ESTIMATION OF POPULATION DENSITY AND INVESTIGATION OF SOCIO-SPATIAL ORGANIZATION OF OCELOTS (*LEOPARDUS PARDALIS*) FROM COMPARISON AND INTEGRATION OF TWO NONINVASIVE METHODS

BY

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THESIS

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ABSTRACT

Due to their elusive nature, most carnivore species are difficult to monitor and study, and thus data are often insufficient to guide appropriate conservation action or to test hypotheses regarding species ecology (Nowell and Jackson 1996, Macdonald and Loveridge 2010, Rodgers and Janečka 2013). Among the 37 extant species in the family Felidae, 26 are listed as endangered, near threatened, or vulnerable, and over 86% have population numbers that are either decreasing or unknown (IUCN Red List 2011.2). The majority of felid species are secretive and solitary (Nowell and Jackson 1996, Sunquist and Sunquist 2002, Macdonald and Loveridge 2010), often making research based on visual observation impossible. Studying felids using traditional capture methods can be costly, labor intensive, or invasive. Telemetry studies yield the greatest level of information on behavior and ecology, however these methods require live capture that may cause stress and disturbance, or potentially injury or mortality if done improperly, raising ethical concerns (Greenwood 1996, Piggott and Taylor 2003, Kelly et al. 2012). Moreover, the significant effort or cost needed to track individuals via radio or GPS collars leads to few studies at a small number of sites, often with small sample sizes (Kelly et al. 2012).

In response to these challenges, two noninvasive methods have become more commonplace in carnivore research: camera trapping (Carey 1926, Karanth 1995), and noninvasive genetics (Hoss et al. 1992, Kohn and Wayne 1997). These two methods do not require capture or direct observation of study animals, greatly increasing the amount of data that can be collected, while also alleviating some of the ethical concerns involved in animal capture. To date, most studies employing these techniques have examined species distributions or population abundance, while fewer have used them to study aspects of species ecology and behavior (Rodgers and Janečka 2013). The research contained in this thesis first compares the use of these two techniques for estimation of population density (chapter 1), and then integrates the two techniques to study aspects of species ecology and behavior (chapter 2) in ocelots \textit{(Leopardus pardalis)} on Barro Colorado Island, Panama.

Ocelots are a medium-sized felid that ranges from northern Argentina to the southern United States. Although ocelots are currently listed as least concern throughout their range (IUCN 2013), some
populations (e.g. southern Texas) are critically endangered, and many populations are in decline due to habitat loss. Ocelots are an ideal species for use of noninvasive techniques because they are elusive and difficult to directly observe or capture. Additionally, they possess unique spot patterns that make it possible to identify individuals from camera trap photographs, and they use communal latrines, making it relatively easy to obtain scat samples for noninvasive genetic analyses. Barro Colorado Island is also an ideal study site, because it contains a population of ocelots that is relatively closed geographically, making it possible to include the entire population in analyses.

In chapter 1, I compare camera trapping with noninvasive genetics for the estimation of ocelot population density. Camera trapping has been used extensively to estimate abundance and population density in many carnivore species, and is generally considered as a reliable technique (O'Connell et al. 2011, Foster and Harmsen 2012). Use of noninvasive genetics to estimate population density has been increasing, however, few studies have examined the accuracy and precision of these techniques by comparing them with more established methods (Rodgers and Janečka 2013). My goal was to evaluate the accuracy and precision of noninvasive genetics for estimating population density in elusive carnivores relative to estimates from camera trapping in the same study population during the same time period. I found that the two techniques were comparable, supporting the validity of noninvasive genetic techniques for estimating density in elusive species.

In chapter two, I integrated camera trapping and noninvasive genetics to examine patterns of spatiotemporal overlap and kin structure in ocelots. Historically, small felids were considered territorial, with one or both sexes maintaining exclusive territories, and this pattern of spatial organization has been observed in some ocelot populations (Tewes 1986, Ludlow and Sunquist 1987, Laack 1991). Recent research, however, has revealed considerable flexibility in spatial organization among population of many felid species, possibly as a result of differences in ecological factors such as resource availability and population density (Sandell 1989, Goodrich et al. 2010). As the ocelot population on Barro Colorado Island is at extremely high density, I hypothesized that ocelots in this population would not maintain
exclusive territories due to the high costs of defending a territory from many competitors. To examine spatiotemporal overlap between ocelots of both sexes, I used three years of camera-trap data encompassing the entire adult population. If individual ocelots overlap considerably in their use of space at high density, one mechanism that could reduce the cost of competition for shared resources is inclusive fitness (Hamilton 1964). To examine this possibility, I used noninvasive genetics to examine if individuals who overlapped in their use of space were more closely related than the population as a whole. I documented extensive intersexual and intrasexual overlap within both sexes of ocelots, however, intrasexual overlap between males was much stronger than between females. I also found a positive relationship between spatiotemporal overlap and genetic relatedness, supporting the hypothesis that kin structure plays a role in structuring ocelot spatial organization.
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CHAPTER 1: SUPPORT FOR FECAL NONINVASIVE GENETICS AS A VALID TECHNIQUE FOR ESTIMATING POPULATION DENSITY OF ELUSIVE ANIMALS

Abstract

Estimates of population density are essential for the effective conservation and management of any species. For elusive animals, however, accurately estimating density can pose a serious challenge. One approach to meet this challenge is integration of DNA collected noninvasively from feces with capture-recapture modeling. To date, the bias and precision of this technique has seldom been evaluated in the field. We conducted a study to compare density estimates of ocelots (Leopardus pardalis) derived from fecal noninvasive genetic techniques to density estimates from camera trapping in the same population on Barro Colorado Island, Panama, during the same study period. We found that density estimates from the two techniques were comparable, especially when using spatially explicit capture-recapture models. Population density estimated using the program DENSITY was 1.74/km² (SE = 0.584) from noninvasive genetics and 1.56/km² (SE = 0.331) from camera trapping. These estimates also represent the highest reported ocelot population density within the species range.

Introduction

Population density is a parameter of vital importance for species conservation. For rare or elusive species, however, acquiring reliable density estimates based on visual observation or capture can be difficult or impossible. As a result, two noninvasive approaches are now commonly used to estimate abundance or density of elusive animals in conjunction with capture-recapture (CR) models: camera trapping (Karanth 1995, Karanth and Nichols 1998), and noninvasive genetics (Kohn et al. 1999, Perez et al. 2006). To obtain CR density estimates from camera trapping, individual animals are identified from photographs based on morphological differences such as unique pelage patterns (Karanth and Nichols 1998, Trolle and Kery 2003) allowing researchers to construct capture histories for each individual. For the many elusive species without unique individual markings, however, the only realistic option for
estimating population density using CR methods may be noninvasive genetics, in which individuals are identified from remotely collected DNA using genetic markers (Sollmann et al. 2013).

Since its first application by Karanth (1995), camera trapping has been widely used, reviewed, and evaluated. To date >100 published studies have used camera trapping in conjunction with CR techniques to estimate abundance or density, including >50 since 2010 (Web of Knowledge accessed 11-June-2013). Additionally, the accuracy of abundance and density estimates from camera trapping has been evaluated through comparisons with populations of known size (Balme et al. 2009), comparisons with estimates from telemetry (Soisalo and Cavalcanti 2006, Dillon and Kelly 2008, Balme et al. 2009, Sharma et al. 2010, Nunez-Perez 2011), and via simulation (Blanc et al. 2013, Tobler and Powell 2013). As a result camera trapping is considered a reliable method for estimating abundance and density in rare or elusive species (O'Connell et al. 2011, Foster and Harmsen 2012).

