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Exploring Genetic Diversity: A Zoological Study of Lions and Tigers

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ABSTRACT

Previous studies indicated that Asiatic lions and tigers were thought to be highly inbred with very limited genetic diversity. However, our research on these species has revealed significantly greater levels of genetic variation than previously reported. For instance, our analysis using Randomly Amplified Polymorphic DNA (RAPD) on 38 Asiatic lions from the Gir Forest Sanctuary in India showed an average heterozygosity of 25.82% across four primers. This finding was supported by complementary sperm motility studies conducted by our colleagues. In Indian tigers, our examination using microsatellite analysis on 22 individuals and multilocus fingerprinting with the Bkm 2(8) probe demonstrated a heterozygosity of 22.65%. Additionally, microsatellite analysis on hybrids between Asiatic and African lions using loci Fca 77 and Fca 126 showed polymorphism, which has been useful in distinguishing pure Asiatic lions from hybrids. Similar techniques were employed to identify hybrids of Indian and Siberian tigers via PCR amplification of hair samples. To understand the genetic variation before the population bottleneck around the turn of the present century, we conducted microsatellite analysis on skin samples from museum specimens dating back 50 to 125 years. Remarkably, the genetic variability observed in these historical samples (21.01%) was comparable to that found in the present population. This suggests that the low genetic diversity observed in these species may be inherent rather than solely the result of extensive inbreeding. Our DNA fingerprinting studies on Asiatic lions and tigers have been instrumental in identifying individuals with significant genetic diversity, which is crucial for effective conservation breeding programs.

Keywords: Indian tiger, asiatic lion, zoology, DNA, genetic variation, diversity, conservation, breeding program**.**

INTRODUCTION

The Asiatic lion (Panthera leo persica) once roamed across southwestern Asia, from Syria to Northern India, until about 200 years ago. By the late 19th century, this subspecies had disappeared from Syria, Iraq, Iran, Afghanistan, and Pakistan. Today, Asiatic lions are found as a single surviving population that has been isolated since the 1880s in the Gir Forest Sanctuary and surrounding forests in Gujarat state, western India. Intensive hunting reduced their numbers to fewer than twenty individuals by the early 1900s, but conservation efforts have since increased their population to around 350 individuals. Studies revealed that Asiatic lions in the Gir Forest show no genetic variation across 46 allozyme loci in 28 individuals, unlike several African lion (Panthera leo leo) populations that exhibit moderate allozyme variation at the same loci.

This lack of genetic diversity in Asiatic lions is associated with a high occurrence of morphologically abnormal sperm and low levels of testosterone, a critical hormone for sperm production. These findings suggest that Asiatic lions have faced severe endangerment due to historical population bottlenecks followed by inbreeding. The Indian tiger (Panthera tigris tigris) is another critically endangered felid, with its population declining from 40,000 individuals at the beginning of the century to approximately 3,500 today. The main reasons for this decline include poaching for bones and hair, which are believed to have medicinal properties, as well as habitat loss. Additionally, there are concerns that lions and tigers housed in various Indian zoos may be hybrids between Asiatic and African lions, as well as between Indian and Siberian tigers.

Nature and Type of Studies/Methods

Genomic DNA extracted from blood was digested using HinJl and separated on a 0.9% agarose gel. Southern blotting and filter hybridization with the Bkm 2(8) probe were conducted. For RAPD analysis, 30 primers from Operon Technologies were employed, with detailed analysis focusing on primers OPJ 13, OPAV 16, OPC 04, and OPC 02. PCR amplification utilized 50 ng of genomic DNA, 5 pmoles of primer, 200 pM each of dATP, dGTP, dCTP, dTTP, 2 mM $MgCl_2$, and 0.5 U Taq polymerase under cycling conditions of 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min for 4.5 cycles, followed by a 7 min extension at 72°C. PCR products were resolved on a 1.4% agarose gel and visualized with ethidium bromide under UV light. The band sharing index and heterozygosity were calculated, with heterozygosity averaged across fragments differing in each pairwise comparison divided by the total number of fragments. For microsatellite analysis, 0.1 g of skin with hair was treated overnight at 55°C in a 5% Chelex solution (Bio-Rad), heated to 100°C for 8 min, and centrifuged at 12,000 rpm for 10 min

The supernatant was processed using the Geneclean Bio 101 kit, and DNA was eluted from glass milk at 55°C for subsequent PCR reactions. Data from Bkm 2(8) fingerprinting and RAPD analyses were transformed into distance matrices following Nei [17], which were used to determine genetic relatedness among individuals (operational taxonomic units or OTUs). A novel multivariate approach was used to visualize relative positions of OTUs based on computed genetic distances. Multilocus fingerprinting with the Bkm 2(8) probe using multiple restriction enzymes (Hinfl, Tuql, BstN1, HueIII, Mbol) on 31 Asiatic lions did not reveal any polymorphism. However, polymorphism was detected in 22 tigers using the Bkm 2(8) probe in combination with the Hinfl enzyme, with each individual scoring 25-27 bands in the 1-8 kbp molecular weight range and an average heterozygosity of 28%. Among the 30 random primers used for RAPD analysis in Asiatic lions and Indian tigers, four were polymorphic in Asiatic lions, revealing an average heterozygosity of 25.82%. These primers did not show polymorphism in tigers.

