RESEARCH ARTICLE SUMMARY

RUMINANT GENOMICS

Biological adaptations in the Arctic cervid, the reindeer (*Rangifer tarandus*)

Zeshan Lin*, Lei Chen*, Xianqing Chen*, Yingbin Zhong*, Yue Yang*, Wenhao Xia*, Chang Liu, Wenbo Zhu, Han Wang, Biyao Yan, Yifeng Yang, Xing Liu, Kjersti Sternang Kvie, Knut Håkon Røed, Kun Wang, Wuhan Xiao, Haijun Wei, Guangyu Li, Rasmus Heller, M. Thomas P. Gilbert, Qiang Qiu†, Wen Wang†, Zhipeng Li†

INTRODUCTION: Reindeer (*Rangifer tarandus*) are naturally distributed across the Arctic and subarctic regions. Consequently, these animals have evolved to face numerous challenges, including exposure to severe cold, limited food availability in winter, and extremely prolonged light or dark periods. Unlike all other cervid species, both male and female reindeer annually grow deciduous antlers. Furthermore, reindeer are the only fully domesticated species among the Cervidae. However, little is known about the underlying genetic causes of these traits.

RATIONALE: We performed comparative genomic analyses between reindeer, other ruminant species, and a number of mammalian outgroups to identify rapidly evolving genes, positively selected genes, and reindeer-specific mutants. We further resequenced the genomes of three domestic reindeer from northern China and three wild reindeer from Northern Europe to validate that the reindeer-specific mutations are fixed in the species rather than individual polymorphisms. To support our computationally derived insights, we subsequently conducted in vitro functional experiments to investigate possible functional consequences of some of the reindeer-specific mutated genes.

RESULTS: We found two genes (*CYP27B1* and *POR*) involved in the vitamin D metabolism pathway to be under positive selection in reindeer. Furthermore, our functional experiments validated that the two key enzymes (CYP27B1 and POR) exhibit much higher catalytic activity than that of the orthologs in goats and roe deer. We also identified fixed reindeer-specific mutations in genes that play a role in fat metabolism, including *APOB* and *FASN*. We showed that a mutation upstream of the reindeer *CCND1* gene endows an extra functional binding motif to the androgen receptor and thus may result in female antler growth. In the circadian rhythm pathway, we observed that eight genes have reindeer-specific mutations and that four genes have been rapidly evolving. Among them, the Pro^{1172}→Thr (P1172T) mutation in the reindeer PER2 causes loss of binding ability with CRY1, which can cause arrhythmicity. Finally, we found reindeer-specific mutations in 11 genes relating to development, migration, and differentiation of neural crest cells, probably accounting for the tameness of reindeer.

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CONCLUSION: Our results reveal the genetic basis of a broad spectrum of the Arctic deer's traits and provide a basis for understanding mammalian adaptive strategies to the Arctic. Our comparative genomic studies and functional assays identify a number of genes that exhibit functionality related to circadian arrhythmicity, vitamin D metabolism, docility, and antler growth, as well as genes that are uniquely mutated and/or are under positive selection. Our results may provide insights relevant to human health, including how the genetic response of vitamin D in reindeer affects bone and fat metabolism and how genes can affect circadian arrhythmicity. ■

The list of author affiliations is available in the full article online.
*These authors contributed equally to this work.
†Corresponding author: lizhipeng01@caas.cn (Z.L.); wwang@mail.kiz.ac.cn (W.W.); qiuqiang@lzu.edu.cn (Q.Q.)
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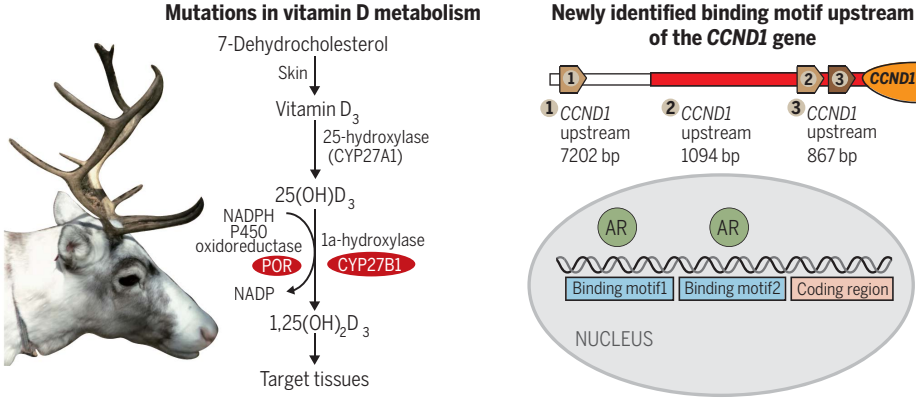

Unique mutations explain the biological adaptations of reindeer. (Left) Two genes (*POR* and *CYP27B1*) play an important role in vitamin D metabolism in reindeer. (Middle) A newly identified binding motif of the androgen receptor (AR) evolved upstream of a key antler *CCND1* gene, which may result in female antler growth. bp, base pairs. (Right) A key reindeer-specific mutation (P1172T) in PER2 results in loss of binding ability with CRY1, which can cause the observed circadian arrhythmicity in reindeer. The circadian genes highlighted in red are positively selected in reindeer; the ones shown in blue are rapidly evolving genes. PACAP, pituitary adenylate cyclase activating polypeptide; SCN, suprachiasmatic nucleus; ER, endoplasmic reticulum; CREB, cAMP response element–binding protein; nNOS, neuronal nitric oxide synthase; P, phosphorus; G, G protein.

