

Genomic insights into ruminant evolution: from past to future prospects

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ABSTRACT

Ruminants (Ruminantia) are among the most successful herbivorous mammals, exhibiting wide-ranging morphological and ecological characteristics (such as headgear and multichambered stomach) and including various key livestock species (e. g., cattle, buffalo, yak, sheep, and goat). Understanding their evolution is of great significance not only in scientific research but also in applications potential for human society. The rapid growth of genomic resources provides unprecedented opportunities to dissect the evolutionary histories and molecular mechanisms underlying the distinct characteristics of ruminants. Here we summarize our current understanding of the genetic, morphological, and ecological diversity of ruminants and provide prospects for future studies.

Keywords: Ruminantia; Genome evolution; Phylogenomics; Traits; Adaptive evolution

INTRODUCTION

Ruminantia mammals are among the most successful herbivores, and include six families: i. e., Antilocapridae,

Bovidae, Cervidae, Giraffidae, Moschidae, and Tragulidae. Ruminantia comprises at least 200 extant species, with Bovidae the most species-rich, consisting of at least 143 species (Heller et al., 2013), including important livestock such as cattle (*Bos taurus*), yak (*Bos grunniens*), sheep (*Ovis aries*), and goat (*Capra hircus*). Ruminants are distributed across extensive habitats, including different latitudes (from tropical to Arctic regions), different altitudes (from plains to plateaus), and different ecological environments (from deserts to rainforests). Moreover, ruminants exhibit several distinct anatomical features, such as headgear and multichambered stomach. Compared with other herbivores, such as horses, the rumen and omasum in ruminants enable more efficient utilization of plant cellulose (Clauss & Rössner, 2014; Janis, 1976; Russell & Rychlik, 2001). This efficient procurement of food energy may be an important reason why ruminants are so prosperous. Ruminants also possess specialized dentition, speed, and considerable variation in body size. Furthermore, they have played an important role in human civilization due to the domestication of several species, such as cattle, buffalo, yak, sheep, and goat. In modern society, these domestic animals remain important for human diet and utilization. Therefore, understanding the evolution and origin

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of ruminants is of great importance for scientific research and for application prospects in human society.

In the past decade, we have witnessed the rapid development of genome sequencing technologies and a resultant wealth of knowledge from animal genomes. To date, the genomes of 73 ruminant species have been reported (Table 1), with increasing availability of resequencing and transcriptome data for representative species, like cattle (Chen et al., 2018; Daetwyler et al., 2014; Medugorac et al., 2017; Soubrier et al., 2016), goat (Alberto et al., 2018; Wang et al., 2016), sheep (Alberto et al., 2018; Hu et al., 2019; Naval-Sanchez et al., 2018), and yak (Qiu et al., 2015; Wang et al., 2019a). Most previous studies have only focused on the evolution of individual species by decoding individual genomes and utilizing large-scale resequencing data; e. g.,

high-altitude adaptation studies on yak and Tibetan antelope (Ge et al., 2013; Qiu et al., 2012; Qiu et al., 2015). Nowadays, large-scale sequencing studies of multiple species across high classification orders have provided extraordinary biological discoveries, e.g., avian phylogenomics (Jarvis et al., 2014). We recently launched the Ruminant Genome Project and conducted a large-scale genomic study of ruminants based on integrated analysis of 51 ruminant genomes (44 newly assembled genomes and seven public genomes) and corresponding transcriptome, resequencing, and functional experiments (Chen et al., 2019; Lin et al., 2019; Wang et al., 2019b). The availability of large-scale genomic resources should provide new insights into our understanding of ruminant diversity, evolution, biogeographic patterns, and adaptation mechanisms.