Use of noninvasive genetics to estimate abundance has recently become more common, but few studies have compared estimates derived from noninvasive genetics with estimates from other sources to evaluate the accuracy or potential bias of the technique (but see Solberg et al. 2006, Mondol et al. 2009, Janečka et al. 2011a, Hedges et al. 2013). In addition, most noninvasive genetics studies have only estimated abundance and not population density. Abundance by itself is a less valuable parameter for conservation as it makes comparisons with other studies and other populations difficult (Rodgers and Janečka 2013). Estimating abundance with CR models is relatively straightforward. Estimating density can be more difficult, however, because it requires accurate estimation of the effective survey area (ESA), which is a challenge in geographically open populations and in areas with patchy habitat. In the past, most camera trap and noninvasive genetics studies that estimated density have used derivations of the mean maximum distance moved (MMDM) between captures to estimate ESA, however the validity of this method has been debated (Soisalo and Cavalcanti 2006, Dillon and Kelly 2008, Pesenti and Zimmermann 2013, Tobler and Powell 2013). More recently, sophisticated Bayesian and maximum-likelihood spatially explicit capture-recapture (SECR) models have been developed that estimate density directly by modeling
ESA explicitly using the geographic locations of captures (Efford 2004, Borchers and Efford 2008, Royle and Young 2008).

To our knowledge, no published study has directly compared estimates of population density derived from noninvasive genetics with estimates from another source in the same population during the same time period to evaluate the accuracy or potential bias of the technique. Thus, we conducted a study to directly compare density estimates from noninvasive genetics with estimates from camera trapping for ocelots (*Leopardus pardalis*) on Barro Colorado Island (BCI) in Panama. We also compared a variety of different CR models for estimating population density from our noninvasive data, including both traditional CR and SECR models. Ocelots are an ideal organism for such a study, because individuals can be identified for CR analyses based both on unique spot patterns (Trolle and Kery 2003) and unique DNA genotypes collected noninvasively from fecal samples. Our island study site is also an ideal setting for such a study because it is relatively closed geographically. For our density estimates from camera trapping, we did not face the same problems with estimating ESA inherent in other studies as we were simply able to use island area as our ESA. Thus, we think our camera-trapping results reflect a relatively unbiased estimate of population density, making them an excellent standard for validating estimates from fecal noninvasive genetic techniques.

**Materials and Methods**

**Study site**

Field work was conducted on BCI, a 1,543-ha island in the Panama Canal waterway, at a research station operated by the Smithsonian Tropical Research Institute (Fig. 1.1). BCI (9°10′N, 79°51′W) sits within Gatun Lake, an artificial body of water created in 1912 by the damming of the Chagres River to create the Panama Canal, and is part of the protected 54-km² Barro Colorado Nature Monument. Vegetation is tropical moist forest, and topography is dominated by hills that reach a maximum elevation of 165 m above sea level. Mean temperature is 27 °C with an average annual precipitation of 2600 mm, with 90% of rainfall occurring from May through November.
**Scat surveys**

Ocelots commonly defecate at latrine sites that are used by multiple individuals of both sexes (Moreno and Giacalone 2008). These latrines are typically located in large cavities or overhanging buttresses of large trees, underneath buttresses of fallen trees, or underneath human structures (Fig. 1.2). Thus, the primary objective of our surveys was to locate as many ocelot latrines as possible. All scats used in this study were found at such latrines. Seven latrines were located during a pilot study in 2011, five of which were still active in 2012. To find additional latrines in 2012, we walked all 39.5 km of trails on BCI (Fig.1.1) a minimum of 3 times (once every 33 days). In addition, we walked >390 km of random, off-trail transects throughout the island in search of latrines. Once a latrine was located, it was revisited every 4-7 days for the remainder of the study to collect additional scats. Scat collection was conducted over 99 days from 29-January – 6-May 2012. The study length was chosen as a balance between being long enough to obtain sufficient data to estimate density, while still being short enough to satisfy assumptions of demographic closure (Tobler and Powell 2013). Scats decompose quickly in the warm and tropical environment of Panama, so all collected scats were deposited by animals during the study period or shortly beforehand (within several days).

**Camera trapping**

We conducted camera trapping using an array of 21 Reconyx PC900 and RC55 trail cameras (Reconyx Inc., Holmen, Wisconsin). Cameras were distributed evenly throughout the entire island so that gaps between cameras that could contain an ocelot home range were highly unlikely (Fig. 1.1), ensuring all individuals on the island had a capture probability > 0. The grid size of camera traps was several times larger than an ocelot home range as suggested for unbiased density estimates (Noss et al. 2012, Tobler and Powell 2013). We placed all cameras along trails to maximize capture probability. Eighteen cameras were part of an ongoing, multi-year camera trap survey of the BCI mammal community; three additional cameras were placed to fill gaps. Each camera station consisted of one camera and thus could photograph only one side of an animal at a time. For all individuals, however, right and left sides were observed in the
same photo sequence at least once either before or during our study period, allowing right and left side
profiles to be paired together for individual identification. Cameras were active for the same 99-day
period that scat surveys were conducted. Seven cameras failed for short durations of the study, and this
censoring was accounted for in the analyses when possible (Foster and Harmsen 2012).

**DNA extraction and species identification**

We extracted DNA from scats using the Quiagen QIAamp DNA stool mini kit (Quiagen, Valencia,
California) following the manufacturer’s recommendations. For species identification, we amplified a
126-bp fragment of the mitochondrial gene ATP6 by polymerase chain reaction (PCR) using primers
ATP6-DF3 and ATP6-DR1 following conditions from Chaves et al. (2012). PCR products were
sequenced on an Applied Biosystems 3730xl DNA analyzer, and resulting sequences were compared to
reference sequences using the online tool DNA Surveillance Carnivora (Chaves et al. 2012).

**Individual identification**

Scat samples were genotyped at 4 microsatellite loci (FCA075, FCA077, FCA088, and FCA132)
originally developed for the domestic cat (*Felis catus*) (Menotti-Raymond et al. 1999). We initially
screened 22 loci previously found to be variable in ocelots (Janečka et al. 2011b). The 4 loci used for
individual identification were selected based on degree of variability, success of amplification, and ease
and clarity of allele scoring. As DNA from noninvasively collected fecal samples is often low quality and
prone to genotyping errors such as allelic dropout and false alleles (Taberlet et al. 1999, Broquet et al.
2007), we used a multiple tubes approach (Taberlet and Fumagalli 1996) whereby each sample was
genotyped 3-9 times until reliable consensus genotypes were obtained. Genotypes were only accepted as
reliable if a minimum of 3 identical heterozygote profiles, or 5 identical homozygote profiles, were
observed (see Appendix A for PCR conditions). Checks for departure from Hardy-Weinberg equilibrium,
and probability of individual identity (Waits et al. 2001) were calculated using the program GENEALEX
(Peakall and Smouse 2006).
Sex identification

For sex identification, we used felid-specific primers that amplify a 200-bp segment of the AMELY gene (Murphy et al. 1999), which is only present on the Y-chromosome of males (See Appendix A for PCR conditions). PCR amplifications were performed in triplicate along with male and female positive controls and a negative control, and PCR products were visualized on agarose gel. Samples were identified as male if they showed amplification of the Y-linked marker for all 3 replicates, and were identified as female if no amplification was observed.

Density estimation

We used a broad suite of different CR models to estimate density from both our noninvasive genetic and camera trap data to provide a comprehensive comparison of common approaches. We used multiple models because we wanted to compare newer, more sophisticated models with older, more widely used models to determine which models performed the best. With our genetic data, we ran traditional closed CR analyses in the program CAPTURE (Otis et al. 1978) to estimate abundance. CAPTURE was implemented within the program MARK (White and Burnham 1999). We also derived abundance estimates from the program CAPWIRE (Miller et al. 2005), a maximum-likelihood estimator designed specifically for use with noninvasive genetic data collected in one pooled sampling occasion. CAPWIRE was implemented in the programming environment R (R Core Team 2012). To convert abundance estimates to density, we buffered sample locations with MMDM as well as ½-MMDM (Wilson and Anderson 1985, Karanth and Nichols 1998), which can be conceptualized as a proxy for home range radius. We then used the sum of the resulting buffers as an estimate of ESA (Karanth and Nichols 1998, Janečka et al. 2011a). MMDM and ESA estimates were calculated in the program DENSITY (Borchers and Efford 2008). Density estimates were then derived by dividing abundance estimates by ESA.

