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Full Length Research Paper

Noninvasive genetic assessment of population status of tigers (*Panthera tigris tigris*) in Buxa Tiger Reserve, West Bengal, India

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Population size estimation is a prerequisite for assessment of conservation status of a species in its natural habitat. With a global scenario of vanishing local population of tigers in many parts of its range distribution, estimation of the minimum number of individuals is vital for prioritizing conservation attention to specific areas with long term possibility of survival of the species. In the present study, DNA based techniques were used to identify species, individuals and sex from 72 faecal samples collected from Buxa Tiger Reserve of West Bengal. Present study confirms the presence of 15 individual tigers with 3 male, 9 female and 3 with unknown sex identity. Genetic capture recapture estimate based on single session sampling using CAPWIRE yields population size of 25 (95% CI 19 to 31) following the even capture probability model. Results obtained from the present study indicate possible movement of tigers from contiguous forest patches of Bhutan to the north and Manas Tiger Reserve on the east of Buxa. The present study shows that noninvasive genetics can be an effective tool in monitoring elusive species such as tiger, especially in areas of low population density.

Key words: *Panthera tigris tigris*, noninvasive genetics, microsatellite, population estimation, CAPWIRE, Buxa Tiger Reserve.

INTRODUCTION

The Royal Bengal tiger (*Panthera tigris tigris*) is listed in the Schedule I species of the Indian Wildlife Protection Act, 1972, also categorized as Endangered by IUCN Red List (IUCN, 2010). Of the current global estimate of 3000 to 3500 tigers (Sanderson et al., 2006), Indian subcontinent harbours nearly 60% of the world population (Jhala et al., 2008). Population monitoring of tigers are difficult to conduct because they are rare and roam over large distances and remote areas (Schipper et al., 2008). Physical tagging methods for population estimation are not possible for tigers because of the logistic difficulties and low capture possibilities (Karanth and Nichols, 2002). Over a decade, remotely triggered camera based photographic capture recapture has been in for determining population size of tiger (Karanth, 1995; Karanth and Nichols, 1998; Jhala et al., 2008). However, photographic methods have disadvantages, such as requirement of large number of cameras and trained manpower, low capture probabilities in low density areas, risks theft and animal damage to the cameras.

Studies have demonstrated the feasibility of using the non-invasive genetic technique for counting tiger individuals through genetic identification of individuals from field collected faecal samples of wild tigers in India (Bhagavatula and Singh, 2006) and for species identification (Mukherjee et al., 2007) and sex identification (Sugi-

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moto et al., 2006). The process of obtaining genetic data from natural populations has been enhanced by the development of noninvasive genetic techniques in past two decades (Hoss et al., 1992; Kohn and Wayne, 1997; Goossens et al., 1998; Fernando et al., 2003) and has been increasingly applied to a wide variety of species (Taberlet et al., 1997; Kohn et al., 1999; Garnier et al., 2001; Eggert et al., 2003; Goossens et al., 2003; Vidya and Sukumar, 2005; Smith et al., 2006). Capture-recapture based genetic population estimation of wild tigers has successfully been carried out in India (Mondol et al*.,* 2009), thus opening a new era of population monitoring of this species in the country. Genetic capture-recapture based on single session sampling has been carried out on tigers in India, with reports of overestimates following both the models of capture probability, possibly due to lower average recapture rate (Borthakur et al., 2010).

Buxa Tiger Reserve (BTR) with an area of 761 km^2 in the state of West Bengal represents part of the Terai ecosystem and lies in the Indo-Malayan biogeographical region, at the confluence of three major Biogeographic Zones, viz. Lower Gangetic plains, Central Himalayas and Brahmaputra Valley. This unique geographic location of BTR, along with seasonal flooding of plains creates a dynamic ecosystem allowing existence of a multiple floral and faunal regime (Mathur et al., 2011). BTR is considered as part of Buxa-Manas Tiger population, one of the three major tiger populations in northeast India described by Jhala et al. (2008). This Buxa-Manas population extends from BTR to the Manas Tiger Reserve (MTR) in the state of and Royal Manas National Park in Bhutan Assam in the east. To the north, BTR shares international boundary with Bhutan as a contiguous forest with Phipsu Wildlife Sanctuary in the other side of the boundary. Overall, BTR is part of a contiguous forest extent of 7 200 km^2 (Jhala et al., 2008). In the present study, carnivore faecal (scat) samples collected from BTR were analyzed in the laboratory to determine the species, individual and sex identity using genetic markers and to use the individual identity data for estimating population size of tigers in the area, following a single session sampling based capture recapture design.

