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REVIEW

Applications and techniques for non-invasive faecal genetics research in felid conservation

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Abstract Non-invasive genetic techniques utilising DNA extracted from faeces hold great promise for felid conservation research. These methods can be used to establish species distributions, model habitat requirements, analyse diet, estimate abundance and population density, and form the basis for population, landscape and conservation genetic analyses. Due to the elusive nature of most felid species, non-invasive genetic methods have the potential to provide valuable data that cannot be obtained with traditional observational or capture techniques. Thus, these methods are particularly valuable for research and conservation of endangered felid species. Here, we review recent studies that use non-invasive faecal genetic techniques to survey or study wild felids; provide an overview of field, laboratory and analysis techniques; and offer suggestions on how future non-invasive genetic studies can be expanded or improved to more effectively support conservation.

Keywords Conservation · Elusive species · Faecal DNA · Felidae · Non-invasive genetics

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Introduction

Due to their elusive nature, wild cats are remarkably 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). Of the 36 extant felid species, 25 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, labour intensive or invasive. Telemetry studies yield the greatest level of information on behaviour and ecology; however, these methods require live capture that may cause stress and disturbance, or potential injury or mortality if done improperly, raising ethical concerns (Greenwood 1996; Piggott and Taylor 2003; Kelly et al. 2012). More importantly, the significant effort needed to track individuals via radio/GPS collars leads to few studies at a small number of sites, often with small sample sizes (Kelly et al. 2012). A broader suite of tools is needed to monitor felid populations and improve understanding of basic ecology and behaviour in order to generate effective conservation strategies.

Non-invasive methods utilising DNA from faeces (Hoss et al. 1992; Kohn and Wayne 1997) provide an alternative approach and are becoming more commonplace in felid studies (Table 1). Although several excellent reviews have been written on general non-invasive genetic wildlife research (see Waits and Paetkau 2005; Schwartz et al. 2007; Kelly et al. 2012), we feel that use of non-invasive genetics in felid biology warrants special attention. Our goal is to stimulate more extensive research initiatives by providing

Table 1 Published studies that have used non-invasive faecal genetic techniques to monitor or study felid species

Analysis type	Species	Citation
Species ID	Amur tiger *Panthera tigris altaica*	Nagata et al. (2005), Sugimoto et al. (2012)
	Andean cat *Leopardus jacobita*	Perovic et al. (2003), Cossios and Angers (2006), Cossios et al. (2007), Walker et al. (2007), Napolitano et al. (2008)
	Arabian leopard *Panthera pardus nimr*	Perez et al. (2006)
	Bengal tiger *Panthera tigris tigris*	Bhagavatula and Singh (2006), Sharma et al. (2008), Mondol et al. (2009b), Reddy et al. (2010), Sharma et al. (2011), Borthakur et al. (2011)
	Bobcat *Lynx rufus*	Ruell and Crooks (2007), Ruell et al. (2009)
	Canadian lynx *Lynx canadensis*	McKelvey et al. (2006)
	Cheetah *Acinonyx jubatus*	Busby et al. (2009)
	Geoffroy's cat *Leopardus geoffroyi*	Cossios and Angers (2006)
	Iberian lynx *Lynx pardinus*	Palomares et al. (2002), Pires and Fernandes (2003), Fernandez et al. (2006), Oliveira et al. (2010)
	Jaguar *Panthera onca*	Farrell et al. (2000), Novack et al. (2005), Haag et al. (2009), Roques et al. (2011), Michalski et al. (2011)
	Jaguarondi *Herpailurus yagouaroundi*	Roques et al. (2011)
	Jungle cat *Felis chaus*	Mukherjee et al. (2010)
	Leopard *Panthera pardus*	Nagata et al. (2005), Busby et al. (2009), Lovari et al. (2009)
	Leopard cat *Prionailurus bengalensis*	Kurose et al. (2005), Mukherjee et al. (2010), Shehzad et al. (2012b)
	Lion *Panthera leo*	Tende et al. (2010)
	Margay *Felis wiedii*	Roques et al. (2011), Michalski et al. (2011)
	Ocelot *Leopardus pardalus*	Farrell et al. (2000), Miotto et al. (2007), Roques et al. (2011), Michalski et al. (2011)
	Pampas cat *Leopardus pajeros*	Cossios and Angers (2006), Walker et al. (2007), Napolitano et al. (2008)
	Puma *Puma concolor*	Farrell et al. (2000), Leberg et al. (2004), Novack et al. (2005), Cossios and Angers (2006), Onorato et al. (2006), Miotto et al. (2007), Napolitano et al. (2008), Haag et al. (2009), Michalski et al. (2011), Roques et al. (2011)
	Snow leopard *Panthera uncia*	Janečka et al. (2008), Lovari et al. (2009), Janečka et al. (2011a), Karmacharya et al. (2011), Shehzad et al. (2012a)
Individual ID	Amur tiger *Panthera tigris altaica*	Sugimoto et al. (2012)
	Arabian leopard *Panthera pardus nimr*	Perez et al. (2006)
	Bengal tiger *Panthera tigris tigris*	Bhagavatula and Singh (2006), Mondol et al. (2009b), Reddy et al. (2010), Borthakur et al. (2011)
	Bobcat *Lynx rufus*	Ruell and Crooks (2007), Ruell et al. (2009)
	Leopard *Panthera pardus*	Mondol et al. (2009a), Lovari et al. (2009)
	Ocelot *Leopardus pardalus*	Miotto et al. (2007)
	Puma *Puma concolor*	Ernest et al. (2000), Miotto et al. (2007)
	Snow leopard *Panthera uncia*	Janečka et al. (2008), Lovari et al. (2009), Janečka et al. (2011a, b), Karmacharya et al. (2011)
Sex ID	Amur tiger *Panthera tigris altaica*	Sugimoto et al. (2012)
	Bengal tiger *Panthera tigris tigris*	Bhagavatula and Singh (2006), Reddy et al. (2010), Borthakur et al. (2011)
	Bobcat *Lynx rufus*	Ruell et al. (2009)
	Leopard *Panthera pardus*	Perez et al. (2006), Lovari et al. (2009)
	Snow leopard *Panthera uncia*	Janečka et al. (2008), Lovari et al. (2009), Janečka et al. (2011a), Karmacharya et al. (2011)

Table 1 (continued)

Analysis type	Species	Citation
Diet analysis	Andean cat *Leopardus jacobita*	Walker et al. (2007)
	Jaguar *Panthera onca*	Farrell et al. (2000), Polisar et al. (2003), Novack et al. (2005), Weckel et al. (2006a, b), Foster et al. (2010)
	Leopard cat *Prionailurus bengalensis*	Shehzad et al. (2012b)
	Ocelot *Leopardus pardalus*	Farrell et al. (2000)
	Pampas cat *Leopardus pajeros*	Walker et al. (2007)
	Puma *Puma concolor*	Farrell et al. (2000), Foster et al. (2010), Novack et al. (2005), Polisar et al. (2003)
	Snow leopard *Panthera uncia*	Anwar et al. (2011), Shehzad et al. (2012a)
Habitat modelling	Andean cat *Leopardus jacobita*	Napolitano et al. (2008), Marino et al. (2011)
	Iberian lynx *Lynx pardinus*	Fernandez et al. (2006)
	Jungle cat *Felis chaus*	Mukherjee et al. (2010)
	Leopard cat *Prionailurus bengalensis*	Mukherjee et al. (2010)
	Pampas cat *Leopardus pajeros*	Napolitano et al. (2008)
Genetic diversity	Amur tiger *Panthera tigris altaica*	Russello et al. (2004)
	Andean cat *Leopardus jacobita*	Napolitano et al. (2008)
	Bengal tiger *Panthera tigris tigris*	Sharma et al. (2008, 2011)
	Pampas cat *Leopardus pajeros*	Napolitano et al. (2008)
Phylogeography	Bengal tiger *Panthera tigris tigris*	Sharma et al. (2008, 2011)
	Jungle cat *Felis chaus*	Mukherjee et al. (2010)
	Leopard cat *Prionailurus bengalensis*	Mukherjee et al. (2010)
Gene flow	Jaguar *Panthera onca*	Haag et al. (2010)
	Puma *Puma concolor*	Ernest et al. (2003)
Mating systems	Cheetah *Acinonyx jubatus*	Gottelli et al. (2007)

an overview and synthesis of applications and recent research utilising non-invasive genetic sampling of faeces to monitor, study and conserve wild felids.

Genetic data collected non-invasively from faeces has numerous applications, many of which can aid in the conservation of elusive wild cats. These applications include: species identification from scat to establish species distributions (Palomares et al. 2002; Cossios et al. 2007), habitat requirements (Fernandez et al. 2006; Mukherjee et al. 2010) and diet (Farrell et al. 2000; Walker et al. 2007); determination of the sex of individuals within a population (Bhagavatula and Singh 2006; Janečka et al. 2008); and identification of individuals within a population, allowing estimates of population abundance (Perez et al. 2006; Mondol et al. 2009b) and density (Ruell et al. 2009; Janečka et al. 2011a). Additionally, faecal DNA can be used to investigate evolutionary, population, landscape or conservation genetic hypotheses such as rates of gene flow (Ernest et al. 2003; Haag et al. 2010), genetic diversity (Russello et al. 2004; Napolitano et al. 2008) or phylogeography (Mukherjee et al. 2010; Sharma et al. 2011). All of this can be done without having to capture or directly observe the study species, greatly increasing the amount of data that can be collected.

Species identification

Species distributions

The most common application of species identification data from scat DNA is determination of species distributions (Palomares et al. 2002; Cossios et al. 2007). Distribution studies based solely on scat morphology can be misleading (see Davison et al. 2002) because sympatric carnivore species often have overlapping or indistinguishable scat morphologies (Farrell et al. 2000). In most studies, only scats thought to be from target species based on morphology are collected; however, DNA analysis frequently reveals many collected samples are from non-target species (Farrell et al. 2000; Perez et al. 2006; Janečka et al. 2008; Sugimoto et al. 2012). Accurate knowledge of species distribution is imperative for conservation and management, and scat misidentification leading to presumed species presence in an area where it does not occur may waste limited conservation resources. Thus, studies inferring species distribution from scat surveys must use genetic techniques to verify species identity. Additionally, species ID from scat can be used to identify which species are responsible for conflicts

with humans, can improve understanding of space partitioning amongst felid species living in sympatry and could provide data for modelling of species occupancy (MacKenzie et al. 2002).

