

Conservation Genetics of the Far Eastern Leopard (*Panthera pardus orientalis*)

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The Far Eastern or Amur leopard (*Panthera pardus orientalis*) survives today as a tiny relict population of 25–40 individuals in the Russian Far East. The population descends from a 19th-century northeastern Asian subspecies whose range extended over southeastern Russia, the Korean peninsula, and northeastern China. A molecular genetic survey of nuclear microsatellite and mitochondrial DNA (mtDNA) sequence variation validates subspecies distinctiveness but also reveals a markedly reduced level of genetic variation. The amount of genetic diversity measured is the lowest among leopard subspecies and is comparable to the genetically depleted Florida panther and Asiatic lion populations. When considered in the context of nonphysiological perils that threaten small populations (e.g., chance mortality, poaching, climatic extremes, and infectious disease), the genetic and demographic data indicate a critically diminished wild population under severe threat of extinction. An established captive population of *P. p. orientalis* displays much higher diversity than the wild population sample, but nearly all captive individuals are derived from a history of genetic admixture with the adjacent Chinese subspecies, *P. p. japonensis*. The conservation management implications of potential restoration/augmentation of the wild population with immigrants from the captive population are discussed.

The Far Eastern or Amur leopard (*Panthera pardus orientalis*; Schlegel 1857), one of the world's most endangered cat subspecies, is classified as "critically endangered" in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Animals (1994), and is listed in Appendix I of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES 1984). *Panthera pardus orientalis* differs from other subspecies of *P. pardus* by a large body size, a thick coat, and large, widely spaced, thick-rimmed black rosettes (Nowell and Jackson 1996; Pocock 1930). Until the late 19th century, the Far Eastern leopard was distributed across the southern stretches of the Amur-Ussuri region in Russia (Arseniev 1914; Cherkasov 1884; Przhevalskiy 1870), Manchuria, North China, and the Korean Peninsula (Pocock 1930) (Figure 1a), reaching as far south as Beijing (Heptner and Sludskiy 1972).

The range of the Far Eastern leopard was reduced dramatically during the 20th century primarily due to habitat

loss, hunting, intensive logging, elimination of prey base, poaching, and a demand for body parts used in Asian traditional medicines. In China, recent leopard surveys suggest that fewer than 10 Far Eastern leopards remain in Jilin Province (Yang et al. 1998), and few, if any, remain in the more northern Heilongjiang Province (Baogang et al. 1999). The status of leopards in North Korea is unknown, although it is possible that some still occur in high mountainous areas (Korean People's Democratic Republic Academy of Science Institute of Geography 1998; Won and Smith 1999).

In Russia, the leopard's range had become fragmented into three isolated populations by the 1970s, two of which became extinct by the mid-1980s (Pikunov and Korkishko 1992). The last known population survives in southwest Primorskiy Krai, about 30 km west of the city of Vladivostok, on a narrow strip of land that borders China to the west and North Korea to the south (Figure 1b,c). This relict population has been relatively stable over the last few decades, with recent surveys estimating 25–40 leopards

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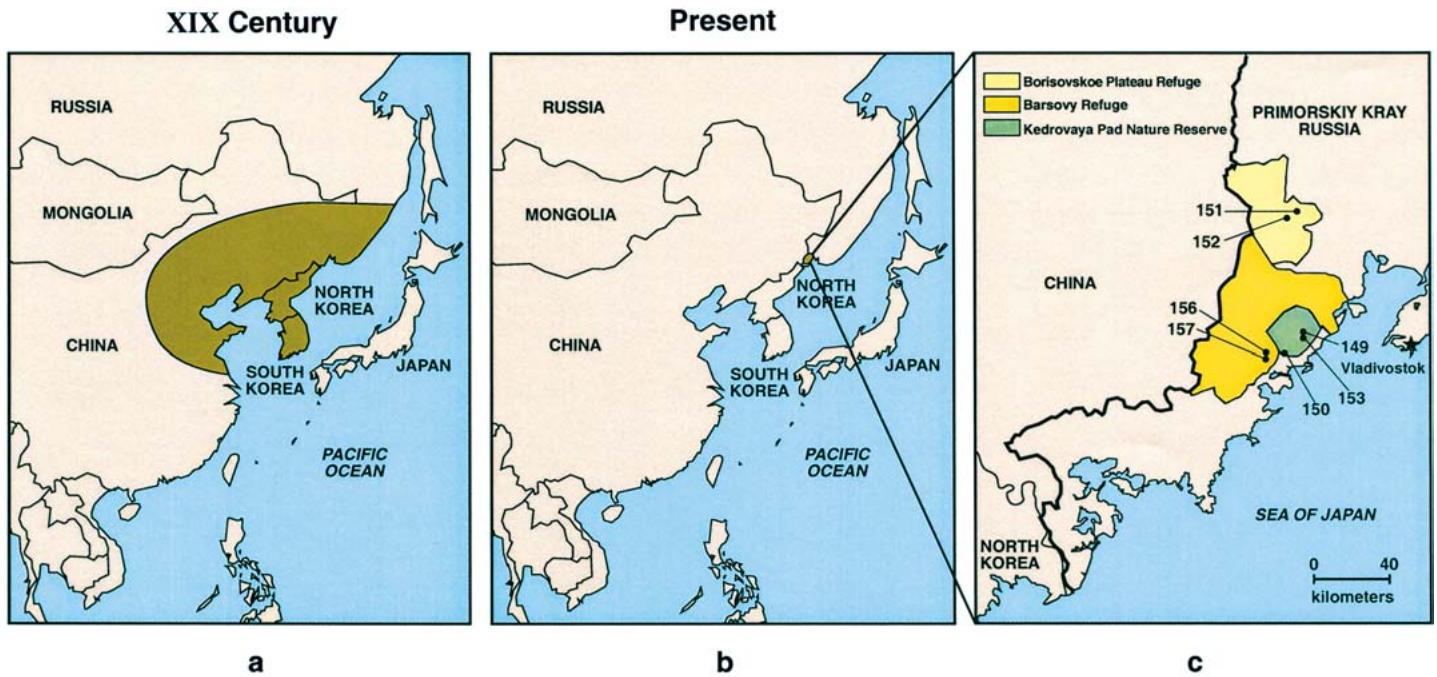


Figure 1. Geographic distribution of the Far Eastern leopard: (a) 19th century, (b) and (c) present.

