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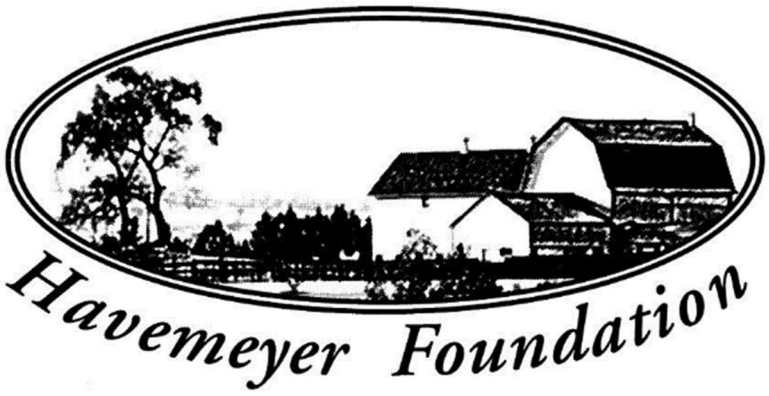

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Specificities of French horse industry

Guillaume Blanc[1], Sophie Danvy[1], Emilie Goulas[1]

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French horse industry is multifaceted, from draught horse in farmlands to thoroughbreds or French trotters in race courses, from leisure native breeds to olympic horses, over 1 million horses, ponies and donkeys are present in all French regions. The economic model is centered on two principal stakeholders in racing and sport. Pari Mutuel Urbain (PMU) represents the French betting activity, generates about 10B€ and 1,5 B€ finances all equine sectors. The French equestrian federation with its specific model of equestrian centers is the third sport by number of licenced riders.
IFCE is the only institute dedicated to horse industry, working for and with the different stakeholders in research, education programs, traceability of horses and on the promotion of French equitation.
The traceability system is based on an unique zootechnic and sanitary database (SIRE). This unique model of centralized and mutualized database, combined with an identification and control activity in the field, optimizes traceability and selection of equidae. This database is linked to the different equine sectors and used as a hub to connect and share all informations. IFCE implemented the Universal Equine Life Number (UELN) system to simplify data exchanges all over the world.
This desire for universality is also reflected in the multiplicity of high-level research centres on all topics (genetics, genomics, biomechanics, behaviour, health, reproduction, performance).

Some references for further information:

Les races locales menacées d'abandon en France – Ethnozootechnie : actualisation des listes et extension de la démarche à de nouvelles espèces https://hal.inrae.fr/hal-04077969

French equine statistic review 2023 – IFCE : https://equipedia.ifce.fr/fileadmin/bibliotheque/6.Statistiques/6.1.Ecus-depliant/Annuaire-ECUS-2023.pdf

Online statistics: https://statscartes.ifce.fr/

Access to French horses database: https://infochevaux.ifce.fr/en/info-chevaux

History of the Havemeyer Horse Genomics Workshop

Ernest Bailey[1], Doug F. Antczak[2]

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In 1990, scientists initiated an international project to sequence the human genome. The project changed the way that genetic studies were conducted and led to development of powerful and inexpensive approaches to evaluating the genome of any species. Horse geneticists and veterinarians met in 1995 in Lexington, KY and agreed to collaborate in a workshop. This was not a symposium, but rather, a workshop to collaborate on a common project, one with a scope beyond the capacity of any one laboratory.

During the next ten years these researchers met frequently under the auspices of the Dorothy Russell Havemeyer Foundation in California, Sweden, South Africa, Australia, Ireland and United Kingdom and they identified genomic homologies between humans and horses with Zoo-Fish, created hundreds of microsatellite DNA markers, created two genetic linkage maps using these markers and known genetic variants, created BAC libraries, mapped cytogenetic landmarks, made synteny maps and created an extensive radiation hybrid map. Along the way, the scientists used the new genomic resources in local projects to identify genetic variants for coat colors and diseases in horses. Even though these applications were not technically part of the workshop, the scientists found that they enjoyed working together, and many of the projects were done as collaborations among many laboratories. The success of the community was noticed and led to the decision by the National Human Genome Research Institute (NHGRI) to sequence the horse genome in 2006. NHGRI reasoned that insights from the investigations by the equine genome scientists would benefit human genetic research.