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RESEARCH ARTICLE

RUMINANT GENOMICS

Biological adaptations in the Arctic cervid, the reindeer (*Rangifer tarandus*)

Zeshan Lin[1]*, Lei Chen[1]*, Xianqing Chen[1]*, Yingbin Zhong[2,3]*, Yue Yang[1]*, Wenhao Xia[1]*, Chang Liu[1], Wenbo Zhu[1], Han Wang[2,3], Biyao Yan[1], Yifeng Yang[4], Xing Liu[5], Kjersti Sternang Kvie[6], Knut Håkon Røed[6], Kun Wang[1], Wuhan Xiao[5], Haijun Wei[4], Guangyu Li[4], Rasmus Heller[7], M. Thomas P. Gilbert[8,9], Qiang Qiu[1]†, Wen Wang[1,10,11]†, Zhipeng Li[4]†

The reindeer is an Arctic species that exhibits distinctive biological characteristics, for which the underlying genetic basis remains largely unknown. We compared the genomes of reindeer against those of other ruminants and nonruminant mammals to reveal the genetic basis of light arrhythmicity, high vitamin D metabolic efficiency, the antler growth trait of females, and docility. We validate that two reindeer vitamin D metabolic genes (*CYP27B1* and *POR*) show signs of positive selection and exhibit higher catalytic activity than those of other ruminants. A mutation upstream of the reindeer *CCND1* gene endows an extra functional binding motif of the androgen receptor and thereby may result in female antlers. Furthermore, a mutation (proline-1172→threonine) in reindeer PER2 results in loss of binding ability with CRY1, which may explain circadian arrhythmicity in reindeer.

Reindeer (*Rangifer tarandus*) are naturally distributed across the Arctic and subarctic regions. They face challenges such as severe cold and limited food availability during the winter and prolonged periods of both light and darkness during the year (*1*) and have thus had to evolve strategies and features to address these environmental obstacles. For example, reindeer do not exhibit 24-hour activity rhythms, and evidence suggests weak or even absent circadian organization (*2*–*4*), indicating that their internal biological clock may be adapted to the extreme light periodicity of the Arctic. These animals have also developed a particular fat metabolism process, limit heat loss by peripheral vasoconstriction, and maintain a low resting metabolic rate (*1*). A further challenge is that despite having to cope with low levels of solar energy, reindeer need to meet the calcium demands of rapid antler growth, therefore implying that they must have evolved a particularly high capacity for metabolizing vitamin D (*5*). Additionally, whereas archaeological evidence indicates that humans have exploited cervid species for millennia (*6*), *R. tarandus* is the only fully domesticated species within the Cervidae family (*7*). In this regard, the emergence of extensive reindeer husbandry during the last centuries was of considerable importance for the development of human civilization in the high Arctic of Asia and Europe. Reindeer are maintained in large domestic herds (*7*) that exhibit differences in activity patterns, tameness, and mitochondrial sequences from those of their wild relatives (*7*–*9*). However, despite the combination of these features, little is currently known about their underlying genetic causes, something we aimed to address in this study.

[1]Center for Ecological and Environmental Sciences, Northwestern Polytechnical University, Xi'an 710072, China. [2]Center for Circadian Clocks, Soochow University, Suzhou 215123, China. [3]School of Biology and Basic Medical Sciences, Medical College, Soochow University, Suzhou 215123, China. [4]Department of Special Animal Nutrition and Feed Science, Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China. [5]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China. [6]Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences, Oslo 0102, Norway. [7]Section for Computational and RNA Biology, Department of Biology, University of Copenhagen, Copenhagen N 2200, Denmark. [8]Section for Evolutionary Genomics, Department of Biology, University of Copenhagen, Copenhagen N 2200, Denmark. [9]Norwegian University of Science and Technology, University Museum, Trondheim 7491, Norway. [10]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China. [11]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China.
*These authors contributed equally to this work.
†Corresponding author: lizhipeng01@caas.cn (Z.L.); wwang@mail.kiz.ac.cn (W.W.); qiuqiang@lzu.edu.cn (Q.Q.)

