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ORIGINAL ARTICLE

Genetic structure of the Amur tiger (P*anthera tigris altaica*) population: Are tigers in Sikhote-Alin and southwest Primorye truly isolated?

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Abstract

We used molecular genetic analyses to noninvasively identify individual Amur tigers and define subpopulations of tigers in the Russian Far East. We identified 63 individuals after genotyping 256 feces, 7 hair and 11 blood samples collected within southern, central and northern Sikhote-Alin, as well as Southwest Primorye. Analysis of nuclear DNA at 9 microsatellite loci demonstrated greater genetic similarity between animals from southern and northern Sikhote-Alin (some 500 km apart) than between animals from Ussuriskii State Nature Reserve and Southwest Primorye (less than 10 km apart at their nearest point), suggesting that a true barrier exists preventing movements of tigers between Southwest Primorye and the southern Sikhote-Alin Mountains.

Key words: Amur tiger, fecal DNA, noninvasive genetic sampling, *Panthera tigris altaica*

INTRODUCTION

The current range of Amur tigers (*Panthera tigris altaica* Timminck, 1884) includes the Sikhote-Alin Mountain Range from the cites of Vladivostok and Ussuriisk (southern Primorskii Krai) to the Amur River of Khabarovskii Krai in the north (Matyushkin *et al*. 1996). There were an estimated 428–502 tigers in this region in 2005 (Miquelle *et al*. 2007). Henry *et al.* (2009) suggest that a small group of 10–20 animals in southwest Primorye and in the adjacent province of Jilin in China (Miquelle & Pikunov 2003; Sugimoto *et al*. 2012) are genetically differentiable from the larger Sikhote-Alin population. The territory from southern Primorskii Krai (including Ussuriskii State Nature Reserve) to Khabarovsk is a nearly continuous habitat (Hebblewhite *et al.* 2014) referred to as the Sikhote-Alin Mountain ecosystem, whereas southwest Primorye appears to have been separated from the Sikhote-Alin Mountains by human development in the Razdolnaya River Basin over the past 25 years (Henry *et al.* 2009; Miquelle *et al.* 2015). This conclusion seems at odds with the well-known capacity of Amur tigers to disperse long distances (Heptner and Sludskii 1992; Hernandez-Blanco *et al*. 2015; Wang *et al*. 2015). Hence, we sought to test the conclusions of Henry *et al*. (2009) that tigers from southwest Primorye represent a subpopulation isolated from the Sikhote-Alin Mountains (including Ussuriskii State Nature Reserve, Udegeyskaya

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Legenda National Park and Khabarovskii Krai), and whether there truly is genetic isolation between tigers in Southwest Primorye and the nearby Ussuriskii State Nature Reserve. Non-invasive molecular genetic analysis has become a useful tool in studies of wildlife populations because samples (feces, urine and hair) can be easily collected (Ernest *et al.* 2003; Fernando *et al*. 2003; Perez *et al.* 2006; Murphy *et al.* 2007) and combined with tissue or blood samples collected during capture or handling of animals, along with museum specimens (Kholodova *et al*. 2005; Sorokin *et al.* 2005; Sorokin *et al*. 2009).

Recent developments in molecular technology have considerably extended the capacity of non-invasive samples as a source of DNA for identification of not only species but also individuals and sex. This development greatly improves the reliability of abundance assessments, can provide additional insights into many ecological and behavioral parameters (Piggott & Taylor 2003), and can precisely define relationships within family groups (Rozhnov *et al*. 2009), as well as identify animals and their derivatives for forensic purposes (Coomber *et al.* 2007). However, non-invasive methods do have some drawbacks: they are labor-intensive and expensive, and errors can occur due to inadequate DNA preservation.

The goal of the present study was to use microsatellite genotypic data to identify individual tigers in the Russian Far East and to test for genetic isolation of animals from different localities across their range. To achieve this goal, we: (i) identified individual tigers (via "fingerprinting") in the study area; and (ii) analyzed the genetic structure and the genetic diversity of tigers from different regions in the Russian Far East.

Figure 1 Distribution map of wild Amur tiger subpopulations, including locations where samples were collected for this study (black dots). *N* = number of individuals.

