Noninvasive tracking of jaguars (*Panthera onca*) and co-occurring feline species in Belize by combining molecular scatology, remote camera trapping and GIS: the impact of fragmentation

Final Report

2008

Submitted to: Programme for Belize

Prepared by:

Claudia Wultsch
Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, VA-24061-0321, US. Email: wultschc@vt.edu.

1. Background

The jaguar (*Panthera onca*) is the largest Neotropical feline species and the only member of the *Panthera* genus in the Americas. Jaguars are elusive and extremely difficult to study due to their wide ranging behavior, their occurrence in low population densities in often dense forest habitats, and their nocturnal and crepuscular activity peaks (Nowell and Jackson 1996). As a result, little is known about jaguars and thus they are considered the least studied of all "great" cats in the world. Throughout their range in Central and South America, jaguars are also listed as an endangered Appendix I species under CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) and as near threatened on the IUCN (International Union for the Conservation of Nature and Natural Resources) Red List (IUCN 2008).

Habitat loss and fragmentation, which has severely increased over the last 100 years in the Americas, represent two of the main threats to these wide-ranging carnivores and has resulted in the disappearance of more than fifty percent of the jaguar's historic range (Sanderson et al. 2002). Due to increased human presence, forest areas have disappeared and/or have been reduced to small, isolated patches. Jaguars now exist in fragmented populations across their range. Although jaguars are a generalist species which occur in a variety of habitat types, they prefer areas with tree cover (Crawshaw and Quiqley 1991), close to water bodies (Swank and Teer 1989, Sunquist 1987), far from human settlements and transport corridors. Due to their large home-range sizes which range from 33.4 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 and well-connected forest areas which also have the potential to support healthy populations of prey species. The degree of isolation, the presence of man-made constructions (roads, human settlements, agricultural developments) between forest patches, and also natural landscape features (high and steep mountains, wide rivers, etc.) could represent non-permeable barriers for wildlife, potentially confining jaguar populations to isolated suitable habitat fragments. These isolated populations may have decreased levels of dispersal of breeding individuals and thus reduced gene flow between them. Low levels of gene flow can cause small populations to suffer from genetic drift, decreasing genetic diversity and increasing the genetic divergence of populations thereby lowering the populations' viability and ability to adapt to a changing environment (Lacy 1993). Increasing the connectivity among remaining prime habitat patches is crucial for the long-term survival of jaguars as this supports genetic exchange between jaguar populations, thus maintaining the genetic diversity enhances fitness and enables adaptability. The identification, creation, and maintenance of corridors, which are defined as areas of natural habitat facilitating dispersal and migration of species between prime habitats (Bennett 2003), would certainly promote connectivity between jaguar populations and their remaining forest patches.

Besides habitat loss and fragmentation, jaguar populations are also threatened by conflicts with cattle ranchers as some cats prey on livestock and are hunted and killed as a result (Medellin et al. 2000, Ruiz-Garcia et al. 2006). One of the biggest threats for jaguars however, is the lack of knowledge we have about this species. In order to prevent further decline and ensure their long-term survival in fragmented and human-dominated landscapes, population level studies on jaguars are needed. Our ability to provide for survival of these felids is hampered by our inability to obtain reliable information on basic ecology, demography, and especially genetics, which is crucial for successful wildlife management and conservation actions in the future (Ruiz-Garcia et al. 2006).

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. 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). Yet this technique has limitations. It tends to photograph a disproportionate number of males (Maffei et al. under review) which might result in inaccurate population estimates. Therefore, there is a great need for other non-invasive approaches to monitor these cat populations more accurately and to estimate the impact of habitat loss and fragmentation on jaguars. Population genetics studies may provide the means to address camera trapping inadequacies for population size and sex-ratio estimation. In addition, genetic analysis can determine whether jaguar populations suffer from a reduction in genetic variation, an alteration in population structure, and low levels of gene flow between disconnected and fragmented reserves.