Reindeer genomes exhibit vitamin D–specific mutations

Although the Ruminantia is an important group of terrestrial herbivores and its members have adapted to a wide range of terrestrial habitats, *R. tarandus* is the only species within the Cervidae that is widely distributed across the Arctic and subarctic regions. Because both reindeer sexes grow large antlers while inhabiting regions that undergo extended periods of low or no solar energy, it is conceivable that this species requires efficient calcium metabolism and reabsorption. Levels of active vitamin D [1α,25-$(OH)_2D_3$] in deer blood have been associated with antler growth rate (*10*, *11*). We therefore recovered all 28 genes from the reindeer reference genome (*12*) that are involved in the vitamin D metabolism pathway and compared them to their orthologs in other ruminant species from our ruminant genome project (*13*) as well as other mammalian outgroups (*14*), including the pig (*Sus scrofa domesticus*) (*15*), horse (*Equus caballus*) (*16*), and human (*Homo sapiens*) (*17*).

We found two genes (*CYP27B1* and *POR*) to be under positive selection in reindeer [branch-site model in phylogenetic analysis by maximum likelihood (PAML), chi-square test, $P < 0.05$] (Fig. 1A, fig. S1, and tables S1 and S2) (*14*). Furthermore, six genes (*ACAT2*, *CYP51A1*, *EBP*, *CYP2R1*, *BK2*, and *TRPV5*) exhibited reindeer-specific mutations (Fig. 1A, fig. S2, and table S3), and *APOB* was identified as a rapidly evolving gene [branch model in PAML, chi-square test, $P < 0.05$, a higher K_a/K_s ratio (i.e., the ratio of the number of nonsynonymous substitutions per nonsynonymous site to the number of synonymous substitutions per synonymous site)] (Fig. 1A and tables S4 and S5) (*14*). To explore whether these alleles were fixed in reindeer, we resequenced three domesticated reindeer from the Greater Khingan Mountains of the Inner Mongolia Autonomous Region, China, and three wild reindeer from the Snøhetta mountain area of Norway (*14*) and found that the mutant alleles were identical in all seven individuals (fig. S2). Note that all mutations described hereafter in other genes are also fixed in all seven reindeer.

CYP27B1 and POR are enzymes that play key roles in triggering the active 1α,25-$(OH)_2D_3$ from vitamin D (*18*). Our genomic comparisons of reindeer against other ruminants [including the closest cervid genome available, which is from the Siberian roe deer (*Capreolus pygargus*), GenBank Assembly accession: GCA 000751575.1] and outgroups revealed reindeer-specific mutations at three positions in both the *CYP27B1* (Fig. 1B and table S3) and *POR* genes (fig. S2 and table S3). We also conducted three-dimensional (3D) structure simulations to examine the possible effects of these mutations on the enzyme structure using Phyre2 (*19*). We found that only the K282→N (K282N) mutation in CYP27B1 was close to the P450 functional domain (Fig. 1C), whereas the other mutated amino acids are not (Fig. 1C and fig. S3). Although these mutations are neither located in nor close to known functional domains, they may also affect the catalytic activities (*20*).

To explore the functional relevance of these mutations, we synthesized reindeer, roe deer, and goat (*Capra hircus*) orthologs in vitro and tested their enzyme catalytic activities by measuring the activities in a reconstituted system consisting of the enzyme, substrate, and NADPH (the reduced form of $NADP^+$) (*14*). For CYP27B1, the reindeer protein variant exhibited significantly higher metabolic efficiency than that of goat and roe deer proteins (~2- and ~1.5-fold increase,

