

Population Genomics of Ungulates



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Abstract Humans have long relied on ungulates for food, clothing, manual labor, and transportation. Ungulates were among the first species to be domesticated and managed in the wild, but more than one-third of species are currently of conservation concern. Starting in the late twentieth century, ungulate research and management began employing genetic tools to assess attributes like the degree of population structure, inbreeding, and variation in functionally important genes. As sequencing technology advanced, research on ungulates shifted to now assay variation across the entire genome. More than 20 ungulates have had their genome assembled with a mean length of 2.6 Gb and N50 of 26 Mb. Genomic studies have provided deeper insights into the evolutionary relationships among giraffes and bovinds, while camelids and horses have had their entire species demographic histories reconstructed using novel Markovian coalescent models. Moreover, artificial and natural selection has left clear signatures on ungulate genomes with high-throughput sequencing techniques being used to identify the genetic basis to important phenotypic traits. Novel assembly strategies and genomic assays are regularly being employed on ungulates, and research on this ecological and economically valuable group will help chart the course of the emerging field of wildlife genomics.

Keywords Adaptation · Cross-species · Demography · Domestication · Genome assembly · SNP chip

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1 Introduction to Ungulates

Terrestrial ungulates are a diverse assemblage of species inhabiting ecosystems from the arctic to the desert (Fig. 1a). A defining characteristic of ungulates are their terminal phalanges enclosed in thick hoofs (Fig. 1b), thereby excluding cetaceans that are genetically embedded within the clade (Shimamura et al. 1997). Taxonomically, ungulates are represented by two orders, Cetartiodactyla or even-toed ungulates, such as pigs, goats, and camels, and Perissodactyla or odd-toed ungulates such as horses, tapirs, and rhinoceroses (Groves and Grubb 2011). Ungulates are under threat from stressors such as climate change, habitat loss, and overharvest (Ripple et al. 2015). In addition, most species exhibit some degree of migration or require large home ranges, with many of these territories and migration routes impacted by environmental perturbations (Dou et al. 2013; Post et al. 2008) and landscape modification (Gedir et al. 2015). Currently, 95 out of 235 ungulate species (numbers excluding the Cetacea order) are listed as critically endangered, endangered, or vulnerable by the International Union for the Conservation of Nature (IUCN 2017).

Humans have had a relationship with ungulates for thousands of years as they are critical sources of food, clothing, and general tools that assist in manual labor and transportation. Ungulates were among the first species to be domesticated starting with sheep and goats approximately 10,000 years ago (Chessa et al. 2009). Wild ungulates are an important subsistence source in indigenous communities (Vors and Boyce 2009) while also generating large revenues through sport hunting and ecotourism (Gordon et al. 2004). The first known efforts of game management



Fig. 1 Example of ungulates (a) and their characteristic enclosed phalanges, or hoofs (b). All images fall under Creative Commons attribution

involving ungulates can be traced to the Mongol Empire in the thirteenth century (Leopold 1987). Some semblance of game management appeared in Europe a century later where there were defined hunting seasons (Leopold 1987), while efforts at management in North America can be traced back to the late seventeenth century when a hunting ban on deer was enacted in Rhode Island (Brown 2013). To put their economic importance in perspective, in 2011 alone, 14 billion USD was spent hunting deer and elk in the United States (U.S. Fish and Wildlife Service 2012). Ungulates are also a keystone species in many ecosystems, with grazing enhancing ecosystem productivity by increasing the nutritional quality of forage and supporting higher plant biodiversity (Truett et al. 2001). While anthropogenic impacts have contributed to the aforementioned conservation listing of many ungulates, some species have benefited from human interventions in the form of habitat modification and the removal of predators, notably cervids (i.e., the deer family), where populations have required controlled culling to reduce numbers (Gordon et al. 2004).

Ungulate research and management changed in the late twentieth century, and a large number of individual-based, long-term ungulate research projects collecting samples for DNA analysis were established during this time (Table 1). Here, genetic tools have been vital for inferring parentage within the study populations, and more broadly, documenting population structure and quantifying patterns of gene flow and isolation (Scribner 1993). For example, reintroductions of species are often done with a limited number of individuals (Rhodes and Latch 2010; Scribner 1993); therefore monitoring genetic processes in populations has become a critical element of gauging the success of management efforts (Hogg et al. 2006; Miller et al. 2012). Genetic tools have also been instrumental for improving our understanding of life history characteristics, particularly mating structure. Targeting functional and immune genes like the major histocompatibility complex (MHC) has been commonplace in ungulate research. This is because variability in vertebrate MHC is associated with higher pathogen resistance (Janeway 2001; Quéméré et al. 2015) and often linked to individual fitness characteristics, such as body size, weight and parasite load, and secondary sexual traits such as antlers (e.g., Ditchkoff et al. 2001; Paterson et al. 1998; Wintemitz et al. 2013). These genotype-phenotype relationships (see Box 1), however, are nuanced, and, for example, in red deer some MHC variants were associated with higher resistance to some parasites but not others, and there was no relationship with antler size (Buczek et al. 2016). These genetic queries of ungulates have benefitted from recent advances in sequencing technology that have facilitated the transition from genetic to genome-wide datasets, resulting in numerous genome assemblies and resources for ungulates.