(Korkishko and Pikunov 1994; Miquelle et al. 1996; Pikunov et al. 1999a). Evidence of reduced litter size (average 1.75 in 1973 to 1.0 in 1991) (Pikunov and Korkishko 1992; Pikunov et al. 1999b) and small population size renders this tiny population at high risk for demographic and genetic depletion. A captive population of *P. p. orientalis*, established in 1961 from nine wild-born founders, has expanded to a worldwide managed population of 170 leopards; however, the origin of the most prolific founding male (studbook SB-2; Figure 2) is unknown and of questionable subspecies origin (Christie and Arzhanova 1999a,b).

A recent molecular genetic assessment of leopard subspecies based on mitochondrial and microsatellite genotypes has affirmed subspecies-level genetic distinctiveness of *P. p. orientalis* (Miththapala et al. 1996; Uphyrkina et al. 2001). In this article we examine the extent and phylogenetic pattern of DNA variation in wild-born and captive populations of *P. p. orientalis* and compare these to those discovered in other leopard subspecies and select Felidae species. The study expands previous analyses to assess diversity, population subdivision, and population affinities among limited samplings of two wild populations of *P. p. orientalis* (RFE, Russian Far East, and NK, North Korea) with the captive population. The data revealed genetically impoverished free-ranging populations with more extreme genetic

depletion than any other leopard subspecies, comparable to the genetically depauperate Asiatic lion (*Panthera leo persica*) and Florida panther (*Puma concolor coryi*) populations (Roelke et al. 1993; Wildt et al. 1987). In addition, we present evidence for inadvertent subspecies mixing in the founders of the captive Amur leopard population. Finally, we review the management implications of these findings with other background in hopes of developing a successful plan for conservation of this critically endangered subspecies of leopard.

Materials and Methods

Samples

Samples of seven animals from the Russian Far East population were collected during capture and immobilization of leopards associated with a radiotelemetry study during 1993–1996 (Augustine et al. 1996). Five of the animals were caught within or in close proximity to the Kedrovaya Pad Reserve, but two were captured about 40 km to the north in the Borisovskoe Plateau Refuge (Figure 1c). Since female and male leopards are territorial, and the average diameter of female home ranges is approximately 6–8 km (Augustine et al. 1996), it is unlikely that all those leopards are first-order relatives.

Samples from five leopards brought to captivity from North Korea were obtained from the Moscow Zoo, Tallin

Zoo, and Berlin Tierpark Zoo. Reliable information concerning their origins is absent: they could represent another wild population of *P. p. orientalis* leopards in North Korea, or they may have been captive bred in the Pyongyang Zoo (Christie and Arzhanova 1999a). In either case, we consider these animals as representative of wild *P. p. orientalis* from North Korea (Table 1).

Twenty-two samples from captive *P. p. orientalis* leopards were used (Table 1); 21 of them were descendants of founder SB-2 (Table 1 and Figure 2) (Shoemaker 1997). In addition, 63 leopard samples of *P. p. pardus*, *P. p. saxicolor*, *P. p. fusca*, *P. p. kotya*, *P. p. delacourii*, and *P. p. japonensis* subspecies were used for phylogenetic analysis and comparative estimation of genetic diversity (Miththapala et al. 1996; Uphyrkina et al. 2001). Twenty-two samples of tigers (*Panthera tigris*) were included in the phylogenetic analysis as an outgroup (Wentzel et al. 1999).

Mitochondrial DNA Sequence Analysis

The mitochondrial gene sequence of NADH-5 (611 bp) and control region (116 bp) homologous to regions previously determined for 77 leopards sampled across their range were assessed for 16 leopards from the captive populations. Primers, sequence alignment, and phylogenetic analysis were as described previously (Uphyrkina et al. 2001).