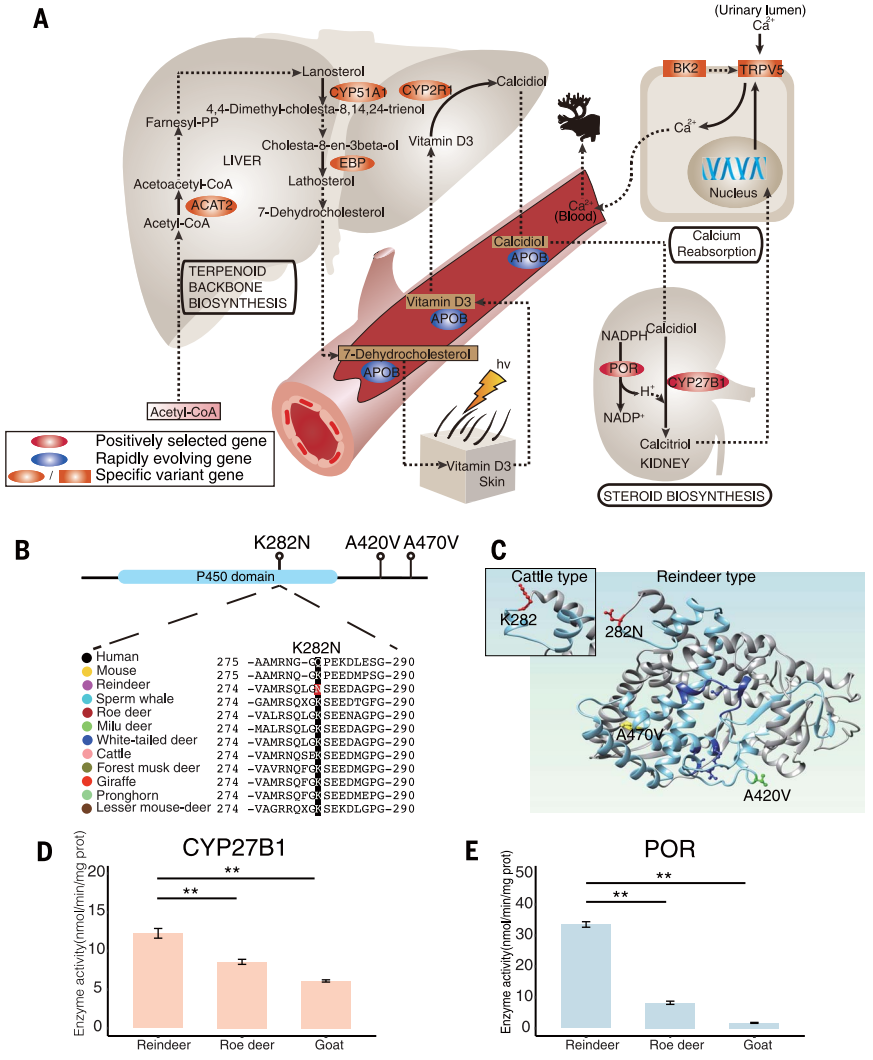

Fig. 1. Vitamin D metabolism in reindeer. (**A**) Alterations in the vitamin D metabolism pathways of reindeer. The genes involved in this pathway are labeled with different colors. Red genes are under positive selection, orange genes exhibit specific mutations in reindeer, and blue genes exhibit increased K_a/K_s values in reindeer. CoA, coenzyme A; h, Planck's constant; ν, frequency. (**B**) Specific mutations in the *CYP27B1* gene. There are three specific mutations (table S3), including one (K282N) in the P450 domain of CYP27B1. The CYP27B1 protein sequences of multiple species (indicated with different colors) were aligned, and the alignments of the K282N adjacent region are shown here. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**C**) 3D structure simulation of reindeer CYP27B1 compared with that of cattle. The 3D structure of POR is provided in fig. S3. (**D** and **E**) Enzyme activities of reindeer CYP27B1 and POR compared with those of roe deer and goat in vitro. $^{**}P < 0.01$ calculated from the *t* test. Error bars indicate SD. prot, protein.

respectively; *t* test, $P < 0.01$) (Fig. 1D). Surprisingly, the efficiency of the reindeer POR was ~20 and ~6 times that of the goat and roe deer POR, respectively (Fig. 1E; *t* test, $P < 0.01$). These results indicate that reindeer have evolved a more efficient vitamin D metabolism pathway than that of other mammals. This change likely enables reindeer to procure the high levels of active vitamin D needed to sustain their metabolism and calcium absorption and to promote body fat oxidation required to survive in the Arctic and subarctic regions (*21*). We also noted that the Ankyrin repeat region of the calcium receptor coding gene *TRPV5*, which is activated by active vitamin D and thus affects the reabsorption of calcium, exhibited reindeer-specific mutations (fig. S2). This indicates that both the up- and downstream pathways associated with vitamin D metabolism evolved in reindeer to secure the supply of calcium and energy.

Reindeer-specific mutations related to fat metabolism

We further identified fixed reindeer-specific mutations in genes that play a role in fat metabolism. These genes include *APOB*, which participates in the transport of low-density lipoproteins, and *FASN*, which encodes fatty acid synthase, an essential enzyme for de novo lipogenesis (*22*) (fig. S2 and table S3). These genes have previously been reported as targets of positive selection in both polar bears (*Ursus maritimus*; *APOB*) and Adélie penguins (*Pygoscelis adeliae*; *FASN*) (*23*). Although the selected sites were different from that seen in polar bears and Adélie penguins, this observation suggests that fat metabolism pathways have undergone convergent evolution across homothermal polar animals via the independent modification of key fat metabolism genes in a species-specific manner.

Mutations related to female antler growth

It is believed that the growth of cervid antlers is either driven by or strongly regulated by androgens (*24*). Notably, the reindeer is the only cervid species in which females (Fig. 2A) as well as males grow antlers, and in contrast to other species of deer, the removal of the gonads of either sex soon after birth does not prevent the growth and subsequent seasonal replacement of the antlers (*5*). We therefore hypothesized that this phenomenon may be related to the increased sensitivity of some genes that regulate antler growth to low-level androgens. The characteristic 5′-TGTTCT-3′ motif has been identified as the androgen receptor binding site in mammals (*25*). We thus examined the promoter regions of 30 highly expressed genes associated with cervid antlers (fig. S4) (*26*) and identified three 5′-TGTTCT-3′ motifs upstream of the antler-specific *CCND1* gene (Fig. 2B), which regulates cell cycles (*27*) and is required for chondrocyte proliferation (*28*) and thus is closely associated with antler growth (*29*) (Fig. 2B). In particular, we noted that reindeer contain a third reindeer-specific motif. To validate its role in gene regulation, we used chromatin immunoprecipitation coupled with quantitative polymerase chain reaction (ChIP-qPCR) to determine the binding capacity of the androgen receptor to the motifs in the blood samples of five female reindeer. Samples from three male roe deer served as controls. The results show that the androgen receptor binds to the second and third motif upstream of the antler-specific *CCND1* gene in reindeer, but only to the second motif in roe deer (Fig. 2C). These findings suggest that this extra motif might enhance the expression of *CCND1* in the presence of low androgen levels, thus enabling female reindeer to grow antlers.

