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Perspectives provided by the leopard and other cat genomes: **how diet determined the evolutionary history of carnivores, omnivores, and herbivores**

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Keywords: leopard, Felidae, wild species genome, evolution, dietary adaptation

Abbreviations: CBD, Convention on Biological Diversity; SNV, Single nucleotide variation; PSMC, Pairwise sequentially Markovian coalescent

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Abstract

Recent advances in genome sequencing technologies enabled humans produce and investigate wild species genomes. This included a very large family of cats such as tiger, lion, and leopard. We have performed an in-depth comparative analysis to identify the genomic signatures in the evolution of felid to become the top carnivore predators on land. Our focus was on how carnivore genomes, compared to omnivore or herbivore, shared evolutionary adaptations in genes associated with nutrient metabolism, muscle strength, agility, and other traits responsible for hunting and meat digestion. We found genetic evidence of genomes represent what animals eat through modifying genes. There were highly conserved such genetically relevant regions at the genomes of cat family level. Also, felid family genomes showed a low level of genetic diversity associated with decreased population sizes, presumably because of their strict diet, suggesting their vulnerability and critical conservation status. Our findings can not only be used for animal studies but they can be used for human health enhancement as we share the same genes as the cats, but with some variation. This is an example how wild life genomes can be a critical resource for human evolution providing key genetic marker information for disease treatment.

Since the Convention on Biological Diversity (CBD) entered into force in 1993, conservation and sustainable use of biodiversity has become an essential issue for the survival of living entities including human in the rapidly changing current ecosystems. Biodiversity traditionally includes species diversity, genetic diversity, and ecosystem diversity. Recently, genomic diversity has been added as one of the most fundamental layers of biodiversity in addition to the three components.

Recent advances in genome sequencing technologies, and the resulting decrease in cost assisted by the refinement of bioinformatics tools to interpret genomic codes, made genomics readily available to biodiversity researches in non-model, wild species. The genome sequences of wild animal species are rapidly accumulated and provide rich resources for the study of adaptation, trait evolution, species divergence, and population structure analyses. Currently, more than 120 genome assemblies and many more whole genome re-sequencing data are available for the mammalian taxa. These data will be used for furthering conservation effort and good management practices of endangered wild species.

Felidae, the family of cats, includes the most iconic and much threatened wild species such

as tiger, lion, cheetah, and leopard. They have been the top predator and eat only meat to survive. As a hyper-carnivore, Felidae developed several key diet-related traits such as digestive enzymes, shortened digestive tracts, and alteration of taste bud sensitivities to sugar. This extreme genetic adaptation endows us to generate invaluable insight and practical bio-markers for human disease and health study in the future as a genome diversity resource. The morphology of cats is highly adapted for hunting, powered by flexible bodies, fast reflexes, and strong muscular limbs. They also possess highly developed senses of night vision, hearing, and smell. The leopard, *Panthera pardus*, is among the most widely distributed big cats, inhabiting from Africa to Russian Far East. Of the nine genetically distinguished subspecies, Amur leopard is facing the most serious threats of extinction with only 60-70 individuals in wild, mostly due to the increasing human population expansion, habitat loss, hunting, and poaching.

Felidae is a well-studied group from a genomic perspective. Genome assemblies have been available for four species in Felidae, i.e., domestic cat (*Felis catus*), tiger (*Panthera tigris*), cheetah (*Acinonyx jubatus*), and lion (*P. leo*). In addition, whole genome sequences for snow leopard *(P. uncia*) and additional individuals of tiger and lion have been released.

Recently, we have produced and added the first reference of a leopard genome to increase the number of high quality cat genome repertoire. It is from a captive Amur leopard from Daejeon Zoo of Korea. We sequenced it to 310× average depth using Illumina HiSeq platforms and assembled into 50,400 scaffolds (N50 length of 21.7 Mb) resulting 2.58 Gb in length which is the highest quality among big cat genomes. A total of 19,043 protein-coding genes were annotated and 39.04% of the genome turned out to be various types of repeats. Additionally, we produced two whole genome re-sequences at the depth of 35× from free ranging wild leopards obtained from “land of leopard” National Park in Russian Far East, and one wild leopard cat from South Korea.

The genetic diversity of Felidae, assessed with heterozygous single nucleotide variation (SNV) rates against domestic cat genome, on average were very low reflecting small population sizes with a high propensity for extinction. The genetic diversities of three leopards were similar to those of snow leopard, cheetah, and white lion, which have extremely low diversity due to habitat isolation or inbreeding. Demographic history of leopards, calculated using a pairwise sequentially Markovian coalescent (PSMC) model inference, showed a rapid decline of effective population size since its divergence 10 million years ago with traces of a severe genetic bottleneck between two million to 900K years ago.

Extensive comparative analyses with publicly available mammalian genomes were conducted to identify genomic signatures of Felidae evolution mainly focusing on its hyper-carnivorous life style. At first, evolutionary aspects were analyzed at the amino acids level with 8,648 orthologous gene families among 18 mammalian genome assemblies of three dietary groups, i.e., eight carnivores (leopard, cat, tiger, cheetah, lion, polar bear, killer whale, and Tasmanian devil), five omnivores (human, mouse, dog, pig, and opossum), and five herbivores (giant panda, cow, horse, rabbit, and elephant). Felidae or carnivore specific gene expansion and contraction, positively selected genes, and convergent amino acid changes well reflected its phenotype, in which gene families associated with muscle activities were among the expanded while those with starch metabolism and detoxification of plant-derived toxicants were contracted. Molecular signatures for adaptation were further verified from the search of highly conserved regions across species. Certain homozygous genomic regions strongly conserved among 14 individuals of the seven species within Felidae stretched 1.13 Gb in length containing genes functionally enriched in sensory perception of light stimulus and synaptic transmission reflecting the characteristic features of Felids such as eminent night vision and fast reflexes. These genomic signatures are not verified experimentally. However, the genetic variation information clearly indicates evolutionary adaptation of the family. A far reaching conclusion of our study is that carnivory is a rather special adaptation compared to omnivory and herbivory. It shows that while cows and humans can eat plants and meat if necessary, cats cannot have an option for plants. One thing we did not investigate was the microflora of the guts of the three different groups. It is likely that there are microbiomes that co-evolved with the hosts to digest plants and meat. This hologenome, the combination of the host and symbionant genomes, can provide another layer of insight on how animals evolved to extreme dietary selection. In the future, when all the major kingdom’s reference genomes are available, it will be necessary to iterate our study to include insects, fish, birds, reptiles, and amphibians to draw a much more complete picture of genomic diversity of dietary adaptation.

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