We also estimated density directly using two SECR models. We used the program SPACECAP (Gopalaswamy et al. 2012), which estimates density directly using a Bayesian approach (Royle and
Young 2008, Royle et al. 2009), and the program DENSITY, which estimates density directly using a maximum-likelihood approach (Efford 2004, Borchers and Efford 2008, Efford et al. 2009). For both SECR models, we considered ocelot latrines as one might consider camera traps or hair snares, as discrete geographic locations where animals could be passively detected. Both SPACECAP and DENSITY allow detectors to be considered as ‘open’ or ‘closed’ during each sampling occasion. Thus, latrines known before commencement of the study were considered as ‘open’ during the entire study period, and latrines discovered in the interim of the study were considered as ‘closed’ prior to discovery and ‘open’ thereafter. Sampling occasions were 3 days, giving a total of 33 occasions during the study. The program SPACECAP was implemented in R (R Core Team 2012). We modeled trap-response as absent, and the detection function as half-normal. Markov Chain Monte Carlo simulations were run for 50,000 iterations, with a burn-in period of 1,000 iterations. Convergence of chains was verified using Geweke’s diagnostics (Gopalaswamy et al. 2012). In the program DENSITY, all models were run under full likelihood with a Poisson distribution and the half-normal detection function. We ran multiple model combinations in DENSITY including various levels of individual heterogeneity, and the best-fit model was chosen using the Akaike Information Criterion adjusted for small sample size (AICc).

For our camera trapping data, we also estimated abundance using the program CAPTURE. As both the MMDM and the $\frac{1}{2}$-MMDM buffer areas were larger than the area of the entire island, we used island area (15.432 km$^2$) as our ESA for estimating density. We also estimated density directly using both SPACECAP and DENSITY under the same parameters described above.

**Results**

**Noninvasive genetic sampling**

We collected 63 scats from 19 ocelot latrines on BCI, and we genetically confirmed 55 of these (87%) as being from ocelots. From these 55 scats, we obtained complete 4-locus genotypes from 43 samples (78%), which consisted of 12 unique genotypes and 31 recaptures from 16 latrines. Six individuals were
identified as male, and six as female. All 4 loci were in Hardy-Weinberg equilibrium and the number of alleles per locus was 3-6. Based on allele frequencies within the sampled population, the probability of two different individuals sharing the same genotype (Probability of individual identity; Waits et al. 2001) was 0.00031 among unrelated individuals (\(P_{\text{ID}}\)), and 0.038 among siblings (\(P_{\text{ID,sibs}}\)).

**Camera trapping**

Twenty-one cameras were active during the 99-day sampling period resulting in 1,824 camera days after accounting for cameras that failed for short periods. This effort resulted in 398 independent photo sequences of 28 individual ocelots. Of these, 4 individuals were kittens photographed with their mother, which were excluded from further density analyses. Of the 24 photographed adult ocelots, 9 were males, and 15 were females. The number of individuals detected from camera trapping was greater than the number detected from noninvasive genetics because of the difficulty of finding active ocelot latrines. All detected latrines were constricted to a portion of the island, while cameras were distributed evenly throughout the entire island (Fig. 1.1). As a result, raw abundance estimates were not directly comparable between our two sampling approaches, however the models which we use to estimate density account for these spatial differences.

**Density estimation**

For traditional CR analyses of our noninvasive genetic data, the discriminant function model selection algorithm in CAPTURE selected model \(M_0\) as the most appropriate model (selection score of 1), with model \(M_{\text{h-jackknife}}\) a close second (selection score of 0.94). As model \(M_0\) is highly sensitive to violations of the assumption of homogeneous capture probability (Otis et al. 1978), and as some heterogeneity in capture probability at ocelot latrines is probable, we report results from both model \(M_0\) and \(M_h\) (Table 1). Model \(M_0\) produced an abundance estimate of 12 (SE = 0.918) and model \(M_h\) produced an abundance estimate of 15 (SE = 2.433). Capture probability was 0.189 for model \(M_0\) and 0.152 for model \(M_h\). In the program CAPWIRE, the ‘tirm’ model with two innate rates of capture (heterogeneity in capture
probability) was selected as the best-fitting model, resulting in an abundance estimate of 13 (95% CI = 12-16). MMDM from noninvasive genetic sampling was 632m (SE = 117), resulting in ESA buffers of 9.760 km$^2$ (MMDM) and 3.114 km$^2$ ($\frac{1}{2}$-MMDM). The program SPACECAP estimated an ocelot density of 1.303/km$^2$ (SE = 0.272), and summary statistics indicated a good model fit (Bayesian $p$-value = 0.5410). The program DENSITY selected the null model $g0(.)\sigma(.)$ as the best-fitting model (Appendix B, Table B.1) resulting in a density estimate of 1.740/km$^2$ (SE = 0.584).

From our camera trapping data, CAPTURE selected model $M_{h\text{-jackknife}}$ as the best-fitting model of those available (Table 1). Model $M_h$ is also the most commonly used model in camera trapping studies (Harmsen et al. 2011). Model $M_h$ resulted in an abundance estimate of 28 (SE = 2.12) ocelots, with a capture probability of 0.262. MMDM from camera trapping was 1509m (SE = 289). The program SPACECAP estimated an ocelot density of 1.576 (Posterior SD = 0.743), but summary statistics indicated poor model fit (Bayesian $p$-value > 0.999). Because SPACECAP is a relatively new program that provides little diagnostic power to determine causes of poor model fit, this result should be interpreted with caution. The program DENSITY selected model $g0(.)\sigma(.)$ as the best fit resulting in a density estimate of 1.561/km$^2$ (SE = 0.322). Model $g0(.)\sigma(h2)$ also had some support ($\Delta AICc = 1.21$), however we decided to forego model averaging as differences in density estimates and 95% confidence intervals from the 2 models were trivial ($\leq 0.003$/km$^2$ for both density and CI’s; Appendix B Table B.2).

Discussion

Our estimates of ocelot population density derived from noninvasive genetics and camera trapping were highly comparable. Point estimates were similar, and 95% confidence intervals from all methods overlapped, except those that used $\frac{1}{2}$-MMDM to estimate ESA. Estimates from camera trapping generally had greater precision than those from noninvasive genetics, but these differences were not substantial (Table 1). Because we obtained density estimates from noninvasive genetics that were comparable, despite a relatively small sample size, to estimates from camera trapping, from which we had a large
sample with many recaptures, our results support the validity of noninvasive genetic techniques for estimating density of elusive species.

All CR models used to estimate density from camera trapping provided similar estimates, whereas estimates from different CR models for noninvasive genetics were more variable. Density estimates from noninvasive genetics that used ½-MMDM to calculate ESA were substantially higher than other estimates, in some cases by more than a factor of 3. Thus, we agree with other researchers that use of ½-MMDM can greatly overestimate density (Soisalo and Cavalcanti 2006; Dillon and Kelly 2008; Sharma et al. 2010; Janečka et al. 2011a; Tobler and Powell 2013). Our density estimates that used full MMDM to estimate ESA were more in line with our other estimates, however this result is likely coincidental rather than biologically meaningful. Although MMDM methods have been used to estimate density in many past studies, we agree with recent criticisms that use of MMDM methods has little theoretical or biological justification (Pesenti and Zimmermann 2013; Tobler and Powell 2013). This shortcoming is evident in our study, as MMDM distances calculated from camera trapping (1509 m) and noninvasive genetics (632 m) were substantially different even though they were derived from the same population during the same time period. The underestimation of ocelot movements from noninvasive genetics can likely be attributed to two factors. First, our camera array covered the entire island, whereas ocelot latrines had a more clumped distribution (Fig. 1.1). Second, our sample size from camera trapping was larger, and contained a far greater number of recaptures. It seems logical that a large number of recaptures are needed to effectively estimate movement distances (Tobler and Powell 2013), an issue widely acknowledged for estimation of home range size from telemetry data (Harris et al. 1990, Seaman et al. 1999). Another problem with using MMDM to estimate survey area is that area buffers may contain both suitable and non-suitable habitat. For example, when we used MMDM and ½-MMDM to estimate ESA from camera trapping, both were larger than the area of the entire island, and thus contained large portions of water, which is obviously not suitable ocelot habitat.