Circadian rhythm regulation in Arctic light conditions

Reindeer experience extended daylight fluctuations and a markedly different seasonality than do cervids from temperate, subtropical, or tropical habitats (*2*, *3*). The effect of the environment on reindeer circadian rhythmicity leads these animals to lose daily rhythmic activity in winter and summer (*2*) and exhibit an unusual internal rhythm of melatonin secretion (*2*–*4*). However,

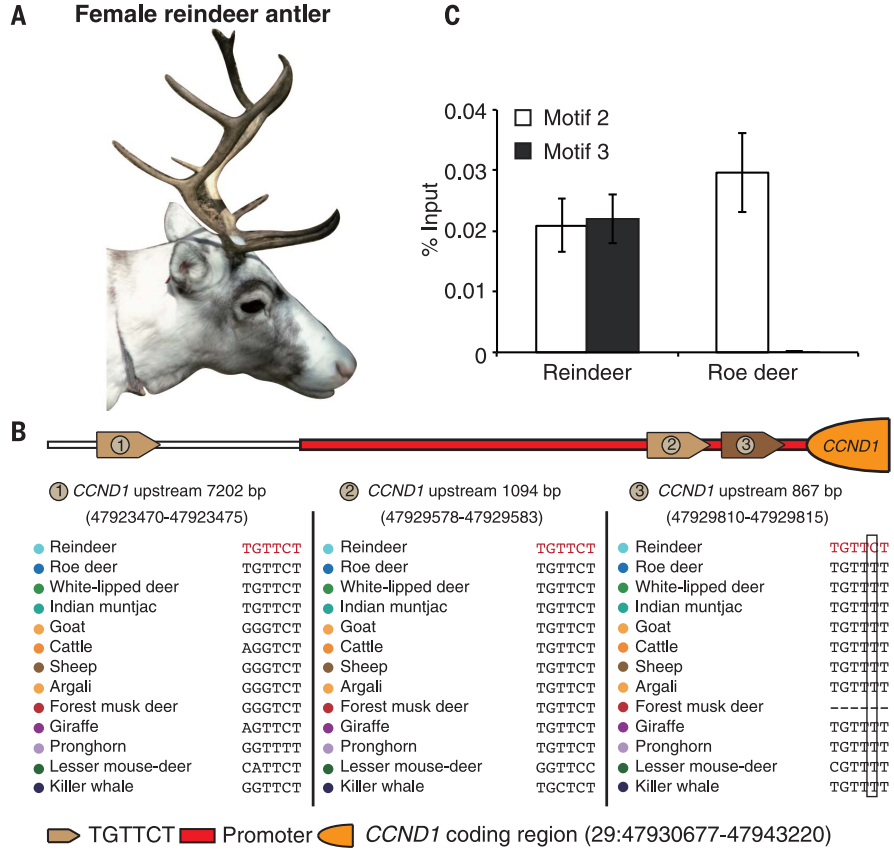

Fig. 2. Androgen receptor affinity sequence 5′-TGTTCT-3′ upstream of *CCND1*. (**A**) Characteristic antler of a female reindeer. (**B**) Three 5′-TGTTCT-3′ motifs were identified upstream of the reindeer *CCND1* gene. Motif 1 exists only in deer, whereas motif 3 exists only in reindeer. bp, base pairs. (**C**) ChIP-qPCR assays validated that the androgen receptor binds to both motif 2 and motif 3 of reindeer, but only to motif 2 of roe deer. Error bars indicate SD.

the genetic mechanism underlying this loss of a robust circadian clock remains unresolved. In other mammals, light regulates the biological clock in the suprachiasmatic nucleus (SCN) of the hypothalamus via a photic signal transduction pathway (*30*) by which light-induced neurotransmitters [PACAP (pituitary adenylate cyclase activating polypeptide) and glutamate] alter Ca^{2+} concentrations and trigger the phosphorylation of cAMP response element–binding proteins (CREBs) (Fig. 3A). We therefore retrieved 165 genes in reindeer that have been identified associated with the circadian rhythm pathway in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (*31*) and Reactome databases (*32*) and found eight genes that exhibit reindeer-specific mutations in functional domains (*PER2*, *NOCT*, *GRIA1*, *GRIN2B*, *GRIN2C*, *ITPR3*, *ADCY5*, and *NOS1AP*) (Fig. 3A and fig. S5). Moreover, among four genes (*ADCY2*, *ADCY8*, *CALML4*, and *CAMK2*) identified as rapidly evolving (table S4), reindeer-specific mutations were also observed in *ADCY8* and *CALML4* (fig. S5).

