

EQUINE

VOLUME XIII
Number 2
FALL 1999

RESEARCH & SERVICE REPORT

UNIVERSITY OF KENTUCKY COLLEGE OF AGRICULTURE

HORSE GENE MAPPING

Improving Research of Genetic & Health Traits

In the Spring 1996 issue of the *Equine Research and Service Report* we reported on the First International Equine Gene Mapping Workshop, which was held in October 1995 at the Maxwell H. Gluck Equine Research Center. In 1995, only seven genes had been mapped to chromosomes.

Today, four years later, the horse gene map has taken shape. The workshop participants recently published a manuscript identifying 161 mapped markers and they are preparing a second manuscript reporting 302 mapped markers.

"The success of this effort is due to the unselfish contributions and collaborations by many scientists from around the world," says Dr. Ernest Bailey, professor at the Gluck Equine Research Center and coordinator for the workshop. "With a 300-marker map, we are finally in a strong position to investigate genetic traits in horses."

The success is also due to independent research activities by scientists including Dr. Bailey and Dr. Teri Lear at the M.H. Gluck Equine Research Center and Drs. E. Gus Cothran and Kathryn Graves at the Equine Blood Typing and Research Laboratory.

The work of Drs. Bailey and Lear has focused on development of new genetic markers, mapping those markers to chromosomes and comparing the organization of the horse gene map to that of other species. Their graduate students are using the map to study health traits in horses.



Dr. Ernest Bailey and graduate student Michelle Mousel discuss the sequence for a gene.

Drs. Cothran and Graves and their students have helped map genetic markers, have begun studying diseases in horse families and also have led the way in studying the extent of genetic variation among diverse breeds of horses. With this information and the gene map in hand, the hereditary aspect of important horse diseases can be investigated.

What is a gene map?

Horses have 64 chromosomes—32 pairs of small bodies within the nucleus of cells that carry genes and are composed largely of DNA. All horses have the same number of chromosomes. The organization of these genes, for the

most part, is the same in all breeds of horses. The forms of the genes influencing certain traits differ from breed to breed.

In gene mapping, the chromosome location—or its relationship to another chromosome—is identified.

Having detailed genetic information, such as the map of all 32 pairs of chromosomes, will help researchers learn what genes influence the occurrence of equine diseases, as well as where a particular gene is located and what its DNA sequence is.

Approaching the questions in this manner has led researchers to use a new

GENE MAPPING continued on page 4

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**EQUINE RESEARCH
& SERVICE REPORT**

Produced by UKERF in co-operation with Agricultural Communications and UK Publishing Services.

Published twice a year on behalf of all equine researchers and veterinarians and the many others in the horse industry who are committed to continued improvements in equine research technology.

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Amerman Family Foundation to Fund New EPM Research

The Maxwell H. Gluck Equine Research Center has received a grant from the Amerman Family Foundation to fund a new area of Equine Protozoal Myeloencephalitis (EPM) Research.

The grant will allow Dr. Daniel K. Howe, the most recent member to join the Equine Research Center's faculty, to study the disease from a parasitology standpoint, looking closely at the protozoan pathogen *Sarcocystis neurona*, the primary cause of EPM.

What is EPM?

Equine protozoal myeloencephalitis (EPM) is a non-contagious infection of the horses's central nervous system that can cause a wide range of neurological problems.

Horses come in contact with the parasite that causes EPM through feed or water that has been contaminated by an infected animal.

The organisms then migrate through the body of the horse's nervous system, where they can cause a wide range of dysfunctional problems manifested by incoordination, lameness, difficulty swallowing or chewing, blindness and muscle weakness.

The Molecular Aspects of the Parasite

Macro- and micro-organisms, including humans, are made of up many different proteins. Although some proteins can be shared between different organisms, many are unique to a species or organism.

"What molecular studies allow you to do," Dr. Howe said, "is pick out those parasite-specific proteins, which will allow you to distinguish between two organisms."

Once these proteins are identified, they can be exploited to develop a diagnostic test for a particular parasite.

