Soothing music in the barn not only promotes horse welfare, but may even improve racing performance, according to a new study.

Anna Stachurska and colleagues at the University of Life Sciences in Lublin, Poland, have been examining the effect of music on horse welfare and racing performance.

The study, which extended over two years, looked at 3-year-old Purebred Arabian horses in their first race season. Each year the horses were divided at random into two groups. The experimental group were housed in a barn at the Race Course in Warsaw-Służewiec, where they were exposed to specially composed music for 5 hours a day each afternoon. The control group were housed in a separate barn with no music.

Ten different music compositions, described as movie-like soundtracks of New Age genre, played on a ten-string Guitar, were used.

The horses’ response to the music was assessed at rest, during saddling, and during warm-up ridden walk, by measuring cardiac variables (such as heart rate and heart rate variability). This was done six times, approximately at monthly intervals. The researchers also noted each horse’s racing performance during the study.

They found a positive influence of the music on the emotional state of the horses. A difference between the two groups was apparent as early as the first month and became more marked in the second and third months. After the third month, the cardiac variables began to return to initial levels. Despite this, the researchers found that music had a positive effect on prizes won by the horses in the experimental group compared with the control group.

“The results display that the music positively influences the nervous system only temporarily. Later, the horse presumably gets accustomed to the music, and the nervous system does not react or tends to come back to the normal state” they write.

They suggest that, rather than using music continuously, it might be better to use it in a strategic manner: “The results suggest that using the music in the barn as a means of improving the welfare of horses is not justified for the whole race season. Trainers may apply the music with regard to the needs, for example, for 2 months in the period before an important race or at the beginning of the racing season.”

Curly horses for allergic riders

Curly horses may provide the solution for riders who are allergic to horses.

Having or developing an allergy to horses at the very least interferes with the rider’s enjoyment of horse riding. At worst, it could prove life threatening.

Dr. Wolfgang Mitlehner, a private medical practitioner from Klappholz, Germany, is particularly interested in respiratory medicine and allergies.

Since 2009, he has addressed the question of whether riders who are allergic to horses can ride so-called "hypoallergenic horses" - Curly horses.

So far he has studied 50 allergic riders with positive results. Interestingly, it seems that not only are the riders able to ride the Curly horses without problems, but overtime, they may lose their allergy to other horses as well.

Two young women and one adult used to experience both asthma attacks and skin, nose and eye symptoms when in-contact with horses. The adult rider even suffered allergic shock on contact with a horse. After 40 Curly-horse-riding lessons, all three riders can now ride and do so regularly. They have not experienced any more allergic symptoms, as they used to do previously.

Dr. Mitlehner has recently published the results of a study, which he carried out in conjunction with Prof Bodo Niggemann of the Dept. of Pediatric Pneumology and Immunology, at the University Children’s Hospital Charité, Berlin.

They investigated 40 horse allergic riders over a period of 37 months. They monitored their patients allergic response using skin prick tests and by measuring airflow in their upper or lower airways. (Constriction of the airways that may occur in allergic responses leads to reduction in airflow.)

Three of forty patients showed an initial significant reduction in airflow. This was reversed by single inhalation of salbutamol, and did not occur on subsequent riding sessions.

Further regular exposure to the Curly horses resulted in abolition of the mild allergic reactions that were noticed initially. Dr. Mitlehner suggests that this may be due to hypoallergenic properties of these horses, as they produce a weaker reaction to the skin prick test than do other horses.

He concludes that, after a period of three years, a loss of reactivity to normal horses could be confirmed in some of the riders.

He suggests that Curly horses may be a suitable alternative for horse allergic riders if the methodological precautions of this study are followed.

For more details, see:

Horse Allergy: Curly Horses Allow Horse Allergic Riders To Ride Again.

Horse allergy in Iranian riders

On a similar topic, researchers in Iran have found that horse riders are more likely to be sensitized to horses than are people who do not ride.

Horse allergy is mainly characterized by rhinitis, conjunctivitis, asthma and urticaria; however, anaphylaxis (allergic shock) has been reported in some cases.