Phosphorylated CREB activates the transcription of period (*PER1*, *PER2*, and *PER3*) and cryptochrome (*CRY1* and *CRY2*) genes, which are the central elements for maintaining circadian rhythms (*33*). Three reindeer-specific mutations were found in the *Per2* gene of reindeer (fig. S5); of these, the P1172T mutation was predicted, through 3D structure simulations, to affect the binding domain between PER2 and CRY (Fig. 3B). To test the effect of this P1172T reindeer-specific mutation, T1172 was mutated to the corresponding wild-type (WT) amino acid (P), and then a coimmunoprecipitation (Co-IP) experiment was performed (*14*). Although WT PER2 (P1172, P-type) bound to CRY1, the reindeer-mutated PER2 (1172T, T-type) could not bind to CRY1 (Fig. 3C). Studies have documented that the binding of PER2 and CRY1 is required for sustained photic-induced circadian rhythms in peripheral tissues and cells, whereas CRY2 only modestly weakens rhythms (*34*). Together, these results show that the reindeer P1172T mutation caused the loss of the binding ability of PER2 with CRY1, which can lead to circadian arrhythmicity in reindeer.

Among the other seven circadian rhythm genes with reindeer-specific mutations, the *NOCT* gene serves as a key posttranscriptional regulator in the circadian control of many metabolic processes, including lipid metabolism (*35*). Notably, we observed several reindeer-specific mutations in this gene (Fig. 3D). Furthermore, among reindeer-specific mutated genes, three genes (*GRIA1*, *GRIN2*, and *ITPR3*) affect Ca^{2+} concentrations in neuron cells, with another four genes (*ADCY5*, *ADCY8*, *CAMK2*, and *NOS1AP*) affecting the phosphorylation of CREB. The *GRIA1* and *GRIN2* genes encode glutamate receptors, the activation of which is a critical step in the transmission of photic information to the SCN (*36*). The *ITPR3* gene encodes an intracellular calcium channel receptor (*37*). CREB is phosphorylated via ADCY-mediated pathways or CAMK2-, CALML4-, and NOS1AP-mediated pathways (*38*). Together, these results indicate not only that several core proteins and genes in the circadian clock are altered in reindeer but also that changes have occurred in their associated upstream and downstream pathways. We therefore hypothesize that these changes may be important components in facilitating the adaptation of reindeer to the Arctic's arrhythmic light conditions.

Mutations related to the docility of reindeer

Although most cervids have evolved sensitive and vigilant behavior to avoid predators, including humans (*39*), reindeer are quite docile. Since approximately the start of the first millennium CE, reindeer have been used by humans for transport, as decoy animals, and even for milking. This characteristic docility potentially explains why the reindeer is the most successfully domesticated cervid (*7*). It has been hypothesized that neural crest cells (NCCs) may play an important role in docility and domestication (*40*). Evidence in support of this hypothesis has been found in cats (*Felis catus*) (*41*) and dogs (*Canis lupus familiaris*) (*42*). We investigated reindeer-specific mutations in genes related to NCCs and found reindeer-specific mutations in 11 genes that relate to development of NCCs (*MSX2*, *ID3*, *BCAT1*, *CAD6*, and *CAD11*) (Fig. 4 and fig. S6), migration of NCCs (*TCOF1*, *BCAT1*, *NOTCH2*, and *NOTCH3*), and differentiation of NCCs (*COL2A1*, *KIT*, and *SI*) (Fig. 4 and figs. S6 and S7). In addition, *GEM* (*43*) and *ASCL1* (*44*) were identified as rapidly evolving genes that have been reported to be associated with the development and differentiation of NCCs, respectively. The *COL2A1* gene related to the differentiation of NCCs was also positively selected in reindeer (Fig. 4). Studies have reported mutations in *KIT* genes, which are associated with white spotting on the body (*40*, *45*), in domestic cats (*41*), horses (*46*), and pigs (*47*). Notably, white-spotted reindeer generally exhibit elevated levels of docility and can be easily approached by herders even when the rest of the herd is in a state of general excitement (*48*). Thus, we speculate that these specific NCC gene mutations may be related to the particular docility trait of reindeer.

Discussion

Our results reveal the genetic basis of a broad spectrum of Arctic deer biology and provide a foundation for understanding the adaptive strategies of mammals to the Arctic environment. Our comparative genomic studies and functional assays identify a number of genes that exhibit functionality related to circadian arrhythmicity,

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vitamin D metabolism, docility, and antler growth; are uniquely mutated; and/or are under positive selection in reindeer. Furthermore, these studies may provide insights relevant to human health, such as how the genetic response of vitamin D in reindeer affects bone and fat metabolism and how the genes related to circadian arrhythmicity could enhance our knowledge of seasonal affective disorders, insomnia, and depression.