Molecular population genetics approaches on jaguars are rare (Eizirik et al. 2001, Ruiz-Garcia et al. 2006) due to the difficulties in obtaining large enough DNA sample sizes from wild jaguar populations. Molecular scatology is a relatively recent advancement in non-invasive genetic monitoring, where individuals are genotyped from intestinal epithelial cells found in their feces (Höss et al. 1992, Kohn and Wayne 1997, Kohn et al. 1999). There has not been any molecular scatology study conducted on wild jaguar populations. This technique, however, seems to especially suitable for wild cats as feces are the most prominent sign left behind from cats from which DNA can be obtained without capturing and handling. Molecular scatology is applicable for species identification (Reed et al. 1997, Dalen et al. 2004, Mukherjee et al. 2007), and identification of individuals, and hence can be also used for population size estimation and monitoring (Ernest et al. 2000, Bhagavatula and Singh 2006, Sugimoto et al. 2006). Individuals are genetically "tagged" and genotyping feces can form the basis of genetic capture-mark-recapture (CMR) (Miller et al. 2005). Furthermore, molecular scatology also provides the means to analyze genetic variation and population structure which might reveal the degree of isolation of cat populations to habitat loss and fragmentation.

Finding scat can be difficult, particularly in wet, humid, tropical climates. Scat detector dogs (originally drug or bomb detection dogs) are an efficient way to successfully detect scat in the field (Long et al. 2007a and b) and significantly increase the sample size for population genetics studies. Scat dogs locate the target scats faster and more successfully than an experienced person searching for scat visually (Smith et al. 2001, Smith et al. 2003). They have been already used for the detection of several other carnivore species such as bobcats (Harrison 2006), kit foxes (Smith et al. 2005), and grizzly and black bears (Wasser et al. 2004).

To date, this project has used a scat detector dog and applied the molecular scatology approach in order to genotype jaguars in Belize, Central America using and microsatellites and to estimate population size and density, and to determine sex ratio, genetic structure and variability of populations both within and among study sites. Co-occurring cat species, puma (*Puma concolor*), ocelot (*Leopardus pardalis*), margay (*Felis wiedii*), jaguarundi (*Herpailurus yaguarondi*) were also genotyped for the same purposes and this will provide additional information on the coexistence of these elusive cat species. The cats were simultaneously

monitored by remote sensing camera traps which will allow for a pioneering comparison of density estimates obtained from these two non-invasive techniques.

The ultimate results will provide important information on how habitat fragmentation affects genetic diversity of jaguars and other felines, and the techniques and analyses developed will be widely applicable to studies of other elusive felid species around the world.

2. Study Area

Rio Bravo Conservation Management Area (RBCMA):

1,050 km^2 of subtropical moist broadleaf and marsh forest disconnected by lowland savanna and agricultural areas in northern Belize. Altitude ranges from 40 to 160 m and annual rainfall averages from 1,550 to 1,600 mm per year. Average temperatures fluctuate between 21 and 31.5°C over the year.

3. Study Objectives

a. Develop a standardized protocol for large-scale molecular scatology studies of elusive cat species in tropical environments

b. Use molecular scatology approach to estimate population densities and sex-ratios of feline species across multiple protected areas in Belize

c. Comparison of two noninvasive monitoring techniques in cat conservation: genotyping feces versus remote camera tracking

d. Genetic structure and variation of cat populations in Belize

e. Connectivity of wild feline populations across Belize: combining GIS and conservation genetics

4. Methods

4.1. Molecular Scatology

Nothing is known yet about population genetics of felid species in Belize and worldwide there have been only a few genetic cat studies conducted in tropical climates using noninvasive genetic techniques. Although molecular scatology studies have been applied successfully before in the Northern Hemisphere, there is a great need to standardize this technique for carnivore studies in warmer and more humid climates.

