The Brave New World of Genomics

The sequencing of the Quarter Horse genome is the key to a new understanding of the biology of the horse in relation to disease *and* performance.

By Lindsay Day

With a single blood sample from a Quarter Horse mare named Sugar, researchers at Texas A&M University recently achieved an important milestone in equine genetics research. Earlier this year they published their report of the first complete sequencing of a Quarter Horse genome. Only one other horse to date bears such an honour – the Thoroughbred mare Twilight, whose DNA contributed to the first-ever mapping of a horse genome in 2007, two years after the completion of the human genome project.

"Sugar's genome represents all her DNA sequence," says Dr. Scott Dindot, Assistant Professor in the Department of Veterinary Pathobiology at Texas A&M University and lead researcher of the study. This genetic code, which she inherited in equal parts from both her parents, and which includes all of her roughly 21,000 genes, can be found tucked away inside the cell nucleus of just about all of the nearly trillion cells within Sugar's body.

Though biologically microscopic in size, when this DNA is sequenced so that it can be "read" by scientists, Sugar's genome translates to roughly 500 gigabytes of data – enough to max out the storage of a brand new Mac laptop. Seven years after the first map of a human genome was created, identifying and compiling all this data is still no insignificant feat. With today's "next-generation" technology, however, the task has certainly become less daunting. Whereas the first genomes took years and millions of dollars to sequence, Sugar's DNA was rendered readable in about a month.

"This technology has brought about a paradigm shift in genetics research," says Dindot. "In the age of genomics, we're not just interested in single genes, we're interested in the entire genome of an individual. In the past three or four years we've gone from looking at little pieces of the DNA to being able to look at the whole picture."

Genomic Variation

"All mammals share a surprising amount of genetic material," says Dr. Ernest Bailey, a researcher at the University of Kentucky's, Gluck Equine Research Center. "Different species do not have different sets of genes. Rather, it appears that minor differences in the way those genes are expressed and small variations in the DNA sequences result in the profound differences we see among animals."

A single blood sample from Sugar, a Quarter Horse mare, allowed researchers at Texas A&M University to achieve the first complete sequencing of a Quarter Horse genome. Sugar follows the Thoroughbred mare, Twilight, whose DNA contributed to the first-ever mapping of a horse genome in 2007, two years after the completion of the human genome project.

It's these sorts of minute differences in the DNA code that the Texas A&M researchers were particularly interested in looking at in their study. "We wanted to look at places where the sequence of the Quarter Horse genome varied from the sequence of the Thoroughbred," explains Dindot. "One of reasons we chose Sugar for the study is because her pedigree is heavily influenced by stock and racing Quarter Horses and does not have any breeding with Thoroughbred lines at least four generations back. She is very much a Quarter Horse and that was our intent."

While there was, as expected, much overlap between the genomes, some interesting differences were uncovered. "We found that much of the genetic variation was associated with genes that are involved in sensory perception," says Dindot. Though he can only speculate at this point, Dindot suggests that some of these differences may contribute to the characteristic behaviours associated with the breeds. "It's possible that the thoroughbred's more flighty disposition, for example, could be imbedded in the genes that are involved in taking in the senses from the environment."

Other variations in the DNA sequence were found in areas related to cellular processes and communication between cells. These sorts of variations might account for differences in metabolism and in how an animal responds to certain drugs. "These variations involve very diverse sorts of genes, so it's not possible to determine their effect with this type of study," explains Dindott. "But we do know, primarily from studies with mice and humans, that much of the variation in drug response between individuals has an underlying genetic component."

Genetic mutations and disease

In some instances, small variations in the DNA sequence of a particular gene can be responsible for causing disease. Referred to as genetic mutations, these changes in the genetic code affect the function of the associated gene, ultimately resulting the in the symptoms that are observed. In Quarter Horses, five such genetic diseases have been identified in the breed to date (see box for a complete list).

"Quarter Horses don't necessarily have any more genetic diseases than you'd expect to see in a breed of their size," notes Dr. James Mickelson an equine genetics researcher at the University of Minnesota. "We just happen to know more about

them, and I think a large part of that is because the American Quarter Horse Association has been willing to invest in the research."

Among the breed, the frequency of genetic disease will vary depending on which population of Quarter Horses you are looking at, adds Mickelson. "HYPP, for example, is well known to be in halter horses, where as HERDA is not as prevalent in that line, but is in the cutting horses." Barrel and racing Quarter Horses appear to be less at risk for these genetic diseases, in part, Mickelson suspects, due to breeding selection for a highly functioning athlete.