The study, led by Mozghan Moghtaderi of Shiraz University of Medical Sciences, Shiraz, Iran, examined 42 horse riders (30 males, 12 females) and 50 healthy individuals (27 men, 23 women) who had not worked with horses. The researchers used skin prick tests with a panel of horse and common animal allergens (including epithelial allergens of...
There can be few places that have not encountered benzimidazole anthelmintic resistance amongst cyathostomins. The small roundworms are the most common cause of parasite-related problems in horses in many parts of the world.

A recent report describes the occurrence of resistance in Uttar Pradesh, in northern India. The research also revealed that intensively managed horses had a significantly higher proportion of resistant alleles among their cyathostomins than did more extensively managed working horses.

The molecular mechanism involved in benzimidazole resistance in cyathostomins has been identified. A mutation at codon 200 of the 1 β-tubulin gene (F200Y) has been shown to confer resistance. This discovery has allowed the development of tools to identify the presence of the resistant alleles (versions of the gene). These tools, such as “Allele Specific PCR” (AS-PCR) provide a very sensitive method of detecting resistant worms in a population.

Sunil Kumar and colleagues at Division of Parasitology, ICAR-Indian Veterinary Research Institute, Uttar Pradesh used both AS-PCR and the more traditional faecal egg count reduction test (FECRT) to look for benzimidazole resistance in cyathostomins in Uttar Pradesh, a state with a humid subtropical climate.

Of the nine locations where FECRT was performed, the researchers found benzimidazole resistance in four. These included an intensively managed equine farm in the mid-western plains (Bareilly) and in extensively managed working horses at three locations (Shahjahanpur, Hardoi, Unnao) in the central plains.

Using the AS-PCR test to detect the benzimidazole resistant β-tubulin gene, the researchers found that homozygous resistant (rr) individuals were significantly more common at the intensively managed farm at Bareilly and in working horses at Shahjahanpur and Hardoi than in other places.

They suggest that, because of the widespread prevalence of benzimidazole resistant alleles in equine cyathostomins in Uttar Pradesh, it would be prudent to restrict the therapeutic use of this family of anthelmintic.

They conclude that proper pasture management and strategic treatment programs may offer a solution for anthelmintic resistance in horses in India.

For more details, see:

Researchers in Germany have had promising results in laboratory tests using betulinic acid to kill equine melanoma cell cultures. Further tests have shown that the compound was well tolerated in two horses.

Melanomas are common in grey horses, affecting up to 80% of them by the time they are 15 years old.

Melanomas are found most often under the tail or around the genital areas. Less commonly they can appear around the head, especially in the salivary glands or in the lips. Melanomas tend to gradually increase in size, and may become ulcerated or infected.

Occasionally, melanomas in grey horses will spread to other organs and may eventually cause death. This more aggressive course is more likely to occur when melanomas occur in horses of colours other than grey, in which they tend to behave more like they do in humans and dogs.

Current treatment options are limited, and often not very successful.

In human medicine, betulinic acid, and related compounds, have shown promise for treating cancer. Betulinic acid is found in the bark of various species of tree, in particular the white birch (Betula pubescens).

Now, recent research has assessed the effect of betulinic acid and its two derivatives B10 and NVX-207, (which are both more soluble in water than is betulinic acid), on two equine melanoma cell lines and one human melanoma cell line.

The work, published in the journal Chemico-Biological Interactions, is the result of collaboration between researchers at the Martin Luther University Halle-Wittenberg, and the Equine Clinic at the Foundation University of Veterinary Medicine Hannover.

They found that all three compounds, but especially NVX-207, showed high cytotoxicity on both equine melanoma cell lines. Cytotoxic effects included damage to both the cell membrane and the nucleus.

They managed to prepare a form of the most active compound (NVX-207) that could be used to treat horses, without any loss of activity. They report that this formulation was well tolerated when administered to two-melanoma affected horses once a week for 19 consecutive weeks.

They conclude: “Our results indicate that the apoptosis (cell death) is induced in the equine melanoma cells by all three compounds. Furthermore, we succeed in encapsulating the most active compound NVX-207 in 2-Hydroxyproyl-β-cyclodextrine without a loss of its activity. This formulation can be used as a promising antitumor agent for treating grey horse melanoma.”

For more details:

In vitro anticancer activity of Betulinic acid and derivatives thereof on equine melanoma cell lines from grey horses and invivo safety assessment of the compound NVX-207 in two horses.