For all of the reasons above, we think recently developed SECR models such as SPACECAP and DENSITY are preferable for estimating population density in noninvasive studies. This is especially true
for studies with small sample sizes, small survey areas (Sollmann et al. 2012), or in study locations with heterogeneous habitat. A key advantage is that SECR models do not require contentious post-hoc estimates of survey area such as use of MMDM or ½-MMDM to convert estimates of abundance to density. Furthermore, SECR models allow the user to specify suitable and non-suitable habitat for inclusion in the model. Additionally, SECR models allow users to designate which detectors were operational during each sampling occasion, avoiding bias introduced by occasional camera trap failure common in traditional CR models (Foster and Harmsen 2012). Of the two SECR programs that we used, DENSITY provided the greatest concordance between density estimates from noninvasive genetics and density estimates from camera trapping. DENSITY may also be preferable to some users because run time for model estimates is minutes, compared to many hours for SPACECAP. DENSITY also provides greater flexibility in comparing models, and allows inclusion of heterogeneity in capture probability, which is an option not yet available in SPACECAP.

Despite our efforts to obtain scat samples from throughout BCI, the spatial extent of our effective sampling area was unequal between camera trapping and noninvasive genetics due to the difficulty of finding ocelot latrines. This difference reflects the reality that most researchers will face when conducting studies that rely on collection of scats. With camera trapping, researchers pre-define the sampling area by choosing the placement of camera traps. With fecal noninvasive genetic sampling, effective sampling area is instead defined by where study animals defecate, and by the ability of researchers to recover scat samples (Rodgers and Janečka 2013). Fortunately, SECR models take differences in sampling area into account when estimating density, making it possible to compare density estimates from different spatial extents. We think that our ability to estimate similar densities from noninvasive genetics and camera trapping, despite differences in spatial extent, lends further support to noninvasive genetics as a viable method.

Our estimated ocelot densities were higher than estimates reported from anywhere else in the species range (Di Bitetti et al. 2008, Kolowski and Alonso 2010). Even our density estimates based on minimum
number known alive (a conservative lower bound estimate) are 1.6 times greater than the densest ocelot population previously reported from the northwestern Amazon of Peru (0.947/km2; Kolowski and Alonso 2010). These unusually high densities could be the result of numerous factors including high prey availability (Terborgh 1992), frustrated dispersal due to the high cost of dispersing from island to mainland across open water, or the vigilant protection BCI receives from poaching of both ocelots and their prey. High densities could also be the result of mesopredator release, as jaguars (*Panthera onca*) and pumas (*Puma concolor*) were resident in the BCI area in the early 20\textsuperscript{th} century, but are now only infrequent visitors to the island (Wright et al. 1994, Moreno et al. 2006).

Our study provides evidence that noninvasive genetic techniques can generate accurate estimates of population density especially when used in conjunction with SECR models. We do caution, however, that further studies should be conducted on a variety of species in diverse environments to further verify the accuracy of these techniques. Our study was conducted in a high-density population of a species that defecates at latrine sites, and thus we were able to find scats relatively easily. For low density populations, it may be necessary to use aids such as scat detection dogs, which although expensive can greatly increase detection rates (Kelly et al. 2012). In such cases, modified SECR models that account for unstructured spatial sampling can be used (Russell et al. 2012, Thompson et al. 2012). In sum, we think that fecal noninvasive genetics provides a promising tool for the estimation of density in elusive animals, especially for species in which individuals cannot be identified from camera trapping. These techniques have great potential to aid in the conservation of the many elusive species worldwide for which population density and conservation status is poorly known.
CHAPTER 2: SOCIO-SPATIAL ORGANIZATION AND KIN-STRUCTURE IN OCELOTS (*LEOPARDUS PARDALIS*) INFERRED FROM INTEGRATION OF CAMERA TRAPPING AND NONINVASIVE GENETICS

Abstract

We used 3 years of camera trapping to examine the spatial organization of an entire ocelot (*Leopardus pardalis*) population on Barro Colorado Island in Panama. We also placed camera traps on ocelot latrines to match photographs of individual ocelots with microsatellite genotypes from feces. We then examined the relationship between spatiotemporal overlap and genetic relatedness to determine if kin selection plays a role in structuring ocelot populations. Strengths of spatiotemporal overlap between individual ocelots were calculated using a half-weight association index based on how often individuals were photographed at the same camera within 30 days of one another. We calculated relatedness between individuals based on 11 variable microsatellite loci. Male ocelots overlapped with ≤ 11 females, and females overlapped with ≤ 7 males. We detected no clear evidence of strict territoriality in either sex. Mean overlap between males was more than 5 times greater than overlap between females; however, spatiotemporal overlap was strong between some female pairs. Overall, overlapping individuals were more related to one another than was the sample population as a whole, which supports the hypothesis that kin-selection influences ocelot spatial organization. Additionally, our results contribute to emerging evidence that small felids are flexible in their social and spatial organization.

Introduction

Space use by individual animals is dictated by the need to acquire the resources necessary to maximize fitness, such as food, mates, or den sites. Consequently, the spatial organization of individuals at the population level is determined by intraspecific competition for these resources (Sandell 1989, Nicholson et al. 2011, Luhrs and Kappeler 2013). If the fitness benefits of defending these resources outweigh the costs, then animals will defend exclusive territories. Conversely, if the fitness costs of territory defense outweigh the benefits, individuals should overlap in their use of space (Davies and
At low population density the cost of territoriality may be low because there will be few competitors from which a home-range must be defended. At high population density, however, the cost of defending a territory from many competitors may be high, resulting in overlap in space use among individuals.

Ocelots (*Leopardus pardalis*), like most felids, are typically described as territorial and solitary (Sunquist and Sunquist 2002); however, reported patterns of spatial overlap between individuals vary. Nearly all studies report intersexual overlap in which males overlap with several females. As for intrasexual overlap, studies have reported that both females and males maintain exclusive territories (Tewes 1986, Emmons 1988, Laack 1991), males maintain exclusive territories with female home ranges overlapping one another (Ludlow and Sunquist 1987), or intrasexual overlap within both sexes (Dillon and Kelly 2008). This plasticity in spatial organization could be the result of differences in resource availability or population density (Sandell 1989). Alternatively, observed differences between ocelot populations could be an artifact of small sample sizes from telemetry studies that only followed a portion of the population, thus underestimating true spatial overlap (Sandell 1989, Johnson and Franklin 1991, Dillon and Kelly 2008).

If individuals overlap considerably in their space use, and are competing for shared resources, one mechanism that could minimize fitness costs due to competition is kin selection. Kin-selection theory predicts that organisms will be more tolerant of competition from relatives than from unrelated individuals because when relatives share limited resources, inclusive fitness benefits offset the individual fitness costs of competition (Hamilton 1964, Wade and Breden 1987). Male-biased dispersal and female philopatry are typical among mammals (Greenwood 1980) including solitary carnivores (Waser and Jones 1983), thus relatedness among individuals sharing space is often higher among females than among males. Relatedness among individuals sharing space has been reported for many mammals including carnivores (Kitchen et al. 2005, Moyer et al. 2006, Nicholson et al. 2011), but has seldom been investigated for small, solitary felids (but see Janečka et al. 2006).
Barro Colorado Island (BCI) in the Panama Canal offers a unique opportunity to investigate the relationship between spatial overlap and genetic relatedness in a high-density population of ocelots. Since the formation of BCI by the creation of Gatun Lake in 1912, ocelots have become the apex predator on the island and have reached the highest density ever reported for this species (>1.5 individuals/km$^2$; Chapter 1), likely due to an abundant prey base and release from competition from larger predators.

We had two goals in this study. First, we investigated patterns of intersexual and intrasexual overlap between individuals in a high-density population of ocelots. We hypothesized that ocelots of the same sex would overlap in their use of space and would not defend territories because of the high cost of defending a home range from many competitors at high population density. To test this hypothesis, we conducted a 3-year camera-trap study of the ocelot population on BCI. Camera trapping enabled us to sample the entire adult population in our study area throughout the study period, which would have been logistically unfeasible with radio telemetry. To our knowledge, this is the first study to examine overlap in space use in a carnivore species with camera trapping.

