Neutral and adaptive genetic variation in Indian snow leopards, *Panthera uncia*

Tirupathi Rao Golla[1,2,3], Laura Tensen[2,4], Vipin[1], Kesav Kumar[1], Satish Kumar[5] and Ajay Gaur[1,6,*]

[1]Laboratory for the Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology, Annexe I, Attapur Ring Road, Hyderabad 500 048, India
[2]Centre for Ecological Genomics and Wildlife Conservation, Department of Zoology, University of Johannesburg, Auckland Park, 2006, South Africa
[3]Oceanographic Research Institute, South African Association for Marine Biological Research, 1 King Shaka Avenue, Point, Durban 4001 KwaZulu-Natal, South Africa
[4]Institute for Integrated Natural Sciences, Zoology, University Koblenz-Landau, Koblenz, Germany
[5]CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India
[6]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201 002, India

In this study, we reveal patterns of genetic variation in snow leopards (*Panthera uncia*) by combining neutral (mtDNA, microsatellites) and adaptive (MHC II-DRB) genes. We collected 56 faecal samples from three locations in India. We observed moderate levels of microsatellite diversity (N = 30; A = 5.6; HO = 0.559). Nine unique MHC II-DRB sequences were identified in four snow leopard samples, of which 8 were novel. We found low levels of polymorphism in MHC class II-DRB exon, which was higher in captive (V_A = 9.4%) compared to wild individuals (V_A = 7.8%), likely as a result of a population bottleneck.

Keywords: Adaptive evolution, balancing selection, captive breeding, genetic diversity, major histocompatibility complex.

ASSESSING patterns of genetic variation in species is essential for identifying management units and setting conservation priorities[1]. The risks of inbreeding and genetic drift are also raised, which result in reduced heterozygosity and allelic richness[2]. The combined losses of genetic variability have several negative consequences at the population level, such as lessened adaptive potential, reduced individual fitness, high frequencies of detrimental gene variants and reduced reproductive fitness[3]. These genetic complications, as well as adaptation to captivity are also a concern for captive breeding programmes, as they could interfere with a potential contribution to conservation[4]. Another genetic concern of captive breeding that complicates its use for species conservation is the hybridization of established subspecies[5].

When assessing the genetic diversity of wild or captive populations, neutral markers such as microsatellites are often used because they effectively detect recent changes in population structure[6]. However, adaptive genes are more informative for the evolutionary potential of species, as they have a direct impact on fitness and are under strong selection[7]. The best documented adaptive genes belong to the major histocompatibility complex (MHC), which involves immune responses and disease resistance[8]. MHC variability is maintained by balancing selection and therefore reflects evolutionary potential[3]. In carnivores, high MHC diversity is often maintained even when neutral variation is depleted due to a population bottleneck[9,10].

Assessing patterns in adaptive variation is particularly important for rare species with a limited geographic range because anthropogenic factors can alter the relative importance of neutral and selective forces that shape genetic variation[11]. Snow leopards (*Panthera uncia*) are vulnerable to extinction because of their limited distribution and low population densities[12,13]. The estimated population size of snow leopards in the wild is 2500 to 3500 adults[14], and roughly 600 snow leopards are kept in zoos, of which eleven are in India (Central Zoo Authority, India Inventory 2019–20). Approximately 500 snow leopards live in the Indian Himalayas[15], of which 73 occur in Himachal Pradesh[16]. Based on deep genetic divergence and lack of admixture, three subspecies have been proposed[17]: (i) Northern/Altai region (*P. u. irbis*), (ii) Western/trans-Himalaya region (*P. u. uncia*) and (iii) Central Himalaya/Tibetan Plateau (*P. u. uncioides*).