MATERIAL AND METHODS

We conducted molecular genetic analysis of DNA isolated from 256 samples of feces, 7 samples of hair, and 11 blood samples collected in winter from tigers from 4 parts of their range in the Russian Far East: Ussuriskii State Nature Reserve in the southern Sikhote-Alin Mountains (60 samples), Udegeyskaya Legenda National Park in the central Sikhote-Alin Mountains (5 samples), Khabarovskii Krai in the northern Sikhote-Alin Mountains (70 samples), and southwest Primorye (139 samples) (Fig. 1). Feces were collected frozen during winter surveys in 2009–2013. Blood samples were taken from 11 animals captured and tagged with GPS collars. Hair samples were collected from barbed wire near the international boundary between Russia and China. Samples of feces and hair were preserved in 96% ethanol. Blood samples were collected in tubes with K_3EDTA. We used reagents of the kit Diatom DNA Prep 200 (Izogen, Russia) for isolation of DNA from blood and QIAamp DNA Mini Kit for isolation of DNA from hair, and QIAamp Stool Mini Kit (Qiagen, United States) for isolation of DNA from feces. We tested 6 microsatellite loci with primers E7, Fca304, Fca43, E6, E21 and D10, previously used for Bengal tigers (Bhagavatula & Singh 2006; Rozhnov *et al.* 2009), and 7 polymorphic loci with primers hdz57, hdz64, hdz463, hdz700, hdz859, hdz993 and hdz481, used for Amur tigers in the Russian Far East (Henry *et al*. 2009). We also tested 5 loci with primers fca5, fca161, fca91, fca304 and fca211, which are considered useful for subspecies recognition (Luo *et al.* 2008), and 8 loci with

primers designed for snow leopard (pun229, pun124, pun935, pun1157, pun894, pun132, pun272 and pun834) (Karmacharya *et al*. 2011; Rozhnov *et al.* 2011). The following 8 loci had only 1 allele for tested samples (tested samples, $n = 8$): fca211 (Menotti-Raymond *et al.* 1999); hdz57, hdz64, hdz463, hdz700, hdz859, hdz993 and hdz481 (Williamson *et al*. 2002). All primers for snow leopard except pun935 did not work with Amur tiger samples. Loci with primers E6 and D10 had only 2 alleles (tested samples $n = 32$). Consequently, we used 9 microsatellite loci with primers e7, e21b (Bhagavatula & Singh 2006); fca304, fca43, fca5, fca161, fca91, fca441 (Menotti-Raymond *et al.* 1999) and pun935 for analysis of nuclear DNA. We also used zinc-finger genes with fluorescent dye as sex primers (Pilgrim *et al.* 2005).We performed polymerase chain reaction (PCR) with Bio-Rad Tetrad 2 Thermal Cycler (Bio-Rad, USA) in 10-μL volumes with final concentration: 0.05 mM of each dNTP, 2.5 mM of $MgCl_2$, 0.5 picomoles of the forward and reverse primers, 1 unit of Hot Start Taq DNA polymerase (SibEnzyme, Russia), 1× PCR buffer and 1.0 μL of DNA extract. Parameters for the PCR were: 1 cycle of 93 °C for 3 min, 10 cycles of 94 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s, 20 cycles of 89 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s, and 1 cycle of 72 °C for 30 min. The size of microsatellite fragments were estimated on an ABI 3130 genetic analyzer (Applied Biosystems, USA) with the addition of the Liz 500 size standard and the Gene Mapper v 4.0 program (Applied Biosystems). PCR with all primers was performed 4 times. The consensus genotype was accepted if it was found at least twice for a heterozygote and 3 times for a homozygote. The individual recognition and the probability of identity for unrelated animals (P_{id}) and for siblings ($P_{id\text{-}sib}$) (Waits *et al.* 2001) were calculated using the program GeneCap (Wilberg & Dreher 2004). We used analysis of molecular variance and pairwise genetic distances (R_{st} values) to analyze population genetic structure. Molecular variability for microsatellite loci was assessed with the software Arlequin 3.5.2. (Excoffier & Lischer 2010). To test for genetic isolation of animals from the 4 localities within tiger range, we used the model-based clustering method as implemented in the program Structure 2.3.1. (Pritchard *et al.* 2000). Run parameters included 1 million iterations following a burn-in period of 100 000 using correlated allele frequencies under a straight admixture model and Locprior model, which is optimal for analysis of data with a small number of loci and limited sample size (Hubisz *et al.* 2009). The most appropriate K value was obtained based on the method described by Evanno *et al*. (2005) by varying the number of clusters K from 1 to 10 with 4 iterations per value of K. We carried out Hardy–Weinberg equilibrium and null allele frequency tests using the software package CERVUS 3.0.3 (Kalinowski *et al*. 2007). We used the program Gimlet 1.3.3 for calculating false and dropout allele rates (Valière 2002).

