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Population size estimation of Amur tigers in Russian Far East using noninvasive genetic samples

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The Amur tiger, *Panthera tigris altaica*, is currently distributed across the southern part of the Russian Far East and parts of northeastern China. Most Amur tigers are found in Russia, where their range is fragmented into at least 3 populations (a large population centered in the Sikhote-Alin Mountains and 2 smaller populations in northwest and southwest Primorye Krai). Traditionally, track-based techniques have been used for surveys of tigers in Russia. However, such techniques involve problems such as misinterpretation of track sizes due to snow degradation, and thus, other survey protocols have been needed. This study aimed to identify individuals and estimate population size using noninvasive genetic samples, such as feces, hairs, and saliva, collected from southwest Primorye Krai during 4 winters (2000–2001, 2001–2002, 2002–2003, and 2004–2005). During these winters, we identified 12 tigers (5 males and 7 females) using 10 microsatellite markers. Population size estimated from the 2002–2003 samples was 12 (95% confidence interval = 9–19), which was comparable to the estimate from the track count survey. Of the 3 types of noninvasive genetic samples we collected, feces were the most useful in terms of genotyping success rate and sampling efficiency. The noninvasive genetic methods developed in this study can contribute to population monitoring and management assessment of tiger conservation in the Russian Far East.

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

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The Amur (Siberian) tiger, *Panthera tigris altaica*, the northernmost subspecies among 6 extant subspecies (Luo et al. 2004), was formerly distributed across northeastern China, the Korean Peninsula, and the southern part of the Russian Far East. Habitat destruction and intensive hunting have virtually eliminated the tiger from most of northeastern China (Ma 2005). In Russia, intensive hunting and capture of young animals for world zoos decreased the tiger population to $\leq$50 individuals in the 1940s (Matyushkin et al. 1996). Since then, various conservation efforts have been put into practice, leading to an increase in population size and distribution of the tiger in Russia (Miquelle et al. 2005). However, recent surveys in China revealed that only 4–6 tigers remain in Jilin Province and 4–7 in Heilongjiang Province (Sun et al. 1999; Yang et al. 1998), with most tracks located along the Russian border. Therefore, the Amur tigers in China are largely considered to be dispersers from Russia, where >95% of the tiger population resides (Miquelle et al. 2005).

Total population sizes in Russia were estimated to be 415–476 in 1996 and 428–502 in 2005 (Matyushkin et al. 1996; Miquelle et al. 2005), suggesting that population size and distribution have recovered dramatically since the 1940s. Currently, the Russian population is composed of 3 populations (Fig. 1). The 1st population, comprising the largest number of tigers, is centered in the Sikhote-Alin Mountains. The 2nd population, located in northwest Primorye Krai, exists in an isolated forest tract just west of Lake Khanka, with space sufficient to retain a few tigers on the Russian side of the border, and perhaps some that extend into China (Miquelle et al. 2005). The 3rd population, located in southwest Primorye Krai, is isolated from the main Sikhote-Alin population by roads, railway, and development, and recent