Table 1 Currently available genome assemblies for ruminant species

Family	Subfamily	Species	Common name	Genome size (Gb)	Scaffold N50 (bp)	Contig N50 (bp)	Assembly level	Reference	
Tragulidae		<i>Tragulus javanicus</i>	Java mouse-deer	2.59	14 082 842	80 230	Scaffold	*	
		<i>Tragulus kanchil</i>	Lesser mouse-deer	3.05	243 497	13 850	Scaffold	Chen et al., 2019	
Antilocapridae		<i>Antilocapra americana</i>	Pronghorn	2.96	18 845 065	61 698	Scaffold	Chen et al., 2019	
Giraffidae		<i>Giraffa camelopardalis</i>	Giraffe	2.47	3 153 258	25 056	Scaffold	Chen et al., 2019	
		<i>Giraffa tippelskirchi</i>	Masai giraffe	2.71	212 164	47 894	Scaffold	Agaba et al., 2016	
		<i>Okapia johnstoni</i>	Okapi	2.88	111 538	39 571	Scaffold	Chen et al., 2019	
Cervidae	Cervinae	<i>Axis porcinus</i>	Hog deer	2.68	20 764 858	172 761	Scaffold	*	
	Cervinae	<i>Cervus albirostris</i>	White-lipped deer	2.69	3 769 372	39 627	Scaffold	Chen et al., 2019	
	Cervinae	<i>Cervus elaphus</i>	Red deer	3.44	107 358 006	7 944	Chromosome	Bana et al., 2018	
	Cervinae	<i>Elaphurus davidianus</i>	Pere David's deer	2.58	2 844 142	59 950	Scaffold	Zhang et al., 2018	
	Hydropotinae	<i>Hydropotes inermis</i>	Chinese water deer	2.53	13 818 975	131 446	Scaffold	Wang et al., 2019	
	Muntiacinae	<i>Muntiacus crinifrons</i>	Black muntjac	2.68	1 305 444	8 265	Scaffold	Chen et al., 2019	
	Muntiacinae	<i>Muntiacus muntjak</i>	Indian muntjac	2.7	1 258 210	23 470	Scaffold	Chen et al., 2019	
	Muntiacinae	<i>Muntiacus reevesi</i>	Chinese muntjac	2.6	1 221 377	72 382	Scaffold	Chen et al., 2019	
	Odocoileinae	<i>Capreolus capreolus</i>	Western roe deer	2.79	10 458	4 167	Scaffold	Kropatsch et al., 2013	
	Odocoileinae	<i>Odocoileus hemionus</i>	Mule deer	2.34	838 758	113 295	Scaffold	Russell et al., 2019	
	Odocoileinae	<i>Odocoileus virginianus</i>	White-tailed deer	2.38	850 721	122 019	Scaffold	Seabury et al., 2011	
	Odocoileinae	<i>Rangifer tarandus</i>	Reindeer	2.9	89 062	77 671	Scaffold	Li et al., 2017	
	Moschidae		<i>Moschus berezovskii</i>	Forest musk deer	2.81	2 509 225	57 706	Scaffold	Chen et al., 2019
			<i>Moschus chrysogaster</i>	Alpine musk deer	4.97	100 428	3 769	Scaffold	Wang et al., 2019
		<i>Moschus moschiferus</i>	Siberian musk deer	3.07	11 728 851	34 785	Scaffold	*	

Continued

Family	Subfamily	Species	Common name	Genome size (Gb)	Scaffold N50 (bp)	Contig N50 (bp)	Assembly level	Reference
	Aepycerotinae	<i>Aepyceros melampus</i>	Impala	2.63	344 542	82 459	Scaffold	Chen et al., 2019
	Alcelaphinae	<i>Alcelaphus buselaphus</i>	Hartebeest	3.88	12 034	889	Scaffold	Chen et al., 2019
	Alcelaphinae	<i>Beatragus hunteri</i>	Hirola	2.7	69 303	57 444	Scaffold	*
	Alcelaphinae	<i>Connochaetes taurinus</i>	Blue wildebeest	2.64	366 224	70 608	Scaffold	Chen et al., 2019
	Alcelaphinae	<i>Damaliscus lunatus</i>	Topi	3.13	1 166 796	38 843	Scaffold	Chen et al., 2019
	Antilopinae	<i>Procapra przewalskii</i>	Przewalski's gazelle	2.69	5 522 907	10 405	Scaffold	Chen et al., 2019
	Antilopinae	<i>Antidorcas marsupialis</i>	Springbok	3.02	694 905	42 749	Scaffold	Chen et al., 2019
	Antilopinae	<i>Eudorcas thomsonii</i>	Thomson's gazelle	2.9	1 581 717	137 476	Scaffold	Chen et al., 2019
	Antilopinae	<i>Litocranius walleri</i>	Gerenuk	2.98	3 126 223	62 351	Scaffold	Chen et al., 2019
	Antilopinae	<i>Madoqua kirkii</i>	Kirk's dik-dik	2.65	27 730	27 722	Scaffold	Chen et al., 2019
	Antilopinae	<i>Nanger granti</i>	Grant's gazelle	3.03	528 456	7 454	Scaffold	Chen et al., 2019
	Antilopinae	<i>Neotragus moschatus</i>	Suni	2.68	952 090	59 277	Scaffold	Chen et al., 2019
	Antilopinae	<i>Neotragus pygmaeus</i>	Royal antelope	2.9	363 895	30 751	Scaffold	Chen et al., 2019
	Antilopinae	<i>Oreotragus oreotragus</i>	Klipspringer	3.25	339 390	42 127	Scaffold	Chen et al., 2019
	Antilopinae	<i>Ourebia ourebi</i>	Oribi	2.52	1 259	1 259	Scaffold	Chen et al., 2019
	Antilopinae	<i>Raphicerus campestris</i>	Steenbok	3.14	537 161	25 050	Scaffold	Chen et al., 2019
	Antilopinae	<i>Saiga tatarica</i>	Saiga antelope	2.88	6 453	6 406	Scaffold	*
	Bovinae	<i>Bison bison</i>	American bison	2.83	7 192 658	19 971	Scaffold	*
Bovidae	Bovinae	<i>Bison bonasus</i>	European bison	2.58	4 690 000	14 530	Scaffold	Wang et al., 2017a
	Bovinae	<i>Bos frontalis</i>	Gayal	2.85	2 737 757	14 405	Scaffold	Wang et al., 2017b
	Bovinae	<i>Bos grunniens</i>	Domestic yak	2.83	114 386 978	44 716 738	Chromosome	Qiu et al., 2012
	Bovinae	<i>Bos indicus</i>	Zebu cattle	2.67	106 310 653	28 375	Chromosome	Canavez et al., 2012
	Bovinae	<i>Bos indicus x Bos taurus</i>	Hybrid cattle	2.68	104 466 507	26 764 281	Chromosome	*
	Bovinae	<i>Bos mutus</i>	Wild yak	2.65	1 407 960	22 822	Scaffold	*
	Bovinae	<i>Bos taurus</i>	Cattle	2.72	103 308 737	25 896 116	Chromosome	Bovine Genome et al., 2009
	Bovinae	<i>Bubalus bubalis</i>	Water buffalo	2.66	117 219 835	22 441 509	Chromosome	Mintoo et al., 2019
	Bovinae	<i>Syncerus caffer</i>	African buffalo	2.93	2 316 376	52 316	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus buxtoni</i>	Mountain nyala	3.27	7 367	1 286	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus eurycerus</i>	Bongo	2.96	12 574	1 974	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus imberbis</i>	Lesser kudu	4.04	1 775 046	12 858	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus oryx</i>	Common eland	2.84	4 043 025	1 262	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus scriptus</i>	Bushbuck	2.87	890 554	28 350	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus spekii</i>	Sitatunga	3.73	46 427	9 477	Scaffold	Chen et al., 2019
	Bovinae	<i>Tragelaphus strepsiceros</i>	Greater kudu	2.88	511 483	33 649	Scaffold	Chen et al., 2019
	Caprinae	<i>Ammotragus lervia</i>	Barbary sheep	2.65	1 301 762	52 017	Scaffold	Chen et al., 2019
	Caprinae	<i>Capra aegagrus</i>	Wild goat	2.83	91 317 560	19 347	Chromosome	Dong et al., 2015