MATERIALS AND METHODS

Buxa Forest Department undertook sampling in the study area for one month period between 11 February, 2010 to 11 March, 2010 and scat samples were sent to the laboratory. The shortened time of sampling allows better approximate demographic closure required for population estimation. Scat samples were kept in air tight plastic bags along with silica gel and Global Positioning System (GPS) coordinates were recorded during sample collection and their map locations established. In the laboratory, these scats were dried in a hot air oven and transferred to air tight plastic containers, following proper precautionary measures to prevent sample cross contamination. DNA extractions for all the scats collected were performed by using commercial Stool DNA isolation kit (QIAamp DNA Stool Kit, QIAGEN Ag., Germany) following minor modifications described by Borthakur et al. (2010). Extracted DNA were run on 1% agarose gel and visualized by ethidium bromide staining on an UV transilluminator.

Genetic markers developed by Mukherjee et al. (2007) were used for identification of genuine tiger scats collected from BTR, thus avoiding analysis of scats of other sympatric carnivores. Here, the species identity is based on the presence or absence of tiger specific mitochondrial Polymerase Chain Reaction (PCR) products of specific size, determined through agarose gel electrophoresis. PCR reactions were carried out using QIAGEN Multiplex PCR Kit (QIAGEN, Germany) following conditions described by Mukherjee et al. (2007) in reaction volume of 10 µL including 2.5 µL of scat DNA. Genuine tiger scat samples were sexed using primers to amplify the Y chromosome linked SRY (sex determining region) loci as demonstrated in the domestic cat individualization panel, MEOWPLEX (Butler, 2002; Butler et al., 2002). This SRY primer pair gives a single PCR product of 99 base pairs in the male and no amplification in the female. The efficacy of these primers in sex identification of tigers has already been tested elsewhere (Borthakur et al., 2010).

Microsatellite loci screened by Borthakur et al. (2010) were taken into consideration in selecting a panel of polymorphic loci for individual identification of tigers from scat samples. However, changes were made to the final panel of polymorphic loci selected by to Borthakur et al. (2010), on the basis of a pre-screening of ten randomly selected fresh tiger scat samples from the study area. The selected panel of loci were screened further on five wild collected reference tissue samples to demonstrate their reliability in individual identification. All the PCR were carried out in two sets of multiplex of 10 µL reaction, each locus labelled with a separate fluorescent label. Multiplexing was carried out using QIAGEN Multiplex PCR Kit (QIAGEN, Germany) following standard kit protocols for reagent concentration with 0.2 µM of each primer and 2.5 µL template DNA in a 10 µL PCR reaction. The thermal cycling was performed with 95°C initial denaturation/activation of 15 min, followed by 40 cycles of 94°C for 45 s, 54°C for 45 s and 72°C for 45 s followed by a 72°C for 15 min final extension step. Each sample and loci were typed at three or more replicates depending on the genotype discrepancy (Navidi et al., 1992) and consensus genotypes were created from these repeat results. Per locus and per multilocus genotype error rates were estimated as a measure of the reliability of the genotypes obtained, which is important in genetic individual identification (Bellemain, 2004; Pompanon et al., 2005). Standard laboratory precautions mentioned by Borthakur et al. (2010) were followed, in order to minimize genotyping errors.

Allele sizing was carried out by using automated allele-calling software (GENEMAPPER v3.7, Applied Biosystems, USA) and later through visual inspection of each allele per locus per sample, in order to detect novel alleles outside of the expected range of a locus, stochastic amplifications within the size range, and potential mistypes due to stutter or large-allele dropout (Pompanon et al., 2005; Dewoody et al., 2006). Program GIMLET v 1.3.3 (Valiere, 2002) was used to generate consensus genotypes and to estimate genotyping error rates. Quality index value was assigned to each genotype as per Miquel et al. (2006), in order to select a final set of samples for individual identification. For the purpose of selection of samples for final data analysis, quality index of 0.667 was kept as the cut-off value.