The genomic diversity of snow leopards is nearly half that of the other *Panthera* species[18], due to a bottleneck event that took place in the Holocene[17,19]. An earlier study concluded that the genetic diversity in Indian snow leopards was moderate to low[20], although little remains known about the adaptive genetic variation of this population. No data are available to relate low diversity to genetic deficiencies, but it has been remarked that mortality in captive snow leopards has been substantial due to diseases such as the common feline virus[21]. Parasitic infections also appear to be more common in captive snow leopards (28%) compared to other felids (4–23%)[22]. Furthermore, captive snow

*For correspondence. (e-mail: agaur@ccmb.res.in)

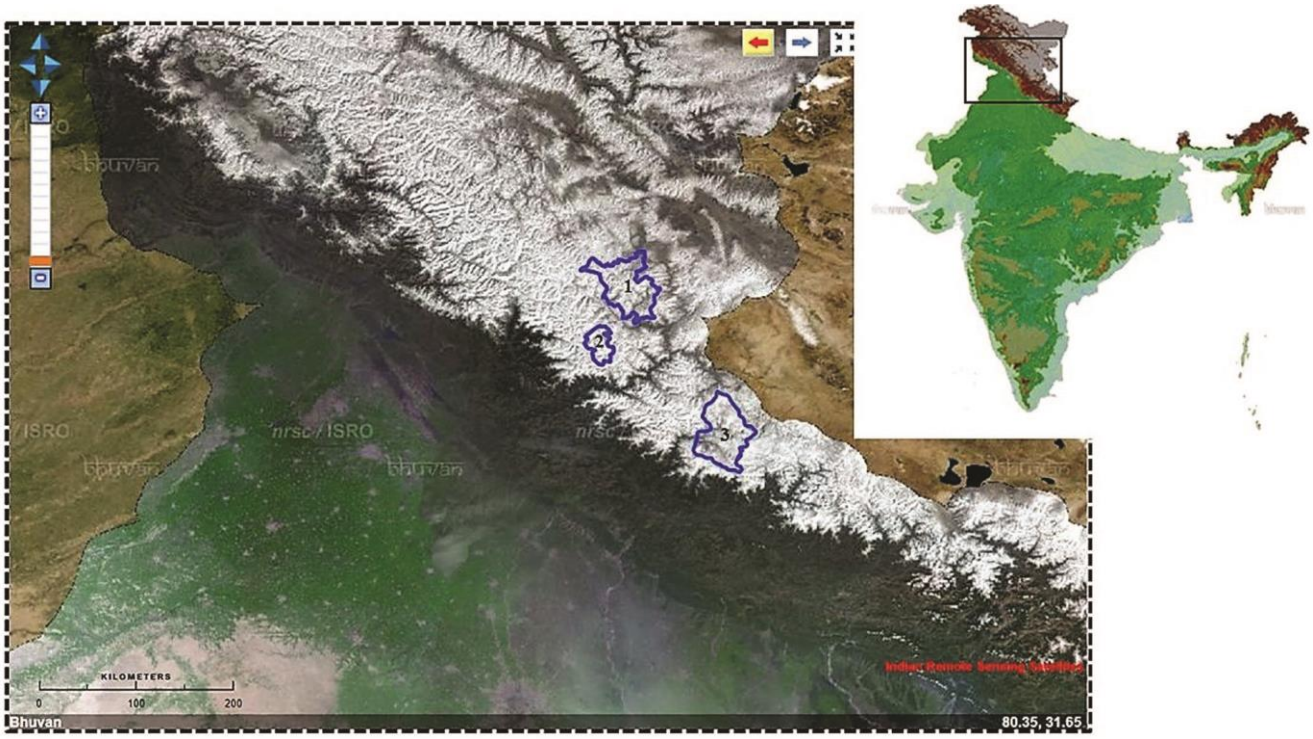

Figure 1. Snow leopard samples collected from the Kibber Wildlife Sanctuary (1) and Pin Valley National Park (2), and Himachal Pradesh and Gangotri National Park (3), Uttarakhand, India. The boundaries of the national parks and the wildlife sanctuary are shown in blue colour. Black dotted lines show the area represented by the black colour rectangle in the inset. The modified images from 'Bhuvan', the Indian Geo-Platform of Indian Space Research Organisation (ISRO) (https://bhuvan.nrsc.gov.in/home/index.php) were used to make the figure.

leopards have a high proportion of malformed spermatozoa[23], which can be a sign of inbreeding depression.

