Zweig

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A report from the Harry M. Zweig Memorial Fund for Equine Research at the College of Veterinary Medicine at Cornell University

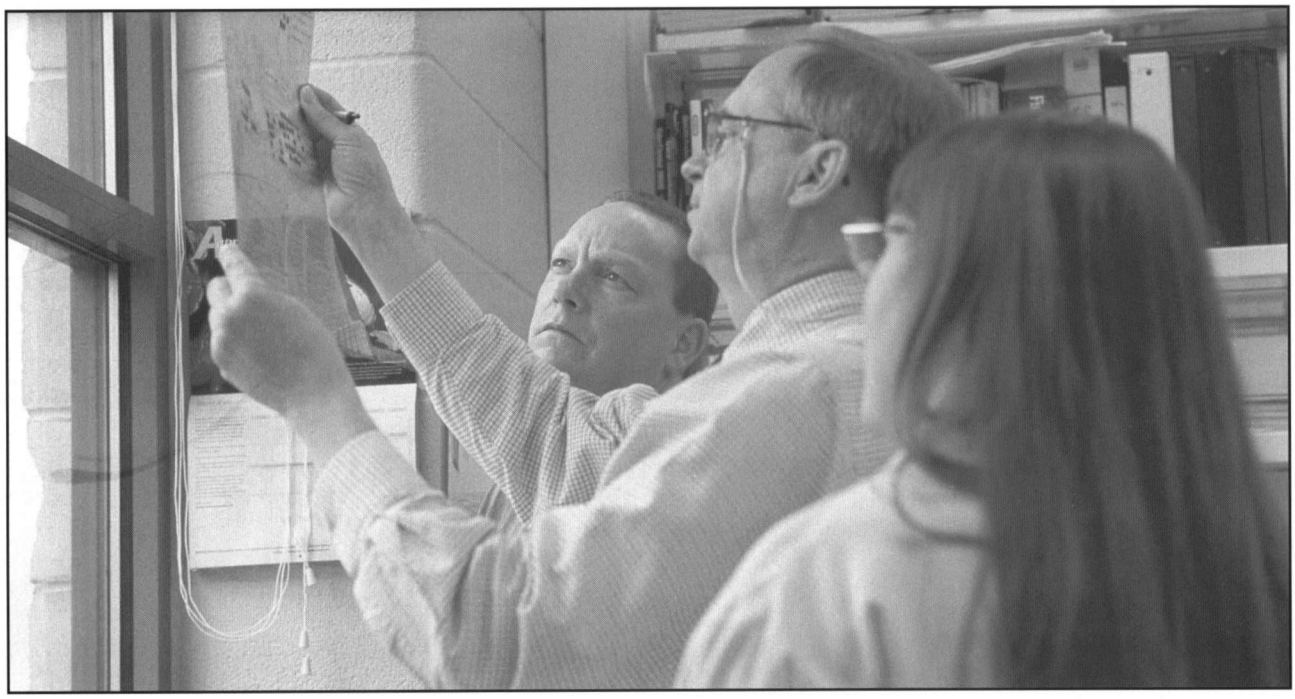

Photo credit: Dede Hatch

The Cornell component of the Horse Genome Project, led by Doug Antczak, is currently undertaking mapping and characterization of genes of the immune defense system. Results are used to develop new assays for immune function.

CORNELL

INSIDE

The Promise of the Horse Genome Project

Less than 10 years ago, horse geneticists from around the world agreed to work together toward a common goal: to map the horse genome. In a world that's usually very competitive, these researchers divvied up the job among their laboratories and came to the common understanding that they would share and integrate their data.

"We tried to parse the genome to a manageable size, and we've done it by breaking it down by chromosomes, of which there are 32 pairs in the horse," says Douglas Antczak, VMD, PhD, director of the Baker Institute for Animal Health. "Although each horse chromosome has about 100 million bits of information, it only contains about 1,000 genes, so it's a manageable number."

In 1995 only about 20 genes had been identified and mapped.

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Antczak

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"By understanding how multiple genes act together to control certain traits, we think the horse genome will allow us to identify disease-causing genes and determine which genes produce characteristics that are valued by horsemen."

—Douglas Antczak

"There was no understanding of the overall organization of the horse genome and no comparisons possible with gene maps of other species. But today, just eight years later, there are a number of linkage markers on each chromosome," says Antczak.

