Haplotype-resolved and chromosome-scale genomes provide insights into co-adaptation between the Amur tiger and Amur leopard

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DEAR EDITOR,

Big cats, such as Amur tigers (*Panthera tigris altaica*) and Amur leopards (*P. pardus orientalis*), are top predators that have evolved specialized traits for hunting and carnivory (Fernandez Moya et al., 2021). They play a crucial role in maintaining biodiversity and ecosystem integrity by balancing prey-predator dynamics. However, human activities, including habitat fragmentation and environmental changes, have led to the persistence of these big cats in small and isolated populations. Currently, all living big cats are categorized as endangered or threatened. Amur tigers and Amur leopards share overlapping geographic ranges, habitats, and some prey in the forests of Northeast Asia (Jiang et al., 2015). To reduce conflict between the two species, they exhibit differentiated dietary and temporal niches. Tigers prefer large ungulates, while leopards hunt small to medium-sized prey (Kerley et al., 2015; Sugimoto et al., 2016). They also occupy different niches temporally, with tigers being active at night and leopards active during the day. Despite spatial and temporal partitioning, interspecific competition between Amur tigers and Amur leopards remains inevitable. Tigers have a competitive advantage due to their larger size, and competition takes various forms, including occasional leopard predation by tigers and declines in leopard populations with increasing tiger density (Donadio and Buskirk, 2006; Jiang et al., 2015). Tigers also exclude leopards from marginal habitats in nature reserves where they coexist.

The coexistence and competition between these big cats involve not only ecological factors but also genetic factors underlying physiological traits. However, the genetic basis for co-adaptation and competition has not been well studied. In this study, we assembled a haplotype-resolved and chromosome-scale genome for a wild Amur leopard without the parental information and conducted comparative genomic analysis with an Amur tiger genome to elucidate the genetic basis for coexistence and competition. This research has implications for the future conservation of these two endangered species.

Comparative genomics aims to understand the similarities and differences between genomes of different organisms. Genome assembly provides the necessary scaffolding for downstream comparative genomic analyses, facilitating the identification of genetic variations and the exploration of functional elements in the genome. By collecting blood samples from a rescued Amur leopard in the wild, we successfully assembled a high-quality genome for this species (hereafter PpoHapG), with a genome size of 2.42 Gb and a scaffold N50 value of 146.6 Mb (Figure 1A, B; Supplementary Table S1, S2). More than 2.40 Gb scaffolds (~99.34%) were anchored to 19 chromosome-scale pseudomolecules with a complete X chromosome identified (Supplementary Figure S1 and Table S3). The hybrid genome was then phased by a hifiasm assembler into two groups of haplotigs, with 19 chromosomes generated in each set of haplotigs (hereafter referred to as the PpoHapGH1 and PpoHapGH2) (Supplementary Figure S2). The genome completeness measured by Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis also showed high scores for all three genomes, while the base-level quality evaluation showed that all three genomes we prepared here have high QV scores (Supplementary Table S4 and S5). Subsequently, we conducted a comprehensive analysis to identify repetitive sequences and protein-coding genes (Supplementary Figure S3, Table S6-S10). Additionally, the collinearity analysis exhibited consistency in the leopard genome, both within haplotypes and among closely related species (Supplementary Figure S4 and S5). Furthermore, we utilized the published Amur tiger genome (hereafter PtaHapG) for subsequent comparative analyses (Tianming et al., 2023).

The genomes were complete with few haplotype-specific sequences. 2586 structural variants were identified, including deletions, duplications, translocations, and inversions, supported by genome mapping (Figure 1A; Supplementary Table S11). These structural variants affected genes related to thyroid and parathyroid function, heart function, insulin secretion, growth hormone synthesis, and olfactory receptors (Supplementary Figure S6, S7 and Table S12). Only a few pseudogenic genes were specific to one haploid genome, related to reproduction and energy metabolism (Supplementary Table S13).