Habitat modelling

Distributional information from faecal DNA can further be used to model species environmental and habitat requirements. Knowledge of habitat requirements is imperative for identifying habitat for protection, restoration and reserve planning. Fernandez et al. (2006) utilised presence–absence data from genetic scat sampling to model habitat requirements for critically endangered Iberian lynx (*Lynx pardinus*) using an information theoretic approach in combination with Geographic Information Systems (GIS) landscape data. Because Iberian lynx have relatively small home ranges, they were able to gather both presence and absence data during intensive field surveys based on detection of genetically identified lynx scat. Correlation between lynx presence vs. absence and environmental variables enabled them to generate a habitat model that was 85 % accurate in predicting Iberian lynx distribution in other areas.

For wider ranging species, generating accurate data showing a species to be absent from a certain habitat may be unrealistic, and presence-only habitat modelling approaches may be required. Maximum entropy modelling (MaxEnt) (Phillips et al. 2006; Phillips and Dudik 2008), a presence-only habitat modelling method, shows promise for modelling habitat requirements and potential geographic distribution from faecal genetic data (Mukherjee et al. 2010; Marino et al. 2011). For example, Mukherjee et al. (2010) used MaxEnt to model habitat suitability based on climatic variables for leopard cats (*Prionailurus bengalensis*) using presence data from scat sampling and other sources. The model they generated classified central India as unsuitable habitat for leopard cats, despite the previous belief that leopard cats were continuously distributed throughout the Indian subcontinent (Sunquist and Sunquist 2002; Nowell and Jackson 1996). This prediction was supported by analyses of genetic structure from scat sampling, which showed that leopard cats in India are distinctly divided into disconnected northern and southern populations (Mukherjee et al. 2010).

Laboratory methods for species identification

Several methods can be used to identify species from scat, most of which rely on the amplification of mitochondrial DNA (mtDNA). The most accurate and unambiguous are direct sequencing methods (Farrell et al. 2000; Perez et al. 2006; Janečka et al. 2008) in which mtDNA products amplified by polymerase chain reaction (PCR) using carnivore-specific primers are sequenced, aligned with reference sequences and placed in species clades using a maximum likelihood or neighbour-joining tree re-construction (Fig. 1). Direct sequencing methods are advantageous because both target and non-target carnivore species present in the study area can be identified. The main downside is that this method is time consuming, and sequencing costs (roughly 5–10 USD/sample) may be prohibitive for large-scale studies or for those that do not have access to sequencing equipment. Short fragments of mtDNA are typically used for direct sequencing because mtDNA is at a higher copy number than nuclear DNA and is, thus, more easily amplified from low yield faecal samples (but see Oliveira et al. 2010 for a method based on nuclear DNA). Additionally, short fragments are preferable because they are more likely to amplify in degraded faecal DNA than are longer fragments (Broquet et al. 2007). A 110 base-pair sequence from the mitochondrial gene cytochrome *b* has been shown to reliably distinguish between felid species and between felids and other carnivores, with the exception of wildcats (*Felis silvestris*) and domestic cats (*Felis catus*) (Farrell et al. 2000; Janečka et al. 2008). This sequence has been shown

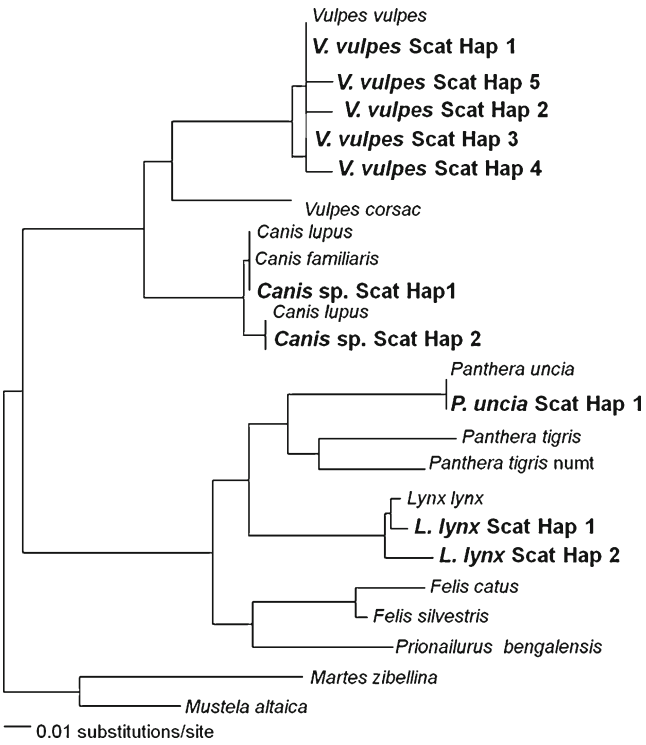

Fig. 1 Cytochrome *b* tree re-constructed using the neighbour-joining method and the Kimura two-parameter file for species identification of unknown carnivore scats. Faecal DNA haplotypes can be identified by clustering with reference sequences. Scat sequences are represented in *bold font*, whilst reference sequences are represented in *italic font*. Species identification of an unknown sample is determined when its haplotype clusters with a reference sequence and has <3 % sequence divergence (which is representative of cytochrome *b* variation between mammals species). (Bradley and Baker 2001)

to occasionally amplify in prey DNA along with target DNA in a few studies (Onorato et al. 2006; Adams et al. 2007); however, it has been successfully used for species ID in many felid studies. Chaves et al. (2012) propose development of a standardised DNA barcode for carnivore species identification worldwide so that direct comparisons can be made between studies. To this end, they tested and compared three different short mtDNA fragments from the genes ATP6, cytochrome oxidase I (COI), and cytochrome *b* in 66 different carnivore species including 12 felids. Chaves et al. (2012) discuss the strengths and weaknesses of these three markers and conclude the ATP6 has the greatest discriminatory power and the greatest amplification success from non-invasive samples and does not amplify prey DNA. Currently, however, there are only ATP6 reference sequences available for 12 felid species on GenBank, compared with 27 for cytochrome *b*, so more reference sequences will have to be gathered from tissue or blood if the ATP6 marker is to be widely used for non-invasive species ID across Felidae in the future.

For studies with only one or few focal species, multiplex diagnostic PCR methods can be used (Palomares et al. 2002; Bhagavatula and Singh 2006). These methods use species-specific primers that amplify only DNA fragments from the target species. Two other methods, PCR-restriction fragment length polymorphism (PCR-RFLP) assays (Nagata et al. 2005; Cossios and Angers 2006; Mills et al. 2000b) and rapid classificatory protocol–PCR (RCP–PCR) (Dalen et al. 2004; Roques et al. 2011) can be designed to distinguish between a suite of previously determined sympatric species. In PCR-RFLP methods, restriction enzymes that target regions that differ between species are used to digest mtDNA amplicons. This results in size differences in the remaining fragments that vary between species and that produce species-specific banding patterns on agarose gel (Foran et al. 1997). A downside of this approach is that failure of enzymatic reactions may result in false negatives or false positives (Dalen et al. 2004). Additional caution should be taken if this method is used in a location other than where the assay was designed, as within-species sequence variation between populations may alter restriction enzyme digestion sites, and other sympatric carnivores that have not been tested may potentially share the same restriction pattern with the target species. This is particularly problematic for studies in tropical areas such as South Asia or Central Africa, where there are many carnivore species that have yet to be thoroughly studied. In such cases, labs may not have all the necessary genetic information on the carnivore community to accurately design these diagnostic tests, in which case direct sequencing methods are preferable.

RCP–PCR methods utilise PCR reactions that include one common reverse primer that binds to an mtDNA site shared by all species, along with a set of species-specific forward primers that bind at varying distances from the reverse primer site in each species. This results in sequence fragments that vary in length between species, producing species-specific banding patterns on agarose gel. One recent study (Roques et al. 2011) used RCP–PCR methods to distinguish between scats of the sympatric Mesoamerican felids jaguars (*Panthera onca*), pumas (*Puma concolor*), jaguarondis (*Herpailurus yagouaroundi*), and ocelots (*Leopardus pardalis*) or margays (*Felis wiedii*). Once properly designed, this approach is less labour-intensive and more cost-effective than those previously discussed; thus, it could prove useful for large-scale or long-term distributional studies that otherwise would be prohibitively expensive. In the latter three methods (multiplex diagnostic PCR, PCR-RFLP and RCP–PCR), it is important to test positive control samples from all sympatric species potentially occurring in the study area, as well as samples from potential prey species, to ensure that the primers used do not amplify in non-target species.

Diet analysis

After DNA analysis has been used to identify scats to species, prey remains from scats can provide information regarding dietary habits. As felids are carnivorous and many species are keystone predators, knowledge of diet and prey requirements is important for understanding of their ecosystem function and for conservation planning. Diet analyses can be used to examine niche partitioning between sympatric species (Walker et al. 2007; Foster et al. 2010), to evaluate diet in hunted vs. non-hunted areas (Novack et al. 2005) or to evaluate predation on livestock, an often important aspect of human–felid conflict (Farrell et al. 2000; Anwar et al. 2011; Shehzad et al. 2012a).

Killing of felids in retribution for livestock mortality perceived to be the result of predation is a major cause of mortality for many felid species and is of significant conservation concern (Inskip and Zimmermann 2009). Diet analysis from genetically identified scats can elucidate the relationship between felids and livestock mortality by determining if and to what extent certain felid species prey on livestock in a given area. For example, Farrell et al. (2000) used species identification from scat DNA to distinguish amongst scats of jaguars, pumas, ocelots and crab-eating foxes (*Cerdocyon thous*), four sympatric carnivores from Venezuela. They then used morphological identification of undigested prey remains within scats to examine which species were responsible for preying on domestic animals. In another study, in Baltistan, Pakistan, Anwar et al. (2011) used faecal DNA to identify snow leopard (*Panthera uncia*) scats and then examined prey remains. They found that

70 % of snow leopard prey biomass consisted of domestic livestock including sheep, goats, cattle and yaks. These findings highlighted the importance of programmes such as Project Snow Leopard, a programme initiated by the Baltistan Wildlife Conservation and Development Organization to provide herdsman a way to insure their livestock and recover some of their losses from snow leopard predation (Hussain 2000, 2003; Anwar et al. 2011). Use of non-invasive genetic methods to determine the extent to which felids prey on livestock will improve decisions that affect local conservation and management.