He goes on to explain: "If you think of a chromosome like an interstate highway, the linkage markers are like the exit signs. They're important for making our maps of the horse genome. There are over 500 mapped markers so far that indicate the order and placement of genes on chromosomes; they provide insights into which traits are inherited together. About 500 more are being characterized. We have mapped more than 300 genes on our physical map, with more being mapped weekly, and we've sequenced more than 3,000 genes, with more completed daily."

The researchers have also developed a comparative map, which compares the organization of genes in the human and horse chromosomes. The strong similarity the horse genome has with the genomes of other animals—particularly humans, cattle, and pigs—has fast-forwarded much of the equine genomics work. The project also has generated a comprehensive map that integrates the information from the other maps to create a unified view of the organization, complexity, and diversity of the equine genome.

The goal of this group of some 120 scientists, from laboratories in 12 countries, is to fill in as many dots as possible on the equine gene map. New technologies have made mapping and sequencing easier, more efficient, and less expensive, so the finer details are getting filled in at an ever faster pace. The final portrait of the genome map of the horse will provide keys to unlocking information on the genetic underpinnings for all kinds of equine traits and genetic predispositions. This information has broad application in diagnostics, breeding, physiology, performance, and the prevention and treatment of disease.

Applications of the Equine Gene Maps

Unlike most other scientific endeavors that don't have practical applications for decades after their initial work, the horse genome has already reaped benefits for equine scientists, clinicians, and horse breeders. Genetic tests are now commercially available for Severe Combined Immunodeficiency Disease (SCID) in Arabians, hyperkalemic periodic paralysis (HYPP) of quarter horses, and the Lethal White Syndrome in paint horses. But these genetics tests are just a peek at what's to come.

"Very rapid progress is expected in the coming years, and this progress holds promise to transform many aspects of equine medicine," Antczak says. "By understanding how multiple genes act together to control certain traits, we think the horse genome will allow us to identify disease-causing genes and determine which genes produce characteristics that are valued by horsemen.

"Unraveling the horse genome will not only improve breeding and performance but will also be used for a wide range of applications. Among these are: developing better vaccines for infectious diseases (such as West Nile virus), preventing cancers with a genetic predisposition (such as equine sarcoid), and developing gene therapies to help horses get back to racing after they've been injured or become arthritic."

Findings of the Horse Genome Project may lead to tests to diagnose diseases such as Chronic Obstructive Pulmonary Disease (COPD or heaves), wobbles, strangles, swayback, and other developmental bone and muscle diseases. They may also be used to identify the genetic code for traits ranging from coat color to muscle physiology. Also they may lead to strategies to prevent certain reproductive problems, such as Mare Reproductive Loss Syndrome. Too, genome data can be used to breed sounder horses by making it possible to screen stallions and mares for certain vulnerabilities such as bone and muscle weaknesses, allergies, exercise-induced pulmonary hemorrhage ("bleeding"), and other problems that prevent horses from performing at peak.

Researchers are already working on ways to identify the genes for a host of disorders, including cerebellar hypoplasia, megacolon disease, epitheliogenesis imperfecta, muscle diseases such as tying-up syndrome, and bone disorders.

"On a more global scale, there are the areas of nutritional genomics and physiological genomics of performance that can help us understand how to condition and train horses better so as to keep them sounder for longer," Antczak adds. "All of these medical advances mean more economical breeding, training, and maintenance of horses."

The equine gene maps also will be used in ways that are not closely tied to traditional genetic studies. Researchers can now test thousands of genes in a single experiment through the use of so-called "microarrays."

"These arrays will find use in almost every aspect of equine medical research: from bone and joint physiology through immunology to reproduction. Rather than concentrating on a single gene of interest, gene array experiments allow us to monitor very large numbers of genes at the same time," Antczak explains. "Such an approach can help determine how infectious agents affect many aspects of an animal's physiology—not only its immune system—and uncover unexpected activities of pharmacologic agents. For complex conditions that may be nonhereditary but involve gene expression (such as laminitis, developmental bone diseases, and colic), microarrays may

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hold the key to the development of new modalities of treatment through a more complete understanding of pathogenesis."

Although the genome maps may be only a sketch now, researchers are filling in more details on a weekly, sometimes daily, basis. The promise of the Horse Genome Project for those who care for and love horses is staggering.

"Its use has already begun, and its widespread application awaits only our imaginations," Antczak concludes.