Materials and methods summary

We aligned the genome sequences of 51 ruminant species (*13*) and 12 outgroup species to the goat (*C. hircus*) chromosomal genome (*49*) using lastal (version 867) (*50*). We then merged aligned sequences using Multiz (version 11.2) (*51*) and identified orthologous genes on the basis of synteny with the cattle genome (*52*), which is so far the best-annotated genome in ruminants. The whole-genome tree was obtained from our ruminant genome project (*13*), which was generated by ExaML (version 3.0.17) (*44*) and RaxML (version 8.2.10) (*53*). We used the orthologous gene sets of 11 species: white lipped deer (*Cervus albirostris*), milu deer (*Elaphurus davidianus*) (*54*), black muntjac (*Muntiacus crinifrons*), reindeer (*R. tarandus*) (*12*), white-tailed deer (*Odocoileus virginianus*), roe deer (*C. pygargus*), cattle (*Bos taurus*) (*52*), giraffe (*Giraffa camelopardalis tippelskirchi*) (*55*), pig (*S. scrofa domesticus*) (*15*), horse (*E. caballus*) (*16*), and human (*H. sapiens*) (*17*), in the following analyses. The Codeml module in the PAML software package (*56*) was used to identify the rapidly evolving genes and positively selected genes. KOBAS (version 2.0) was used to conduct the KEGG and Gene Ontology (GO) enrichment analysis (*57*). Protein sequences of selected genes were compared between reindeer and other animals to identify reindeer-specific mutations. Pfam (version 1.6) (*58*) was applied to determine whether the mutations were located in the domain region of the protein. To validate whether these identified mutations are specific for reindeer, three domestic reindeer samples from the Greater Khingan Mountains, Inner Mongolia Autonomous Region, China, and three wild reindeer samples from the Snøhetta mountain area in south-central Norway (*7*) were whole-genome resequenced using an Illumina HiSeq 2500 instrument. The high-quality reads were aligned to our previously released reindeer reference genome (*12*) using BWA-MEM (version 0.7.12) (*59*). SAMtools (version 0.1.19) (*60*) was used to sort and merge the alignment results. Reads around indels were realigned by the Genome Analysis Toolkit (GATK version 3.5) with the Realigner Target Creator and Indel Realigner programs (*61*). The protein 3D structure of interaction between PER2 and CRY1 was downloaded from the Protein Data Bank (*62*). The

