EQUUS KIANG (MOORCROFT, 1841) WHAT'S IN THE LITERATURE, WHAT WE NEED TO KNOW

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ABSTRACT

The Tibetan/Asiatic Wild Ass, *Equus kiang* currently inhabiting the Tibetan plateau and the Ladakh region in India is the only odd-toed ungulate belonging to the order- Perissodactyla, the family- Equidae, and the genus- *Equus*. It has been categorized as a Least Concern species by IUCN, but the subspecies *Equus k. kiang* found in the Changthang Wildlife Sanctuary of Ladakh is classed as Data-Deficient by IUCN. To validate this status of *E. k. kiang*, I searched for all the relevant pieces of literature on PUBMED (NCBI) and Google Scholar using various strings like *Equus kiang*, Asiatic Wild ass, Tibetan wild ass, and kiang. The results indicated a majority of the literature focus on the ecological and behavioral aspects of kiang and very few papers were on the genetics of kiang typically estimating its genetic diversity and phylogenetic analysis without the population structure. Despite having the population of kiang only in India and Tibet, to date, no study at the molecular level has been conducted, and not even a single nucleotide has been deposited in the GenBank from India. From the result, I infer that the species *E. kiang* is a Least-Concern species and the subspecies *E. k. kiang* a data-deficient. I recommend the study of this species with molecular markers like mtDNA D-loop markers, MSAT, and SNP markers to guestimate the population structure of this robust equid.

Keywords Equus kiang, Tibetan/Asiatic Wild Ass, Review, Changthang Wildlife Sanctuary, Ladakh, India

INTRODUCTION

Equus kiang (Tibetan/Asiatic Wild Ass) in the Literature

The Tibetan/Asiatic Wild ass, *Equus kiang* belongs to the family- Equidae, order- Perissodactyla, and the genus- *Equus*. In India, it is found only in the Ladakh region and globally it has a population distribution in China, Nepal, Bhutan, Tibet, and Pakistan. The kiang, *Equus kiang* was earlier considered as a subspecies of *Equus hemionus*, but now it is considered as a separate species (Ryder and Chemnick, 1990). There are three subspecies of *E. kiang* identified on geographical, cranial structures, and morphological basis without any proper genetic clarification (Shah, 2002; Groves and Mazak, 1967). The three subspecies are *Equus kiang* (Western kiang), *E. k. polyodon* (Southern kiang) and *E. k. holdereri* (Eastern kiang). Of the three subspecies, *E. k. kiang* is the only subspecies found in Ladakh and it has been confirmed by the skull investigation (Spasskaya, 2007). In India, the ultimate refugia for *E. kiang* is the Ladakh region (75° 50' to 75° 80' E and 32° 30' N to 32° 37' N). Within Ladakh, the Changthang Wildlife Sanctuary (~33.96°N to 78.20°E and extending in an area of 4000 km²) is the only conserved area for kiang. The other two subspecies are found along the borders of Sikkim and China and some portions of Pakistan (Duncan, 1992; Shah, 1994).

According to IUCN, the genus- Equus includes all the extinct and extant horse-like species including the domestic horses (Equus caballus), Przewalski's horses (Equus przewalskii), African wild asses (Equus africanus), Asiatic wild asses (Equus hemionus and E. kiang), mountain zebra (Equus zebra), plains zebra (Equus quagga) and Grevyi's zebra (Equus grevyi). The domestic donkey (E. africanus asinus) has been considered a subspecies of the African wild asses and its domestication was found to occur in Northern America 5000 years ago (Blench, 2000).

IUCN Status and Conservation

According to Schaller (1998), *E. kiang* is one of the least studied animals across the globe. The IUCN Red List of Threatened Species was created to provide scientific information on the current threatened status of global biodiversity (IUCN 2015). IUCN has categorized the Asiatic/Tibetan wild ass (*E. kiang*) as a "Least Concern" species in the 2011 IUCN Red List of Threatened Species. Based on their morphology and karyotype, two main species of the Asiatic wild ass, *E. hemionus* and *E. kiang*, have been described (Groves and Mazak, 1967; Ryder and Chemnick, 1990); however, molecular data failed to distinguish these species (McCue *et al.*, 2008; Vilstrup *et al.*, 2013). *E. hemionus* has been categorized as "Endangered" by IUCN.

According to Oakenfull and Ryder (2002), although defining any subspecies is often a difficult and controversial task, still they pointed out certain factors that should be noticed while defining any subspecies. The various factors are; geographical location, habitat, the amount of interaction that occurs between the populations, behavioral characteristics, coat color, and other morphological features. They also inferred that the recent use of genetic markers to analyze any variation in the DNA sequences is also proving beneficial in defining various subspecies.

Nonetheless, many debatable subspecies of *E. kiang* (three subspecies) and *E. hemionus* (five subspecies) have been geographically described for each species. At present, five subspecies of *E. hemionus* (*E. h. hemionus*, *E. h. onager*, *E. h. kulan*, *E. h. luteus*, and *E. h. khur*) have been identified (Groves and Mazak, 1967; Schaller, 1998; Shah, 2002), however, recently emerged molecular information failed to justify the variation in the subspecies (Vilstrup *et al.*, 2013; Rosenbom *et al.*, 2015; Khaire *et al.*, 2016).

Similarly, three subspecies of *E. kiang* (*E. k. kiang*, *E. k. holdereri*, and *E. k. polyodon;* corresponding to the Western, Eastern, and Southern kiang respectively) have been recognized (Shah, 2002; Grubb, 2005) which differ in their cranial structures, external features and geographical distribution (Groves and Mazak, 1967; Shah, 2002). However, genetic variation among the three subspecies of *E. kiang* has not been investigated.

The subspecies Western kiang, *Equus k. kiang* found in Ladakh has been categorized by IUCN as "Datadeficient". The other two subspecies of *E. kiang*, i.e. *E. k. holdereri* and *E. k. polyodon* has been recognized as "Least Concern" and "Data-deficient" respectively by the IUCN.

Kiang is now legally protected in many countries. In China it is in the 1st class protection category; in India, it is in Schedule-I of the Indian Wildlife Protection Act, 1972, and in Pakistan, it is conserved under the Northern Areas Wildlife Preservation Act, 1975. It is on Appendix II of CITES (IUCN SSC Equid Specialist Group).

Phenotypic Characters

From the morphological standpoint, *Equus kiang* has a large head with a convex nose, a thick rostrum, and a short snout. It has a relatively short body with long limbs and broad horse-like hooves. It bears a closer resemblance to horses than donkeys. The average body length of *Equus kiang* is about 210 cm (7 feet), with a tail length of 50 cm and a shoulder height of 140 cm (4.6 feet). The chestnut-brown coat covers the upper part of the thighs, back, upper flanks, upper part of the hind legs, dorsal part of the neck, cheeks, and forehead. The undersides, throat, and insides of the ears are all white, while the mane, tips and edges of the ears are black. The legs are generally white, but they can be light brown on the front (St-Louis and Côté, 2014). Their pelage changes with the seasons, becoming shorter and thinner in the summer and longer and thicker in the winter. During the summer, the hairs are about 14-16 mm long, while in the winter, they can reach up to 35-46 mm (Groves, 1974; Maza'k, 1962). Kiang is well-adapted to cold and hypoxic conditions in Ladakh and is the largest wild ass following Bergmann's rule.

The *E. kiang* has a dental formula of i 3/3, c 0-1/0-1, p 3-4/3, m 3/3. They have a total of 36-42 teeth, with the possibility of the canine and fourth premolars being absent or present. Similar to other equids, *E. kiang* has long molar and premolar teeth with complex enamel patterns visible on the grinding surface, as described by (Hodgson, 1847a). Adult kiangs can live up to 20 years in the wild, as reported by comparative tooth wear studies with zebras (Schaller, 1998). However, the average lifespan of kiang in most cases has

been reported to be around 7 to 11 years. Despite numerous attempts in the past, the kiang has never been successfully domesticated (Schaller, 1998).