Determining which populations suffer genetic declines is critical for long-term survival by identifying dispersal corridors in the wild and selection of founders in captivity[24]. To this end, we collected non-invasive faecal samples from three regions in northern India (Figure 1) and screened for variation at both neutral (mitochondrial DNA (mtDNA) and microsatellite) and adaptive (MHC) genetic markers. We also used cloning and sequencing of the class II-DRB gene of the MHC to identify the genetic diversity and putative phylogenetic relationship in wild and captive snow leopards. This allowed us a better understanding of the impact of captivity on polymorphism in adaptive genes. This study is the first to assess MHC diversity in snow leopards to date. In total, this study addressed three questions: (i) what is the level of genetic variation in Indian snow leopards; (ii) how are neutral and adaptive diversity in snow leopards spatially structured; and (iii) is there evidence of genetic differentiation, possibly as a result of different selective pressures, between wild and captive snow leopards?

Methods

In total, 56 faecal samples were collected in three different locations in India: (i) Kibber Wildlife Sanctuary (N = 20) and (ii) Pin Valley (N = 20) in Himachal Pradesh, and (iii) Gangotri National Park (N = 16) in Uttarakhand (Figure 1). The scats were collected between an altitude of 3000 and 4600 m. Blood samples were also collected from two captive individuals housed at the Padmaja Naidu Himalayan Zoological Park in India. Genomic DNA was isolated from the samples and stored at –20°C. The quality of the DNA was checked using agarose gel electrophoresis and in a NanoDrop ®ND-1000 spectrophotometer (Wilmington, USA), and the concentration was equalized between faecal and blood samples before downstream analysis.

We amplified 4 segments of the mitochondrial control region Panthera uncia-hyper variable sequence (PUN-HVS) and cytochrome *b* Panthera uncia-central conserved region (PUN-CCR) and genotyped 7 physically unlinked microsatellite loci, which have been used by previous studies[17] and thus allowed for comparison. We also amplified exon 2 (β1 domain) of the MHC class II-DRB loci[25]. Between 5 and 10 recombinant plasmids were screened for each captive and wild individual. Each clone was subsequently used for colony PCR, and 8–10 randomly picked clones were sequenced in a 3730 DNA analyser (ABI, USA). Nucleotide polymorphisms found within clones derived from a single individual are caused by the multigenic nature of MHC[25]. For laboratory procedures and primer details, see supplementary material.

mtDNA sequences and microsatellite loci were checked and aligned with Geneious 6.1.5 (Biomatters Ltd). Microsatellite alleles were tested for genotypic errors, linkage

Table 1. Genetic diversity indices of snow leopard (*Panthera uncia*) in India, based on microsatellite data, compared to previous studies from India[20] and Nepal[13]

	N	*A*	A_P	H_O	H_E	F_{IS}	*r*
India	30	5.6	–	0.559 ± 0.978	0.684 ± 0.050	0.185	0.105
Kibber Wildlife Sanctuary	15	4.6	0.77	0.516 ± 0.172	0.688 ± 0.092	0.257	0.106
Pin Valley	12	3.4	0.33	0.549 ± 0.236	0.597 ± 0.091	0.084	0.125
Gangotri National Park	3	3.6	1.17	0.786 ± 0.284	0.729 ± 0.23	–0.100	0.000
India[20]	34	5	–	0.539 ± 0.038	0.723 ± 0.18	0.242	0.124
Nepal[13]	20	8.2	–	0.475 ± 0.072	0.642 ± 0.076	0.276	–

A, Number of alleles; A_P, Private allelic richness, H_O, Observed heterozygosity; H_E, Expected heterozygosity; F_{IS}, Inbreeding coefficient ($1 - H_O/H_E$); *r*, Relatedness; ±, Standard deviation.