Liebscher G, Vanchangiri K, Mueller T, Feige K, Cavalleri JV, Paschke R.

The University of Florida Large Animal Hospital is currently recruiting gray horses with melanoma for a clinical research trial. The aim is to develop a vaccine to prevent or treat melanoma in horses.

Melanomas are common in gray horses and rare in other breeds. They start to appear when the gray skin coloration is complete (usually after 5 years of age). By 15 years of age, 80% of gray horses have visible malignant melanomas.

Most melanomas are found under the tail, around the anus, or around the genital areas. A less common location is around the head, especially on the lips and the salivary glands inside the mouth. Melanomas increase in size, become infected, and bleed, and eventually may obstruct the anus.

A minority of melanomas in grays, and most melanomas in other horses, will spread to other organs and eventually cause death.

Dr. Rowan Milner at the University of Florida College of Veterinary Medicine has developed a vaccine for treating melanoma in dogs, in which the condition is often highly fatal. So far, after several years of studies, the results look promising.

Dr Milner has also created the vaccine used in horses which is a different version of that used in dogs. “Our expectation is that the melanoma(s) will not continue to enlarge after vaccination. It is our hope that they will in fact shrink or disappear.”

Continued...
British horses sought for laminitis study

Horse owners across Britain are urged to join the fight against laminitis by enrolling their horses and ponies in the CARE about laminitis study.

The CARE study’s aim is to determine how common laminitis is and to identify equine lifestyle factors that affect its development. PhD student Dee Pollard, based at the Animal Health Trust in Newmarket, is coordinating the project, with help from colleagues at the Royal Veterinary College, the Animal Health Trust and Rossdales Equine Hospital. The study is funded by World Horse Welfare.

Dee Pollard said: “We need a collaborative effort by all horse owners, professionals and researchers to both increase awareness about laminitis and, in time, help make early recognition of laminitis less deceptive.

“The success of this project depends on gathering data from over 3000 British horses and ponies. Therefore we need to reach and recruit as many owners nationwide as possible and keep them enrolled for up to two years.”

It is not just horses with laminitis that are needed. Information is needed from healthy animals, as well as from laminitis cases, to provide meaningful results.

“Not only will this give an estimate of the frequency of laminitis in the British equine population (the proportion of horses and ponies that have laminitis vs. those that don’t) it will also reveal how many new cases of laminitis occur during the study period.”

The project will also investigate differences in management and other factors that affect the risk of developing laminitis.

CARE members submit regular information online about their horses’ health and management. An initial baseline questionnaire covers all aspects of the horse’s environment, activity, nutrition and daily care. Members are then asked to check-in on a regular basis and, if required, update these details. They also have access to an online weight tracker to monitor their horse’s weight and body condition.

For more information and to register your horse or pony, go to: 
www.careaboutlaminitis.org.uk/
Horses can read human facial expressions, according to researchers at the University of Sussex.

Sussex psychologists studied how 28 horses reacted to seeing photographs of positive (“happy”) and negative (“angry”) human facial expressions. The study has been published in Biology Letters.

Horses were recruited from five riding or livery stables in Sussex and Surrey, UK, between April 2014 and February 2015. They were shown happy and angry photographs of two unfamiliar male faces. The tests examined the horses’ spontaneous reactions to the photos, with no prior training.

The experimenters were not able to see which photographs they were displaying so they could not inadvertently influence the horses. They monitored the horses’ behavioural responses and heart rate changes to each image.

The right side of the brain seems to be important for processing "negative" emotions such as fear, while the left hemisphere is involved in processing “positive” emotions. Because most of the nerves from the eye cross to the visual cortex on the opposite side of the brain, horses process signals from their left eye with the right side of the brain, and vice versa.

The researchers found that when viewing angry faces, horses looked more with their left eye, demonstrating bias towards processing the images with the right side of the brain, a behaviour associated with perceiving negative stimuli. Their heart rate also increased more quickly and they showed more stress-related behaviours.

They conclude that this response indicates that the horses had a functionally relevant understanding of the angry faces they were seeing. The effect of facial expressions on heart rate has not been seen before in interactions between animals and humans.

Amy Smith, a doctoral student in the Mammal Vocal Communication and Cognition Research Group at Sussex, co-led the research. She said: “What's really interesting about this research is that it shows that horses have the ability to read emotions across the species barrier. We have known for a long time that horses are a socially sophisticated species but this is the first time we have seen that they can distinguish between positive and negative human facial expressions.

