New heights of inquiry: Untangling roaring’s genomic origins

By Lauren Cahoon Roberts

Thanks in part to Zweig funding, Cornell veterinarian and scientist Dr. Dorothy Ainsworth, professor of large animal medicine, and a team of collaborators have begun to unveil the genomic underpinnings of recurrent laryngeal neuropathy (RLN), or roaring.

Roaring is a common and economically important cause of exercise intolerance, and tends to occur more frequently in taller horses. The left side of a horse’s laryngeal muscles become ‘sluggish’ due to the degeneration of the nerve controlling those muscles, causing issues with breathing during exercise. Treatments usually involve a surgery that ‘ties back’ the ineffective muscle, or more experimental techniques to re-innervate the atrophying larynx (see “Rethinking roaring treatment” on page 4).

As a veterinary internist, Ainsworth knew the clinical aspects of the disease very well, including the anecdotal evidence that taller horses were usually more likely to suffer from roaring. However, very little was known about why this was the case. This mystery led her to collaborate with Michigan State University scientists Dr. Ed Robinson and Ms. Ashley Braman, and Cornell geneticists Dr. Adam Boyko and Dr. Samantha Brooks (now at the University of Florida), to learn more about the disease on a genetic level. They first looked at the disease in Thoroughbreds, which have a prevalence of roughly 11 percent of RLN within the breed.

Thanks to help from numerous equine practitioners, horse owners/trainers and scientists at the Cornell Veterinary Biobank (Drs. Rory Todhunter, Marta Castelhano and Ms. Liz Corey), Cornell Biotechnology Resource Center (Dr. Peter Schweitzer), and GeneSeek (Dr. Jeremy Walker), the team analyzed over 500 samples of Thoroughbred DNA in a genome-wide association study (GWAS) to determine what genomic factors might be at play in the condition — and to tease out how horses’ heights were involved. In a study published in BMC Genomics in 2014, the group reported that the two traits were strongly aligned in the genetic code — each associated with a single locus on chromosome three (ECA3). This association could mean several things, Ainsworth explained. One explanation could be that the genes cause a horse to be tall, thus making their laryngeal nerves extra-long and potentially (but not inevitably) more vulnerable to wear and tear. A second explanation is that the genes on ECA3 not only code for growth but allow for the transcription of other genes affecting diverse physiological functions, including nerve function. A third explanation could be that there are two separate genes on the ECA3 locus that independently underwent mutations (affecting height and nerve function) but have been ‘linked’ due to the limited genetic diversity in Thoroughbreds.

To further investigate the relationship between height and RLN, and at the suggestion of Cornell colleague Dr. Rory
Todhunter, the team opted to examine another breed that frequently suffers from RLN — the American Belgian draft horse, which experiences a prevalence of roaring as high as 40 percent.

With help from Jean Young, an LVT at the college, Ainsworth and a second team (her collaborators at MSU Drs. Robinson, Stick, Ms. Braman), independently traveled around the Northeast and Midwest conducting laryngeal endoscopic examinations, measuring wither heights and collecting blood samples from over 500 American Belgian draft horses. They published their results in the journal Physiological Genomics in fall of 2018. “Just like with the Thoroughbreds, we found that roaring was associated with a specific chromosome,” said Ainsworth. Yet in this breed, it was chromosome 15, not chromosome three. “The association of RLN with the genetic marker on ECA15 was strongest for males, suggesting that estrogen may have a protective effect on the associated candidate gene function,” Ainsworth explained. “However, this is only a hypothesis that requires further investigation.”

To complicate matters further, when the team conducted a GWAS for height in the American Belgian draft horses, they did not see a neat one-to-one genotypic correlation to roaring as they had in Thoroughbreds (even though taller draft horses are more likely to have RLN). Instead, a strong association pattern emerged on an entirely different chromosome — chromosome one. “This indicates that, for American Belgian draft horses, a growth trait correlated with height, but not necessarily absolute height itself, increases the risk of roaring,” said Ainsworth.

As the genetic plot thickens for roaring, Ainsworth would like additional investigations of chromosome 15 in draft horses to be conducted: Perhaps a custom-designed genetic array would allow investigators to further tease out possible associations in the genetic code, and pave the way to more clarity on the multi-layered link of equine height and roaring.