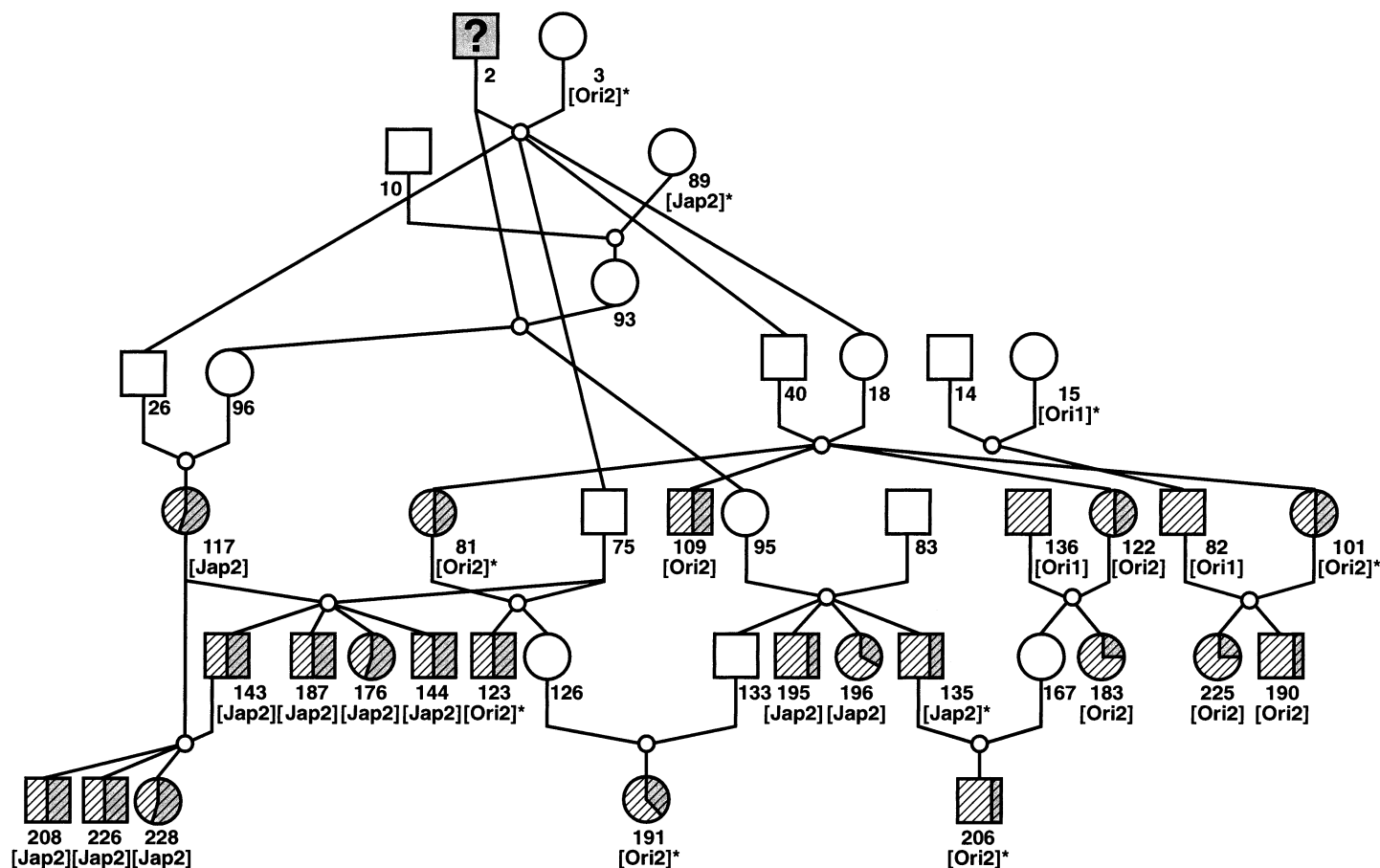


Figure 2. Pedigree of captive *P. p. orientalis* leopards. Hatched circles and squares show leopards genotyped and/or sequenced in present study; shading shows their proportionate relationships to founder SB-2. Studbook numbers are assigned to individual leopards by international and European studbooks (Christie and Arzhanova 1999a; Shoemaker 1997). Studbook numbers and mitochondrial DNA haplotypes [in brackets] assigned to each leopard are listed in Table 1. Asterisks indicate imputed mitochondrial DNA haplotypes from female lineage inference.

Microsatellite Locus Variation

Samples from 22 captive-bred leopards were amplified and genotyped for 25 nuclear microsatellite (STR) loci, originally designed for the domestic cat (*Felis catus*) (Menotti-Raymond et al. 1999). The efficacy of these loci (*FCA 008*, *FCA 026*, *FCA 043*, *FCA 075*, *FCA 077*, *FCA 090*, *FCA 094*, *FCA 096*, *FCA 097*, *FCA 098*, *FCA 105*, *FCA 123*, *FCA 126*, *FCA 139*, *FCA 161*, *FCA 211*, *FCA 220*, *FCA 224*, *FCA 229*, *FCA 247*, *FCA 310*, *FCA 391*, *FCA 441*, *FCA 453*, *FCA 678*) had been demonstrated for leopards (Uphyrkina et al. 2001). Polymerase chain reaction (PCR) amplifications for each microsatellite locus and DNA sequencing were performed as described (Menotti-Raymond et al. 1999; Uphyrkina et al. 2001).

Neighbor-joining (NJ) phylogenetic trees were constructed using the proportion of shared allele genetic distances (*Dps*) and kinship coefficient genetic distances (*Dkf*) (Bowcock et al. 1994) using MICROSAT (Minch et al. 1995).

Assessment of pairwise differences between populations were calculated using F_{ST} (Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) values and significance tests were performed using the ARLEQUIN (Schneider et al. 1997) software package for population genetic analysis. Genetic variation was estimated using the following parameters: percentage of polymorphic loci (*P*), observed heterozygosity (H_o), average (*A*) and minimum-maximum number of alleles, average effective number of alleles (*E*), average range of microsatellite repeats (*R*), and average variance (*V*). Correlation analysis between the percentage of genetic representation of the founder SB-2 in each captive leopard and its distance from the wild-born *P. p. orientalis* group (as averaged across all individuals) was done using STATISTICA for Windows (StatSoft, Inc. 1995).

Estimation of relatedness values between individual leopard pairs (r_{xy}) and relatedness of whole populations (R_{xy})

was performed by using RELATEDNESS 5.0 (Queller and Goodnight 1989). Samples from seven wild-born *P. p. fusca* leopards (Ppa 91–97) from northern India and samples from seven wild-born *P. p. kotiya* leopards (Ppa 102, Ppa 104–106, Ppa 116, Ppa 118, and Ppa 128) from Sri Lanka, genotyped previously for the same microsatellite loci (Uphyrkina et al. 2001), were used for comparison with seven samples from the remaining *P. p. orientalis* in the Russian Far East population. Twenty-two captive-bred *P. p. orientalis* were compared by r_{xy} and R_{xy} with five *P. p. orientalis* from North Korea and seven *P. p. orientalis* from the Russian Far East. Individuals in each group that had unknown relationships were assumed to be unrelated.

Genetic variation in the 7 wild *P. p. orientalis* from the Russian Far East was also compared with genetic variation in 10 Florida panthers (*Puma concolor coryi*), 10 Gir Forest lions (*Panthera leo persica*), 10 Ngorongoro lions (*Panthera*

Table 1. Wild-caught and captive-born *P. p. orientalis* samples used in the study

| Ppa no. ^a | Status/studbook no. ^b | SB-2 genes (%) ^c | mtDNA haplotype ^d | Place of origin | Source |
|----------------------|----------------------------------|-----------------------------|------------------------------|------------------|---|
| 149 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 150 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 151 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 152 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 153 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 156 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 157 | W | — | Ori2 | Russian Far East | Hornocker Wildlife Institute, H. Quigley |
| 138 | NK/136 | 0.00 | Ori1 | North Korea | Moscow Zoo, V. Spitsin |
| 140 | NK/211 | 0.00 | Ori2 | North Korea | Moscow Zoo, V. Spitsin |
| 142 | NK/212 | 0.00 | Ori2 | North Korea | Moscow Zoo, V. Spitsin |
| 144 | NK/193 | 0.00 | Ori2 | North Korea | Tallin Zoo, V. Fainstein |
| 158 | NK/142 | 0.00 | Ori2 | North Korea | Berlin Tierpark Zoo, Blaszkiewitz |
| 46 | C/196 | 31.25 | Jap2 | Captive born | St. Louis Zoological Park, R. Junge |
| 47 | C/187 | 53.12 | Jap2 | Captive born | St. Louis Zoological Park, R. Junge |
| 55 | C/143 | 53.12 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 56 | C/117 | 56.25 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 58 | C/81 | 50.00 | [Ori2] | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 59 | C/123 | 50.00 | [Ori2] | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 60 | C/144 | 53.12 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 61 | C/228 | 54.69 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 62 | C/226 | 54.69 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 63 | C/208 | 54.69 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 64 | C/176 | 53.12 | Jap2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 65 | C/190 | 25.00 | Ori2 | Captive born | Mulhouse Zoological Park, J. M. Lernoould |
| 68 | C/82 | 0.000 | Ori1 | Captive born | Frankfurt Zoological Park, R. Faust |
| 69 | C/101 | 50.00 | [Ori2] | Captive born | Frankfurt Zoological Park, R. Faust |
| 72 | C/225 | 25.00 | Ori2 | Captive born | Frankfurt Zoological Park, R. Faust |
| 139 | C/206 | 28.12 | [Ori2] | Captive born | Moscow Zoo, V. Spitsin |
| 141 | C/135 | 31.25 | [Jap2] | Captive born | Moscow Zoo, V. Spitsin |
| 143 | C/122 | 50.00 | Ori2 | Captive born | Moscow Zoo, V. Spitsin |
| 145 | C/109 | 50.00 | Ori2 | Captive born | Tallin Zoo, V. Fainstein |
| 146 | C/183 | 25.00 | Ori2 | Captive born | Tallin Zoo, V. Fainstein |
| 207 | C/195 | 31.25 | Jap2 | Captive born | Denver Zoological Park, R. Cambre |
| 208 | C/191 | 40.62 | [Ori2] | Captive born | Denver Zoological Park, R. Cambre |