“The reaction to the angry facial expressions was particularly clear – there was a quicker increase in their heart rate, and the horses moved their heads to look at the angry faces with their left eye.”

She continued: “It’s interesting to note that the horses had a strong reaction to the negative expressions but less so to the positive. This may be because it is particularly important for animals to recognise threats in their environment. In this context, recognising angry faces may act as a warning system, allowing horses to anticipate negative human behaviour such as rough handling.”

Horses may have adapted an ancestral ability for reading emotional cues in other horses to respond appropriately to human facial expressions during their co-evolution.

"Alternatively, individual horses may have learned to interpret human expressions during their own lifetime. What’s interesting is that accurate assessment of a negative emotion is possible across the species barrier despite the dramatic difference in facial morphology between horses and humans.”

“Emotional awareness is likely to be very important in highly social species like horses – and our ongoing research is examining the relationship between a range of emotional skills and social behaviour.”

Amy Smith and Professor McComb are based in the School of Psychology at Sussex. The study is co-authored by Sussex colleagues Dr Leanne Proops, Kate Grounds and Dr Jennifer Wathan. This research is part of an ongoing project into emotional awareness in horses that is funded by the Leverhulme Trust and the University of Sussex.

For more details, see:

Functionally relevant responses to human facial expressions of emotion in the domestic horse (Equus caballus)

Amy Victoria Smith, Leanne Proops, Kate Grounds, Jennifer Wathan, Karen McComb

For many years, tapeworms were thought to be harmless. Now they have been implicated in several types of colic, such as intussusception ('telescoping') of the caecum, caecal perforation, peritonitis and intestinal obstruction. Many horses are infected. Indeed, some reports suggest the number of horses carrying the infection is increasing.

Horse tapeworms (Anoplocephala perfoliata) are not easy to identify in faecal samples. Unlike roundworms, they don't release eggs regularly, so examining fecal samples may give an unreliable estimate of the level of infection. Faecal samples may appear negative even though tapeworms are present in the horse's gut.

Tests for antibodies to tapeworm antigens in blood (and, more recently, saliva) have been developed, which can be used to monitor the level of infection.

Tapeworms also differ from the common horse roundworms in that they spend part of their life cycle in an intermediate host. Eggs in the horses' faeces are ingested by oribatid mites on the pasture, in which they develop to the mature larval stage (known as a cysticercoid) over a period of up to 5 months. The life cycle is completed by a horse ingesting the infected mite when grazing.

There is still much we do not know about the biology of the parasite in its intermediate host, and the factors that affect its transmission to the horse.

For example, does the mite population on the pasture differ with the seasons, and does the proportion of infective mites change through the year? Does regularly clearing manure from the pasture influence the transmission of the parasite? Do infected mites behave differently to parasite-free mites?

To answer questions like these, a new research project is being set up, entitled: "Improved control of the equine tapeworm, Anoplocephala, through new insights into the biology of the oribatid mite intermediate host."

Professor Mark Fox, Dr Damer Blake, Kim Stevens (Royal Veterinary College, London), Professor Arancha Meana (University of Madrid), Professor Paul Davis, Dr Corrine Austin (Austin Davis Biologics Ltd) and Professor John Pickett (Rothamsted Research) have been awarded a grant by the Petplan Charitable Trust to carry out the research.

The research team say “This study will enable us to:

(1) monitor seasonal changes in exposure of grazing horses to stages of the tapeworm, Anoplocephala, in the mite intermediate hosts;

(2) further understand the role of horse mucosal antibody responses in the complex balance between horse, parasite and the environment; and

(3) develop more effective parasite control programmes by establishing (a) the optimum time(s) of treatment for tapeworm; (b) the value of removing dung from pastures in tapeworm control and the extent to which such measures result in a reduction in frequency of deworming treatments required; and (c) whether possible changes in infected pasture mite behaviour necessitate changes in turnout times and dates.”

For more details, see:
http://www.rvc.ac.uk/research/news/general/anoplocephala

For more information on the tapeworm intermediate hosts, see:
Merijo Eileen Jordan
MSc thesis 2001 University of Florida
http://etd.fcla.edu/UF/UFE0000324/Thesis.PDF
Researchers in Iran have developed a blood test to predict whether a foal will be male or female.