Continued

Family	Subfamily	Species	Common name	Genome size (Gb)	Scaffold N50 (bp)	Contig N50 (bp)	Assembly level	Reference
	Caprinae	<i>Capra hircus</i>	Goat	2.92	87 277 232	26 244 591	Chromosome	Bickhart et al., 2017
	Caprinae	<i>Capra ibex</i>	Alpine ibex	2.7	61 905 114	380 983	Scaffold	Chen et al., 2019
	Caprinae	<i>Capra sibirica</i>	Siberian ibex	2.73	15 190 720	376 582	Scaffold	*
	Caprinae	<i>Hemitragus hylocrius</i>	Nilgiri tahr	2.72	85 340	66 552	Scaffold	*
	Caprinae	<i>Ovis ammon</i>	Argali	2.64	72 289 505	151 569	Scaffold	Chen et al., 2019
	Caprinae	<i>Ovis aries</i>	Sheep	2.87	107 697 089	2 572 683	Chromosome	Jiang et al., 2014
	Caprinae	<i>Ovis canadensis</i>	Bighorn sheep	2.86	69 397	55 973	Scaffold	*
	Caprinae	<i>Pseudois nayaur</i>	Bharal	2.58	21 385	20 979	Scaffold	Chen et al., 2019
	Cephalophinae	<i>Cephalophus harveyi</i>	Harvey's duiker	2.82	365 466	61 368	Scaffold	Chen et al., 2019
Bovidae	Cephalophinae	<i>Philantomba maxwellii</i>	Maxwell's duiker	3.15	383 899	11 377	Scaffold	Chen et al., 2019
	Cephalophinae	<i>Sylvicapra grimmia</i>	Common duiker	3.15	583 330	9 720	Scaffold	Chen et al., 2019
	Hippotraginae	<i>Hippotragus niger</i>	Sable antelope	2.6	4 586 323	45 501	Scaffold	Koepfli et al., 2019
	Hippotraginae	<i>Oryx gazella</i>	Gemsbok	2.76	1 579 191	19 498	Scaffold	Chen et al., 2019
	Pantholopinae	<i>Pantholops hodgsonii</i>	Tibetan antelope	2.7	2 772 860	18 674	Scaffold	Ge et al., 2013
	Reduncinae	<i>Kobus ellipsiprymnus</i>	Defassa waterbuck	2.9	779 552	26 169	Scaffold	Chen et al., 2019
	Reduncinae	<i>Redunca redunca</i>	Bohor reedbuck	2.72	423 407	39 382	Scaffold	Chen et al., 2019

*: These genomes can be accessed from the NCBI website (<https://www.ncbi.nlm.nih.gov/assembly/>).