Table 2. Nucleotide diversity metrics at MHC II-DRB loci in wild and captive snow leopards

	N	*A*	*V*	*C*	*S*	π	V_N (%)	V_A (%)
Wild	2	2	9	185	9	0.026 (SD 0.004)	4.6	7.8
Captive	2	8	11	183	11	0.025 (SD 0.003)	5.7	9.4

N, Number of individuals; *A*, Number of alleles; *V*, Variable sites; *C*, Conserved sites; *S*, Parsimony-informative sites; π, Nucleotide diversity; SD, Standard deviation; V_N, Nucleotide variation; V_A, Amino acid variation.

disequilibrium and deviation from Hardy–Weinberg equilibrium (HWE) and significance after Bonferroni correction. We calculated the inbreeding fixation index (F_{IS}) and other genetic diversity indices, as well as pairwise relatedness. We also conducted population structure analysis and principal component analysis (PCA). For MHC II-DRB, sequence analysis was carried out using the ABI (USA) software Sequencing Analysis v5.2 and CodonCode Aligner v5.0.1. The sequences were aligned with the published MHC II-DRB region of snow leopard available in Genbank (accession number FJ210684)[26]. New alleles were named based on a four-letter code of the species' scientific name (Paun for *Panthera uncia*) plus a unique code. To estimate the phylogenetic relationship between captive and wild snow leopards, as well as among other Felidae species, we applied a Bayesian Inference (BI) method, and measured the number of synonymous (dS) and non-synonymous substitutions (dN) per site for wild and captive snow leopards separately (for detailed data analysis and genetic software, see Supplementary Material).

Results

Of the 56 faecal samples that were collected, we could distinguish 30 unique individuals. These 30 individuals were successfully genotyped for 4 mtDNA segments and 7 microsatellite loci (see Supplementary Table 3 for microsatellite scores). We did not observe linkage disequilibrium between the microsatellite loci; however, we observed deviations from the HWE ($P < 0.05$) in 3 out of 7 loci. This has been observed in snow leopards before and was concluded to be a result of the Wahlund effect. We also found possible null alleles in one locus (PUN100). After removing PUN100, we did not observe major differences in our results. Due to the limited number of loci used in this study, we decided to include them for downstream analysis. As a result of the low DNA quantity, which is common for non-invasive samples, only two individuals were successfully cloned for MHC II-DRB, which were compared to two captive snow leopards. We acknowledge that our sample size is limited, so the results should be interpreted carefully.

Genetic diversity

We obtained 30 sequences of 4 mtDNA segments but found no haplotype variability. All microsatellite loci were polymorphic, with 4 to 7 alleles per locus (Supplementary Table 4) across all populations. We found moderate levels of genetic variation in Indian snow leopards ($A = 5.6 \pm 1.3$; $H_O = 0.559 \pm 0.978$) and significant inbreeding coefficients ($F_{IS} = 0.185$). It is notable that despite the small sample size, Gangotri National Park has the highest private allelic richness and heterozygosity (Table 1). The average relatedness in the study population was 0.105 ± 0.168, which is typical for half-siblings.

We identified 9 unique MHC II-DRB sequences for snow leopards, of which 8 were novel because Paun-DRB*201 was previously described (FJ210684)[26], which were added to the Genbank database (Supplementary Table 2). The first of the Paun*GNP2 variants (i.e. sequence variants among clones) was unique to wild snow leopards, whereas all other variants were found in captive individuals only. The number of variants was 2 in wild individuals and 8 in captive individuals, and the nucleotide diversity did not vary between the two samples (Table 2). For all alleles, 9 and 11 nucleotide sites were polymorphic in wild and captive snow

