MMDM	3388	309.00	11.00	0.45	3.56	0.15		0.00	0.00	0.04
		N/*ETA* - CAPTURE	MMDM	6777	586.79	11.00	0.45	1.87	0.08		0.00	0.00	0.04
		N/*ETA* - MARK	½ MMDM	3388	309.00	11.21	0.56	3.63	0.18		3.56	4.73	0.05
		N/*ETA* - MARK	MMDM	6777	586.79	11.21	0.56	1.91	0.10		1.88	2.49	0.05
		ML - SECR	---	---	---	---	---	1.36	0.53		0.65	2.84	0.39
		B - SECR	---	---	---	---	---	1.45		0.51	0.58	2.42	0.35
		CAPWIRE	½ MMDM	3388	309.00	13.00		4.21			3.56	5.18	
		CAPWIRE	MMDM	6777	586.79	13.00		2.22			1.87	2.73	

Fig. 1 Map of survey sites across Belize, Central America, including Mountain Pine Ridge Forest Reserve (MPR), Rio Bravo Conservation and Management Area (RB), Cockscomb Basin Wildlife Sanctuary (CBWS), Chiquibul Forest Reserve and National Park (CFRNP), Fireburn/Balam Na Nature Reserve (FB) in Belize, Central America, 2007 – 2009.

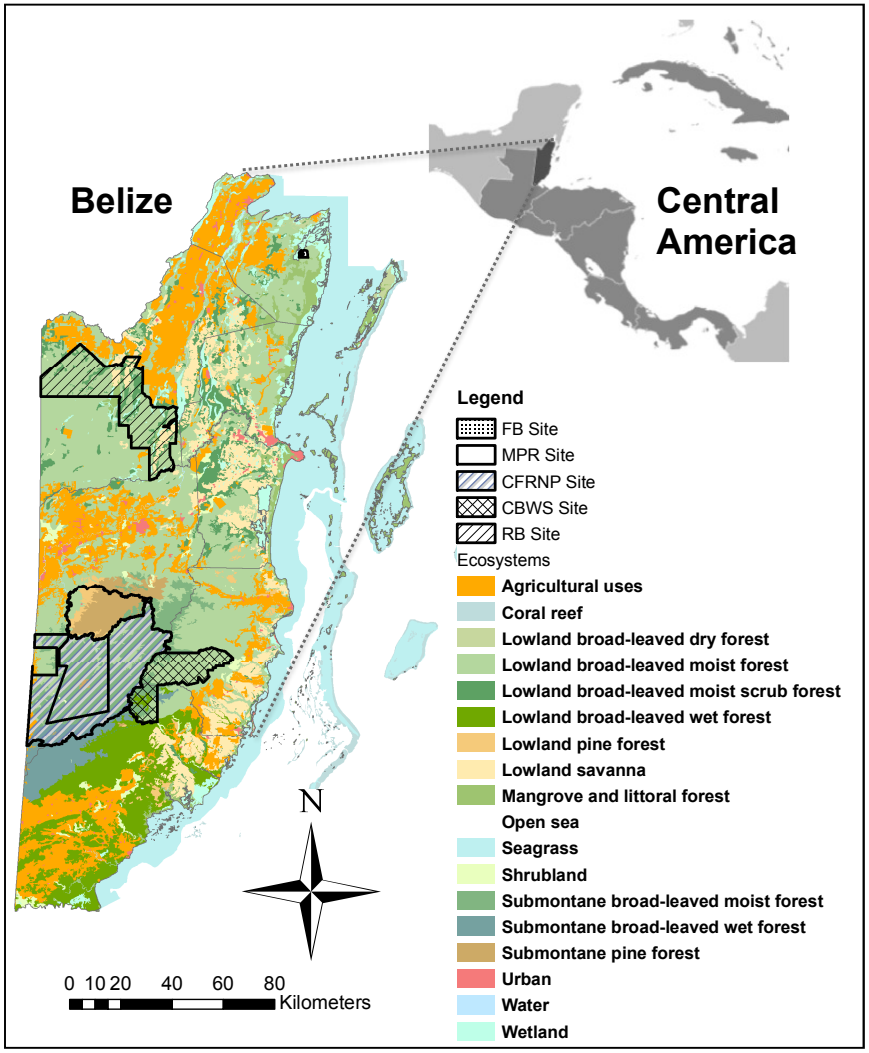

Fig. 2 Survey design for noninvasive monitoring of jaguars (*Panthera onca*) and pumas (*Puma concolor*) by simultaneously using remote cameras and noninvasive genetic sampling/scat surveys (with scat detector dogs) across five study sites (1 – 5) in Belize, Central America, 2007 - 2009.