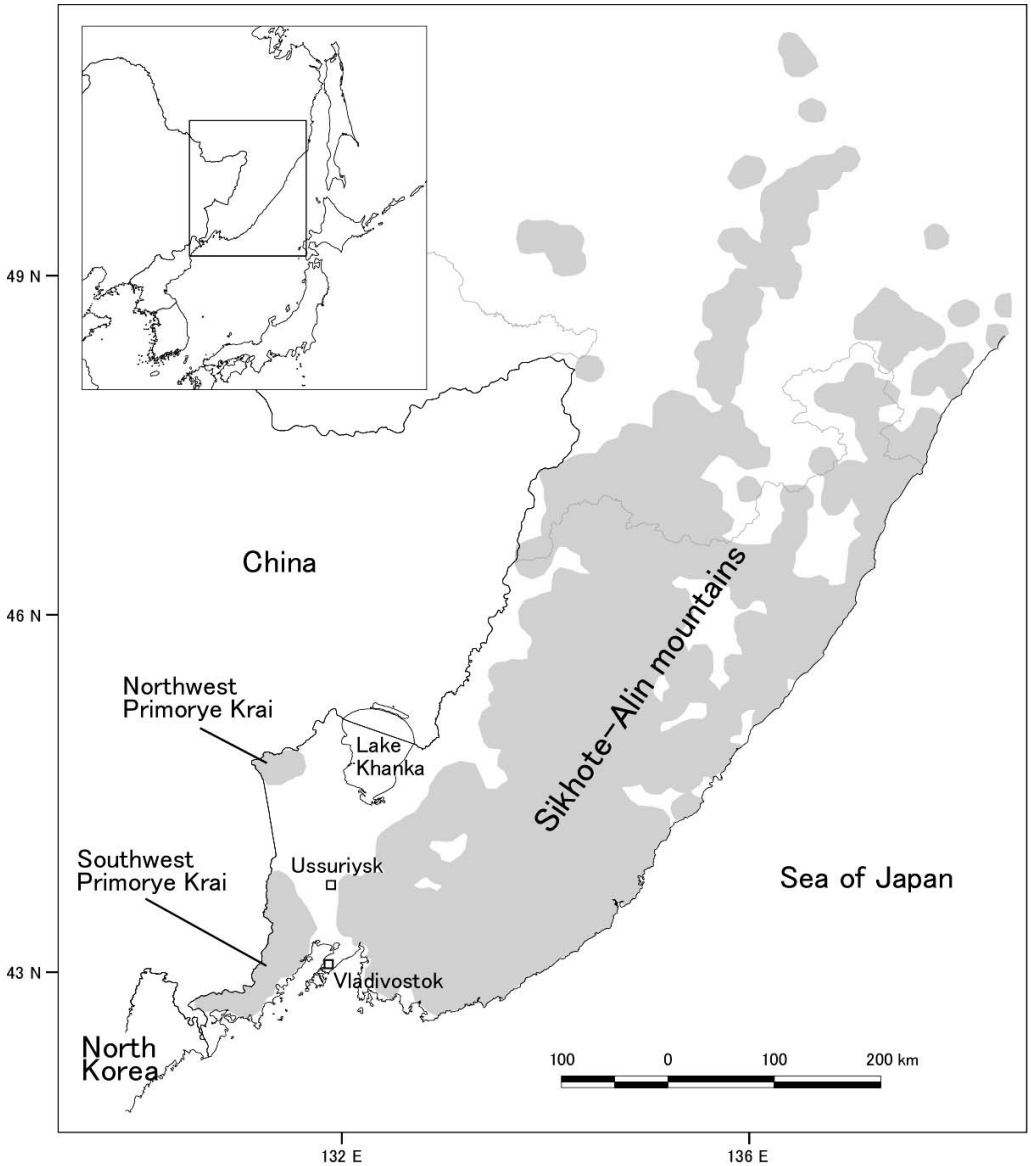

Fig. 1.—Distribution of the Amur tiger based on the 2004–2005 winter survey (Miquelle et al. 2005).

analyses suggest that this population is genetically distinguishable from the main Sikhote-Alin population (Henry et al. 2009). The population in southwest Primorye Krai represents the primary source for the recovery of tigers in northeastern China (Miquelle et al. 2010) and is therefore important despite its relatively small size in comparison to the main Sikhote-Alin population.

Surveys of tigers in Russia have traditionally been conducted by interpreting the spatial distribution and relative size of tracks to estimate tiger numbers (Miquelle et al. 2006). Multiple problems exist with this approach, including snow degradation that results in changes in track size, little standardization in interpreting track data, and few comparisons of the snow-track approach to other rigorous survey protocols (Miquelle et al. 2006).

Noninvasive genetic sampling provides an alternative approach for estimating population size of elusive or rare species such as tigers (e.g., Mondol et al. 2009). Through individual identification, the population size has been estimated for many species (Bellemain et al. 2005; Frantz et al. 2003; Piggott et al. 2006) using several different approaches, such as accumulation curve methods (Eggert et al. 2003; Kohn et al. 1999) and mark–recapture–based methods (e.g., Miller et al. 2005; Otis et al. 1978). In addition to estimates of the population size, noninvasive genetic samples provide information on the genetic diversity, relatedness, and genetic structure of a population, which are difficult or impossible to obtain by conventional field-based methods such as track-based surveys. However, noninvasive genetic samples require careful treatment, because extracted DNA is usually low in quantity and quality, which can cause amplification failure and genotyping errors (i.e., allelic dropout and false alleles). These errors directly affect the accuracy of the data and can lead to erroneous outcomes, such as a significant

overestimation of the population size (Creel et al. 2003; Waits and Leberg 2000). It may be difficult to eliminate these errors, but careful laboratory protocols and adequate genotyping criteria can minimize errors and enhance the precision of the research.

In this study, we aimed to identify individuals and estimate population size from noninvasive genetic samples collected from the population in southwest Primorye Krai (Fig. 1). We compared our estimate with that of the track count survey (Pikunov et al. 2003) to determine whether these 2 different estimation methods provide consistent outcomes.