So, does breeding horses to be tall condemn them to a fate of roaring? Not necessarily, said Ainsworth. “RLN is a complex genetic trait, and the genomic analysis only explains 45 percent of the variation in the RLN type in Thoroughbreds, and only 26 percent in the American Belgian Draft Horse.” Clearly, despite genetic predisposition, environmental factors also play a role — but what those factors are remains to be determined. ■
As an equine surgeon, Michelle Delco ’98, D.V.M. ’03, Ph.D. ’16, frequently treats horses with arthritis and fractures that go into the joint. As a researcher, she hopes to figure out how to help her patients much sooner. “There are many reasons why horses break down on the track,” said Delco, an assistant research professor in the Department of Clinical Sciences, “but in many cases, the first domino to fall is probably a low-grade cartilage injury.”

Joints that have suffered even mild damage often develop arthritis, but it can take months, years, sometimes decades for the first, painful symptoms to show up. At that point, the joint damage is irreversible. Currently no replacement exists for healthy cartilage, which cushions the ends of bones and ensures frictionless joint movement. When cartilage is lost, the underlying bone is exposed to repeated microtrauma and may become brittle and ripe for fracture.

“So my motivation is to try to find out why we get ongoing tissue damage after a joint injury,” Delco explained. “What can we do to prevent and treat it before it’s too late?” The answers, she believes, lie in the mitochondria.

Often referred to as “the powerhouses of the cell,” mitochondria evolved from bacteria that lived symbiotically in other organisms, converting nutrients and oxygen into energy for normal tissue functioning and repair. “I totally geek out when I talk about mitochondria, because they’re just amazing little organelles,” Delco said. “They even have their own genome, which is separate and different from the DNA in our chromosomes.”

This unique attribute, however, can cause problems when mitochondria dysfunction and release mitochondrial damage-associated molecular patterns (mDAMPs). The body’s immune system interprets mitochondrial DNA as a foreign invader and fights back, creating inflammation and ongoing tissue damage.

While this process is known to take place in other tissues, Delco’s research on mDAMPs in cartilage is new. The concept stems from her previous work. “We know that mitochondria in cartilage cells dysfunction when you already have arthritis, but we were the first to look at mitochondria immediately after a joint injury,” she said. These earlier studies showed that impact injury to live cartilage causes chondrocytes (cartilage cells) to undergo mitochondrial dysfunction. The next piece of the puzzle is to determine how this cell dysfunction turns into a joint-wide problem with self-perpetuating tissue damage — that’s where mDAMPs come in.

Delco is running four experiments to assess the role of mDAMPs in early joint injury and arthritis: First, she is stressing chondrocytes on a dish with chemicals that cause mitochondria to fail in different ways. “I’ll see what makes them spit out mitochondrial DNA, which will tell me some of the mechanisms going on inside the cell,” she explained. Zooming out, another study will determine if mitochondrial dysfunction caused by mechanical injury to chunks of cartilage results in mDAMP release. Delco hopes these studies will reveal the most sensitive indicators of mitochondrial dysfunction and early cartilage injury.

To gauge the relationship between cartilage injury and mDAMPs in live animals, Delco is drawing on joint fluid samples collected a few years ago for her doctoral work on experimental high-speed injuries to articular cartilage in horses. Finally, she plans to look at mDAMPs in samples collected from horses being treated for naturally occurring joint injuries.

Ultimately, Delco hopes her research will help turn mDAMPs in joint fluid into a useful biomarker — a non-invasive diagnostic and prognostic tool after joint injury. “If mDAMPs are signaling ongoing inflammation and tissue damage, acting as the fuel of arthritis, can we figure out how to cut this off at the pass?” Delco said. “If we can understand some of these early links in the chain, it gives us new ways to intervene — not only in horses but also in humans.”
Jonathan Cheetham, Ph.D. ‘08, associate professor of large animal surgery, is pushing the boundaries of roaring therapy thanks to the Zweig Foundation.

His research focuses on accelerating the recovery of laryngeal nerve grafts — a procedure used to treat roaring, a major cause of poor athletic performance in race and sport horses. The work may also benefit human patients as well.

“The treatment for recurrent laryngeal neuropathy in horses has remained unchanged for decades with minimal refinements,” says Cheetham. “This proposal allows us to transform the way we treat these cases, improve the recovery time, and will help avoid common complications.”

**Touching a nerve**

Roaring, also known as recurrent laryngeal neuropathy (RLN) causes a gradual degradation of the nerves that control the airway muscle known as the cricoarytenoid dorsalis, or CAD.