To investigate the genomic basis of the biological characteristics of the tiger and leopard as the apex predator, we first constructed a phylogenetic tree and performed the gene expansion analysis. 171 and 280 gene families in the PtaHapG and PpoHapG were found to be expanded, respectively (Supplementary Figure S8). In both KEGG and GO enrichment analysis, we found similar enriched terms and pathways shared by these two species, and energy metabolism related GO terms were predominant in both PtaHapG and PpoHapG (Supplementary Figure S9-S12). Interestingly, we found that the long-term potentiation (LTP) (map04720), was significantly enriched in the expanded gene families in the PtaHapG based on the KEGG pathway analysis (Figure 1C). The LTP is a long-lasting increase in the strength of connections between neurons in the hippocampus. LTP is thought to be a cellular molecular mechanism underlying learning and memory (Dong et al., 2015; Martinez and Derrick, 1996; Morris, 2003). In this pathway, the calmodulin (*CaM*) gene family was found to expand in both PtaHapG and PpoHapG when compared with other Felidae species (Figure 1D). The CaM is a ubiquitous Ca2+ binding protein, and the complex of Ca2+/CaM further activates the Ca2+/CaM-dependent kinase II (CaMKII), which is critical for synaptic plasticity that underlies learning and memory (Chang et al., 2019). The *CaM* gene family expansion, which may enhance the expression level of the *CaM* gene and potentially impact the functionality of LTP pathway in Amur tiger.

The analysis of positive selection in comparative genomics is commonly employed to unravel adaptive evolution and comprehend the genetic basis of species-specific traits. In this study, we found 119 PSGs and 401 REGs in the

PtaHapG, 139 PSGs and 370 REGs in the PpoHapG. In both PtaHapG and PpoHapG, we found a large number of genes were closely related to physical requirements of big cats as apex predators (Supplementary Table S14, S15), including energy metabolism, vision, muscle, teeth and bone, immunity, lipid metabolism, cardiovascular, and other shared positively selected genes related with hearing, smell, claw, and adrenal glands (Figure 1E, Supplementary Figure S13). Similarly, we found more species-specific amino acid changes in the tiger than in the leopard genome (Figure 1F-1H). Strikingly, we identified a rapidly evolving gene, *MEF2C*, in PtaHapG, but not in PpoHapG (Figure 1E). This gene negatively regulates the excitatory synapse number and function to further regulate basal and evoked synaptic transmission, which finally facilitates hippocampal-dependent learning and memory. In mice, the overexpression of *MEF2C* in the adult prefrontal cortex also improves working memory and cognition like the *KMT2A* gene (Supplementary Table S16). Similarly, we also identified the gene, *ADCY9*, was under strong positive selection in the PtaHapG but not in the PpoHapG. The expression of this gene was reduced in the hippocampus of aged mice but increased in after spatial learning, which suggested that this gene may regulate learning, memory and cognition (Supplementary Table S16). The rapidly evolving gene *MEF2C* and the positively selected gene *ADCY9* suggest that the tiger may have the superior cognitive ability to the leopard. Furthermore, the tiger has been reported to have a larger brain size than the leopard and other large cats, and larger pyramidal neurons containing more complex dendrites with disproportionally greater dendritic measures relative to body/brain size in prefrontal, motor, and visual cortices that play important roles in higher cognitive functions such as planning, problem-solving, reasoning, and episodic memory retrieval, musculoskeletal functions, and visual information processing (Supplementary Table S16). These findings, along with our own research, suggest that there may be a genetic basis for the biological differences observed between the Amur tiger and Amur leopard.

The Amur tiger and Amur leopard share the most recent ancestor and largely overlap in niches as top predators in habitats they coexist. Thus, we expected genomic signatures of parallel evolution between them when compared with other species. We identified 17 parallel evolutionary genes between PtaHapG and PpoHapG, many which were found to be closely related with their survival in the wild, including wound healings (*PEAR1* and *MEGF10*), bone development and healing (*FARP2*), hearing (*PKHD1L1*), muscle development and energy metabolism (*SLC25A25* and *MYO18B*) (Supplementary Table S17 and Figure S14). This indicates that the Amur tiger and Amur leopard may have parallelly evolved on those above-mentioned aspects as required in the physiology of top predators. To further explore adaptively parallel genes (APG), we screened these 17 genes that were simultaneously under positive selection. One APG (*SLC25A25*) and five APGs (*FREM2, L1CAM, MEGF10, PEAR1, SLC5A9*) were found in the PpoHapG and PtaHapG genomes, respectively (Figure 1E). As in the gene family expansion and natural selection analysis, we also found a parallelly evolved gene, *KMT2A*, which may play a role in memory improvement. Simultaneously, we found a parallel evolving gene, *KMT2A*, between the PtaHapG and PpoHapG. This gene is required for the formation of working memory and memory consolidation (Jakovcevski et al., 2015; Kerimoglu et al., 2017). Working memory is connected or related to intelligence, information processing, executive function, comprehension, reasoning, problem-solving, planning, and learning in humans and all sorts of animals (Cowan, 2014). These indicated that the Amur tiger and leopard may have superior cognitive ability than other Felidae species.