Materials and Methods

Sampling and DNA extraction.—During the 4 winters of 2000–2001, 2001–2002, 2002–2003, and 2004–2005, we collected noninvasive genetic samples in southwest Primorye Krai, Russian Far East (Fig. 1). This area shares borders with China in the west and North Korea in the south, and is dominated by the eastern slopes of the eastern Manchurian Mountains. The area contains 3 wildlife refuges (Barsovy, Borisovskoe Plateau, and Poltavski) and 1 nature reserve (Kedrovaya Pad). However, the Poltavski wildlife refuge in the north revealed no evidence of tiger tracks in a recent survey (Pikunov et al. 2003). Most of the settlements are located along the coast, and there were more human-caused forest fires closer to the settlements and roads (Miquelle et al. 2004). A federal highway, as well as local small roads, run through southwest Primorye Krai, and thus all tiger habitats are easily accessible to people. Samples were collected by project personnel, rangers of the nature reserve and wildlife refuges, inspectors of the state hunting inspection, and local hunters. We did not establish a priori transects for sampling. Instead, to efficiently collect samples, we surveyed trails that are regularly used by tigers or Far Eastern leopards (*Panthera pardus orientalis*). We chose this sampling method because, similar to brown bears (*Ursus arctos*—De Barba et al. 2010), the long-term field experiences of our project members, local rangers, and specialists indicated that transect sampling is not practical or efficient for fecal sampling. Feces were collected opportunistically and by following tracks of large cats such as Amur tigers and leopards discovered on the trails. Hair samples were collected from trees, bushes, fences of deer farms, and military barbed wire. Saliva samples were collected using cotton swabs from wounds of prey killed, such as sika deer (*Cervus nippon*) and roe deer (*Capreolus capreolus*). Most of the sample locations were recorded on a 1:100,000 topographic map with an estimated error <1 km, and some locations were recorded using a handheld global positioning system (Garmin International, Inc., Olathe, Kansas). Twenty-four samples were received without location data.

Samples collected between mid-January and early March 2001, between mid-November 2001 and late March 2002, between late November 2002 and late February 2003, and between mid-January and early April 2005 were classified as 2000–2001, 2001–2002, 2002–2003, and 2004–2005 winter samples, respectively. Two samples collected on 1 May 2001 also were classified as 2000–2001 winter samples. We could collect only 15 samples in the winter of 2000–2001; however, in the winters of 2001–2002 and 2002–2003, we successfully collected more than 100 samples. In these 2 winters, approximately 30 people spent approximately 30 days at 8 h/day collecting samples in southwest Primorye Krai, covering most of the tiger and leopard habitats except the southern periphery of their distributions. We estimated the total sampling effort per winter as $\geq$7,000 h. In the winter of 2004–2005, we attempted to collect hairs using hair snares. We directly nailed a small synthetic carpet (10 $\times$ 20 cm) on trees (30–40 cm from the ground) along the trails (McDaniel et al. 2000) or to a piece of plywood (20 $\times$ 20 cm) that was nailed to the ground. We placed a Bobcat Gland Lure (Minnesota Trapline Products, Pennock, Minnesota) on plywood or on trees near the snares to attract the carnivores. Hair trapping was performed at 25 sites situated in areas of high track density (Pikunov et al. 2003). The sites were spaced 2–7 km apart, and approximately 5 snares were placed at each site within a distance of 15–30 m. We checked the snares every 7–10 days. A new snare was placed every time hair samples were obtained on the older snare. Feces also were collected during these regular visits to the snares.

All sample types were preserved in situ in a zip-closure plastic bag at ambient temperature and transported to the freezer (-20°C) at a field base normally within 1–4 h. When transporting the samples from the field base to the laboratory, we used an ice chest and then stored samples at -20°C in the laboratory until DNA extraction. Fecal DNA was extracted by the GuSCN/silica method (Boom et al. 1990; Höss and Pääbo 1993) using approximately 200 mg of fecal material eluted in 200 µl of tris-ethylenediamintetraacetic acid buffer. DNA was extracted from hair samples using ISOHAIR (Nippon Gene, Tokyo, Japan). The number of hairs per sample varied from those with several scores of hairs (in which case approximately 10 hairs with follicle ends were used) to samples in which only a few hairs (e.g., 2–5 hairs) were available. DNA was extracted from saliva samples using the QIAamp DNA mini kit (Qiagen, Tokyo, Japan). DNA extracted from hair and saliva samples was eluted in 50 µl of tris-ethylenediamintetraacetic acid buffer. DNA extraction of all samples, including negative controls, was conducted in a dedicated space using dedicated micropipettes with aerosol-resistant tips to avoid contamination.

Species and sex identification.—Feces, hairs, and saliva collected in the field included those from other sympatric carnivores such as the Far Eastern leopard; therefore, the tiger samples required identification. Species and sex identification were conducted following the method of Sugimoto et al. (2006).

Microsatellite genotyping.—For individual identification, 10 microsatellite loci (6HDZ089, FCA043, FCA077, FCA090, FCA094, FCA105, FCA123, FCA161, FCA224, and FCA441) were selected from 18 loci developed by Williamson et al.