RESULTS

High-quality samples of DNA suitable for individual identification were successfully extracted from 163 out of 256 samples of feces (64%), and all blood and hair samples. Multilocus genotypes at 6 to 9 loci were successfully determined in 152 tiger samples (Table 1). In total, 63 individual tigers were identified, including 29 individuals (11 males, 18 females) from Khabarovskii Krai, 19 individuals (9 males, 10 females) from Southwest Primorye, 3 individuals (2 males, 1 females) from Udegeyskaya Legenda National Park and 12 individuals (8 males, 4 females) from Ussuriskii State Nature Reserve. Data on frequencies of 9 microsatellite loci were obtained (Table 2). The resultant probability of identity for unrelated individuals (P_{id}) and siblings ($P_{id\text{-}sib}$) was 1.14×10^{-6} and 2×10^{-3}, respectively. Given the population size in the study area, we determined this probability of identity sufficient to accurately identify individuals. The mean percent of dropout alleles for each locus ranged from 0.010 to

Table 1 Frequency of false allele (FA) and dropout allele (DA) among samples, and proportion of samples without dropout loci, with 1 dropout locus, with 2 dropout loci and with 3 dropout loci

	FA among samples	DA among samples	0 dropout locus	1 dropout locus	2 dropout loci	3 dropout loci
Uss-Kha	0.020	0.067	0.63	0.28	0.06	0.03
Sw Pri	0.016	0.065	0.60	0.21	0.13	0.06

Sw Pri, southwest Primorye subpopulation; Uss-Kha, Ussuriskii State Nature Reserve and Khabarovskii Krai.

Table 2 Allele frequency distributions (% for 9 microsatellite loci) in 2 tiger sub-populations (Southwest Primorye and Sikhote-Alin Mountains) in the Russian Far East (Allele sizes are noted in base pairs

Locus	Alleles	Uss-Kha (Sikhote-Alin)	Sw Pri (SW Prim)
e7	150	6.10	2.78
	152	90.24	63.89
	156	3.66	33.33
fca304	128	33.33	42.11
	134	34.52	23.68
	136	32.14	34.21
fca43	117		15.79
	119	11.36	2.63
	123	59.09	42.11
	127	29.55	39.47
e21b	160	63.95	69.44
	162	13.95	2.78
	164	22.09	27.78
pun935	102	62.50	15.79
	108	36.11	31.58
	120	1.39	2.63
	124		50.00
fca5	139	38.64	31.58
	141	36.36	42.11
	143	25.00	26.32
fca161	184	4.76	10.53
	186		21.05
	190	71.43	23.68
	192	23.81	44.74
fca91	134	2.56	11.76
	140	73.08	58.82
	144	24.36	29.41
fca441	144	15.85	2.63
	148	30.49	7.89
	152	32.93	31.58
	156	3.66	
	160	15.85	34.21
	164	1.22	23.68

Sw Pri, southwest Primorye subpopulation; Uss-Kha, Ussuriskii State Nature Reserve and Khabarovskii Krai.

0.232, and the average allelic dropout rates for Sikhote-Alin and Southwest Primorye subpopulations were 0.081 and 0.65, respectively (Table 3). Thus, 4 repeat amplifications can effectively minimize the errors. One locus (fca304) deviated from HWE expectations and 3 loci (fca304, e21b, fca161) had a high probability of null allele in the Southern Primorye population (Table 3), possibly due to PCR-related competitive amplification of 1 allele over another in heterozygotes for loci e21b and fca161 or family structure for loci fca304.

We tested all combinations of samples from the 4 regions to determine which samples were genetically closest to each other (Table 4). The most statistically significant result was obtained when the samples were combined into 2 subpopulations: the first including tigers from the Ussuriskii State Nature Reserve, Udegeyskaya Legenda National Park and Khabarovskii Krai (i.e. the Sikhote-Alin subpopulation), and the second including all tigers from Southwest Primorye. The level of genetic isolation was estimated as R_{st} = 0.219 ($P < 0.05$). This result was in agreement with clusters obtained by the software Structure 2.3.1. Analyses using different assumptions about the number of subpopulations (K) (from 1 to 10) produced a modal value of ΔK when K = 2. Four individuals geographically located in southwest Primorye had over 90% probabilities of belonging to the Sikhote-Alin subpopulation, suggesting that these individuals may have dispersed from Sikhote-Alin. There were no samples genetically associated with the Southwest Primorye cluster collected in the Sikhote-Alin Mountains (Fig. 2).