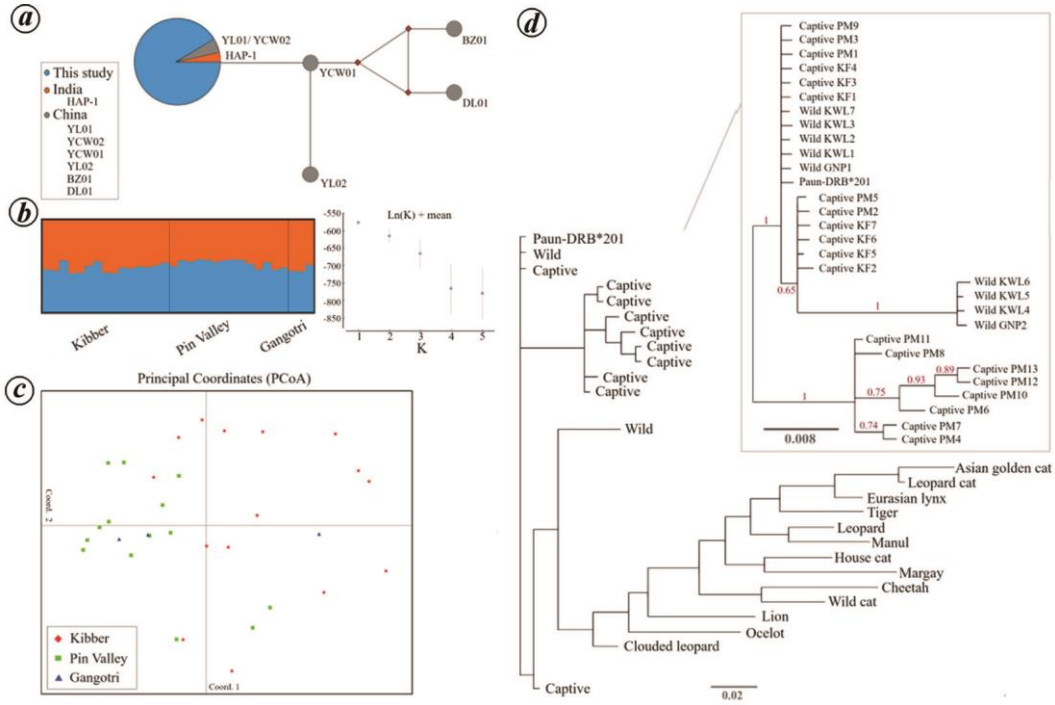

Figure 2. Spatial population structuring of snow leopard. ***a***, Haplotype network using mtDNA control region, including haplotypes from the Lahaul and Spiti region, India[20] and the Anhui Province, China[35]. ***b***, Bayesian clustering patterns of snow leopards in India at $K = 2$, based on STRUCTURE analysis, ***c***, Principal coordinates analysis (PCoA) of three sampling localities, ***d***, Bayesian phylogeny of snow leopard using MHC II-DRB alleles of wild and captive snow leopards, and a comparison with other Felidae species.

leopards respectively. Of the MHC sequences, a putative amino acid translation was made, which involved 64 codon positions (Supplementary Figure 1). Of these, 5 and 6 amino acid codons were variable for wild and captive snow leopards respectively. In total, 5 out of the 10 codon variations were positioned in peptide-binding sites (PBS; Supplementary Figure 2). The amino acid variation was lower in wild individuals ($V_A = 7.8\%$) compared to captive individuals ($V_A = 9.4\%$).

Population structure

We did not detect population genetic structure in Indian snow leopards based on 643 bp mtDNA control region and 139 bp of cytochrome *b*. When a haplotype network was built using the control region, we could see that the haplotype found in this study had been previously found in India (Figure 2 *a*). Based on microsatellite loci, we did not detect any population structure using either the Bayesian clustering method (Figure 2 *b*) or PCA (Figure 2 *c*). As for the PCA, the first coordinate explained 15.5% of the genetic variation, and the second coordinate explained 13.6% of the variation.

A Bayesian tree was constructed to clarify the phylogenetic relationship of MHC II-DRB alleles in wild and captive snow leopards (Figure 2 *d*). Even though the most common allele (Paun-DRB*201) was shared among both samples, all other alleles were unique to either wild or captive individuals. Out of 20 mutations, only one was shared between the populations. The average number of nucleotide differences between wild and captive snow leopards was 7.26, and the genetic distance (F_{ST}) between the populations was 0.332 ($P = 0.04$).

Selection

The rates of non-synonymous (d_N) and synonymous (d_S) substitutions were calculated for wild and captive snow leopards separately, and *Z* tests of selection at the MHC II-DRB loci were performed (Supplementary Table 5). For wild individuals, no selective hypothesis was tested significant, but purifying selection appeared more probable than positive selection. For captive individuals, significant positive selection was observed (d_N–$d_S = 1.86$; $P = 0.03$). We found 4 amino acids under positive selection in snow leopards, of which 2 codon sites were confirmed by all methods (Supplementary Table 6).