(2002) and 25 loci used by Uphyrkina et al. (2001) by amplifying DNA derived from 3 captive tigers and 6 good-quality fecal samples, which were presumably from different individuals based on their sampling locations. Marker selection was based on the following criteria: amplifying short lengths of DNA, ease of amplification, the presence of many alleles, and ease of scoring. We conducted genotyping of noninvasively collected samples using multiplex polymerase chain reaction with the following loci combinations: 6HDZ089; FCA123; FCA043 and FCA161; FCA090 and FCA094; FCA077 and FCA105; and FCA224 and FCA441. The microsatellite loci were amplified in 10-μl volumes including 1× polymerase chain reaction buffer, 0.2 mM of each deoxynucleoside triphosphate, 3.0 mM of $MgCl_2$, 0.4 μM of each primer, 4 μg of bovine serum albumin, 0.3 units of Taq DNA polymerase (Applied Biosystems, Foster City, California), and 1.0 μl of DNA extract. The reaction conditions were as described by Menotti-Raymond et al. (1999) although we increased the number of 2nd cycles from 20 to 30, resulting in 40 cycles in total. Polymerase chain reaction products were analyzed on an ABI 3100 automatic sequencer (Applied Biosystems), and genotype data were collected using GeneScan version 3.1 (Applied Biosystems). We used a modified multitube approach based on Taberlet et al. (1996). Three polymerase chain reaction amplifications were performed initially for each sample, and when the sample consistently showed positive amplifications, no ambiguous electropherograms (i.e., clear peaks), and no allelic dropout or false alleles at every locus, genotyping for the sample was considered complete. Under any other circumstance, 2 or more additional amplifications were performed and a heterozygous genotype was determined when both alleles were detected at least 3 times; a homozygous genotype was determined when 1 allele was detected at least 5 times after ≥5 polymerase chain reaction amplifications.

Genotyped data reliability.—We calculated the probability of identity for unrelated individuals and siblings (Waits et al. 2001) using the program GIMLET (Valière 2002) from genotyping data of the noninvasive genetic samples to assess whether the number of markers was sufficient to distinguish between different individuals. The genotyping error rate per locus was calculated using equations 1 and 3 from Broquet and Petit (2004). Compared with the consensus genotypes obtained through repeat amplification, allelic dropouts were recorded when 1 allele was not detected at a heterozygous locus, and false alleles were recorded when an additional allele was detected at both homozygous and heterozygous loci.

Genotyping data were grouped using the program GIMLET (Valière 2002). If partial genotypes (≥8 loci) were present, we performed additional genotyping at the missing loci using relevant markers and examined whether the data obtained met our genotyping criteria (stated above). If a genotype differed from other genotypes only in 1 or 2 alleles, we performed polymerase chain reaction amplification repeatedly at the relevant locus. Furthermore, additional DNA extraction and a series of genotyping were performed to verify the genotype in instances where a sample represented a distinct genotype. In this 2nd multilocus genotyping, polymerase chain reaction amplification was performed at least 3 times, and a heterozygote was determined when both alleles were detected at least 2 times, whereas a homozygote was determined when 1 allele was detected at least 3 times.

After individual identification, we observed a pair of individuals with genotypes that differed only by 1 locus, which we termed as 1-mismatch-pair (Paetkau 2003). We also observed two 2-mismatch-pairs. To determine how these 1-mismatch-pairs or 2-mismatch-pairs changed as the number of examined loci increased, we analyzed 2 additional loci, FCA310 (Menotti-Raymond et al. 1999) and 6HDZ481 (Williamson et al. 2002), for those pairs of individuals. If the given individuals were identified from multiple samples, we randomly selected 3 samples and genotyped them. However, if the given individuals were identified from a single sample, we genotyped the 1st and 2nd DNA extracts of those individuals. The abovementioned polymerase chain reaction condition and program were used, and polymerase chain reaction amplification was performed at least 3 times per sample.

After individual identification, we found that the average number of fecal samples per male and female tigers were different (see ''Results'') and, thus, the difference was statistically examined with a Brunner–Munzel test using the R computer package (http://www.R-project.org/, accessed 2 March 2009).