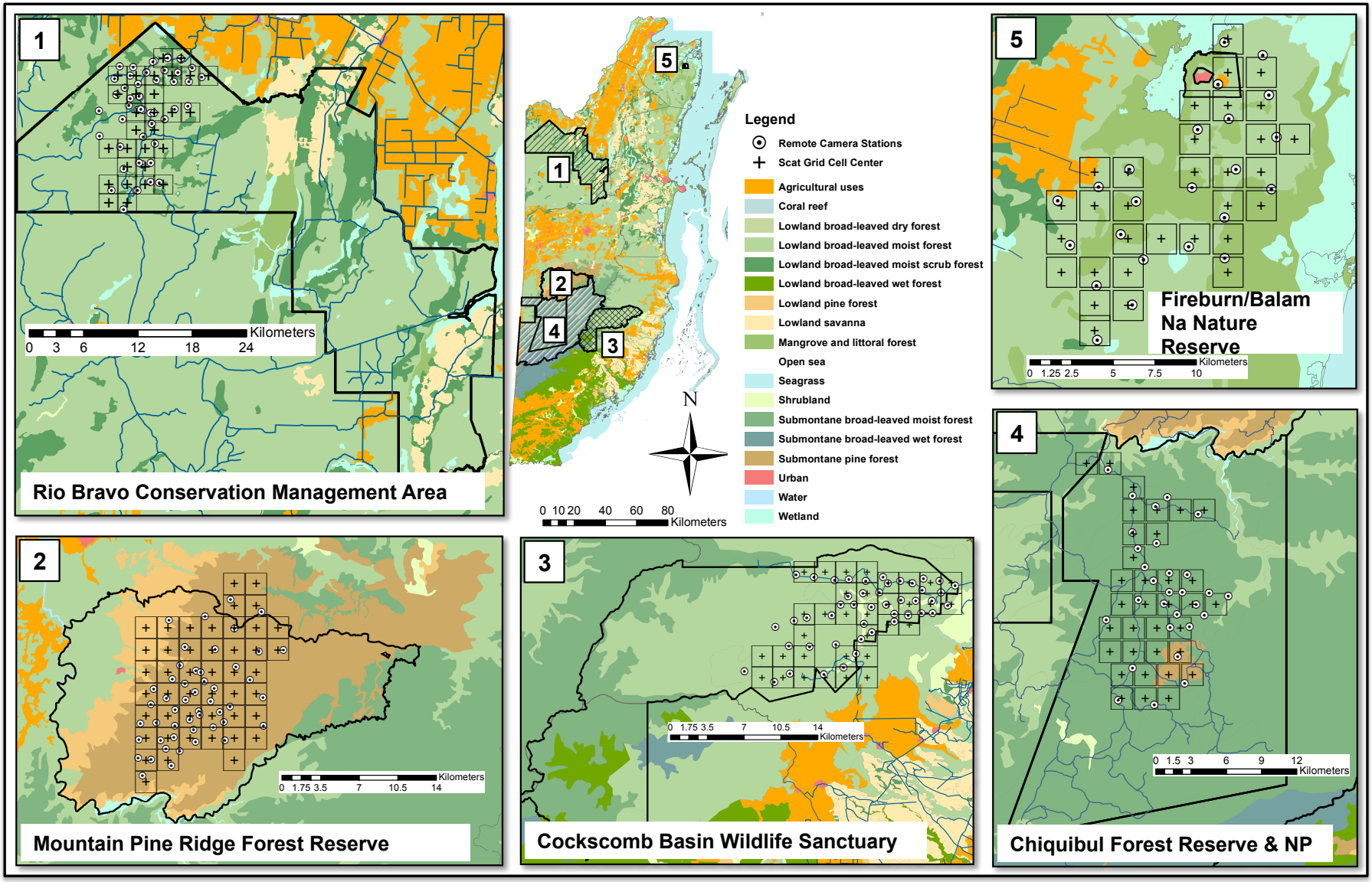

Fig. 3 Effective trapping area (ETA) calculation using mean maximum distances moved (1/2 and full MMDM). For remote camera trapping survey, buffer areas were created around camera stations, whereas the molecular scatology study used center locations of scat survey grid cells. Buffer areas were dissolved and total ETAs were calculated in km2 across all five study sites for both target species, jaguars (*Panthera onca*) and pumas (*Puma concolor*) (ETA calculations for jaguars at the MPR site pictured here as example).