Racing Quarter Horses may also benefit from the influence of Thoroughbred bloodlines. One study that looked at 106 racing Quarter Horses in the United States found only the presence of the genetic mutation for PSSM in the group – two horses each had one copy of the defective gene.

In the case of PSSM, a muscle disorder that provokes tying up symptoms, one copy of the mutation is sufficient to confer susceptibility. "It will increase the horse's propensity to develop the active disease state," says Mickelson, "but other factors like nutrition and management, the level of exercise, and perhaps the rest of the genetic makeup of the horse, will also play a role."

Other genetic diseases require inheritance of two copies of the mutation (ie. one from each parent) to manifest. Sugar's genome, for example, revealed that she had one copy of the mutation for HERDA, a debilitating skin disease. Though she herself does not suffer from the condition, if she were bred to another carrier there would be a 25 percent chance that the foal would inherit two copies and would, therefore, develop the disease.

Genetic testing for these known mutations can, therefore, be a particularly valuable tool in making informed breeding decisions, or in the case of PSSM, to help confirm a diagnosis. For $125 USD (or less if you are a member), the AQHA offers a panel test for the five known mutations through the Veterinary Genetics Laboratory at the University of California-Davis.

Complex Diseases

In contrast to these single-gene disorders, many common diseases are going to be influenced by the interaction of a number of genes in combination, as well as environmental factors like nutrition and management, or exposure to certain hazards or risk factors. These sorts of conditions – such as osteoarthritis, recurrent airway obstruction, laminitis and muscle conditions like tying up disorders – pose a greater challenge to scientists when trying to unravel underlying genetic components.

"The extent to which a particular trait or disease process is influenced by inherited DNA is a question that has been around a long time," says Dr. James MacLeod, a researcher at the University of Kentucky's Gluck Equine Research Center. "There's pedigree analysis and controlled breeding experiments, but those studies were done decades ago. Now, with the advances in genomics technology, we can go back and re-examine these sorts of questions with much more powerful research methods."

Taking a genome-wide approach has enabled scientists to identify when there are correlations between particular variations in the DNA sequence and the presence of a disease or other trait of interest. "When you read in the headlines about the discovery of a new gene associated with addictive behaviour or a particular kind of cancer, or schnizophrenia, those reports are frequently based on this same type of study," says MacLeod.

In horses, the approach has been used to identify genes associated with different coat colours, performance traits, swayback (in Saddlebreds) and susceptibility to Equine Viral Arteritis infection, with a number of other studies underway. "These studies rarely tell us directly what the specific mutation or gene is, but they do usually narrow the region down and point us in the right direction," says MacLeod. "And that's something we couldn't have done with anything near the same level of efficiency three years ago."

Genetics of Performance

While training and management will always play a significant role in racing success, it has also long been accepted that there are underlying genetic factors that can affect a horse's athletic potential, says Emmeline Hill, a researcher at University College Dublin, and Director of Equinome Ltd, a company that provides genetic profiling for Thoroughbred racehorses in Ireland. "Every time a pedigree is consulted by a breeder it represents an attempt to capture and evaluate the genetic contributions from ancestors within that pedigree."

Given all the physiological factors that

Hereditary Equine Regional Dermal Asthenia (HERDA) is a severe skin disease, most often associated with cutting horses, that undermines the strength and durability of the skin. Two copies of the genetic mutation must be inherited, and with no effective treatments available, the majority of diagnosed horses are euthanized.

Hyperkalemic Periodic Paralysis (HYPP) is a muscle disorder that predisposes a horse to sporadic attacks of muscle tremors, weakness or paralysis. Tracing back to the American Quarter Horse sire Impressive, one copy of the genetic mutation is sufficient to cause the disease, while horses with two copies appear to be more severely affected.

Polysaccharide Storage Myopathy (PSSM) is a muscle disorder that causes an abnormal accumulation of polysaccharide and glycogen in the muscles, resulting in symptoms associated with tying-up such as muscle tremors, stiffness and reluctance to move. One copy of the mutation predisposes a horse to the condition, which can be managed through nutrition and an appropriate exercise regimen.

Glycogen Branching Enzyme Deficiency (GBED) is a fatal genetic defect that prevents effective glycogen storage such that it is not available in sufficient quantities for the muscles, heart and brain to function properly. Two copies of the mutation must be inherited, and will result in abortion, stillbirth or early death of the foal. It is estimated that approximately five percent of Quarter Horses are carriers of one copy of the mutation.