Feeding Habits of Equus kiang

Being a member of the Equidae family, *Equus kiang* is also a hindgut fermenter like all other equids with the majority of the microbial digestion taking place in the caecum (Janis, 1976), which allows kiang to feed on coarse and fibrous forages with a high stem-to-leaf ratio (Duncan, 1992; Janis, 1976; McNaughton, 1985). The chief feeding grass of *E. kiang* is the graminoids (Harris and Miller, 1995; Schaller, 1998). A study conducted in Tibet's Chang Tang Nature Reserve by Schaller (1998) revealed that kiang's summer and winter diets vary based on the availability of food. During the summer, approximately 65% of their diet consists of *Stipa spp.*, followed by *Kobresia, Carex, Poa, Elymus*, and small amounts of a few forbs and shrubs. In comparison, their winter diet consists of a higher proportion (>90%) of *Stipa spp.* in the same area. Occasionally, kiangs have been observed digging *Oxytropis* roots, mainly in the winter when there is little availability of dry grasses.

According to research conducted by Harris and Miller (1995) and Schaller (1998), the diet of kiang in the Changtang Wildlife Sanctuary is mostly composed of grass and does not overlap much with the diets of other wild ungulate species in the Tibetan plateau. This can be attributed to coevolution and ecological niche partitioning over a long period of time. However, domestic livestock such as sheep, goats, yaks, and horses, which were recently introduced, may pose a greater potential for competition with the wild ungulate-kiang, as suggested by Namgail *et al.*,(2008).

A study conducted by St-Louis and Cote (2012) explored the foraging behaviour of *E. kiang* using a multiscale approach. They investigated the instantaneous forage intake rate at a fine scale and the proportion of time spent foraging at a coarse scale. The researchers also examined the foraging behaviour of different group types, sex differences in adults, and differences between females of different reproductive stages. Their results indicated that kiangs are not strict feeders and instead employ flexible foraging behaviour that varies with vegetation quality, abundance, and seasonal variations. The authors suggest that this could be due to the extreme seasonal conditions encountered on the Tibetan plateau.

In a separate study, also conducted by St-Louis and Cote (2014), the resource-selection pattern in *E. kiang* was determined. The researchers examined resource-selection patterns at three spatial levels, namely habitat, feeding site, and plant, during the summer and fall seasons. The study found that kiang preferred both mesic and xeric habitats in summer and only xeric (plain) habitats during winter at the habitat level. At the feeding site levels, the researchers found that the feeding sites with higher plant biomass and percentage of green foliage were selected over the random sites in the same habitat. At the plant level, the results indicated that the kiang chose green plants over forbs and shrubs. Overall, the results suggest that the resource-selection pattern in kiang is greatly influenced by the quality and abundance of forage and varies across scales.

Very little has been documented regarding the water requirements and water intakes of E. kiang. A study by Schaller (1998) documented that the E. kiang inhabiting the Tibetan plateau fulfills its water requirements from the dietary metabolism and snow. Occasionally, they have been reported to drink from waterholes, lakes, and streams.

Reproductive Features

Kiang exhibits mild sexual dimorphism with larger males (weighing between 350-400 kg) compared to females (250-300 kg). They attain sexual maturity at around 2-3 years of age, mate between late July to the end of August, and the gestation period can vary from 7-10 months (Hayssen et al., 1993), 355 days (Shah, 2002), to 365 days (Groves and Willoughby, 1981), with an average of 11 months (Schaller, 1998). The newborns measure up to 90 cm at the shoulders and weigh \leq 36 kg. They are weaned within a year after birth and soon reach adult size. Infanticide, which is the killing of infants by adult members of the same

species, has been observed in captive *E. kiang* (Berger, 1986), as well as in other equid species as observed by Cameron *et al.*,(2003), Duncan (1982), and Linklater *et al.*,(1999).

In captivity, kiangs are known to mate with donkeys, onagers, horses, and Burchell's zebras. However, like any other equine hybrid, the offspring produced by these interbreeding are sterile. According to Chandley *et al.*, (1974), the reason for infertility of the hybrid offspring could be due to meiosis failure in the primary spermatocytes during the synaptic stage in male offspring, as they are unable to pair properly. In the case of female hybrids, there are very few oocytes. It is interesting to note that despite being able to produce hybrids in captivity, kiang seems to be prevented from interbreeding with *E. h. hemionus* and *E. h. khur* in the wild, possibly due to geographical barriers (Groves and Maza'k, 1967).

Hybrids of *Equus kiang*

It has been documented that horse-donkey hybrids have existed for at least 3000 years (Brosnahan, 2019). Mules, which are a type of hybrid, were used for various tasks in ancient Greece, from farming to harness racing. They were also popular for military and agricultural purposes in North America starting in the 1700s. In captivity, E. kiang can mate with other equid species such as E. caballus, E. asinus, E. hemionus (E. h. hemionus and E. h. khur), and E. burchelli. However, in the wild, geographical barriers most likely prevented interbreeding between E. kiang and other equid species, particularly E. h. khur and E. h. hemionus (Grover and Mazak, 1967). Even if crossbreeding occurs, there is no evidence that the resulting hybrids are fertile. In fact, they are sterile like any other equid hybrid. Nevertheless, many hybrids between E. kiang and E. caballus, E. asinus, E. hemionus, and E. burchelli have been recorded in captivity (Gray, 1972; Hay, 1859; Kinloch, 1869). Although these hybrids are infertile, they can still live for a fair amount of time. For instance, a female hybrid produced between E. kiang and E. caballus, born in the Jardin des Plantes, Paris, France, lived for over 36 years. Similarly, another female hybrid between E. kiang and E. burchelli also lived for over 32 years (Gray, 1972). Additionally, E. kiang's hybrid between E. h. hemionus and E. h. khur has been reported (Gray, 1972). Although almost all the equine hybrids are infertile, a notable exception is the hybrid of the domestic horse (Equus caballus) and the Przewalski's horse (Equus przewaslki) whose hybrid is consistently fertile (Chandley et al., 1974).

Population Density

Kiang populations are sparse and widely separated and there is a need to reassess the taxonomic status and the relationship among other equid populations. Due to the remoteness and the geographical extent of the Tibetan plateau (Ladakh), it is difficult to conduct wildlife surveys, since no intensive survey of *E. kiang* has been taken over its entire ranges. The number of kiangs is estimated in several regions of its distribution range using different methods, hence estimating actual population size is a difficult task (Lious and Cote, 2009). Also, the animal E. kiang is a trans-border species and due to its high tendency of large-scale movements makes any effort for its exact number to be determined in any area quite difficult (Shah, 1996). However, rough estimates have been documented for the individual range, like Tibet- 37,000-48,000 animals, Xinjiang- 4,500-5,000 individuals, Qinghai and Gansu- 15,000 specimens (Schaller, 1998), Pakistan-15-25 individuals (Rasool, 1992; Shafiq and Ali, 1998), and Nepal-37-500 animals (Shah, 2002; Sharma et al., 2004). The total estimate of E. kiang's worldwide population was found to be 60,000-70,000 individuals (Schaller, 1998). In India, the population of E. kiang is estimated to be around 1,600-2,200 individuals (Shah, 2002) and found to be restricted in Ladakh. A census conducted by the Jammu and Kashmir Wildlife Department, Leh, in 1988, estimated a total count of 1,500 kiangs, and in 1994 counted 1,518 kiangs in East Ladakh (Ladakh Wildlife Department, Jammu, and Kashmir State Forest Department). Results concluded later by (Shah, 2002) remained similar to that of the census. The population densities at Ladakh were deduced at 0.03-0.86 individuals/km² (Bhatnagar et al., 2006). A study documented by (Shah, 1996) depicted the encounter rate of kiangs in the major kiang inhabiting areas like, Pangong Tso, Hanley, Tso Kar, Chushul, Tso Moriri, and Demchok. They found an encounter rate of 1.17 kiang individual/km in these areas (497 kiangs in 426 km area).