Sometimes it can be more than mere curiosity that drives owners to know the sex of a foal before it is born. In particular, the value of in-foal mares at sales may be affected by the gender of the foal they are carrying.

Foetal sexing is possible using an ultrasound scan around 60 to 70 days’ gestation to ascertain the position of the genital tubercle, the precursor of the penis or clitoris. However, the accuracy of the technique depends very much on the experience of the operator.

Ali Kadivar and colleagues at the Shahrekord University in Iran have developed a test that requires a single blood sample from the mare. A report of the research is published in the Journal of Equine Veterinary Science.

The test, a nested real-time PCR assay, looks for the SRY (sex determining region Y) gene in the circulating cell-free fetal DNA. Cell-free fetal DNA (cffeDNA) is genetic material that is released by the placenta and circulates in the mare’s blood during pregnancy.

The sex-determining region Y protein (also known as testis-determining factor) produced by the SRY gene controls the processes that make a fetus develop male gonads (testes) and prevent the development of female reproductive structures (uterus and fallopian tubes). So fetuses with the SRY gene will develop into males, and those without will be female.

The research team tested a single plasma sample from each of 28 pregnant Arabian mares between 8 and 20 weeks of gestation. At the time of sampling, the gender of the fetus was unknown. This was only confirmed by direct clinical examination after birth.

Three samples failed to yield sufficient cffeDNA to test. From the remaining 25 samples, the test correctly identified 12 of 14 male pregnancies and 10 of 11 female pregnancies, resulting in a sensitivity of 85.7%, and a specificity of 90.9%. Overall, the accuracy of the test was 88%.

The researchers conclude: “Detection of cffeDNA in maternal plasma with a nested real time PCR assay can be used to determine equine fetal sex with a good accuracy by analyzing SRY gene just after 8 weeks of gestation.”

For more details, see:
Developing a nested real-time PCR assay for determining equine fetal sex prenatally
Ali Kadivar, Rohollah Dehghani Tafti, Heidar Heidari Khoei, Mahboobeh Heidari Nasirabadi, Naser Shams Esfandabadi, Narjes Cheraghi
doi:10.1016/j.jevs.2016.02.229

Mysteries of the inner workings of the horse’s digestive tract are set to be revealed by scientists at the University of Saskatchewan using innovative imaging technology.

The horse’s digestive tract may be up to 20 metres long, most of it inaccessible to current imaging methods. A 3 metre long video-endoscope can inspect the horse’s digestive tract as far as the stomach, reaching to (and just through) the pylorus, the muscular valve at the entrance to the small intestine. Beyond that, the small and large intestines have remained out of reach. Laparoscopy (a small camera inserted through the body wall) or surgical exploration of the abdomen can be used. But neither technique gives a view inside the digestive tract.

Now a swallowable camera “pill” has allowed scientists to look inside the horses intestines. Such techniques have been used in human medicine before, but the technology has not been applied to horses.

“Whenever I talk to students about the horse abdomen, I put up a picture of a horse and put a big question mark in the middle,” said veterinary researcher Dr. Julia Montgomery in the U of S Western College of Veterinary Medicine.

Montgomery worked with equine surgeon Dr. Joe Bracamonte and Khan Wahid, a specialist in health informatics and imaging in the College of Engineering. The team used an endoscopy capsule about the size and shape of a vitamin pill—a sort of “mini submarine” with a camera—to have a look inside a horse.
“This is really a cool way to look at the entire small intestine,” Montgomery said.

She explained that capsule endoscopy offers a powerful new tool to diagnose diseases such as inflammatory bowel disease and cancer, or to check surgical sites. Researchers could use it to see how well drugs to stimulate bowel action are working, or to answer basic questions such as determining what “normal” small intestine function looks like.

Wahid has long worked with endoscopy capsule technology for humans and has even patented algorithms and data compression technology for their improved performance. The “camera pills” have been in use for human medicine for some time, he explained, but have yet to be applied in equine health.

“We thought, ‘why not try it for veterinary medicine?’” Wahid said.

On March 1, 2016, they did just that, administering the capsule through a stomach tube directly to the horse’s stomach. For the next eight hours, the capsule and its camera made its way through the horse’s small intestine, offering a continuous picture of what was going on inside.