Clinicians have treated this issue by placing a permanent laryngoplast suture (a “tie-back”) that holds the larynx open — or they attempt to restore function by grafting functioning nerves into the denervated airway muscles.

“This method only innervates a small portion of the CAD muscle,” says Cheetham, “so recovery is slow and limited, and the technique has not yet gained widespread acceptance.”

Cheetham wants to change that rate of recovery through a new technique. They would adopt the nerve graft approach, but instead of innervating one portion of the atrophied muscle, he would graft onto the pre-existing nerve root so that it would reach all portions of the CAD muscle.

“This change alone should produce a much faster recovery than previous nerve-grafting approaches,” says Cheetham.

**Tuning up the healing process**

In addition to this new surgical process, Cheetham wants to pair the technique with the application of immune cells that can fight inflammation and speed healing (known as immunomodulation).

“Over the last three years, thanks to support from the Harry M. Zweig Memorial Fund and the National Institutes of Health, we have begun to understand the basic mechanisms behind the role of a particular type of immune cell — the macrophage — in peripheral nerve repair,” Cheetham explains.

“These cells are the major cell type migrating to the repair site and are the ‘conductors of the orchestra,’ laying down tiny capillary networks along which other cell types can migrate.”

Cheetham’s team developed a sophisticated technique to
isolate macrophages from the site of peripheral nerve injury.

Using this technique, they evaluated how genes expressed by these macrophages change over time after injury and how genes that control the types of macrophages at the injury site affect repair after nerve graft.

“We have also shown that these cells change when there is a delay between injury and nerve graft, leading to modification of the microenvironment at the injury site and decreased recovery,” Cheetham says. “Fortunately, we’ve found that these effects can be reversed using a small molecule, the cytokine interleukin-10, that reduces inflammation, and that this reversal leads to improved recovery.”

In his latest efforts with this line of inquiry, Cheetham wants to change the type of macrophages at the site of the nerve graft to improve healing by applying a stable hydrogel containing interleukin-10, which will alter the type of macrophages present at the injury site.

“We hypothesize that by manipulating the microenvironment at the site of nerve graft and changing the function of macrophages, this will allow re-growing nerve axons to cross the repair site more rapidly and functional recovery will be faster and better,” Cheetham says.

**Benefits on the horizon**

Cheetham’s research team has already tested and proven this approach in mice, rats and dogs. With his latest Zweig grant Cheetham will now test this hypothesis in horses. His hope is that this new approach will result in a quicker healing time.

If successful, this equine therapy could also eventually benefit human patients who suffer from peripheral nerve injury. “The ability to alter the type of macrophages at the injury site and improve recovery would be of great benefit to these patients,” Cheetham says.
Cornell scientists help reduce racetrack fatalities
By Olivia Hall

New York’s four Thoroughbred racetracks (Aqueduct, Belmont, Saratoga and Finger Lakes) provide not only popular entertainment, but – with an estimated impact of $4.2 billion per year — an important economic engine to the state. Equine fatal injuries, however, put a damper on the sport’s finances and reputation and threaten its very existence.

For the past five years, scientists at the Cornell University College of Veterinary Medicine have partnered with the New York State Gaming Commission (NYSGC) to tackle this issue, a top priority shared by the racing industry. The researchers — the core team consists of associate professor Sean McDonough; assistant professor Heidi Reesink, Ph.D. ’16; professor Hussni Mohammed; adjunct professor and NYSGC Equine Medical Director Scott Palmer, Ph.D. ’08; as well as regulatory racetrack veterinarians — have conducted three Zweig-funded, complementary epidemiological studies to identify the types of prior injuries, changes to bone structure and other factors that increase the risk for catastrophic breakdowns. “Identifying horses at increased risk for catastrophic injury before it happens provides an opportunity for intervention,” said Palmer.

The Cornell-NYSGC collaboration began after a well-publicized rash of 21 fatalities at the Aqueduct Racetrack’s 2011-2012 winter meet prompted the Governor’s Office to appoint the New York Task Force on Racehorse Health and Safety. Under Palmer’s leadership, the Task Force recommended the creation of a mandatory necropsy program, which brought all horses that died at New York racetracks to Cornell’s Animal Health Diagnostic Center (AHDC) for a postmortem examination. For the past five years, this program has documented the immediate cause of death as well as other suspected predisposing factors.