In this review, we first summarize current knowledge of ruminant evolution learned from the genomic era, especially phylogenetic relationships, genetic basis and evolution of complex traits, genetic basis underlying local adaptations, and domestication of livestock ruminants. We then provide an outlook for future research on ruminants given the rapid advances in the accumulation of genomic resources and increasing efforts in functional genomics.

PHYLOGENETIC RELATIONSHIPS OF RUMINANTS

Phylogeny is essential to biology not only because it reflects the evolutionary history of species or lineages and divergent events, but also provides a framework for tracing the evolution of distinct complex characteristics. In previous studies, phylogenetic trees of ruminants were constructed based on morphological data (e.g., teeth and skeletons), fragments of nuclear DNA, or complete mitochondria DNA (Bibi, 2013; Decker et al., 2009; Gatesy et al., 1992; Hassanin & Douzery, 2003; Hassanin et al., 2012). However, controversies in these phylogenetic trees remain, resulting in different placements at the genus, subfamily, and even family level. To resolve these issues, Chen et al. (2019) provided a robust phylogenetic tree based on whole genome data from 51 ruminants, clarifying

most of the controversies in ruminant phylogeny. Here, using all available genomes (Table 1), we reconstructed a simplified phylogenetic tree (Figure 1). In previous studies, Antilocapridae was considered an outgroup to all other pecorans (all ruminants, excluding Tragulidae) based on mtDNA (Bibi, 2013; Hassanin et al., 2012; Hernández Fernández & Vrba, 2005). Moschidae was placed at the base of Pecora (Janis, 1987; Janis & Theodor, 2014; Webb & Taylor, 1980) or as a sister group to Bovidae based on morphological data (Sánchez et al., 2010), or proposed as a sister group of Cervidae or Bovidae based on mtDNA (Bibi, 2014; Dos Reis et al., 2012; Hassanin & Douzery, 2003; Hassanin et al., 2012). The whole genome tree confirms the sister-group relationship between Antilocapridae and Giraffidae, and Moschidae as a sister group with Bovidae. In addition, some controversies in Bovidae (Hernández Fernández & Vrba, 2005) are also resolved, with Reduncinae confirmed as a sister group of Caprinae, Alcelaphinae, and Hippotraginae.

The above phylogenetic controversies may be due to incomplete lineage sorting (ILS) or to introgression between lineages. A high level of phylogenetic heterogeneity is observed at the family level of ruminants, showing that only 21.5% of sample genomic windows are consistent with the topology of

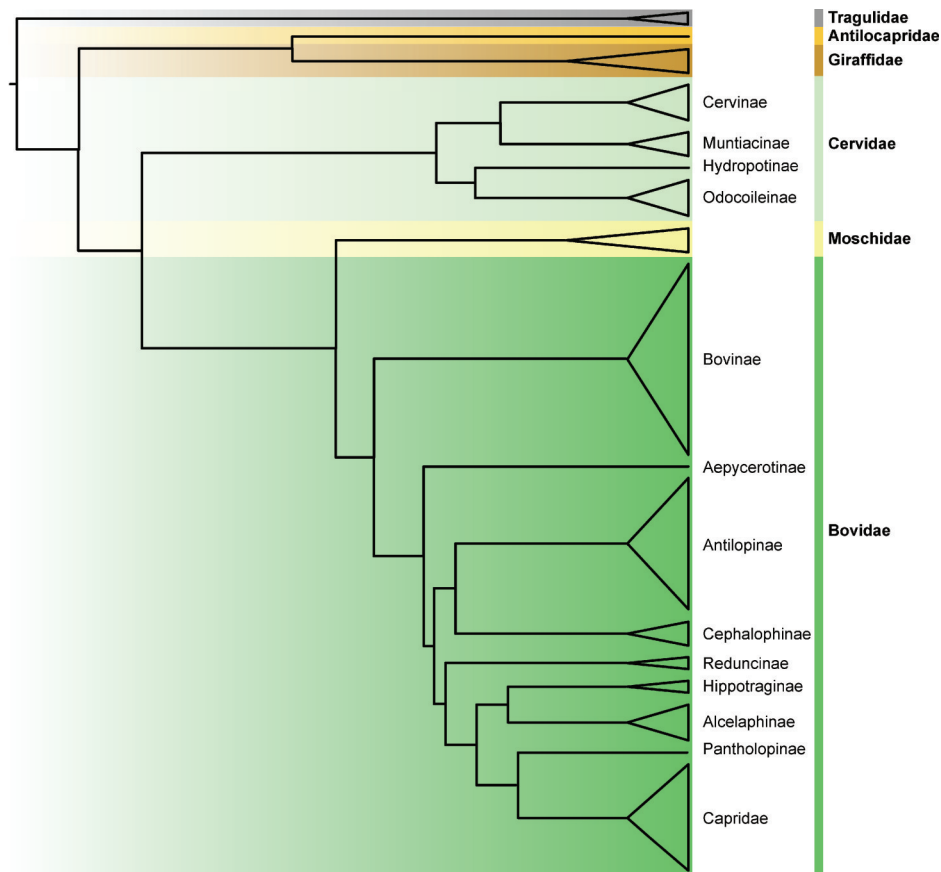


Figure 1 Phylogenetic relationship among ruminants

Phylogenetic tree of ruminants is presented with species within same families and subfamilies collapsed. Species used in this tree are listed in Table 1, and the phylogenetic relationship is mainly based on Chen et al. (2019).