Table 1 Ongoing and long-term ungulate research projects involving genetic sampling

Species name	Common name	Location	Year
<i>Ovis canadensis</i>	Bighorn sheep	Ram Mountain, Alberta, Canada	1972
<i>Ovis canadensis</i>	Bighorn sheep	Sheep River, Alberta, Canada	1981
<i>Ovis canadensis</i>	Bighorn sheep	Bison Range, Montana, USA	1979
<i>Ovis aries</i>	Soay sheep	St. Kilda, Scotland	1985
<i>Oreamnos americanus</i>	Mountain goat	Caw Ridge, Alberta, Canada	1988
<i>Cervus elaphus</i>	Red deer	Isle of Rum, Scotland	1972
<i>Cervus elaphus</i>	Red deer	Trondelag, Norway	1987
<i>Equus ferus caballus</i>	Feral horses	Sable Island, Nova Scotia, Canada	2007
<i>Odocoileus virginianus</i>	White-tailed deer	Anticosti Island, Quebec, Canada	2002
<i>Capreolus capreolus</i>	Roe deer	Trois Fontaines, France	1975
<i>Capreolus capreolus</i>	Roe deer	Chize, France	1977
<i>Rangifer tarandus</i>	Svalbard reindeer	Svalbard archipelago, Norway	1994, 2002
<i>Bison bison</i>	Bison	Konza Prairie, Kansas, USA	1987
<i>Bison bison</i>	Bison	Wind Cave, South Dakota, USA	1960s
<i>Ovis gmelini</i>	European mouflon	Caroux-Espinouse Massif, France	1985
<i>Rupicapra rupicapra</i>	Alpine chamois	Les Bauges, France	1985
<i>Capra ibex</i>	Alpine ibex	Belledonne, France	1985
<i>Dama dama</i>	Fallow deer	Phoenix Park, Ireland	1985
<i>Dama dama</i>	Fallow deer	San Rossore, Italy	1984
<i>Tragelaphus strepsiceros</i>	Greater kudu	Kruger Park, South Africa	1984–1993
<i>Alces alces</i>	Moose	Vega, Norway	1992
<i>Antilocapra americana</i>	Pronghorn antelope	Bison Range, Montana, USA	1981
<i>Rupicapra pyrenaica</i>	Pyrenean chamois	Orlu, France	1985
<i>Rupicapra pyrenaica</i>	Pyrenean chamois	Parc National des Pyrenees, France	1993

2 A Brief History of Whole-Genome Assemblies in Ungulates

The first assemblies of ungulate genomes started with domestic species of high interest and economic importance. The first completed ungulate draft genomes were of the cow (*Bos taurus*) in 2004 and domestic horse (*Equus caballus*) in 2007, both of which used early forms of whole-genome shotgun sequencing (Elsik et al. 2009; Wade et al. 2009). This method randomly fragments genomic DNA and inserts it into either fosmid or bacterial artificial chromosomes (BAC) vectors. The DNA is then clonally amplified, and random clones are selected and sequenced using Sanger sequencing. With technological improvements, subsequent ungulate genomes applied hybrid approaches that combined BAC-end or fosmid-end sequencing with newer high-throughput sequencing (HTS) techniques. In this approach, library

preparation occurred in a cell-free system, rather than cloned in vectors, and produced significantly more data in less time. Both domestic goat (*Capra hircus*) and domestic pig (*Sus scrofa*) used BAC and fosmid sequencing to create long read pairs that facilitated the placement of the newer high-throughput but short-read data into contigs and scaffolds (Dong et al. 2015; Groenen et al. 2012). Improvements in assembly continued with the addition of mate-pair library preparations that circularized long fragments of DNA, thereby creating long insert reads without the use of vectors. This approach has been used to assemble both the domestic yak (*Bos mutus*) and sheep genome (*Ovis aries*; Jiang et al. 2014; Qiu et al. 2012).

Most genome sequencing projects to date have been concentrated on model or domestic species. With the improvement and lowered cost in sequencing technologies, that focus has shifted to include more wildlife species (Ellegren 2014). Current genome assemblies rely on whole-genome sequencing of short-read and mate-pair fragments (or alternative long-read sequencing strategies) with HTS technology. For ungulate assemblies, the most popular sequencing technology is Illumina sequencing by synthesis that includes, for example, the wild goat (*Capra aegagrus*; Dong et al. 2015), giraffe (*Giraffa camelopardalis*; Agaba et al. 2016), and Przewalski's horse genomes (*Equus przewalskii*; Huang et al. 2014). A trend has emerged toward using multiple sequencing technologies to improve genome assembly; for example, the Bactrian camel (*Camelus ferus*; Jirimutu et al. 2012) used both Illumina and SOLiD sequencing, while the donkey (*Equus asinus*; Huang et al. 2015) assembly used both Illumina and 454 pyrosequencing technologies. The bioinformatics combination of high-coverage sequencing of a wild species and use of a closely related reference are also commonplace. Both the okapi (*Okapia johnstoni*; Agaba et al. 2016) and bighorn sheep (*Ovis canadensis*; Miller et al. 2015) assemblies relied on closely related [domestic] genomes. Overall we collected data on 25 ungulate genomes; these showed high variation in N50 scaffold size (range from 10,458 to 100,310,653) with a mean N50 of 27,074,698. Genome size estimates were more consistent ranging from 1.99 to 3.31 Gb, with a mean length of 2.61 Gb (Table 2).