The team plans to run more tests in the next few months on different horses to gather more data. With this in hand, they plan to pursue funding to further develop equine capsule endoscopy.

“We thought, ‘why not try it for veterinary medicine?’” Wahid explained. “Once we know more about the requirements, we can make it really customizable, a pill specific to the horse.”

Kentucky horse owners and farm managers with pregnant mares are warned to be on the lookout for Eastern Tent Caterpillars.

Eastern Tent Caterpillars are found throughout the eastern United States. They feed on various wild and cultivated trees. The wild black cherry tree, which is common in woods and fences around paddocks on stud farms, is their favourite. The caterpillars build silken webs or tents at forks in the branches.

Ingestion of large numbers of the mature, hairy, caterpillars by pregnant mares can cause Mare Reproductive Loss Syndrome.

Lee Townsend, University of Kentucky College of Agriculture, Food and Environment extension entomologist, warned owners of pregnant mares to monitor fence lines containing wild cherry and other host trees and look for small tents produced by developing caterpillars.

“UK researchers conducted studies that revealed horses will inadvertently eat the caterpillars, and the caterpillar hairs embed into the lining of the horse’s alimentary tract. Once that protective barrier is breached, normal alimentary tract bacteria may gain access to and reproduce in sites with reduced immunity, such as the fetus and placenta.”

“If practical, farm managers should plan to move pregnant mares from areas where these trees are abundant to minimize the chance of caterpillar exposure. The threat is greatest when the mature tent caterpillars leave trees and wander to find places to pupate and transform to the moth stage.”

For more details, see:
http://news.ca.uky.edu/article/eastern-tent-caterpillar-egg-hatch-begins-central-kentucky-0
Abdominal hernia repair

Kick wounds to the lower abdomen of the horse can result in hernias. Although the skin remains intact, the underlying muscle layers may rupture, resulting in a soft swelling, with the intestines lying just beneath the skin. Adhesions may occur, and the intestines can become trapped and cause colic.

Such wounds are challenging to treat because of the weight of the abdominal contents bearing down on the surgical repair. The damaged muscle layers may not be strong enough to withstand the tension on the sutures used to repair the defect.

It is often necessary to implant some mesh to strengthen the damaged muscle layers and repair the defect. Even then there is a danger that the tension on the stitches will disrupt the wound. One such case was reported in BMC Veterinary Research. Carla Faria Orlandini and others describe the successful use of polyester buttons and polypropylene mesh to repair an abdominal hernia, after a previous surgical repair had broken down.

The patient, a three-year-old male Appaloosa weighting 500 Kg, had developed a swelling of the lower abdomen following a collision with a farm fence. The authors suggest that the recurrence of the hernia after the first surgery was probably related to the weakness presented by the lacerated muscles, with high friability and inflammation.

The second surgical repair used polypropylene mesh, to reinforce the suture line, and polyester buttons to prevent the sutures pulling through the muscle. The animal returned to its normal routine 30 days after the procedure.

The authors say that “the materials used to reinforce the abdominal suture were easy to apply, had good interaction with the suture and excellent resistance to traction, even when inserted in heavily lacerated muscle.”

They conclude that the use of polyester buttons and polypropylene mesh, “provided effective reinforcement to the abdominal tension and was deemed an effective option for reconstruction of lacerated muscles after post-traumatic eventration in a horse.”

For more details, see:

Surgical treatment of traumatic eventration with polyester button and polypropylene mesh to strengthen the suture technique in equine. Carla Faria Orlandini, Denis Steiner, André Giarola Boscarato, Gabriel Coelho Gimenes and Luiz Romulo Alberton

BMC Veterinary Research

DOI: 10.1186/s12917-016-0686-8

The horse undergoing surgery for treatment of traumatic eventration demonstrating the polyester buttons and polypropylene mesh used for reconstruction of the obliquus and transverse abdominis. ©Orlandini et al.
Equine Science Update

Equine Science Update

Most horses today are treasured for their ability to run, work, or be ridden, but have lost their wild-type camouflage: pale hair with zebra-like dark stripes known as the Dun pattern. Now an international team of scientists has discovered what causes the Dun pattern and why it is lost in most horses. The results, published recently in Nature Genetics, reveal a new mechanism of skin and hair biology, and provide new insight into horse domestication.