the whole genome tree (Chen et al., 2019). These phylogenetic disagreements can primarily be explained by ILS, resulting from the rapid radiation of ruminant families during the Miocene (Chen et al., 2019). In addition, a strong gene flow signal has been reported among Giraffidae, Cervidae, Bovidae, and Moschidae (Chen et al., 2019), implying ancestral introgressions may also partially account for the phylogenetic debates. In particular, pervasive introgression among *Bos* species has been reported from other studies (Wang et al., 2018a; Wu et al., 2018), which provides support for introgression effects on the phylogenetic relationships of ruminants.

EVOLUTION OF IMPORTANT TRAITS

Most traits are consequences of long-term evolution and form the basis of biodiversity. As one of the most successful mammalian lineages, ruminants have evolved extensive and unique morphological traits, such as headgear, multichambered stomach, substantial variation in body size, cold (Arctic) adaptation (reindeer), and high-altitude adaptation. The availability of large-scale genomic data,

together with transcriptomics and functional genomics, has enabled investigations into the genetic basis and evolution of complex traits in ruminants.

Evolution of headgear

Ruminants are the only mammalian group with osseous headgear (Davis et al., 2011). Headgear is not only used in self-defense against predators but also in intraspecific competition for mates and territories (Bubenik & Bubenik, 2012; Davis et al., 2011). Four families of ruminants have headgear, including Giraffidae, Antilocapridae, Cervidae, and Bovidae. Headgear in different families exhibits distinct morphological characteristics, such as ossicones in giraffids, pronged horns in pronghorns, antlers in cervids, and horns in bovids (Bubenik & Bubenik, 2012). The genetic mechanisms involved in headgear have long been a topic of interest to evolutionary biologists (Aristotle, 1991; Goss, 1983).

The expression profiles, phylogenies, and convergent headgear losses support a single evolutionary origin of ruminant headgear (Chen et al., 2019; Wang et al., 2019b). The highly or specially expressed genes in horns and antlers are mostly co-expressed in bone, skin, nerve tissue, and testis, and many positively selected genes are associated with

conserved elements involved in neural functions (Wang et al., 2019b). The headgear in ruminants likely originated from neural crest stem cells, which diverged into horns, antlers, ossicones, and pronged horns in the bovids, cervids, giraffids, and pronghorns, respectively. The loss of headgear in Moschidae and Hydropotinae was likely due to convergent pseudogenization of the gene *RXFP2* (Wang et al., 2019b).

Interestingly, antlers in the Cervidae have evolved further remarkable features. Professor Richard Goss from the University of Brown wrote, "*The antlers of deer are so improbable that if they had not evolved in the first place they would never have been conceived even in the wildest fantasies of the most imaginative biologists*" (Goss, 1983). Deer antlers are the only completely regenerative organ found in mammals. They exhibit extremely rapid growth rates (~1.7 cm/day in red deer) that can even surpass cancerous tissue growth in cell proliferation (Goss, 1983), although deer experience low rates of cancer (Griner, 1983; Lombard & Witte, 1959). Wang et al. (2019b) showed that antler-specific expressed genes are enriched in the axon guidance pathway, suggesting that antler growth may be involved in neural processes. Strikingly, their results also revealed that proto-oncogenes (*FOS*, *REL*, *FAM83A*) and tumor suppression genes are positively selected in cervids, thus explaining the high growth rate of deer antlers but low cancer rate in cervids. Understanding the genetic basis of rapid antler regeneration could provide new insights into the regeneration of mammalian organs and oncogenesis.

Evolution of multichambered stomach

The ruminant digestive system plays important ecological and functional roles in evolution. The appearance of the rumen and its close interaction with microorganisms allows ruminants to gain energy efficiently, thus providing unique evolutionary advantages and promoting the prosperity and diversity of ruminant groups. Compared with non-ruminants (e.g., horses), ruminants can achieve higher utilization of plant fiber due to their unique forestomach fermentation system (Janis, 1976). Most previous studies have focused on the interaction between microorganisms and the rumen (Bovine Genome Sequencing and Analysis Consortium et al., 2009; Seshadri et al., 2018; Stewart et al., 2018; Zhang et al., 2016). However, little is known about the origin and evolution of the multichambered stomach.

