



Applying molecular genetic tools to the conservation and action plan for the critically endangered Far Eastern leopard (*Panthera pardus orientalis*)

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Abstract

A role for molecular genetic approaches in conservation of endangered taxa is now commonly recognized. Because conservation genetic analyses provide essential insights on taxonomic status, recent evolutionary history and current health of endangered taxa, they are considered in nearly all conservation programs. Genetic analyses of the critically endangered Far Eastern, or Amur leopard, *Panthera pardus orientalis*, have been done recently to address all of these questions and develop strategies for survival of the leopard in the wild. The genetic status and implication for conservation management of the Far Eastern leopard subspecies are discussed. **To cite this article:** O. Uphyrkina, S.J. O'Brien, C. R. Biologies 326 (2003).

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Résumé

Application des outils de génétique moléculaire à la conservation et au plan de sauvegarde d'une espèce en grave danger, le léopard d'Extrême-Orient (*Panthera pardus orientalis*). Le rôle des approches de la génétique moléculaire dans la conservation des espèces menacées d'extinction est généralement reconnu. L'analyse génétique, qui assure la compréhension nécessaire du statut taxinomique, détermine la viabilité actuelle et prédit le destin futur des taxons menacés, est dans l'agenda de presque tous les programmes de la conservation et la restauration. Pour répondre à ces questions et élaborer la stratégie de la survie dans l'état naturel du léopard d'Extrême-Orient ou d'Amur (*Panthera pardus orientalis*), qui représente une espèce gravement menacée d'extinction, l'analyse génétique a été effectuée. L'état génétique et la gestion ensuite dans des programmes de la conservation et la restauration concernant des sous-espèces sont discutés. **Pour citer cet article :** O. Uphyrkina, S.J. O'Brien, C. R. Biologies 326 (2003).

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1. Introduction

The Far Eastern leopard (*Panthera pardus orientalis*) is one of nine recently recognized subspecies of leopard [1,2], yet is almost extinct in the wild. This subspecies, that only a hundred years ago occupied North-eastern China, the southern part of the Russian Far East and the whole Korean peninsula today survives in the wild as only a single population of 25–40 animals in the southwestern part of the Primorsky Kray, Russia [3]. Habitat loss and alteration resulting from direct human developments, poaching and illegal markets have led to dramatic consequences. The subspecies is well represented in captivity; however all but ten of the almost 200 captive leopards descend from a founder mixture of *P. p. orientalis* that also included at least one male of another subspecies [4]. The other ten leopards were brought recently to European zoos from the Zoo of Pyongyang and are thought to possibly represent another population of the subspecies in the wild in North Korea, although reliable information concerning the origin of these animals is lacking.

To prevent the immediate extinction of this leopard in the wild, the subspecies survival and conservation program were developed by group of international cat specialists during their first international meeting in Vladivostok, Russia in November 1996 [5]. The Far Eastern leopards from the wild had never been studied genetically; and its taxonomic status has remained unsolved after a recent genetic survey that used the captive individuals has remained uncertain [1]. The size of the Primorsky Kray population has been stable for the 30 years since it became isolated from another two populations that disappeared by end of 1970s [6]. Following the common fate of all small isolated populations, the leopards must have undergone a close inbreeding that may have resulted in inbreeding depression as a direct consequence of genetic depletion. Detailed physiological and reproductive studies of the wild leopards have not been undertaken, partly due to enormous difficulties in catching these remaining animals in the wild, partly due to disagreement among ecologists concerning the conservation strategy and necessity of such an analysis. Meanwhile, a decrease in litter sizes over the years (from about 2 in 1973 to 1.0 in 1997) has been noted by field biologists [6]. Therefore, for conservation and restoration efforts it was essential to resolve the taxonomic sta-

tus of the Far Eastern leopard and its relation to other leopard subspecies and to estimate the level of genetic depletion in the remaining population. A captive population, established in zoos and private collections throughout the world, represents a back-up population of the subspecies, but is suspected to be of hybrid origin [4]. Thus the origin of the founders also had to be determined by genetic analysis.