^a Ppa: identification number of leopard individuals as they are listed in the specimen collection at the Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD, 21702.

^b Status of leopard: W, wild caught and released; C, captive bred; NK, North Korea, status unknown; studbook number of each captive bred leopard (Shoemaker 1997).

^c Percent of genome estimated to be derived from leopard SB-2 from pedigree in Figure 2.

^d mtDNA haplotype assigned to each sample sequenced in the present or previous (Uphyrkina et al. in press) studies. Haplotypes in brackets were imputed from maternal lineages or offspring as in Figure 2 based on *International Amur Leopard Studbook* (Shoemaker 1997).

leo leo), and 20 African cheetah (10 *Acinonyx jubatus raineyi* and 10 *A. j. jubatus*), species known to show low genetic variation and fitness loss (Packer et al. 1991; Roelke et al. 1993; Wildt et al. 1987). Genetic variation among five species was compared in terms of polymorphism (P), expected heterozygosity (H_e), average number of alleles (A), average range of microsatellite repeats (R), and microsatellite variance (V) across 16 microsatellite loci (*FCA 008*, *FCA 026*, *FCA 43*, *FCA 75*, *FCA 77*, *FCA 90*, *FCA 94*, *FCA 98*, *FCA 105*, *FCA 126*, *FCA 139*, *FCA 161*, *FCA 224*, *FCA 229*, *FCA 247*, *FCA 310*) amplified in all five species (Driscoll et al. 2002; Uphyrkina et al. 2001).

Results

Phylogenetic Analysis of

P. p. orientalis Populations

Three separate groups of *P. p. orientalis* were examined: ORI-RFE, 7 wild-caught

individuals from the wild population in the Russian Far East; ORI-NK, 5 North Korean leopards held in European zoos originating from North Korea; and ORI-C, 22 individuals from the captive population. ORI-RFE plus ORI-NK are considered as the wild population, ORI-W. Composite microsatellite genotypes of each of these individuals were analyzed using phylogenetic algorithms in the context of 63 leopards from other leopard subspecies (Uphyrkina et al. 2001). Minimum evolution trees (using *Dps* and *Dkf* genetic distance estimators) are presented in Figure 3.

The phylogenetic analyses of 25 microsatellite loci demonstrate monophyletic cluster distinctions among subspecies corresponding to geographic locale, as previously reported (Uphyrkina et al. 2001). In addition, there occurred a monophyletic clustering of the captive (ORI-C) plus wild-born (ORI-W) *P. p. orientalis* individuals relative to other subspecies

(Figure 3). The ORI-RFE leopards clustered together as a sister cluster with ORI-NK (Figure 3B). (A single Russian leopard, Ppa-151, clustered within the ORI-NK group; Figure 3B).

The ORI-C group fell as a polyphyletic grouping positioned between the ORI-W group and the Chinese subspecies *P. p. japonensis* (Figure 3). The phylogenetic distinction between ORI-C and ORI-W was evident in F_{ST} (0.19; $P < .01$) and R_{ST} (0.15; $P < .01$) estimates between the populations. Within the captive population, the percent genetic representation of founder SB-2 in each individual increases with phylogenetic distance from the wild-born *P. p. orientalis* group (correlation index, $\beta = 0.604$, $P < .05$; Figure 3B). These observations are consistent with the suspicion that captive population founder SB-2 (Figure 2) was not an authentic *P. p. orientalis* (Christie and Arzhanova 1999a).

Sequence of mtDNA in Captive-Born

P. p. orientalis

Two mitochondrial regions, NADH-5 gene (611 bp) and control region (116 bp), were sequenced in 16 captive *P. p. orientalis* leopards (Table 1). Three mtDNA haplotypes, all previously described (Uphyrkina et al. 2001), were found among captive-born *P. p. orientalis* leopards: (1) Ori1, previously seen in one wild-born leopard (Ppa-138) originating from North Korea, now found in a single captive leopard (Ppa-68) whose parents were born in the Russian Far East; (2) Ori2, a common haplotype found in all wild-born leopards from the Russian Far East (Shoemaker 1997) and in most leopards from North Korea; and (3) Jap2, a haplotype identical to one of two found previously in *P. p. japonensis* leopards. Thus, the three haplotypes found in captive-born *P. p. orientalis* were identical to those seen in wild-born *P. p. orientalis* (Ori1, Ori2) and *P. p. japonensis* (Jap2).

The Jap2 mitochondrial genotype is present in several family groups of the captive population and by female lineage tracking can be traced to the female founder SB-89 (Figure 2). Thus, at least two founders of the sampled captive pedigree (SB-2 male and SB-89 female) have genetic information more consistent with *P. p. japonensis* than any wild-born specimens of *P. p. orientalis*. This observation lends support to a history of genetic admixture between *P. p. orientalis* and *P. p. japonensis* in the captive pedigree.