Sarcocystis neurona and *Toxoplasma gondii*, an AIDS-associated pathogen, are two closely related protozoan pathogens that share some of the same proteins. There are also other parasites that share proteins with and are closely related to *Sarcocystis neurona*. These closely related parasites, according to Dr. Howe, can mislead a person who is evaluating the result of a diagnostic test from an animal. The

"The initial emphasis of my research is going to be breaking the parasite down with a view to characterize proteins that are unique to it and those that are shared with other organisms."

evaluator may think the animal has been exposed to one parasite when it actually may have been exposed to a similar parasite that cannot cause EPM.

"These are problems which you can tackle by taking a molecular approach," Dr. Howe said. "The initial emphasis of my research is going to be breaking the parasite down with a view to characterize proteins that are unique to it and those that are shared with other organisms."

Questions to be addressed

Dr. Howe believes there are a lot of questions to be answered about EPM from what he calls a classical parasitologic standpoint. For instance, what other animals, along with the opossum, may serve as a host for the parasite in nature. Knowing this will help horse owners develop practices to prevent the disease.

"We know that the opossum is one of the primary definitive hosts, so people are actively trying to eliminate them from their farms," Dr. Howe said. "It would also help to know if there are other hosts so that horse owners adopt measures to minimize contact with those animal species as to better prevent the disease from occurring."

Dr. Howe also says that molecular studies are another area where research needs to be carried out.

"It's not something that circumvents normal classical work. It's just an additional field of study that really helps in development of improved diagnostics, drug treatments, and hopefully vaccines," Dr. Howe added.

Other areas of interest

Dr. Howe also hopes that molecular studies will help in other areas, not just in diagnostic testing for EPM. He is also interested in working with Dr. Tom Tobin to find new drugs to treat EPM.

"Obviously, there is a big need for new and improved drugs for treating EPM," Dr. Howe said. "Often you'll treat an animal, and it will seem to get better, and you'll think it's cured. Then you discontinue treatment and, after a period of time, it relapses and comes down with the disease again. It's not really understood why it relapses."

"One advantage of molecular research is that you can better identify how the drug kills the parasite—what the drug is attacking and acting on to kill the parasite. When you have the target of the drug, you can often make modifications to it to improve it, so that it's more effective."

Currently, Dr. Tobin is looking at the effects of treating horses for EPM. Dr. Howe said one of his interests would

AMERMAN FAMILY continued on page 6

GENE MAPPING continued from front page

term, "genome." A genome is the whole body of information about the location, organization, sequence and regulation of genes and chromosomes.

"Genomics is essential to future research success because scientists can conduct better experiments with this information in hand," says Bailey. "If we don't have the gene sequences for the horse, we will be limited in the science we can do in the twenty-first century. This will be a major tool for doing research in the future."

In the Beginning

In 1980 Dr. David Botstein, a geneticist concerned with hereditary diseases of humans, theorized that a human gene map could be made with a relatively small number of genetic markers. He noted that the genome is 3,000 centiMorgans long and if he had 150 evenly spaced markers, they would be 20 centiMorgans apart. A 20-centiMorgan map can be very effective for mapping genetic traits in families.

Suddenly the idea of a gene map became feasible, according to Dr. Bailey. "We expect that the horse genome contains 100,000 genes. Understanding the organization seems a daunting task. However, Botstein's premise suggested that we could make an effective gene map using a small number of markers."

But there were problems. Before the late 1980s, it was expensive and tedious to identify new genetic markers. Indeed, less than 30 genetic markers had been characterized for the horse at that time.

Furthermore, at least 300 markers would have to be developed and tested since randomly selected genetic markers would not necessarily be evenly spaced. However, the discovery and development of a new class of DNA markers, called microsatellite DNA, allowed scientists to quickly develop the requisite 300 markers and more.

The International Equine Genome Project

In October of 1995, 70 scientists from around the world participated in the first International Equine Gene Mapping Workshop in Lexington. From this workshop, approximately 25 laboratories signed on to participate in the Equine Genome Project.

In the project, the laboratories work together, sharing information from their research to help make a complete map of the horse. Each lab has taken on part of the work in developing the hundreds of DNA markers and acquiring DNA samples from horses to study inheritance.