Finding scat of wide-ranging species can be difficult, particularly in hot, humid, tropical environments which also affect the genetic data generation. Generally, feces samples decompose much faster in hot, humid, and rainy regions (Chame 2003). The DNA obtained from fecal samples in the tropics is much more likely to be degraded which means that samples might contain low DNA concentrations or fragmented DNA strands. Swanson and Rusz (2006) described this type of DNA as low copy DNA (lcDNA), which has, according to Miller et al. (2002), a much higher potential to show genotyping error caused by allelic dropout, PCR failure,

or contamination with non-target DNA. PCR inhibitors which are also present in fecal samples are another source of genotyping error as false alleles get produced. The genetic analysis of scat samples needs to apply efficient techniques to decrease genotyping errors and obtain reliable information from often highly-degraded fecal DNA samples. Pompanon et al. (2005) strongly recommended accounting for genotyping error by carrying out a pilot study in order to evaluate the theoretical and real genotyping error.

Because there is no established protocol for locating, collecting and storing scat samples from wild cat populations, I conducted a pilot study in June-August 2007 to determine the feasibility of this molecular scatology approach for genetic studies of feline species in tropical climates.

a. Locating scat samples: using a scat detector dog to maximize the scat detection rate and accuracy

Finding scat is difficult, particularly in dense and humid tropical forests. Therefore, I used a scat detector dog (originally a drug/bomb detection dog) to increase the probability of locating fecal samples more efficiently. The scat detector dog and me, the dog handler, were professionally trained by Packleader Dog Training (Gig Harbor, WA; http://www.packleaderdogtraining.net) to locate scats of all occurring cat species (jaguar, puma, ocelot, margay, and jaguarundi) in Belize. Morphological identification of scats by the dog handler and even olfactorial identification of scats from very similar species by the scat detector dog is controversial and often not accurate (Packleader, pers. comm.). Therefore, cat species identification through scat samples has been conducted genetically.

During the pilot field season (June to August 2007), scat detection took place within an effective survey area of approximately 216 km^2 based on the location of the simultaneous remote camera trap survey. In 2008 I surveyed Rio Bravo Conservation Management Area. Three different transect designs based on previous work with scat detector dogs were employed (Long 2006, P. McKay personal comm.). Design A: 1 km linear transects starting at camera stations along roads and trails or starting at random points within the effective survey area. Design B: 1 km diamond shaped transects starting at camera stations along trails and roads or at random points within the effective survey (following Long 2006). Design C: Opportunistic search along roads, trails, game-trails, off-trail, and in various habitat types. In the pilot study, designs A and B proved to be inefficient at collecting scats. Therefore, I decided to use design C as the standard survey design. For this transect design, each study area is superimposed with a grid consisting of 10-13 (4 x 4 km) cells and each cell was visited once during each sampling period. Each sampling period took about 8-10 days to complete and represented one encounter occasion in capture histories which will be created for each identified individual in the study. The study site was sampled 5 times to create 5 encounter occasions. The area may need to be sampled longer if scat detection is low (e.g. < 100 scats).

Transect surveys took place about 6 days per week starting early in the morning to prevent overheating of the scat detector dog and covering as much distance as possible. Throughout the day I also gave the dog many breaks while collecting the scat samples or at least every hour for about ten minutes (Fig.3.).

The scat detector dog was initially trained and continuously re-trained throughout the field season to avoid the most abundant venomous snakes such as Fer de Lances (*Bothrops asper*), jumping vipers (*Bothrops nummifer*) and tropical rattlesnakes (*Crotalus terrificus*) in

Belize (Fig.4.).

Fig.3. Students from UB helping with dog work

Fig. 4. Snake avoidance training

b. Collecting scat samples:

DNA samples can be potentially collected from different locations on the scat sample which each might vary in DNA quality and quantity due to various factors such as mold, UV-light, contamination with prey DNA etc. Thus, DNA samples were collected from four different scat locations (top, bottom, side, and inside) and analyzed based on success rates of PCR amplification of certain microsatellite loci.

In the field, each scat sample collected was additionally categorized in different classes based on age, moisture, odor strength, and presence and absence of mold (Wasser et al. 2004). We recorded time, location (through a hand held GPS unit), habitat type, and whether the sample was detected by the dog or not during each scat sample event. The weather and the temperature were recorded separately on a daily basis.