Second, we evaluated whether kin-structure was evident within the population. Our hypotheses for females and males differed because space use for females is most strongly tied to allocation of resources necessary for rearing offspring, whereas space use for males is influenced most strongly by access to females (Perrin and Mazalov 2000). For females, we hypothesized that inclusive fitness benefits can reduce costs of sharing the resources necessary for rearing offspring. Therefore we predicted that female ocelots that overlap in their use of space would be more closely related than females in the population as a whole. Conversely for males, we hypothesized that local mate competition with close relatives decreases inclusive fitness. This is because males are promiscuous and compete for access to females, and suppressing mating success of close relatives decreases inclusive fitness (Perrin and Mazalov 2000). Therefore, we predicted that overlapping males would be less related than males in the population as a whole. Finally, we predicted that relatedness between males and females that overlap with one another would be low to avoid inbreeding (Pusey 1987). To test these hypotheses, we used a novel approach that integrated camera trapping with noninvasive genetics.
Materials and Methods

Study site

Field work was conducted on BCI, a 1,543-ha island in the Panama Canal waterway, at a research station operated by the Smithsonian Tropical Research Institute (Fig. 2.1). BCI (9°10’N, 79°51’W) sits within Gatun Lake, an artificial body of water created in 1912 by the damming of the Chagres River to create the Panama Canal, and is part of the protected 54-km² Barro Colorado Nature Monument. Vegetation is tropical moist forest, and topography is dominated by hills that reach a maximum elevation of 165 m above sea level. Mean temperature is 27 °C with an average annual precipitation of 2600 mm, with 90% of rainfall occurring from May through November.

Camera trapping

To examine spatial overlap between individual ocelots, camera trapping was conducted from 6 February 2010 through 31 December 2012 using an array of 17 Reconyx PC900 and RC55 trail cameras (Reconyx Inc., Holmen, Wisconsin). Cameras were distributed evenly throughout the island so that gaps between cameras that could contain an ocelot home range were unlikely (Fig. 2.1). We placed all cameras along trails to maximize capture probability. Cameras were checked a minimum of every 6-7 months, and replaced or repaired if no longer functioning. Some camera failures occurred due to ant colonization, tree-falls, or flooding, especially during the wet season, resulting in minor gaps in data collection for most cameras. We added four additional cameras to the array for shorter periods in 2011 and 2012. Additionally, 18 cameras were placed on ocelot latrines between 29 January and 5 May 2012 to photograph defecating ocelots in order to match photographed individuals with genotypes from noninvasive genetics. We identified individual ocelots from photographs based on unique spot patterns (Trolle and Kery 2003). Each camera station consisted of one camera and thus could photograph only one side of an animal at a time. For all individuals, however, we observed right and left sides in the same
photo sequence at least once either before or during our study period, allowing us to pair right and left
side profiles for individual identification.

Scat surveys

Ocelots commonly defecate at latrine sites used by multiple individuals of both sexes (Moreno and
Giacalone 2008). These latrines are typically located in large cavities or overhanging buttresses of large
trees, underneath buttresses of fallen trees, or underneath human structures (Fig. 1.2). All scats used in
this study were found at such latrines. Seven latrines were located during searching from 16 March to 15
April 2011, 5 of which were still active in 2012. To find additional latrines in 2012, we walked all 39.5
km of trails on BCI (Fig. 2.1) a minimum of 3 times (once every 33 days) from 29 January to 6 May. In
addition, we walked >390 km of random, off-trail transects throughout the island in search of latrines.
Once a latrine was located, it was revisited every 4-7 days to collect additional scats until 6 May 2012.

Species identification and microsatellite genotyping

We extracted DNA from scats using the Quiagen QIAamp DNA stool mini kit (Quiagen, Valencia,
California) following the manufacturer’s recommendations. For species identification, we amplified a
126-bp fragment of the mitochondrial gene ATP6 by polymerase chain reaction (PCR) using primers
ATP6-DF3 and ATP6-DR1 following conditions from Chaves et al. (2012). PCR products were
sequenced on an Applied Biosystems 3730xl DNA analyzer, and resulting sequences were compared to
reference sequences using the online tool DNA Surveillance Carnivora (Chaves et al. 2012).

To identify individuals and determine genetic relatedness, we used 11 polymorphic microsatellite loci.
To choose informative loci for our study, we initially screened 22 microsatellites originally developed for
the domestic cat (Felis catus) (Menotti-Raymond et al. 1999). To avoid linkage disequilibrium, we
selected these loci for their broad distribution throughout the genome based on linkage maps for the
domestic cat (Janečka et al. 2011b). The family Felidae has >95% chromosomal conservation between
species (Davis et al. 2009) so we assumed genomic position of loci to be shared between the ocelot and
domestic cat genomes. Of these initial 22 loci, we ultimately chose the 11 used in our study (Appendix B Table B.3) based on success of amplification, degree of variability, and ease and clarity of allele scoring.

To identify individuals, all scat samples were initially genotyped at 4 microsatellite loci (FCA075, FCA077, FCA088, and FCA132). We then genotyped one sample from each identified individual at an additional 7 loci (FCA008, FCA117, FCA124, FCA126, FCA171, FCA229, and FCA082) for relatedness analyses. As DNA from noninvasively collected fecal samples is often low quality and prone to genotyping errors such as allelic dropout and false alleles (Taberlet et al. 1999, Broquet et al. 2007), we used a multiple tubes approach (Taberlet and Fumagalli 1996) whereby each sample was genotyped 3-9 times until reliable consensus genotypes were obtained. Genotypes were considered reliable if a minimum of 3 identical heterozygote profiles, or 5 identical homozygote profiles, were observed (see Appendix A for PCR conditions). Probabilities of individual identity (Waits et al. 2001) and checks for departure from Hardy-Weinberg equilibrium were calculated using the program GENEALEX (Peakall and Smouse 2006).

**Sex identification**

To determine sex of ocelots from scat samples, we used felid-specific primers that amplify a 200-bp segment of the AMELY gene (Murphy et al. 1999), which is only present on the Y-chromosome of males (see Appendix A for PCR conditions). PCR amplifications were performed in triplicate along with male and female positive controls and a negative control, and PCR products were visualized on agarose gel. Samples were identified as male if they showed amplification of the Y-linked marker for all 3 replicates, and were identified as female if no amplification was observed.

**Spatial overlap analyses**

We used the 3 years of camera trap data to quantify the degree of spatiotemporal overlap between individual ocelots. For this analysis, we used the program SOCPROG (Whitehead 2009) to calculate the strengths of association between individuals based on how often they were photographed at the same
camera traps. We used a half-weight association index (Whitehead and Dufault 1999) in which pairwise association values can range from 0 (individual A and individual B were never photographed at the same camera) to 1 (individual A and individual B were photographed at the same camera during every 30-day sampling period in which both individuals were photographed at least once). This index can be conceptualized as the proportion of time during the study that a pair of individuals spent using the same area, and thus as a measure of spatiotemporal overlap. The choice of 30-day sampling periods was a balance between being long enough to contain sufficient data (median for females was 1.7 sequences/30 days; median for males was 3.5 sequences/30 days) and being short enough to be biologically meaningful (i.e., individuals in the same area within 30 days of one another could compete for the same resources). We restricted these analyses to individuals present in the study area all 3 years, and who were either adults or sub-adults in 2010, and mature adults in both 2011 and 2012.

Next, we tested whether observed spatiotemporal associations between individuals differed from a null model in which associations between individuals were random. Because association index values between individuals are non-independent, we used the Bejder et al. (1998) permutation test in SOCPROG to permute our matrix of association values within samples (Whitehead 2008). We ran 20,000 permutations of our matrix with 1,000 trials per permutation. We used the difference in the coefficient of variation (CV) between our observed association matrix and randomly generated association matrices as a test statistic to determine if our observed data differed from random. A non-random matrix would be expected to have significantly greater variation than random (Whitehead 2008). We also used this test to determine which pairs of individuals showed associations or disassociations greater than random expectations. Last, we used a Mantel test (Mantel 1967) with 10,000 random permutations to determine if males had significantly larger mean association index values than did females.