Surveys in the Hanle Valley of eastern Ladakh between 2001 and 2004 yielded densities of 0.56 individuals/km² (Bhatnagar *et al.*, 2006). Human presence has been observed to have a negative impact on the densities of *E. kiang*, as evident from the densities estimated from the Chang Tang Nature Reserve (Tibet), where there were 1.06-1.53 individuals/km² under low human interventions and only 0.88 individuals/km² under moderate human influences (Fox and Bardsen, 2005). In Ladakh, densities were estimated at 0.25 individuals/km² (Fox *et al.*, 1991). In Ladakh, the population of *E. kiang* was greatly reduced during the 1962 war with China (Bhatnagar *et al.*, 2006) and the subsequent buildup of armed forces across their habitats (Fox *et al.*, 1991). In China, a survey carried out by (Turghan M. *et al.*, 2013) in the Arjin Mountain Nature Reserve China, recorded an overall population density of 0.63 ± 0.23 animals/km² for a total population of 8500-9500 kiangs in the reserve of 45,000 km².

A survey for the distribution and the population estimation of the ungulates in the Changthang region was performed by (Shrotriya *et al.*, 2015). In their survey, they addressed all the ungulates present in the region. They included the kiang also in their study and recorded the distribution and population estimates of the kiang (*E. kiang*) using the MaxEnt model for the distribution. In their results, they demonstrated that the slope and elevation were the main factors for the distribution with the highest percent contributions. The other factors like distance to water and ruggedness were not that important, because once areas with high slope and elevation were excluded, there remained no variation within these two variables. They identified a total of ~5200 km² area as kiang habitat, which demonstrates a reduction in the habitat area from the earlier census conducted by Chundawat and Qureshi (1999), which depicted an area of 7400 km² as kiang habitat. These results indicate a fair amount of habitat reduction for kiang over a period of just 16 years which will ultimately harm the species' survival. They found a population density of 0.86 individuals per sq. km.

Kiang densities were maximum in large open meadows with adequate resources and minimum human interventions. Nevertheless, kiangs were also spotted in a good number at extremely high altitudes (18680 ft. above the sea level) with scarce resources (barren land) and hail storms during the month of May. Such versatile characters of the kiang exhibit the efficacy of this species to survive in the most diverse and extreme conditions (Mumtaz *et al.*,2023). With such highly efficient surviving ability, this robust wild ass still needs intense care and study for proper conservation.

Our understanding of the mortality rates of *E. kiang* is very less as there are negligible studies on it, and hence, poaching remains a major threat to the wild ass because they often get hunted by the nomadic communities for their subsistence (Huber, 2005). However, religious beliefs in ancient Tibet prohibited kiang and other wild equid hunting, but the killing of kiang increased drastically during the mid-20th century when the accessibility to the remote areas became easier and also due to the availability of modern weapons (Leslie and Schaller, 2008; Schaller *et al.*, 2005). In China, *E. kiang* was killed indiscriminately during the great famine of 1958-1961 and also during the socioeconomic changes (Schaller, 1998; Schaller *et al.*, 2005; Harris, 2008). Researchers like King, S. R. (2007) are of the opinion that without proper quantification of the dangers of putting Asiatic wild ass populations in peril, it is impossible to find ways to combat them.

Territory Marking Behaviour in Kiang

In Ladakh, the territories of *E. kiang* ranged from 0.5-5 km² (Denzau and Denzau, 1999). The males have been observed to defend their territories by aggressively chasing an intruding male (i.e. the male kiangs are territorial in nature). The chase is often preceded by an alarming posture with its head up, ears laid back, and tail horizontal, often grunting or making a "wheezing bray" at the same time (Schaller, 1998). Male kiangs have been observed to defend their territories and occupy the same area for several months (Denzau and Denzau, 1999). Documentation by (Shah *et al.*, 2015) observed males with natural marks occupied the same area for above 3 years in eastern Ladakh. During the estrus phase, females of the same reproductive stage are often associated and males are often solitary and spaced in such a fashion as to appear territorial (Schaller, 1998).

In most cases, aggressive fighting may occur by kicking and biting the mane, neck, and even the tail, resulting in scars. The territorial marking behaviour of male *E. kiang* was studied by Paklina (2007) and it was found to be somewhat different from that of the feral horses (*E. caballus*) and Przewalski's horses (*E. przewalksi*) in the following manners; kiang didn't make big defecation piles, but marked the territorial borders with single defecation and urination marks; didn't sniff own excrements after defecation; and didn't mark urination and defecation marks of females.

Social Structure of *E. kiang*

Kiangs are usually solitary animals or are found only in small herds. However, many reports suggest that there are no permanent herdings, rather the groups are temporary and territorial mainly for reproduction and the most stable social group is that of mother-foal (Groves and Willoughby, 1981; Schaller, 1998). Studies reveal that the old males are displaced from the herd and live an isolated life (Klingel, 1975; Rubenstein, 1986; Boyd and Keiper, 2005). Two types of social organizations are found in the wild equids. The 1st type- the harem-forming equids with female defense polygyny, the permanent group with few males and several females and young ones; the males are not territorial, e.g., in *E. burchellii, E. caballus, E. przewalskii, E. zebra*. The IInd type- the territorial equids with resource defense polygyny, permanent association is only between mother and foal, i.e. rather a temporary grouping comprising few females and their young ones or only males (bachelor groups); adult males are often territorial (Klingel 1975, 1977).

Although limited observations have been documented regarding the social behavior of *E. kiang*, whatever data available indicates that the *E. kiang*, like any other arid wild equid, such as Grevyi's zebra (*E. grevyii*), African wild ass (*E. africanus*) and Asiatic/Indian wild ass (*E. hemionus*), exhibits the second type of social organization (Klingel, 1977; Groves and Willoughby 1981; Schaller, 1998). Some instances of large congregations of kiang have also been observed in the good pasture habitats during the fall and winter, with the finding of a herd congregate of about 300-400 individuals at times during the autumn and winter (Schaller, 1998). Moreover, another study (Shah and Huibin, 2000) reported the presence of 500 kiangs in one group in December 1999.

In general, equids communicate with each other using their ears, facial expressions, voices, tails, and postures. However, the way they communicate audibly differs between species. For example, horses neigh, Grevyi's zebra and asses bray, and the plains zebra barks (Policht *et al.*, 2011). *Equus kiang*, on the other hand, communicates through a unique sound referred to as a *"shrieking bray,"* (Lydekker, 1904b), which is different from the neighing of the horses and the braying of the asses (Hay, 1859). When faced with danger, the male *Equus kiang* gives out a signal, usually a short but loud exhale, and runs in a specific trot known as the "wooden steps." During this trot, the male raises his head and turns it slightly to either side to keep an eye on the potential threat. If the danger is behind him, like when running away from a car, he runs in a zigzag manner, turning his head to the left and right (Paklina and Van Orden, 2007).

The Complete Mitogenome of E. kiang

The complete mitochondrial DNA genome, also known as the mitogenome, of *E. kiang*, was sequenced by Luo Y *et al.*,(2011). They discovered that the mitogenome of kiang is composed of 16,634 bp, containing two strands - the heavy (H) strand and the light (L) strand - encoding all 37 genes found in typical vertebrates. Out of these 37 genes, 22 were tRNA genes (16 on H-strand and 6 on L-strand), 13 were protein-coding OXPHOS genes (12 on H-strand and 1- ND6 on L-strand), and 2 were rRNA genes (both on H-strand). They found the mtDNA of *E. kiang* to be 36 bp shorter than that of *E. asinus* due to the shorter D-loop. The overall nucleotide content had a higher composition of A+T content, which is typical for all vertebrate mitochondrial genomes. They also found that the non-coding control region, also known as the D-loop, of kiang was 1161 bp-long, which was 45 bp shorter than that of *E. asinus*. The D-loop of *E. kiang* was found to be bounded by tRNA-Pro and tRNA-Phe owing to the circular structure of mtDNA.

