CUSTOMER INTERVIEW

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Researchers use Illumina's Equine80k Array to reveal a surprising discovery about Kazakhstan horses

Genomic analysis showed that traditional Kazakh horses, long thought to be six distinct breeds, actually represent one single breed

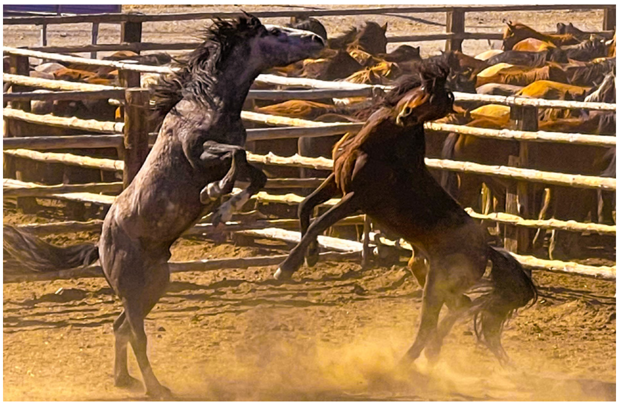

Photo *Shamekova Malika at the Institute of Plant Biology and Biotechnology and Zhengir Khan West-Kazakhstan Agrarian Technical University. Shamekova contributed this project by organizing the sample collection and phenotype description.*

Horses have played a vital role in the culture and economy of Kazakhstan for thousands of years. Historians have traced the first known use of horses in the country all the way back to the Bronze Age, and these animals are still central in Kazakh life today. They provide a source of milk and meat, serve as working and saddle horses, and are given as gifts at important events such as anniversaries. "The horse is the symbolic animal of our country, it is the most important animal in Kazakhstan," says Dr Dilyara Gritsenko, a research scientist at Institute of Plant Biology and Biotechnology in Kazakhstan.

Horse farmers and breeders have long thought that Kazakh horses represent six different breeds: three traditional types and three derivative breeds. Because of the cultural and economic importance of horses in Kazakhstan, Dr Dilyara Gritsenko, her colleague Mr Alexandr Pozharskiy, and their team at the Institute of Plant Biology and Biotechnology and Zhengir Khan West-Kazakhstan Agrarian Technical University decided to use genomic analysis to verify that the breeds were distinct.

They performed microarray-based SNP genotyping for over 2,000 Kazakh horses using Illumina's Equine80k array, which allowed the researchers to evaluate the animals' genetic structure. Their study, published in the journal Animal in September 2023, revealed that the six supposed breeds of Kazakh horses actually represent one single breed. "We just have one big population of horses that cross with each other," explains Dr Gritsenko. The team's findings have important implications for horse breeding and selection both in the Kazakhstan and internationally.

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iCommunity spoke with Dr Gritsenko and Mr Pozharskiy about their new study and findings, why they used the Illumina Equine80k array and how it helped them make the discovery, how farmers could use this research to improve livestock productivity, and what the future of livestock genomic selection in Kazakhstan might hold.

Q: What does the Institute of Plant Biology and Biotechnology do? What is the Institute's focus and mission?

Dilyara Gritsenko (DG): Our institute focuses on the investigation and research of plants and all other subject related to plants. We investigate different crops — their genetics and genomics, interactions with pathogens, the soil, and so on. Our institute has seven laboratories, and our lab focuses not only on crops and plants, but also animal research. We're currently investigating horse genomics and previously, we did research on honeybees, sheep, and camels. We look at the genetic parentage of animals as well as their population genetics.

Q: What are your thoughts on genomics adoption in Kazakhstan, specifically in the livestock space?

DG: The genomics of livestock is a newer area for Kazakhstan, it's just at the beginning. Local farmers currently only use phenotypic data to breed their animals, and they are trying to understand what they can get from genomics. They want to know how they can elevate their animals' productivity in terms of meat and milk, and as scientists, we try to explain the importance of genomics to them. The government needs to provide financial support in order to introduce genomics because it isn't cheap to do genomic analyses for animals. In near future, there will likely be more livestock genomics adoption in Kazakhstan.

> "We're currently investigating horse genomics and have used Illumina's Equine80k array to genotype over 2000 Kazakh horses."

Q: Why are horses important in Kazakhstan? What role do they play?

Alexandr Pozharskiy (AP): Horses have thousands of years of history in Kazakhstan. All nomadic peoples who inhabited this territory used horses for transport and as a source of food. This started from the Bronze Age — at least that is the first confirmed date. So in the history of Kazakhstan, horses have been a special animal in the local economy.

DG: Horses are a traditional source of meat and milk. Horse milk is fermented and made into what in the Kazakh language is called "kymyz". Kazakh nomads used to drink it because it was hard to access water. But horses are not only used for meat and milk production, they're part of the culture and history of Kazakhstan. And this connection continues up to today. We have a tradition where for important events, such as anniversaries and holidays, the most beautiful horse is given as a gift. It's a symbol of respect. The horse is the symbolic animal of our country, it is the most important animal in Kazakhstan.

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Q: What is the history of Kazakh horse breeding?

AP: The way Kazakh people use horses implies a very special way of breeding and maintaining the horses, unlike methods of Western breeding or in Arab countries. Here, breeders have traditionally had limited impact on how horses breed. Horses typically pasture mostly free in big herds. In some respects, their way of living is similar to wild horses, with only limited supervision by the owners.