3 The Importance of Domestic Genomes

Ungulate research has benefited from genome projects on domestic animals more so than most other taxonomic groups. Resources like primers, SNP chips, and annotated genomes generated in domesticated species including cow, sheep, goat, pig, and horse can be applied to related ungulates, resulting in so-called “genome-enabled” taxa (Kohn et al. 2006). While there are limits to how distantly related a species one can use (Cosart 2013; Miller et al. 2012), and concerns related to potential biases (Powell et al. 2016; Shafer et al. 2016), the genome-enabled nature of ungulates has permitted addressing genome-scale questions in ungulates well before other taxonomic groups. The application of cross-amplified markers has been used to generate relatively dense linkage maps (Poissant et al. 2010; Slate et al. 2002), assess population structure (Miller et al. 2011; Haynes

Table 2 Whole-genome assemblies in ungulates

Species name	Common name	Assembly length (Mb)	N50 (scaffold)	Coverage	Sequencing technology
<i>Camelus ferus</i>	Wild Bactrian camel	2,009	2,005,940	30×	Illumina GAIIx; 454 GS-FLX Titanium; SOLiD 3
<i>Camelus dromedarius</i>	Arabian camel	2,004	4,188,677	65×	Illumina HiSeq2000
<i>Camelus bactrianus</i>	Bactrian camel	1,993	8,812,066	79×	Illumina HiSeq2000
<i>Bison bison</i>	American bison	2,828	7,192,658	60×	454; Illumina HiSeq
<i>Bubalus bubalis</i>	Water buffalo	2,836	1,412,388	70×	Illumina GAIIx; Illumina HiSeq; 454
<i>Capra aegagrus</i>	Wild goat	2,829	91,317,560	84×	Illumina HiSeq2000
<i>Capra hircus</i>	Domestic goat	2,923	87,277,232	50.0×	PacBio
<i>Bos mutus</i>	Wild yak	2,645	1,407,960	130×	Illumina HiSeq; Illumina GA
<i>Ovis aries</i>	Sheep	2,616	100,009,711	166×	Illumina GAII; 454; PacBio RSII
<i>Ovis canadensis</i>	Bighorn sheep	2,591	100,190,483	12×	PacBio
<i>Capreolus capreolus</i>	Western roe deer	2,785	10,458	50×	Illumina HiSeq
<i>Odocoileus hemionus</i>	Mule deer	3,310	156,284	50×	Illumina HiSeq
<i>Okapia johnstoni</i>	Okapi	2,878	111,538	30×	Illumina HiSeq
<i>Giraffa camelopardalis</i>	Giraffe	2,705	212,164	37×	Illumina HiSeq
<i>Sus scrofa</i>	Pig	2,809	576,008	24×	Illumina GAII
<i>Ceratotherium simum</i>	Southern white rhino	2,464	26,277,727	91×	Illumina HiSeq
<i>Equus asinus</i>	Ass	2,391	3,776,412	42×	Illumina MiSeq; 454; Illumina HiSeq
<i>Equus caballus</i>	Horse	2,475	46,749,900	6.8×	ABI 3730
<i>Equus przewalskii</i>	Przewalski's horse	2,396	513,800	86×	Illumina HiSeq
<i>Loxodonta africana</i>	Elephant	3,197	46,401,353	7×	Sanger
<i>Bos taurus</i>	Cow	2,650	6,806,220	19×	Sanger, PacBio RS II
<i>Bos indicus</i>	Zebu	2,674	106,310,653	52×	SOLiD
<i>Vicuna pacos</i>	Alpaca	2,014	5,303,709	73×	Illumina HiSeq2000
<i>Pantherops hodgsonii</i>	Chiru	2,697	2,772,860	67×	Illumina
<i>Elaphurus davidianus</i>	Père David's deer (Milu)	2,580	2,850,000	114×	Illumina HiSeq2000

Assembly length is reported in megabases (Mb) and N50 scaffold is equal to the length of the longest sequence where the sum of the lengths is greater than or equal to half the length of the genome being assembled

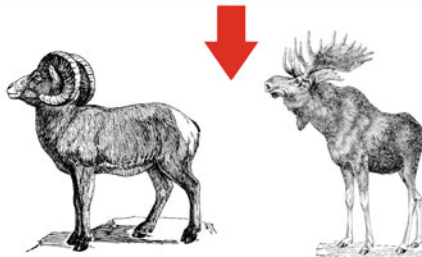
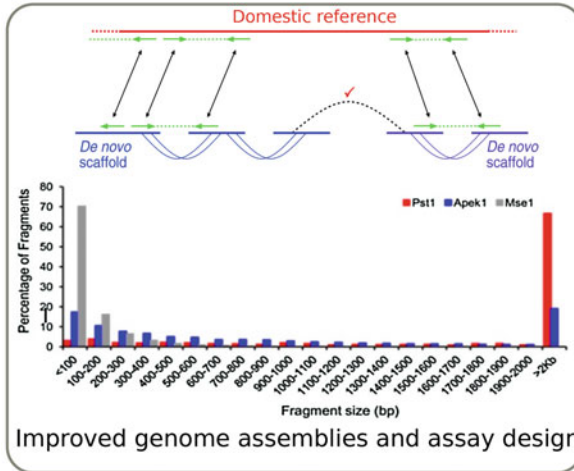
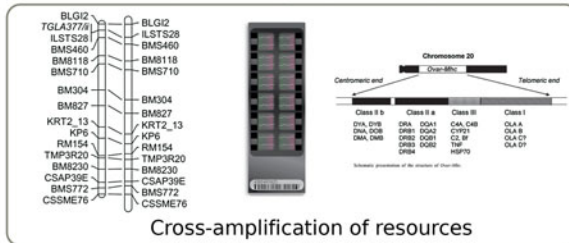
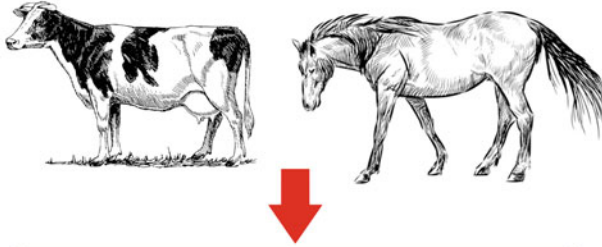
and Latch 2012; Iacolina et al. 2016), scan for adaptive loci (Sim et al. 2016; Powell et al. 2016; Roffler et al. 2016a, b), and assay functional gene variation (Slate et al. 2009; Shafer et al. 2012). Moreover, both population monitoring and management of wild ungulates have routinely relied on markers developed in the non-focal species (Corti et al. 2011; Ogden et al. 2012; Olson et al. 2012). The cross-species application of resources remains prevalent in basic and applied research of ungulates (Fig. 2).