Two recent studies, one in snow leopard (Shehzad et al. 2012a) and the other in leopard cat (Shehzad et al. 2012b), used molecular techniques to identify prey items in scat, as opposed to the conventional approach of using morphological identification of undigested prey. These studies first used molecular techniques to identify scats to species and then used next generation sequencing to target a 100-bp fragment of the mtDNA 12S rRNA gene from prey DNA for prey identification. Shehzad et al. (2012b) conclude that their DNA-based approach has better resolution for prey identification than the conventional approach.

Sex identification

Knowledge of sex ratios enhances understanding of population demography, and determination of sex can be used to investigate behavioural differences between sexes such as sex-biased dispersal and territoriality. To identify sex from scat, the most common method is to use felid-specific primers to amplify a DNA segment from the Y chromosome using PCR (Janečka et al. 2008; Borthakur et al. 2011). As the Y chromosome is only present in males, amplification success will identify samples as male, and samples that do not amplify may be regarded as female. When assigning individuals as female with this method, it is important to ensure that males are not misclassified as females due to PCR amplification failure. Thus, all samples should be run in triplicate along with male and female positive controls and a negative control. If at least two of the three samples show amplification success, individuals are assigned as male, and if no amplification occurs in all three samples, individuals are assigned as female. If available, analyses can also be performed on multiple samples from the same individual to increase reliability.

Pilgrim et al. (2005) developed an alternative non-invasive method of sex identification for felids that provides positive identification of both males and females based on the size of amplicons resulting from insertions/deletions observed in the X and Y chromosome orthologues of the zinc finger (ZFY vs. ZFX) and amelogenin (AMELY vs. AMELX) genes. With this method, males produce a PCR product derived from the Y chromosome (male only) that differs in size from the product derived from the X chromosome (male and female). Pilgrim et al. (2005) caution, however, that this method may be problematic for faecal non-invasive studies, as these regions also amplified in some prey species could lead to contamination and misidentification of sex.

Individual identification and abundance estimation

The most common application of individual identification from scat DNA is estimating population abundance (Mondol et al. 2009b; Ruell et al. 2009). For many threatened or endangered felid populations, abundance is unknown, which strongly hampers species conservation (Nowell and Jackson 1996). Monitoring population trends over time using non-invasive genetic methods can enable managers to better identify population declines and threats of local extinction and to monitor the effectiveness of conservation actions. Individual identification from non-invasively collected faecal DNA could additionally be used to monitor the status of endangered felid re-introduction programmes. Individual identification can also be used to examine ecological and behavioural parameters such as home range size, spatial overlap between individuals and population turnover, as well as to identify ‘problem animals’ responsible for attacks on livestock or conflict with humans. Finally, individuals must be identified among the scat sampled before population, landscape and conservation genetics analyses can be undertaken (Haag et al. 2010).

Abundance estimation

Once individuals are identified, the simplest approach is to determine minimum population size (Ernest et al. 2000; Miotto et al. 2007); however, capture–recapture (CR) approaches can provide more rigorous estimates of abundance (Lukacs and Burnham 2005b). If sampling is conducted over multiple, independent sampling occasions, then traditional CR models can be used (Mondol et al. 2009b; Ruell et al. 2009). As capture probability often varies between individual animals according to sex, age, social status or other variables and capture heterogeneity can bias abundance estimates, attempts should be made to minimise or at least quantify capture heterogeneity whenever possible (Ebert et al. 2010; Marucco et al. 2011). Capture probability can also be affected by probability of identity (Mills et al. 2000a). Thus, CR models specifically designed to deal with capture heterogeneity should be used when appropriate (Mills et al. 2000a; Lukacs and Burnham 2005b). Such models include but are not limited to M_h-jackknife (Burnham and Overton 1979) and M_h-Chao (Chao 1988), within the programme CAPTURE (Otis et al. 1978; Mills et al. 2000a;

Miller et al. 2005). Additionally, there are models in the programme MARK (White and Burnham 1999) which allow flexibility when accounting for capture heterogeneity using covariates and mixture models (Lukacs 2009).

If samples cannot be collected over multiple sampling occasions, other CR algorithms can be used to estimate abundance from samples collected in one pooled sampling occasion. Kohn et al. (1999) proposed using rarefaction curve methods to estimate abundance by plotting the number of individual genotypes accumulated as a function of the number of scats analysed. The asymptote of the resulting curve provides an estimate of population size (Eggert et al. 2003; Perez et al. 2006). The rarefaction curve formula in Kohn et al. (1999) significantly overestimates abundance (Eggert et al. 2003; Miller et al. 2005); however, the formula proposed by Eggert et al. (2003) appears to perform much better (Miller et al. 2005). The software programme CAPWIRE (Miller et al. 2005) provides a maximum-likelihood model designed specifically for genetic data that allows input of different capture probabilities. This model performs best in small populations with heterogeneous capture probabilities. Finally, Petit and Valiere (2006) developed a method for estimating abundance from non-invasive CR data based on a single sampling occasion using a Bayesian estimator. Strengths and weaknesses of these numerous models are discussed in greater detail in Miller et al. (2005), Lukacs and Burnham (2005b), Bellemain et al. (2005) and Petit and Valiere (2006). Which type of model is most appropriate will depend upon how the study was designed and on the temporal and spatial distribution of the samples collected (Marucco et al. 2011). We recommend that researchers explore multiple competing models and choose the one that is least biased according to their sampling methods and the ecology of their focal species.

Two studies have directly compared population abundance estimates from faecal non-invasive genetic surveys and camera-trapping surveys in felids (Mondol et al. 2009b; Janečka et al. 2011a). Mondol et al. (2009b) found that abundance estimates from both approaches were very similar for a Bengal tiger population in India. Janečka et al. (2011a) caution that estimates from the two approaches may be difficult to compare because of differences in the distribution of observations and because sub-adults are typically excluded from population estimates derived from camera-trapping, whilst differentiating between adults and sub-adults is not possible using non-invasive genetic techniques. Janečka et al. (2011a) do, however, conclude that costs of estimating abundance using non-invasive genetic sampling were lower than for camera-trapping for their study primarily because a much larger area could be covered in a shorter period of time. Therefore, scat sampling has the potential to enable large-scale distribution and abundance surveys of felids at a lower cost than camera trap surveys. Non-invasive genetic studies may also be preferable to camera trapping in some cases because the resulting genetic data can provide valuable information concerning population, landscape and conservation genetics that could not be gathered using camera trapping.

Laboratory methods for individual identification

DNA extracted from faeces can be used to identify individuals within a population using variable microsatellite markers (Kohn et al. 1999; Ernest et al. 2000). Over 300 microsatellite primers and other molecular markers have been developed for domestic cat, and their locations in the genome have been determined by linkage, radiation hybrid and fluorescence *in situ* hybridization (FISH) maps (Menotti-Raymond et al. 1999; Menotti-Raymond et al. 2003a; Menotti-Raymond et al. 2003b; Davis et al. 2009; Menotti-Raymond et al. 2009). The majority of these markers (>90 %) amplify and are variable in other felids, and many have been successfully used for individual identification across Felidae (Ernest et al. 2000; Schwartz et al. 2002; Waits et al. 2006; Ruell and Crooks 2007; Mondol et al. 2009a). A genomic study of the location of markers has revealed >90 % conservation of the order and structure of chromosomes across Felidae, making the domestic cat chromosome maps a valuable resource for all wild species (Davis et al. 2009). In addition, the 2.8X felCat4 domestic cat whole genome assembly, which is available on the University of California Santa Cruz (UCSC) Genome Browser website, can be used to design additional primers in regions of interest. Thus, the time and costs associated with developing new microsatellite primers for each species are greatly reduced.

To identify individuals, microsatellite markers that are polymorphic within the population of interest are used. For any two individuals, the probability that both will share the same allele at a given microsatellite locus is dependent upon the frequency of that allele in the population. As more loci are analysed, the probability that two individuals will possess the same alleles at all loci decreases multiplicatively (Mills et al. 2000a). The number of microsatellite loci needed to confidently distinguish between individuals with high probability varies depending on the amount of genetic diversity within the population and the expected heterozygosity of the loci used (Paetkau 2003). For example, Ruell and Crooks (2007) determined that using only four microsatellite loci was sufficient in a genetically diverse bobcat (*Lynx rufus*) population in southern California because the probability that unrelated individuals would share the same genotype (P_{id}) was <0.0001, and the probability of siblings sharing the same genotype (P_{sibs}) was 0.02. In contrast, in a study of Arabian leopards (*Panthera pardus nimr*), a subspecies that suffers from low genetic diversity due to the effect of small and inbred remnant populations, Perez et

al. (2006) found that seven of the 11 microsatellite loci that were amplified in their samples were monomorphic, and the remaining four had few alleles. Thus, Perez et al. (2006) could only achieve a P_{id} of 0.05 and P_{sibs} of 0.221 using the four polymorphicloci available for their analyses. These findings allude to the reality that selecting loci that will identify individuals with confidence may be especially difficult in small inbred populations that have undergone recent bottlenecks due to genetic drift.

The number of microsatellite loci that should be used to identify individuals is a balance between achieving sufficiently low probability of individual identity, whilst minimising costs by using the least number of loci necessary (Mills et al. 2000a). Typically, a criterion of $P_{sibs}<0.05$ is used (Waits et al. 2001); however, in studies where large numbers of individuals are sampled (>100), additional loci may be required (Paetkau 2003). Use of more loci than necessary, however, may upwardly bias abundance estimates as a result of increased potential for genotyping error (Waits and Leberg 2000). Probability of identity values can be calculated from genotype data using numerous software packages including Cervus (Marshall et al. 1998), Gimlet (Valiere 2002) and GenAlEx (Peakall and Smouse 2006).