Population size estimation.—Population size was estimated using a mark–recapture model that assumed closed populations. We used the program CAPTURE (Otis et al. 1978) for the M_h-jackknife and M_h-Chao estimates and the program CAPWIRE (Miller et al. 2005) for the Capwire estimate. M_h-jackknife and M_h-Chao allow for variation in capture probability due to individual heterogeneity, which is commonly observed in genetic mark-recapture studies (Banks et al. 2003; Piggott et al. 2006). We used the 2 innate rates model (TIRM) in CAPWIRE because more feces were collected from male than female animals (see ''Results''). We estimated population size from the samples collected in 2002–2003 only, because the samples were well dispersed spatiotemporally. Furthermore, the largest number of samples was successfully genotyped in this winter among the 4 winters sampled. The sampling session was divided into 3 periods for CAPTURE estimation: November–December, January, and February. The models used for population size estimates assume that the study population is closed over the sampling period. However, our sampling period extended >3 months, and tigers are capable of traveling long distances during this time. Therefore, our data sets probably violated the assumption of no immigration and emigration, leading to an upward bias in estimating population size. According to the distribution of tiger tracks (Miquelle and Murzin 2000), possible migration sites outside the sampling area were the southernmost edge of Russia, where sampling was not conducted, and across the border into China. In the Chinese area along the

Table 1.—Results of individual identification for samples collected during the 4 winters. The individuals were labeled as MT (male tiger no.) and FT (female tiger no.) in the order of their sampling date. Numbers in parentheses below the winters observed indicate the number of tiger samples revealed from species identification.

Individual	Total no. observations	Winters observed			
		2000–2001 (n = 6)	2001–2002 (n = 14)	2002–2003 (n = 42)	2004–2005 (n = 17)
MT1	12	1	1	7	3
MT2	2	1	0	1	0
MT3	10	0	4	6	0
MT4	1	0	0	1	0
MT5	5	0	0	0	5
FT1	1	1	0	0	0
FT2	1	1	0	0	0
FT3	6	0	1	3	2
FT4	3	0	0	1	2
FT5	1	0	0	1	0
FT6	2	0	0	2	0
FT7	2	0	0	2	0
Total (n feces/n hairs)	46 (40/6)	4 (4/0)	6 (5/1)	24 (23/1)	12 (8/4)

Russian border of southwest Primorye Krai, the tracks of a few individuals were observed in the 1998 survey (Yang et al. 1998), and it is possible that tigers moved across the border into China during winter sampling in 2002–2003. In addition, a few individuals may have moved in and out of the sampling area in the south, which is the southern periphery of distribution of the Amur tiger. The few occurrences of migration events are unlikely to have led to significant overestimation of population size, although these events should be taken into consideration.

Results

Species and sex identification.—A total of 286 samples (243 feces, 37 hairs, and 6 saliva) were collected during 4 winters; 15 samples (13 feces, 1 hair, and 1 saliva) in 2000–2001, 102 samples (91 feces, 6 hairs, and 5 saliva) in 2001–2002, 104 samples (102 feces and 2 hairs) in 2002–2003, and 65 samples (37 feces and 28 hairs) in 2004–2005. Seventy-nine of the 286 samples were identified as being from tigers, 157 were from leopards, and the remaining 50 could not be identified due to nonamplification. Sex could be determined for 53 of these 79 samples (98 for the leopard). The success rate for sex identification for both species was highest in 2002–2003 (78.0%; 71.4% for tigers), followed by 2004–2005 (62.5%; 70.6% for tigers), 2001–2002 (49.3%; 50.0% for tigers), and 2000–2001 (46.7%; 66.7% for tigers). For fecal samples, the success rate was 66.2%, whereas it was 54.5% for hair samples. Of the 6 saliva samples, species identification was successfully performed in 4 samples (1 tiger and 3 leopards). However, sex identification was not successful in any these samples.

Individual identification.—Multilocus genotypes at 10 loci were successfully determined in 46 tiger samples (40 feces and 6 hairs) and none of the saliva samples could be genotyped. The 46 multilocus genotypes were compared with each other, resulting in the identification of 12 tigers (5 males and 7 females; Table 1). The resultant probability of identity for unrelated individuals and siblings was 1.15×10^{-7} and 1.96×10^{-3}, respectively (Table 2). Given the small population size in the study area, we recognized this probability of identity sufficient to accurately identify individuals. Genotyping error rates per locus ranged from 0 to 0.141 (Table 3), and the average allelic dropout rate was

Table 2.—Genetic variation at 10 microsatellite loci for 12 tigers identified during 4 winters. A: observed number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity; $P_{ID\text{-}sib}$/locus: probability of identity for siblings per locus; and $P_{ID\text{-}sib}$ product: cumulative product of $P_{ID\text{-}sib}$/locus value. Loci are ranked from low to high values of $P_{ID\text{-}sib}$/locus. 6HDZ089 is from Williamson et al. (2002) and the other markers are from Menotti-Raymond et al. (1999).