The creation of the horse genome sequence was only the beginning. The Workshop continued to meet in places such as Minnesota, California, Portugal, Italy, Cornell, and France to share methods and discoveries. This international collaboration led to development of SNP arrays for use in GWAS, creation of a new reference genome, and the FAANG initiative to characterize the landmarks of functional genomics. At the same time, scientists continued to use the resources to make discoveries about coat colors, hereditary diseases and even performance in horses. The cost of whole genome sequencing continued to drop. By 2018, the cost of whole genome sequencing had dropped so much that it became a standard approach when studies involved genotyping horses. This would have been large surprise to the group of scientists in 1995, whose objective was only to create a genetic map and never expected to see a whole genome sequence for horses. The challenge for the future is to continue finding ways to collaborate for the benefit of all scientists studying horse genetics, and ultimately, the benefit of the horse owner.

The horse before and after our shared history

Ludovic Orlando

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Horses revolutionised human history with fast mobility. Yet, the timeline encompassing their initial domestication and widespread adoption as a mode of transportation across human societies is a topic of contention. The breeding practices that supported the global demand for horses during early domestication stages and have engendered the diversity of modern domestic bloodlines existing around the world remain also largely unknown. The last 4,000+ years of human-driven selection, fragmentation and admixture left a rather limited genetic diversity in contemporary horse populations, which stands in stark contrast with the multitude of divergent wild lineages prevailing before domestication. However, the complex evolutionary history and environmental conditions that accompanied the emergence and extinction of those wild lineages are still poorly understood. This presentation will highlight our latest breakthroughs in unraveling the intricate interplay between human influence and climate change, and their consequences on the horse genome, following 50,000 years of evolution.

A survey of genetic variation in today's US Thoroughbred with application to predicting the diversity of tomorrow

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Thoroughbreds represent hundreds of years of selection for performance and fitness traits. With a closed population, selection increases the frequency of beneficial alleles and decreases that of detrimental alleles; resulting in a decrease in genetic variation over time. The goal of this work was to characterize the genetic structure of the US Thoroughbred and provide data for development of tools to monitor and model future changes in the population. DNA samples were obtained from 1,091 Thoroughbreds residing in the US. Pedigrees were used to identify 102 horses (born 2000 to 2020) to represent the diversity of the breed; whole-genome sequence data was obtained on each. Samples from 83 horses banked at the University of Kentucky (born 1965 to 1986) were also sequenced. In total 14.5 million SNP variants were identified across these horses, 12.9 million in autosomes. Mitochondrial sequence (excluding D-loop) placed the horses across 9 of 18 previously characterized clades. Individual inbreeding estimated by runs of homozygosity (ROH) averaged 0.262 (0.143 to 0.350). Horses born 2000-2020 had greater (P<0.001) inbreeding (FROH) than horses born between 1965 and 1986. The more recent cohort, however, did not have a greater proportion of inbreeding attributed to "long" (>2 Mb) ROH than horses born 2-4 generations prior. Genomic estimates of inbreeding were weakly correlated (r=0.449) with pedigree-based estimates. Genomic variation identified is being used to develop haplotype imputation panels to quantify diversity in a larger, random sample using low-pass sequencing. All sequence data are publicly available (PRJNA993255).

A Thoroughbred T2T Reference Genome

Kai Li[1], Donald Miller[2], Douglas Antczak[2], Nahla A. AbouEl Ela[1], Lauren Johnson[1], Julia L. Ciosek[1], Brian P Walenz[3], Brandon Pickett[3], Sergey Koren[3], Adam M. Phillippy[3], Shavahn C. Loux[1], Elizabeth Hudson[4], Melissa Laird-Smith[4], Brian Davis[5], Sian Ann Durward-Akhurst[6], Molly E McCue[6], Jessica L. Petersen[7], and Ted Kalbfleisch[1]