This study indicates a comprehensive understanding of the competition between Amur tigers and Amur leopards through comparative genomics analysis. The two big cats, occupying the same niche as top predators, evolve in parallel with shared genomic characteristics related to hearing, smell, claw and muscle formation, immunity, reproduction, and detoxification. These shared functions contribute to their predatory traits and survival abilities. Furthermore, our results identify specific genes and amino acid changes in the two species that are associated with bone, cardiovascular function, reproduction, and immunity. Overall, our findings indicate that both species have evolved similar physiological traits to adapt to their current habitats, allowing for coexistence and competition.

However, we also reveal genomic differences that give tigers an advantage over leopards in competition. The Amur

tiger possesses genes related to olfactory receptors, reproduction, and energy metabolism that are absent in the leopard, suggesting weaker functionality in these aspects for the latter. Additionally, the tiger demonstrates advantages in expanded gene families associated with food digestion, mineral absorption, and vision. Moreover, the study finds tiger-specific amino acid changes in certain genes, potentially enhancing the tiger's adaptability as the apex predator. The Amur tiger also exhibits a higher number of immune-related and cardiovascular genes compared to the Amur leopard, indicating better adaptivity in these areas. Conversely, the leopard shows a higher number of energy metabolism-related genes, reflecting adaptation to a higher metabolic rate due to its smaller body size (White and Seymour, 2003). Furthermore, the leopard genome exhibits a higher enrichment of disease-related pathways compared to the tiger, suggesting potentially greater susceptibility to certain diseases in the leopard.

Overall, our study provides a comprehensive and well-annotated genomic analysis of the Amur leopard and Amur tiger, revealing a greater presence of genomic signatures associated with top-predator traits in the Amur tiger compared to the Amur leopard, which sheds light on their one-sided competition from a genetic perspective. To ensure the co-conservation of these top predators, strategies that facilitate effective niche partitioning should be implemented. Understanding ecological thresholds such as climate changes, habitat availability, vegetation, prey base, competitors, and anthropogenic disturbances can help inform management approaches that promote habitat resources favoring niche partitioning between tigers and leopards.