The average expected heterozygosity for the Sikhote-Alin subpopulation, H_e= 0.52 ± 0.06, was slightly less than for tigers from Southwest Primorye, H_e = 0.62 ± 0.03 (Table 5). The average number of alleles per locus was similar: 3.33 ± 1.00 versus 3.56 ± 0.73, respectively.

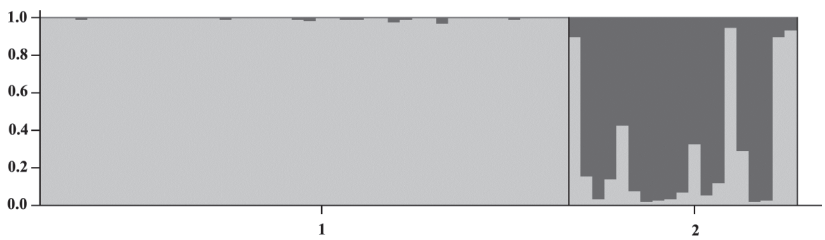

Figure 2 Genetic composition of 63 Amur tigers, expressed in terms of assignment probability to one of two populations, with population 1 including northern Sikhote-Alin (Khabarovskii Krai), central Sikhote-Alin (Udegeskaya Legenda National Park, and southern Sikhote-Alin (Ussuriskii State Nature Reserve), while population 2 includes only southwest Primorye.

Table 3 Results of tests for deviations from Hardy–Weinberg equilibrium with Bonferroni correction (HW), the mean percent of dropout allele (DA) and false allele (FA), and the null allele frequency estimate (NA) for 2 subpopulations at every studied locus

Locus	HW (Uss-Kha)	HW (Sw Pri)	DA (Uss-Kha)	DA (Sw Pri)	FA (Uss-Kha)	FA (Sw Pri)	NA (Uss-Kha)	NA (Sw Pri)
E7	ND	NS	0.159	0.147	0.070	0	−0.043	−0.027
Fca304	NS	S	0.138	0.232	0.028	0.026	−0.018	0.163
Fca43	NS	NS	0.074	0.083	0	0.075	−0.042	−0.036
E21b	NS	ND	0.057	0.047	0.069	0.012	−0.073	0.224
Pun935	NS	NS	0.068	0.058	0	0	−0.102	−0.072
Fca5	NS	NS	0.010	0.039	0	0	−0.05	−0.059
Fca161	NS	NS	0.111	0.055	0.009	0.015	0.016	0.095
Fca91	NS	NS	0.061	0.022	0.019	0.017	0.02	−0.116
Fca441	NS	NS	0.064	0.076	0	0.015	−0.126	−0.083
Total			0.081	0.065	0.015	0.016		

ND, not performed; NS, not significant; S, significant.

Table 4 The level of genetic isolation was estimated as the sum of squared size difference (R_{ST})

	Kha	ULNP	Uss	Sw Pri
Kha	0			
ULNP	0.052	0		
Uss	0.016	−0.062	0	
Sw Pri	0.166*	0.287*	0.253*	0

*P-value < 0.05. Kha, Khabarovskii Krai; Sw Pri, southwest Primorye; ULNP, Udegeyskaya Legenda National Park; Uss, Ussuriskii State Nature Reserve.

DISCUSSION

Using 9 microsatellite loci we were able to differentiate 63 tigers. Analyses suggested that genetic variability of the subpopulation from Southwest Primorye is similar to that of the main Sikhote-Alin subpopulation, but that these subpopulations are distinguishable. Despite extensive human development between Vladivostok and Ussuriisk (Miquelle *et al*. 2015), our data suggest that at least some tigers are moving between southwest Primorye and the main population in the Sikhote-Alin Mountains. These results

Table 5 Values of expected (H_e) and observed (H_o) heterozygosity, and the polymorphism information content (PIC) and probability of identity for siblings ($P_{id\text{-}sib}$) for 2 subpopulations at every studied locus