Discussion

This study is the first to compare levels of neutral and adaptive diversity in Indian snow leopards. We found no diversity at the mitochondrial markers, as also reported by Janecka *et al.*[17]. It was hypothesized that this could be the

result of adaptation to high altitudes or a sign of adaptive introgression after hybridization of diverged lineages[12]. A study by Singh *et al.*[20] also found only one haplotype in northern India, which corresponded to ours. We detected moderate microsatellite variation in snow leopards, likely due to their restricted range and low population densities, and a population bottleneck that occurred approximately 6000–8000 years ago[17,20]. We found no population structure, which is not surprising considering their high dispersal ability that extends far over 500 km (ref. 27). Gangotri National Park had the highest private allelic richness and heterozygosity, which could be due to its eastern location bordering a distinct population in Nepal[13].

The moderate level of neutral genetic diversity in snow leopards was consistent with adaptive MHC variability, for which we found only 5% nucleotide and 8% amino acid variation. When comparing the MHC diversity metrics of snow leopards to other large cats, snow leopards appeared relatively poor in diversity. For instance, in Indian leopards, the nucleotide variation was on an average 22%, and amino acid variation 38% (ref. 25). Furthermore, nucleotide diversity was 4 times higher in tigers ($\pi = 0.10$)[28] when compared to our estimate of snow leopards ($\pi = 0.025$). Genetic diversity was also found to be low in other large cat species that have undergone a bottleneck, such as the Amur leopard[29] and cheetah[30]. However, polymorphism varies strongly between different species, and evolutionary processes are very different in adaptive and neutral genes, due to which MHC diversity metrics cannot be directly compared[31].

Our results show higher polymorphism at MHC II-DRB in captive individuals compared to wild individuals, which contradicts the expected trend[5]. Captive populations normally have lower levels of genetic diversity because of the small number of founders, genetic drift and inbreeding[4]. A possible explanation is that hybridization of distinct evolutionary lineages would have occurred in captivity[32]. It is unclear where captive snow leopards have originated from, but it is possible that all lineages are represented in the zoo population, which would increase the number of alleles in captive individuals[11]. However, our results could also be an artefact of the small sample size and possible allelic dropout due to differences in DNA quality between samples. Future research is required to clarify whether subspecies hybridization has occurred in captivity or not.

When inferring the phylogenetic relationships of the MHC II-DRB sequences, we found 2 major clades and 4 distinct subclades that were most often sample specific. Hence, genetic differentiation appeared to have occurred between captive and wild snow leopards ($F_{ST} = 0.33$; $P = 0.04$). However, due to the small sample size, it could also be the result of copy number variation. Only the most common allele (Paun*201) was present in both samples (i.e. wild versus captive). All felid species were clustered and closely related to the MHC II-DRB allele (Paun*GNP2), which showed a common origin[33]. The tree topology also suggested the occurrence of trans species polymorphism, which indicates that balancing selection occurs at MHC II-DRB[8]. Balancing selection is an evolutionary process in which alleles that are important for gene functioning are maintained without fixation[34]. Balancing selection of MHC II-DRB alleles has previously been observed in large cats[25,26].

In the two captive snow leopards, the overall number of non-synonymous substitutions was higher than synonymous substitutions, which could be evidence of positive selection acting on MHC II-DRB loci[31]. In wild snow leopards, we did not find evidence of positive selection, possibly due to the small sample size. For wild individuals, we found indications of purifying selection in MHC II-DRB, which was also observed in Bengal tigers[28]. This indicates that conserved amino acid residues could also be essential for MHC functioning in snow leopards[34]. With respect to PBS, which is vital for MHC functioning and disease resistance, 50% of polymorphisms occurred in PBS in both populations. The low level of MHC variation observed in snow leopards does not necessarily mean that their immune competence is compromised, as the impact of MHC polymorphism on the long-term survival of bottlenecked species remains largely unclear[34]. Furthermore, the low sample size of this study makes it plausible that we might have overlooked MHC II-DRB alleles. We did find possible signs of genetic differentiation between wild and captive snow leopards, potentially as a result of subspecies hybridization, which highlights the need for more comprehensive research when the aim is to safeguard genetic diversity.