Fig.5. Detected jaguar scat

Fig.6. Collecting an off-trail sample

c. Preserving scat samples: DET buffer versus 95% Ethanol

From each scat four 0.5 ml samples were collected and stored in 2.0 ml storage vials following different protocols: (1) scat was soaked in 95% ethanol at 1:≥4 ratio by volume (Murphy et al. 2002), and (2) scat was stored in 1:4 ratio with DET buffer (L. Waits, personal communication).

d. Analyzing DNA scat samples:

In the fall 2007, I was trained in DNA extraction and analysis by Dr. Lisette Waits in the Conservation Genetics Laboratory at the University of Idaho, US.

DNA extraction

Faecal DNA extractions were conducted in a separated room to avoid contamination while working with low concentration DNA samples. Extraction negatives were included in all extraction runs to monitor for possible contaminations. The QIAamp® DNA Stool Mini Kit protocol (Quiagen, Inc., Valencia, CA, US) was followed to extract DNA from all faecal samples.

PCR amplification and DNA sequencing

Carnivore specific mitochondrial cytochrome b primers (Farrell et al. 2000) were used initially to verify the feline origin of the scat samples.

Furthermore, a set of 14 highly variable microsatellite loci was identified which can be used as genetic primers to genotype all five Belizean wild cat species (jaguar, puma, ocelot, margay, jaguarondi) and used to conduct PCR amplification. The primers were fluorescently labeled and applied in a multiplex approach in order to minimize the genotyping error. All PCR runs included a positive and negative (sterile water and PCR mixture) control. DNA was sequenced using an Applied Biosystems 377 automated sequencer. Sequences were analyzed with the program Sequencher 3.0 and compared with sequences listed in Gene Bank.

Genetic mark-recapture: Population densities and sex-ratios

Demographic parameters will be determined from patterns of multi-locus variation (Aspi et al. 2006): (1) species identification by observation of genetic profiles consistent with a certain species (Swanson and Rusz 2006), (2) individual identity determined by the observation of multilocus genotypes distinguishing among individuals (Frantz et al. 2003), and (3) gender as determined by DNA markers associated with the Y sex chromosome carried by males, but not by females (Pilgrim et al. 2005).

For mark-recapture analysis, each scat sample (where DNA can be extracted successfully) represents a single capture, which will then be used to develop a capture history for individuals. Furthermore, I will use the program CAPTURE to calculate abundance estimates (Otis et al. 1978, White et al. 1982, Rexstad and Burnham et al. 1991).

Density and sex-ratio estimates will be calculated per species and study site.

Genetic structure and variation of wild feline populations: the impact of fragmentation

Genetic diversity (the amount of genetic variation), genetic differentiation (the distribution of genetic variation within and among populations) and genetic distance (amount of genetic variation compared between different pairs of populations) (Lowe et al. 2004) of wild cat populations in across Belize will be determined based on the levels of heterozygosity and allelic diversity of cat populations obtained from fecal DNA samples.

4.2. Remote Camera Trapping

Camera traps were arrayed in trapping grids systematically spaced at 3 km intervals for jaguars

and pumas (and at 1.5 km intervals for the smaller cats). The spacing for the large cats is based on the smallest home range recorded for 1 female radio-collared jaguar of 10 km^2 (Rabinowitz and Nottingham 1986) and that for ocelots based on 2 km^2 from Emmons (1988) and results from Dillon and Kelly (2007). Therefore, every 9 km^2 or 2 km^2 contained a camera trap; hence no individual cat should be missed due to gaps in the trapping grid and every animal has a probability of being captured, a necessary assumption of the mark-recapture models (Otis et al. 1978). The same spacing was used for both pumas and jaguars assuming that pumas have similar spatial requirements.

Cats will be identified based on individual spot patterns, scars and other characteristics. Individual capture histories will be built for each the felines and data will then be analyzed in program CAPTURE to calculate abundance estimates (Otis et al. 1978, White et al. 1982, Rexstad and Burnham et al. 1991). The abundance will then be divided by the effective area (km^2) surveyed to generate density estimates for each site. The effective trap area will be determined by calculating a buffer value around each camera trap or around each scat sample, equal to ½ the mean maximum distance moved (½ MMDM) among all individuals recaptured (either photographically or genetically recaptured) at least once (Wilson and Anderson 1985, Karanth and Nichols 1998). Buffers will be dissolved and total area calculated from a GIS. The results will be summarized per species and study area.