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Telomere-to-Telomere reference genomes have become the goal of genetic and genomic research communities. Here, we introduce a telomere-to-telomere reference genome generated from a female mule with a Thoroughbred dam and a donkey sire. The genome was constructed from PacBio HiFi, Oxford Nanopore ultra-long reads, and Dovetail Omni-C sequence data. Twenty four of the 31 autosomal chromosomes and chromosome X are T2T, 13 of those including X are gapless. The remaining 7 have telomeres at one end. This genome is ~10% larger than the current horse reference genome EquCab3 with content being added mostly at the centromeres. Our effort represents the first T2T assembly for the horse and will be foundational in the equid pangenome work currently underway. In addition to the genome sequence, tissues were collected from this mule for 142 tissues to facilitate annotation. This genome will be submitted to NCBI and Ensembl. We are also submitting this genome to the equine genomics community for consideration as the primary reference for the horse.

Equine pangenome graph identifies novel structural and single nucleotide variants

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Single-haplotype reference genomes have been foundational to identify genetic variation for decades. However, this approach has significant limitations regarding the genetic diversity that exists between populations, resulting in considerable bias where genome structure and sequence divergence between horse breeds cause a misrepresentation of the true variation in sequenced individuals aligned to the current thoroughbred single-haplotype reference (EquCab3). Recent advances in long read technologies have afforded the ability to create multiple, highly contiguous reference genomes that represent a diversity of populations. We generated 32 diploid reference genomes from 12 horse breeds and non-caballine equids, including one near telomere-to-telomere thoroughbred reference to facilitate heritable disease and genome evolution studies. We combined these 64 haplotypes with all published reference genomes into one pangenome graph, allowing for a flexible structure accommodating multiple variants at a given position. We identified novel structural variants in every breed represented in the graph such as Peruvian horse, Clydesdale, Shire, Arabian, Icelandic, Quarter horse, Morgan, and Shetland pony. This allowed more accurate mapping of short read genomes, resulting in over 10 million more single nucleotide and small insertion-deletion variants detected than aligning to EquCab3. This proved particularly effective in regions of the genome with high variability, such as gene families and copy number variation. We identify a reduction in reference bias over 20% across breeds, even in those not represented in the graph. We believe this approach will be the new paradigm for variant identification across diverse horse breeds and facilitate finding mutations associated with phenotypes and heritable disease.

A Rosetta Stone for the Equine Major Histocompatibility Complex

Donald C. Miller and Douglas F. Antczak

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The Major Histocompatibility Complex (MHC) is one of the most complicated regions of the genome. It is very gene-dense, containing nearly 200 genes in about 3-5 megabases, and also highly polymorphic. Genetic variation in the MHC is often assessed at haplotype level because of the strong linkage disequilibrium in the MHC region. MHC haplotypes have been linked with a variety of traits in horses in disease association studies, and MHC haplotyping is also useful in regenerative medicine in the selection of MHC matched stem cell donors. There are also applications in reproductive biology and in studies of immunity to infectious diseases. Various molecular platforms can be used for MHC haplotyping, and this can lead to difficulties in comparing results across platforms. Here we have integrated data from three independent methods to assess MHC variation at the haplotype level. We compared MHC haplotypes determined using intra-MHC SNPs and intra-MHC microsatellites on over 500 horses of several breeds and observed over 90% correlation between SNP- and microsatellite-determined MHC haplotypes. From this work we also provide evidence for intra-MHC recombination as a major mechanism in driving haplotype diversification within breeds. Finally, we determined cDNA sequences of selected MHC class I and class II genes from MHC homozygous horses and found a high correlation between structural gene polymorphism and anonymous SNP and microsatellite markers. SNPs and microsatellites thus represent a good proxy for assessing MHC class I and class II gene variation for a variety of applications in equine medicine, surgery, and husbandry.