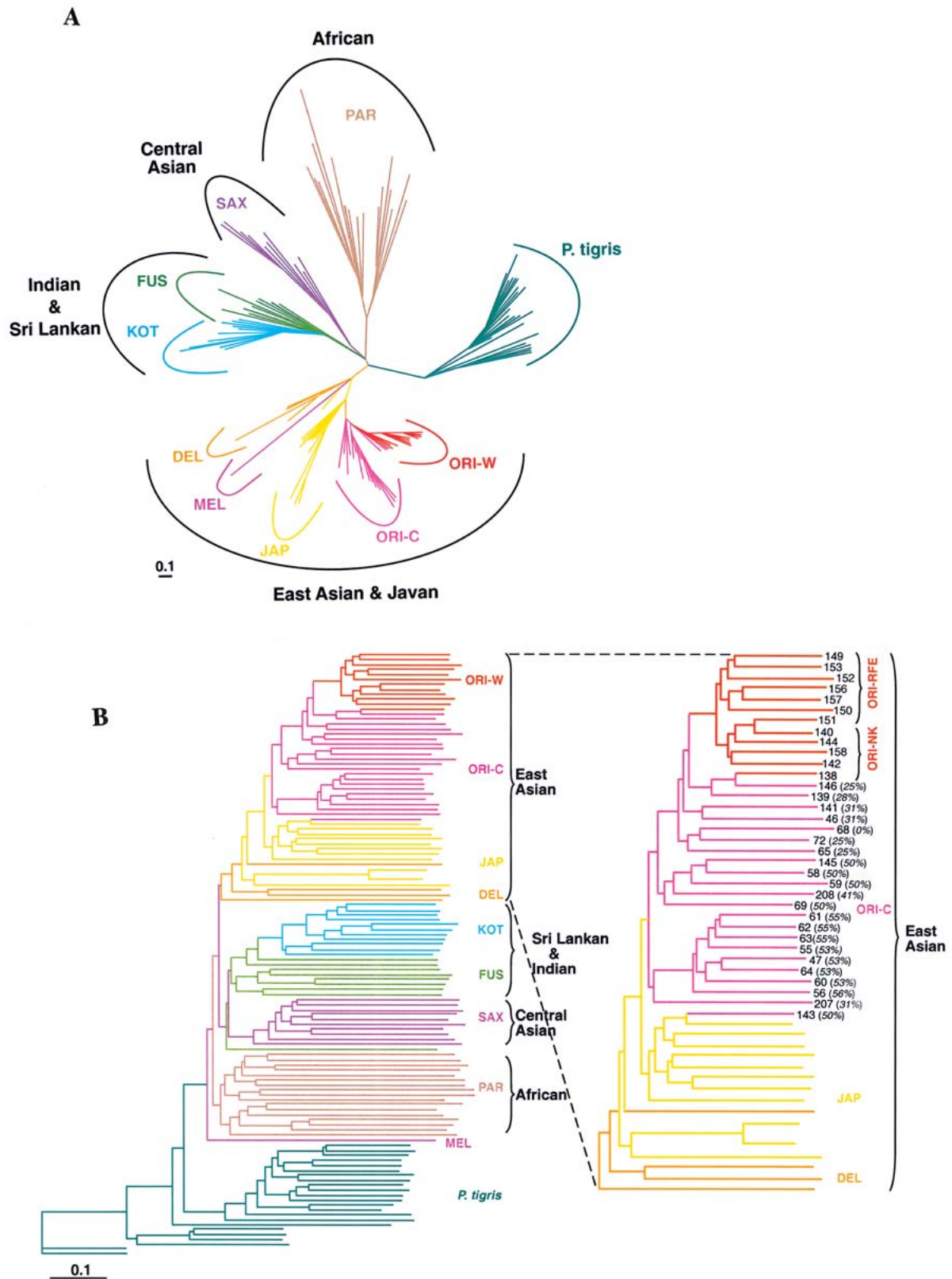


Figure 3. Phylogenetic relationships among the individual leopards based on composite genotype of 25 microsatellite loci. The same color branches represent leopard individuals of a particular subspecies shown by three-letter codes (see Table 2): **(A)** tree constructed based on proportion of shared allele genetic distances (D_{ps}) using $-\ln(p_s)$ transformation; **(B)** tree constructed based on kinship coefficient genetic distances (D_{kf}) using $1-(k_f)$ transformation. Relationships between wild *P. p. orientalis* (shown in red), captive *P. p. orientalis* (shown in pink), *P. p. japonensis* (shown in yellow), and *P. p. delacourii* (shown in orange) are highlighted in the larger view. Numbers shown are Ppa numbers assigned to each leopard (Table 1). Percentages in parentheses indicate the percentage of genetic complement derived from founder SB-2 calculated based on pedigree (Figure 2).

Table 2. Genetic variation in *P. pardus* populations across 25 microsatellite loci^a

| Subspecies ^b | Population | No. leopards | Polymorphic loci (%) (<i>P</i>) | Mean <i>H_o</i> (SE) | Mean no. alleles/locus (<i>A</i>) | Min-max no. alleles | Mean effective no. alleles (<i>E</i>) | Mean range rep/locus (<i>R</i>) | Microsatellite variance (<i>V</i>) |
|------------------------------------|------------|--------------|-----------------------------------|--------------------------------|-------------------------------------|---------------------|---|-----------------------------------|--------------------------------------|
| <i>P. p. orientalis</i> (RFE + NK) | ORI-W | 12 | 92 | 0.365 (0.222) | 2.60 | 1–4 | 1.8 | 2.84 | 1.71 |
| | RFE | 7 | 80 | 0.402 (0.235) | 2.32 | 1–4 | 1.7 | 2.72 | 1.59 |
| | NK | 5 | 76 | 0.320 (0.245) | 2.20 | 1–4 | 1.7 | 2.36 | 1.70 |
| <i>P. p. orientalis</i> (captive) | ORI-C | 21 | 100 | 0.490 (0.129) | 3.12 | 2–6 | 2.3 | 3.64 | 2.38 |
| <i>P. p. japonensis</i> | JAP | 15 | 100 | 0.478 (0.171) | 3.76 | 2–7 | 2.6 | 4.44 | 3.70 |
| <i>P. p. delacouri</i> | DEL | 4 | 100 | 0.850 (0.126) | 4.20 | 2–6 | 3.4 | 5.56 | 5.70 |
| <i>P. p. kotiya</i> | KOT | 11 | 96 | 0.500 (0.202) | 3.52 | 1–7 | 2.3 | 4.58 | 4.25 |
| <i>P. p. fusca</i> | FUS | 9 | 100 | 0.685 (0.144) | 5.52 | 2–9 | 3.9 | 6.20 | 5.38 |
| <i>P. p. saxicolor</i> | SAX | 10 | 100 | 0.610 (0.083) | 4.24 | 2–7 | 3.0 | 5.12 | 4.28 |
| <i>P. p. pardus</i> | PAR | 17 | 100 | 0.783 (0.076) | 8.52 | 5–15 | 5.7 | 9.72 | 7.28 |

^a *P*, percent polymorphic loci; *H_o*, average observed heterozygosity; *A*, average number of alleles per locus observed; *E*, average effective number of alleles per locus; *R*, range of allele size expansion in repeat motif number; *V*, average microsatellite variance in allele expansion breadth.