Nine labs provided DNA from equine family members, 12 labs developed genetic markers to place on the map, and other labs worked on the physical mapping of the genes to chromosomes.

"This collaboration works well because we each need a good gene map to pursue our research," Dr. Bailey said. "Furthermore, each of us has a different genetic trait that we want to investigate, such as muscle diseases, diseases of bone, as well as infectious and allergic diseases.

"In effect, we have agreed not to compete on map construction, so that we can more quickly develop the map and move on to solving the problems that have resisted previous technologies," he said.

The first workshop map is in press, reporting 161 markers. The second publication, now in preparation, reports accomplishment of the 300-marker goal.

"It's particularly satisfying that our workshop has been successful in working together since October 1995. It's one thing to work in your laboratory where you control everything that goes on, but quite another to work with people from diverse institutions, all of them with different goals, pressures and constraints of their own. It's an exciting time to study the genetics of the horse."

GENE MAPPING continued on page 5



Dr. Kathryn Graves of the Equine Blood Typing and Research Laboratory, pictured with her Quarter Horse, Invest in Penny.



Dr. Gus Cothran of the University's Equine Blood Typing and Research Laboratory types the international reference families to help build the workshop linkage map.

GENE MAPPING continued from page 4

Application of the Gene Mapping to Health Problems

Many genetic studies are directed at identifying genetic markers for adverse traits. Once a marker is identified, then horse breeders can select against adverse traits.

Genetic tests are already available for severe combined immunodeficiency disease (SCID) and for hyperkalemic periodic paralysis (HYPP) in horses. The laboratories developing the gene map are in the forefront of this effort.

Dr. Cothran and one of his graduate students are interested in using the map to look at a particular skin disease in Saddlebred horses. Likewise, a group in Minnesota wants to look at muscle diseases in horses. Many laboratories have indicated an interest in investigating a developmental bone disease and allergic diseases.

In effect, each lab is developing and placing markers on the map, all the while keeping their specific research applications in mind.

However, using genetic markers for selection is probably less valuable than other applications. "Once gene sequences are identified, we can assay tissues to characterize gene expression," says Bailey. "We can use genetics to better understand the physiology, biochemistry and even early development of horses."

Cells from different tissues express different sets of genes. The genes expressed in each cell are characteristic of the cell function but may be modified by physiological, immunological or pharmacological stimulation.

One of the early steps in gene function is to transcribe the DNA into another molecule, RNA, that can be used to make protein. Scientists can assay the types and amounts of RNA in a cell to determine function. For example, if a horse is treated with a particular drug, researchers can sample the tissue to discover what genes are being

expressed and what the result of a certain drug would be.

Studies could also be done to show what genes are "up-regulated" or "down-regulated" by various vaccines or exposure to infectious agents. Knowing how gene expression can be affected may allow us to intervene to benefit the health of the horse.

Genomics at the Gluck Equine Research Center

Health research involves genetics to a greater extent than ever before. Strong programs in immunogenetics and cytogenetics have been developed at the Gluck Equine Research Center. These programs focus research on health and welfare of horses. This is due in part to the greatly improved genetic tools that have become available. Drs. Bailey and Lear train students and conduct horse research in the area of genetics at the Gluck Center.

Immunogenetics

During the past 5 years, development of the horse gene map has been a major priority. However, Dr. Bailey and his students have also been actively investigating the genes controlling the immune system. One of his students, Dr. Darrilyn Fraser, recently completed a doctorate, characterizing genes involved in the production of antibody responses.

Catherine Wagner is a new graduate student who is taking up where Dr. Fraser left off. One area of her project involves studying the impact of these genes on populations with endemic viral infections.

Another student, Michele Mousel, is interested in genes that may play a role in resistance to bacterial infections. Her project has begun by applying the information from the gene map to investigate different genes.

Bailey said, "Ten years ago we could not have considered pursuing these studies. The gene map and other technological developments make it

possible for us to pose and answer questions about genetics and diseases in horses."

Cytogenetics

Dr. Lear has established a strong program in cytogenetics at the Gluck Center. In connection with this program she investigates chromosomal abnormalities in horses as well as using molecular cytogenetics to study horse genomics.