From the microsatellite genotype data, allele frequency, observed and expected heterozygosity and probability of identity (P_{ID}) and Probability of identity among siblings (P_{ID}-sibs) were calculated using the software GIMLET v 1.3.3 (Valiere, 2002). Tests for linkage disequilibrium and Hardy-Weinberg equilibrium were performed using the software Arlequin V3.0 (Excofier et al., 2005). The unique multilocus microsatellite genotypes, that is, individual tigers were identified using the identity analysis module of the program CERVUS (Marshall et al*.,* 1998). Here, incomplete multilocus genotypes were also considered, that is, samples with identical genotypes for at least five common loci are considered as same individual.

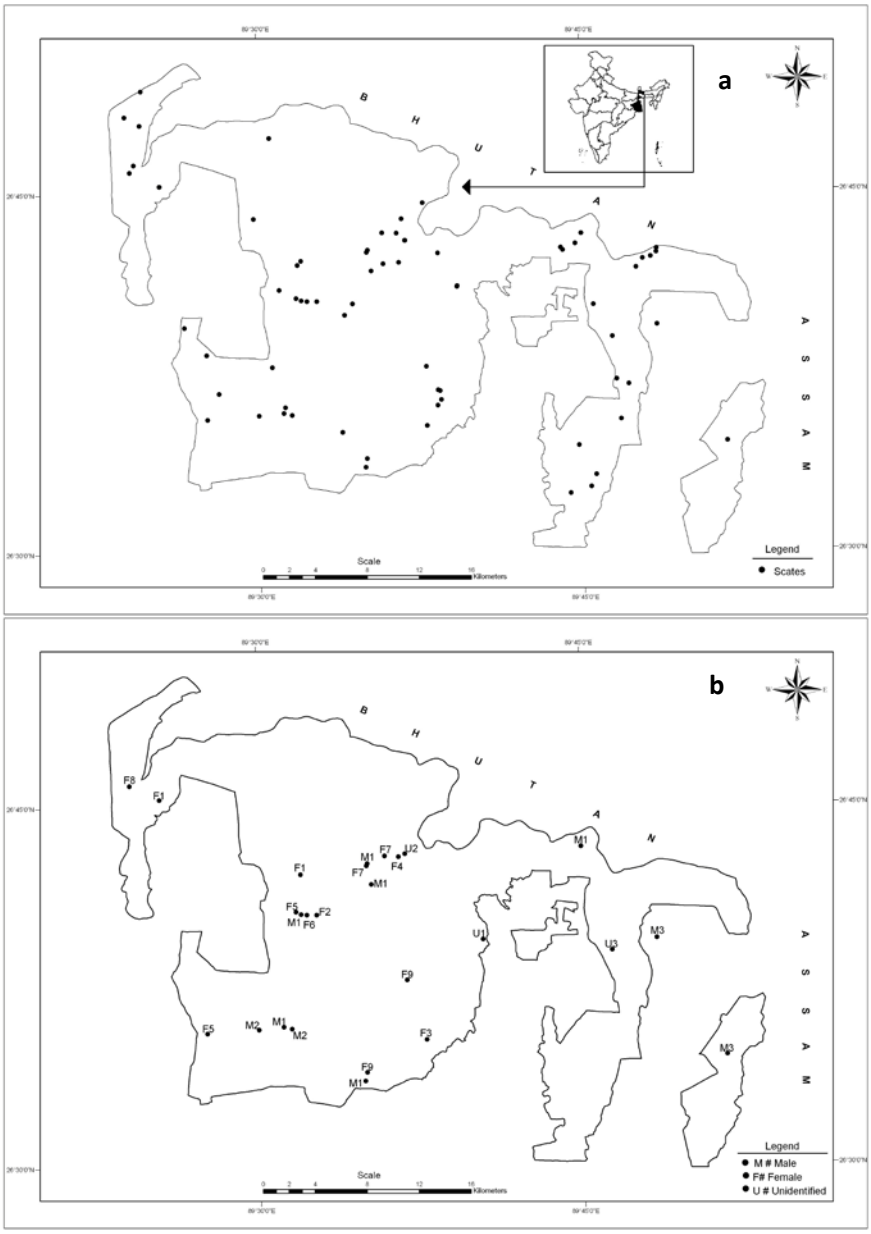

Figure 1. Map showing a, locations of Buxa Tiger Reserve in India and distribution of 72 scats collected in the area; b, sample locations of 15 genetically identified individual tigers.