Adaptations to the Ladakh and Tibetan Plateaus

Being a good dweller of the plateau habitat (cold, arid, hypoxic, and low-energy diets) of the Tibetan plateau and the adjoining Ladakh region, *E. kiang* shows some interesting adaptations that allow them to thrive on such adverse habitats. The main challenge is the availability of fresh, green, and energy-rich foods and to combat it kiang shows the adaptation in their gut microbiota. Research by (Gao *et al.*, 2020) demonstrated the plateau adaptability of kiang from their findings of the gut microbiota, i.e. Bacteroidetes and Firmicutes as the predominant gut microbial phyla and suggested that the structure and abundance of the gut microbiota differed significantly between seasons and environments. These gut microbial phyla enable kiang to digest and ferment the fibrous forage (Flint and Bayer, 2008). These phyla are the major components of all faecal microbial communities, and their presence is vitally important for host animals (Brice *et al.*, 2019; Gao *et al.*, 2019). Numerous bacteria of Bacteroidetes have a degrading function of organic molecules with high molecular weight (Abdallah Ismail *et al.*, 2011), such as fat, plant cell walls, and complex carbohydrates (Coelho *et al.*, 2018). In herbivores, the gut flora converts the plant biomass into fermentable sugars, free fatty acids, and other absorbable forms (Janis, 1976).

In a study conducted by Zhang *et al.*, (2020), the density of gut microbiota in the Tibetan wild ass (*E. kiang*) was investigated based on altitude- high altitude and low altitude. The study found that the high-altitude kiang specimens had predominating phyla of Bacteroidetes and Firmicutes in comparison to the low-altitude ones. Additionally, the study discovered that the genera *Ruminococcus flavefaciens*, *rumen_bacterium_YS2*, *Acinetobacter baumannii*, and *Bacillus anthracis* were only present in the high-altitude specimens. The researchers inferred that high altitudes cause the gut microbes of the species to evolve more efficiently, enabling them to digest diets with more cellulose and hemicellulose content more efficiently, converting it to low-chain fatty acids to adapt to the high-cold, low-oxygen, and low-energy diets (Zhang et al., 2016).

Luo et al., (2013) conducted a study on E. kiang to analyze the adaptations at the mitochondrial regulatory levels. They discovered sixteen amino acid substitutions, including three in the subunits ND4 and ND5 of NADH dehydrogenase (Complex-I of the mitochondrial respiratory chain), which were unprecedented as compared to those found in donkeys and horses. They concluded that these substitutions are essential for regulating the mitochondrial respiratory complexes and electron transport chain efficiency. Additionally, they may contribute to the cold-hypoxic plateau adaptations. The adaptations of equids can also be attributed to the changes in reprogramming transcriptional expressions, especially for the genes involved in the metabolism of carbohydrates and lipids, hair development, and limb morphogenesis (Orlando, 2015). Researchers have investigated various biological mechanisms and gene admixture in addition to the adaptations required for high altitude and hypoxic conditions on the Tibetan plateau. According to a study by Zeng et al., (2021), there has been a selective sweep between kiangs and donkeys for high-altitude adaptation. The study inferred that kiangs have adapted in the EPAS1 (Endothelial PAS domain-containing protein-1) gene, which is also known as Hypoxia-inducible factor 2-alpha (HIF-2α), while Tibetan donkeys have adapted through the EGLN1 gene. Several studies conducted by Huerta-Sánchez et al., (2014), Miao et al., (2017), and VonHoldt et al., (2017) suggest that humans and dogs in Tibet acquired the EPAS1 allele essential for hypoxic adaptation through interbreeding with closely related lineages that were already adapted to the Tibetan Plateau. However, the adaptation mechanisms of Tibetan donkeys and kiangs are different, despite being closely related and residing in the same habitat. This suggests that adaptations for high-altitude in E. kiang and Tibetan donkeys didn't occur through admixture (i.e. adaptive introgression) but through a different biological pathway (selective sweep).

Threats to E. kiang

The species *E. kiang* faces various threats both natural and anthropogenic which constantly challenge their survival. Some of the prominent threats that the species is currently facing are rapid climatic changes, anthropogenic activities like changes in land use (Goyal *et al.*, 1999), poaching (Reading *et al.*, 2001; Feh *et al.*, 2002; and Turghan *et al.*, 2013), iron and asbestos mining in Qinghai province, China (Turghan *et al.*, 2013)

al., 2013), and more importantly the human-wildlife (human-kiang) conflicts due to forage and pasture competition with the livestock (Bhatnagar et al., 2006). Being the largest of all the asses, and because of their non-compartmented digestive tracks (St-Louis and Côté, 2014) equids (kiang) require large forage consumption, hence they invade the pastures and give direct competition to the livestock and cashmere goat (Fox et al., 1991; Reading et al., 2001; and Turghan et al., 2013). In the Changthang Wildlife Sanctuary, approximately 5000 wild animals compete with ~2,15000 domestic livestock for pasture, and due to rapid commercialization of pashmina encouraged by the Jammu and Kashmir government by providing incentives to the residents has created an intolerance for kiang among them. The changes in rangeland use policy have resulted in human activities and movements into important wildlife areas, which caused the conversion of many rangelands into private ranches owned by many families leading to intensified competition for pastures (Fox et al., 2008). Many instances of wired and walled fences around key resources in the major kiang inhabiting areas were found (Mumtaz et al., 2023 and Bhatnagar et al., 2006). Similar barbed wire fencings were also found to be erected around key resources in the Changthang Wildlife Sanctuary between Loma and Hanle areas to keep kiang away from the grazing lands (Naoroji and Sangha, 2011). Fences above 150 cm have been proven to be a hindrance to the movement of large mammals (Bendeguz et al., 2020) leading to habitat fragmentation and thus restricting small populations in discrete isolated areas. In the long run, it leads to a reduced mating choice which causes mating between closely related kins. Such mating brings about a loss in genetic variabilities and increases the chance of producing inbred offspring, thus producing offspring that may not be as fit to adapt to the ever-changing environment and also resist pathogenic infections.

Besides these prominent threats, *E. kiang* also faces some other threats like the construction of roads for strategic purposes and tourism developmental activities of visiting Mount Kailash directly from Ladakh through major kiang inhabiting areas of Dungti and Fukche (Pfister, 1998). According to research (Bhatnagar et al., 2006), many instances of chasing kiang on horseback or setting dogs upon kiang and driving them away from the pastures have also been recorded.

Some other threats that kiang have faced in the past were the killing of kiang for their hides to produce ejiao (donkey-hide gelation or ass hide glue) and the male kiangs were killed their penis in earlier times as it was supposed to increase virility (Reading *et al.*, 2001). All these reasons in addition to the Indo-Chinese War (1962) and the Great Famine of China (1958-61) have brought their numbers to a worrisome level, thus encouraging research related to their conservation.

Cytogenetic Features

A study (Schaller, 1998) suggested that little genetic information exists for extant populations of *E. kiang*. From the genomic point of view, kiangs are diploid animals with 2N = 51 or 52 chromosomes, this polymorphism in diploid numbers in both males and females can be accredited to Robertsonian translocation, i.e. centromere-centromere fusion (Ryder and Chemnick, 1990). The X chromosome is submetacentric and the Y chromosome is acrocentric.

Centromeric fusion, also known as centromere-centromere fusion or centromere repositioning, is a frequent occurrence among equids and has played a role in their speciation. It is the most common type of translocation involved in the evolution of equids. One such translocation event took place between chromosome 5 and chromosomes 23 and 24 of the domestic horse and the Przewalski's horse, which distinguishes the karyotype of these two species (Myka *et al.*, 2003).

One of the most peculiar features of the Perrisodactyla order, as studied by Ryder *et al.*, (1978), is their rapid rate of chromosomal evolution, which results in highly variable chromosome numbers. For example, the Mountain zebra (*E. zebra*) has 2N = 32, while the Przewalski's horse (*E. przewaslki*) has 2N = 66. Although equid species exhibit significant karyotypic variability, they can interbreed and produce healthy and viable offspring, with the resulting hybrids mostly being sterile. A unique structural feature of equid

chromosomes is their satellite-free centromeres, which facilitate the study of biological principles related to chromosomal structure (Giulotto *et al.*, 2017).