After a ten-year search for variation on the horse Y chromosome: what we have learned about the history of stallions and what still remains hidden

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The Y chromosome is an important part of human and animal population history, genealogy, and forensic studies due to its male specificity, haploidy, and escape from recombination. However, the sequence diversity in domestic horse Y is extremely low and meaningful variants could only be defined with the advent of high-throughput sequencing technology. Hence, the first (five!) population informative Y markers were described in 2013. From this starting point, our continuous efforts improved the resolution of the horse Y phylogeny.
The most current Y tree, ascertained from 170 male horses, consists of 153 haplotypes (HTs) which are determined by 2,966 SNPs (Bozlak et al., 2023). We further interpreted the HT pattern in the horse population and clarified the origin of several HTs by implementing data from ancient horses. In addition, we delineated the Y-signature of numerous influential historical breeding sires based on their offspring with documented ancestry.
Last, we carried out extensive genotyping, and thanks to the generous support of many researchers who provided sample material, we have determined Y HTs in more than 3,800 horses from 251 breeds/populations across the globe. At the meeting we share these results and our interpretation of origin, dissemination and influence of breeding stallions. We present the male mediated history of several horse breeds beyond pedigree information but also point out open questions. Furthermore, we discuss practical applications of Y chromosomal analysis, its potential and possible pitfalls.

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Assessing stakeholder needs to inform outreach efforts and research priorities from the equine genomics community

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The collaborative Horse Genome Workshop contributed to many scientific achievements aimed at improving the welfare of equids around the world. Yet, shifts in communication technology, industry goals, and the political environments surrounding scientific funding will present our group with new challenges. We are now organizing the collective horse genome projects in the USA into an equine-centric USDA Multistate Research Program, "S1094: Genomic tools to improve equine health, wellbeing, and performance", approved in 2023. The project's objectives encompass pan-genomic variability/genetic diversity, improving genomic resources for equine performance and disease, availability of genetic diagnostic testing and education and data sharing platforms.

Multistate projects are intended to encourage research efforts aimed at addressing large-scope industry needs, "stakeholder" education, and the application of scientific findings to address practical challenges. Thus, we have constructed a short online survey for assessing the needs of stakeholders in the USA and internationally. This survey asks simple questions on the perceived critical concerns in health and genetics from horse industry participants. With a planned launch in early 2024, results from this survey are pending and will be presented here.

The intent of S1094 is to provide the framework for a scientific community. To meet its objectives, strong international participation is desirable. Further, we will need to pursue collaborative funding mechanisms to support our scientific and educational activities; this will likely be facilitated through increased interaction with our stakeholders. The results of our stakeholder needs assessment survey will hopefully help guide these interactions and outreach activities in the future.

Standards and Guidelines for Equine Genetics Research

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An important goal of equine genetics research is the identification of causal variants for inherited diseases, enabling genetic testing to reduce or eliminate the disease occurrence. Genome-wide association analyses (GWA) allow for the identification of regions of the genome associated with diseases or traits of interest. Care must be taken in these studies to address population stratification appropriately and not over-interpret GWA results. Human GWA guidelines that can guide the equine community have been developed (1). Due to extensive linkage disequilibrium, association to a genomic region can be obtained with enough carefully collected individuals and markers. Even with a region identified, identifying causative variation can remain challenging since many variants will be perfectly associated. It is, therefore, essential to provide a means of classifying the evidence towards causality of a variant. Stringent standards in human genetics have been developed; however, similar criteria can be used to categorize the evidence available regarding causality in animal genetics (2). The American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP) have defined criteria for Mendelian diseases, including the definition of functional classes of variants: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign (3). We suggest a round table discussion that would have as its goal a summary of best practices for GWA and a classification system of variants to be used for equine genetics. This could culminate in a publication outlining best practices and explaining the system to a broader audience of users.

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Equine Pangenome Workshop

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Technologies have emerged making it straightforward to build very accurate, nearly complete, fully phased genomes for both haplotypes of diploid mammals. There are several efforts underway in horses and other equids to build better reference genome resources.
One such effort has built phased genomes for a Grevy's zebra, donkey, Thoroughbred, Quarter Horse, Shire, Arabian, Clydesdale, and Peruvian horses. Also, Telomere-to- Telomere phased assemblies have been derived from a female mule with a Thoroughbred dam and a donkey sire. Other efforts are in process, or nearing completion.
In this workshop we discuss how the community can engage the project beyond simply using the assemblies. Specifically, how they can contribute samples, and/or their own high-quality equine genome assemblies. This will involve establishing thresholds for quality and contiguity, also best practices for generating and assembling data into genomes suitable for annotation, and inclusion into the pangenome.
We will describe the genomes built to this point, and efforts underway to create an equid pangenome from them. We will also describe the utility of pangenomes for understanding population-specific variation and how proper annotation can lead to more accurate and specific findings. We will also discuss the large catalog of short read sequencing that we are amassing to further understand genetic variation across equine populations.
Although at its inception, this effort was limited to a few members, the equine pangenome is being created as a community resource whose development and growth should be guided by community needs, and bolstered by input, samples, and data the community offers.