Despite multiple *de novo* assemblies for ungulates, quality varies as evidenced by the N50 scaffold estimates (Table 2). This is because for mammals, *de novo* genome assembly and annotation are still prohibitive in terms of cost, computational resources, and required genetic material for a high-quality draft genome (Ekblom and Wolf 2014). There are multitudes of less direct benefits of being genome-enabled taxa. For example, *de novo* genome assemblies can use the domestic reference as a backbone to make assemblies more tractable, as was the case in bighorn sheep (Miller et al. 2015) and elk (Brauning et al. 2015). Similarly, genome annotation can take advantage of protein-based homology approaches using the wide array of domesticated ungulate data (Fitak et al. 2016). *In silico* digests of domestic genomes also provide important information for genomic assay design (Van Tassel et al. 2008; Wiedmann et al. 2008) and techniques like optical mapping (Dong et al. 2013): this information can easily be incorporated into experimental designs and analyses of wild populations with limited resources. From a practical standpoint, sequencing facilities require basic genome information that more often than not can simply be borrowed from the most closely related wild or domestic relative (Fig. 2; Table 2).

4 Alternatives to Whole-Genome Sequencing

Reduced representation strategies have emerged as valuable alternatives to whole-genome sequencing (Narum et al. 2013), though they have been used sparsely in ungulates. By targeting a subset of the genome for resequencing, reduced representation strategies facilitate HTS in non-model species even in the absence of genomic information. Reduced representation approaches largely fall into two categories, genotyping by sequencing (GBS) approaches that target for DNA variants distributed across the genome and transcriptomes that provide insight into functional variation in transcribed DNA. Transcriptome sequencing has typically focused on domestic species (Jäger et al. 2011; McLoughlin et al. 2014) or farmed species to identify differentially expressed genes associated with disease or economically valuable traits (Box 1, Marfell et al. 2013; Yao et al. 2012a). A transcriptome of white-tailed deer has also been generated (Genomic Resources Development Consortium et al. 2014) that will be useful for annotation and assay designs. GBS assays allow for a targeted subset of genome to be sequenced across multiple individuals or populations and can be used to target anonymous loci, candidate loci, or a combination of both, each coming with important considerations for implementation

Domestic species



Wild species

Fig. 2 The genome-enabled nature of ungulate genomics. Genomic resources developed in domestic animals (top) have been, and still are, applied to wild ungulate species (bottom). This includes cross-amplification of markers for building linkage maps, SNP chips for genotyping, and candidate genes for sequencing. Domestic reference genomes can also be used to improve de novo assemblies of wild ungulates and help with the design assays (i.e., in silico digest of domestic genome to select restriction enzymes for a reduced representation sequencing approach)

(Andrews et al. 2016; Jones and Good 2016). Surprisingly few studies focusing on an ungulate taxon have actually employed a GBS assay. To our knowledge GBS assays have been limited to cattle (De Donato et al. 2013), pygmy hippo (Senn et al. 2014), and addax (Ivy et al. 2016). In part, readily available SNP chips from domesticated species have fettered the de novo development of GBS assays in wild ungulates.

Candidate genes of interest can be surveyed through targeted resequencing. Here, cross-species exon capture, in which coding sequences from a model species are used to create hybridization probes to sequence homologous exons in non-model species, has seen recent application in ungulates. Cosart et al. (2011) first demonstrated the utility of this approach using the cattle genome to capture exons in bison and zebu. Powell et al. (2016) used the cattle genome to capture exons in mule deer (*Odocoileus hemionus*), while Roffler et al. (2016a) used probes designed from the domestic sheep genome to capture exons in Dall's sheep (*Ovis dalli dalli*). In recent applications where no genome was available in the target species, over half of the probes designed in cattle successfully hybridized in mule deer (~30 million years divergent; Powell et al. 2016), and 35% of probes hybridized to pigs (50–60 million years divergent; Cosart 2013). Overall, both reduced representation and exon capture data provide useful insight into population processes and adaptive and functional variation in ungulates (Cosart et al. 2011; Hodges et al. 2007; Powell et al. 2016; Roffler et al. 2016a) that can be informative for basic research and conservation and management programs.