Fetlock breakdown injuries emerged as the most common cause of New York equine exercise fatalities, especially biaxial proximal sesamoid bone (BPSB) fractures. Fracture of both of these two tiny, triangular bones at the back of the fetlock causes a mechanical disruption of the joint and generally requires the horse to be humanely euthanized, due to the extensive degree of both bone and soft tissue injury.

“One of the most significant pathologic findings of this study was subchondral bone sclerosis associated with many of the fetlock fracture cases,” Palmer said. Making trainers and veterinarians aware of the risk of training horses with this pathology in the joint was a first step toward improving fatality rates, though identifying associated clinical signs and early radiographic changes remains a challenge.

Working with computer scientists at The Jockey Club, the researchers went on to develop an algorithm to tease out links between the exercise histories of horses with BPSB fractures and specific exercise protocols or other confounding factors. “With this information in hand, we were able to begin to identify horses at increased risk for this most common type of fatal musculoskeletal injury, such as horses that experienced an unusually high amount of high-speed training in the interval between their first official recorded workout and their first race,” Palmer said.

A parallel research effort has been taking a closer look at the gross and histologic anatomy of the proximal sesamoid bones (PSB) of horses that have experienced PSB fractures. So far, the bones’ size and shape appear to play a significant role in their propensity to fracture, furthered by the presence of osteoarthritic changes. PSB bone volume fraction is also influenced by horses’ exercise regimens.

With multiple pre-existing conditions contributing to fatal injuries, the pre-race inspection of Thoroughbred racehorses by regulatory veterinarians prior to competition emerges as a critical tool for mitigating risk. In a third
study comparing the pre-race inspections of 90 horses that experienced fatal BPSB fractures with those of 180 control animals, horses with abnormal clinical findings of the fetlock joint and gait abnormalities were not only more likely to suffer a BPSB fracture, but the probability increased with the number of abnormalities recorded.

Palmer and his colleagues are pleased with the positive impact their close collaboration with the NYSGC has had on the racing industry. Their research findings have made their way directly into regulatory policy and have provided content for a required continuing education program for horse trainers. “Racing fatalities are no longer considered to be the inevitable result of a ‘bad step’; rather they are subject to intervention,” Palmer said.

But Palmer and his colleagues aren’t stopping there. In future studies, they plan to define a “healthy horse” training profile for Thoroughbreds — comparing exercise regimens for fatally injured and uninjured horses — to provide additional objective criteria to more accurately identify horses at increased risk for catastrophic injury and help provide trainers with guidelines that will reduce that number even further.

Thanks to these efforts, combined with implementation of many other Task Force recommendations, the number of Thoroughbred fatalities at New York racetracks has fallen by 42 percent since the fateful Aqueduct winter meet, bringing the state’s losses in line with or below the national average.

To mark the 40th anniversary of the Zweig Fund and its partnership with Cornell University, we have many events planned for late 2019, including research presentations by faculty supported by the fund, lectures on other equine-related research topics and a reception to commemorate the event.

**Wednesday, November 13, 2019 | Cornell University College of Veterinary Medicine | Ithaca, New York 14853**

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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 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.
Welcome to our new Zweig Committee member

Louis M. Jacobs

Jacobs, who holds both a bachelor’s degree and master’s degree in business administration from Harvard University, is Co-Chief Executive Officer of Delaware North, a family-owned, global leader in hospitality and food service. Delaware North has annual revenue of $3 billion and more than 55,000 employees, and it serves half a billion customers annually on four continents.

Jacobs has worked for Delaware North for over thirty years, having served as executive vice president and president of Delaware North Companies International, and chairman of the Australian division. His initial focus was on Delaware North’s pari-mutuel and gaming operations, with responsibilities for operations, business strategy and expansion of the company’s pari-mutuel holdings.

Jacobs has long been active in equestrian sports and has represented the United States in several international events. He currently serves as vice president of the board of directors for the National Horse Show, as well as vice chairman of the board of directors for the Lake Placid Horse Show Association. He is a member of the board of directors for the Hampton Classic Horse Show and the United States Equestrian Team Foundation. He is also a member of the finance committees for the United States Hunter Jumper Association and its charitable foundation.

Our site provides information on the projects and publications resulting from the Zweig Memorial Fund, and demonstrates the objectives of the Fund in promoting equine health in the racing industry. The Zweig News Capsule is published twice a year, and can be downloaded at bit.ly/ZweigNews. Please encourage other equine enthusiasts to visit the site.

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