Fig. 7. Map of Belize representing the cost surface and resistance values level for dispersing jaguars according to our classification; least -cost path analysis between two protected areas in Belize (Mountain Pine Ridge Forest Reserve and Rio Bravo Conservation Area)

4.3. GIS-based Spatial Analysis

Landscape genetics: combining conservation genetics and GIS

I will use two different landscape genetics approaches to study the gene flow among feline populations in Belize. First, I will focus on individuals and analyze the least cost paths and the most probable dispersal route between protected areas this approach identifies (methods see above). Various resistance surfaces will be created in order to test for several scenarios dispersing felines might encounter depending on the weight landscape features (elevation, land-cover, type, roads, water, anthropogenic factors) will be assigned. Causal modeling based on Mantel tests will indicate which landscape features provides most resistance (possible dispersal barriers) to the movement of feline species in Belize.

Second, assignment tests will be conducted to assess current dispersal patterns of felines between protected areas across Belize. Through allele frequencies per population and maximum-likelihood statistics, individuals will be assigned to populations of best fit. The effect of various landscape characteristics and anthropogenic disturbances on current dispersal rates and genetic structure of cat populations will be further analyzed.

5. Progress and Preliminary Results

5.1. Molecular Scatology

a. Locating scat samples

During our field survey (January to March 2008) the total number of scat samples detected in Rio Bravo with the scat detector dog is 251. For doing 376 km of scat survey I walked a total distance of 510 km with the scat detector dog within a 45 effective working days. The overall detection rate for scat samples is 1.5 km survey per scat sample and 2.03 km of actual walking distance.

Scat samples were detected on roads, trails, game trails and also off-trail. Based on the high sample size, we strongly believe that professional trained scat detector dogs are a very effective conservation tool to help improving monitoring of highly elusive species such as felines in hot and humid tropical environments.

b. Analyzing DNA scat samples

Using 14 highly polymorphic microsatellites we analyzed the scat samples collected in Rio Bravo and could successfully detect jaguars 34 times, pumas 32 times, ocelots 30 times and margays once. Out of the samples collected we could identify 36 individual cats. 9 jaguars (6 males, 3 females), 18 cougars (7 males, 11 females), 9 ocelots (2 males, 7 females) and one margay (gender unknown yet). The technique used is highly efficient in obtaining DNA from wild cats without ever capturing them. The number of detected wild cats in Rio Bravo is striking and definitely identifies the area as high priority for cat conservation.

5.2. Remote Camera Trapping

In progress

5.3. GIS-bases Spatial Analysis

In progress

6. Involvement of Local People and Education

Local people are also involved in this project through the continued recruitment and training of additional guides. I relied on the company of one or more guides to navigate and to traverse the difficult roads and trails. Their experience has proven invaluable to past research efforts in Belize and will also be an integral part of this project. During our field work we also trained many local students (from the University of Belize) and international students and gave numerous talks about our project at the Da Silva Research station, lodges, schools etc. (Fig.9. and 10). In addition, three Natural Resource students (Silverio Marin, Raquel Chun, and Yahaira Urbina) from the University of Belize participated in this project as part of their undergraduate research project. They learned to collect data in the field and were trained in various research aspects of the project including scat detection with a working dog, scat collection, camera trapping, habitat surveying and navigation. I am continuing to advise Yahaira and Raquel on

their independent projects, the results of which will be presented at the 3rd NRM Symposium at the University of Belize in Belmopan, in Belize in 2009.

Fig.8. Dog demo at Gallon Jug Primary School

Fig. 9. Workshop on scat detection

9. References

Aspi, J., Roininen, E., Ruokonen, M., Kojola, I., and C. Vila. 2006. Genetic diversity, population structure, effective population size and demographic history of the Finnish wolf population. Molecular Ecology 15: 1561-1576.

Bennett, A.F. 2003. Linkages in the Landscape: The role of corridors and connectivity in wildlife conservation. Second Edition. Gland, Switzerland and Cambridge, UK: World Conservation Union.