2. Material and methods

The complete genetic status of the Far Eastern leopard in the wild and captivity has become clear through a few steps of genetic assessment. First, a molecular genetic survey of all leopard subspecies was performed using 25 microsatellite feline-specific loci and sequence comparison of two mtDNA segments (NADH-5, 611 bp and CR, 116 bp). This analysis revealed subspecies level distinctiveness for the wild Far Eastern leopards [2]. The study has provided the basis for recognition of nine leopard subspecies distinguished at evolutionary and genetic levels, with the Far Eastern leopard, *P. p. orientalis*, being one of the nine. Further *P. p. orientalis* was shown to be close genetically to the North Chinese leopard, *P. p. japonensis*, likely evolving from it rather recently [2,7].

As the next step, we have undertaken genetic analysis of seven leopards available from the Primorsky Kray population, 5 leopards from North Korea and 22 leopards from the captive zoo population maintained worldwide [9].

3. Results and discussion

We found that all seven leopards from the Primorsky Kray population possessed a single mitochondrial haplotype, designated Ori2. The North Korean leopards had two haplotypes, Ori1 and Ori2. Twenty-two captive leopards (21 of which were descended from the leopard-founder with unknown origin – SB2, or studbook number 2) had three haplotypes: Ori1, Ori2, and Jap2. The third captive haplotype, Jap2, was identical to a common haplotype found in *P. p. japonensis* leopards [2]. A pedigree analysis of the haplotype Jap2 transmission in the captive population indicated its origin from a single founder-female SB-89.

Table 1
Microsatellite diversity in leopard (*Panthera pardus*) and some other cat populations

Subspecies	Polymorphism	Average heterozygosity	Average number alleles/locus	Average range repeat/locus	Microsatellite variance	Relatedness, R_{xy} (%)
(1) <i>P. p. orientalis</i> (a)	0.80/0.88*	0.341/0.340*	2.32/2.25*	2.72/1.66*	1.59/1.4*	77.5
(2) <i>P. p. orientalis</i> (b)	0.76	0.322	2.20	2.36	1.70	75.8
(3) <i>P. p. orientalis</i> (c)	1	0.521	3.12	3.64	2.38	57.5
(4) <i>P. p. pardus</i>	1	0.803	8.52	9.72	7.28	–
(5) <i>P. p. fusca</i>	1	0.696	5.52	6.2	5.38	40.7
(6) <i>P. p. kotiya</i>	0.96	0.485	3.52	4.58	4.25	66.2
(7) <i>P. p. japonensis</i>	1	0.549	3.76	4.44	2.70	–
(8) <i>Puma concolor coryi</i>	0.63	0.240	1.88	1.19	1.49	–
(9) <i>Panthera leo persica</i>	0.19	0.087	1.37	0.62	0.25	–
(10) <i>Panthera leo leo</i> **	1	0.567	3.37	3.13	4.36	–

(a) Seven leopards from the Primorsky Kray population, the Russian Far East; (b) five leopards from North Korea; (c) 22 leopards from the managed zoo (captive) population; see the text.

(1)–(7) Diversity summarized across the same 25 microsatellite loci [2].

(1)*, (8)–(10) Diversity summarized across the same 16 microsatellite loci [7].

** Ngorongoro population.

By analysing 25 microsatellite loci in all three *P. p. orientalis* populations in a phylogenetic context with leopards from other subspecies, we found that wild leopards from the Primorsky Kray population and North Korean leopards comprise together a monophyletic group and genetically are not distinguished between each other [7]. This suggests that the two groups have become separated in the very recent past, perhaps less than a hundred years ago. The phylogenetic position of all captive leopards was intermediate between wild *P. p. orientalis* and *P. p. japonensis*. Additionally, leopards carrying a greater portion of leopard SB2's genes clustered close to *P. p. japonensis* and leopards carrying a lesser portion of leopard SB2's genes clustered close to wild *P. p. orientalis*. These data have led us to the hypothesis that the leopard with unknown origin, SB2, most probably belonged to the *P. p. japonensis* subspecies. Analysis of STR allele distribution in the captive leopards allocated all alleles to within wild *P. p. orientalis*, or within *P. p. japonensis* or within both of them, confirming our hypothesis. Thus, we clarify another very important question for the conservation program – genetic status of the captive population. We confirmed that the population has a mixed origin, but this mixture is due to interbreeding founders of two subspecies, *P. p. orientalis* and its close neighbor *P. p. japonensis*.