Population density estimation

Once abundance is estimated, the next logical step is to quantify population density so that estimates can be compared between studies and sites, and so estimates can be extrapolated to other environmentally and geographically similar areas. This step can be difficult, however, as it requires accurate estimation of the geographic extent from which the abundance estimate was derived. The most common approach is to create buffers around sample locations that are representative of species home ranges within the study population and use the sum of these buffered areas to estimate total survey area. This approach has been commonly used in camera-trapping studies (Karanth and Nichols 1998; Balme et al. 2009; Nunez-Perez 2011). If home range estimates from telemetry are available, these can be used to determine buffer size; however, such data are available for few felid populations. Home range estimates should be derived directly from the study population, as many felid species exhibit vastly different home range sizes in different environments or locations (Dillon and Kelly 2008; Goodrich et al. 2010). Where home range estimates from telemetry are not available, studies have used derivations of the mean of the maximum distance moved between captures (MMDM) to estimate home range and provide buffers for population density estimates (Wilson and Anderson 1985; Karanth and Nichols 1998; Dillon and Kelly 2008). For camera trapping, some felid studies have concluded that 1/2 MMDM buffers provide a reasonable estimate of home range size (Balme et al. 2009; Nunez-Perez 2011), whilst others have argued that this metric underestimates distances moved and inflates density estimates (Soisalo and Cavalcanti 2006), and that 1 MMDM buffers are a better estimator (Dillon and Kelly 2008). Which buffer size is most biologically meaningful is still debated.

Few non-invasive faecal genetics studies have yet attempted to estimate population density from scat survey data. Ruell et al. (2009) buffered their scat search transects with both the radius and the diameter of a male bobcat home range derived from a telemetry study in their study area. They, however, do not discuss which buffer estimator, home range radius or home range diameter was a more meaningful representation of effective survey area. Janečka et al. (2011a) found that although 1/2 MMDM distances from scat surveys were similar to 1/2 MMDM distances from camera trapping in their study of snow leopards, the total effective areas estimated from camera trapping and scat surveys differed greatly, resulting in a threefold higher density estimate from scat surveys. They attribute this bias to the fact that the scats they collected were clustered on only a few transects with a limited distribution, which reduced the size of their effective survey area calculated from the sum of 1/2 MMDM buffers around sample data points. By contrast, their camera trap array was more widely distributed in a manner so as to detect the maximum number of snow leopards, resulting in a larger survey area estimate from 1/2 MMDM buffers. Janečka et al. (2011a) recommend that to minimise such bias, non-invasive scat survey transects should be uniformly distributed and oriented to maximise the area surveyed. They also suggest that it may be preferable to sample a greater number of shorter transects, as opposed to a few long transects.

In camera trapping and non-invasive genetics studies using hair snares, camera and snare sites are generally distributed in a uniform grid intended to maximise the area surveyed (Boulanger et al. 2004; Maffei and Noss 2008). On the other hand, in non-invasive genetic studies using faeces, samples are generally collected opportunistically along transects (Mondol et al. 2009b; Ruell et al. 2009) or from latrines or scrape sites that are used by multiple individuals (Janečka et al. 2011a). As a result, sample data points from scat surveys are often clumped together on transects or at latrine sites. This clumping results in underestimation of total survey area after buffers are applied and thus overestimation of population density. Overestimation of population density may be a detriment to conservation if management decisions are based on inflated density estimates. Further research evaluating methods to more accurately estimate total effective survey area from non-invasive scat surveys in order to minimise upward bias of density estimates is needed.

A new modelling approach that may hold promise for estimating population density from non-invasive genetic data from faeces is spatially explicit CR (SECR) (Borchers and Efford 2008; Royle and Young 2008). SECR models do not require estimation of effective survey area, but instead estimate density directly using maximum likelihood (Borchers and Efford 2008) or hierarchical Bayesian (Royle and Young 2008) approaches. These models use spatial information (capture location) in conjunction with the capture probability of individuals to estimate the number of sampled individuals with activity centres within the sampling area (somewhat like a combination of CR and line distance sampling). Additionally, these models are robust to individual capture heterogeneity, and they do not require geographic closure, a problematic assumption with many other CR models. These models have been used in camera-trapping studies (Royle et al. 2009; Sollmann et al. 2011) and in non-invasive genetic studies using hair snares (Obbard et al. 2010; Kery et al. 2011). One recent paper (Thompson et al. 2012) has adapted a SECR model for use in non-invasive faecal sampling by accounting for variable search intensities, non-standard survey routes and lack of fixed 'trap' locations. The model described in Thompson et al. (2012) could prove very useful for estimating felid population density from faecal samples collected opportunistically; however, future studies are needed for validation.

Of the studies that have estimated abundance from faeces using non-invasive genetic methods in felids (Table 2), few have been conducted over large spatial scales with a spatially or temporally systematic design that allows sophisticated modelling. Even fewer have attempted to estimate population density. The tools are now available to conduct such studies. It is important to utilise consistent methodology across studies so that comparisons can be made regarding the status of populations across a species range. We urge researchers to focus efforts towards larger scale, systematically designed studies (see Marucco et al. 2011) with the aim of estimating population density. When population density is estimated, comparisons can be made amongst studies and estimates can be applied to other geographically and environmentally similar areas in order to more effectively support management and conservationat a broad scale.

Population, landscape and conservation genetics

Genetic information from faecal DNA can be used as the basis to test population, landscape and conservation genetic hypotheses. As sample size is often a limiting factor for felid genetic studies, non-invasively collected faecal DNA holds great promise for improving the power of such studies. Relatively few felid studies, however, have taken advantage of faecal DNA for these types of analyses. Two studies have used DNA from scat in addition to more traditional sources to examine gene flow in pumas (Ernest et al. 2003) and jaguars (Haag et al. 2010). Scat DNA has also been used in phylogeographic studies to determine evolutionarily significant units for conservation in jungle cat (*Felis chaus*) and leopard cat (Mukherjee et al. 2010), and Bengal tiger (Sharma et al. 2011), as well as to determine appropriate source populations for Bengal tiger translocation programmes (Sharma et al. 2008). One study used faecal DNA to examine mating systems in cheetahs (*Acinonyx jubatus*), which revealed promiscuity amongst females (Gottelli et al. 2007). Non-invasive scat sampling could also be used to examine relatedness and social structure (Janečka et al. 2006; Onorato et al. 2011), rates and sex bias of dispersal (Janečka et al. 2007a), effective population size (Spong et al. 2000; Janečka et al. 2007b) and genetic diversity within populations of conservation concern (Russello et al. 2004; Janečka et al. 2011b). Assessment of genetic diversity is especially important for small isolated populations where inbreeding depression may threaten population viability (Frankham and Ralls 1998; Reed et al. 2007).

Methodological considerations

Sample collection, storage and DNA extraction

Many felid species (such as tigers, bobcats, pumas, leopard cats and jungle cats, to name just a few) defecate along trails and dirt roads as these are common routes of movement (Macdonald 1980). Thus, scats often can be collected along such paths (Miotto et al. 2007; Mondol et al. 2009b; Ruell et al. 2009; Mukherjee et al. 2010). In some instances, it may be possible to follow animal tracks until scats are encountered, especially when snow cover is present, making tracks readily visible (McKelvey et al. 2006). Additionally, some felid species such as ocelots, Andean cats (*Leopardus jacobita*) and snow leopards defecate at common latrines or scrape sites (Walker et al. 2007; Janečka et al. 2011a). Such latrines may harbour numerous scats, and once latrine sites are located, fresh scats can be routinely collected. Use of detection dogs can greatly increase the efficiency of collecting samples (Smith et al. 2003; Wasser et al. 2004; Kelly et al. 2012), especially where vegetation is particularly dense, such as in many tropical regions. Several dog-training facilities including PackLeader and Working Dogs for Conservation can provide trained detection dogs with human handlers for research purposes. The largest constraint concerning use of detection dogs is the high cost (up to 2,000 USD/week), which may be well in access of the budget of many researchers.

Because heat, moisture, UV radiation and mould lead to DNA degradation, samples should be collected as fresh as

Table 2 Studies of felid species that used non-invasive faecal genetic techniques to identify individuals and estimate population abundance. Systematic study design refers to studies that were designed specifically with the intent of estimating abundance with capture–recapture models; opportunistic study design refers to studies where scats were collected opportunistically. Species ID amplification success refers to success rate of genetic assays to identify species from scat, and genotyping success refers to determination of the correct genotype for individual identification. *# loci* used refers to number of microsatellite loci used for individual identification. *PID* is the probability that two individuals within the population will possess the same microsatellite genotype; *PIDsibs* is the probability that two siblings within the population will possess the same microsatellite genotype based on allele frequencies within the population. *Minimum number detected* refers to the number of individuals identified. *Population abundance estimate* refers to total population size estimated using capture–recapture models (model type used in *parentheses*). *Blank fields* were not reported in the given study