Bergl, R.A., and L. Vigilant. 2007. Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). Molecular Ecology 16: 501-516.

Bhagavatula J., and L. Singh. 2006. Genotyping faecal samples of Bengal tiger (*Panthera tigris tigris*) for population estimation: A pilot study. BMC GENETICS 7: 48.

CEPF. 2004. Northern Region of the Mesoamerica Biodiversity Hotspot. Latin America Forum, Rio de Janeiro, Brazil.

Chame, M. 2003. Terrestrial mammal feces: a morphometric summary and description. Mem. Inst. Oswaldo Cruz 98(1): 71-94.

Crawshaw, P.G., and H.B. Quigley. 1991. Jaguar spacing, activity and habitat use in a seasonally flooded environment in Brazil. Journal of Zoology 223: 367-370.

Dalen L., Gotherstrom A., and A. Angerbjorn. 2004. Identifying species from pieces of faeces. Conservation Genetics 5(1): 109-111.

Dillon, A.G. and M.J. Kelly. 2007. Ocelot activity, trap success, and density in Belize: the impact of trap spacing and animal movement on density estimates. Oryx 41: 469-477.

Eizirik, E., J.-H. Kim, et al. 2001. Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). 10: 65-79.

Emmons, L.H. 1988. A field study of ocelots (*Felis pardalis*) in Peru. Revue d'Ecologie (Terre et la Vie), 43, 133–157.

Ernest, H.B., Penedo M.C.T., May B.P., Syvanen M., and W.M. Boyce. 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. Molecular Ecology9: 433–441.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Applications to human mitochondrial DNA restriction data. Genetics 131:479-491.

Farrell, L.E., Roman, J., and M.E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9(10): 1583-1590.

Felsenstein, J. 1993. PHYLIP-phylogeny inference package, version 3.5. University of Washington, Seattle, WA. http://evolution.genetics.washington.edu/phylip.html

Frantz, A.C., Pope, L.C., Carpenter, J., Roper, J., Wilson, G.J., Delahays, R.J., and T. Burke. 2003. Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Molecular Ecology 12: 1649-1661.

Gompper M.E., Kays R.W., Ray J.C., et al. 2006. A comparison of noninvasive techniques to survey carnivore communities in northeastern North America. Wildlife Society Bulletin 34 (4): 1142-1151.

Guo, S.W., and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Haldane, J.B.S. 1954. An exact test for randomness of mating. Journal of Genetics 52: 631-635.

Harrison, R.L. 2006. A Comparison of Survey Methods for Detecting Bobcats. Wildlife Society Bulletin 34(2): 548-552.

Höss, M., Kohn, M., Pääbo, S., Knauer, F., and W. Schröder. 1992. Excrement analysis by PCR. Nature 359:199-199.

IUCN 2008. 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 25 November 2008.

Karanth, K.U., and J.D. Nichols. 1998. Estimation of tiger densities in India using photographic captures and recaptures. Ecology 79(8): 2852-62.

Kelly, M. J. 2003. Jaguar monitoring in western Belize. Caribbean Geography 13: 19-32.

Kohn, M.H., York, E.C., et al. 1999. Estimating population size by genotyping faeces. Proceedings of the Royal Society B: Biological Sciences 266(1420): 657-657.

Kohn, M.H., and R.K. Wayne. 1997. Facts from feces revisited. Tree 12: 223-227.

Lacy, R.C. 1993. Impacts of inbreeding in natural and captive populations of vertebrates: implications for conservation. Perspectives in Biology and Medicine 36: 480–496.

Long, R.A., Donovan, T.M., MacKay, P., Zielinski, W.J., and J.S. Buzas. 2007a. Effectiveness of Scat Detection Dogs for Detecting Forest carnivores. The Journal of Wildlife Management 71(6): 2007-2017.

Long, R.A., Donovan, T.M., MacKay, P., Zielinski, W.J., and J.S. Buzas. 2007b. Comparing Scat Detection Dogs, Cameras, and Hair Snares for Surveying Carnivores. The Journal of Wildlife Management 71(6): 2018-2025.