One aspect of molecular cytogenetics involves cloning genes then using the



Dr. Teri Lear and graduate student Patrick Gallagher study chromosomal abnormalities in horses.

clones to determine exactly where they are on the chromosomes.

Molecular cytogenetics involves identifying a particular gene's location on a chromosome. Dr. Lear isolates horse genes then "hybridizes" them, allowing the genes to "stick" to their complementary DNA sequence on a chromosome, thus identifying the gene's location.

Dr. Lear has strong collaborative ties with scientists in Australia, Texas, France, and elsewhere to help map genes of mutual interest.

Patrick Gallagher is a graduate student investigating repetitive ele-

GENE MAPPING continued on page 6

GENE MAPPING continued from page 5

ments of DNA which may play a role in chromosome breakage and rearrangement. This program has also been of increasing interest to younger students. During the past two years, students from the Dunbar High School (Lexington, KY) Math and Science program have worked on projects with Dr. Lear.

Horse genes can also be hybridized to other exotic species, including donkeys and zebras. "Comparing the genetics of the different equids is very interesting to me, because each of the equid species has a different number and arrangement of chromosomes. There's been a lot of change and rearranging of chromosomes during evolution," Dr. Lear said.

"Comparing exotic equids with the domestic horse is valuable. Donkey and zebra species react differently to disease when compared to the domestic horse," says Dr. Lear. "The differences are, by definition, genetic. Identifying those differences will ultimately help researchers improve on treatments for diseases of domestic horses. Comparing the genome organization is an important first step."

Genomics and the Equine Blood Typing and Research Laboratory

Drs. Cothran and Graves are primarily responsible for providing parentage testing for horses at the University of Kentucky Equine Blood Typing and Research Laboratory. This laboratory is one of the three divisions of the Department of Veterinary Science. While providing this valuable service to the horse industry, they also conduct research in the area of horse genomics.

Drs. Cothran and Graves have been involved in the workshop activity from the beginning by typing the international references families to build the workshop linkage map. In addition they developed another mapping method called synteny mapping to identify the relationships of chromosomes among DNA markers. This work led to a Master of Science degree for graduate student Nancy Raney, who

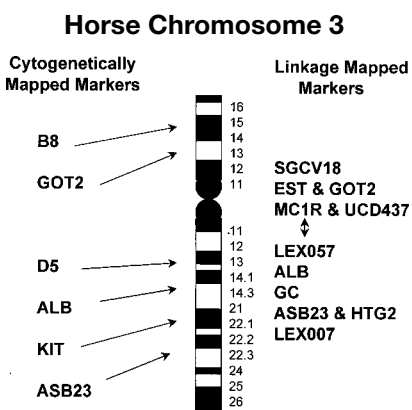
currently works in a genetics laboratory at Michigan State University.

More recently scientists in the laboratory have begun identifying new DNA markers based on genes expressed in skin tissue.

One of their students, Louis Lieto, is developing new genetic markers called expressed sequence tagged sites (ESTs)—pieces of the sequence of genes that are expressed in specific tissues and can serve as markers—and the laboratory is hunting for a single nucleotide polymorphisms (SNPs)—single sites in the sequence of DNA that vary among individuals. This work may help identify a gene mutation causing a skin disease in Saddlebred horses called epitheliogenesis imperfecta.

Yet another student, Rebecca Terry, is interested in the genetics of coat color. The genetics of Appaloosa appear similar to a coat color system already identified in mice. Terry is using the horse gene map to study homologous genes in the horse and determine whether or not they are involved in the production of Appaloosa horse color.

These activities simultaneously use the existing genomic information and also expand that knowledge. The principal responsibility of the laboratory is service, but, as the name implies, part of that service is realized through research.



In 1995, only 7 genes had been mapped to chromosomes and only a handful of linkage groups identified. Today over 300 genetic markers have been mapped to horse chromosomes. This figure identifies the 14 genes that have been mapped to horse chromosome 3 through the efforts of scientists at the University of Kentucky and elsewhere. The acronyms reflect the gene name (ALB stands for albumin) or the laboratory that identified the marker (LEX for Lexington, Kentucky).