5 From Phylogenomics to Population Demography

Examining newly constructed ungulate genomes has allowed for determination of the most recent common ancestor (MRCA) and reconstructing evolutionary relationships. This is particularly relevant for ungulate groups like the Caprinae, where even 6,000 base pairs of DNA sequence data could not resolve deeper phylogenetic relationships (Shafer and Hall 2010). Whole-genome approaches must consider the influence of incomplete lineage sorting (Nater et al. 2015), although this appears to be only a minor cause of phylogenetic discrepancies within mammals (Scornavacca and Galtier 2017). Among ungulates, whole-genome sequencing of the okapi and giraffe showed that giraffe species shared a MRCA ~2 mya, with the okapi roughly 11.5 mya, and with cattle 28 mya (Agaba et al. 2016). Glanzmann et al. (2016) showed that the African buffalo shared MRCA with the cow (*Bos taurus*) approximately 5–10 mya based on the two species' genome sequences.

Genomic data also offer the prospect of reconstructing population histories from a single contemporary genome, a complex task that is virtually impossible with patchy or nonexistent observational and fossil data. Two particularly exciting approaches are the pairwise and multiple sequentially Markovian coalescent models (SMCs; Li and Durbin 2011; Schiffels and Durbin 2014 see also Salmona et al. 2017) that were developed for whole-genome data and have recently been applied to GBS data

(Liu and Hansen 2017). Using SMC models, Orlando et al. (2013) compared the genomes of five domestic horse breeds, a Late Pleistocene horse, Przewalski's horse, and a donkey to reconstruct the demographic history of the modern horse. Horse population fluctuations coincided with favorable and adverse climatic conditions and were confirmed by population size estimates resulting from analysis of ancient mitochondrial DNA genomes (Orlando et al. 2013). Recent reductions correspond to the last glacial maximum with no evidence of recovery (Fig. 3a; Der Sarkissian

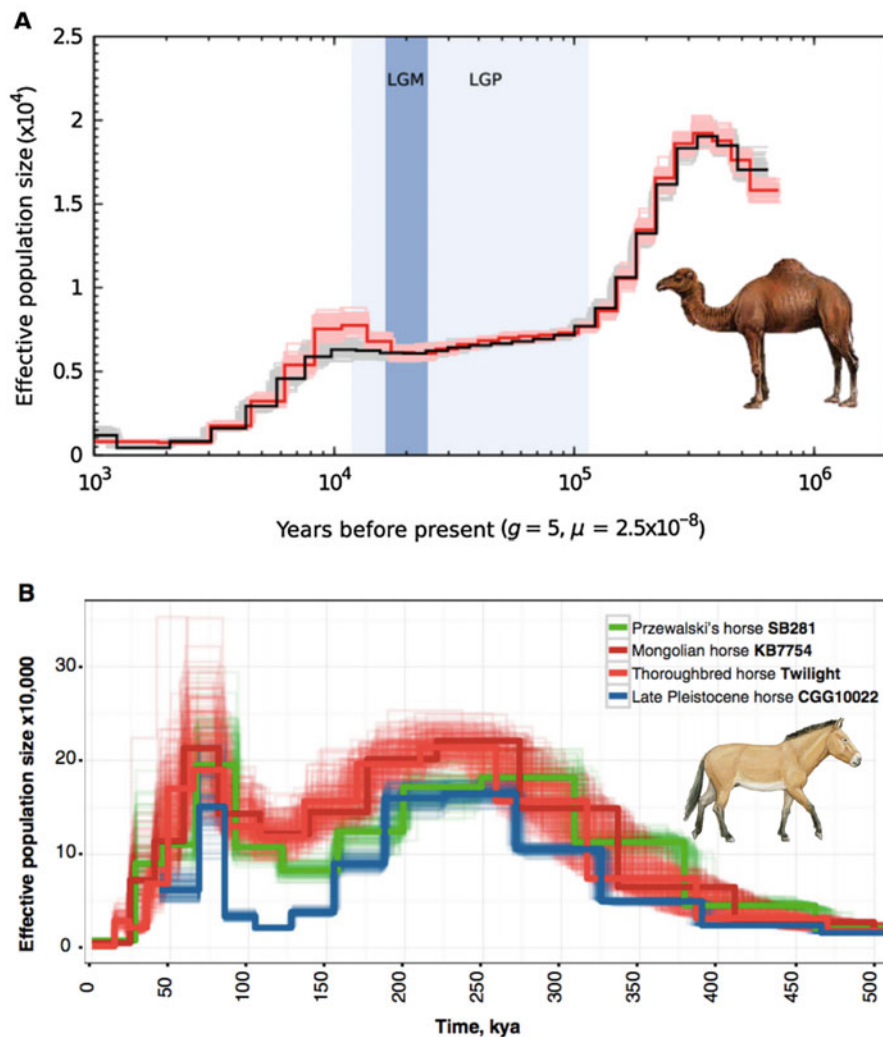


Fig. 3 Demographic inferences using pairwise sequentially Markovian coalescent (PSMC) models in (a) dromedary (camel) and (b) horse species. PSMC plots are from Fitak et al. (2016) and Der Sarkissian et al. (2015)

et al. 2015; Librado et al. 2015). Similarly, Wu et al. (2014) characterized the demographic history of three Old World camel species over the last million years (only dromedary shown in Fig. 3b). The three camel species experienced sharp drops in the effective population size (up to 70%) from 100,000 to 20,000 ya due to climatic changes during the last glacial period (Burger 2016; Fitak et al. 2016) with the reasons for a second, recent reduction hypothesized to be from habitat loss and increased hunting pressure (Burger 2016).