The Horse Genome Project is linked to various web sites and two databases that hold the information on the equine genome. These web sites will be continually updated as more information on genome organization of the horse is made available. ■

Web Site Contents	Web Address
Horse Genome Project (HGP)	www.uky.edu/Ag/Horsemap/
HGP database (France)	http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl?BASE=horse
U-Cal Davis Equine Genetics Laboratory	www.vgl.ucdavis.edu/~lvmillon/default.htm#top
VetGen Corporation (commercial genetic testing company)	www.vetgen.com/
University of Kentucky Equine Genetics Service Laboratory	www.uky.edu/Agriculture/VetScience/EBT.HTM#DNA%20Testing

PROFILE

Dr. Lisa Fortier

Nicola Kountoupes/University Photography

The basic knowledge about cellular development that Lisa Fortier discovers is a first step in understanding the cause of arthritis in cartilage cells.

Lisa Ann Fortier, DVM, PhD, grew up on a farm in North Dakota with six brothers and sisters. Like many westerners, she participated in rodeo events, such as roping and barrel racing. Having first enrolled as a theater major at Moorhead State University, Fortier soon realized theater was not her future.

"I just couldn't stay out of the chemistry and biology classes," she recalls.

While human medicine didn't appeal to her, animal care did. She switched into a pre-veterinary program, then went on to earn her DVM at Colorado State University.

Although Fortier is now an equine surgeon, she never planned it that way. The path of her career, she says, has been continuously molded by the influence of great mentors. In veterinary school, for example, she was exposed to faculty members who were pioneering the latest advances in arthroscopic surgery. Instead of going immediately into private practice, as many of her classmates did, Fortier decided to pursue an equine internship at the Illinois Equine Clinic in Naperville, Illinois. While there she gained knowledge about the surgical management of diseases of racehorses from the extensive experiences of the veterinary staff.

Her lifelong interest in horses and cattle steered her toward equine surgery. "At that point, I knew Cornell had the best equine surgery program, but I wanted to go back west, not to New York," she recalls.

Fortier visited Cornell when applying for her surgical residency and never looked back. "The faculty at Cornell were clearly the most supportive and collegial of any that I encountered, and they were outstanding in equine surgery," Fortier says.

After becoming board certified in large animal surgery and completing her PhD at Cornell in veterinary science, Fortier worked for a year as a postdoctoral researcher. In 2000 she joined the faculty as an assistant professor of clinical sciences. Her appointment is 75 percent research.

Fortier's long-term research goal is to understand how cells develop from their embryonic stage to maturity and then go on to become diseased, so she can then apply that knowledge to determining the cause of arthritis in cartilage cells.

A first step is to establish and characterize a line of equine stem cells. This

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Fortier

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work has already been done for humans, some primates, and two strains of mice, but not for horses.

Fortier obtains her stem cells from embryos that are flushed from pregnant mares, just as they would be for embryo transfer. While research on human stem cells is strictly limited by federal regulations (because in the process the embryo is destroyed), there is no such restriction on the study of equine stem cells. Such cells are totipotent, that is, they can potentially be stimulated to develop into any kind of cell such as heart, lung, or nerve cells. Down the line, these custom-made tissues could be used to repair damaged ones.

In the meantime, an established line of equine stem cells would be invaluable to other researchers.

"Because these cells can be turned into any cell type in the body, they are particularly useful for researchers studying diseases for which tissue is difficult to obtain: placenta cells for early pregnancy/abortion studies or nerve cells for scientists trying to understand why nerves die and how they can be regenerated," she explains.

In the future, Fortier also expects to use the horse as a model for stem cell transplantation studies, which are not currently allowed in human beings.

Initially, Zweig funding supported her basic research for a year, which resulted in a career development award from the National Institutes of Health and a New Investigator Award from the Orthopaedic Research Society. A current Zweig grant supports the establishment of her initial line of equine embryonic stem cells.

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—Lisa Fortier

Fortier is the co-author on almost two dozen scientific journal articles, serves on the review boards of *Veterinary Surgery* and *The American Journal of Veterinary Research*, and speaks internationally about her research findings and surgical techniques. In addition, she teaches equine lameness and surgery to DVM students. Fortier is married to another equine surgeon, Alan Nixon. They have two daughters.

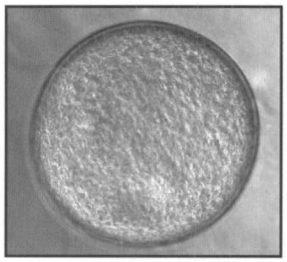

Photo credit: Lisa Fortier

Embryonic stem cells are derived from blastocysts, which develop about one week after fertilization of the egg. In this eight-day-old equine blastocyst, the totipotent stem cells are the lighter-appearing mass located at 6:00 on the image. All the other cells will become the placenta.