Programme CAPWIRE (Miller et al., 2005) was used to estimate population size in single session sampling scheme. Likelihood Ratio Test (LRT) incorporated in CAPWIRE was used to select one of the two models of capture probability. The even capture model (ECM) assumes there is no capture heterogeneity in the data set while the two innate rates model (TIRM) assigns individuals as having either a high or a low capture probability. Population estimate was derived using the selected model with 95% confidence intervals with parametric bootstrap of 10 000 replicates. The samples collected at the same time and location (coordinates) were considered as a single observation, as adopted by Zhan et al. (2006). Samples of Individuals with missing coordinates in this study were not included in the final capture-recapture analysis.

RESULTS

A total of 72 scat samples were collected (Figure 1a) and sent to the laboratory. DNA extractions were performed on all the 72 scat samples. Of the total scats, 38 could be confirmed as tiger scats through genetic analysis. Twenty six of the samples were confirmed as non-tiger origin, whereas 8 samples failed to produce any results.

A total of 6 polymorphic loci were used in the present study (Table 1) out of which five were used previously by Borthakur et al. (2010). Locus Fca441, HDZ700 and F53

Table 1. Results of 6 polymorphic loci used in individual identification from 27 tiger scat samples

Locus Name	Number of alleles	Allele Range	% PCR success	ADO	FA	H_{exp}	H_{obs}
Fca628	6	91-125	100	0.038	0	0.71	0.67
HDZ170	7	202-230	96	0.022	0	0.54	0.41
Pati09	5	111-129	100	0	0	0.62	0.56
Ple51	6	163-175	92	0	0	0.68	0.15
Fca304	7	126-198	73	0.04	0	0.68	0.19
Ple55	6	149-165	92	0	0	0.8	0.81

were not used here due to high allele dropout in the study population, whereas Locus Fca628 screened by Borthakur et al. (2010) on test scat samples was used in the present study. Figure 2 shows a graphical representation of product P_{ID} and P_{ID}-sibs value of six selected loci on five reference tissue samples and 10 randomly selected fresh scat samples. Product P_{ID} value of 4.7×10^{-5} and P_{ID}-sibs value of 1.4×10^{-2} were obtained for six selected loci in tiger reference samples, which denote high resolving power of this set of markers in individual identification of tigers. Loci Fca628, Fca304, HDZ170, Ple51 and Ple55 showed significant deviation from Hardy-Weinberg Equilibrium, with no Linkage disequilibrium.

As per quality criteria mentioned in the methodology, genotype data for 27 tiger scat samples were selected for identification of unique multilocus genotypes and determining the number of individual tigers. These 27 samples retained ten randomly selected fresh scats from the study population used for loci selection. The number of alleles, allele size range, percentage PCR success rate, ADO and FA, H_{exp} and H_{obs} in 27 tiger scat samples from BTR are presented in Table 1. The mean observed heterozygosity of the six loci in 27 tiger scat samples is found to be 0.47. Calculate genotyping error rate was 1.7% for the 27 final scat samples. Cumulative P_{ID} value of 8.21×10^{-6} and P_{ID}-sibs value of 8.32×10^{-3} were obtained for six loci in 27 scat samples. Fifteen unique multilocus genotypes were obtained based on available genotype data with at least 5 or more loci. Locations of scats of all 15 individual tigers are shown in Figure 1b. Sex identification data was incorporated for 24 genotyped scats and different samples from the same individual tiger shared the same sex identity, thus confirming further the individual identity of matching samples. However, for three samples, sex identity could not be confirmed. These 3 samples were single representatives of 3 individual tigers, thus sex identity for these individuals could not be ascertained. In total, identity of 3 male and 9 female tigers could be confirmed in the dataset, with 3 individuals of unknown sex. The population size estimate obtained by capture-recapture analysis in CAPWIRE was 25 (95% CI 19 to 31) with ECM model. The average observation per individual in this analysis was 1.8.