**Genetic relatedness analyses**

To determine if individuals who overlapped in their space use were more closely related than the population as a whole, we first used our microsatellite genotype data to calculate pairwise relatedness
between all sampled individuals using the regression estimator of Lynch and Ritland (1999) with the program COANCESTRY (Wang 2011). This estimator calculates relatedness coefficients (R) between pairs of individuals based on the number of identical alleles the two individuals share by descent, while also taking into account allele frequencies in the entire sampled population. R-values can range from -1 to 1, with positive R-values indicating 2 individuals are more related than expected by chance, and negative R-values indicating the opposite. We then tested if mean R-values of individuals who overlapped in their use of space were larger or smaller than mean R-values in the sampled population as a whole for all individuals, as well as for males and females only. To test for significance, we used COANCESTRY to carry out 1,000,000 bootstrapping permutations, and then compared our observed values with the distribution of permuted values (Fig. 2.6). Due to our moderate sample sizes, we chose to use an alpha of 0.10 in order to balance type I and type II errors. Because genotypes were only obtained from a segment of the population, and not all genotyped individuals could be matched with photographed individuals, we defined overlapping pairs as individuals that used the same latrine during our scat sampling period, or for ocelots with genotype-photograph matches, as individuals who were photographed at the same camera during the same 30-day sampling period.

Results

Spatiotemporal overlap

Our 3 years of camera trapping resulted in a 12,015 camera nights, during which we recorded 2,376 independent sequences of 37 individual ocelots. Nineteen of these individuals were females, 15 were males, and 3 were kittens that died before sex could be determined. We captured 126 sequences of juveniles, and 2,250 sequences of adults or sub-adults. For our analyses of spatiotemporal overlap we used 19 core individuals (7 males and 12 females) chosen because they were adults in all 3 years, with the exception of 2 males and 2 females who were sub-adults in 2010, but were mature adults by 2011. All other individuals were born, died, or disappeared during the study period, and were excluded from
analyses to avoid biasing of overlap results. The 19 core individuals accounted for 2,004 independent photo sequences, with individual males captured a median of 123 times (range 106-300), and individual females captured a median of 60 times (range 28-312).

Male ocelots overlapped on average with 8.14 (SE = 0.83) females and 6.71 (SE = 0.18) other males, whereas females overlapped on average with 4.75 (SE = 0.64) males and 3.41 (SE = 0.47) other females (Fig. 2.2). Based on half-weight association indices, strength of intrasexual overlap between males (mean = 0.299; SE = 0.048) was > 5 times the overlap between females (mean = 0.057; SE = 0.015; P<0.0001; Fig. 2.3). The distribution of association index values also differed for males versus females (Fig. 2.4). The CV of our real overlap matrices was greater than the mean CV of our randomly permuted matrices for both females and males (P<0.0001), indicating both matrices differed from the null model of overlap being entirely random (Whitehead 2008). For females, the CV for real data was 2.192 versus 1.460 for the mean of random permutation, whereas for males, the CV of real data was 0.694 versus 0.420 for the mean of random permutations. Five of 66 (7.5%) female dyads showed overlap significantly greater than random expectations, whereas 5 of 21 (23.8%) male dyads showed overlap significantly greater than random expectations (P<0.05; Fig. 2.5).

Genetic relatedness and kin structure

We collected 80 scats from 23 ocelot latrines on BCI, and we genetically confirmed 68 of these (85%) as being from ocelots. From these 68 scats, we obtained complete 4-locus genotypes from 55 samples (81%), which consisted of 13 unique genotypes and 42 recaptures. The probability of two different individuals sharing the same genotype (Probability of individual identity; Waits et al. 2001) from our 4 initial loci was 0.00031 among unrelated individuals (P(ID)), and 0.038 among siblings (P(ID)sibs). Six individuals were identified as male and 7 as female. Eight of 9 individuals detected in 2011 were detected again in 2012. We matched genotype data with photographic data from our latrine cameras for eight individuals (4 males and 4 females). For our 13 identified individuals, all were successfully genotyped at
all 11 loci, except for 2 individuals which were genotyped at 10 loci. Mean number of alleles per loci was 5 (range 3-6). None of the 11 loci deviated significant from Hardy-Weinberg equilibrium.

Mean R for the entire sampled population was -0.0832 (Var = 0.0422). There was no difference in mean relatedness between the sexes (female R = -0.0983, Var = 0.0203; male R = -0.0754, Var = 0.0372; P = 0.375). When both sexes were considered together, overlapping dyads were more related to one another (R = -0.0307, Var = 0.0399) than the population as a whole (R = -0.0832, Var = 0.0422; P = 0.022; Fig. 2.6a). Overlapping male dyads were less related to one another (R = -0.1193, Var = 0.0321) than were all male dyads (R = -0.0754, Var = 0.0772), but this difference was not significant (P = 0.190; Fig. 2.6b). Overlapping female dyads were more related to one another (R = -0.0250, Var = 0.0135) than were all female dyads (R = -0.0983, Var = 0.0203; p = 0.085; Fig. 2.6c). Overlapping intersexual dyads (male-female) were more closely related (R = -0.0142, Var = 0.0469) than were all intersexual dyads (R = -0.0784, Var = 0.0548; P = 0.0257; Fig. 2.6d).

**Discussion**

**Spatiotemporal overlap**

We documented extensive spatiotemporal overlap between ocelots of opposite sex, as well as intrasexual overlap for both males and females. Males overlapped with up to 11 different females during the study. Females also typically overlapped with several males (range =1-7), potentially allowing females to exert mate choice. For intrasexual overlap, mean strength of overlap between males was much greater than that between females. Males overlapped with nearly all other males on the island to some extent, most males overlapped strongly with several other males (Fig 2.5), and nearly a quarter of male-male dyads had stronger spatiotemporal association than expected by chance. All females overlapped with at least 2 other females during the study, however, the strength of this overlap was typically small, suggesting most females overlapped only in peripheral portions of their home ranges. Several female-female dyads showed strong overlap in their use of space throughout the study, however, and associations for 5 of these dyads were greater than expected by chance (Fig 2.5). The degree of intrasexual overlap
was more extensive than most previous reports for ocelots (Sunquist and Sunquist 2002), which supports our hypothesis that at high density it is costly for individuals to maintain territories and thus intrasexual overlap is the norm, especially for males.

The difference in mean strength of intrasexual overlap between males and females is likely the result of different strategies between the sexes in terms of maximizing fitness. For females, home range size should be determined by availability of nutritional resources for rearing young. For males, home range size is more likely to be determined by access to females for mating (Sandell 1989, Perrin and Mazalov 2000). If prey availability is high, as in our study area (Terborgh 1992; and personal observations from camera trapping), females may contract their home range to a small size to minimize aggressive encounters with other females, as long as the home range is still large enough to contain sufficient resources for reproduction. For males however, decreasing home range size would also decrease the number of females with which they overlap, potentially decreasing fitness. Thus, the costs of overlapping and competing with other males could be less than the fitness benefits gained by overlapping with many females (Sandell 1989). This tradeoff may explain why males display a greater degree of overlap than females, but it fails to explain why some (albeit fewer) females still maintain strong overlap with other females.

Small felids exhibit substantial variation in their socio-spatial organization between species, ranging from exclusive home range defense in one or both sexes, to considerable home range overlap in one or both sexes (reviewed in Macdonald et al. 2010). In the genus *Leopardus*, margays (*Leopardus weiedii*) in Tamaulipas, Mexico displayed extensive home-range overlap among males (Carvajal-Villarreal et al. 2012). In the Argentinian Pampas, both male and female Geoffroy’s cats (*Leopardus geoffroyi*) displayed considerable intrasexual overlap (Manfredi et al. 2006), whereas in Chilean Patagonia, female Geoffroy’s cats had overlapping home-ranges, but males did not (Johnson and Franklin 1991). As many telemetry studies of small felids rely on small sample sizes, representing only a subset of individuals in the
population, spatial overlap has likely been underestimated often (Sandell 1989, Johnson and Franklin 1991, Dillon and Kelly 2008).