Locus	A	H_E	H_O	$P_{ID\text{-}sib}$/locus	$P_{ID\text{-}sib}$ product
6HDZ089	5	0.76	0.67	0.413	4.13×10^{-1}
FCA161	4	0.70	0.83	0.456	1.88×10^{-1}
FCA043	3	0.68	0.75	0.470	8.85×10^{-2}
FCA441	3	0.64	0.58	0.501	4.43×10^{-2}
FCA224	3	0.57	0.58	0.552	2.45×10^{-2}
FCA123	3	0.54	0.67	0.568	1.39×10^{-2}
FCA077	4	0.49	0.50	0.594	8.25×10^{-3}
FCA094	2	0.51	0.50	0.603	4.97×10^{-3}
FCA105	3	0.49	0.42	0.606	3.01×10^{-3}
FCA090	2	0.43	0.42	0.651	1.96×10^{-3}
Total	3.2	0.58	0.59		

Table 3.—Frequencies of genotyping error rates per locus.

Locus	Allelic dropout	False allele
6HDZ089	0.037	0.005
FCA043	0.038	0.011
FCA077	0.038	0.006
FCA090	0.022	0.006
FCA094	0.041	0.012
FCA105	0.019	0.006
FCA123	0.049	0.018
FCA161	0.016	0
FCA224	0.141	0.022
FCA441	0.083	0.011
Total	0.048	0.010