To investigate the origin and evolution of the ruminant stomach, Chen et al. (2019) compared gene expression profiles from 516 samples covering 50 tissues of sheep, together with large-scale genomic data. The expression profiles suggested that the rumen, reticulum, and omasum may have originated from the esophagus, as also suggested by Warner (1958) and Xiang et al. (2016), whereas the abomasum was closer to the intestine. Newly evolved genes also seem to have played an important role in the evolution of the rumen. Seven newly evolved genes identified at the ancestor of ruminants are specially expressed in the rumen, two of which, *PRD-SPRR1* and *TCHHL2*, are structural genes (Chen et al., 2019; Jiang et al., 2014). *PRD-SPRR1* is related

to the cornification of the keratin-rich surface of the rumen, and *TCHHL2* plays a role in cross-linking keratins at the rumen surface (Jiang et al., 2014). The omasum is a newly evolved organ in pecorans, resembling the rumen in structure and function (Millen et al., 2016). Among the 75 genes specifically and highly expressed in the omasum compared with other organs, one gene is newly evolved (*LOC101107119*) and another (*SCNN1D*) exhibits pecoran-specific amino acid changes (Chen et al., 2019). In addition, four genes (i.e., *SIM2*, *PAX9*, *KCNK5*, and *DENND2C*) show pecoran-specific conserved non-exonic elements (CNEs) within their immediate upstream/downstream 10 kb regions (Chen et al., 2019). Based on gene family analysis, the lysozyme *c* family is also expanded in ruminants, containing ten or more copies (Chen et al., 2019; Irwin, 2015), whereas other outgroup mammals have only one or a few copies. Furthermore, most expanded copies of lysozyme *c* are expressed in the abomasum, suggesting adaptation to its microbe-rich environment.

Evolution of body size

Ruminants exhibit extensive variation in body size, from 2 kg (e.g., lesser mouse deer, *Tragulus kanchil*) to 1 200 kg (e.g., giraffe, *Giraffa camelopardalis* and African buffalo, *Syncerus caffer*) (Castelló, 2016). At the family level, body size is largest in Giraffidae, followed by medium-sized Antilocapridae and Cervidae, and relatively small-sized Tragulidae and Moschidae (Clauss et al., 2003). Bovidae exhibits varied body size, with Bovinae generally being the largest (Castelló, 2016). Ruminants living in open grasslands also tend to be larger, which can help in resisting predators and adapting to changes in the environment. Ruminants living in the jungle tend to be smaller in size, which allows for more flexible movement and thus predator avoidance.

Genes associated with body size have significantly higher non-synonymous substitution rates in large- and small-sized bovid species (Chen et al., 2019). Specifically, six genes (i.e., *CXCL13*, *RNF115*, *NPNT*, *KL*, *SLC9A3R1*, and *MSTN*) are associated with increased body size and five genes (i.e., *SBDS*, *BMP3*, *LRRN3*, *NFATC3*, and *SMARCAL1*) are related to reduced body size via regulation of bone and muscle development. Several genes also have functional support from other studies. For example, *SLC9A3R1* plays a role in osteogenesis by mineralizing osteoblasts, with disruption of this gene resulting in reduced body weight in mice (Shenolikar et al., 2002). *MSTN* regulates muscle cell growth and differentiation (Sartori et al., 2013), and mutations in *MSTN* can affect muscle mass in livestock species such as goat, sheep, and cattle (Grobet et al., 1997; Li et al., 2016; Luo et al., 2014; Wang et al., 2018b) and other mammals (Bi et al., 2016; Gu et al., 2016). *SBDS* serves an important role in cell proliferation and is a causal gene of Shwachman-Diamond syndrome in humans (Boocock et al., 2003), which is characterized by short stature and skeletal abnormalities. For individual species, the special stature of giraffes enables access to food and is an adaptation to tropical grassland. Those genes associated with bone development with giraffe-specific

mutations are involved in the transforming growth factor- β (TGF- β), Hedgehog, Notch, Wnt, and FGF signaling pathways (Agaba et al., 2016; Chen et al., 2019).

Reindeer adaptation to Arctic regions

Reindeer (*Rangifer tarandus*) are naturally distributed across Arctic and sub-Arctic regions, and consequently face numerous challenges, including severe cold, limited food availability, and prolonged periods of light or darkness (Blix, 2016). To adapt to these environments, reindeer have evolved several strategies, including special lipid metabolism to avoid heat loss and to induce circadian arrhythmicity for long periods of light or dark (Lu et al., 2010; Stokkan et al., 2007; Van Oort et al., 2005). Based on detailed comparative genomic analysis, Lin et al. (2019) identified two positively selected genes (i. e., *CYP27B1* and *POR*) involved in the vitamin D metabolism pathway. Functional experiments have also shown that enzymes encoded by these two genes exhibit much higher catalytic activity than that of their orthologs in goat and roe deer (Lin et al., 2019). Eight genes with reindeer-specific mutations have been reported to be involved in the circadian rhythm pathway, with four being rapidly evolving genes. Among these genes, Pro1172Thr (P1172T) mutation in *PER2* results in loss of binding ability with CRY1, which can cause arrhythmicity (Lin et al., 2019).