Musilova *et al.*, (2009) conducted a comparative chromosome mapping of the *Equus kiang* and *E. h. kulan* to examine the various chromosomal discrepancies/polymorphism using horse (*E. caballus*) chromosome arm-specific probes. They found that the karyotype of kulan is identical to that of onager, but the only difference in their karyotypes is in the fusion/fission of chromosomes homologous to *E. caballus*- 2q/3q, resulting in chromosome numbers of 2N=54 in kulan and 2N=56 in onager. These chromosomes are involved in the fusion/fission polymorphism of many other equid species, including the Asiatic asses (Myka *et al.*, 2003).

As far as the karyotype of *E. kiang* is concerned, it differs from the *E. hemionus* karyotype by two additional fusions- *E. caballus* 8q/15 (*E. kiang* 3) and 7/25 (*E. kiang* 4). Moreover, in the kiang individuals with 2N = 52 chromosomes, the chromosomes that are equivalent to horse 2q and 3q are not fused (Musilova *et al.,* 2009).

It is widely accepted that today's equid species evolved from a common ancestor that lived roughly 4 million years ago (MYA), and the period of their diversification from the common lineage can be determined (Musilova et al., 2013)

- The domestic horses were the first to diverge around this period (i.e. 4 MYA),
- Followed by the African wild asses at approximately 2.1 MYA, and
- Then, the final split between the Asiatic asses and the zebra clades arising about 1.9 MYA.

Although, from the geological aspect, these timeframes are quite recent, subsequent chromosomal evolution occurred comparatively rapidly (Musilova *et al.*, 2013). The wide variation in the number and structure of the karyotypes of each surviving species may be due to the cumulative effects of chromosomal rearrangements, including centromere repositioning, translocations, and inversions (Jónsson *et al.*, 2014; and Trifonov *et al.*, 2012).

In a study conducted by Trifonov *et al.*, (2008), the rate of evolutionary chromosomal rearrangements was estimated using cross-species banding. The study found that the equid lineage had the highest rate of chromosomal rearrangements, ranging from 2.92 to 22.2 rearrangements per million years, while the ceratomorphs had the lowest rate (<0.3 rearrangements per million years). Based on their findings, the researchers concluded that the equid genome evolution is the most rapid among non-rodent mammals. Despite the rapid chromosomal evolution that leads to different karyotypes, gene flow is not prevented, as evidenced by a study conducted by Jónsson *et al.*, (2014). Interestingly, the karyotypic changes are not expressed in the phenotype of extant equid species, rather, the species strongly resemble each other, which indicates the accelerated evolution evident in the sequence and implies the rapid radiation among the members of Equidae, as reported by Vilstrup *et al.*, (2013).

Studies Related to the Phylogenetic Analysis of E. kiang

The hemiones have been a matter of debate for the taxonomists. Some hypotheses segregate *Equus* species into three genera- *Plessipus, Allohippus,* and *Equus,* but the monophyly of the genus *Equus* has been elucidated by Cirilli *et al.,* (2021), by their cladistical analysis combined with a morphological and morphometrical comparison of cranial anatomy. They also defined the following evolutionary steps directly related to the evolution of the extant zebras and asses *E. simplicidens–E. stenonis–E. koobiforensis–E. grevyi* -zebra-ass clade.

The split time of the equid populations was determined by (Jónsson *et al.*, 2014) using the first divergence date in the demographic profiles of sister species and coalescent-based simulations. They found the early population split between the African and Asiatic asses occurred ~1.7 Mya. They inferred that the onager and the kiang divergence took place more recently ~266-392 Kya and the three populations of the extant zebra divergence occurred ~1.1Mya. The extinct quagga split from the plain's zebra population of only ~233–356 Kya. These findings show that the earliest equine divergence (speciation) took place with gene

flow in northern America and that the ancestors of the modern asses and zebras were dispersed into the Old World \sim 2.1- 3.1Mya.

From the aspect of the gene flow in the equid branch, (Jónsson *et al.*, 2014) found shreds of evidence of four main episodes of gene flow among three contemporary equid species despite large chromosomal variations (2n = 16-31)

- One during the earliest equid divergence,
- One from kiang (2n = 51-52) into the donkey lineage (2n = 62-64),
- One between the Somali wild ass (2n = 62) and Grevy's zebra (2n = 46), and
- One between African asses (2n = 62 64) and the mountain zebra (2n = 32).

According to (Feh *et al.*, 2002), the IUCN Action Plan for Asiatic wild asses states that though we do have some data on the genetics and the behavioural ecology of the wild asses still there are loopholes in our knowledge.

Some of the reasons for the taxonomic uncertainties and controversies of the members of Equidae are insufficient data on the hemiones themselves, extinction of the geographically northern and southern populations, varied taxonomic philosophies, lack of phylogenetic insight, and insufficient data in the palaeoenvironment

Being a member of the family- Equidae, the taxonomic status and phylogenetic relationship of *E. kiang* within and among species is also uncertain. The mtDNA analysis of *E. kiang* was carried out by (Rosenbom *et al.*, 2015) using non-invasive sampling approaches. They found a haplotype diversity ranging from 0.00 to 0.667 ± 0.132 and a nucleotide diversity of 0.0052 ± 0.0013 that reveals a considerable genetic diversity among the kiang population. From their phylogenetic analyses, they identified two major clades, i.e. the clade grouping *E. h. onager* haplotypes and the other clade grouping together the *E. h. hemionus* and *E. kiang*. These results were not in accordance with the findings of other phylogenetic analyses (Oakenfull *et al.*, 2000; Steiner *et al.*, 2012; Vilstrup *et al.*, 2013) in which a separate clade was obtained for the hemionus subspecies and a monophyletic clade of the kiang haplotypes were formed inside the wider variation of *E. hemionus*. Such inconsistent results show the phylogenetic uncertainties and encompass the need to resolve the phylogenetic status of *E. kiang*.

The population structure of the Indian wild ass, *E. h khur* from the Little Rann of Kutch, a closely related species of kiang was determined by (Khaire *et al.*, 2016 and 2017) using mtDNA D-loop, Cyt-b, and various MSAT markers. In their studies, they inferred that khur possessed low mtDNA genetic diversity and the species experienced a recent population bottleneck despite a decent population number due to the deadly protozoic infection, *Surra* (1980) caused by *Trypanosoma evansi*, hence emphasizing the need to conserve this species.

CONCLUSION

From the available pieces of literature, I conclude that now we have a fair understanding of the adaptations of *E. kiang* to the Ladakh and Tibetan xeric plateau conditions which facilitates the species to thrive in adverse habitats. We also have a good understanding of ecology and behaviour like diet preferences, resource selection patterns, reproductive features, and also the various threats this species currently faces. But our understanding of the species from its genetic and phylogenetic aspects is still in the doldrums. Shreds of literature found during this review validate the IUCN Least-Concern and Data-deficient statuses of the species *Equus kiang* and the subspecies *Equus k. kiang* respectively, especially in the molecular studies.

Despite the whole mitogenome sequencing, as of now, no study has estimated the population structure of the Tibetan Wild Ass, *E. kiang*. Like the Indian wild ass, *E. h. khur*, the species *E. kiang* has also faced numerous past catastrophic events like the great famine of China (1958-61) and the Indo-Chinese War (1962) that caused a drastic decline in its population. Also, there are pieces of literature that indicate a significant population reduction during and after the war (Bhatnagar *et al.*, 2006). Since kiang is a transborder animal, it faced the maximum war consequences and experienced a significant population

decline. These past catastrophic events in addition to the constant present-day pressures, especially the human-kiang conflicts, and the habitat fragmentations impose a potential threat to the continued existence of *E. kiang* in the wild. Through this review, I urge studies on this robust wild equid to estimate its population structure, especially any recent genetic bottleneck and past population demography using highly polymorphic genetic tools like MSAT, SNP, and mtDNA markers (D-loop and Cyt-b markers). Studies using median-joining network analysis may be employed to detect any haplotype sharing between various kiang populations, thus validating the trans-border movement of the species.