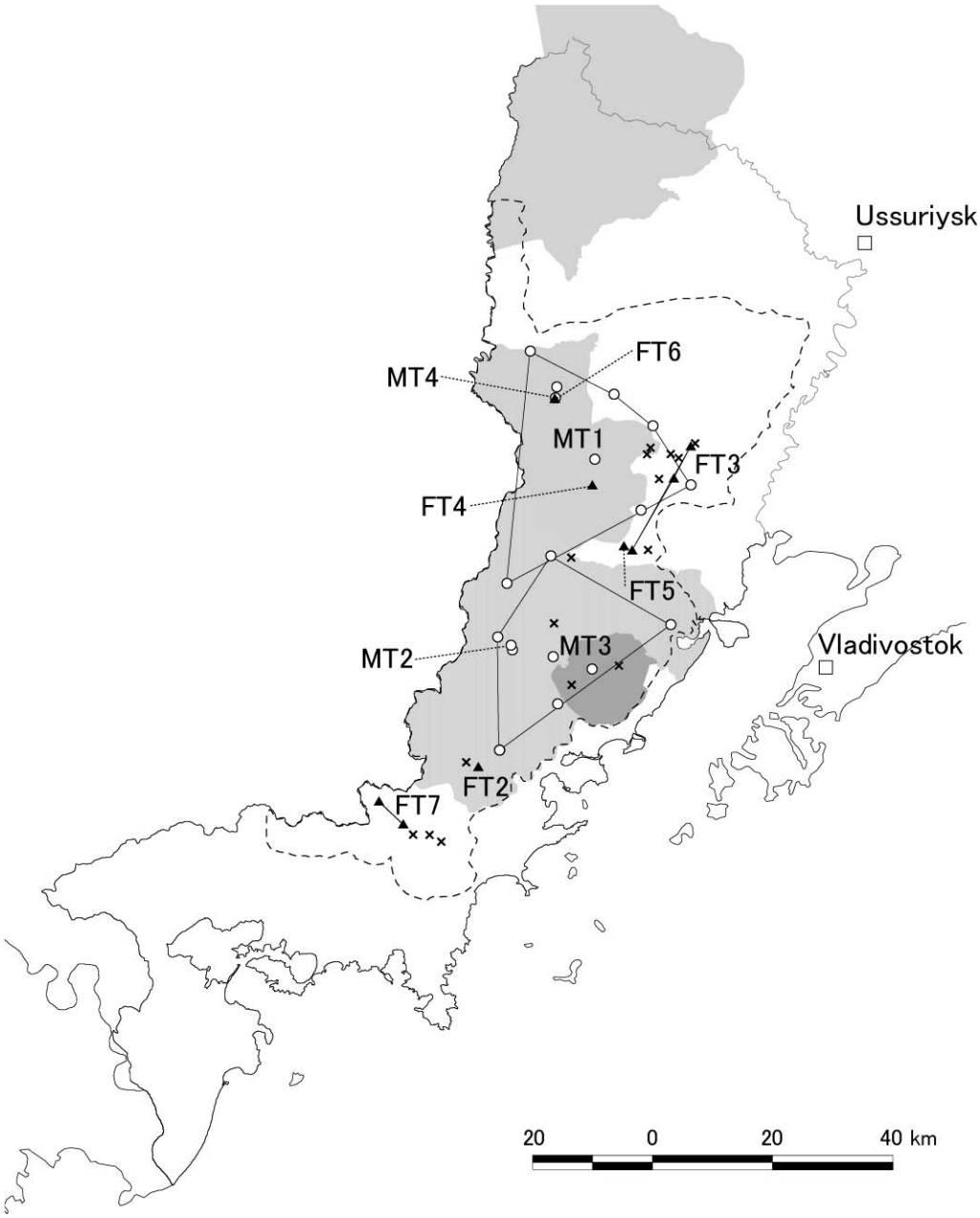

Fig. 2.—Distribution of identified tiger individuals during the first 3 winters (2000–2001, 2001–2002, and 2002–2003) in southwest Primorye Krai. Males are indicated with white circles and females with black triangles with individual identification numbers, and tiger samples in which individual identification was unsuccessful are indicated as crosses. FT1 is not shown on the map due to lack of location data. For individuals with >2 different locations, home ranges were obtained by a minimum convex polygon, and movement paths are drawn by a line for individuals with 2 different locations. The broken line indicates the sampling area in the winter of 2002–2003. The shaded area indicates protected areas and the darker area indicates the Kedrovaya Pad Nature Reserve.

0.048. Thus, 3 or 5 repeat amplifications can effectively minimize the errors.

When we examined the similarity between pairs of individuals, we observed a 1-mismatch-pair (MT1/MT4) and two 2-mismatch-pairs (MT3/FT5 and MT4/FT4). The other pairs differed by more than 2 loci. Given that MT4 represented a single-capture individual with the same sex as MT1 and located in the range of MT1 (Fig. 2), we could consider MT4 to have been created by genotyping errors of MT1. However, a consistent and clear difference was observed at the given locus (6HDZ089), and following genotyping of 2 additional loci (FCA310 and 6HDZ481), we found a 2nd consistent mismatch at FCA310. Thus, we recognized MT4 as a distinct genotype. We also concluded that both of the 2-mismatch-pairs were unique individuals because they differed in sexes and in alleles at FCA310 and 6HDZ481.

The number of genotyped samples of each individual ranged from 1 to 12; 4 individuals (1 male and 3 females) were

identified from a single sample (Table 1). The mean number of fecal samples per individual was higher for males than for females, although there was no significant difference ($\bar{X} \pm SD$: 5.0 ± 4.2 in males; 2.1 ± 1.5 in females; $P = 0.245$). The distribution of individually identified samples in the first 3 winters, excluding those with no location data, is shown in Fig. 2. MT1 and MT3 appeared to have nonoverlapping ranges from each other, and these 2 male tigers alone occupied most of the protected areas in our sampling area. For females, however, small sample sizes precluded the defining of home ranges.

Population size estimate.—M_h-Chao produced the largest estimate with the widest confidence interval, 27 (95% confidence interval [95% *CI*] = 12–136), whereas the M_h-jackknife and Capwire estimates were intermediate, 14 (95% *CI* = 11–25), and lowest, 12 (95% *CI* = 9–19), respectively.

Discussion

The noninvasive genetic analysis resulted in the identification of 12 tigers with potentially a slight female-biased sex ratio during the 4 winters of this study. In 2002–2003, a relatively large number of tigers was identified compared with the previous and subsequent winters. This simply reflects larger spatial sampling coverage and higher sampling intensity together with higher success rate of species identification in 2002–2003. The minimum number known alive in this winter was 9 and the population size estimates were different depending on the models used. Of the 9 tigers identified in 2002–2003, only 3 were recaptured during the sessions. This small number of recaptures probably resulted in higher estimates for the M_h-jackknife and M_h-Chao models and an extremely wide confidence interval for the M_h-Chao model (95% *CI* = 12–136). The advantage of the Capwire model over the others is that estimates can be developed without having to create multiple sampling sessions during the survey period (Miller et al. 2005). When the expected population size is small ($N < 100$) and capture heterogeneity is substantial, the Capwire model works better than the M_h-jackknife and M_h-Chao models, according to the simulation study of Miller et al. (2005). The Capwire estimate of 12 (95% *CI* = 9–19) appeared to provide the best estimate of the tiger population size in southwest Primorye Krai. However, our estimate was affected by a potential upward bias derived from violation of the closed assumption. In addition, the presence of tigers that were undetected because of insufficient sampling or genotyping failure may have resulted in an underestimate of population size. Of the 2 factors though, we believe that the latter factor is more important in our study. Of the 42 tiger samples collected in the winter of 2002–2003, 18 samples could not be genotyped (Table 1). Additionally, the lower track density and limited available habitat in the most probable migration site (Miquelle and Murzin 2000; Pikunov et al. 2003; Yang et al. 1998) makes immigration or emigration events less likely.

