Highlight: A Massively Parallel Sequencing Approach Uncovers Ancient Origins and High Genetic Variability of Endangered Przewalski’s Horses

Danielle Venton

*Corresponding author: E-mail: danielle.venton@gmail.com.

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Przewalski’s Horses Not Ancestors of Modern Domestic

For millions of years, the stout, muscular Przewalski’s horse freely roamed the high grasslands of Central Asia. By the mid-1960s, these, the last of the wild horses, were virtually extinct: a result of hunting, habitat loss, and cross breeding with domestic horses.

Recovering from a tiny population of 12 individuals and only four purebred females, there are now nearly 2,000 Przewalski’s horses around the world. Once again, the light-colored horses, standing about 13 hands, or 1.3 meters, tall, are beginning to graze on the Asian steppe, thanks to captive breeding and reintroduction programs.

Protecting Przewalski’s horses, listed as critically endangered by the International Union for Conservation of Nature, will require far more than protecting their habitat. Understanding and safeguarding their genetic diversity is key, said Kateryna Makova, an evolutionary genomicist at Pennsylvania State University. In a new study (Goto et al. 2011), Makova and her colleagues Hiroki Goto, Oliver Ryder, and others report on the most complete genetic analysis of Przewalski’s horses to date, clarifying previous genetic analyses that were inconclusive.

Because Przewalski’s horses are the only remaining wild horses, many people have hypothesized that they gave rise to modern domestic horses. The Australian Brumbies or the American Mustangs, sometimes referred to as wild horses, are actually feral domestic horses, adapted to life in the wild. Przewalski’s horses and domestic horses come from different evolutionary gene pools, so breeders should avoid crosses with domestic horses, they advise. Przewalski’s horses and domestic horses have a different number of chromosomes (66 for the former, compared with 64); yet their offspring are fertile (with 65 chromosomes). The hybrids are viable because they differ only by a centric fusion translocation, also called a Robertsonian translocation. The process of pairing chromosomes during meiosis is not disrupted. Cross breeding should be a last resort, if too few Przewalski’s horses are available. Their analysis also suggests that, since diverging, Przewalski’s and domestic horses have both retained joint ancestral genes and swapped genes between populations. One of the two current major blood lines, the “Prague” line, is known to have a Mongol pony as one of its ancestors. The other primary line, the “Munich” line, is believed to be pure. However, because the two groups have historically mixed, keeping “pure” Przewalski’s horses from Przewalski’s horses with known domestic horse contributions might not be necessary, the authors write.

Additionally, a full sequencing of the mitochondrial genomes from the four surviving maternal lineages revealed surprising diversity: three mitochondrial haplotypes, a set of genes generally inherited as a single unit. Haplotypes I and II were very similar, whereas haplotype III stemmed from a division that is between 0.12 and 0.19 Myr old; this division is at least a hundred thousand years before horses were first domesticated.

“Przewalski’s horses have a lot more diversity than anyone was expecting,” said Makova. The Przewalski horses used in

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the study had a mean autosomal diversity of 0.195%, higher than the Thoroughbred horse sequence (0.05%). "The advice to breeders is that, in carefully selecting which individuals should be bred and which should be introduced to the wild, we can keep and maximize this diversity."

Inbreeding is one of the biggest threats to Przewalski's horses, reducing genetic diversity and exposing recessive, disease-causing alleles. Therefore, in selecting horses for reintroduction into a wild area, both primary haplotypes (I/II and III) should be represented, they advise.

Their analysis of the nuclear genome, which analyzed between 24.5 and 106.2 million bases for autosomes and from 0.8 to 5.6 million bases for the X chromosome, revealed a slightly different phylogenetic tree. Instead of two distinct Przewalski's haplotype groups, both branches of which shared mitochondrial DNA similarities with different domestic horse breeds, based on the nuclear genome, Przewalski's horses grouped into single-sourced clade. However, these results should be interpreted with caution, the authors write, as more complete follow-up studies are needed.

"This is a major advance over previous studies of Przewalski's horse genetics, and has quite likely solved the issue of its complicated relationship to domesticated horses," said Robert Fleischer, head of the Center for Conservation and Evolutionary Genetics at the Smithsonian Conservation Biology Institute.

Fleischer was not surprised, he said, that an analysis of mitochondrial DNA would yield a slightly different family tree than the nuclear genes. Mitochondrial DNA remains intact in matrilineal lineages, whereas autosomal DNA becomes crossed in hybridization.

As a next step, Makova would like to see the mitochondrial DNA of more domestic horse breeds characterized, currently only six are available. "It's possible that if 20 domestic horses more were added, we might find horses split even more ancestrally," she said.

An analysis of DNA from preserved Przewalski's specimens, dating before the bottleneck, would reveal if genetic diversity was once even higher, Makova said. And a tracing of the exact maternal haplotype of all captive Przewalski's horses would give zoos the power to make the most diverse breeding matches.

"I think we should build upon genomics studies like ours. It's not just an academic pursuit anymore. It can really change the fate of these animals long-term."

**Literature Cited**

Goto H, Ryder OA, Fisher AR, Schultz B, Pond SK, Nekrutenko A, Makova KD. 2011. A massively parallel sequencing approach uncovers ancient origins and high genetic variability of endangered Przewalski's horses. Genome Biol Evol. doi:10.1093/gbe/evr067.

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