The epigenome of male germ cells: role in male fertility and in the programming of offspring phenotype

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Mature spermatozoa are transcriptionally inactive and represent the ultimate form of male germ cell differentiation. Their fate is to survive outside the organism and contribute to a new individual after fertilization of an oocyte. In support to these functions, the epigenome of spermatozoa is unique (Carrell, 2012; Champroux et al., 2016). The sperm-specific epigenome is acquired during the differentiation of male germ cells into mature spermatozoa, a process that starts at the embryonic stage and is only achieved after puberty has been reached and during each cycle of spermatogenesis. In particular, the DNA methylation landscape of male germ cells is massively remodeled during the in utero life of the male, and these genome-wide changes are highly sensitive to environmental factors. In cattle and especially dairy breeds, where bull semen is widely used for artificial insemination, several selection and breeding practices may interfere with proper establishment of the sperm epigenome and with the future fertility of the bull. The selection of artificial insemination bulls relies on their genetic merit, and they are usually obtained from the breeding of high breeding value sires and high-producing dairy cows. These cows are more likely to experience a negative energy balance in the event of concurrent lactation and gestation, which may lead to an unfavorable in utero environment for the developing fetus (Wu and Sirard, 2020). Otherwise, practices to reduce the generation interval and accelerate genetic gain, such as hormonal treatments of the mothers, embryo technologies, or the hastened growth and puberty of male calves, may have a long-term impact on the sperm epigenome (Rivera, 2019). Finally, bull semen is extensively processed before its use for artificial insemination, which according to data obtained in other species, may affect the chromatin structure (Aurich et al., 2016). Because bull semen has a widespread diffusion potential, with dozens of offspring potentially being generated per batch, it is important to understand the impact of these practices on the epigenetic landscape of spermatozoa and the degree to which variations in the epigenome might affect the phenotype of offspring. This talk will provide an overview of recent knowledge regarding the epigenome of male germ cells, its roles in male fertility and in the programming of phenotypes, with particular emphasis on cattle and in light of the knowledge accumulated in other species.

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Embryonic and foetal programming in the equine: a need for epigenetic evaluation

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Epigenetic plasticity during early development in mammals is influenced by environmental cues. This leads to the apposition of epigenetic marks that support the Developmental Origins of Health and Disease. In horses, maternal nutrition, age and parity as well as reproductive technologies have been shown to affect offspring birthweight, post-natal growth, metabolism, testicular maturation, juvenile osteoarticular pathologies and performance (Chavatte-Palmer et al. 2017; Palmer et al. 2018). These observations have prompted changes in broodmare management recommendations in order to optimise fetal programming (Robles et al. 2021). The placenta, of foetal origin, is a key organ at the interface between dam and foetus. In response to maternal environment, modifications in placental function, whether in gene expression or cellular organisation, affect materno-foetal exchanges. These changes lead to foetal adaptation that will modify offspring long-term phenotype (Robles et al. 2022). Although epigenetic mechanisms were demonstrated to underlie developmental programming in many species, it has not yet been demonstrated in horses. There is an urgent need to explore the interactions between genome, epigenome, and environment in equine species, so as to develop complementary strategies to combine with genetic selection to produce offspring with optimal phenotypic capacities. Indeed, additional epigenetic information could improve phenotype prediction obtainedfrom genotype alone, as already demonstrated in cattle for bull selection (Costes et al. 2022). In terms of age, the economic and ethical need of longevity of sport horses could also benefit of epigenetic programming strategies during development for adult health.