Locus	H_e (Uss-Kha)	H_e (Sw Pri)	H_o (Uss-Kha)	H_o (Sw Pri)	Pic (Uss-Kha)	Pic (Sw Pri)	$P_{id\text{-}sib}$ (Uss-Kha)	$P_{id\text{-}sib}$ (Sw Pri)
E7	0.18	0.49	0.19	0.5	0.17	0.39	0.815	0.6
Fca304	0.67	0.67	0.69	0.47	0.59	0.57	0.453	0.477
Fca43	0.56	0.66	0.59	0.68	0.48	0.57	0.523	0.476
E21b	0.53	0.45	0.60	0.28	0.46	0.36	0.542	0.627
Pun935	0.48	0.64	0.58	0.74	0.38	0.56	0.577	0.478
Fca5	0.66	0.67	0.73	0.74	0.58	0.58	0.457	0.471
Fca161	0.44	0.71	0.43	0.58	0.37	0.64	0.6	0.443
Fca91	0.41	0.57	0.38	0.71	0.34	0.48	0.619	0.552
Fca441	0.76	0.74	0.95	0.84	0.70	0.67	0.4	0.422
Total	0.52	0.62	0.57	0.61			0.004	0.002

support the findings of Henry *et al.* (2009) who report 3 potential migrants (2 individuals apparently moving from the Sikhote-Alin to southwest Primorye, and one in the opposite direction). Currently, it is still unclear whether full isolation occurred for the Southwest Primorye population, and when dispersal of individuals took place. Given that some movement is occurring, it is also unclear to what extent the Razdolnaya development corridor currently represents an obstacle for movement of tigers. On the one hand, our data indicate that some movement of tigers is occurring between the 2 populations, but allelic frequencies and their distribution for all but dispersing animals from southwest Primorye suggest that the genetic composition of this subpopulation differs from that of tigers from the Sikhote-Alin Mountains (Table 2). Most strikingly, tigers from Ussuriskii State Nature Reserve are more genetically similar to tigers some 500 km away in Khabarovskii Krai than they are to tigers in southwest Primorye, some 10 km away at its nearest point (Table 4). It is possible that differentiation derived from isolation of a small number of tigers in Southwest Primorye at some time in the past. Despite the fact that the level of genetic differentiation may be due to using a different set of microsatellite markers and sample composition, our estimate of genetic isolation (R_{st} = 0.219) is much lower than that of Henry *et al*. (2009) (R_{st} = 0.47).

Our results suggest that movement of tigers is still occurring between southwest Primorye and southern Sikhote-Alin, but the continuing development along the Razdolnaya River basin represents a threat to future exchange between these 2 populations. Moreover, the clear genetic differentiation between 2 subpopulations so geographically close to each, in stark contrast to tigers scattered across the much larger Sikhote-Alin subpopulation, is a strong indicator of the difficulty of movement between these 2 subpopulations. These results are, thus, a clear warning of the need to secure an ecological corridor across the Razdolnaya River before continuing development completely separates the 2 tiger subpopulations of Southwest Primorye and Sikhote-Alin.

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REFERENCES

Bhagavatula J, Singh L (2006). Genotyping faecal samples of Bengal tiger *Panthera tigris tigris* for population estimation: A pilot study. *BMC Genetics* **7**, 1–12.

Coomber N, David VA, O'Brien SJ, Menotti-Raymond M (2007). Validation of a short tandem repeat multiplex typing system for genetic individualization of domestic cat samples. *Croatian Medical Journal* **48**, 547–55.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology* **14**, 2611–20.

Excoffier L, Lischer H (2010). Arlequin suite ver 3.5: y new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–7.

Fernando P, Vidya TN, Rajapakse C, Dangolla A, Melnick DJ (2003). Reliable noninvasive genotyping: fantasy or reality? *Journal of Heredity* **94**, 115–23.

Hebblewhite M, Miquelle DG, Robinson H *et al*. (2014). Including biotic interactions with ungulate prey and humans improves habitat conservation modeling for endangered Amur tigers in the Russian Far East. *Biological Conservation* **178**, 50–64.

Henry P, Miquelle D, Sugimoto T, Mccullough DR, Caccone A, Russello MA (2009). *In situ* population structure and ex situ representation of the endangered Amur tiger. *Molecular Ecology* **18**, 3173–84.

Heptner VG, Sludskii AA (1992). Carnivora (hyaenas and cats). In: Heptner VG, Naumov NP, eds. *Mammals of the Soviet Union*, Volume II, Part 2. Amerind Publishing. New Delhi, pp. 95–202.

Hernandez-Blanco HA, Naidenko SV, Chistopolova MD *et al.* (2015). Social structure and space use of Amur tigers (*Panthera tigris*) in southern Russian Far East based on GPS telemetry data. *Integrative Zoology* **10**, 365–75.

Hubisz M, Falush D, Stephens M, Pritchard J (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* **9**, 1322–32.

Kalinowski ST, Taper ML, Marshall TC (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**, 1099–106.