6 Adaptive Divergence in Ungulates

Ungulates inhabit some of the most inhospitable environments on earth ranging from the Arctic to desert. These selective pressures have driven genetic changes by favoring certain phenotypes in one environment, thereby increasing corresponding allele frequencies over generations (Whitehead 2012). A plurality of genome sequencing approaches can be used to identify locally adapted genes (Hoban et al. 2016), with many being applied to ungulates. Desert species, like camels and some goat and sheep populations, have several adaptations to deal with excessive sun exposure and hot and dry conditions (Table 3). To combat low water availability in the desert, both camels and sheep have adaptations that promote vasodilation and water reabsorption (Jirimutu et al. 2012; Yang et al. 2016). Camels also show evidence for selection on genes that affect photoreception and visual protection, presumably related to high exposure to UV radiation in the desert (Wu et al. 2014). Similarly, goat and sheep populations showed selection on genes that have likely contributed to thermotolerance by regulating melanogenesis and coat color (Kim et al. 2016).

Genome sequencing of the Tibetan yak and goat has detected genetic evidence for adaptation to high-altitude environments (Qiu et al. 2012; Song et al. 2016). Both yak and goats show increased blood hemoglobin concentrations (Song et al. 2016), whereas sheep in the Himalayas appear to have evolved enhanced lung capacity (Gorkhali et al. 2016). Ungulates inhabiting the Arctic and sub-Arctic, including the Yakutian horse and even woolly mammoth (although a member of the Proboscidea order), have shown evidence for changes altering the regulation of epidermis and hair development, creating thicker, warmer hair (Librado et al. 2015; Lynch et al. 2015). These two species also have adaptations associated with their circadian clock in order to maintain normal rhythms despite the lack in daily light and dark cycles in the Arctic (Librado et al. 2015; Lynch et al. 2015).

Not all adaptations are due to natural selective pressures but rather domestication events and selective breeding. The domestication and selective breeding of goats, pigs, and horses have led toward increased productivity (e.g., milk production, body size, and fecundity; National Research Council et al. 2002). Fertility is an important factor when breeding, and genes associated with increased litter sizes have

Table 3 Candidate genes identified in ungulates, the proposed selection pressure, and adaptive response

Selection pressure	Adaptive response	Species	Candidate genes
Desert/low water availability	Increased water reabsorption/salt concentration	Camel, sheep	CYP2J, CYP2E, GPX3
Desert/airborne dust	Increased respiratory health/lung development	Camel	FOXP3, CX3CR1, CYSLTR2, SEMA4A
Desert/high UV exposure	Increased visual protection	Camel	OPN1SW, CX3CR1, CNTFR
High altitude/low oxygen availability	Increased hypoxia response	Yak, horse, sheep	Adam17, Arg2, Mmp3
High altitude/low oxygen availability	Increased hemoglobin concentration	Horse, yak	EPAS1
Desert/high temperature	Increased thermotolerance (melanogenesis)	Sheep, goat	FGF2
Arctic/extended periods of light and dark	Altered circadian clock response	Woolly mammoth, horse	LECT2, FBXL21, PER2
Arctic/low temperature	Increased hair and epidermis development	Woolly mammoth, horse	BARX2, TRPV3
Domestication	Increased fat production/composition	Pig	FASN, MOGAT2
Domestication	Nervous system	Pig, horse, goat	VDAC1, GRID1, CACNA1C, CACNA1D
Domestication/racing	Increased speed	Horse	MSTN
Agriculture/transport	Equine physiology	Horse	NR3C2, SCPEP1, ACAD8, SGCD
Domestication	Higher fertility	Pigs	AHR
Intra-male competition and female choice	Larger horns	Bighorn sheep, Soay sheep, sheep	RXFP2
Low food availability	Increased energy storage/metabolism	Camel, sheep, goat, horse, woolly mammoth	ACC2, DGKZ, GDPD4, GLUL, GCNT3, MYH, NOS3, eNOS, CPS1

signatures of selection (Groenen 2016). Both horses and goats have been under selection for serotonin release, as this is involved in the taming of animals (Dong et al. 2015; Schubert et al. 2014). There are also optimal or desired traits in domesticated species that have been selected for by breeders. For example, horses show selection for an allele in the MSTN gene, also known as the speed gene in

thoroughbreds (Schubert et al. 2014), and there is evidence for selection of genes like NR3C2, SCPEP1, ACAD8, and SGCD that are associated with the increased energy demand required agriculture, transport, and racing (Schubert et al. 2014). Pigs show signatures of selection due to breeding and domestication, specifically in fat composition (Molnár et al. 2014); the gene FASN, which encodes a fatty acid synthase, increases the total body weight and fat, and the gene MOGAT2 affects the absorption and digestion of fat (Molnár et al. 2014).

7 Future Perspectives

More than one-third of ungulate species are of conservation concern, with threats ranging from climate change to overharvest (IUCN 2017). Disease and other changing selective pressures can lead to rapid population declines in wildlife populations – often threatening population persistence, as evidenced by several emerging wildlife diseases and the increased transfer of disease between domestic and wild ungulate populations (e.g., see Forde et al. 2016; Kutz et al. 2004; Martin et al. 2011). Selective forces exerted by infectious diseases can rapidly influence the distribution of adaptive genetic variants associated with disease susceptibility over short time scales (Gallana et al. 2013). This process can result in an evolutionary rescue of a species (Carlson et al. 2014; Maslo and Fefferman 2015), where disease-resistant animals increase in number subsequent to initial population declines from strong selective sweeps from disease. Supporting this would be scans of immune genes showing signatures consistent with selection in buffalo (Lane-deGraaf et al. 2015) and more broadly ungulates (Schaschl et al. 2006). The prion gene (PRNP) is of particular relevance for screening in cervids because of its link to chronic wasting disease resistance.