DISCUSSION

The present study uses six highly polymorphic microsatellites with 5 to 7 alleles per locus, from a pool of loci suggested by Borthakur et al. (2010). The P_{ID} and P_{ID}-sibs values for these 6 loci were considerably low in randomly selected tiger scats, as well as, reference tissue samples of wild origin, which is comparable to the values obtained previously by Bhagavatula and Singh (2006), Mondol et al. (2009) and Borthakur et al. (2010). The approach of assigning quality index as per Miquel et al. (2006) to genotype data obtained from carnivore scats were first being used by Mondol et al. (2009) and later by Borthakur et al. (2010). In this study, 71% of the genetically identified tiger scats were retained for further analysis based on quality index cut off value of 0.667, as followed by Borthakur et al. (2010). A high mean PCR success rate of 92% from the tiger scat samples was obtained in this study, which is comparable to 91.5% obtained by Borthakur et al. (2010) and higher compared to 85% obtained by Mondol et al. (2009) and 60% obtained by Bhagavatula and Singh (2006). The genotyping error rate observed in this study was below 2%, which is lower compared to 3.4% obtained by Borthakur et al. (2010) using 8 loci and 3.3% by Bhagavatula and Singh (2006) using 6 loci.

The results of the present study confirming the presence of a minimum of 15 individual tigers are of high importance, in view of the lack of information on tiger population status in BTR. Prior to this study, survey undertaken by Jhala et al. (2008) in 596 km^2 of tiger occupancy in Northern West Bengal comprising of Buxa Tiger Reserve, Jaldapara Wildlife Sanctuary and Gorumara National Park estimated 10 individuals. However, results of the present study cannot directly be compared to that of Jhala et al. (2008), with differences in sampling approach and coverage area. BTR is a part of a contiguous forest extent of 7 200 km^2, which has estimated tiger occupancy of 1051 km^2, along with MTR of Assam in the East (Jhala et al., 2008). According to Jhala et al. (2008) the source population of Royal Manas in Bhutan is maintaining tiger occupancy in BTR. In the present study, a sample of one male tiger individual M3