Long, R. 2006. Developing predictive occurrence models for carnivores in Vermont using data collected with multiple noninvasive methods. Doctoral Thesis, University of Vermont, USA.

Lowe, A., Harris S., and P. Ashton. 2004. Ecological genetics: design, analysis and application. Blackwell Publishing, Oxford, United Kingdom.

Maffei, L., Noss A. Silver, S. and M.J. Kelly. Under review. Abundance/density case study: Jaguars in South and Central America.

Maffei, L., Cuéllar, E., and A. Noss. 2004. One thousand jaguars (*Panthera onca*) in Bolivia's Chaco? Camera trapping in the Kaa-Iya National Park. Journal of Zoology, London 262: 295-304.

Medellin, R.A., Chetkiewicz, C., Rabinowitz, A., Redford, K.H., Sanderson, E., and A. Taber.

2000. Jaguars in the new millennium. A status assessment, priority detection and recommendation for the conservation of jaguars in the Americas. Mexico: Universidad Nacional Autonoma de Mexico/Wildlife Conservation Society.

Menotti-Raymond, M., David, V.A., Lyons, L.A., Schaffer, A.A., Tolmlin, J.F., Hutton, M.K., and S.J. O'Brien. 1999. A genetic linkage map of microsatellites in the domestic car (*Felis catus*). Genomics 57: 9-23.

Menotti-Raymond, M., and S.J. O'Brien. 1995. Evolutionary conservation of ten microsatellite loci in four species of Felidae. The Journal of Heredity 86: 319-322.

Meerman, J.C., and J.R. Wilson. 2005. The Belize National Protected Areas Systems Plan. National Protected Areas Policy & System Plan Project, Belmopan, Belize.

Miller, C.R., Joyce P., et al. 2005. A new method for estimating the size of small populations from genetic mark-recapture data. 14: 1991-2005.

Miller, C.R., Joyce, P., and L.P. Waits. 2002. Assessing Allelic Dropout and Genotype Reliability Using Maximum Likelihood. Genetics 160: 357-366.

Mills, L.S., Citta J.J., et al. 2000. Estimating animal abundance using noninvasive DNA sampling: Promise and pitfalls. Ecological Applications 10(1): 283-294.

Minch, E., Ruiz-Linares, A., Goldstein D., Feldman, M., and L.L. Cavalli-Sforza. 1995. MICROSAT version 1.4d: a computer program for calculationg various statistics on microsatellite allele data. http://hpgl.stanford.edu/projects/microsat/

Mukherjee, N., Mondol, S., Andheria, A., and U. Ramakrishnan. 2007. Rapid multiplex PCR based species identification of wild tigers using non-invasive samples. Conservation Genetics 8: 1465-1470.

Murphy, M.A., Waits, L.P., Kendall, K.C. et al. 2002. An evaluation of long-term preservation methods or brown bear (*Ursus arctos*) faecal DNA samples. Conservation Genetics 3(4): 435-440.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nowell, K. and Jackson, P. (compilers and editors) 1996. Wild Cats. Status Survey and Conservation Action Plan. IUCN/SSC Cat Specialist Group. IUCN, Gland, Switzerland.

Otis, D.L., Burnham, K.P., White, G.C., and D.R. Anderson. 1978. Statistical inference from capture data on closed populations. Wildlife Monographs 62: 1–135.

Page, R.D.W. 1996. TREEVIEW: an application to display phylogenetic trees on personal computer. Computer Applications in the Biosciences 12: 357-358.

Pilgrim, K.L., McKelvey, K.S., Riddle, A.E., and M.K. Schwartz. 2005. Felid sex identification based on noninvasive genetic samples. Molecular Ecology Notes 5: 60-61.

Pritchard, J.K., Stephens, M., and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Rabinowitz, A.R., and B.G. Nottingham. 1986. Ecology and behavior of the jaguar (*Panthera onca*) in Belize, Central America. Journal of Zoology 210:149-159.

Reed, J.Z., Tollit, D. J., et al. 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. Molecular Ecology 6**:** 225-234.

