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Evaluation of non-invasive genetic sampling methods for estimating tiger population size

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ABSTRACT

There is often a conservation need to estimate population abundances of elusive, low-density, wide-ranging carnivore species. Because of logistical constraints, investigators often employ non-invasive 'captures' that may involve 'genetic' or 'photographic' sampling in such cases. Established capture–recapture (CR) methods offer a powerful analytical tool for such data. In this paper, we developed a rigorous combination of captive, laboratory and field-based protocols for identifying individual tigers (*Panthera tigris*) from fecal DNA. We explored trade-offs between numbers of microsatellite loci used for reliable individual identifications and the need for higher capture rates for robust analyses. Our field surveys of scats were also specifically designed for CR analyses, enabling us to test for population closure, estimate capture probabilities and tiger abundance. Consequently, we could compare genetic capture estimates to results of a 'photographic capture' study of tigers at the same site. The estimates using the heterogeneity model (M_h-Jackknife) for fecal DNA survey were [M_{t+1} = 26; $\hat{p} = 0.09$ and $\hat{N}$ ($S\hat{E}[\hat{N}]$)=66 (12.98)] in close agreement with those from the photographic survey [(M_{t+1} = 29; $\hat{p} = 0.04$ and $\hat{N}$ ($S\hat{E}[\hat{N}]$) = 66 (13.8)]. Our results revealed that designing field surveys of scats explicitly for CR data analyses generate reliable estimates of capture probability and abundance for elusive, low density species such as tigers. The study also highlights the importance of rigorous field survey and laboratory protocols for reliable abundance estimation in contexts where other approaches such as camera-trapping or physical tagging of animals may not be practical options.

1. Introduction

Abundance (population size) is a state variable of primary interest to scientists and conservationists because of its decisive influence on ecological and behavioral attributes, and, thus the potential viability of any animal population (Williams et al., 2002). However, abundance is a particularly difficult parameter to estimate for low density, wide-ranging, elusive, carnivore species, most of which are threatened (Schipper et al., 2008). For example, wild tigers (*Panthera tigris*) have suffered a massive range contraction of ~93% in historical times, with a global population size <3000–4000 animals surviving in fragmented local populations (Sanderson et al., 2006; Morell, 2007; Ranganathan et al., 2008). However, it is not practical to employ invasive physical tagging methods to derive tiger density estimates because of logistical difficulties, high costs and small numbers of captures possible (Karanth and Nichols, 2002). Consequently, non-invasive, photographic capture–recapture methods were initially developed by Karanth (1995) and thereafter refined (Karanth and Nichols, 1998, 2002) for estimating tiger numbers. Since then photographic CR sampling has been successfully employed on tigers (Karanth et al., 2004b; Wegge et al., 2004; Kawanishi and Sunquist, 2004; Simchareon et al., 2007) and other elusive felids like leopards (Henschel and Ray, 2003), jaguars (Silver et al., 2004) and ocelots (Trolle and Kery, 2003). The photographic CR method takes advantages of the fact that in all these species, individuals are identifiable from natural markings. However, they do not work for species in which individuals are not uniquely identifiable. Other potential disadvantages of photographic capture methods include: requirement of large number of camera traps, scarcity of skilled personnel, vulnerability of cameras to theft, vandalism, animal damage or adverse weather, and, potentially low animal densities or wariness resulting in small number of detections.

In this context, non-invasive genetic typing of DNA extracted from animal hair or scat has emerged as an alternative option for

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capture–recapture sampling of populations of such rare, endangered or cryptic species (Waits, 2004). Genetic identification methods have often been simply used to count the minimum number of individuals or as part of pilot studies (e.g. Sloane et al., 2000; Creel et al., 2003; Fickel and Hohmann, 2006; Bhagavatula and Singh, 2006). However, since not all individuals in the population may be detected, such estimates are of limited utility (Williams et al., 2002).

Several different approaches have been used to estimate animal abundance from genetic data, in the face of such imperfect detections. Principally, these include rarefaction curve from unique genotypes (Kohn et al., 1999; Eggert et al., 2003; Wilson et al., 2003; Frantz et al., 2003, 2004), genetic capture–recapture method (Banks et al., 2002; Mowat and Paetkau, 2002; Boersen et al., 2003; Solberg et al., 2006) using Jackknife estimators (Flagstad et al., 2004), Lincoln–Peterson estimator (Triant et al., 2004) under closed (Rudnick et al., 2008) or open population (Prugh et al., 2005) capture–recapture (CR) models or under 'capture with replacement' methods (Miller et al., 2005; Zhan et al., 2006; Puechmaille and Petit, 2007). Different estimation methods have been reviewed from both theoretical (McKelvey and Schwartz, 2004; Lukacs and Burnham, 2005) and empirical (Petit and Valiere, 2006; Piggott et al., 2006; Dreher et al., 2007) perspectives. Among these, closed and open model capture–recapture (CR) methods have been the most well-founded theoretically and empirically (Williams et al., 2002; Amstrup et al., 2005).