Genomic data can also guide the identification of individuals most suited for translocation and introduction to genetically depauperate populations. For example, in case of a disease outbreak, genome scans can test for pathogen presence in the putative source (see also Box 2). Extending this idea, translocation candidates can also be selected based on specific genomic markers that increase fitness or just to introduce more variation to the target population (Shafer et al. 2015). Genetic and genomic profiles of endangered oryx species (Hedrick et al. 2000; Ogden et al. 2012) are being factored into breeding programs and eventual reintroduction plans. Further, monitoring the success of a translocation or reintroduction can be bolstered by genome data, where Miller et al. (2012) used genomic data to document the positive effect of introductions, including the reversal of deleterious effects of inbreeding, in a population of bighorn sheep.

Box 1 Genetic Basis to Horns and Antlers

Multiple ungulate species have evolved cranial appendages in the form of horns and antlers (Fig. 4). Understanding the genetic basis to these traits is important for our understanding of both sexual selection (Poissant et al. 2008) and artificial selection (Hengeveld and Festa-Bianchet 2011), with work on the impact of trophy hunting (Coltman et al. 2003) and the downstream management consequences being particularly polarizing. However, pedigree (Coltman et al. 2003) and harvest data (Pigeon et al. 2016) data show an unequivocal genetic component to horn growth. Likewise, antler characteristics are heritable, although estimates vary (Lukefahr and Jacobson 1998; Michel et al. 2016; Williams et al. 1994). Targeted sequence of immune genes and their influence on antler development have produced mixed results (Buczek et al. 2016; Ditchkoff et al. 2001), but genomic studies have identified multiple candidate genes (Johnston et al. 2011; Poissant et al. 2008), with the relaxin-like receptor 2 (RXFP2) linked to horn size in Soay sheep (Johnston et al. 2013) and bighorn sheep (Kardos et al. 2015; Roffler et al. 2016a, b). Transcriptome sequencing has also identified candidate genes associated with antler development, with genes involved in signaling (growth) pathways and extracellular matrix proteins upregulated (Yao et al. 2012b). While genome scans for genes underlying traits in natural populations will have limitations (Kardos et al. 2016), the array of long-term studies with pedigree information (Table 1) hold considerable promise for elucidating the genomic architecture of horns and antlers. Once detected, management and conservation agencies could easily screen such genes – especially in scenarios of suspected artificial selection – to inform harvest quotas and breeding designs.



Fig. 4 Many ungulate studies have focused on detecting the genetic basis to (a) antlers and (b) horns. Antlers consist of bone and are shed annually by members of the cervid family; horns have a permanent bony core that is covered by keratin and is found in the bovid family. Images courtesy of Peter Mills

Box 2 Metagenomic Assessments of Ungulate Microbiomes and Viromes

Metagenomes are defined as all the genetic material found within an environmental sample but have a general focus on microbiota (Marchesi and Ravel 2015). As such, the source of this genetic material falls into two groups: viral, including bacteriophages and RNA and DNA viruses that make up the virome, and microbiota that include bacteria, archaea, and fungi that make up the microbiome. Genomic tools include targeted amplicon sequencing of conserved genes, such as 16S in bacteria, and shotgun sequencing techniques that rely on the similarity of sequences to define operational taxonomic units (OTUs). These molecular approaches to assessing metagenomes are important in that many taxa are either very difficult or too dangerous to culture, and even when cultured, can be very difficult to identify phenotypically (Budowle et al. 2007).

The importance of elucidating the diversity of different microbiomes and viromes is related to the fact that this nonhost genetic material is integral to species health and can be commensal, symbiotic, and pathogenic in nature. The roles of these nonhost taxa are as fundamental as conveying an ability to process and obtain nutrients from food to protecting or harming the host organism through varied immune function. Specifically, ungulate microbiomes play central roles in digestion by providing the metabolic capabilities required to digest the ingested plant material (Yoon et al. 2015). Importantly, understanding the microbial communities of commercial ungulates is front and center when it comes to improving production (Alexander and Plaizier 2016). It is not surprising then, that much of the literature on ungulate metagenomics is focused on applications within animal husbandry. For example, bovine respiratory disease is a costly yet poorly understood disease for the cattle industry. Mitra et al. (2016) characterized the virome of cattle and found that a suite of both previously known and uncharacterized viruses contribute to the etiology of this disease. Magistrelli et al. (2016) investigated the influence of different diets on the microbial populations of pig fecal matter using targeted oligonucleotide probes to gain insight into the respective bacteriomes and infer health. Similar work on captive musk deer identified a shift in microbial communities with age (Hu et al. 2017).

Metagenomic approaches have been used to illustrate not only the diversity but also the evolutionary history, geographic range, and spread of various pathogens. Schirtzinger et al. (2015) gained insight into the evolutionary history and spread of viruses associated with respiratory syndrome in pigs through metagenomic sequencing, whereas Dupuy et al. (2015) documented

(continued)