Rexstad, E., and K.P. Burnham. 1991. User's guide for interactive program CAPTURE. Abundance estimation of closed populations. Colorado State University, Fort Collins, Colorado, USA.

Rousset, F.M, and M. Raymond. 1995. GENEPOP, version 1.2: A population genetics software for exact tests and ecumenism. Journal of Heredity 86:248-249.

Ruiz-Garcia, M., Payan E., et al. 2006. DNA microsatellite characterization of the jaguar

(*Panthera onca*) in Colombia. Genes & Genetic Systems 81(2): 115-127.
Sanderson, E.W.; Redford, K.H.; Chetkiewicz, C-L. B.; Mendellin, R.A.; Rabinowitz, A.R.; Robinson, J.G., and A.B. Taber. 2002. Planning to save a species: the jaguar as a model. Conservation Biology 16: 58-72.CEPF. 2004. Northern Region of the Mesoamerica Biodiversity Hotspot. Latin America Forum, Rio de Janeiro, Brazil.
Silver, S.C.; Ostro, L.E.T.; Marsh, L.K.; Maffei, L.; Noss, A.J.; Kelly, M.J.; Wallace, R.B.; Gomez, H. and G. Ayala. 2004. The use of camera traps for estimating jaguar abundance and density using capture/recapture analysis. Oryx 38:148-154.
Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.
Smith, D. A., Ralls, K., et al. 2005. Assessment of scat-detection dog surveys to determine kit fox distribution. "Wildlife Society Bulletin 33(3): 897-904.
Smith, D.A., Ralls, K., Hurt, A., Adams, B., Parker, M., Davenport, B., Smith, M. C. and J. E. Maldonado. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6: 339–346.
Smith, D.A., Ralls, K., Davenport, B. Brice, A., and J.E. Maldonado. 2001. Canine Assistants for Conservationists. Science, New Series 5503: 435.
Sokal R.R., and F.J. Rohlf. 1994. Biometry: the principles and practice of statistics in biological research, third edition. W.H. Freeman, New York.
Sugimoto T., Nagata J., Aramilev V. V., et al. 2006. Species and sex identification from faecal samples of sympatric carnivores, Amur leopard and Siberian tiger, in the Russian Far East. Conservation Genetics 7 (5): 799-802.
Sunquist, F. 1987. Kingdom of Cats. National Wildlife Federation, Washington, DC, USA.
Swank, W.G., and J.G. Teer. 1989. Status of the jaguar – 1987. Oryx 23: 14-21.
Swanson, B.J., and P.J. Rusz. 2006. Detection and classification of cougars in Michigan using low copy DNA sources. American Midland Naturalist 155: 363-372.
Paetkau, D., Slade, R., Burden, M., and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation- based exploration of accuracy and power. Molecular Ecology: 13: 55-65.
Pompanon, F., Bonin, A., Bellemain, E. et al. 2005. Genotyping errors: Causes, consequences, and solutions. Nature Reviews Genetics 6: 847-859.
Wallace, R.B., Gomez, H., Ayala, G., and F. Espinoza. 2003. Camera trapping for jaguar (*Panthera onca*) in the Tuichi Valley, Bolivia. Mastozoological Neotropical 10: 5–11.
Wasser, S.K., Davenport, B., Ramage, E.R., Hunt, K. E., Parker, M., Clarke, C. and G. Stenhouse. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead ecosystem, Alberta, Canada. Canadian Journal of Zoology 82: 475–492.
Weir, B.S. 1996. Genetic data analysis II. Sinauer Associates, Sunderland, MA.
Weir, B.S., and C.C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370.
White, G.C., Anderson, D.R., Burnham K.P., and D.L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory Publication. LA-8787-NERP. Los Alamos, New Mexico. USA.
Wilson, K.R., and D.R. Anderson. 1985. Evaluation of nested grid approach for estimating density. Journal of Wildlife Management 49(3):675-678.
Wright, S. 1965. The interpretation of population structure by *F*-statistics with special regard to

systems of mating. Evolution 19:355-420.