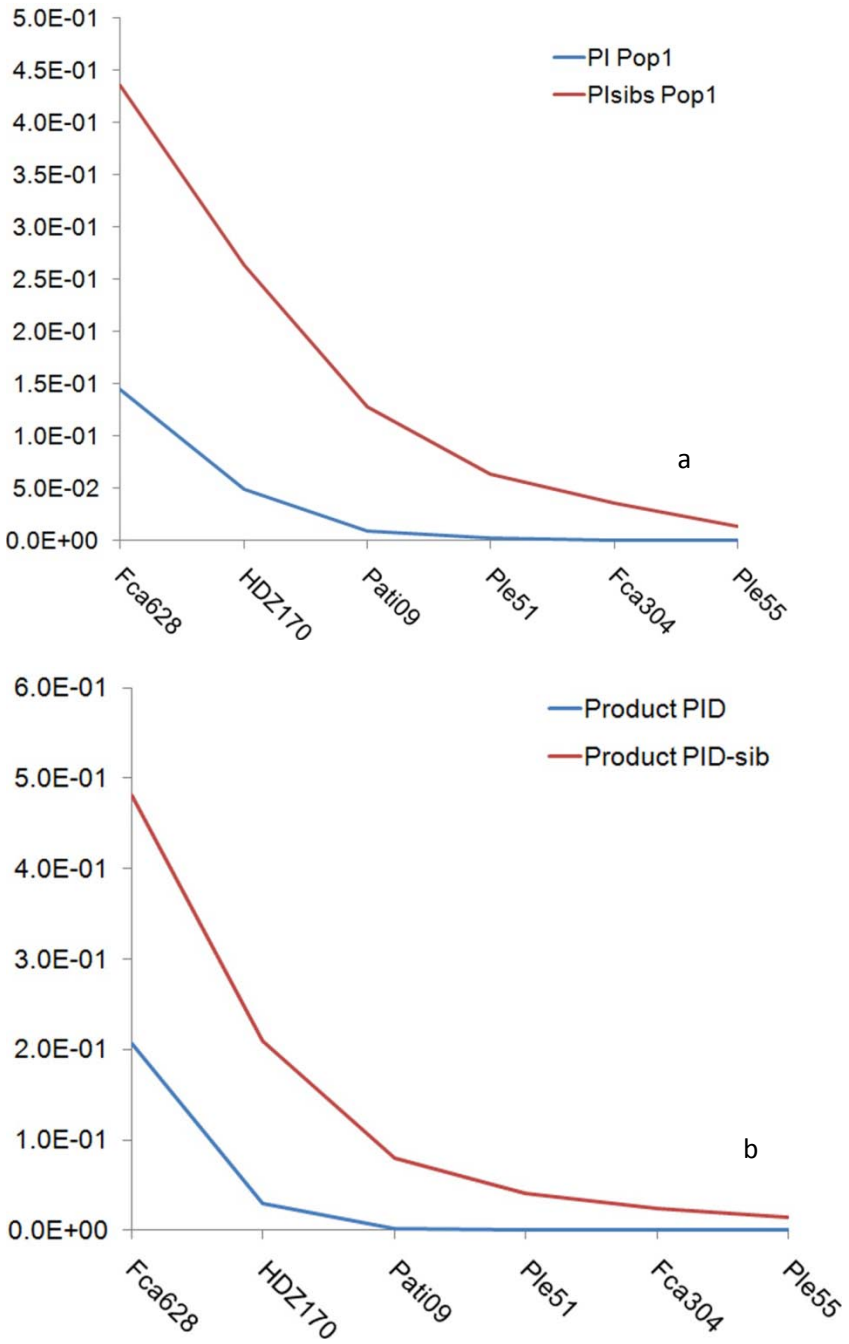

Figure 2. Graphical representation of product P_{ID} and P_{ID}-sibs value of six selected loci on a, 10 randomly selected fresh scat samples;b five reference tissue samples.

was found at the bordering areas of BTR and MTR. Thus there is a possibility of movement of tigers between BTR and MTR, which needs to be investigated further.

The present study gives concrete evidence of the presence of tigers in BTR, which has been questioned recently in the context of lack of sighting information fromthe area. Multiyear assessment of population status and connectivity of BTR along with the contiguous forest extents of MTR in Assam and Royal Manas in Bhutan is required, in order to formulate any long term conservation strategy for tigers in the region.

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REFERENCES

Bellemain E (2004). Genetics of the Scandinavian Brown Bear (Ursus arctos): implications for biology and conservation. PhD thesis submitted to Agricultural University of Norway, Norway and Université Joseph Fourier, France.

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

Borthakur U, Barman RD, Das C, Basumatary A, Talukdar A, Ahmed MF, Talukdar BK, Bharali R (2010). Noninvasive genetic monitoring of tiger (*Panthera tigris tigris*) population of Orang National Park in the Brahmaputra floodplain, Assam, India. European J. Wildlife Res. DOI: 10.1007/s10344-010-0471-0.

Butler JM (2002). Information on the Cat STR Multiplex Developed at NIST and a Protocol for its Use on the ABI 310 and ABI 3100 Instruments. A Technical Report submitted to the National Institute of Justice, USA.

Butler JM, David VA, O'Brien SJ, Menotti-Raymond M (2002). The MeowPlex: A New DNA Test using Tetranucleotide STR Markers for the Domestic Cat. In Profiles in DNA, Promega Corporation, September 2002, Pp 7-10.

Dewoody J, Nason JD, Hipkins VD (2006). Mitigating scoring errors in microsatellite data from wild populations. Mol. Ecol. Notes, 6:951-957.

Eggert LS, Eggert JA, Woodruff DS (2003). Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. Mol. Ecol. 12: 1389-1402.

Excoffier L, Estoup A, Cornuet JM (2005). Bayesian analysis of an admixture model with mutations and arbitrarily linked markers. Genetics, 169: 1727-1738.

Fernando P, Vidya TNC, Rajapakse C, Dangolla A, Melnick DJ (2003). Reliable noninvasive genotyping: fantasy or reality? J. Hered. 94(2): 115-123.

Garnier JN, Bruford MW, Goossens B (2001). Mating system and reproductive skew in black rhinoceros. Mol. Ecol., 10: 2031-2041.

Goossens B, Waits LP, Taberlet P (1998). Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. Mol. Ecol. 7: 1237-1241.