Box 2 (continued)

the spread of contagious caprine pleuropneumonia that infects both wild and domestic goats in Africa and Asia. As such, metagenomic tools have been identified as being critical to enhanced surveillance and control measures for these devastating diseases and begin to highlight the real threats of transmission of infectious disease between wildlife and domestic ungulates (Martin et al. 2011; Maclachlan and Mayo 2013). For wild populations, beyond identifying health parameters, varying metagenomic profiles have the potential to give higher spatial and temporal resolution to the movements of their host species and spread of emerging diseases, although to date, such approaches have been limited. Forde et al. (2016) used bacterial genomics to understand the epidemiology of *Erysipelothrix rhusiopathiae* in Arctic and boreal ungulates, including caribou, moose, and muskox. In yaks, further insight was gleaned from bacterial genomic analyses of a diarrhetic disease (Chen et al. 2015). Beyond applications toward enhanced understanding of animal health and enhancing commercial applications, ungulate metagenomics, specifically those from pigs, are also often used as surrogates for human research given the similarity of their internal flora and fauna relative to humans. McIntyre et al. (2016) used 16S rDNA profiling to identify the cutaneous microbiome of pigs on healthy skin and at the sites of wounds and found subsets of bacteria accelerate healing from injury. Human health is also heavily impacted by zoonoses, such as identified by Roth et al. (2016) where hepatitis E viral infections in humans were traced back to wild boar strains. The applications and implications of metagenomics work are largely nascent, but the aforementioned spectrum of studies provides insight into the importance of continued research in this field.

Genome-wide SNP arrays can be useful for estimating relationships among individual animals and for reconstructing pedigrees, which has advanced conservation and management of both captive and wild ungulates (Box 3). Genomic data also provide accurate estimates of inbreeding and can identify the specific genes contributing to inbreeding depression (Kardos et al. 2016). This can have important consequences, as, for example, in Soay sheep, the estimated rate of inbreeding detection was higher based on genomic data compared to more traditional molecular markers (Bérénois et al. 2016). In red deer only three fitness components were significantly correlated with the pedigree inbreeding estimate compared to six based on SNP data (Huisman et al. 2016). Genomic data provide some key advantages over pedigree estimates in terms of the sensitivity and accuracy of inbreeding estimates while permitting the potential identification potential genes underlying inbreeding depression (Kardos et al. 2016).

Box 3 Genomics for Conservation and Management of Captive and Intensively Managed Ungulates

Genomic data has improved the management of captive and intensively managed ungulates. Zoo-based captive breeding programs typically rely on accurate pedigrees to maintain genetic variation and prevent close inbreeding over the long term. Breeding strategies that minimize the average kinship (coancestry) in a population are an effective way to retain diversity and limit the accumulation of inbreeding (Lacy 1995; Fernández and Toro 1999; Sonesson and Meuwissen 2001). In the absence of complete data on parentage, molecular data can be used to resolve unknown relationships. High-throughput sequencing methods have the potential to revolutionize the genetic management of populations with incomplete or poorly known pedigrees, because we can use a large number of DNA markers to calculate very accurate estimates of kinship between animals (Jones et al. 2010; Santure et al. 2010; Skare et al. 2009). In captive ungulates, genome-wide SNPs have been used to accurately estimate relationships among individuals (Bosse et al. 2015; Ivy et al. 2016). Ivy et al. (2016) used both the BovineHD BeadChip and RAD-derived SNPs to resolve unknown relationships within the addax (*Addax nasomaculatus*) captive breeding program. Both approaches produced sufficient data to accurately estimate relationships, even in this severely bottlenecked population. Molecular coancestry estimates are also improving genetic diversity retention in intensively managed wild populations, where managers cannot dictate breeders but can remove less valuable individuals from the breeding pool (Eggert et al. 2010; Giglio et al. 2016, 2018). In bison, a kinship-based removal strategy outperformed alternative removal strategies at retaining genome-wide variation over the long term and limiting the accumulation of inbreeding (Giglio et al. 2016). Long-term population viability relies on the maintenance of genome-wide variation, and HTS has the potential to revolutionize the genetic management of captive and wild populations by supplying a very large number of markers distributed throughout the genome to calculate very accurate empirical estimates of genetic variation within and among populations and genetic relationships between individual animals.

Conservation and management of ungulates stands to benefit from identifying geographic patterns of local adaptation, the environmental drivers of divergent selection among populations, and genes and their variants involved in local adaptation, especially in the context of changing environmental conditions. Individuals with adaptive genetic variants leading to higher fitness in local environments could be used in conservation actions such as breeding programs, assisted gene flow, genetic rescue, or reintroduction programs to help ensure success of those programs. Managers could monitor the frequency of adaptive variants over time to assess the genetic health of a population following management interventions (Schwartz et al. 2007; Shafer et al. 2015; Flanagan et al. 2017).

8 Summary

Ungulates are a diverse taxonomic group that can be found in some of the most extreme environments. For most of human history, people have relied on ungulates for food, clothing, and transportation, with more contemporary uses including sport hunting and ecotourism. In the late twentieth century, ungulate management programs began to incorporate genetic data; with technological advancements and lowering costs of genome sequencing, it has become possible to address more detailed questions about the evolution, conservation, and management of ungulates, both in captivity and the wild. The first ungulate genomes sequenced were those of domestic animals due to economic importance. Wild ungulate genomes have started to emerge, and in many cases rely on domestic genome assemblies. Genomic data allows for reconstruction of the evolutionary and population histories from a few contemporary genomes while providing the tools to uncover the genes underlying adaptive divergence. In particular, both natural selection and artificial selection have left distinct signatures on ungulate genomes, and there is a wide array of candidate genes identified. For conservation and management, genomic data provides more accurate estimates of inbreeding, allows for the reconstruction of pedigrees in wild populations, and facilitates the design of breeding and management programs to retain genetic variation, based on markers distributed at high density across the genome. As more ungulate genomes are sequenced and the existing assemblies are improved, our understanding of population processes and the genetic basis of key traits will steadily improve, impacting regional economies, and the conservation and management of this important group.

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