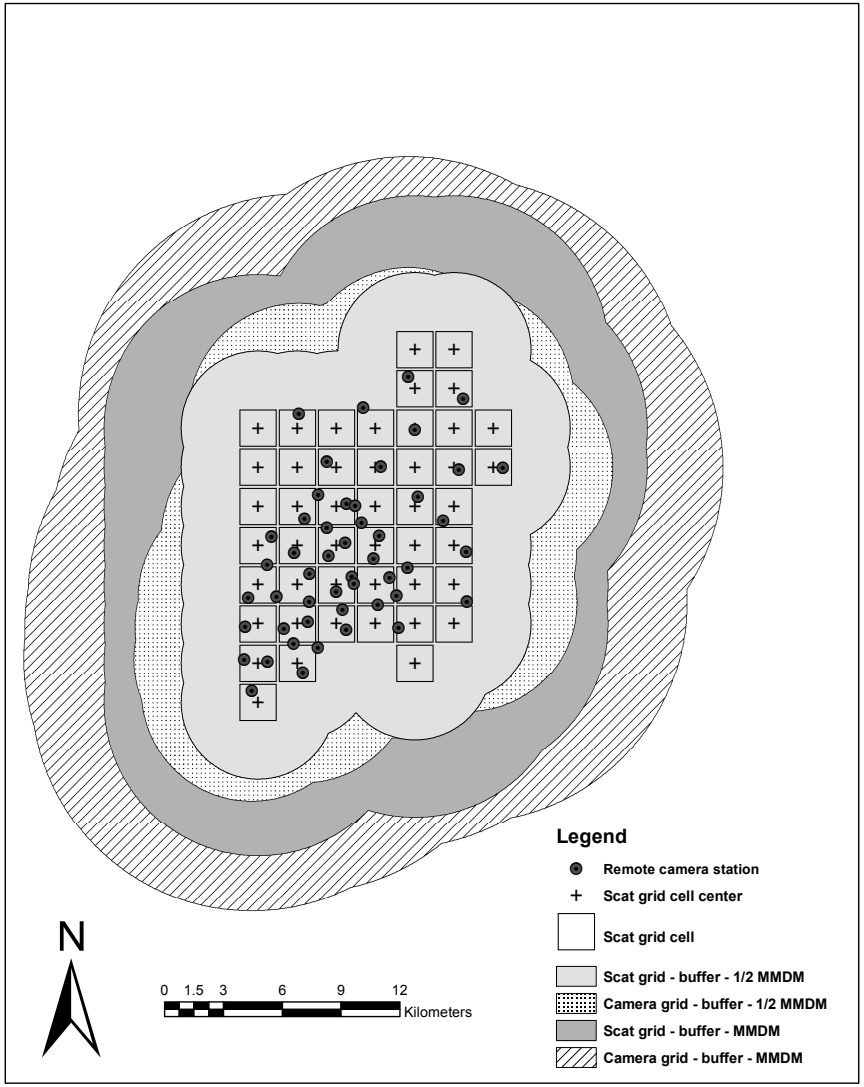

Fig. 4 Genetic mark-recapture density estimates and 95% confidence limits for jaguars (*Panthera onca*) across five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize using eight methods of density estimation (CAPTURE – ½ MMDM, $\hat{N}$ /*ETA* with half MMDM as buffer distance; CAPTURE - MMDM, $\hat{N}$ /*ETA* with MMDM as buffer distance; MARK – ½ MMDM, $\hat{N}$ /*ETA* with half MMDM as buffer distance; MARK – MMDM, $\hat{N}$ /*ETA* with MMDM as buffer distance; ML – SECR, maximum likelihood based spatially explicit mark-recapture in program DENSITY; B – SECR, Bayesian spatially explicit mark-recapture in program SPACECAP; CAPWIRE – ½ MMDM, genetic-based $\hat{N}$ /*ETA* with half MMDM as buffer distance; CAPWIRE – MMDM, genetic-based $\hat{N}$ /*ETA* with MMDM as buffer distance).