Citation	Species	Country	Study site	Survey design	Transect length surveyed	Sample size (# scats)	Species ID amplification success	% of scat target species	Genotyping success	# loci used	PID, PIDsibs	Minimum number detected	Population abundance estimate	Survey area	Density per 100 km^2
Ernest et al. (2000)	Puma	United States	Yosemite Valley	Opportunistic	_____	32	_____	34 %	47 %	12	_____	9	_____	_____	_____
Perez et al. (2006)	Arabian leopard	Israel	Judean desert, Nagev Highlands	Opportunistic	_____	268	41 %	48 %	76 %	4	0.05, 0.221	7	5–8 (rarefaction)	_____	_____
Miotto et al. (2007)	Puma	Brazil	Sao Paulo State	Opportunistic	_____	20	60 %	83 %	100 %	4	0.0001,–	9	_____	_____	_____
Ruell and Crooks (2007)	Bobcat	United States	Orange County, CA	Opportunistic	_____	259	87 %	56 %	87 %	4	<0.0001, ≈0.02	30	_____	_____	_____
Lovari et al. (2009)	Snow leopard	Nepal	Sagarmartha NP	Opportunistic	130 km	30	70 %	76 %	69 %	9	0.00002, 0.00832	4	_____	_____	_____
Mondol et al. (2009a, b)	Bengal tiger	India	Bandipur	Systematic, 42 days	255.4 km	63	86 %	92 %	76 %	5	–, 0.024	26	66 (M_h-Jackknife)	671 km^2	_____
Ruell et al. (2009)	Bobcat	United States	Santa Monica Mountains	Systematic, 16 days	128 km	262	83 %	43 %	89 %	4	_____	38	54 (M_h-Jackknife) 59 (CAPWIRE)	196.6 km^2	25–42
Reddy et al. (2010)	Bengal tiger	India	Kawal Wildlife Sanctuary	Opportunistic	_____	47	_____	45 %	24 %	4	_____	4	_____	_____	_____
Borthakur et al. (2011)	Bengal tiger	India	Orang NP	Opportunistic	_____	57	88 %	96 %	92 %	8	<0.0001, 0.00285	17	27 (CAPWIRE)	_____	_____
Janečka et al. (2011a)	Snow leopard	Mongolia	Gobi Desert	Semi-systematic, 8 days	37.74 km	180	81 %	33 %	65 %	7	0.00016, 0.01801	15	19 (CAPWIRE), 16–17 (rarefaction)	314.3 km^2 (1/2 MMDM)	4.9–5.9
Karmacharya et al. (2011)	Snow leopard	Nepal	Kangchenju-nga NP SheyPhoksundo NP	Opportunistic	_____	71	69 %	39 %	53 %	6	0.00014, 0.0182	9	_____	_____	_____
Sugimoto et al. (2012)	Amur tiger	Russia	Primorye Krai	Opportunistic	_____	286	82 %	33 %	58 %	10	<0.0001 0.00196	12	14 (M_h-Jackknife) 12 (CAPWIRE)	_____	_____

possible. In some instances, however, even very old samples may yield useable DNA (Janečka et al. 2011a). It may also be wise to time collection in accordance with seasons that will decrease degradation, such as the dry season in tropical areas or in winter in temperate areas. It is also important to avoid cross-contamination of samples, as this will result in unreliable genotypes and will not permit individual identification. We recommend conducting a pilot study to determine the feasibility of locating enough faecal samples to address proposed research questions, to ascertain what sampling strategy is most appropriate, and to assess sample DNA quality, genotyping errors, and potential sources of capture heterogeneity (Valiere et al. 2007; Marucco et al. 2011).

After collection, samples are typically stored on silica gel desiccant (Janečka et al. 2008; Ruell et al. 2009), or in 95 % ethanol (Mondol et al. 2009b) or DET buffer. For DNA extraction, numerous kits designed specifically for faecal DNA are available (Qiagen Stool DNA Extraction Kit, Qiagen, Valencia, CA, USA; PSP Spin Stool DNA Kit, Invitek, Berlin, Germany; Stool DNA Isolation Kit, Norgen Biotek, Thorold, ON, Canada). These kits are specially designed to remove PCR inhibitors commonly found in faeces. Hebert et al. (2011) found that putting faecal DNA extracted with a standard stool kit through a second step termed Concentrated Chelex Treatment to further remove PCR inhibitors increased PCR amplification success by nearly 30 %. In our experience, ≤80 % of scat samples collected will yield DNA suitable for species ID using mtDNA markers. Of those that are positively identified, only≈70 % may be from the target species, and of those ≤70 % may yield DNA suitable for individual identification using microsatellite genotypes. For example, if the target is to have 30 usable samples from which to identify individuals, at least 76 scats may need to be collected.

Dealing with genotyping errors

DNA from scat is typically more degraded and of lower quality than DNA extracted from tissue (Broquet et al. 2007). As a result, genotyping errors such as allelic dropout and false alleles are more frequent, and these errors can bias results if care is not taken (Taberlet et al. 1999; McKelvey and Schwartz 2004). To overcome the problem of genotyping errors, a multiple-tubes approach is typically used (Taberlet and Fumagalli 1996; Miller et al. 2002; Frantz et al. 2003), though other less costly alternative approaches have been suggested (Paetkau 2003; McKelvey and Schwartz 2004). For the multiple-tubes approach, each sample is re-amplified at each locus multiple times until consensus genotypes are obtained independently from each sample. As genotyping errors occur randomly and are non-reproducible, consensus genotypes obtained multiple times from the same sample should verify the genotypes accuracy. The number of replicates necessary will depend upon rates of genotyping error; however, three or more replicates are typically required. This approach increases costs considerably but is typically necessary for attaining reliable results. Tests have been developed to check for genotyping errors (McKelvey and Schwartz 2004) and rates of error can be quantified and should be reported along with the results of any study (Bonin et al. 2004; Broquet and Petit 2004). Also, the programme DROPOUT (McKelvey and Schwartz 2005) can be used to identify loci and samples that likely contain genotyping errors. Several CR models have been developed that incorporate genotyping errors (Lukacs and Burnham 2005a; Knapp et al. 2009b). Knapp et al. (2009a) propose a model they call the Genotyping Uncertainty Added Variance Adjustment (GUAVA), which corrects for genotyping errors and shadow effect (Mills et al. 2000a) and minimises bias of abundance estimates derived from only one round of genotyping. Although the multiple-tubes approach is preferable, GUAVA may provide a more cost-effective alternative for estimating abundance from very large sample sizes whilst still maintaining accuracy.

Microsatellite primers should be directly labelled with fluorescent dyes (e.g., 6-FAM, NED, PET or VIC) rather than indirectly labelled using the more economical m13 labelling system (Guo and Milewicz 2003). This will increase genotype signal and lead to greater genotyping success from low quality scat DNA, and will help minimise genotyping errors such as allelic dropout. In a small pilot study using faeces from ocelots, we found that genotyping success was 36.8 % higher using direct-labelled primers compared to m13-labelled primers (χ^2=43.152, p<0.0001). A more in-depth discussion of quality considerations for non-invasive genetic data can be found in Waits and Paetkau (2005).

Conclusions

The applications for felid conservation research afforded by non-invasive genetic sampling of faeces are numerous and are only beginning to be realised. Although many felid species are threatened or endangered, and many play important roles in ecosystem dynamics through top-down predatory effects (Terborgh et al. 1999; Estes et al. 2011), they remain some of the least understood of all mammalian species due to their elusive behaviour. Traditional capture and telemetry methods often provide indispensable data regarding species ecology; however, non-invasive techniques utilising DNA from scat offer a promising tool to contribute to the body of knowledge regarding felid biology and conservation. Non-invasive genetic techniques should continue to be utilised and expanded in research, conservation and management of wild cat species in order to help insure the persistence of healthy populations.

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References

Adams JR, Lucash C, Schutte L, Waits LP (2007) Locating hybrid individuals in the red wolf (*Canis rufus*) experimental population area using a spatially targeted sampling strategy and faecal DNA genotyping. Mol Ecol 16(9):1823–1834. doi:10.1111/j.1365-294X.2007.03270.x

Anwar MB, Jackson R, Nadeem MS, Janecka JE, Hussain S, Beg MA, Muhammad G, Qayyum M (2011) Food habits of the snow leopard *Panthera uncia* (Schreber, 1775) in Baltistan, Northern Pakistan. Eur J Wildl Res 57(5):1077–1083. doi:10.1007/s10344-011-0521-2

Balme GA, Hunter LTB, Slotow R (2009) Evaluating methods for counting cryptic carnivores. J Wildl Manag 73(3):433–441. doi:10.2193/2007-368

Bellemain E, Swenson JE, Tallmon O, Brunberg S, Taberlet P (2005) Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. Conserv Biol 19(1):150–161. doi:10.1111/j.1523-1739.2005.00549.x

Bhagavatula J, Singh L (2006) Genotyping faecal samples of Bengal tiger *Panthera tigris tigris* for population estimation: a pilot study. BMC Genet 7:48. doi:10.1186/1471-2156-7-48

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13(11):3261–3273. doi:10.1111/j.1365-294X.2004.02346.x

Borchers DL, Efford MG (2008) Spatially explicit maximum likelihood methods for capture–recapture studies. Biometrics 64 (2):377–385. doi:10.1111/j.1541-0420.2007.00927.x

Borthakur U, Barman RD, Das C, Basumatary A, Talukdar A, Ahmed MF, Talukdar BK, Bharali R (2011) Noninvasive genetic monitoring of tiger (*Panthera tigris tigris*) population of Orang National Park in the Brahmaputra floodplain, Assam, India. Eur J Wildl Res 57(3):603–613. doi:10.1007/s10344-010-0471-0

Boulanger J, McLellan BN, Woods JG, Proctor MF, Strobeck C (2004) Sampling design and bias in DNA-based capture–mark–recapture population and density estimates of grizzly bears. J Wildl Manag 68(3):457–469. doi:10.2193/0022-541x(2004)068[0457:sdabid]2.0.co;2

Bradley RD, Baker RJ (2001) A test of the genetic species concept: cytochrome-*b* sequences and mammals. J Mammal 82(4):960–973. doi:10.1644/1545-1542(2001)082<0960:atotgs>2.0.co;2

Broquet T, Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. Mol Ecol 13(11):3601–3608. doi:10.1111/j.1365-294X.2004.02352.x

Broquet T, Menard N, Petit E (2007) Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conserv Genet 8(1):249–260. doi:10.1007/s10592-006-9146-5

Burnham KP, Overton WS (1979) Robust estimation of population-size when capture probabilities vary among animals. Ecology 60 (5):927–936. doi:10.2307/1936861

Busby GBJ, Gottelli D, Wacher T, Marker L, Belbachir F, De Smet K, Belbachir-Bazi A, Fellous A, Belghoul M, Durant SM (2009) Genetic analysis of scat reveals leopard Panthera pardus and cheetah *Acinonyx jubatus* in southern Algeria. Oryx 43(03):412. doi:10.1017/s0030605309001197

Chao A (1988) Estimating animal abundance with capture frequency data. J Wildl Manag 52(2):295–300. doi:10.2307/3801237