What about that longing to return to the West? "We're fairly embedded here," Fortier says. "There are a lot of smart people at Cornell who are putting it all together. As a scientist, the package is unbeatable." ■

Harry M. Zweig Memorial Fund for Equine Research—2003 Research Awards

New

$57,700 to Dorothy Ainsworth for "Airway Epithelial Cell Contributions to the Immunopathogenesis of Heaves—Preliminary Investigations"

$50,000 to Lisa Fortier for "The Establishment of Equine Embryonic Stem Cell Lines"

$54,750 to Nikolaus Osterrieder for "The Importance of Equine Herpesvirus Type I Glycoproteins for Replication, Virulence, and Vaccine Efficacy"

$16,314 to J. Brett Woodie and Brad Njaa for "The Role of Cecal Histamine in the Pathogenesis of Acute, Alimentary Laminitis"

$69,457 to Norm Ducharme and J. Brett Woodie for "Laryngeal Hemiplegia: Do We Have the Wrong Treatment?"

Renewal

$52,577 to Douglas Antczak for "Horse Genome Project: Functional Genomics of the Equine Immune System"

$55,000 to Alan Nixon for "Growth Factor Gene Enhanced Cartilage Repair Using Adeno-Associated Viral Vectors"

Inventors of the DDSP Collar File for a Patent

Testing of the Cornell DDSP Collar—an external collar that prevents dorsal displacement of the soft palate (DDSP)—has been so promising that its inventors, Norm G. Ducharme, DMV, MSc, Dipl ACVS, J. Brett Woodie, DVM, MS, Dipl ACVS, and Richard P. Hackett, DVM, MS, Dipl ACVS, have filed for a patent on the collar's design and use with the U.S. Patent Office.

DDSP, also known as choking down, is an upper airway problem common in racehorses and other types of performance horses. During exercise the palate shifts position and the horse can't breath sufficiently. The horse may open its mouth and slow down rapidly or stop to swallow. Sometimes, it may make a gurgling sound when the palate is displaced. Because DDSP disrupts airflow, horses with DDSP can't exercise adequately. The condition is a major problem for the racing industry.

For years, veterinarians have treated DDSP with surgery but it's only 60 percent effective. One day while exercising horses on a treadmill to evaluate how the larynx (voice box) contributes to DDSP, veterinary surgeons Ducharme and Woodie made an interesting observation.

"We found that we could prevent palate displacement by manually holding their voice box while they were running on the treadmill," Ducharme recalls.

With support from the Zweig Memorial Fund, they designed and tested a collar to perform the same function. Their research on the laryngohyoid stabilizer (LHS), commonly known as the Cornell DDSP Collar, was presented at the American College of Veterinary Surgeons meeting on October 10, 2003, in Washington, D.C.

"We think this collar could have widespread application for the racing industry, because nearly 90 percent of racehorses wear tongue-ties, at least partly, to stabilize the upper airway," says Ducharme. "This collar could be used widely during training and racing to prevent palate displacement. If the tongue-tie alternative collar is not approved for racing, the collar could be used to identify horses that will respond to surgery."

To test the collar's effects on airflow, ventilation, and its ability to prevent DDSP in exercising horses, Ducharme, Woodie, and Hackett evaluated ten adult horses in a two-phase study. In phase one, a control group of healthy horses exercised on a high-speed treadmill with and without the collar. In phase two, the surgeons performed a bilateral resection of the thyrohyoideus muscles (TH) to create exercise-induced DDSP. These horses were tested twice with and without the collar. The researchers used videoendoscopy, analyzed arterial blood gas samples, and analyzed static tracheal and pharyngeal pressures. In separate trials, they measured airflow and tracheal pressure. In each trial exercise, data were collected while the horses exercised on the treadmill at 75, 90, and 100 percent of maximum heart rate.

"From these measurements, we calculated if the airway was impeded," says Woodie. "While the collar had no effect on exercising horses with a normal upper airway, the LHS prevented the DDSP from occurring in 13 out of 14 experimental trials. In other words, the LHS, when placed in proper position, does not reduce the integrity of the upper airway and can prevent experimentally created exercise-induced DDSP."

The researchers now are field testing the collar on Thoroughbred racehorses at New York racetracks, thanks to funding from the New York Thoroughbred Horseman's Association, Inc. ■

"This collar could be used widely during training and racing to prevent palate displacement."

Norm Ducharme

Photo credit: J. Brett Woodie

The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state's equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State legislature created the Harry M. Zweig Memorial Fund to promote equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

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