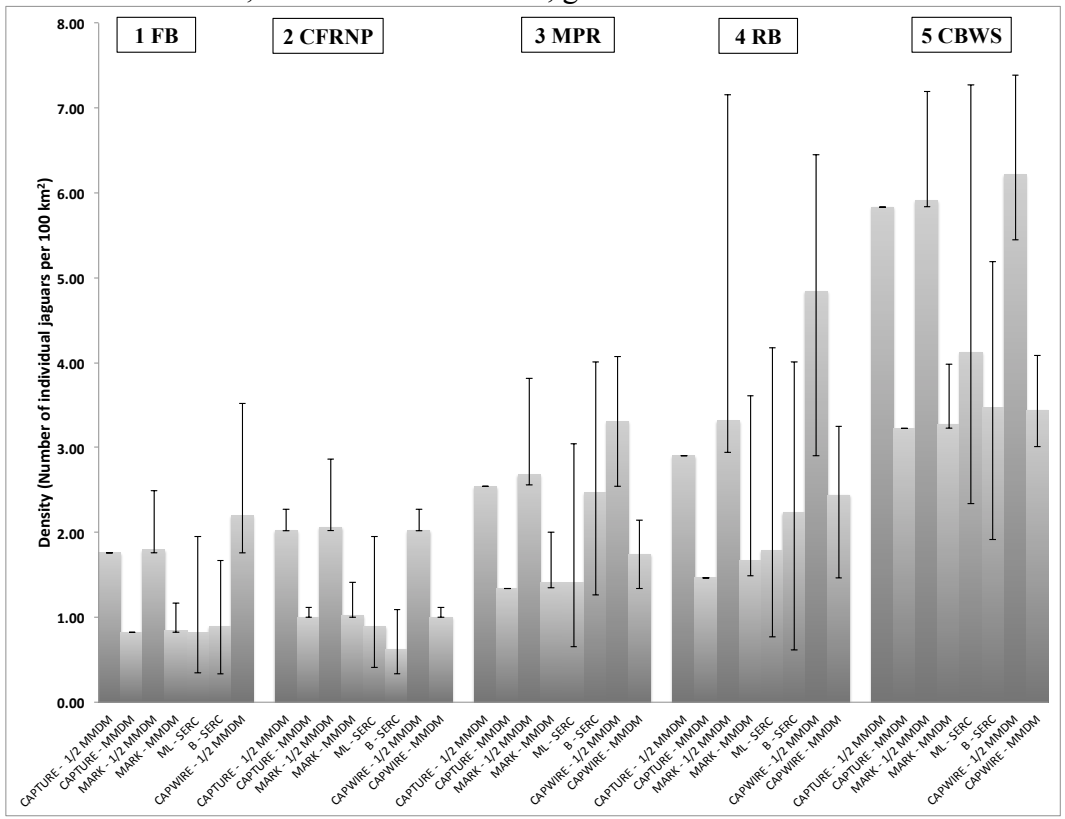

Fig. 5 Genetic mark-recapture density estimates and 95% confidence limits for pumas (*Puma concolor*) across four study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize using eight methods of density estimation (CAPTURE – ½ MMDM, $\hat{N}$ /*ETA* with half MMDM as buffer distance; CAPTURE - MMDM, $\hat{N}$ /*ETA* with MMDM as buffer distance; MARK – ½ MMDM, $\hat{N}$ /*ETA* with half MMDM as buffer distance; MARK – MMDM, $\hat{N}$ /*ETA* with MMDM as buffer distance; ML – SECR, maximum likelihood based spatially explicit mark-recapture in program DENSITY; B – SECR, Bayesian spatially explicit mark-recapture in program SPACECAP; CAPWIRE – ½ MMDM, genetic-based $\hat{N}$ /*ETA* with half MMDM as buffer distance; CAPWIRE – MMDM, genetic-based $\hat{N}$ /*ETA* with MMDM as buffer distance).