AMERMAN FAMILY continued from page 3

be taking existing drugs and using them on parasites in the lab to try to figure out what the actual target of the drug is.

"The more you find out about how the drug is killing the parasite, the more you can improve on the drug," Dr. Howe added. "Finding how a drug kills an organism often tells you something about the basic biology of the organism—how it goes through life, how it's different from other bacteria, protozoa, plants and animals, and how they've evolved."

The Holy Grail

The ultimate goal for most researchers of any disease is to develop an effective and safe vaccine, so that an animal could be protected against ever coming down with the disease.

And such is the case with EPM as well.

"A vaccine is kind of the Holy Grail," Dr. Howe said. "The old saying 'an ounce of prevention is worth a pound of cure' is quite true. If you could prevent horses from ever getting the disease, that would be fantastic.

"Even when you come up with a superior treatment, often the damage has been done. If you can completely prevent the disease, that's of much greater advantage. Development of a vaccine is probably the most difficult task that lies ahead. It's tough to develop a good vaccine for a protozoan parasite."

Still, Dr. Howe thinks it's within his reach.

"It's going to be hard, but it's not impossible."

Parasitology— and Howe

When Dr. Daniel Howe decided to become a parasitologist, he wasn't exactly thinking about working with horses.

But he is, and it's an experience he's enjoying.

"Although I hadn't had any ideas of working on equine diseases, EPM is a natural fit for what I had been doing previously, because it's a related organism," Dr. Howe said.

Dr. Howe is one of the newest researchers at the University of Kentucky's Maxwell H. Gluck Research Center. A parasitologist, Dr. Howe's work will focus on molecular aspects of the parasites that can cause Equine Protozoal Myeloencephalitis (EPM).

"Dr. Howe comes to this program with excellent credentials in the field of molecular work on protozoan parasitic infections," said Dr. Peter J. Timoney, director of the Gluck Research Center.

"He has extensive experience in working with parasites closely related to that primarily involved in causing EPM, and this should help him greatly in his research on the biology of the parasite and hopefully to the development of improved diagnostics and a safe and effective vaccine against this economically signifi-



Dr. Daniel Howe primarily studies equine protozoal myeloencephalitis (EPM) from a parasitology standpoint.

cant disease," Timoney said.

After receiving his Ph.D. from Purdue University in West Lafayette, Indiana, Dr. Howe went on to Washington University Medical School in St. Louis for a postdoctoral fellowship where he started working on *Toxoplasma gondii*, a protozoan parasite which is associated with congenital disease in human neonates.

"When AIDS came about, it (*Toxoplasma gondii*) was found much

more often as one of the opportunistic infections that infected or caused disease in AIDS patients, including encephalitis," Dr. Howe said.

After working on *Toxoplasma gondii* for close to six years, Dr. Howe became interested in a closely related parasite, *Neospora caninum*. "*Toxoplasma gondii* and *Neospora caninum* are almost like sibling parasites," Dr. Howe said. "They're that closely related and similar to each other."

Enter EPM, which is primarily caused by the protozoan *Sarcocystis neurona*, another "relative" of *Toxoplasma gondii* and *Neospora caninum*.

"In a research lab, you're often somewhat disconnected from the clinical side," Dr. Howe said of his previous work. "We were in a medical school, but we were strictly a research lab. This is an interesting and unique situation for me, where I am much more closely associated with the people who actually have to deal with the disease.

"That's nice," Dr. Howe added, "because it makes you realize what you're actually doing the work for. It's not just for your own interest. Now you see how you can help people."

Dr. Howe is enjoying his new home in Lexington with his wife, Amy, and sons Wesley (10), Austin (5), and Zachary (4).

Lloyd's continues Support for "Equine Disease Quarterly"

Nick Strong (left), President of Lloyd's Kentucky Agents, and Julian Lloyd (center), chairman of Lloyd's Livestock Committee, present a check from Lloyd's of London Underwriters, Brokers, and their Kentucky Agents, to Dr. Peter J. Timoney, Director of the Maxwell H. Gluck Equine Research Center, for continued support of the Equine Disease Quarterly. Lloyd's has supported the publication since it was first published in 1992.



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