Karmacharya DB, Thapa K, Shrestha R, Dhakal M, Janecka 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**, 1–8.

Kholodova MV, Sorokin PA, Lushchekina AA (2005). Changes in the genetic diversity of european *Saiga tatarica* during a drastic population depression. *Doklady Biological Sciences* **404**, 358–9.

Luo SJ, Johnson WE, Martenson J *et al.* (2008). Subspecies genetic assignments of worldwide captive tigers increase conservation value of captive populations. *Current Biology* **18**, 592–6.

Matyushkin EN, Pikunov DG, Dunishenko YM *et al.* (1996). *Numbers, distribution, and habitat status of the Amur tiger in the Russian Far East.* Final report to the United States Agency for International Development Russian Far East Environmental Policy and Technology Project, Vladivostok, Russia.

Menotti-Raymond M, David V, Lyons LA *et al.* (1999). Genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics* **57**, 9–23.

Miquelle DG, Pikunov DG (2003). Status of the Amur tiger and Far Eastern leopards. In: Newell JP, ed. *The Russian Far East: A Reference Guide for Conservation and Development*, Daniel & Daniel, McKinleyville, CA, pp. 106–9.

Miquelle DG, Pikunov DG, Dunishenko YM et al. (2007). 2005 Amur Tiger Census. *Cat News* **46**, 14–6.

Miquelle DG, Rozhnov VV, Emorshin V *et al*. (2015). Identifying ecological corridors for Amur tigers (*Panthera tigris altaica*). *Integrative Zoology* **10**, 389–402.

Murphy A, Kendall K, Robinson A, Waits P (2007). The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. *Conservation Genetics* **8**, 1219–24.

Perez I, Geffen E, Mokady O (2006). Critically endangered Arabian leopards *Panthera pardus nimr* in Israel: Estimating population parameters using molecular scatology. *Oryx* **40**, 295–301.

Piggott MP, Taylor AC (2003). Remote collection of animal DNA and its application in conservation management and understanding the population biology of rare and cryptic species. *Wildlife Research* **30**, 1–13.

Pilgrim KL, Mckelvey KS, Riddle AE, Schwartz MK (2005). Felid sex-identification based on noninvasive genetic samples. *Molecular Ecology Notes* **5**, 60–1.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–59.

Rozhnov VV, Sorokin PA, Naidenko SV *et al*. (2009). Noninvasive individual identification of the Amur tiger (*Panthera tigris altaica*) by molecular-genetic methods. *Doklady Biological Sciences* **429**, 518–22.

Rozhnov VV, Zvychainaya EY, Kuksin AN, Poyarkov AD (2011). Noninvasive molecular genetic analysis in studying the ecology of the snow leopard: Problems and prospects. *Russian Journal of Ecology* **42**, 439–44.

Sorokin PA, Kirilyuk VE, Lushchekina AA, Kholodova MV (2005). Genetic diversity of mongolian gazelle (*Procapra gutturosa* Pallas. 1777). *Russian Journal of Genetics* **41**, 1101–5.

Sorokin PA, Soldatova NV, Lukarevskiy VS, Kholodova MV (2011). Genetic diversity and relations of the goitered gazelle (*Gazella subgutturosa*) groups from Uzbekistan, Turkmenistan and Azerbaijan: Analysis of the D-Loop of mitochondrial DNA. *Biology Bulletin* **38**, 585–90.

Sugimoto T, Nagata J, Aramilev VV, Mccullough DR (2012). Population size estimation of Amur tigers in Russian Far East using noninvasive genetic samples. *Journal of Mammalogy* **93**, 93–101.

Valière N (2002). GIMLET: A computer program for analyzing genetic individual identification data. *Molecular Ecology Notes* **2**, 377–9.

Waits L, Luikart G, Taberlet P (2001). Estimating the probability of identity among genotypesin natural populations: Cautions and guidelines. *Molecular Ecology* **10**, 249–56.

Wang T, Feng L, Mou P *et al.* (2015). Long distance dispersal of an Amur tiger indicates potential to restore the North-east China/Russian tiger landscape. *Oryx* **49**, 578–9.

Wilberg MJ, Dreher BP (2004). GENECAP a program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. *Molecular Ecology Notes* **4**, 783–5.

Williamson JE, Huebinger RM, Sommer JA, Louis JE and Barber RC (2002). Development and cross-species amplification of 18 microsatellite markers in the Sumatran tiger (*Panthera tigris sumatrae*) *Molecular Ecology Notes* **2**, 110–2.

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