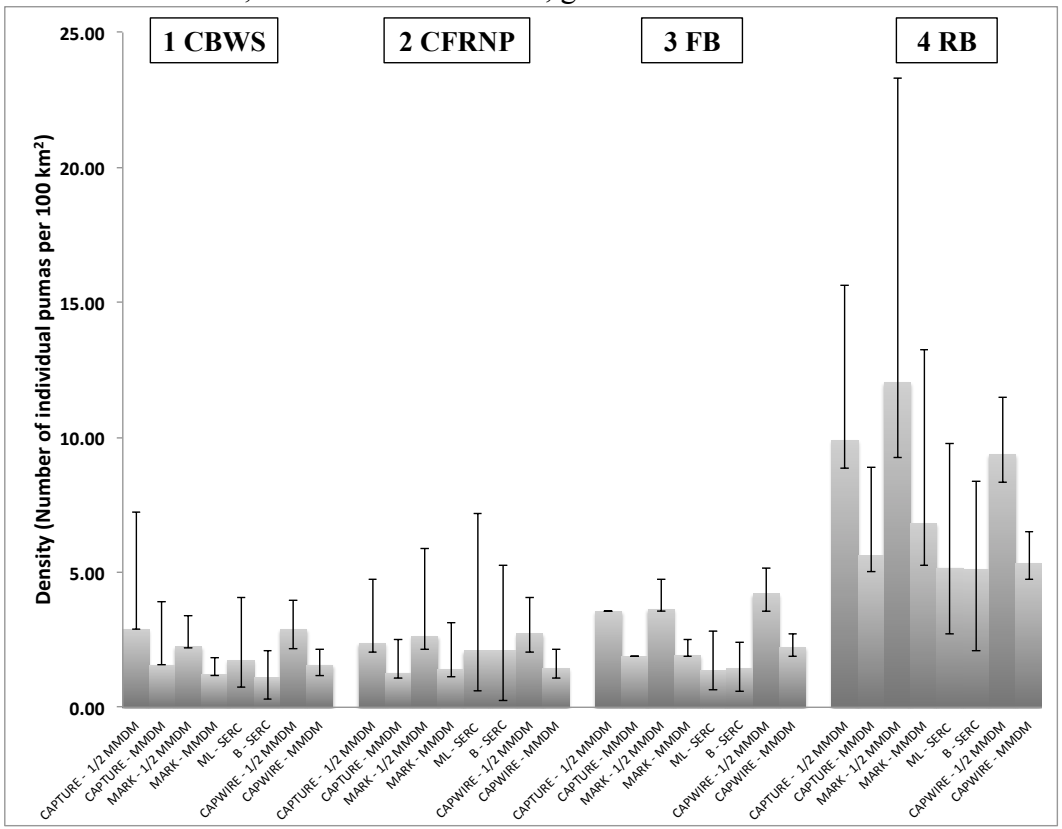

Fig. 6 Mark-recapture density estimates and 95% confidence limits for jaguars (*Panthera onca*) across five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize using molecular scatology, remote camera trapping and four methods of density estimation (CAPTURE – ½ MMDM, $\hat{N}$ /*ETA* with half MMDM as buffer distance; CAPTURE - MMDM, $\hat{N}$ /*ETA* with MMDM as buffer distance; ML – SECR, maximum likelihood based spatially explicit mark-recapture in program DENSITY; B – SECR, Bayesian spatially explicit mark-recapture in program SPACECAP).