^b RFE, Russian Far East; NK, North Korea.

Genetic Variation

Diminished microsatellite genetic diversity relative to other leopard subspecies was apparent in *P. p. orientalis*, that is, in both ORI-NK and ORI-RFE, but not in the captive *P. p. orientalis* (Table 2). For microsatellites, the wild-born *P. p. orientalis* showed the lowest average heterozygosity (0.365), mean number of alleles per loci (2.60), effective number of alleles (1.8), mean range of microsatellite repeats (2.84), and microsatellite variance (1.71) compared to other subspecies. This genetic diminution is also reflected in mtDNA sequence variation, where π for wild *P. p. orientalis* (ORI-W) was 0.02 (SE = 0.04) compared to 1.22 (SE = 0.67) for the African subspecies *P. p. pardus* (Uphyrkina et al. 2001). The captive population of *P. p. orientalis* had greater microsatellite variation than the wild samples in all measures, but examination of the distribution of microsatellite alleles among ORI-W, ORI-C, and *P. p. japonensis* (Figure 4) reveal that the ORI-C population's microsatellite diversity is largely derived from a genetic mixing of *P. p. orientalis* and *P. p. japonensis* alleles. Thus, 13 of the ORI-C captive population's microsatellite alleles are shared with wild *P. p. orientalis*, 17 with *P. p. japonensis*, and 47 with both subspecies, and only one allele is unique to the captive population. These results add further credence to the scenario that the captive population is a genetic admixture of *P. p. orientalis* and another subspecies, likely the adjacent *P. p. japonensis*.

Comparative Relatedness Analysis Among Leopard Populations

To quantify the relative extent of inbreeding among leopard subspecies, levels of relatedness among seven *P. p. orientalis* from the wild Russian Far East population (ORI-RFE) were compared

with seven wild-born *P. p. fusca* (FUS) leopards from northern India and with seven wild-born *P. p. kotiya* (KOT) leopards from Sri Lanka. *P. p. kotiya* leopards have been shown previously to have reduced genetic variation relative to mainland *P. p. fusca*, likely a reflection of an historic island population founder effect (Miththapala et al. 1991). The leopards were compared in terms of pairwise microsatellite genotype relatedness values (r_{xy}) among individuals in each of three subspecies.

The distributions of relatedness values (r_{xy}) for all pairwise combinations within the three subspecies populations are shown in Figure 5A. Pairwise relationships in each population appeared to distribute into three categories: leopards “least related” to each other (the first peak), leopards “more related” to each other (the second peak), and leopards “most related” to each other (the third peak). *P. p. fusca* leopards appear to be the most outbred, *P. p. orientalis* are the most inbred, and *P. p. kotiya* were intermediate. Relatedness values (r_{xy}) between the “least related” *P. p. fusca* ranged from 0.20 to 0.50; between the “least related” *P. p. kotiya* from 0.45 to 0.75, and between the “least related” *P. p. orientalis* from 0.60 to 0.90 (Figure 5A). Distribution of r_{xy} among most closely related *P. p. fusca* leopards (r_{xy} ranged from 0.75 to 0.85) coincided with the distribution of “unrelated” *P. p. orientalis* leopards. Relatedness calculated as average across all possible pairs in each subspecies revealed *P. p. fusca* to be identical by 40.7% (SE = 4.0%), *P. p. kotiya* by 66.2% (SE = 4.7%), and *P. p. orientalis* from the remaining wild population in the Russian Far East by 77.5% (SE = 4.0%).

The same analysis performed for *P. p. orientalis* from the Russian Far East and North Korea analyzed separately pro-

duced a similar distribution of r_{xy} (Figure 5B). The percent microsatellite identity among the last leopards reached 75.8% (SE = 5.2%). The r_{xy} distribution in captive *P. p. orientalis* was much broader (from 0.25 to 0.95) than in wild populations (Figure 5B). Captive leopards were related by an average of 57.5% (SE = 3.2%), consistent with the presumption of their derivation from genetic mixing of founder individuals from different subspecies. These relatedness comparisons should be tempered by the contingency that all the leopards were indeed “unrelated,” a possibility difficult to prove with small sampling of five and seven individuals.

Comparison of Genetic Variation With Other Felidae Species

The estimated genetic diversity in wild *P. p. orientalis* from the Russian Far East was also compared with that observed for 16 microsatellite loci in selected Felidae populations previously shown to be genetically compromised with apparent physiological fitness costs (Driscoll et al. 2002; O'Brien et al. 1985; Roelke et al. 1993; Wildt et al. 1987). The extent of microsatellite genetic variation in *P. p. orientalis* was comparable to that observed in Florida panthers, a severely handicapped population with several physiological correlates that are attributed to inbreeding during population reduction during the 20th century (Roelke et al. 1993) (Figure 6). Measures of genome diversity in *P. p. orientalis* were only slightly greater than the Asian lion population, the most extremely inbred felid population observed to date (Driscoll et al. 2002; Gilbert et al. 1991; Wildt et al. 1987). The relatively high values for *A*, *R*, and *V* in African cheetahs are interpreted as reflecting a much longer period of microsatellite allele reconstitution since the defining genome

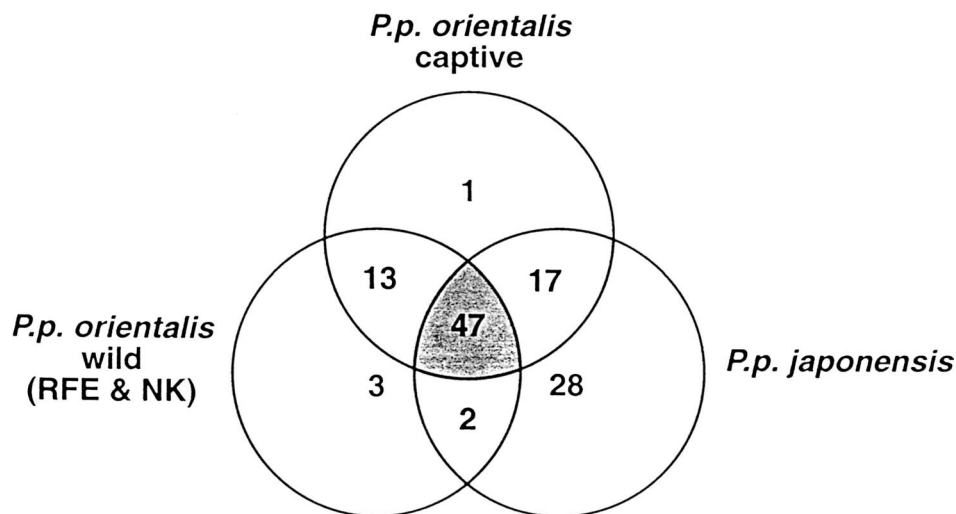


Figure 4. Venn diagram for the number of microsatellite alleles shared or unique from three leopard populations.

homogenizing bottleneck, while the Ngorongoro Crater elevation reflects a recent incomplete founder effect (Driscoll et al. 2002; Packer et al. 1991) (Figure 6).