Chaves PB, Graeff VG, Lion MB, Oliveira LR, Eizirik E (2012) DNA barcoding meets molecular scatology: short mtDNA sequences for standardized species assignment of carnivore noninvasive samples. Mol Ecol Resour 12(1):18–35. doi:10.1111/j.1755-0998.2011.03056.x

Cossios D, Angers B (2006) Identification of andean felid feces using PCR-RFLP. Mastozool Neotrop 13(2):239–244

Cossios ED, Madrid A, Condori JL, Fajardo U (2007) Update on the distribution of the Andean cat *Oreailurus jacobita* and the pampas cat *Lynchailurus colocolo* in Peru. Endanger Species Res 3:313–320. doi:10.3354/esr00059

Dalen L, Gotherstrom A, Angerbjorn A (2004) Identifying species from pieces of faeces. Conserv Genet 5(1):109–111. doi:10.1023/b:coge.0000014060.54070.45

Davis BW, Raudsepp T, Wilkerson AJP, Agarwala R, Schaffer AA, Houck M, Chowdhary BP, Murphy WJ (2009) A high-resolution cat radiation hybrid and integrated FISH mapping resource for phylogenomic studies across Felidae. Genomics 93(4):299–304. doi:10.1016/j.ygeno.2008.09.010

Davison A, Birks JDS, Brookes RC, Braithwaite TC, Messenger JE (2002) On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. J Zool 257:141–143. doi:10.1017/s0952836902000730

Dillon A, Kelly MJ (2008) Ocelot home range, overlap and density: comparing radio telemetry with camera trapping. J Zool 275 (4):391–398. doi:10.1111/j.1469-7998.2008.00452.x

Ebert C, Knauer F, Storch I, Hohmann U (2010) Individual heterogeneity as a pitfall in population estimates based on non-invasive genetic sampling: a review and recommendations. Wildl Biol 16 (3):225–240. doi:10.2981/09-108

Eggert LS, Eggert JA, Woodruff DS (2003) Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. Mol Ecol 12(6):1389–1402. doi:10.1046/j.1365-294X.2003.01822.x

Ernest HB, Penedo MCT, May BP, Syvanen M, Boyce WM (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. Mol Ecol 9(4):433–441. doi:10.1046/j.1365-294x.2000.00890.x

Ernest HB, Boyce WM, Bleich VC, May B, Stiver SJ, Torres SG (2003) Genetic structure of mountain lion (*Puma concolor*) populations in California. Conserv Genet 4(3):353–366. doi:10.1023/a:1024069014911

Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JBC, Marquis RJ, Oksanen L, Oksanen T, Paine RT, Pikitch EK, Ripple WJ, Sandin SA, Scheffer M, Schoener TW, Shurin JB, Sinclair ARE, Soule ME, Virtanen R, Wardle DA (2011) Trophic downgrading of planet Earth. Science 333(6040):301–306. doi:10.1126/science.1205106

Farrell LE, Romant J, Sunquist ME (2000) Dietary separation of sympatric carnivores identified by molecular analysis of scats. Mol Ecol 9 (10):1583–1590. doi:10.1046/j.1365-294x.2000.01037.x

Fernandez N, Delibes M, Palomares F (2006) Landscape evaluation in conservation: molecular sampling and habitat modeling for the Iberian lynx. Ecol Appl 16(3):1037–1049. doi:10.1890/1051-0761(2006)016[1037:leicms]2.0.co;2

Foran DR, Crooks KR, Minta SC (1997) Species identification from scat: an unambiguous genetic method. Wildl Soc Bull 25(4):835–839

Foster RJ, Harmsen BJ, Valdes B, Pomilla C, Doncaster CP (2010) Food habits of sympatric jaguars and pumas across a gradient of human disturbance. J Zool 280(3):309–318. doi:10.1111/j.1469-7998.2009.00663.x

Frankham R, Ralls K (1998) Conservation biology—inbreeding leads to extinction. Nature 392(6675):441–442. doi:10.1038/33022

Frantz AC, Pope LC, Carpenter PJ, Roper TJ, Wilson GJ, Delahay RJ, Burke T (2003) Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Mol Ecol 12(6):1649–1661. doi:10.1046/j.1365-294X.2003.01848.x

Goodrich JM, Miquelle DG, Smirnov EN, Kerley LL, Quigley HB, Hornocker MG (2010) Spatial structure of Amur (Siberian) tigers (*Panthera tigris altaica*) on Sikhote-Alin Biosphere Zapovednik, Russia. J Mammal 91(3):737–748. doi:10.1644/09-mamm-a-293.1

Gottelli D, Wang JL, Bashir S, Durant SM (2007) Genetic analysis reveals promiscuity among female cheetahs. Proc R Soc B Biol Sci 274(1621):1993–2001. doi:10.1098/rspb.2007.0502

Greenwood JJD (1996) Basic techniques. Ecological census techniques: a handbook. Cambridge University Press, Cambridge

Guo DC, Milewicz DM (2003) Methodology for using a universal primer to label amplified DNA segments for molecular analysis. Biotechnol Lett 25(24):2079–2083. doi:10.1023/B:BILE.0000007075.24434.5e

Haag T, Santos AS, De Angelo C, Srbek-Araujo AC, Sana DA, Morato RG, Salzano FM, Eizirik E (2009) Development and testing of an optimized method for DNA-based identification of jaguar (*Panthera onca*) and puma (*Puma concolor*) faecal samples for use in ecological and genetic studies. Genetica 136(3):505–512. doi:10.1007/s10709-008-9347-6

Haag T, Santos AS, Sana DA, Morato RG, Cullen L Jr, Crawshaw PG Jr, De Angelo C, Di Bitetti MS, Salzano FM, Eizirik E (2010) The effect of habitat fragmentation on the genetic structure of a top predator: loss of diversity and high differentiation among remnant populations of Atlantic Forest jaguars (*Panthera onca*). Mol Ecol 19(22):4906–4921. doi:10.1111/j.1365-294X.2010.04856.x

Hebert L, Darden SK, Pedersen BV, Dabelsteen T (2011) Increased DNA amplification success of non-invasive genetic samples by successful removal of inhibitors from faecal samples collected in the field. Conserv Genet Resour 3(1):41–43. doi:10.1007/s12686-010-9280-8

Hoss M, Kohn M, Paabo S, Knauer F, Schroder W (1992) Excrement analysis by PCR. Nature 359(6392):199–199. doi:10.1038/359199a0

Hussain S (2000) Protecting the snow leopard and enhancing farmers' livelihoods: a pilot insurance scheme in Baltistan. Mt Res Dev 20 (3):226–231. doi:10.1659/0276-4741(2000)020[0226:ptslae]2.0.co;2

Hussain S (2003) The status of the snow leopard in Pakistan and its conflict with local farmers. Oryx 37(1):26–33. doi:10.1017/s0030605303000085

Inskip C, Zimmermann A (2009) Review human–felid conflict: a review of patterns and priorities worldwide. Oryx 43(1):18–34. doi:10.1017/s003060530899030x

Janečka JE, Blankenship TL, Hirth DH, Tewes ME, Kilpatrick CW, Grassman LI (2006) Kinship and social structure of bobcats (*Lynx rufus*) inferred from microsatellite and radio-telemetry data. J Zool 269(4):494–501. doi:10.1111/j.1469-7998.2006.00099.x

Janečka JE, Blankenship TL, Hirth DH, William Kilpatrick C, Tewes ME, Grassman LI (2007a) Evidence for male-biased dispersal in bobcats *Lynx rufus* using relatedness analysis. Wild Biol 13 (1):38–47. doi:10.2981/0909-6396(2007)13[38:efmdib]2.0.co;2

Janečka JE, Tewes ME, Laack LL, Grassman LI, Haines AM, Honeycutt RL (2007b) Small effective population sizes of two remnant ocelot populations (*Leopardus pardalis albescens*) in the United States. Conserv Genet 9(4):869–878. doi:10.1007/s10592-007-9412-1

Janečka JE, Jackson R, Yuquang Z, Diqiang L, Munkhtsog B, Buckley-Beason V, Murphy WJ (2008) Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. Anim Conserv 11(5):401–411. doi:10.1111/j.1469-1795.2008.00195.x

Janečka J, Munkhtsog B, Jackson R, Naranbaatar G, Mallon D, Murphy W (2011a) Comparison of noninvasive genetic and camera-trapping techniques for surveying snow leopards. J Mammal 92(4):771–783. doi:10.1644/10-MAMM-A-036.1

Janečka JE, Tewes ME, Laack LL, Caso A, Grassman LI Jr, Haines AM, Shindle DB, Davis BW, Murphy WJ, Honeycutt RL (2011b) Reduced genetic diversity and isolation of remnant ocelot populations occupying a severely fragmented landscape in southern Texas. Anim Conserv. doi:10.1111/j.1469-1795.2011.00475.x

Karanth KU, Nichols JD (1998) Estimation of tiger densities in India using photographic captures and recaptures. Ecology 79(8):2852–2862. doi:10.1890/0012-9658(1998)079[2852:eotdii]2.0.co;2

Karmacharya DB, Thapa K, Shrestha R, Dhakal M, Janečka JE (2011) Noninvasive genetic population survey of snow leopards (*Panthera uncia*) in Kangchenjunga Conservation Area, Shey Phoksundo National Park and surrounding buffer zones of Nepal. BMC Research Notes 4(516):1–8. doi:10.1186/1756-0500-4-516

Kelly MJ, Betsch J, Wultsch C, Mesa B, Mills LS (2012) Noninvasive sampling for carnivores. In: Biotani L, Powell RA (eds) Carnivore ecology and conservation: a hand-book of techniques. Oxford University Press, Oxford, p 47–67

Kery M, Gardner B, Stoeckle T, Weber D, Royle JA (2011) Use of spatial capture–recapture modeling and DNA data to estimate densities of elusive animals. Conserv Biol 25(2):356–364. doi:10.1111/j.1523-1739.2010.01616.x

Knapp SM, Craig BA, Waits LP (2009) Incorporating genotyping error into non-invasive DNA-based mark–recapture population estimates. J Wildl Manag 73(4):598–604. doi:10.2193/2007-156

Kohn MH, Wayne RK (1997) Facts from feces revisited. Trends Ecol Evol 12(6):223–227. doi:10.1016/s0169-5347(97)01050-1