The work is an international collaboration led by groups at Uppsala University, Uppsala, Sweden, and the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama.

Pale hair colour in Dun horses provides camouflage as it makes a horse in the wild less conspicuous. In contrast, domestic horses, as well as many other domestic animals, have been selected over many generations to be more conspicuous, more appealing or simply different than the wild type. The pale hair colour in Dun horses does not affect all parts of the body; most Dun horses have a dark stripe along their back, and often show zebra-like leg stripes. However, the majority of domestic horses are non-dun and show a more intense pigmentation that is uniformly distributed.

"Dun is clearly one of the most interesting coat colour variants in domestic animals because it does not just change the colour but the colour pattern", states Leif Andersson, whose group led the genetic analysis. “We were really curious to understand the underlying molecular mechanism why Dun pigment dilution did not affect all parts of the body.”

The research team started by analysing the distribution of pigment in individual hairs.

"Unlike the hair of most well studied mammals, the dilute coloured hairs from Dun horses are not evenly pigmented the whole way around. They have a section of intense pigmentation along the length of the hair, on the side that faces out from the body of the horse, whilst the rest of the hair has more or less no pigment,” explains Freyja Imsland, the lead author for the genetic analysis, and a PhD student in Andersson's group.

In contrast, the hairs from the dark areas of Dun horses are intensely pigmented all around each individual hair. In spite of scientists having studied hair

Continued

Illustration of the mechanism underlying Dun colour. TBX3 expression (indicated in green) in the hair follicle in Dun horses leads to lack of pigment production in parts of the hair follicle; pigment indicated in balck. In non-dun horses there is no TBX3 expression in the hair follicle, and the entire hair is fully pigmented.

© Kelly McGowan and Freyja Imsland;
pigmentation in detail for a very long time, this kind of pigmentation is novel to science, and quite unlike that seen in rodents, primates and carnivores.

Genetic analysis and DNA sequencing revealed that Dun versus non-dun colour is determined by a single gene that codes for the T-box 3 (TBX3) transcription factor. In humans, inactivation of the TBX3 gene causes a constellation of birth defects known as Ulnar-Mammary Syndrome. But in horses that have lost their Dun colour, TBX3 mutations do not inactivate TBX3 protein function and instead only affect where the gene is expressed in the growing hair.

“Previous studies in humans and laboratory mice show that TBX3 controls several critical processes in development that affect bones, breast tissue, and cardiac conduction,” explains Greg Barsh, whose group led the tissue analysis. “We were surprised to find that TBX3 also plays a critical role in skin and hair development.”

The team discovered two forms of dark, non-dun colour, non-dun1 and non-dun2, caused by different mutations.

“Non-dun horses have much more vibrant colour than Dun horses. Non-dun1 horses tend to show primitive markings similar to Dun horses, whereas non-dun2 horses generally don’t show primitive markings. These primitive markings in non-dun1 horses can sometimes lead horse owners to think that their intensely pigmented non-dun1 horses are Dun,” states Freyja Imsland.

To understand how TBX3 affects hair colour, they measured TBX3 distribution in individual hairs relative to other molecules previously known to regulate pigmentation.

“In growing hairs, TBX3 mirrors the distribution of melanocytes, the cells that produce pigment,” explains Kelly McGowan, a senior scientist in the Barsh group. “Our results suggest that TBX3 affects differentiation of specific cells in the hair, creating a microenvironment that inhibits melanocytes from living in the "inner" half of the hair.”

The group speculates that the signals governing where TBX3 is expressed could help to explain zebra stripes.

“The region of the body where TBX3 is expressed may account for the stripe pattern,” says McGowan, “whereas the region of the hair where TBX3 is expressed may account for colour intensity.”

The results suggest that the non-dun2 variant occurred more recently, most likely after domestication. In contrast, both the Dun and non-dun1 variants were present before domestication; both Dun and non-dun1 variants were found in ancient DNA from a horse that lived about 43,000 years ago, long before horses were domesticated.

“This demonstrates that horse domestication involved two different colour morphs (Dun and non-dun1) and future studies of ancient DNA will be able to reveal the geographic distribution and the abundance of the two morphs, ends Leif Andersson.