Discussion

The small relict populations of *P. p. orientalis*, sampled here from the Russian Far East and from North Korea, displayed remarkably reduced genomic diversity relative to other leopard subspecies similarly studied (Uphyrkina et al. 2001). The low level of population-specific alleles in *P. p. orientalis* ($N_{sp} = 3$; Figure 4) as compared to *P. p. japonensis* ($N_{sp} = 28$) would suggest that this genetic depletion reflects a historic founder effect in isolating *P. p. orientalis*, more recently exacerbated by close inbreeding in the small isolated population. The Russian population is fewer than 40 individuals and has remained at this level for more than 30 years (Korkishko and Pikunov 1994; Miquelle et al. 1996). The estimated relatedness (r_{xy}) between apparently unrelated individuals ($r_{xy} = 60\text{--}90\%$; Figure 5) was comparable to that of close relatives from more outbred subspecies, consistent with a recent history of close inbreeding. A single mitochondrial DNA haplotype in 11 of 12 sampled Far Eastern leopards (Table 1) plus highly diminished quantities of microsatellite allele diversity (Table 2 and Figure 6) reveals a population afflicted with genetic depletion.

The levels of genetic depletion observed among *P. p. orientalis* were as

extreme as those observed in the genetically impoverished Florida panther (*Puma concolor coryi*) and Gir Forest lion (*Panthera leo persica*) subspecies, and lower than the Ngorongoro lions and African

cheetahs (Figure 6). Each of these feline populations has been shown to suffer from varying congenital and reproductive abnormalities that correlate with their history of close inbreeding (O'Brien et al. 1985; Packer et al. 1991; Roelke et al. 1993; Wildt et al. 1987). There is little evidence to date that the wild *P. p. orientalis* population in the Russian Far East displays physiological or reproductive impairments derived from inbreeding (except litter size reduction; Pikunov and Korkishko 1992; Pikunov et al. 1999b); however, detailed physiological assessments have not yet been conducted.

The low genetic diversity and high level of relatedness (Figure 5B) among North Korean leopards may reflect close relationships among the captive-bred founders from North Korea. Alternatively, if the founders were not close relatives, the surviving leopards in North Korea, if they still exist, may also be experiencing inbreeding in a small population. Two of 25 microsatellite loci were monomorphic when Russian and Korean leopards were considered together

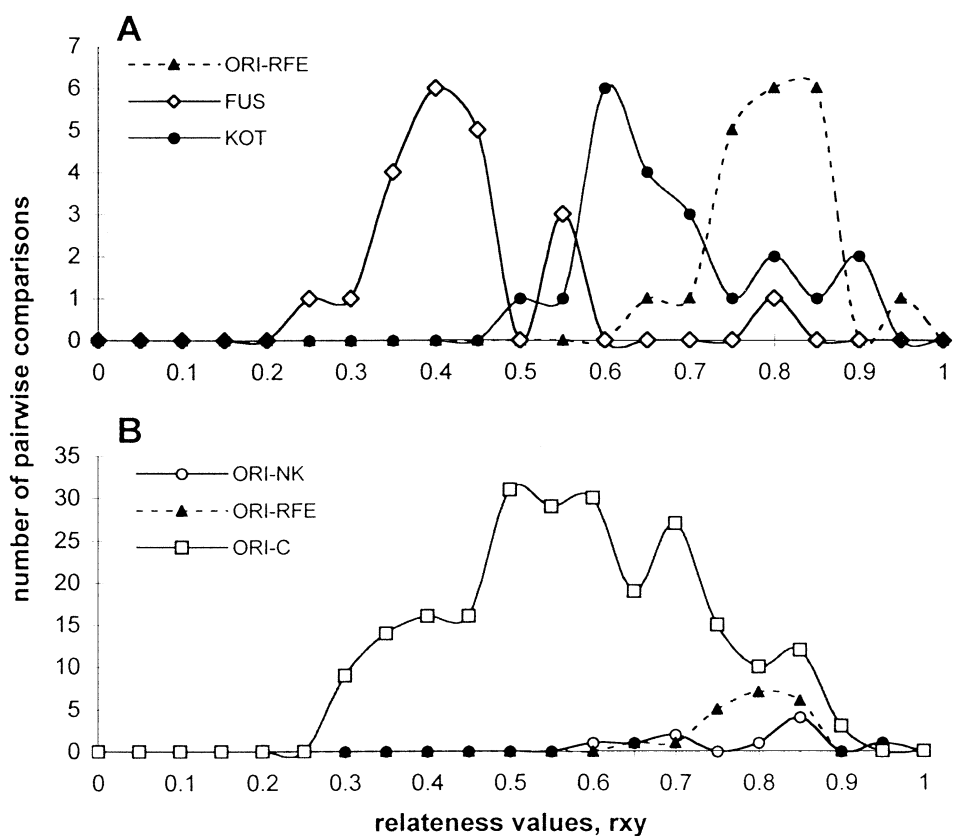


Figure 5. Distribution of composite microsatellite genotype relatedness values, r_{xy} , in pairwise comparisons of leopards: (A) distribution of r_{xy} in *P. p. orientalis* from Russian Far East (ORI-RFE), *P. p. fusca* from the northern part of India (FUS), and *P. p. kotiya* from Sri Lanka (KOT) (Uphyrkina et al. in press); (B) distribution of r_{xy} in *P. p. orientalis* from North Korea (ORI-NK), *P. p. orientalis* from Russian Far East (ORI-RFE), and captive *P. p. orientalis* (ORI-C).