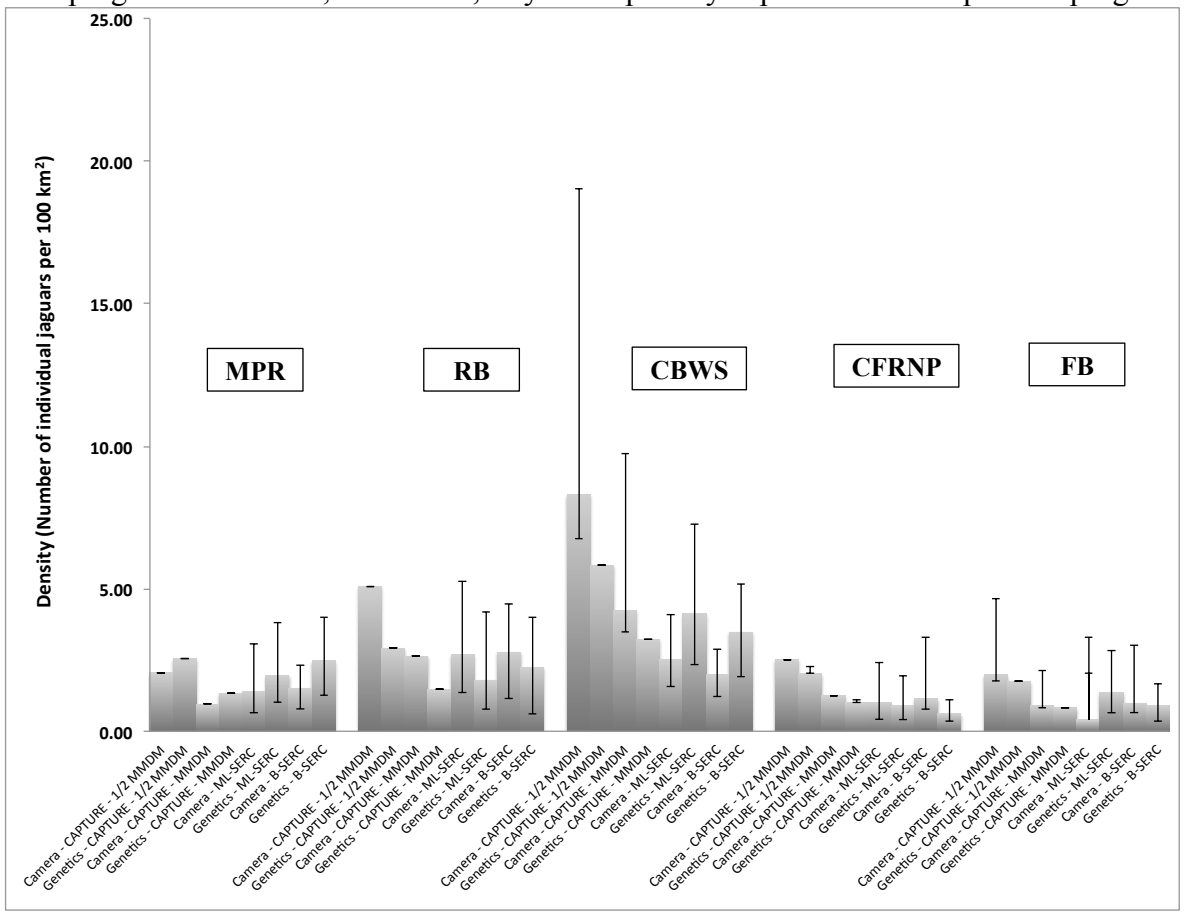

Fig. 7 Capture probabilities for jaguars (*Panthera onca*) across five study sites (MPR, Mountain Pine Ridge Forest Reserve; RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize using molecular scatology, remote camera trapping and several methods of density estimation (CAPTURE – $\hat{N}$ /*ETA* ML – SECR, maximum likelihood based spatially explicit mark-recapture in program DENSITY; B – SECR, Bayesian spatially explicit mark-recapture in program SPACECAP; CAPWIRE – genetic-based $\hat{N}$ /*ETA*).