1. Frankham, R., Ballou, S. E. J. D., Briscoe, D. A. and Ballou, J. D., *Introduction to Conservation Genetics*, Cambridge University Press, Cambridge, 2002.
2. Willoughby, J. R., Fernandez, N. B., Lamb, M. C., Ivy, J. A., Lacy, R. C. and DeWoody, J. A., The impacts of inbreeding, drift and selection on genetic diversity in captive breeding populations. *Mol. Ecol*., 2015, **24**, 98–110.
3. Hedrick, P. W., Conservation genetics: where are we now? *Trends Ecol. Evol*., 2001, **16**, 629–636.
4. Woodworth, L. M., Montgomery, M. E., Briscoe, D. A. and Frankham, R., Rapid genetic deterioration in captive populations: causes and conservation implications. *Conserv. Genet*., 2002, **3**, 277–288.
5. Frankham, R., Genetic adaptation to captivity in species conservation programs. *Mol. Ecol*., 2008, **17**, 325–333.
6. Zimmerman, S. J., Aldridge, C. L. and Oyler-McCance, S. J., An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. *BMC Genomics*, 2020, **21**, 1–16.
7. Janjua, S. *et al.*, Improving our conservation genetic toolkit: ddRAD-seq for SNPs in snow leopards. *Conserv. Genet. Resour*., 2020, **12**, 257–261.
8. Klein, J., *Natural History of the Major Histocompatibility Complex*, Wiley, 1986.
9. Hedrick, P. W. and Kim, T. J., Genetics of complex polymorphisms: parasites and maintenance of the major histocompatibility complex variation. In *Evolutionary Genetics: From Molecules to Morphology* (eds Singh, R. S. *et al.*), Cambridge University Press, Cambridge, 2000, pp. 204–234.
10. Sachdev, M., Sankaranarayanan, R., Reddanna, P., Thangaraj, K. and Singh, L., Major histocompatibility complex class I polymorphism in Asiatic lions. *Tissue Antigens*, 2005, **66**, 9–18.