No study has yet been conducted from India on the Indian population of kiang to unravel its genetic diversity, population genetics, and phylogenetic relationship with other extant equid species. Not even a single nucleotide sequence has been deposited in the GenBank from India, despite having a population of *E. kiang* in one of its wildlife sanctuaries, the Changthang Cold Desert Wildlife Sanctuary. Studies at the genetic level will generate valid molecular data which according to authors like Kefena *et al.*, (2012) is more reliable and authentic as compared to those of the physical measurements of the dental, cranial, or post-cranial data in outlining the evolutionary history and taxonomic classification. The three subspecies of *E. kiang*, i.e. *E. k. kiang*, *E. k. holdereri*, and *E. k. polyodon* have been described purely on the basis of geographical and morphological features without genetic analysis. Hence, the subspecies categorization also needs to be validated through more reliable molecular information. Such studies will not only cast light on the genetic diversity, population genetics, phylogenetic analyses, and past population demography of kiang but also will serve as potential data for framing and implementing various conservation protocols by the conservation authorities like Wildlife Departments, IUCN, Wildlife enthusiasts and other non-profit bodies involved in the conservation of biodiversity.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

Abdallah Ismail N, Ragab SH, Abd Elbaky A, Shoeib AR, Alhosary Y and Fekry D (2011). Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Archives of Medical Science*, **7**(3), 501–507. *https* //doi.org/10.5114/aoms.2011.23418.

Bendegúz M, George W, Zsolt N, Viktor S, and Szilvia K (2020). Effects of natural and artificial barriers on the genetic diversity of wild animal species A REVIEW. *Balkan Journal of Wildlife Research*, **5**(1). https://DOI 10.15679/bjwr.v5i1.61

Berger J (1986). Wild horses of the Great Basin social competition and population size. University of Chicago Press, Chicago, Illinois. https://cir.nii.ac.jp/crid/1130000796705817600

Bhatnagar YV, R Wangchuk, HHT Prins, SE Van Wieren and C Mishra (2006). Perceived conflicts between pastoralism and conservation of the kiang, *E. kiang* in the Ladakh Trans-Himalaya, India. *Environmental Management,* **38** 934–941.

https://doi.org/10.1007/s00267-005-0356-2

Blench RM (2000). A history of donkeys, wild asses and mules in Africa. In Blench RM, and MacDonald KC, editors. The origins and development of African livestock Archaeology, genetics, linguistics and ethnography. *London UCL;* p. 339–354.

https://doi.org/10.4324/9780203984239

Boyd L and Keiper R (2005). Behavioural ecology of the domestic horse The origins, development, and management of its behaviour. *Cambridge University Press, Cambridge, UK*, 55.

Brice KL, Trivedi P, Jeffries TC, Blyton MD, Mitchell C, Singh BK and Moore BD (2019). The Koala (*Phascolarctos cinereus*) faecal microbiome differs with diet in a wild population. *PeerJ*, **7** e6534. https://doi.org/10.7717/peerj.6534

Brosnahan MM (2019). Genetics, Evolution, and Physiology of Donkeys and Mules. *Veterinary Clinics Equine Practice*, **35**(3) 457-467.

https://doi.org/10.1016/j.cveq.2019.08.003

Cameron EZ, WL Linklater, KJ Stafford and EO Minot. (2003). Social grouping and maternal behavior in feral horses (*Equus caballus*) the influence of males on maternal protectiveness. *Behavioral Ecology and Sociobiology*, **53** 92–101.

https://doi.org/10.1007/s00265-002-0556-1

Chandley AC, Jones RC, Dott HM, Allen WR and Short RV (1974). Meiosis in interspecific equine hybrids. *Cytogenetic and genome research*, **13**(4) 330-341. *https://doi.org/10.1159/000130284*

Chundawat RS and Qureshi Q (1999). Planning wildlife conservation in Leh and Kargil districts of Ladakh, Jammu and Kashmir. Report submitted to the *Wildlife Institute of India, Dehradun*.

Cirilli O, Pandolfi L, Rook L and Bernor RL (2021). Evolution of Old World *Equus* and origin of the zebra-ass clade. *Scientific Reports*, **11**(1) 1-11.https //doi.org/10.1038/s41598-021-89440-9

Coelho LP, Kultima JR, Costea PI, Fournier C, Pan Y, Czarnecki- Maulden G and Bork P (2018). Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome*, **6**(1) 72. https://doi.org/10.1186/s4016 8-018-0450-3

Denzau H, Denzau G and Neumann-Denzau G (1999). Wildesel. Thorbecke, Stuttgart, Germany.

Duncan P (1992). Horses and grasses the nutritional ecology of equids and their impact on the Camargue. *Springer-Verlag, New York.*

Feh C, Shah N, Rowen M, Reading R, Goyal SP (2002). Status and action plan for the Asiatic Wild Ass (*Equus hemionus*). – *In*: P. Moehlman (ed.) Equids Zebras, Asses, and Horses. - *IUCN, Gland, Switzerland,* pp. 62-70.

Flint HJ and Bayer EA (2008). Plant cell wall breakdown by anaerobic microorganisms from the Mammalian digestive tract. Annals of the New York Academy of Sciences, 1125 280–288. https://doi.org/10.1196/annals.1419.022

Fox JL and BJ Bardsen (2005). Density of Tibetan antelope, Tibetan wild ass, and Tibetan gazelle in relation to human presence across the Chang Tang Nature Reserve of Tibet, China. *Acta Zoologica Sinica*, 51 586–597. *https://cir.nii.ac.jp/crid/1570291225646239360*

Fox JL, C Nurbu and RS Chundawat (1991). The mountain ungulates of Ladakh, India. *Biological Conservation*, 58 167–190. *https://doi.org/10.1016/0006-3207(91)90118-S*

Gao H, Chi X, Li G, Qin W, Song P, Jiang F and Zhang T (2020). Gut microbial diversity and stabilizing functions enhance the plateau adaptability of Tibetan wild ass (*Equus kiang*). *Microbiology Open*, **9**(6) 1150-1161. *https* //doi.org/10.1002/mbo3.1025

Giulotto E, Raimondi E and Sullivan KF (2017). The unique DNA sequences underlying equine centromeres. *Centromeres and Kinetochores*, 337-354.

https://doi.org/10.1007/978-3-319-58592-5_14

Goyal SP, Sinha B, Shah N, Panwar HS (1999). Sardar Sarovar Project - a conservation threat to the Indian wild ass (*Equus hemionus khur*). – *Biological Conservation*, 88 277-284. https://doi.org/10.1016/S0006-3207(98)00082-2

Gray AP (1972). Mammalian hybrids. 2nd ed. Commonwealth Agricultural Bureaux, Farnham Royal, Slough, United Kingdom.

Groves CP (1974). Horses, asses, and zebras in the wild. Mammalia, 38 221–254.

Groves C P, and D P Willoughby (1981). Studies on the taxonomy and phylogeny of the genus *Equus*, 1 subgeneric classification of the recent species. *Mammalia*, **45** 321–354. *https* //doi.org/10.1515/mamm.1981.45.3.321

Groves CP, Mazák V (1967). On some taxonomic problems of Asiatic wild asses; with the description of a new subspecies (Perissodactyla; Equidae). Zeitschrift für Säugetierkunde, **32** 321–355.

Grubb P (Ed.) (2005). Order Perissodactyla. The Johns Hopkins University Press, Baltimore, Maryland, USA. https://cir.nii.ac.jp/crid/1571980075665438208

Harris RB (2008). Wildlife Conservation in China preserving the habitat of China's Wild West. M. E. Sharpe Inc., *Armonk, New York.* https://doi.org/10.4324/9781315698168

Harris RB and Miller DJ (1995). Overlap in summer habitats and diets of Tibetan plateau ungulates. *Mammalia*, 59 197-212. http://dx.doi.org/10.1515/mamm.1995.59.2.197

Hay WE (1859). Notes on the kiang of Thibet (*E. kiang*). *Proceedings of the Zoological Society of London*, 27 353–357.

Hayssen V, A Van Tienhoven, and A Van Tienhoven (1993). Asdell's patterns of mammalian reproduction a compendium of species-specific data. *Cornell University Press, Ithaca, New York.*

Hodgson BH (1847a). Description of the wild ass and wolf of Tibet, with illustrations. *Calcutta Journal of Natural History*, **7** 469–477.