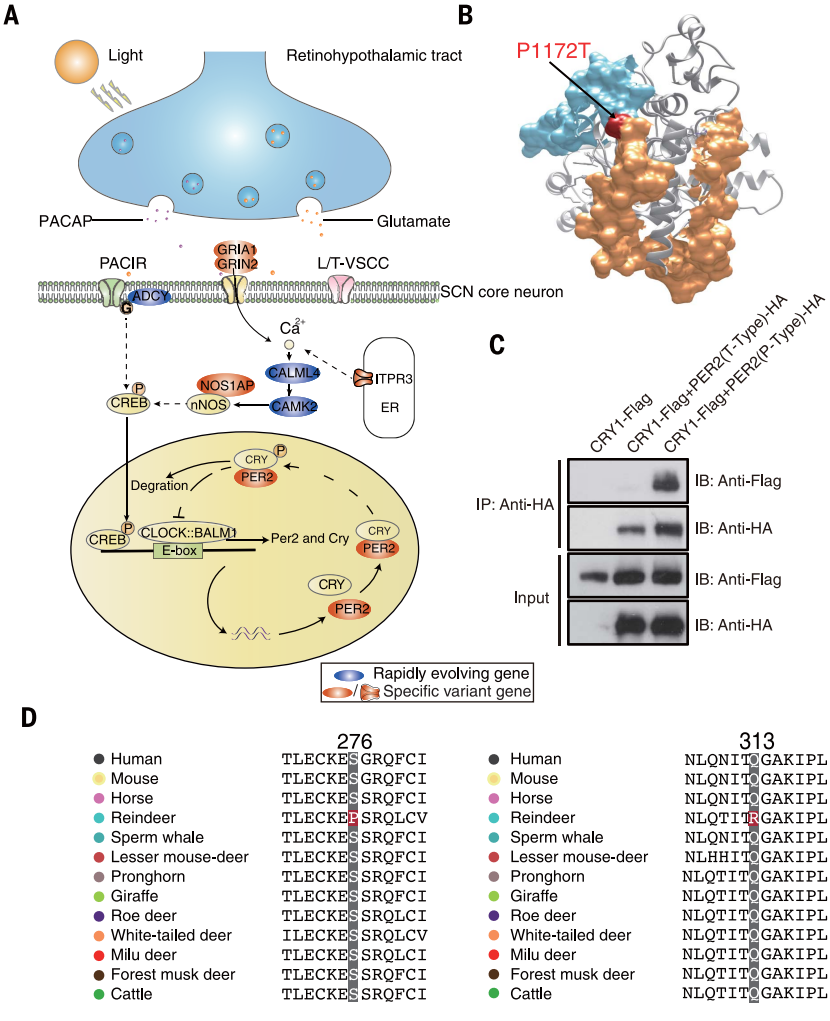

Fig. 3. Mutations in the reindeer circadian rhythm genes and pathways. Orange genes have reindeer-specific mutations; blue genes exhibit increased K_a/K_s values in reindeer. (**A**) Light regulates the molecular clockwork in reindeer SCN neurons. G, G protein; P, phosphorus; nNOS, neuronal nitric oxide synthase; ER, endoplasmic reticulum. (**B**) The reindeer-specific mutation (P1172T) of PER2 is located within the binding domain of the PER2-CRY complex, as determined by 3D modeling. The site of this mutation is marked in red. (**C**) Co-IP assays show that reverted PER2-T1172P (P-type) proteins can bind to CRY1, but reindeer PER2-P1172T (T-type) cannot bind. CRY1-Flag was transfected with or without PER2–hemagglutinin (HA) or PER2-T1172P, and Co-IP was conducted. IB, immunoblot. (**D**) The *NOCT* gene functional domain has specific mutations (fig. S5). The reindeer *NOCT* gene has two specific mutations (S276P and Q313R) in the C-terminal deadenylase domain. The amino acid substitution in reindeer is indicated in red.

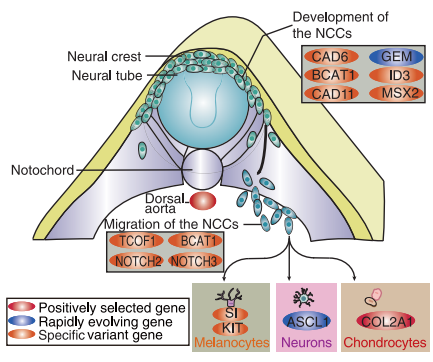

Fig. 4. Genes altered in reindeer play a role in neural crest development, migration, and differentiation. Red genes are under positive selection in reindeer, orange genes have reindeer-specific mutations, and blue genes exhibit increased K_a/K_s values in reindeer. Five genes (*CAD6*, *BCAT1*, *ID3*, *CAD11*, and *MSX2*) participating in the development of NCCs, four genes (*TCOF1*, *BCAT1*, *NOTCH2*, and *NOTCH3*) involved in the migration of NCCs, and two genes (*SI* and *KIT*) related to the differentiation of NCCs into melanocytes were found to have specific mutations in reindeer. In addition, one gene (*ASCL1*) related to the differentiation of NCCs into neurons and one gene (*GEM*) involved in the development of NCCs showed increased K_a/K_s values in reindeer. One gene (*COL2A1*) related to the differentiation of NCCs into chondrocytes was under positive selection in reindeer.

3D structures of the CYP27B1 and POR proteins were predicated using Phyre2 (*19*) and then visualized using UCSF Chimera (*63*). The *CYP27B1* and *POR* genes were synthesized and then cloned into the vector pET-28a by Gibson assembly methods (*64*). The plasmid was transformed into *Escherichia coli* BL21 to express these two genes. The cultured cells were purified and then used to measure enzyme activity as described by Akiyoshi-Shibata *et al.* (*65*). The reindeer *PER2* (P1172T), reindeer *CRY1*, and reverted *PER2* (T1172P) genes were synthesized and then ligated into vector pET-28a by Gibson assembly methods (*64*). Co-IP and immunoblotting were performed as described previously (*66*) to test the interaction between PER2 and CRY1. ChIP-qPCR was applied to the blood samples of five female reindeer and three male roe deer to confirm that androgen receptor binds to the reindeer motif 3 with the reindeer-specific mutation (for details, see the full materials and methods in the supplementary materials) (*14*).

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SUPPLEMENTARY MATERIALS

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Science

Biological adaptations in the Arctic cervid, the reindeer (*Rangifer tarandus*)

Zeshan Lin, Lei Chen, Xianqing Chen, Yingbin Zhong, Yue Yang, Wenhao Xia, Chang Liu, Wenbo Zhu, Han Wang, Biyao Yan, Yifeng Yang, Xing Liu, Kjersti Sternang Kvie, Knut Håkon Røed, Kun Wang, Wuhan Xiao, Haijun Wei, Guangyu Li, Rasmus Heller, M. Thomas P. Gilbert, Qiang Qiu, Wen Wang and Zhipeng Li

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Phylogeny and characteristics of ruminants

Ruminants are a diverse group of mammals that includes families containing well-known taxa such as deer, cows, and goats. However, their evolutionary relationships have been contentious, as have the origins of their distinctive digestive systems and headgear, including antlers and horns (see the Perspective by Ker and Yang). To understand the relationships among ruminants, L. Chen *et al.* sequenced 44 species representing 6 families and performed a phylogenetic analysis. From this analysis, they were able to resolve the phylogeny of many genera and document incomplete lineage sorting among major clades. Interestingly, they found evidence for large population reductions among many taxa starting at approximately 100,000 years ago, coinciding with the migration of humans out of Africa. Examining the bony appendages on the head —the so-called headgear—Wang *et al.* describe specific evolutionary changes in the ruminants and identify selection on cancer-related genes that may function in antler development in deer. Finally, Lin *et al.* take a close look at the reindeer genome and identify the genetic basis of adaptations that allow reindeer to survive in the harsh conditions of the Arctic.

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