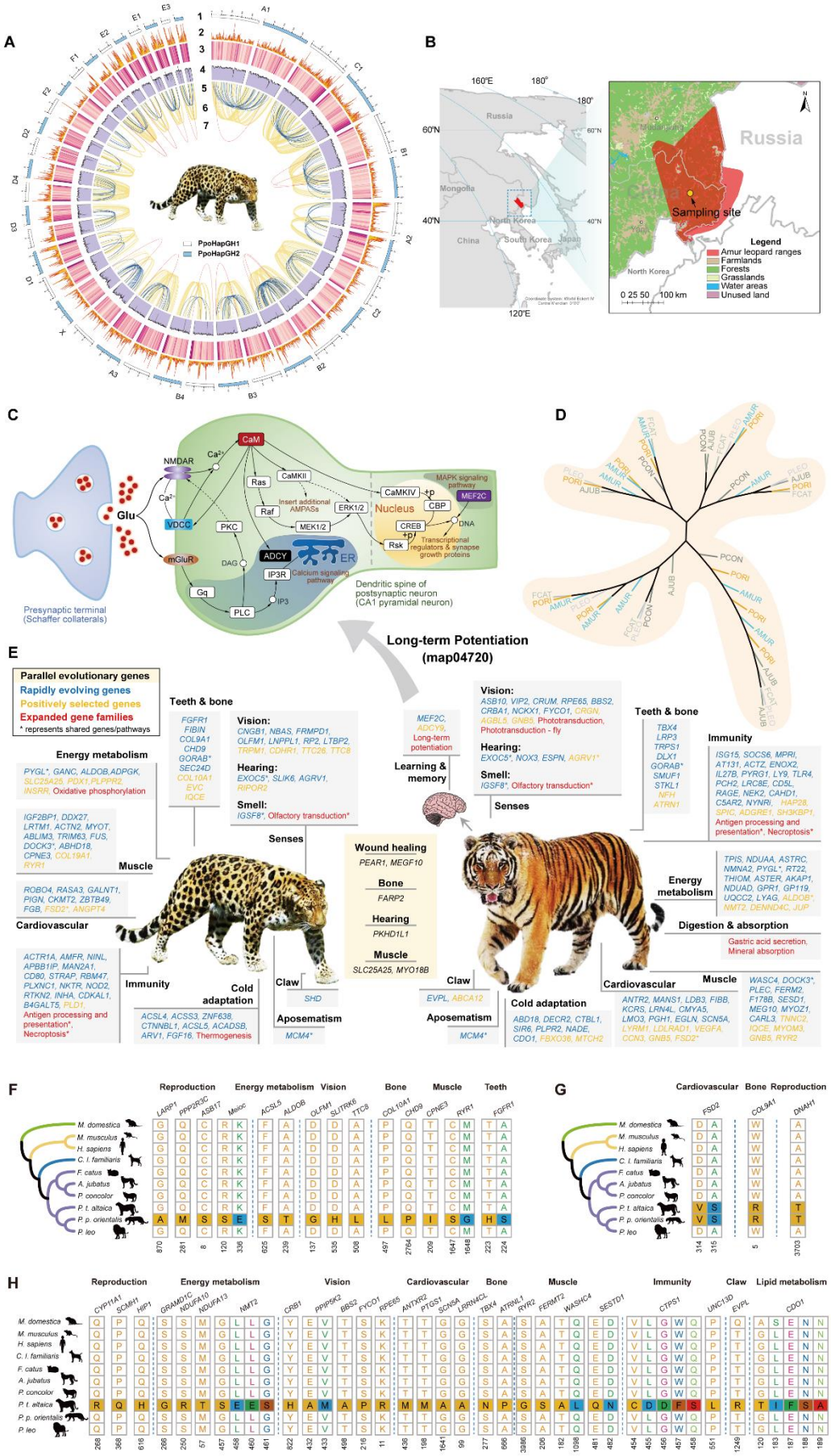

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Figure 1. A: The genomic landscape of the Amur leopard genome. 1. schematic diagram of chromosomes (white: PpoHapGH1; blue: PpoHapGH2); 2. gene density; 3. sequencing depth; 4. GC contents; 5. SV: duplication; 6. SV: inversion; 7. SV: translocation. B: The distribution area and sampling sites of Amur leopard in this study. C: The long-term potentiation pathway. The *CaM* gene family was significantly expanded in both Amur tiger and Amur leopard genome. The rapidly evolving gene, *MEF2C*, and positively selected gene, *ADCY* are shown in purple and black colors, respectively. D: The maximum likelihood phylogenetic tree of *CaM* gene family. The Amur tiger's genes are highlighted in light blue, while the genes of Amur leopard and other Felidae species are displayed in orange and gray colors, respectively. E: Genes and pathways with either shared or unique evolutionary signals in Amur tiger and Amur leopard and their putative adaptive functions. F: The Amur leopard specific amino acid changes in REGs compared with other species. G: Amino acid changes in REGs shared by Amur tiger and Amur leopard. H: The Amur tiger specific amino acid changes in REGs compared to other species.

Declaration of Interest Statement

The authors declare no competing interests.

Author Contributions

These authors contributed equally: T.M.L., H.M.L., Y.C.X. and G.S.J. initiated and designed the project. D.L., Y.Z., W.Y.K., Y.M., B.Y.L. and L.Z. collected the samples. L.Y.C. and S.C.Y. performed the RNA/DNA isolation. H.R.L. performed the DNA libraries construction and sequencing. T.M.L. coordinated the data analysis. H.M.L., M.H.S. B.Y.L. and carried out the data analysis. T.M.L., H.M.L., B.Y.L. and M.H.S. wrote the manuscript. Y.C.X., S.K.S., S.L.L., N.D., L.D. and G.S.J. revised the manuscript. Y.C.X. and G.S.J. provided the supervision of this project.

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Data and Code Availability

The data that support the findings in this study have been deposited into CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0003803.

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