DG: All our horses are like wild horses because they are free gazing and free mating. They are unsupervised crossings between different breeds, between different animals. Consequently, this situation leads to an admixture — it's like one big population of horses, without a specific breed. But farmers and breeders have always thought several different breeds of horses exist in Kazakhstan.

Q: So farmers and breeders thought there were three traditional breeds and three derivative breeds. How did they define them?

AP: The three traditional breeds are called Zhabe, Adai, and Naiman. They are usually described mainly by the regions where they're used, and they have some differences in endurance and stature. Some are more adapted to mountainous regions, whereas others are more universal and better fit to steppe pasturing.

The derivative breeds originated from more recent breeding programs that started at the beginning of the 20th century. For instance, Kushum horses were initially designed for use in the military, and later they were used for meat production.

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Q: Was your study the first time someone looked in-depth at genetic differences between these breeds?

AP: Yes. In collaboration with the West Kazakhstan University, we collected a total of about 2,000 of horse samples of six Kazakh breeds, including the three traditional breeds and three derivative breeds. For the first time, we used SNP genotyping with genome-wide coverage to describe the genetic composition and internal variability of these breeds.

Q: Why did you feel this research was important?

DG: One reason is because the horse is such an important traditional animal in Kazakhstan. Secondly, we knew that we have a good genetic pool for breeding because our horses are resistant to cold weather and they can get their food from the frost. These horses can survive under high stress condition. Understanding the genetics of these horses can help to improve other horse breeds all over the world. But first we needed to understand the genetics of our own horse populations here in Kazakhstan.

AP: Understanding the genetics of local horses is an important and necessary step, so that we can move towards more contemporary methods of breeding based on genomic data.

Q: What were your study methods and what exactly did you find?

AP: We used Illumina's SNP Equine80k array, which contains 80,000 markers. This is an updated and expanded version of the initial 50k array, which was the first SNP array developed for horses based on the Thoroughbred variety. Since then, this array has become one of the most used arrays in the field of horse genome studies. It is important because it allowed us to compare our data to previous data, since the same platform and microarray ensure the compatibility of data.

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Through genotyping, we found no significant differences in the genetic structure underlying populations and breeds of local horses in Kazakhstan. We concluded that Kazakh horses represent one big landrace with some regional variants. Those regional variants don't have enough genetic differences to be considered separate breeds. We were also able to identify some hybrid genotypes that resulted from mating Kazakh horses with the Thoroughbred horses in the twentieth century.

These hybrids had not been documented prior to genotyping, we discovered them during the study.

Compared to worldwide breeds, we found that local horses have a significantly higher level of individual variability — similar patterns to those we identified in data for horses from Mongolia and Tuva. We deduced that Mongolian and Tuvian horses have a close genetic relationship with Kazakh horses, which is not surprising considering the historical relationships between nomadic peoples of Mongolia, Kazakhstan, and Siberia. Perhaps our findings about how Kazakh horses differ from Western horses and others can be applied not only to Kazakh horses, but also to Mongolian, Tuvian, and other horses of the steppe region in Central Asia.

Q: Can you say more about why you chose Illumina's microarray genotyping solution over other methods?

AP: As I mentioned, this array contains about 80,000 SNP and indel markers. In our study, we limited our attention to SNPs. This allowed us to do a comparison with the available compatible data. In addition, the infrastructure of Illumina's platform is more widely available in our country than other alternatives.

DG: The reason we used Illumina's array is because they have just released the new Equine80k array, which includes more SNP markers compared to previous one. It was important to study more SNPs and gain a better understanding of the genetic structure of Kazakh horses' populations.

Q: How could the outcome of your research impact the future of Kazakh horses?

DG: I hope that our research helps farmers understand the important of genomics for their livelihoods. To obtain good traits, it's important to analyze the genetics of breeds. We want to prove to farmers that phenotypic data alone is not sufficient to increase productivity. I hope our research will help to improve the productivity of milk and meat of Kazakh horses, as well as serve as good reference for understanding Kazakh horses' traits.

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Q: What challenges still exist for genomics adoption in Kazakhstan?

AP: Local farmers and breeders have no background in genetics and still use traditional ways of selection; they struggle to understand how it would help them improve the breeding process. Moreover, Kazakh horse genomics have not been studied until recently — ours is the first genomic data and there isn't any other data available. Lastly, genomic technologies remain quite expensive today. Implementation of genomics in Kazakhstan has just started but it's still not economical to carry out at a large scale.

DG: Most institutes depend on financial support from the government. Not a lot of businesses are interested in such scientific research. But the situation is getting better.

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Q: Do you think genomic selection might eventually become mandatory for livestock breeding in Kazakhstan?

DG: More farmers are asking for livestock productivity prediction, so there is growing interest in genomics. I'm sure that in the next three to five years, analyzing the genomics of your livestock in Kazakhstan will be required.

Q: What is next for your research in this area?

DG: Our next step is whole genome analysis. We're just waiting for samples from farmers, and we will sequence all six of our traditional "breeds" — we will compare the diversity between their genomes. With the thousand Zhabe samples that we have, we will investigate both genotypic and phenotypic data and try to reveal new loci for meat productivity, disease, and climate resistance. We're also planning to perform whole genome sequencing for Kazakhstan's endemic honeybee species. Currently there is limited data regarding this species and we do not understand its impact on apiculture practices. This native pollinator is also essential for the reproductive success of wild flora, so it plays a pivotal role in maintaining ecological balance.

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