Huber T (2005). Antelope hunting in northern Tibet cultural adaptations to wildlife behaviour. *Memorie della Societa` Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano*, **23** 5–17.

Huerta-Sánchez E, Jin X, As an, Bianba Z, Peter BM, and Vinckenbosch N, (2014). Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature*, **512**(7513) 194–197. *https* //doi.org/10.1038/nature13408

IUCN (2015). IUCN Categories and Criteria (version 3.1) IUCN, Gland. Available from *http://www.iucnredlist.org/static/categories crietria 3 1.2001*

Janis C (1976). The evolutionary strategy of the Equidae and the origins of rumen and caecal digestion. *Evolution*, **30** 757–774. https://doi.org/10.2307/2407816

Jónsson H, Schubert M, Seguin-Orlando A, Ginolhac A, Petersen L, Fumagalli M, and Orlando L (2014). Speciation with gene flow in equids despite extensive chromosomal plasticity. *Proceedings of the National Academy of Sciences*, **111**(52) 18655-18660.

https://doi.org/10.1073/pnas.1412627111

Kefena E, Mekasha Y, Han JL, Rosenbom S, Haile A, Dessie T and Beja-Pereira A (2012). Discordances between morphological systematics and molecular taxonomy in the stem line of equids A review of the case of taxonomy of genus *Equus*. *Livestock Science*, **143**(2-3) 105-115. https://doi.org/10.1016/j.livsci.2011.09.017

Khaire D, Atkulwar A, Farah S. and Baig M. (2016). Mitochondrial DNA analyses revealed low genetic diversity in the endangered Indian wild ass *Equus hemionus khur*. *Mitochondrial DNA Part A*, **12** 1–6. https://doi.org/10.3109/24701394.2016.1174221

Khaire, D, Atkulwar A, Farah S, and Baig M (2017). Low genetic diversity of the endangered Indian wild ass, *Equus hemionus khur*, as revealed by microsatellite analyses. *Journal of Genetics*, **96**(1) 31-34. https://doi.org/10.1007/s12041-017-0784-9

King SR (2007). Asiatic wild asses in the literature what do we need to know now? *Erforschung biologischer Ressourcen der Mongolei/ Exploration into the Biological Resources of Mongolia*, **97**, ISSN 0440-1298. http://digitalcommons.unl.edu/biolmongol/97

Kinloch A (1869). Large game shooting in Thibet and the northwest. *Harrison and Sons, London, United Kingdom*.

Klingel H (1975). Social organization and reproduction in equids. *Journal of reproduction and fertility*. Supplement, (23), 7-11. PMID 1060868.

Klingel H (1977). Observations on social organization and behaviour of African and Asiatic wild asses (*Equus africanus* and *E. hemionus*). Applied Animal Behaviour Science, 44(3) 323-331. https://doi.org/10.1111/j.1439-0310.1977.tb00999.x

Centre for Info Bio Technology (CIBTech)

Leslie DMJR, and GB Schaller (2008). Pantholops hodgsonii (Artiodactyla Bovidae). Mammalian Species, 817 1–13. https://doi.org/10.1644/817.1

Linklater WL, EZ Cameron, EO Minot and KJ Stafford (1999). Stallion harassment and the mating system of horses. *Animal Behaviour*, **58** 295–306.

https://psycnet.apa.org/doi/10.1006/anbe.1999.1155

Luo Y, Chen Y, Liu F, Jiang C and Gao Y (2011). Mitochondrial genome sequence of the Tibetan wild ass (*Equus kiang*). *Mitochondrial DNA*, **22**(1-2) 6-8.

https://doi.org/10.3109/19401736.2011.588221

Luo Y, Yang X and Gao Y (2013). Mitochondrial DNA response to high altitude a new perspective on high-altitude adaptation. *Mitochondrial DNA*, 24(4) 313-319.

https://doi.org/10.3109/19401736.2012.760558

Lydekker R (1904b). Notes on the specimens of wild asses in English collections. *Novitates Zoologicae*, 11 583–596.

Mazák V (1962). Spring moult in *Equus hemionus kiang* Moorcroft (1841) and a contribution to the phylogenesis of moulting in the subfamily Equinae (Perissodactyla, Mammalia). *Zoologischer Anzeiger*, 168 164-170.

McCue ME, Bannasch DL, Petersen JL, Gurr J, Bailey E, Binns MM, Distl O, Guerin G, Hasegawa T, Hill EW, Leeb T, Lindgren G, Penedo MC, Roed KH, Ryder OA, Swinburne JE, Tozaki T, Valberg SJ, Vaudin M, Lindblad-Toh Wade CM, Mickelson JR (2008). A high-density SNP array for the domestic horse and extant Perissodactyla utility for association mapping, genetic diversity, and phylogeny studies. *PLoS Genetics*. 8 e1002451. https://doi.org/10.1371/journal.pgen.1002451

Mcnaughton SJ (1985). Ecology of a grazing ecosystem the Serengeti. *Ecological Monographs*, 55 259–294. https://doi.org/10.2307/1942578

Miao BP, Wang Z, Li YX. (2017). Genomic analysis reveals hypoxia adaptation in the Tibetan Mastiff by introgression of the grey wolf from the Tibetan Plateau. *Molecular Biology and Evolution*, **34**(3) 734–743. https://doi.org/10.1093/molbev/msw274

Moorcroft W & Trebeck G (1841). Travels in the Himalayan Provinces of Hindustan and the Panjab; in Ladakh and Kashmir; in Peshawar, Kabul, Kunduz, and Bokhara; from 1819 to 1825. *Murray*.1.

Mumtaz Ali, Kishor Patil, Ashwin Atkulwar (2023). Habitat survey of *Equus kiang kiang* from Changthang Wildlife Sanctuary of Ladakh, India. *Romanian Journal of Biology - Zoology*, **68**(1-2) 77–84. Musilova P, Kubickova S, Horin P, Vodicka R, and Rubes J (2009). Karvotypic relationships in Asiatic

asses (kulan and kiang) as defined using horse chromosome arm-specific and region-specific probes. *Chromosome Research*, **17** 783–790.

https://doi.org/10.1007/s10577-009-9069-3

Musilova P, Kubickova S, Vahala J and Rubes J (2013). Sub chromosomal karyotype evolution in Equidae. *Chromosome Research*, **21**(2) 175-187. https://doi.org/10.1007/s10577-013-9346-z

Myka JL, Lear TL, Houck ML, Ryder OA and Bailey E (2003). FISH analysis comparing genome organization in the domestic horse (*Equus caballus*) to that of the Mongolian wild horse (*E. przewalskii*). *Cytogenetic and genome research*, **102**(1-4) 222-225. https://doi.org/10.1159/000075753

Namgail T, Bagchi S, Mishra C and Bhatnagar YV (2008). Distributional correlates of the Tibetan gazelle *Procapra picticaudata* in Ladakh, northern India towards a recovery programme. *Oryx*, **42**(1) 107-112. https://doi 10.1017/S0030605308000768

Naoroji R and Sangha HS (2011). Threats to habitat and wildlife in Changthang and Rupshu areas of Ladakh, a case study at Hanle. *Indian Birds*, 7(1) 2-6.

https://api.semanticscholar.org/CorpusID 209464897

Oakenfull EA and Ryder OA (2002). Genetics of equid species and subspecies. Equids Zebras, Asses and Horses. *Status Survey and Conservation Action Plan*, 108-112.

http://www.equids.org/docs/Moehlman_02_Low.pdf

Oakenfull EA, Lim H, Ryder O (2000). A survey of equid mitochondrial DNA implications for the evolution, genetic diversity and conservation of *Equus. Conservation genetics*, **1** 341–355. https://doi.org/10.1023/A 1011559200897

Orlando L (2015). Equids. Current Biology, 25(20) R973-R978.

https://doi.org/10.1016/j.cub.2015.09.005

Paklina NV and Van Orden C (2007). Territorial Behaviour of Kiang (*Equus kiang* Moorcroft, 1841) in Ladakh (India). *Exploration into the Biological Resources of Mongolia*, ISSN 0440-1298. **10**. https://digitalcommons.unl.edu/biolmongol/87

Pfister O (1998). The breeding ecology and conservation of the Black-necked Crane (*Grus nigricollis*) in Ladakh, India. *Ph.D. Thesis. University of Hull, Hull.*

Policht R, Karadžos A and Frynta D (2011). Comparative analysis of long-range calls in equid stallions (Equidae) are acoustic parameters related to social organization? *African Zoology*, **46**(1) 18-26. https://doi.org/10.1080/15627020.2011.11407474

Rasool G (1992). Tibetan wild ass verging on extinction. Tiger Paper.19 16–17.