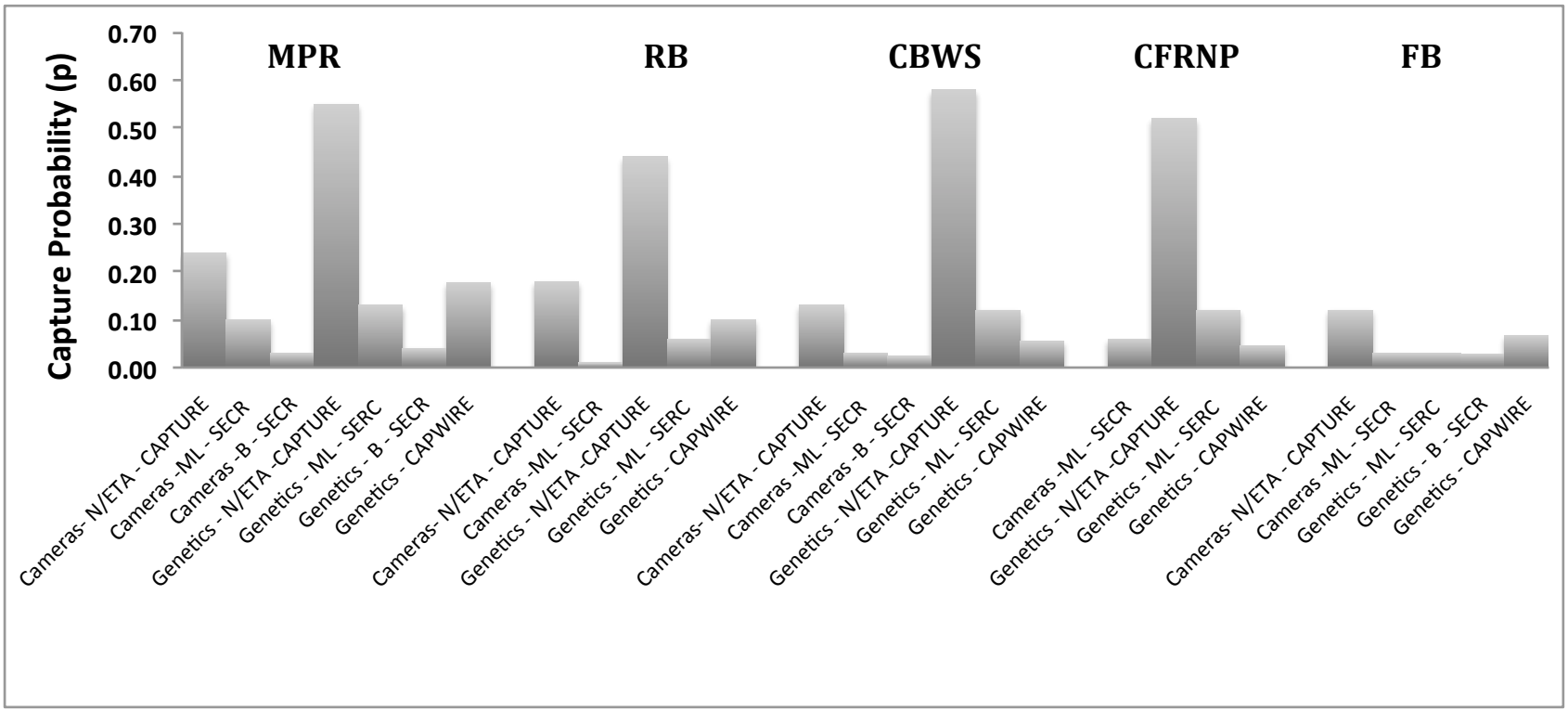

Fig. 8 Capture probabilities for pumas (*Puma concolor*) across four study sites (RB, Rio Bravo Conservation and Management Area; CBWS, Cockscomb Basin Wildlife Sanctuary, CFRNP, Chiquibul Forest Reserve and National Park; FB, Fireburn/Balam Na Nature Reserve) in Belize using molecular scatology, remote camera trapping and several methods of density estimation (CAPTURE – $\hat{N}$ /*ETA* ML – SECR, maximum likelihood based spatially explicit mark-recapture in program DENSITY; B – SECR, Bayesian spatially explicit mark-recapture in program SPACECAP; CAPWIRE – genetic-based $\hat{N}$ /*ETA*).