Adaptation to high altitude

Ruminants are distributed across a wide range of habitats, including high-altitude regions such as the Tibetan Plateau, with risks of hypobaric hypoxia. These ruminants include the yak, Tibetan sheep, and Tibetan goat, which are three key livestock species serving as sources of meat and fiber for Tibetan inhabitants (Hu et al., 2019; Qiu et al., 2012; Wang et al., 2016). In addition, the Tibetan antelope (*Pantholops hodgsonii*) and Marco Polo sheep (*Ovis ammon polii*) also exhibit adaptation to high altitude (Ge et al., 2013; Yang et al., 2017). Therefore, understanding the genetic basis and mechanisms underlying high-altitude adaptation is of great value in many scientific fields.

Genetic variation related to transcription factors, oxygen sensors, and target genes has been exploited to detect signals of selection in ruminant species such as the Tibetan sheep (Hu et al., 2019), Tibetan goat (Wang et al., 2016), yak (Qiu et al., 2012), and Tibetan antelope (Ge et al., 2013). In yak, three genes, identified as positively selected in response to hypoxia, are involved in hypoxia-inducible factor-1 α (Hif-1 α), including two important regulators (Adam17 and Arg2) and one target gene (*Mmp3*) (Qiu et al., 2012). In Marco Polo sheep, several genes associated with hypoxia response have been identified, including ryanodine receptor 1 (*RYR1*) and purinergic receptor P2X and ligand-gated ion channel 3 (*P2RX3*) (Yang et al., 2017). In addition to whole genome analysis in yak, Marco Polo sheep, and Tibetan antelope, resequencing analysis between low- and high-altitude ruminant populations has also identified candidate genes associated with hypoxia response, especially the HIF-1 pathway (Hu et al., 2019; Wang et al., 2016), including one

gene (*NOXA1*) in goats (Wang et al., 2016) and eight genes (*EPO*, *TLR4*, *PIK3CA*, *PRKCA*, *EGLN3*, *EGLN2*, *IFNGR2*, and *CUL2*) with strong signals in sheep (Hu et al., 2019). Although candidate genes under positive selection can contribute to high-altitude adaptation, introgression may also play a crucial role. For instance, genes involved in the response-to-hypoxia pathway (e. g., *EGLN1*, *EGLN2* and *HIF3a*) are introgressed from yak to Tibetan cattle, which may be responsible for their high-altitude adaptation (Wu et al., 2018). Of these genes, *EGLN1* is reported to be associated with high-altitude adaptation in Tibetans (Simonson et al., 2010). Therefore, these positively selected genes may provide valuable insights into plateau medicine.

DOMESTICATION EFFECTS ON RUMINANT LIVESTOCK

The importance of ruminants is also highlighted by several major livestock species, including goat, sheep, cattle, and yak. Domestication of livestock ruminants was a key factor in the transition from hunter-gather societies to agricultural civilizations. Under intense artificial selection, domesticated livestock species have experienced extensive morphological or physiological changes compared to their wild relatives, including shadow color coat, floppy ears, shorter muzzles, smaller brain and cranial capacity, and tameness, which are referred to as domestication syndromes (Wilkins et al., 2014).

It has been hypothesized that domestication syndromes predominantly resulted from mild neural crest cell (NCC) deficits during embryonic development (Wilkins et al., 2014). Several recent studies have revealed that selected candidate regions under domestication of cattle, yak, sheep, and goat are enriched in pathways related to brain development or neurobehavioral functioning, such as NCCs (Alberto et al., 2018; Naval-Sanchez et al., 2018; Qanbari et al., 2014; Qiu et al., 2015). For example, neural genes (e. g., *TMEM132D*, *CACNA1C*, *NRXN1*, and *NPAS3*) show signals of positive selection in cattle (Qanbari et al., 2014). Thirty genes in yak show signals of selection involved in brain and neuronal development, 19 of which are associated with behavior (Qiu et al., 2015). In addition, five genes are associated with the nerve system in sheep and goat, which showed convergent evolution (Alberto et al., 2018). These genes associated with the brain and nerve system under pressure from artificial selection may explain tameness or aggressiveness in livestock species.

In addition to major livestock species, reindeer were also domesticated by sub-Arctic people. Although cervid species are usually cautious and sensitive, reindeer are very tame and are the only domesticated species in the cervid family. Lin et al. (2019) identified reindeer-specific mutations in genes associated with NCCs to explain their tameness, and observed that genes involved in development (*MSX2*, *ID3*, *BCAT1*, *CAD6*, and *CAD11*), migration (*TCOF1*, *BCAT1*, *NOTCH2*, and *NOTCH3*), and differentiation (*COL2A1*, *KIT*, and *SI*) of NCCs were either rapidly evolving or demonstrated reindeer-specific mutations.