In the winter of 2002–2003, Pikunov et al. 2003) conducted a track count survey in southwest Primorye Krai. They investigated 151 transects (12–15 km each) that were established to cover the entire tiger and leopard habitats (Pikunov et al. 2003:figure 2). In the survey, 14 specialists covered the established 151 transects from 4 to 28 February 2003 mostly by foot and also using a vehicle, snowmobile, and by ski on some transects. They identified species and individuals based on the paw shape and size. If the distance between 2 tracks of similar sizes was greater than the known home-range size or possible daily travel distance, the tracks were considered to be from different individuals. In this survey, they estimated the tiger population size to be 16–21, whereas our DNA-based estimate was 12 (95% *CI* = 9–19). Given possible sources of error, such as misidentification of species or individuals, double-counting the same individual on different transects, and failure in detecting an unknown number of tigers in the track-based survey and genotyping errors or diverse potential bias on the estimator in the DNA-based survey, the estimate of our DNA-based approach was quite similar to that of the track-based approach. However, a further noninvasive genetic study with better spatial sampling coverage is required to compare the 2 estimation methods more rigorously.

The average number of genotyped samples per individual in the winter of 2002–2003 was 2.7, which meets the sample size criteria for the Capwire model for which an average of >2.5 observations per individual is necessary for accurate estimation in small populations ($N \leq 25$—Miller et al. 2005). However, a further increase in sampling effort is desirable to improve the precision of the estimate. For instance, Miller et al. (2005) indicated that an average of >3.0 observations per individual is preferred, and Solberg et al. (2006) recommended that the number of fecal samples collected should be 2.5–3.0 times the assumed number of animals, considering the 70–80% success rate of genotyping. Increasing the sample size would also help us to determine home ranges more clearly.

All surveys show that the isolated population in southwest Primorye Krai is small, indicating that unpredictable stochastic events such as demographic and environmental changes could swiftly lead to extinction of this population. Mating among relatives is unavoidable in such a small population, increasing the risk of extinction due to inbreeding depression (Frankham et al. 2002). Henry et al. (2009) indicated that the southwest Primorye Krai is already genetically distinct, but movement of individual tigers appears to occur between the southwest population and the main Sikhote-Alin population. A clustering analysis assigned 2 tigers sampled in the southwest population and 1 tiger sampled in the main Sikhote-Alin population to the opposite population, suggesting that complete separation has not yet occurred (Henry et al. 2009). Nonetheless, to avoid inbreeding and to mitigate genetic differentiation, maintaining connectivity between these 2 populations is important.

In the Russian Far East, the samples obtained in winter are usually frozen, and thus, are preserved naturally in the field; therefore, noninvasive genetic samples collected in winter should be reliable genetic material. In the present study, however, the success rate of sex identification (64%) and of individual identification (58%; Table 1) for tiger samples were not particularly high. There are several possible reasons for this. The samples, especially in the first 2 winters (2000–2001 and 2001–2002), were retained for a relatively long time before DNA extraction in the laboratory (about 3–5 years). This might have led to the lower success rate of sex identification in these samples compared to samples from subsequent winters. In addition, some samples were preserved at ambient temperature for a relatively long time in the field or during transport. We used a freezing method in the present study; however, other methods such as liquid preservation in DETs (dimethylsulfoxide, ethylenediamine-tetraacetic acid, Tris, and NaCl) buffer or 70–100% ethanol and drying preservation using silica (e.g., Frantz et al. 2003; Murphy et al. 2002) may be used to reduce sample degradation in certain situations where freezing equipment is not available or when longer periods of sample transportation occur. DNA analysis immediately after sample collection should also improve the success rate of sexing and genotyping.

In the present study, we used 3 types of noninvasive genetic samples: feces, hairs, and saliva. The most preferred sample is feces, because these samples give the highest success rate of genotyping, and it is possible to collect a relatively large number of samples. Hair samples are also useful for genotyping, and thus, are worth collecting whenever they are found. In 2004–2005, we used hair snares and collected 28 samples, only 8 of which were from tigers (7 from leopards), despite the large number of snares being set (about 130). Thus, the hair snares employed in the present study were not particularly efficient and further improvement is needed. None of the saliva samples were successful in individual identification, but they did provide species information and were useful in identifying species responsible for the livestock depredations that often occur in southwest Primorye Krai (Hötte 2003).

We consider feces to be the most useful samples and believe that increasing the spatial sampling coverage and sample size along with improved sample handling will provide reliable estimates of population size. The use of feces-detection dogs may be an option to increase sample size (Kerley and Salkina 2007). In addition, given the presence of 1-mismatch-pairs and 2-mismatch-pairs in the present study, we recommend use of the 2 additional loci for future work. Continued DNA sampling will provide a means of monitoring demographic changes and permeability of the developing barrier between populations. This same approach should be applied to the other isolated population in northwest Primorye Krai (Fig. 2) to reveal its ecological and genetic status as well as to integrate this population into a similar conservation strategy.

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