Ocelots exhibit a great deal of flexibility in spatial organization between populations. Most studies of ocelots have observed exclusive territories in one or both sexes (Tewes 1986, Ludlow and Sunquist 1987, Emmons 1988, Laack 1991). Contrarily, in broadleaf forest in Belize where ocelot density is more than 5 times lower than the density in our population, and where home-ranges are large compared to most populations, Dillon and Kelly (2008) observed substantial intrasexual overlap in both sexes. Dillon and Kelly (2008) attributed this intrasexual overlap to the high cost of defending a large home range. Paradoxically, we observed intrasexual overlap in our population where density is extremely high (Chapter 1) and where home ranges are nearly an order of magnitude smaller than in Belize (1.5km$^2$ for females and 3.5km$^2$ for males; Moreno et al. 2012). In better-studied large felid species such as pumas ($Puma concolor$), leopards ($Panthera pardus$), and tigers ($Panthera tigris$), degree of intrasexual overlap is highly flexible between populations (Goodrich et al. 2010, Macdonald et al. 2010). Recent evidence from our study and others suggests that social organization in small felids may be highly flexible within species as well.

**Genetic relatedness and kin-structure**

Overall, individuals in the sampled population were less related than would be expected by chance (mean $R<0$), indicating that the island population maintains gene flow with nearby mainland populations. Additionally, females in the sampled population were not more closely related than males, potentially indicating equal dispersal between the sexes in this population, which is contrary to the norm for felids. If dispersal were male-biased, we would expect females in the population to be more closely related than males (Janecka et al. 2007).

Some of our hypotheses regarding relationships between spatial overlap and genetic relatedness were supported, and others were not. Regardless of sex, overlapping individuals were more related than were
all individuals in the sampled population. Contrary to our prediction, overlapping opposite-sex dyads were more related than were all opposite sex dyads. On average, however, overlapping male-female dyads were not closely related (R<0) so mating between overlapping individuals would still be unlikely to cause severe inbreeding. As females overlapped with multiple males during the study, females might avoid mating with close relatives to prevent inbreeding (Pusey and Wolf 1996). Kin recognition occurs in many mammal species (Blaustein et al. 1987, Mateo 2003), and in feral cats (*Felis catus*) females willfully avoid mating with close relatives (Ishida et al. 2001).

Relatedness between overlapping males was lower than between all male-male dyads as predicted, but the trend was not significant. Relatedness between overlapping females was higher than between all female-female dyads, supporting our hypothesis that overlapping females counter costs of sharing resources with gains through inclusive fitness. Nevertheless, mean relatedness values for all classes of overlapping individuals (male-female, male-male, female-female) were still lower than expected by chance (R<0). Thus, on average, even overlapping females were not closely related. In terms of our hypotheses however, the important finding is that overlapping females were more closely related than the population as a whole, and thus are gaining some net benefit from inclusive fitness due to their spatial organization.

Unfortunately, we were only able to obtain genetic data from roughly one-half of our population, and we only obtained photo-genotype matches from a subset of those. As a consequence, we could test if individuals who overlapped were more closely related than all genotyped individuals in the population, but we did not have a complete enough sample to test for a relationship between the strength of overlap and relatedness, which may have been more informative. Even so, we think our novel method of integrating camera trapping with noninvasive genetics by photographing defecating individuals at latrine sites could be a useful tool for future studies of ocelots and other latrine-using carnivores. Our inability to obtain photo-genotype matches from all individuals was mostly a consequence of not finding a sufficient numbers of ocelot latrines distributed throughout our study area. One potential approach to overcome this
limitation is use of scat-detecting dogs (Kelly et al. 2012) to find most latrines in an area, and thus detect all individuals in the population.

In conclusion, both male and female ocelots exhibited overlap in their use of space with individuals of the same sex. For males overlap was strong, indicating that males do not defend territories at high density. For females, although mean intrasexual overlap was low, strong overlap occurred between some pairs of individuals. Our results, in combination with previous research, demonstrate that ocelots, like some other felids, are flexible in their social and spatial organization. Further empirical research is needed to determine which ecological factors truly influence these differences between populations (Macdonald et al. 2010). Finally, we found a positive relationship between spatiotemporal overlap and genetic relatedness, suggesting that kin-selection may play a role in structuring ocelot social and spatial organization.
### Table 1.1 Population density estimates of ocelots (*Leopardus pardalis*) from Barro Colorado Island, Panama.

<table>
<thead>
<tr>
<th>Method and Model</th>
<th>Abundance (N)</th>
<th>Density N/KM²</th>
<th>SE</th>
<th>95% CI</th>
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<tbody>
<tr>
<td><strong>Camera trapping</strong></td>
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<td></td>
</tr>
<tr>
<td>Minimum number alive</td>
<td>25</td>
<td>1.620</td>
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<tr>
<td>CAPTURE $M_{h(jackknife)}$</td>
<td>28</td>
<td>1.815</td>
<td>0.163</td>
<td>1.686 – 2.464</td>
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<tr>
<td>SPACECAP</td>
<td>-----</td>
<td>1.640</td>
<td>0.743</td>
<td>1.639 - 1.641</td>
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<tr>
<td>DENSITY</td>
<td>-----</td>
<td>1.637</td>
<td>0.331</td>
<td>1.106 - 2.423</td>
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<tr>
<td><strong>Noninvasive Genetics</strong></td>
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<td></td>
</tr>
<tr>
<td>Minimum number alive - MMDM</td>
<td>12</td>
<td>1.229</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Minimum number alive - ½ MMDM</td>
<td>12</td>
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</tr>
<tr>
<td>CAPTURE $M_0$ - MMDM</td>
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<td>CAPTURE $M_0$ - ½ MMDM</td>
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<tr>
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<td>0.781</td>
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<td>CAPWIRE - MMDM</td>
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<td>1.229 – 1.639</td>
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<tr>
<td>CAPWIRE - 1/2MMDM</td>
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<td>4.175</td>
<td>-----</td>
<td>3.854 – 5.138</td>
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<td>0.272</td>
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<tr>
<td>DENSITY</td>
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<td>1.738</td>
<td>0.584</td>
<td>0.917 - 3.302</td>
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CAPTURE, SPACECAP, DENSITY, and CAPWIRE are software programs designed to estimate abundance or density using capture recapture models. MMDM is a method of estimating effective survey area based on the mean of the maximum distance moved between captures.
**Fig. 1.1** Map of Barro Colorado Island in Panama, our study area for estimating population density of ocelots (*Leopardus pardalis*) from camera trapping and noninvasive genetics.
Fig. 1.2 An ocelot (*Leopardus pardalis*) defecating at a latrine under the buttress of a large tree on Barro Colorado Island, Panama.
Fig. 2.1. Map of Barro Colorado Island, Panama, showing the location of camera traps placed along trails and at ocelot latrines for investigation of spatiotemporal overlap and kin-structure.
Fig. 2.2. Mean (+/− 1 SE) number of same sex and opposite sex individuals that male and female ocelots overlapped with from 6 February 2010 through 31 December 2012 on Barro Colorado Island, Panama. Overlap is based on the whether 2 individuals were photographed at the same camera trap within the same 30-day sampling period.
Fig. 2.3. Mean (+/- 1 SE) half-weight association index values of intrasexual overlap of ocelots on Barro Colorado Island, Panama. Association index values represent the strength of spatiotemporal overlap between pairs of same-sex individuals based on how often they were photographed at the same camera trap during the same 30-day sampling period.
Fig. 2.4. Distribution of association index values between pairs of ocelots on Barro Colorado Island, Panama. Half-weight association index values range from 0-1, and represent the strength of spatiotemporal overlap between same-sex dyads based on how often they were photographed at the same camera trap during the same 30-day sampling period.
Fig. 2.5. Half-weight association index values between pairs of female (a) and male (b) ocelots from Barro Colorado Island, Panama, shown in both matrix and graphical format. Values and line weights represent the strength (on a scale of 0-1) of spatiotemporal overlap between pairs of individuals based on how often they were photographed at the same camera trap during the same 30-day sampling period. Asterisks represent associations and double asterisks represent dissociations that differed from random expectations ($P < 0.05$).
Fig. 2.6  Observed mean difference in relatedness between dyads of individual ocelots with overlapping space use (vertical bold lines) versus all dyads in the sampled population, shown along with the distribution of simulated differences from 1,000,000 randomly generated bootstrap replicates. The curve represents the distribution of permuted values, and reference lines represent quantiles from the simulated distribution. a) all dyads, b) male-female dyads, c) male-male dyads, and d) female-female dyads.


