

The wolves of India: population genetics and evolutionary history of the world's oldest wolf lineages

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Research Location: (1) Central Indian grasslands, consisting of the Indian states of Maharashtra, Gujarat, Rajasthan, Karnataka, and Madhya Pradesh. (2) Trans-Himalayas consisting of the Indian states Jammu & Kashmir, Himachal Pradesh, and Sikkim. (see Map)

Project Description:

Background: Once found across the Northern Hemisphere, the gray wolf has been extirpated from much of its former range. The southernmost populations of the species, most notably the Himalayan and Indian wolves, harbor most of the genetic diversity and are the most evolutionarily ancient lineages (Figure 1). In fact, the antiquity of their mitochondrial-based (matrilineal) divergence hints that one or both of these wolves could represent distinct species¹, although genomic information (that is, reflecting all genes) is necessary to evaluate this fundamental question. Regardless of their ultimate classification, these unique southern populations also face the greatest danger of extinction.

Despite their evolutionary significance among the world's wolves, little is known about current population trends and distribution of Himalayan and Indian wolves, hindering their conservation. Based on a 1991 estimate, <3,000 Indian wolves remained in the wild, where they inhabit fragmented grasslands interspersed among agricultural landscapes.² Most Indian wolves reside outside of Protected Areas, where natural prey species are rare, resulting in high levels of wolf- livestock conflict and retaliatory killings.³ It is unknown whether subpopulations of Indian wolves are genetically isolated—potentially facing threats of inbreeding—or connected through long-distance movements and, if the latter, facilitated by what habitats or geographic corridors. While the Himalayan wolf faces similar threats, the most pressing issue is the lack in understanding of their distribution and genomic distinctiveness. To date, genetic identification of Himalayan wolves has been based on a single maternally inherited genetic marker and has been attempted in only a few locations in India, Nepal, and China (Figure 2). Consequently, neither their genomic makeup nor their current distribution are well characterized, presenting major obstacles to conservation efforts.

Although maternal genealogical analysis has provided a coarse outline of evolutionary history in wolves, emerging genomic methods are necessary to assess population genetic connectivity across the landscape and to resolve taxonomic questions. Most fundamentally, genomic tools will enable us to determine to what extent and for how long Himalayan and Indian wolves have been reproductively isolated from other wolves and whether particular genes unique to these populations (or species) confer specialized adaptations to their local environments.⁴ The same genetic tools enable measurement of gene flow and inbreeding among geographically disparate subpopulations of these wolves.

Objective: Using genomic methods, my research aims to address the following questions: **(Q1)** How much gene flow and what geographic routes connect Indian wolf subpopulations and how much genetic diversity has been lost to inbreeding within these subpopulations? **(Q2):** How genetically distinct are Himalayan and Indian wolves compared to other Asian wolf populations? The data collected from wolves in India will be part of a larger overarching study on the processes and patterns influencing the timing and pattern of diversification of wolves in Asia.

Study Area: Genetic sample collection will take place in the Central Indian grasslands and the Trans-Himalayas of India (Figure 2). Indian wolf genetic sampling will span the geographic regions of Maharashtra, Rajasthan, Karnataka, and Gujarat. Because the Wildlife Institute of India (WII) has current Himalayan wolf genetic samples from the Western Himalayas, genetic sampling expeditions will take place in the Eastern Himalayas, specifically in the Indian state of Sikkim.

Methods: Phase 1 (*June to Sept 2017*) will consist of building a collection of tissue and fecal genetic samples of Himalayan and Indian wolves for preliminary and future genetic analysis using two types of genomic marker: “microsatellites” (a track of repetitive DNA which varies among individuals) and “restriction-site associated DNA sequencing” (RAD-seq, a method that identifies thousands of single nucleotide polymorphisms across the genome). Specifically for **Phase 1**, I will (1) lead field efforts in collaboration with WII to collect fecal samples of wild Central Indian wolves in Maharashtra, (2) evaluate the quality and location of current fecal and tissue samples stored at WII to select key areas for further sampling, and (3) extract DNA from fecal and tissue samples and genotype DNA at microsatellites at the Conservation Genetics Laboratory on the WII campus. The microsatellite results from this summer and future RAD-seq analysis will involve standard phylogenomic and population genetics analyses to produce estimates of genetic effective populations sizes, gene flow, landscape resistance, time-calibrated divergence estimates, and historical demographic reconstructions.^{3,4} The results of these

analyses will enable us to determine the genetic diversity and genetic distinctiveness of Indian and Himalayan wolves to clarify their population status, taxonomy and evolutionary history.

Collaborators: I have support from Dr. Ben Sacks (my Ph.D advisor) at UC-Davis, who will aid in statistical analyses of genomic data and overall guidance for the Ph.D project. Additionally, I will be a research affiliate with the Wildlife Institute of India and collaborate with WII professors Dr. Bilal Habib and Salvador Lyngdoh. Both have current research projects on Indian and Himalayan wolves and will assist me in planning field logistics, acquiring permits, and incorporate research results into on-the-ground conservation initiatives in India. Additionally, I am collaborating with Geraldine Werhahn, who is a researcher focusing on Himalayan wolf research in Nepal through WildCRU (Oxford) and the Himalayan Wolves Project, which will enable us to evaluate genetic connectivity of Himalayan wolves across the Himalayan range.

References:

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- 2.) Jhala YV, Giles RH. 1991. The status and conservation of the wolf in Gujarat and Rajasthan, India. *Conservation Biology* 5(4):476-483.
- 3.) Singh M, Kumara HN. 2006. Distribution, status and conservation of the Indian gray wolf (*Canis lupus pallipes*) in Karnataka, India. *Journal of Zoology* 270:164-169.
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- 5.) Mondol S, Bruford M, Ramakrishnan U. 2013. Demographic loss, genetic structure, and the conservation implications for Indian tigers. *Proc R. Soc. Lond* 280: 20130496 doi:10.1098/rspb.2013.0496.

Project Budget:

A large majority of the funding provided by UKWCT grant money will be used for transportation, which includes a roundtrip plane ticket to and from U.S.A and India, train tickets to and from my field sites in Maharashtra and Delhi, as well as vehicle transportation while collecting wild Indian wolf genetic samples (i.e. scat and hair) at my

field sites in Maharashtra. In field, I will be recruiting and training residents of remote communities to join my field team to assist in collecting scat, hair, and tissue samples from key areas. Funding from the UKWCT grant will go towards a field salary for these residents, as well as lodging and food for myself and my local field team.

Lastly, I have a strong interest in on-the-ground conservation education programs in these remote communities living alongside wolves. This summer, I plan on giving presentations at rural schools to give awareness of the unique Central Indian grassland fauna, especially Indian wolf. UKWCT grant money will make it possible to purchase educational materials for these sessions, such as awareness posters to distribute at these schools and the children's families.