To estimate the relative genetic health of all *P. p. orientalis* populations we have compared indices of genetic variation in *P. p. orientalis* populations (1) with populations of other leopard subspecies, and (2) with populations of other cat species known to have low genetic variation and loss of fitness. Then, we estimated relatedness values between individuals in *P. p. orientalis* populations and compared to those in some other leopard subspecies. The Primorsky Kray population and the North Korea group both showed lower genetic variation than any of the other leopards subspecies examined: there was no mtDNA genetic variation among living animals; and all STR diversity parameters were considerably decreased (Table 1). A considerable portion of microsatellite alleles had frequencies less than 0.1 or more than 0.9, suggesting that the populations can lose additional variation in a short period of time due to genetic drift. Microsatellite variation in these two groups was comparable to variation in the genetically depauperate populations of Florida panther and Asiatic lions that show the severe fitness cost of genetic depletion caused by close inbreeding [8,9]. The captive population of the Far Eastern leopards, despite its origination from only 9 founders [4], revealed an appreciable level of mtDNA and STR diversity, comparable with populations of some other leopard subspecies (Table 1). The high

fecundity of the captive population may have been explained by subspecies hybridization, the positive outcome of which has been already shown for big cats [10,11].

Relatedness values, estimated based on microsatellite allele frequencies [12], varied in wild *P. p. orientalis* from 60% to 90% confirming our worst expectation: the leopards have been exposed to close inbreeding for a number of generations. The results of this analysis have led to one of the major conclusions in our study: the last viable population remaining in Russia is dominated by very close relatives. Similarly, leopards in North Korea (if they still exist) have been surviving at a very small number and also must have experienced close inbreeding. This extraordinary low level of genetic diversity by parallels to genetically compromised populations [8,10] would raise the prospect that physiological and reproductive abnormalities might be occurring in the remaining leopards. Indeed, three kittens recently born in captivity from North Korean founders had either bone deformity or reproductive abnormalities [13].

In May 2001, the Far Eastern leopard second international workshop took place in Vladivostok, where international cat specialists and local authorities, based on recent ecological and genetic studies, developed further recommendations for conservation of the Far Eastern leopard in the wild. It was agreed that the remaining Primorsky Krai population is at considerable risk due to both genetic impoverishment with associated inbreeding depression and demographic threats of small populations. Primary conservation efforts should be concentrated around creation of additional population(s) in the wild, perhaps in areas of the leopard's former range. A suitable source of potential leopards for reintroduction seemed to be leopards from the managed zoo population that due to accidental hybridization appeared to be more genetically diverse and viable than the animals from the wild. However, because they are hybrid, some argued that they could not be released into the wild. According to them, to preserve the integrity of a morphologically and genetically distinct leopard is a major goal of the conservation program.

The Far Eastern leopard, *P. p. orientalis*, is a genetically unique subspecies of leopard [2]. This leopard is particularly distinguished among the otherwise tropical species, since it is the only subspecies adapted

to a cold snowy climate. From this perspective, conservation efforts should strive to save the integrity of the subspecies. However, the single remaining population is severely threatened by both genetic and demographic impoverishment, and a rescue strategy with genetic augmentation/restoration should be considered as a major priority. The captive population that derived from gene flow of two neighboring subspecies, *P. p. orientalis* and *P. p. japonensis*, should be taken as an acceptable and genetically beneficial source of leopards for potential reintroduction. Perhaps only a hundred years ago the two subspecies had a common border in their natural habitats, and the accidental subspecies interbreeding in the captive population would be equal to natural gene flow between subspecies. The captive leopards would maximize genetic diversity of homogenized wild leopards strengthening their health and fitness and helping to withstand the environmental changes. A similar conservation strategy was used for restoration from near certain extinction of the Florida panther relict population [8,14], and results of such conservation efforts have exceeded many expectations [11].

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