PERSPECTIVES

Traditional biological methods are often unable to solve the genetic basis of the evolution of complex traits across higher taxa at the family, order, or even class level. To address this issue, we propose a new approach called evolutionary genotype-phenotype systems biology (eGPS), which compares the evolution of many species using large-scale genome, transcriptome, regulatome, metabolome, and proteome data and systematically integrates evidence from genetic factors, developmental network evolution, and phenotypes to analyze the genetic basis of complex animal traits, especially those with evolutionary significance. Using this approach, we could resolve the genetic basis of complex traits and determine key regulatory genes or pathways. Functional experiments could verify these genetic factors, and thus facilitate further investigation on the evolutionary mechanisms of complex traits and their potential application. The Ruminant Genome Project has served as a good example of eGPS study with large-scale multi-omics data.

The rapid development of sequencing technology, especially long-read and Hi-C technologies, has enabled researchers to obtain high-quality reference genomes with long continuous contigs and scaffold size, even to the chromosome level. High-quality assemblies should promote additional studies on ruminants, including chromosome evolution, accurate identification of new functional elements (e.g., regulatory elements and new genes), and functional validation using gene editing technology such as *CRISPR/Cas9*. Chromosome numbers in *Muntiacus* species demonstrate considerable variations due to chromosome fusion; for example, *Muntiacus reevesi* has a karyotype of $2n=46$, *Muntiacus vaginalis* has a karyotype of $2n=6$ and 7 , and *Muntiacus crinifrons* has a karyotype of $2n=8$ and 9 in females and males, respectively (Shi, 1983; Wurster & Benirschke, 1967, 1970). High-quality reference genomes will help to reveal the genetic mechanisms underlying the recurrent fusion of chromosomes in muntjacs and its effects on the interaction between genes flanking the fusion regions, and thus facilitate understanding of speciation driven by chromosome variation. New regulatory elements and genes may be crucial in adaptive phenotypic diversification. Availability of high-quality genome data should facilitate identification of these new functional elements, thus revealing the potential genetic basis underlying adaptations. For casual or functional mutations identified using large-scale data among lineages, *CRISPR/Cas9* experiments could be utilized for verification using model organisms or key livestock species, such as sheep and goats.

Additionally, genomic resources and comparative analyses of ruminants are likely to contribute to animal husbandry (Figure 2). Comparative analysis between domestic livestock species and wild species has identified a series of candidate genes associated with domestication or economically valuable traits, such as meat quality and milk production (Alberto et al., 2018). These artificially selected genes can provide new targets for animal breeding and improvement. In the future,

gene editing technology (such as *CRISPR/Cas9*) may be applicable to quickly improve traits of livestock.

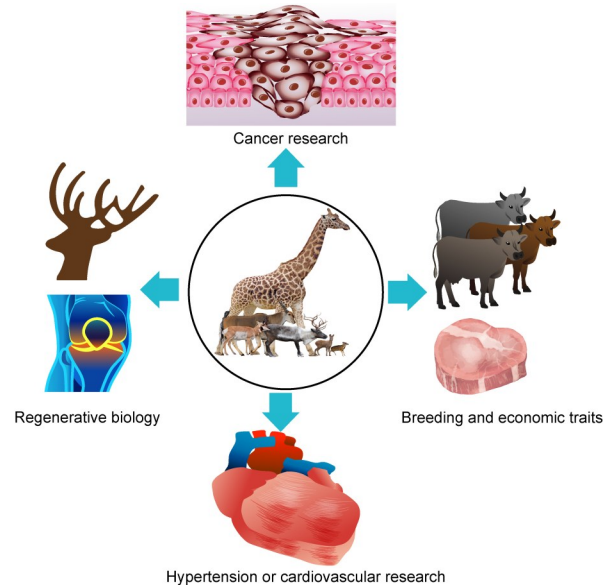


Figure 2 Possible applications of future ruminant research

Studies on ruminants could be applied in cancer research, regenerative biology, hypertension, and cardiovascular research, as well as breeding of livestock.

In addition to application in agriculture, ruminants could serve as animal models for medical research and thus provide valuable insights into medical treatments and even clinical studies (Figure 2). Further research on deer antlers may offer unprecedented clues on regenerative biology and oncogenesis. Moreover, study on giraffes may provide insight into hypertension or cardiovascular research. Overall, increasing our understanding of the genetic basis and mechanisms underlying certain ruminant traits could benefit human society in many ways.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W. W. and L. C. proposed the ideas and revised the manuscript. B. W. collected data, reviewed the literature, and drafted the manuscript. All authors read and approved the final version of the manuscript.

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