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Defining the intolerome of the equine conceptus

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Equine pregnancy loss can occur anytime from conception to birth, with the underlying cause often not identified. We hypothesized that a significant proportion of these unknown losses would be attributed to lethal genetic variants. The overarching goal of the laboratory is therefore to define what we call the intolerome: changes in chromosomes and genes that are incompatible with life. We previously described aneuploidies in clinical cases of early pregnancy loss (EPL) but the relevance of other copy number (CN) errors remained unknown. To address this question, we are investigating 73 new clinical cases of EPL (collected between 2018 and 2023) and 102 abortions using multiple molecular DNA methodologies including medium and high-density genotyping arrays, Short Tandem Repeats (STR), Nextera skim-sequencing and digital droplet PCR. Screening is ongoing, with preliminary analysis providing evidence triploidy is very common in clinical cases of EPL (26/57) but not abortions (0/102). STR analysis of EPLs from pregnancies established following natural mating, revealed 3 alleles in both fetal and placental tissue of most putative triploids suggesting origins in polyspermy or errors in meiosis I. Trisomy 4 and 20 and monosomy 26 were additionally identified exclusively in the EPLs while a segmental aneuploidy was found in one abortion case (1/102) suggesting size of the genomic imbalance is key to outcome. To define specific fetal anomalies associated with CN errors, we have assessed a subset of fetuses by gross morphology and microCT revealing abnormalities of the central nervous system and asymmetrical growth disorders inconsistent with life.

An upstream deletion of the TUBB locus is associated with Early Pregnancy Loss in the Mare

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Early Pregnancy Loss (EPL) in the mare is an important health and economic problem in equine breeding programs and affects 7-15% of horse pregnancies annually. Of these losses, roughly 50% can be linked to copy number genetic aberrations such as aneuploidy (whole chromosome) and Copy Number Variations (CNV) characterized as the loss or gain of genomic segments from 1Kb to 1Mb in length. Data generated in the de Mestre lab using Single Nucleotide Polymorphism (SNP) arrays and Whole Genome Sequencing (WGS) identified a 4.5Kb deletion on chromosome 20 proximal to the start of the Tubulin Beta Class I (TUBB) gene in six EPL samples collected from the 2013-2018 breeding seasons and confirmed by PCR (Shilton Thesis, 2021). In a continuation of those findings, we screened tissue from 31 EPLs from the 2023 breeding season (20-TB, 2-WB, 9-Other). Briefly, 2023 EPLs were diagnosed by attending veterinarians, lavaged from pregnant mares, then sent to the laboratory for gross examination and dissection. DNA was extracted from placental tissue and tested via PCR for the TUBB deletion. We found 9 EPLs (29%) that harbored the deletion (5-TB, 0-WB, 4-Other). Currently we are mapping the upstream and downstream breakpoints of the deletion using primer walking, investigating conservation of the deletion locus between unrelated individuals, and acquiring parental samples to examine inheritance of the deletion. Finally, TUBB's location in the highly polymorphic Major Histocompatibility Complex (MHC) Class I region suggests it may be a bystander in structural rearrangement of this complex locus.

A genome-scan for homozygous haplotype deficiency in the Thoroughbred horse identifies variants for normal embryogenesis

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Deleterious recessive haplotypes tagging rare genomic variants disrupting embryonic and neonatal development have been reported in livestock but have not been reported in the Thoroughbred horse. Here, we tested the hypothesis that the Thoroughbred genome harbours deleterious recessive haplotypes carrying lethal/semi-lethal alleles segregating within the population that may disrupt normal embryonic and/or neonatal development. The objective was to catalogue haplotypes absent/depleted in the homozygous state and to identify candidate genes and variants causative of their depleted homozygosity.

N=14,931 Thoroughbred horses were genotyped on the Illumina Equine SNP70 BeadChip or the Axiom Equine Genotyping Array. An overlapping sliding window approach (0.25-10Mb) identified 3,212 significantly ($P<0.05$) depleted homozygous haplotypes. To further characterise 20 of these haplotypes, whole genome sequence data from n=29 Thoroughbred and n=70 mixed-breed horses identified 668 predicted high/moderate effect variants within +/- 1Mb of the haplotypes.