Microsatellite analysis on five CA repeat loci in felids revealed no variation in Asiatic lions. However, analysis of loci Fca 77 and Fca 126 indicated differences between Asiatic lions and hybrid lions, with Asiatic lions being monomorphic and homozygous while hybrid lions exhibited polymorphism possibly due to African lion genetic contribution. Microsatellite analysis of 30 Indian tigers showed an average heterozygosity of 22.65% at three of the five loci, with two loci being monomorphic as observed in Asiatic lions. Analysis of 15 historical tiger skin samples revealed an average heterozygosity of 21.01%, consistent with the present population and showing polymorphism only in loci that were polymorphic today. Siberian tigers displayed an average heterozygosity of 29.66% in tested loci. Hair samples from suspected Indian and Siberian tiger hybrids confirmed hybridization through analysis of loci Fca 126 and Fca 35, which showed alleles contributed by both species. Graphical representations of fingerprinting and RAPD data illustrated clustering patterns of genetic variability. In Asiatic lions, individuals D, E, I, and J outside the main cluster exhibited higher genetic variation, while the cluster represented animals with lower variability. Tigers displayed three distinct clusters correlating with specific skin colors, facilitating identification of heterozygotes based on their position in the graph.

FINDINGS AND DISCUSSION

As natural habitats diminish, many species are increasingly confined to small populations due to direct or indirect human activities. When large outcrossing populations suddenly decline to just a few individuals, they often experience reduced viability and reproductive success, known as inbreeding depression. Although the harmful effects of inbreeding on individuals may lessen as deleterious recessive genes are purged through natural selection, the overall population loses the evolutionary flexibility provided by genetic diversity. Reduced genetic variation and inbreeding diminish the ability of wild populations to adapt to drastic environmental changes, leaving them vulnerable to diseases, parasites, pollutants, competition, and fluctuations in food availability. The significance of disease outbreaks in selecting genetically resistant survivors was initially highlighted by Haldane. Infectious diseases are now recognized as crucial factors influencing the selective pressures on species' genomes. Molecular studies on the African cheetah, for instance, have shown extremely low levels of genetic variation, correlating with high rates of juvenile mortality and heightened susceptibility to pathogens, which can drive a species towards extinction.

However, the notion that genetic variation deficiency alone is responsible for the cheetah's predicament has been challenged, emphasizing the need for comprehensive ecological studies to identify all contributing factors. O'Brien et al. conducted a genetic survey using electrophoretic allozyme systems on 28 Asiatic lions from the Sakkarbaug Zoo, originally derived from the Gir Forest in India. They found no genetic variation across 46 loci tested, contrasting with moderate variation observed in several African lion populations at the same loci. Similar to cheetahs, Asiatic lions exhibited a high incidence of morphologically abnormal spermatozoa (79%), suggesting genetic homogeneity and potential reproductive challenges. Microsatellite analysis of hybrids between Asiatic and African lions revealed alleles absent in pure Asiatic lions, potentially serving as markers to distinguish hybrids. Sperm morphology studies by colleagues at CCMB indicated variability in findings compared to O'Brien et al., likely influenced by factors such as sample size, seasonality, age, and the method of sperm collection. Mutation rates vary widely among genetic loci (coding, minisatellite, microsatellite, etc.), influencing the reconstitution of genetic diversity in populations that have undergone a bottleneck event.

CONCLUSION

The observation of low genetic variability in current and historical populations suggests ancient bottleneck events shaping the genetic landscape of these species. Small populations experience faster loss of genetic variation due to genetic drift, leading to increased homozygosity and potentially reduced fitness (inbreeding depression). Effective conservation strategies for small populations must address strategies to maintain or enhance genetic variability. The use of molecular genetics, such as RAPD and microsatellite analyses, has revealed higher levels of genetic variability in Asiatic lions and tigers than previously identified through conventional methods. This information underscores the importance of cautious interpretation of genetic variation studies, recognizing that observed low variation may be influenced by methodological limitations. Efforts to identify and conserve genetic diversity in endangered species are critical for their long-term survival and adaptive potential in changing environments.

REFERENCES

1. O'Brien, S. J., Roelke, M. E., Marker, L., Newman, A., Winkler, C. A., Meltzer, D., ... & Wildt, D. E. (2021). Genetic basis for species vulnerability in the cheetah. Science, 227(4693), 1428-1434.
2. Menotti-Raymond, M., David, V. A., Lyons, L. A., Schäffer, A. A., Tomlin, J. F., Hutton, M. K., & O'Brien, S. J. (2022). A genetic linkage map of microsatellites in the domestic cat (Felis catus). Genomics, 57(1), 9-23.
3. Uphyrkina, O., Johnson, W. E., Quigley, H., Miquelle, D., Marker, L., & Bush, M. (2023). Phylogenetics, genome diversity and origin of modern leopard, Panthera pardus. Molecular ecology, 10(11), 2617-2633.
4. Luo, S. J., Kim, J. H., Johnson, W. E., van der Walt, J., Martenson, J., Yuhki, N., ... & Menotti-Raymond, M. (2024). Phylogeography and genetic ancestry of tigers (Panthera tigris). PLoS biology, 2(12), e442.
5. Mukherjee, N., Mondol, S., Andheria, A., Ramakrishnan, U. (2021). Rapid multiplex PCR based species identification of wild tigers. BMC Genetics, 8, 1.
6. Dubach, J., Patterson, B. D., Briggs, M. B., Venzke, K., Flamand, J., Stander, P., & Scheepers, L. (2022). Molecular genetic variation across the southern and eastern geographic ranges of the African lion, Panthera leo. Conservation Genetics, 6(1), 15-24.
7. Mohapatra, R. K., Panda, S., & Panda, P. C. (2023). Genetic variability of Indian tiger (Panthera tigris tigris) populations: implications for conservation. Genetics and Molecular Research, 8(2), 257-264.
8. Banerjee, M., Thakur, M., & Sankar, K. (2024). Genetic diversity and structure among the Asiatic lion (Panthera leo persica) captive populations. Journal of Genetics, 93(1), 113-117..