11. Marsden, C., Verberkmoes, H., Thomas, R., Wayne, R. and Mable, B., Pedigrees, MHC and microsatellites: an integrated approach for genetic management of captive African wild dogs (*Lycaon pictus*). *Conserv. Genet*., 2013, **14**, 171–183.
12. Li, X., Schuler, M. A. and Berenbaum, M. R., Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annu. Rev. Entomol*., 2007, **52**, 231–253.
13. Shrestha, B. and Kindlmann, P., Implications of landscape genetics and connectivity of snow leopard in the Nepalese Himalayas for its conservation. *Sci. Rep*., 2020, **10**, 1–11.
14. McCarthy, T., Mallon, D., Jackson, R., Zahler, P. and McCarthy, K., *Panthera uncia*. *IUCN Red List Threat*, Species, 2017, e-T22732A50664030.
15. Bhatnagar, Y. V. *et al*., South Asia: India. In *Snow leopards*, Elsevier, Academic Press, Amsterdam, The Netherlands, 2016, pp. 457–469.
16. Suryawanshi, K. *et al*., Estimating snow leopard and prey populations at large spatial scales. *Ecol. Solut. Evid*., 2021, **2**, e12115.
17. Janecka, J. E. *et al.*, Range-wide snow leopard phylogeography supports three subspecies. *J. Hered.*, 2017, **108**, 597–607.
18. Aruge, S., Batool, H., Khan, F. M. and Janjua, S., A pilot study – genetic diversity and population structure of snow leopards of Gilgit-Baltistan, Pakistan, using molecular techniques. *Peer J*., 2019, **7**, e7672.
19. Cho, Y. S. *et al*., The tiger genome and comparative analysis with lion and snow leopard genomes. *Nat. Commun*., 2013, **4**, 2433.
20. Singh, V. K., Singh, S. K., Joshi, B. D., Chandra, K., Sharma, L. K. and Thakur, M., Population genetics of the snow leopards (*Panthera uncia*) from the Western Himalayas, India. *Mamm. Biol*., 2022, **102**, 263–269.
21. Munson, L. and Worley, M., Veno-occlusive disease in snow leopards (*Panthera uncia*) from zoological parks. *Vet. Pathol*., 1991, **28**, 37–45.
22. Johnston, L., Armstrong, D. and Brown, J., Seasonal effects on seminal and endocrine traits in the captive snow leopard (*Panthera uncia*). *Reproduction*, 1994, **102**, 229–236.
23. Fitzpatrick, J. L. and Evans, J. P., Reduced heterozygosity impairs sperm quality in endangered mammals. *Biol. Lett.*, 2009, **5**, 320–323.
24. Storfer, A., Gene flow and endangered species translocations: a topic revisited. *Biol. Conserv*., 1999, **87**, 173–180.
25. Parmar, D. R., Mitra, S., Bhadouriya, S., Rao, T., Kunteepuram, V. and Gaur, A., Characterization of major histocompatibility complex class I, and class II DRB loci of captive and wild Indian leopards (*Panthera pardus fusca*). *Genetica*, 2017, **145**, 541–558.
26. Wei, K. *et al*., Lineage pattern, trans-species polymorphism, and selection pressure among the major lineages of feline Mhc-DRB peptide-binding region. *Immunogenetics*, 2010, **62**, 307–317.
27. Riordan, P., Cushman, S. A., Mallon, D., Shi, K. and Hughes, J., Predicting global population connectivity and targeting conservation action for snow leopard across its range. *Ecography*, 2016, **39**, 419–426.
28. Pokorny, I., Sharma, R., Goyal, S. P., Mishra, S. and Tiedemann, R., MHC class I and MHC class II DRB gene variability in wild and captive Bengal tigers (*Panthera tigris tigris*). *Immunogenetics*, 2010, **62**, 667–679.
29. Uphyrkina, O. *et al*., Phylogenetics, genome diversity and origin of modern leopard, *Panthera pardus*. *Mol. Ecol*., 2001, **10**, 2617–2633.
30. Charruau, P. *et al*., Phylogeography, genetic structure and population divergence time of cheetahs in Africa and Asia: evidence for long-term geographic isolates. *Mol. Ecol*., 2011, **20**, 706–724.
31. Yuhki, N. and O'Brien, S. J., Nature and origin of polymorphism in feline MHC class II DRA and DRB genes. *J. Immunol*., 1997, **158**, 2822–2833.
32. Zecherle, L. J. *et al*., Subspecies hybridization as a potential conservation tool in species reintroductions. *Evol. Appl*., 2021, **14**, 1216–1224.
33. Yuhki, N. and O'Brien, S. J., Nature and origin of polymorphism in feline MHC class II DRA and DRB genes. *J. Immunol*., 1997, **158**, 2822–2833.
34. Kaufman, J., Salomonsen, J. and Flajnik, M., Evolutionary conservation of MHC class I and class II molecules – different yet the same. *Semin. Immunol.*, 1994, **6**, 411–424.
35. Zhang, F., Jiang, Z., Zeng, Y. and McCarthy, T., Development of primers to characterize the mitochondrial control region of snow leopard (*Uncia uncia*). In *Genbank Submittion*, Institute of Zoology, Chinese Academy of Science, Beijing, 2007.

ACKNOWLEDGEMENTS. We acknowledge Director, CSIR-CCMB and Scientists In-Charge, LaCONES for their support and facilitation during the course of this study. We also thank Chief Wildlife Wardens, officials and field staff of forest departments of Himachal Pradesh and Uttarakhand for necessary permissions and support during sample collections. Curator and Veterinary Doctors of Padmaja Naidu Himalayan Zoological Park, Darjeeling, India, are also acknowledged for providing the captive snow leopard samples. We also acknowledge the Department of Biotechnology, India for funding this study.

Received 11 November 2022; revised accepted 25 April 2023

doi: 10.18520/cs/v125/i2/204-209