Eight variants within conserved sequences of seven genes with functions essential for normal embryogenesis were identified as candidates underlying the observation of depleted homozygosity. These mutations are located within haplotypes that occur at population frequencies 2.6-15.1%. Experiments to ascertain functional effects of these variants will lead to a better understanding of equine embryonic development. In practice, breeding management decisions to avoid carrier matings will have a positive impact on reproductive efficiencies. This would reduce the overall frequency of unfavourable haplotypes in the population and alleviate the impact of harmful recessive alleles on pregnancy. Analysis to validate these haplotypes in the population is in progress in an independent set of ~5,000 Thoroughbreds.

Molecular studies of Thoroughbred stallion subfertility due to impaired acrosomal exocytosis

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An idiopathic form of subfertility in Thoroughbred (TB) stallions with normal physical and semen parameters has been attributed to impaired acrosomal exocytosis (IAE). This impairment is significantly associated with a double-homozygous A/A-A/A genotype in *FKBP6* exon5 on chromosome 13 (Raudsepp et al. 2012; Castaneda et al. 2021). While this genotype is present across breeds, it is associated with subfertility only in TBs, suggesting *FKBP6* tags TB case-specific haplotypes containing causative variants. Our goal is to find these variants. Using our TaqMan genotyping assay for *FKBP6*, we identified 25 subfertile TB stallions with the susceptibility genotype. Illumina short-read sequencing data has been generated for 23. Initial alignment of TB cases with 428 horses from 46 breeds, including 54 non-case TBs, showed the variant landscape in a 110 kb region around the *FKBP6* IAE susceptibility genotype is fixed only in TBs. Inspection of 8,447 single nucleotide variants (SNVs) in all TBs determined a 171 kb TB case-specific haplotype with 38 implicated SNVs in 5 genes for further investigation. Sequences of all 23 TB cases are aligned to EquCab3 in combination with over 1100 other horses to provide variant landscape of unprecedented resolution for the region. In addition, PacBio Hi-Fi long-read data was generated for two TB cases resulting in a 20 Mb gapless contig encompassing the *FKBP6*-region, including a structurally complex region 5' to the 171 kb TB case-specific haplotype, not assembled in EquCab3. These long-read genomes have been incorporated into our equine pangenome and analysis of the variant landscape is ongoing.

Reference:

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Charting the equine miRNA landscape: an integrated pipeline and browser for annotating, quantifying, and visualizing expression

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MicroRNAs (miRNAs) regulate many biological processes in a temporal and tissue-specific manner via binding to complementary sequences of target mRNAs. Over 60% of human protein-coding genes contain at least one miRNA binding site, [1] and miRNA dysregulation has been implicated in numerous conditions [2–4]. Similar to protein-coding isoforms, a significant proportion of miRNAs have sequence and length variations (isomiRs), conferring potential altered targets and ultimately regulatory functions [5]. However, there are limited available systematically characterized miRNA/isomiR data in the horse. To address this gap, we developed a user-friendly pipeline (FARmiR: Framework for Analysis and Refinement of miRNAs) and expression browser (AIMEE: Animal IsomiR and MiRNA Expression Explorer), leveraging the most comprehensive equine miRNA data collection available. This includes small RNA-seq datasets aggregated from public data repositories, a Quarter Horse cohort, and the FAANG Thoroughbreds, resulting in a quality-controlled assortment of nearly 6000 miRNAs/isomiRs from 461 datasets from 61 different tissues. Here we present a brief overview of the open-source, containerized pipeline, including application, data management, and orchestration tools, together providing a reproducible solution for scalable and efficient data processing. Then we describe the global expression atlas, including tissue specificity, rank aggregation across data sources, and target profiling preceding over-representation analysis. Finally, we highlight multiple practical applications for AIMEE, demonstrating available data exploration and analytical capabilities. The large data collection processed with FARmiR and accessible via AIMEE, provides valuable information about the critical activities miRNAs and isomiRs play in tissue-specific regulatory functions contributing to equine production and health.