Kohn MH, York EC, Kamradt DA, Haugt G, Sauvajot RM, Wayne RK (1999) Estimating population size by genotyping faeces. Proc R Soc Lond Ser B Biol Sci 266(1420):657–663

Kurose N, Masuda R, Tatara M (2005) Fecal DNA analysis for identifying species and sex of sympatric carnivores: a noninvasive method for conservation on the Tsushima islands, Japan. J Hered 96(6):688–697. doi:10.1093/jhered/esi124

Leberg PL, Carloss MR, Dugas LJ, Pilgrim KL, Mills LS, Green MC, Scognamillo D (2004) Recent record of a cougar (*Puma concolor*) in Louisiana, with notes on diet, based on analysis of fecal materials. Southeast Nat 3(4):653–658. doi:10.1656/1528-7092(2004)003[0653:rroacp]2.0.co;2

Lovari S, Boesi R, Minder I, Mucci N, Randi E, Dematteis A, Ale SB (2009) Restoring a keystone predator may endanger a prey species in a human-altered ecosystem: the return of the snow leopard to Sagarmatha National Park. Anim Conserv 12(6):559–570

Lukacs PM (2009) Closed population capture–recapture models. In: Cooch E, White G (eds) Program MARK: a gentle introduction. p 14.1–14.38. http://www.phidot.org/software/mark/. Accessed 22 June 2012

Lukacs PM, Burnham KP (2005a) Estimating population size from DNA-based closed capture–recapture data incorporating genotyping error. J Wildl Manag 69(1):396–403. doi:10.2193/0022-541x(2005)069<0396:epsfdc>2.0.co;2

Lukacs PM, Burnham KP (2005b) Review of capture–recapture methods applicable to noninvasive genetic sampling. Mol Ecol 14 (13):3909–3919. doi:10.1111/j.1365-294X.2005.02717.x

Macdonald DW (1980) Patterns of scent marking with urine and faeces amongst carnivore communities. In: Stoddart DM (ed) Olfaction in mammals. Academic Press, London, p 107–135

Macdonald D, Loveridge A (2010) The biology and conservation of wild felids. Oxford Press, UK

MacKenzie DI, Nichols JD, Lachman GB, Droege S, Royle JA, Langtimm CA (2002) Estimating site occupancy rates when detection probabilities are less than one. Ecology 83(8):2248–2255. doi:10.2307/3072056

Maffei L, Noss AJ (2008) How small is too small? Camera trap survey areas and density estimates for ocelots in the Bolivian chaco. Biotropica 40(1):71–75. doi:10.1111/j.1744-7429.2007.00341.x

Marino J, Bennett M, Cossios D, Iriarte A, Lucherini M, Pliscoff P, Sillero-Zubiri C, Villalba L, Walker S (2011) Bioclimatic

constraints to Andean cat distribution: a modelling application for rare species. Divers Distrib 17(2):311–322. doi:10.1111/j.1472-4642.2011.00744.x

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7(5):639–655. doi:10.1046/j.1365-294x.1998.00374.x

Marucco F, Boitani L, Pletscher DH, Schwartz MK (2011) Bridging the gaps between non-invasive genetic sampling and population parameter estimation. Eur J Wildl Res 57(1):1–13. doi:10.1007/s10344-010-0477-7

McKelvey KS, Schwartz MK (2004) Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. J Wildl Manag 68(3):439–448. doi:10.2193/0022-541x(2004)068[0439:geawpe]2.0.co;2

McKelvey KS, Schwartz MK (2005) DROPOUT: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Mol Ecol Notes 5(3):716–718. doi:10.1111/j.1471-8286.2005.01038.x

McKelvey KS, Von Kienast J, Aubry KB, Koehler GM, Maletzke BT, Squires JR, Lindquist EL, Loch S, Schwartz MK (2006) DNA analysis of hair and scat collected along snow tracks to document the presence of Canada lynx. Wildl Soc Bull 34(2):451–455. doi:10.2193/0091-7648(2006)34[451:daohas]2.0.co;2

Menotti-Raymond M, David VA, Lyons LA, Schaffer AA, Tomlin JF, Hutton MK, O'Brien SJ (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 57(1):9–23. doi:10.1006/geno.1999.5743

Menotti-Raymond M, David VA, Agarwala R, Schaffer AA, Stephens R, O'Brien SJ, Murphy WJ (2003a) Radiation hybrid mapping of 304 novel microsatellites in the domestic cat genome. Cytogenet Genome Res 102(1–4):272–276. doi:10.1159/000075762

Menotti-Raymond M, David VA, Chen ZQ, Menotti KA, Sun S, Schaffer AA, Agarwala R, Tomlin JF, O'Brien SJ, Murphy WJ (2003b) Second-generation integrated genetic linkage/radiation hybrid maps of the domestic cat (*Felis catus*). J Hered 94 (1):95–106. doi:10.1093/jhered/esg008

Menotti-Raymond M, David VA, Schaffer AA, Tomlin JF, Eizirik E, Phillip C, Wells D, Pontius JU, Hannah SS, O'Brien SJ (2009) An autosomal genetic linkage map of the domestic cat, *Felis silvestris catus*. Genomics 93(4):305–313. doi:10.1016/j.ygeno.2008.11.004

Michalski F, Valdez FP, Norris D, Zieminski C, Kashivakura CK, Trinca CS, Smith HB, Vynne C, Wasser SK, Metzger JP, Eizirik E (2011) Successful carnivore identification with faecal DNA across a fragmented Amazonian landscape. Mol Ecol Resour 11 (5):862–871. doi:10.1111/j.1755-0998.2011.03031.x

Miller CR, Joyce P, Waits LP (2002) Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160 (1):357–366

Miller CR, Joyce P, Waits LP (2005) A new method for estimating the size of small populations from genetic mark–recapture data. Mol Ecol 14(7):1991–2005. doi:10.1111/j.1365-294X.2005.02577.x

Mills LS, Citta JJ, Lair KP, Schwartz MK, Tallmon DA (2000a) Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecol Appl 10(1):283–294. doi:10.2307/2641002

Mills LS, Pilgrim KL, Schwartz MK, McKelvey K (2000b) Identifying lynx and other North American felids based on mtDNA analysis. Conserv Genet 1(3):285–288. doi:10.1023/a:1011574209558

Miotto RA, Rodrigues FP, Ciocheti G, Galetti PM (2007) Determination of the minimum population size of pumas (*Puma concolor*) through fecal DNA analysis in two protected cerrado areas in the Brazilian Southeast. Biotropica 39(5):647–654. doi:10.1111/j.1744-7429.2007.00315.x

Mondol S, Navya R, Athreya V, Sunagar K, Selvaraj VM, Ramakrishnan U (2009a) A panel of microsatellites to individually identify leopards and its application to leopard monitoring in human dominated landscapes. BMC Genet 10:79. doi:10.1186/1471-2156-10-79

Mondol S, Ullas Karanth K, Samba Kumar N, Gopalaswamy AM, Andheria A, Ramakrishnan U (2009b) Evaluation of non-invasive genetic sampling methods for estimating tiger population size. Biol Conserv 142(10):2350–2360. doi:10.1016/j.biocon.2009.05.014

Mukherjee S, Krishnan A, Tamma K, Home C, Navya R, Joseph S, Das A, Ramakrishnan U (2010) Ecology driving genetic variation: a comparative phylogeography of jungle cat (*Felis chaus*) and leopard cat (*Prionailurus bengalensis*) in India. PLoS One 5 (10):e13724. doi:10.1371/journal.pone.0013724

Nagata J, Aramilev VV, Belozor A, Sugimoto T, McCullough DR (2005) Fecal genetic analysis using PCR-RFLP of cytochrome *b* to identify sympatric carnivores, the tiger *Panthera tigris* and the leopard *Panthera pardus*, in far eastern Russia. Conserv Genet 6 (5):863–866. doi:10.1007/s10592-005-9038-0

Napolitano C, Bennett M, Johnson WE, O'Brien SJ, Marquet PA, Barria I, Poulin E, Iriarte A (2008) Ecological and biogeographical inferences on two sympatric and enigmatic Andean cat species using genetic identification of faecal samples. Mol Ecol 17 (2):678–690. doi:10.1111/j.1365-294X.2007.03606.x

Novack AJ, Main MB, Sunquist ME, Labisky RF (2005) Foraging ecology of jaguar (*Panthera onca*) and puma (*Puma concolor*) in hunted and non-hunted sites within the Maya Biosphere Reserve, Guatemala. J Zool 267:167–178. doi:10.1017/s0952836905007338

Nowell K, Jackson P (1996) Wild cats: status survey and action plan. International Union for Conservation of Nature and Natural Resources, Gland, Switzerland

Nunez-Perez R (2011) Estimating jaguar population density using camera-traps: a comparison with radio-telemetry estimates. J Zool 285(1):39–45. doi:10.1111/j.1469-7998.2011.00812.x

Obbard ME, Howe EJ, Kyle CJ (2010) Empirical comparison of density estimators for large carnivores. J Appl Ecol 47(1):76–84. doi:10.1111/j.1365-2664.2009.01758.x

Oliveira R, Castro D, Godinho R, Luikart G, Alves PC (2010) Species identification using a small nuclear gene fragment: application to sympatric wild carnivores from South-western Europe. Conserv Genet 11(3):1023–1032. doi:10.1007/s10592-009-9947-4

Onorato D, White C, Zager P, Waits LP (2006) Detection of predator presence at elk mortality sites using mtDNA analysis of hair and scat samples. Wildl Soc Bull 34(3):815–820. doi:10.2193/0091-7648(2006)34[815:doppae]2.0.co;2

Onorato D, Desimone R, White C, Waits LP (2011) Genetic assessment of paternity and relatedness in a managed population of cougars. J Wildl Manag 75(2):378–384. doi:10.1002/jwmg.43

Otis DL, Burnham KP, White GC, Anderson DR (1978) Statistical inference from capture data on closed animal populations. Wildl Monogr (62):1–135

Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. Mol Ecol 12(6):1375–1387. doi:10.1046/j.1365-294X.2003.01820.x