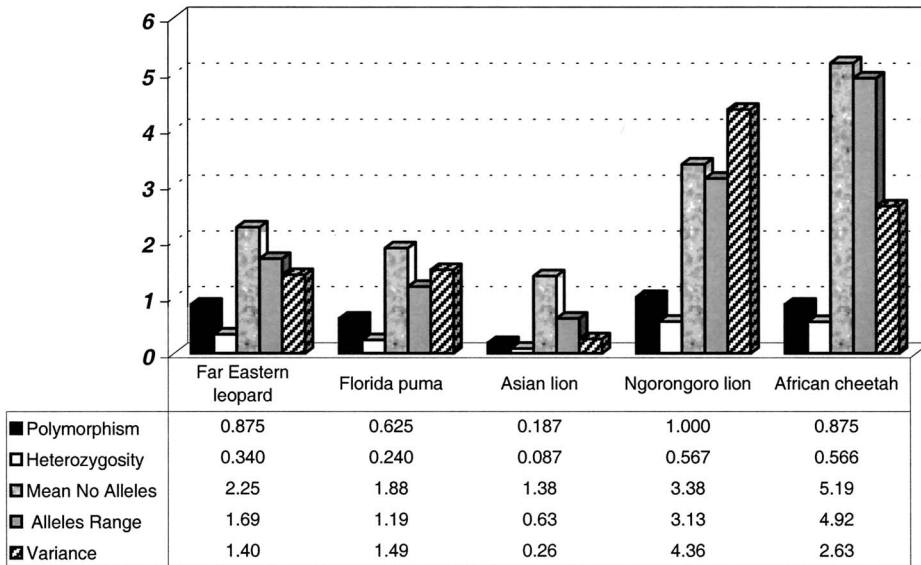


Figure 6. Estimates of microsatellite diversity in *P. p. orientalis* and in other Felidae species populations previously studied with the same microsatellite markers (Driscoll et al. 2002). Genetic indices: *P* (polymorphism), H_e (expected heterozygosity), *A* (average number of microsatellite alleles per locus), *R* (range of microsatellite allele expansion), and *V* (microsatellite variance). Values for each genetic parameter are shown.

er (ORI-W), while 5 and 6 loci were monomorphic when ORI-RFE and ORI-NK were considered separately. Apparently both ORI-RFE and ORI-NK populations show evidence of several generations of close inbreeding subsequent to their separation. Because the physical isolation of these populations is rather recent—that is, during or after World War II and the Korean War (Stephens 1994)—the populations are genetically very similar and not distinct from each other by genetic parameters ($F_{ST} = 0.033$, $P = .138$; $R_{ST} = 0.058$, $P = .108$), although both show evidence of additional allelic loss since their recent isolation.

The Captive Population of *P. p. orientalis*

Several lines of evidence support the suggestion that the relatively healthy captive population is derived from an inadvertent genetic mixing of authentic *P. p. orientalis* and another population, likely *P. p. japonensis*. These include (1) phylogenetic analysis of composite microsatellite genotypes which show the captive individuals not interspersed among wild-caught specimens, rather representing a paraphyletic cluster intermediate between wild *P. p. orientalis* and *P. p. japonensis* (Figure 3); (2) an apparent relationship between the fraction of SB-2 genetic contribution and phylogenetic similarity to *P. p. japonensis*

(Figure 3B); (3) the observation that nearly all (99%) of the microsatellite alleles observed in the captive population are also found in either wild *P. p. orientalis* (17%), *P. p. japonensis* (22%), or both (60%) (Figure 4); (4) the occurrence of three mtDNA haplotypes among captive individuals, two (Ori1, Ori2) found in wild *P. p. orientalis* and the third (Jap2, attributable to a single female founder, SB-89; Figure 2) in *P. p. japonensis* (Table 1); and (5) that relative genetic diversity in the captive population is high compared to wild ORI-RFE or ORI-NK populations (Table 2) and is likely a consequence of the subspecies mixing. Taken together, these data affirm the suspicion that the captive population (Figure 2) included at least two founders (SB-2 and SB-89) potentially derived from subspecies other than *P. p. orientalis*.

Conservation Implications

The fragile remaining population (or populations, if leopards still survive in North Korea; Figure 1) of *P. p. orientalis* is at risk due to both genetic impoverishment with associated inbreeding depression and demographic threats of small populations. Stochastic unpredictable calamities such as severe winters, prey deprivation, human depredation, and disease outbreaks could rapidly drive a small population to extinction. The primary goal is to increase the popula-

tion number and suitable habitat for the threatened subspecies before one or any of these perils destroys them. The native population should not be considered as a source for capture to supplement the captive population because any reduction in situ would be detrimental, and because the wild population would not augment the genetic endowment of the captive group.

The captive population appears to derive from gene flow from two subspecies, *P. p. orientalis* and likely *P. p. japonensis*. The rapid expansion and genetic outcrossing probably has increased relative population fitness as has been observed in both lion and puma subspecies admixtures (McBride 2001; O'Brien et al. 1987; Roelke et al. 1993). In these cases, hybridization among subspecies improved fitness based on reproductive and physiological measurements. A similar assessment of the captive and wild “pure” *P. p. orientalis* would be valuable to assess the physiological consequences of inbreeding.

Whatever such measures reveal, we should consider that very recently, probably less than a few hundred years ago, the native ranges of the two parent subspecies had an overlap and the inadvertent subspecies intercrosses characterizing the captive breeding program will parallel natural gene flow which occurred in situ rather recently. As such, the captive population should be considered as an acceptable representative for the wild leopards of Northeast Asia and not an aberrant mongrel population. The conservation managers of this species should strive to maximize genetic representation within the captive population as well as in the wild population. If an opportunity to supplement the existing population(s) or to establish new populations occurs, captive animals may be considered as suitable migrants that would improve the genetically depauperate wild population.

The Far Eastern leopard (*P. p. orientalis*) is a morphologically and genetically distinct leopard subspecies (Nowell and Jackson 1996; Uphyrkina et al. 2001) and should be considered to be an appropriate legal unit for conservation. From this perspective, conservation efforts should strive to save the integrity of subspecies. However, when a subspecies is severely threatened by both genetic and demographic impoverishment, genetic augmentation/restoration strategies should

be carefully considered as a rescue strategy. A similar consideration and conservation restoration action in the face of near-certain extinction of the Florida panther relict populations (Alvarez 1993; Roelke et al. 1993) would merit close inspection by those who choose to conserve the Amur leopard.

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