Malignant Hyperthermia (MH) is a condition observed in a number of species (including humans), which makes a carrier of a copy of the mutation susceptible to a potentially fatal rise in body temperature associated with abnormal muscle contractions and heart rhythm when undergoing anesthesia with halogenated anesthetics. The MH mutation – estimated to be present in less than one percent of the Quarter Horse population – can also worsen the symptoms of PSSM.

contribute to athletic performance, a whole spectrum of genetic variation is likely to be involved. “Any one performance trait may be influenced by hundreds or even thousands of genes that individually have a small effect, but together are important,” says Bailey.

“During the last century, animal scientists have been very successful in crafting selection strategies to make large improvements in production for cattle, sheep, pigs and chickens, even though they did not know anything about the genes that underlie the traits,” reports Bailey. “They did this using mathematics and an assumption that each trait is the result of hundreds of genes.”

“On the other hand, sometimes geneticists discover “major genes” – genes that have a huge impact, more so than the presumed hundreds of genes that each on their own may only have a small effect.

In horses, the genetic basis of performance is likely the product of lots of genes having a small effect on traits as well as a few "major genes with a big impact,” says Bailey, noting that when there is a major gene, it is likely to be highly selected and become common in a relatively short period of time.

So far, the research on genetic contributions to performance in horses has focused primarily on Thoroughbred racing, and there are currently a handful of companies worldwide that offer genetic profiling services within the industry. "We have seen an eager acceptance of genetic technologies as an additional tool for decision making,” reports Hill.

One gene in particular that has been quite extensively studied in Thoroughbred racehorses is the *myostatin gene*, one version of which is associated with an aptitude for short sprint racing, and another for endurance over longer distances. These alleles (versions of the gene) are present in Quarter Horses as well, who have been found, not surprisingly, to have a higher frequency of the sprinting version amongst the population. Egyptian Arabians, on the other hand, well known for their endurance capabilities, have a higher frequency of the endurance allele. Sugar’s genome, however, demonstrates that the association is not quite absolute: She instead managed to inherit two copies of the endurance version.

Gene Expression

Throughout her life, Sugar’s genome sequence will remain essentially unchanged. But the genes contained within it will function differently depending on which cells of her body they are in, her stage of life, her health and a likely myriad of other factors. “Genes are like a water faucet,” explains Dindot. “They can be turned on just a bit, or a lot, or turned off all the way.”

“One of the things we are learning is that the actual gene sequence is less important than timing when those genes are turned on and turned off,” says Bailey. In other words, it’s not just what DNA you’ve got, but how you use it.

"With the sequencing of the whole genome, we have a powerful tool to study and look at what’s actually going on inside the animal. So beyond the question of inheritance, genomics allows us to ask, for example, what happens if we feed or train the horse differently – what genes are turned on or off?"

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The question is so much more interesting and complicated than we previously thought, continues Bailey. “Of the nearly three-billion base pairs that are present in the genome, only about three percent of this DNA codes for proteins. The other 97 percent does other things – we suspect it probably plays a regulatory role, telling other parts of the genome what should be turned on or off, but we are still almost clueless as to how that works. That's one of the biggest questions in science right now – to figure out how these interactions work.”

It’s a question being explored in labs across the world. MacLeod’s work, for example is examining gene expression in cartilage during development and in joint disease. Bailey, an immunologist, is interested in understanding how pathogens like Equine Herpes Virus are able to get past the horse’s natural defenses. Others like Hill have been investigating gene expression in exercise physiology, while the field of “nutrigenomics” looks at how nutrition affects gene expression.

“The potential for the technology is a lot bigger than finding diagnostic tests for diseases or performance traits,” says Baiely. “Genomics gives us a key to better understand the biology of the horse in relation to disease and performance.”

The road ahead

By all accounts, this is an exciting time in genomics research. “We are really just learning how to harness the potential of this technology,” says Dindot. "Its becoming possible to re-sequence whole genomes, that’s certainly right in front of us, in human medicine and veterinary medicine as well, as the cost of sequencing has plummeted.”

As research continues to shed more light on the inner workings of this genetic code, new approaches in healthcare may be on the horizon. A better understanding of disease processes and infectious pathogens will contribute to the development of improved treatment and prevention strategies, while the anticipated ability to affordably sequence whole genomes may open up the possibility of a more customized health care approach tailored to, and informed by, an individual’s genetic make-up.

“This is really just a start for us,” says Dindot. “It’s a start to understanding the genetic variation that regulates performance traits in horses and to understanding the genetic variation that causes diseases in horses. And then as we go forward I suspect it will also be a tool that we will use to manage the health and well being of horses.

“It’s not science fiction anymore.” RQ

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