Estimation methods based on 'genetic captures' require data from multiple hypervariable loci for unambiguous identification of individual animals (Taberlet and Luikart, 1999; Mills et al., 2000; Lukacs and Burnham, 2005; Waits and Paetkau, 2005; Broquet et al., 2007). However, getting reliable genotypes at sufficient loci for reliable individual identification poses a challenge. For example, amplifying all ten loci from a degraded genetic sample is much harder than amplifying just five loci. This requirement of increased genetic information lowers the number of identifiable individuals counted, thereby reducing numbers of 'individual captures' available for CR analysis, with consequent trade-offs between degree of accuracy of individual identification and statistical needs of robust CR modeling.

In this study, we addressed key methodological issues related to field survey design, laboratory protocols and CR analyses relevant to genetic capture–recapture sampling of tigers based on DNA extracted from scats. Our study was carried out in the 880 km^2 Bandipur Tiger Reserve (76°12′–76°46′ E and 11°37′–11°57′ N) in Karnataka state, India. This rugged landscape is drained by several rivers and varies in altitude (680–1454 m), annual rainfall (625–1250 mm) and mean monthly temperature (18 °C–29 °C). Its deciduous forests support high densities of ungulate prey species (~35.2 animals/km^2), and consequently, a large tiger population (Karanth et al., 2004b). Additionally, long-term photographic CR monitoring in this site provides independent, robust estimates of tiger abundance, allowing us to specifically investigate the possible trade-offs between the individual identification accuracy and CR.

Our objectives were: (1) develop and validate a genetic identification protocol for tigers using known distinct individuals (2) employ field survey protocols explicitly designed for robust capture–recapture analyses under various plausible models (Williams et al., 2002; Karanth et al., 2004a, 2006) (3) evaluate fecal DNA-based tiger abundance estimates in comparison to standard photographic capture–recapture methods, and, (4) explore methodological trade-offs between the twin objectives of attaining greater certainty in individual identifications by using more loci versus achieving higher capture and recapture rates for subsequent analyses. In addition to estimation of tiger numbers, we believe our results have wider relevance for non-invasive, fecal DNA based population assessments in many other scarce, low density, elusive, wide-ranging animal species.

2. Materials and methods

2.1. Field surveys for collection of scats

The study area of 671 km^2, located within Bandipur contained 18 'search routes' spatially distributed to ensure that the scats of each individual tiger within the area had probability of being detected. This was ensured by embedding the surveyed area within a camera trap array area that used in earlier photographic capture–recapture studies (Karanth et al., 2004a,b) designed to avoid large 'holes' in which an individual tiger had no exposure to detection (Fig. 1). These surveys followed dirt roads and trails that are known to be used as regular travel routes and regularly marked by scat deposits by tigers (Smith et al., 1989; Karanth and Sunquist, 2000). Each route was sampled by a team of two trained trackers at seven day intervals. Thus the entire study area was surveyed over six successive days (followed by a day of rest) by three teams, which covered a distance of 235.4 km, collecting 'fresh' tiger scats judged to be less than 7-days-old based on physical appearance and moisture content (Andheria, 2006). The survey was repeated over six consecutive weeks in the same order to ensure that each 'search route' was visited regularly once a week. The survey yielded a total of 63 samples.

In terms of capture-history data structure (Otis et al., 1978; Williams et al., 2002), tiger scats collected during days 1–6 of the survey were assigned to the first 'sampling occasion', days 8–13 to the second sampling occasion, and so on, to attain a total of six sampling occasions over 42 days. Thus our survey met two key requirements of standard capture–recapture analyses: (1) full spatial coverage of the area in each sampling occasion ensuring exposure of all individual tigers present to potential 'captures', and, (2) a sufficiently short survey period that reasonably met the assumption of population closure (Karanth and Nichols, 1998).

2.2. Validation and development of laboratory protocols

Blood samples were collected from 14 captive tigers from three different zoos in southern India. These animals included close relatives such as siblings, and were used for standardization of the final set of loci used for individual identification. Additionally, scats were collected from 13 separately housed tigers to test the effects of different sources of DNA (i.e. blood, or fresh scats <12 h old) on the amplification process and associated error estimates.

To test the efficiency of the microsatellite loci from scats of wild tigers, we collected 10 fresh scats deposited the same night, but sufficiently far apart within the Bandipur–Nagarahole landscape, allowing us to assume reasonably that they came from different individuals within the same genetic population. These scats were used to standardize protocols but not in the abundance estimation. These field scat samples were carefully collected to avoid contamination and preserved in absolute alcohol for DNA extraction. All samples were stored in ethanol as opposed to other storage mediums (e.g silica gel) following recommendations in Murphy et al., 2002.

2.3. DNA extraction and species identification

DNA was extracted in duplicate from all scat samples using commercially available QIAamp DNA Stool mini kit (QIAGEN Inc.) following the manufacturer's instructions with slight modifications. Around 180–200 mg of sample from the outer parts of the faeces was used for each extraction. The first overnight incubation