Moreno, R. S., R. W. Kays, and R. Samudio. 2006. Competitive release in diets of ocelot (Leopardus pardalis) and puma (Puma concolor) after jaguar (Panthera onca) decline. Journal of Mammalogy 87:808-816.


APPENDIX A

PCR Conditions

PCR conditions for species identification can be found in Chaves et al. (2012). PCR conditions for microsatellite amplification and sex ID were as follows. Reactions included 1 ul 10X Buffer, 0.8 ul of MgCl, 0.2 ul of 10 mM DNTPs, 0.2 ul of 20 mM fluorescently-labeled forward primer, 0.2 ul of 15-20 mM reverse primer, 0.04 ul of AmpliTaq Gold® 360 DNA Polymerase (Life Technologies; Grand Island, NY, USA), 0.1ul of BSA, 0.2 ul of GC-enhancer, 5.76 ul of water, and 1.5 ul of template DNA for a total reaction volume of 10 ul. Thermocycling conditions were as follows: initial denaturation at 95°C/10’, followed by 50 cycles of 95°C/5”, 55°C/15”, 72°C/45”, and a final extension of 72°C/10’. Microsatellite forward primers were fluorescently labeled with one of 4 dies (FAM, NED, PET, or VIC). Each microsatellite was amplified individually and then the loci were pooled together and genotyped on an Applied Biosystems 3730xl DNA analyzer.
APPENDIX B

Supplementary tables

Table B.1  AIC table for density estimates from noninvasive genetic sampling from the program DENSITY.

<table>
<thead>
<tr>
<th>Model</th>
<th>#Parameters</th>
<th>ML Log likelihood</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Density</th>
<th>SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>g0[.]s[.]</td>
<td>3</td>
<td>-186.49</td>
<td>381.97</td>
<td>0</td>
<td>1.7403</td>
<td>0.5841</td>
<td>0.917-3.302</td>
</tr>
<tr>
<td>g0[h2]s[.]</td>
<td>5</td>
<td>-186.13</td>
<td>392.27</td>
<td>10.3</td>
<td>1.7397</td>
<td>0.5841</td>
<td>0.917-3.301</td>
</tr>
<tr>
<td>g0[..]s[h2]</td>
<td>5</td>
<td>-186.45</td>
<td>392.91</td>
<td>10.94</td>
<td>1.7407</td>
<td>0.5832</td>
<td>0.919-3.30</td>
</tr>
<tr>
<td>g0[h2]s[h2]</td>
<td>6</td>
<td>-185.78</td>
<td>400.35</td>
<td>18.38</td>
<td>1.7612</td>
<td>0.591</td>
<td>0.929-3.34</td>
</tr>
<tr>
<td>g0[h3]s[.]</td>
<td>7</td>
<td>-185.67</td>
<td>413.34</td>
<td>31.37</td>
<td>1.7482</td>
<td>0.3086</td>
<td>1.240-2.464</td>
</tr>
<tr>
<td>g0[..]s[h3]</td>
<td>7</td>
<td>-186.43</td>
<td>414.85</td>
<td>32.88</td>
<td>1.7338</td>
<td>1.1981</td>
<td>0.510-5.800</td>
</tr>
<tr>
<td>g0[h3]s[h3]</td>
<td>9</td>
<td>-186.12</td>
<td>480.24</td>
<td>98.27</td>
<td>1.9242</td>
<td>0.7769</td>
<td>0.907-4.084</td>
</tr>
</tbody>
</table>

Table B.2  AIC table for density estimates from camera trapping from the program DENSITY.

<table>
<thead>
<tr>
<th>Model</th>
<th>#Parameters</th>
<th>ML Log likelihood</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Density</th>
<th>SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>g0[..]s[h2]</td>
<td>5</td>
<td>-1121.59</td>
<td>2256.34</td>
<td>0</td>
<td>1.6374</td>
<td>0.3308</td>
<td>1.106-2.423</td>
</tr>
<tr>
<td>g0[..]s[.]</td>
<td>3</td>
<td>-1124.78</td>
<td>2256.71</td>
<td>0.37</td>
<td>1.6391</td>
<td>0.3309</td>
<td>1.108-2.425</td>
</tr>
<tr>
<td>g0[h2]s[.]</td>
<td>5</td>
<td>-1123.78</td>
<td>2260.71</td>
<td>4.37</td>
<td>1.6378</td>
<td>0.3308</td>
<td>1.107-2.424</td>
</tr>
<tr>
<td>g0[h2]s[h2]</td>
<td>6</td>
<td>-1123.44</td>
<td>2263.55</td>
<td>7.21</td>
<td>1.6365</td>
<td>0.3307</td>
<td>1.106-2.422</td>
</tr>
<tr>
<td>g0[..]s[h3]</td>
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<td>-1121.59</td>
<td>2263.77</td>
<td>7.43</td>
<td>1.6331</td>
<td>0.0001</td>
<td>1.633-1.633</td>
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<tr>
<td>g0[h3]s[.]</td>
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<td>-1123.83</td>
<td>2268.25</td>
<td>11.91</td>
<td>1.6354</td>
<td>0.0653</td>
<td>1.512-1.768</td>
</tr>
<tr>
<td>g0[h3]s[h3]</td>
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<td>-1121.61</td>
<td>2273.23</td>
<td>16.89</td>
<td>1.6373</td>
<td>0.2846</td>
<td>1.168-2.296</td>
</tr>
</tbody>
</table>
**Table B.3** Summary of 11 microsatellite loci used to estimate genetic relatedness. $H_o$ is observed heterozygosity. $H_e$ is expected heterozygosity under Hardy-Weinberg equilibrium. All loci were in Hardy-Weinberg equilibrium (HWE probability > 0.05).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size</th>
<th># alleles</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>HWE probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCA075</td>
<td>110-128</td>
<td>5</td>
<td>0.769</td>
<td>0.782</td>
<td>0.826</td>
</tr>
<tr>
<td>FCA077</td>
<td>133-137</td>
<td>3</td>
<td>0.615</td>
<td>0.492</td>
<td>0.463</td>
</tr>
<tr>
<td>FCA088</td>
<td>92-110</td>
<td>5</td>
<td>0.846</td>
<td>0.809</td>
<td>0.564</td>
</tr>
<tr>
<td>FCA132</td>
<td>167-179</td>
<td>6</td>
<td>0.769</td>
<td>0.788</td>
<td>0.559</td>
</tr>
<tr>
<td>FCA008</td>
<td>123-137</td>
<td>5</td>
<td>0.846</td>
<td>0.763</td>
<td>0.501</td>
</tr>
<tr>
<td>FCA117</td>
<td>157-165</td>
<td>6</td>
<td>0.769</td>
<td>0.748</td>
<td>0.439</td>
</tr>
<tr>
<td>FCA124</td>
<td>135-144</td>
<td>6</td>
<td>1.000</td>
<td>0.775</td>
<td>0.767</td>
</tr>
<tr>
<td>FCA126</td>
<td>119-129</td>
<td>5</td>
<td>0.923</td>
<td>0.800</td>
<td>0.463</td>
</tr>
<tr>
<td>FCA171</td>
<td>101-111</td>
<td>5</td>
<td>0.846</td>
<td>0.726</td>
<td>0.631</td>
</tr>
<tr>
<td>FCA229</td>
<td>147-166</td>
<td>4</td>
<td>0.583</td>
<td>0.711</td>
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<td>FCA082</td>
<td>233-245</td>
<td>4</td>
<td>1.000</td>
<td>0.745</td>
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<tr>
<td>Mean</td>
<td>na</td>
<td>5</td>
<td>0.811</td>
<td>0.742</td>
<td>0.524</td>
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</table>