Palomares F, Godoy JA, Piriz A, O'Brien SJ, Johnson WE (2002) Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. Mol Ecol 11 (10):2171–2182. doi:10.1046/j.1365-294X.2002.01608.x

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6(1):288–295. doi:10.1111/j.1471-8286.2005.01155.x

Perez I, Geffen E, Mokady O (2006) Critically endangered Arabian leopards *Panthera pardus nimr* in Israel: estimating population parameters using molecular scatology. Oryx 40(03):295. doi:10.1017/s0030605306000846

Perovic P, Walker S, Novaro A (2003) New records of the endangered Andean mountain cat in northern Argentina. Oryx 37(3):374–377. doi:10.1017/s0030605303000644

Petit E, Valiere N (2006) Estimating population size with noninvasive capture–mark–recapture data. Conserv Biol 20(4):1062–1073. doi:10.1111/j.1523-1739.2006.00417.x

Phillips SJ, Dudik M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31(2):161–175. doi:10.1111/j.0906-7590.2008.5203.x

Phillips S, Anderson R, Schapire R (2006) Maximum entropy modeling of species geographic distributions. Ecol Model 190(3–4):231–259. doi:10.1016/j.ecolmodel.2005.03.026

Piggott MP, Taylor AC (2003) Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wildl Res 30 (1):1–13. doi:10.1071/wr02077

Pilgrim KL, McKelvey KS, Riddle AE, Schwartz MK (2005) Felid sex identification based on noninvasive genetic samples. Mol Ecol Notes 5(1):60–61. doi:10.1111/j.1471-8286.2004.00831.x

Pires AE, Fernandes ML (2003) Last lynxes in Portugal? Molecular approaches in a pre-extinction scenario. Conserv Genet 4(4):525–532. doi:10.1023/a:1024762013876

Polisar J, Maxit I, Scognamillo D, Farrell L, Sunquist ME, Eisenberg JF (2003) Jaguars, pumas, their prey base, and cattle ranching: ecological interpretations of a management problem. Biol Conserv 109(2):297–310. doi:10.1016/s0006-3207(02)00157-x

Reddy PA, Kumaraguru A, Yadav PR, Ramyashree A, Bhagavatula J, Shivaji S (2010) Studies to determine presence or absence of the Indian tiger (*Panthera tigris tigris*) in Kawal Wildlife Sanctuary, India. Eur J Wildl Res 57(3):517–522. doi:10.1007/s10344-010-0460-3

Reed DH, Nicholas AC, Stratton GE (2007) Genetic quality of individuals impacts population dynamics. Anim Conserv 10(3):275–283. doi:10.1111/j.1469-1795.2007.00120.x

Roques S, Adrados B, Chavez C, Keller C, Magnusson WE, Palomares F, Godoy JA (2011) Identification of neotropical felid faeces using RCP–PCR. Mol Ecol Resour 11(1):171–175. doi:10.1111/j.1755-0998.2010.02878.x

Royle JA, Young KV (2008) A hierarchical model for spatial capture–recapture data. Ecology 89(8):2281–2289. doi:10.1890/07-0601.1

Royle JA, Nichols JD, Karanth KU, Gopalaswamy AM (2009) A hierarchical model for estimating density in camera-trap studies. J Appl Ecol 46(1):118–127. doi:10.1111/j.1365-2664.2008.01578.x

Ruell EW, Crooks KR (2007) Evaluation of noninvasive genetic sampling methods for felid and canid populations. J Wildl Manag 71 (5):1690–1694. doi:10.2193/2006-061

Ruell EW, Riley SPD, Douglas MR, Pollinger JP, Crooks KR (2009) Estimating bobcat population sizes and densities in a fragmented urban landscape using noninvasive capture–recapture sampling. J Mammal 90(1):129–135. doi:10.1644/07-mamm-a-249.1

Russello MA, Gladyshev E, Miquelle D, Caccone A (2004) Potential genetic consequences of a recent bottleneck in the Amur tiger of the Russian far east. Conserv Genet 5(5):707–713. doi:10.1007/s10592-004-1860-2

Schwartz MK, Mills LS, McKelvey KS, Ruggiero LF, Allendorf FW (2002) DNA reveals high dispersal synchronizing the population dynamics of Canada lynx. Nature 415(6871):520–522. doi:10.1038/415520a

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. Trends Ecol Evol 22(1):25–33. doi:10.1016/j.tree.2006.08.009

Sharma R, Stuckas H, Bhaskar R, Rajput S, Khan I, Goyal SP, Tiedemann R (2008) mtDNA indicates profound population structure in Indian tiger (*Panthera tigris tigris*). Conserv Genet 10(4):909–914. doi:10.1007/s10592-008-9568-3

Sharma R, Stuckas H, Bhaskar R, Khan I, Goyal SP, Tiedemann R (2011) Genetically distinct population of Bengal tiger (*Panthera tigris tigris*) in Terai Arc Landscape (TAL) of India. Mamm Biol 76(4):484–490. doi:10.1016/j.mambio.2010.10.005

Shehzad W, McCarthy TM, Pompanon F, Purevjav L, Coissac E, Riaz T, Taberlet P (2012a) Prey preference of snow leopard (*Panthera uncia*) in South Gobi, Mongolia. PLoS One 7(2). doi:10.1371/journal.pone.0032104

Shehzad W, Riaz T, Nawaz MA, Miquel C, Poillot C, Shah SA, Pompanon F, Coissac E, Taberlet P (2012b) Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. Mol Ecol 21(8):1951–1965. doi:10.1111/j.1365-294X.2011.05424.x

Smith DA, Ralls K, Hurt A, Adams B, Parker M, Davenport B, Smith MC, Maldonado JE (2003) Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Anim Conserv 6:339–346. doi:10.1017/s136794300300341x

Soisalo MK, Cavalcanti SMC (2006) Estimating the density of a jaguar population in the Brazilian Pantanal using camera-traps and capture–recapture sampling in combination with GPS radio-telemetry. Biol Conserv 129(4):487–496. doi:10.1016/j.biocon.2005.11.023

Sollmann R, Furtado MM, Gardner B, Hofer H, Jacomo ATA, Torres NM, Silveira L (2011) Improving density estimates for elusive carnivores: accounting for sex-specific detection and movements using spatial capture–recapture models for jaguars in central Brazil. Biol Conserv 144(3):1017–1024. doi:10.1016/j.biocon.2010.12.011

Spong G, Johansson M, Bjorklund M (2000) High genetic variation in leopards indicates large and long-term stable effective population size. Mol Ecol 9(11):1773–1782. doi:10.1046/j.1365-294x.2000.01067.x

Sugimoto T, Nagata J, Aramilev VV, McCullough DR (2012) Population size estimation of Amur tigers in Russian Far East using noninvasive genetic samples. J Mammal 93(1):93–101. doi:10.1644/10-mamm-a-368.1

Sunquist ME, Sunquist F (2002) Wild cats of the world. University of Chicago Press, Chicago

Taberlet P, Fumagalli L (1996) Owl pellets as a source of DNA for genetic studies of small mammals. Mol Ecol 5(2):301–305. doi:10.1111/j.1365-294X.1996.tb00318.x

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. Trends Ecol Evol 14(8):323–327

Tende T, Ottosson U, Hansson B, Akesson M, Bensch S (2010) Population size of lions in Yankari Game Reserve as revealed by faecal DNA sampling. Afr J Ecol 48(4):949–952. doi:10.1111/j.1365-2028.2009.01196.x

Terborgh J, Estes JA, Paquet P, Ralls K, Boyd-Heger D, Miller BJ, Noss RF (1999) The role of top carnivores in regulating terrestrial ecosystems. In: Soulé, ME, Terborgh, J (eds.) Continental conservation: Scientific foundation of regional reserve networks. Island Press, Washington DC, USA, p 65–98

Thompson CM, Royle JA, Garner JD (2012) A framework for inference about carnivore density from unstructured spatial sampling of scat using detector dogs. J Wildl Manag 76(4):863–871. doi:10.1002/jwmg.317

Valiere N (2002) GIMLET: a computer program for analysing genetic individual identification data. Mol Ecol Notes 2(3):377–379. doi:10.1046/j.1471-8286.2002.00228.x

Valiere N, Bonenfant C, Toigo C, Luikart G, Gaillard J-M, Klein F (2007) Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. Conserv Genet 8(1):69–78. doi:10.1007/s10592-006-9149-2

Waits JL, Leberg PL (2000) Biases associated with population estimation using molecular tagging. Anim Conserv 3:191–199. doi:10.1017/s1367943000000901

Waits LP, Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. J Wildl Manag 69(4):1419–1433. doi:10.2193/0022-541x(2005)69[1419:ngstfw]2.0.co;2

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol Ecol 10(1):249–256

Waits LP, Buckley-Beason VA, Johnson WE, Onorato D, McCarthy TOM (2006) A select panel of polymorphic microsatellite loci for

individual identification of snow leopards (*Panthera uncia*). Mol Ecol Notes 7(2):311–314. doi:10.1111/j.1471-8286.2006.01591.x

Walker RS, Novaro AJ, Perovic P, Palacios R, Donadio E, Lucherini M, Pia M, Lopez MS (2007) Diets of three species of Andean carnivores in high-altitude deserts of Argentina. J Mammal 88 (2):519–525. doi:10.1644/06-mamm-a-172r.1

Wasser SK, Davenport B, Ramage ER, Hunt KE, Parker M, Clarke C, Stenhouse G (2004) Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Can J Zool Rev Can Zool 82 (3):475–492. doi:10.1139/z04-020

Weckel M, Giuliano W, Silver S (2006a) Cockscomb revisited: jaguar diet in the Cockscomb Basin Wildlife Sanctuary, Belize. Biotropica 38(5):687–690. doi:10.1111/j.1744-7429.2006.00190.x

Weckel M, Giuliano W, Silver S (2006b) Jaguar (*Panthera onca*) feeding ecology: distribution of predator and prey through time and space. J Zool 270(1):25–30. doi:10.1111/j.1469-7998.2006.00106.x

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Stud 46:120–139

Wilson KR, Anderson DR (1985) Evaluation of 2 density estimators of small mammal population-size. J Mammal 66(1):13–21. doi:10.2307/1380951