Reading RP, Henry M, Lhagvasuren B, Feh C, Kane DP, Dulamtseren S and Enkhbold S (2001). Status and distribution of khulan (*Equus hemionus*) in Mongolia. *Journal of Zoology*, **254**(3) 381-389. https //doi.org/10.1017/S0952836901000887

Rosenbom S, Costa V, Chen S, Khalatbari L, Yusefi GH, Abdukadir A, Yangzom C, Kebede F, Teclai R, Yohannes H, Hagos F, Moehlman PD, Beja-Pereira A. (2015). Reassessing the evolutionary history of ass-like equids insight from the pattern of genetic variation in contemporary extant populations. *Molecular Phylogenetics and Evolution*, **85** 88-96.https://doi.org/10.1016/j.ympev.2015.01.005

Rubenstein DI (1986). Ecology and sociality in horses and zebras. *Ecological Aspects of Social Evolution*, 282-302. https://cir.nii.ac.jp/crid/1570854174506168064

Ryder O, Epel N, Benirschke K (1978). Chromosome banding studies of the Equidae. *Cytogenetics and Cell Genetics*, **20** 323–350. https://doi.org/10.1159/000130862

Ryder OA, Chemnick LG (1990). Chromosomal and molecular evolution in Asiatic wild asses. *Genetica*, 83 67-72. https://doi.org/10.1007/BF00774690

Schaller, G. B. (1998). Wildlife of the Tibetan steppe. University of Chicago Press, Chicago, Illinois.

Schaller GB, Lu Z, Wang H and Su T (2005). Wildlife and nomads in the eastern Chang Tang Reserve, Tibet. *Memorie della SocietaItaliana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano*, 23 59-67.

Shafiq MM and A Ali (1998). Status of large mammal species in Khunjerab National Park. *Pakistan Journal of Forestry* 48 91–96.

Shah N (2002). Status and action plan for the kiang (*Equus kiang*). Pp. 72–81 in Equids zebras, asses and horses. Status survey and conservation action plan (P. D. Moehlman, ed.). International Union for Conservation of Nature and Natural Resources/Species Survival Commission, *Equid Specialist Group*, *Gland*, *Switzerland*.

Shah NV and Huibin Z (2000). Status of kiang (*Equus kiang*) in East Arjin Mountain Nature Reserve, Xinjiang Province, People's Republic of China. Report (Dec 1999-Jan 2000). *China Exploration and Research Society, Hong Kong.*

Shah N, St Louis A and Qureshi Q (2015). Equus kiang. The IUCN Red List of Threatened Species, 723. Shah NV (1994). Status survey of southern kiang (Equus kiang polyodon) in Sikkim, India. M.S. University of Baroda, and Zoological Society for the Conservation of Species and Populations, Baroda, Gujarat, India and München, Germany.

Shah NV (1996). Status and distribution of western kiang (*Equus kiang kiang*) in Changthang Plateau, Ladakh, India. M. S. University of Baroda, and Gujarat Nature Conservation Society, Baroda, Gujarat, India.

Sharma BD, Clevesr J, Graaf RD and Chapagaln NR (2004). Mapping *Equus Kiang* (Tibetan wild ass) habitat in Surkhang, Upper Mustang, Nepal. *Mountain Research and Development*, **24**(2) 149-156. https://doi.org/10.1659/02764741(2004)024[0149 MEKTWA]2.0.CO;2

Shrotriya S, Reshamwala HS, Mahar N, Habib B, Suhail I and Takpa J (2015). Distribution and Population Estimation of Ungulates in Changthang Region, Ladakh, Jammu and Kashmir India. Technical Report–*Wildlife Institute of India and Department of Wildlife Protection, Govt. of JandK.* (p. 15).

Spasskaya N (2007). Investigation of the kiang (*Equus kiang*, Equidae) skull from Ladakh, India. *Exploration into the Biological Resources of Mongolia, ISSN 0440-1298.* **10** 227-230. http://digitalcommons.unl.edu/biolmongol/90

Steiner CC, Mitelberg A, Tursi R and Ryder OA (2012). Molecular phylogeny of extant equids and effects of ancestral polymorphism in resolving species-level phylogenies. *Molecular Phylogenetic Evolution.* 65 573–581. https://doi.org/10.1016/j.ympev.2012.07.010

St-Louis A and Côté SD (2009). Equus kiang (Perissodactyla Equidae). Mammalian Species, 835 1–11. https://doi.org/10.1644/835.1

St-Louis A and Côté SD (2012). Foraging behaviour at multiple temporal scales in a wild alpine equid. *Oecologia*, **169**(1) 167-176. https://www.jstor.org/stable/41487941

St-Louis A and Côté SD (2014). Resource selection in a high-altitude rangeland equid, the kiang (*Equus kiang*) influence of forage abundance and quality at multiple spatial scales. *Canadian Journal of Zoology*, **92**(3) 239-249. https://doi.org/10.1139/cjz-2013-0191

Trifonov VA, Musilova P and Kulemsina AI (2012). Chromosome evolution in Perissodactyla. *Cytogenetic and Genome Research*, **137**(2-4) 208-217.

https://doi.org/10.1159/000339900

Trifonov VA, Stanyon R, Nesterenko AI, Fu B, Perelman PL, O'Brien PC and Yang F (2008). Multidirectional cross-species painting illuminates the history of karyotypic evolution in Perissodactyla. *Chromosome Research*, **16** 89-107.

https://doi.org/10.1007/s10577-007-1201-7

Turghan M, Ma M, Zhang X, Zhang T and Chen Y (2013). Current population and conservation status of the Tibetan wild ass (*Equus kiang*) in the Arjin Mountain nature reserve, China. *Pakistan Journal of Zoology*, **45**(5), 1249-1255.

Vilstrup JT, Seguin-Orlando, A Stiller, M, Ginolhac, A, Raghavan, M, Nielsen, S.C, Weinstock, J, Froese, D, Vasiliev SK, Ovodov ND, Clary J, Helgen KM, Fleischer RC, Cooper A, Shapiro B, Orlando, L, (2013). Mitochondrial phylogenomics of modern and ancient equids. *PLoS One*, 8 e55950. https://doi.org/10.1371/journal.pone.0055950

VonHoldt B, Fan ZX, Ortega-Del Vecchyo D, and Wayne RK. (2017). EPAS1 variants in high-altitude Tibetan wolves were selectively introgressed into highland dogs. *Peer Journal*, **5** e3522. https://doi.org/10.7717/peerj.3522

Zeng, L, Liu, H. Q, Tu, X. L, Ji, C. M, Gou, X, Esmailizadeh, A, and Sanke, O. J. (2021). Genomes reveal selective sweeps in kiang and donkey for high-altitude adaptation. *Zoological research*, **42**(4) 450. https://doi.org/10.24272%2Fj.issn.2095-8137.2021.095

Zhang, H, Chen, Y, Wu, X, Shang, S, Chen, J, Yan, J, and Zhang, H. (2020). Comparison of the gut microbiota in the Tibetan wild ass (*Equus kiang*) collected from high and low altitudes. *Pakistan Journal of Zoology*, **52**(6) 2281-2291.

Zhang, Z, Xu, D, Wang, L. I, Hao, J, Wang, J, Zhou, X, ... and Shi P *et al.* (2016). Convergent evolution of rumen microbiomes in high-altitude mammals. *Current Biology*, 26(14) 1873-1879. http://dx.doi.org/10.1016/j.cub.2016.05.012

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