Goossens B, Anthony N, Jeffery K, Johnson-Bawe M, Bruford MW (2003). Collection, storage and analysis of non-invasive genetic material in primate biology. In: Setchell JM, Curtis DJ (eds) Field and laboratory methods in primatology, a practical guide. Cambridge University Press, Cambridge, Pp 295-308.

Hoss M, Kohn M, Pääbo S, Knauer F, Schröder W (1992). Excrement analysis by PCR. Nature, 359 (6392): 199.

IUCN (2010). IUCN Red List of Threatened Species. Version 2010.4. www.iucnredlist.org.

Jhala YV, Gopal R, Qureshi Q (eds.) (2008). Status of the Tigers, Co-predators, and Prey in India. National Tiger Conservation Authority, Govt. of India, New Delhi, and Wildlife Institute of India, Dehradun. TR 08/001, Pp 151.

Karanth KU (1995). Estimating tiger *Panthera tigris* populations from camera-trap data using capture recapture models. Biol. Conserv. 71: 333-338.

Karanth KU, Nichols JD (1998). Estimating tiger densities in India from camera trap data using photographic captures and recaptures. Ecology, 79: 2852-2862

Karanth KU, Nichols JD (2002). Monitoring Tigers and their Prey: A manual for researchers, managers and conservationists in Tropical Asia. Centre for Wildlife Studies, Bangalore, India.

Kohn M, Wayne RK (1997). Facts from faeces revisited. Tree, 12: 223-227.

Kohn MH, Eric CY, Denise AK, Gary H, Raymond MS, Robert KW (1999) Estimating population size by genotyping faeces. Proceedings of the Royal Society of London Series B. Biol. Sci. 266: 657-663

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998). Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7: 639- 655.

Mathur VB, Gopal R, Yadav SP, Singa PR (2011). Management Effectiveness Evaluation (MEE) of Tiger Reserves in India: Process and Outcomes. National Tiger Conservation Authority, Government of India, Pp 97 http://projecttiger.nic.in/.

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: 1991-2005.

Miquel C, Bellemain E, Poillot C, Bessiere J, Durand A, Taberlet P (2006). Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. Mol. Ecol. Notes., 6: 985 –988.

Mondol S, Karanth KU, Kumar NS, Gopalaswamy AM, Andheris A, Ramakrishnan U (2009). Evaluation of non-invasive genetic sampling methods for estimating tiger population size. Biol. Conserv., 242, 10: 2350-2360.

Mukherjee N, Mondol S, Andheria A, Ramakrishnan U (2007). Rapid multiplex PCR based identification of wild tigers using non-invasive samples. Conserv. Genet., 8: 1465-1470.

Navidi W, Arnheim N, Waterman MS (1992). A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. American J. Hum. Genet. 50: 347-359.

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005). Genotyping Errors: causes, consequences and solutions. Nat. Rev. Genet., 6: 847-859

Sanderson E, Forrest J, Loucks C et al. (2006). Setting Priorities for the Conservation and Recovery of Wild Tigers: 2005–2015, The Technical Assessment. WCS, WWF, Smithsonian and NFWF-STF, New York-Washington DC, USA.

Smith DA, Ralls K, Hurt A, Adams B, Parker M, Maldonado JE (2006). Assessing reliability of microsatellite genotypes from kit fox faecal samples using genetic and GIS analyses. Mol. Ecol. 15: 387-406.

Sugimoto T, Nagata J, Aramilev VV, Belozor A, Higashi S and McCullogh D (2006). Species and sex identification from faecal samples of sympatric carnivores, Amur leopard and Siberian tiger, in the Russian Far East. Conserv. Genet. 7: 799-802.

Taberlet P, Camarra JJ, Griffin S et al (1997). Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Mol. Ecol. 6: 869-876.

Valiere N (2002). GIMLET: A computer program for analyzing genetic individual identification data. Mol. Ecol. Notes, 10.1046/j.1471-8278 .2002.00228.x.

Vidya TNC, Sukumar R (2005). Amplification success and feasibility of using microsatellite loci amplified from dung to population genetic studies of the Asian elephant (*Elephas maximus*). Curr. Sci., 88(3): 489-492.

Zhan X, Li M, Zhang Z, Goossens B, Chen Y, Wang H, Bruford MW, Wei F (2006). Molecular censusing doubles giant panda population estimate in a key nature reserve. Curr. Biol. 16: 451–452.