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High-quality, haplotype-resolved reference assemblies of the Friesian horse and the Dutch Warmblood horse using trio binning

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In horses, genetic diversity is predominantly observed between breeds, with little variation within breeds. The Friesian horse population is distinct from other horse breeds and suffers from consequences of inbreeding. Increasingly, genomic technologies are used to aid in the conservation of such breeds. The studbooks of the two largest horse populations in the Netherlands, the Dutch Warmblood horse and Friesian horse population, are routinely collecting genotypes. However, when assessing their DNA-profile, breed-specific variation will be missed because all polymorphism detections are biased towards the sequence structure of the current refence genome derived from a Thoroughbred horse. Here, we performed nanopore sequencing (R10.4, Q20+) of an F1 cross between a Dutch Warmblood horse and a Friesian horse to create two breed-specific reference assemblies using trio binning. Triocanu was used to bin the nanopore reads (80X) of the F1 cross using the short reads (50X) of the parents. Then, we used Flye and RagTag software to create and scaffold both assemblies. Scaffolded assemblies were validated with breed-specific linkage maps based on 70K genotypes. This resulted in two high-quality, haplotype-resolved reference assemblies (contig N50 was 35Mb and 37Mb and single copy gene completeness was 99.2% and 99.3%, for the Dutch Warmblood horse and Friesian horse, respectively). Ongoing analyses, are aimed to identify structural variants between the Dutch Warmblood horse and the Friesian horse. These high quality reference assemblies form a baseline for further genetic analysis in both breeds, which will improve breed conservation.

Mendelian and oligogenic variant discovery workshop and round table

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Our team is building an open-source, comprehensive, user-friendly, and validated pipeline to identify pathogenic variants from whole genome sequencing. This pipeline includes variant and candidate gene identification, and prioritization. In addition to building this pipeline, we are developing best practices for each step to optimize candidate gene and variant identification (analytical validity). We are also building a framework for clinical validation and interpretation of genes and variants which incorporates genetic (segregation, association, population allele frequencies), experimental (experimental data implicating the gene or variant) and informatic evidence (variant effect predictors, functional gene annotation, etc.). We propose a workshop that includes four 15-minute abstracts (listed below), hands on demonstration of the pipeline, and a roundtable discussion to gather input on the best practices for analytical validity, and framework for clinical validation and interpretation. Proposed components and timeline:

Genome-wide runs of homozygosity revealed sources of inconsistencies in the Axiom^{TM} Equine Genotyping array

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Runs of homozygosity (ROH), haplotypes identical-by-descent (IBD), are one of the main tools to derive genomic inbreeding (F_{ROH}). In livestock, previously reported concordance rates between F_{ROH} and pedigree derived inbreeding (F_{PED}) were moderate to high. In the framework of a genetic diversity study in Franches-Montagnes horses (FM), we recently observed conspicuously low F_{ROH} values compared to respective F_{PED}. In this study a total of 463 FM horses were genotyped on the 670K Axiom^{TM} Equine Genotyping array (602,131 SNPs mapped to EquCab3.0). Quality of genotyping was considered acceptable with a dish QC (DQC) ≥ 0.82 and QC call rate (CR) ≥ 97. Runs of homozygosity segments were determined with an overlapping window approach in PLINK v1.9 (minimum SNP density of one SNP per 50 kb, maximum gap length of 100 kb, minimum length of homozygous segments of 500 kb, one heterozygote SNP per segment). In general, F_{ROH} was higher than F_{PED}, but 24 horses (5%) had smaller F_{ROH} than F_{PED}. Ten of these horses, albeit purebred, carried even less ROH segments compared to F1 outcrosses. In general, outliers had slightly reduced DQC (between 0.875 and 0.985) and CR values (between 97 and 99). However, there were another 40 horses with the same attributes which did not show noticeable differences in the inbreeding comparison. The parameters DQC and CR fail to successfully identify horses for which genotype errors caused ROH breakdowns. We will further investigate these inconsistencies by re-genotyping the outliers to compile a list of unreliable SNP genotypes for future applications.

Gene Doping and RNA Applications in Human and Animal Models

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This keynote presentation delves into the intriguing realms of gene doping and RNA applications, with a focus on their implications in both human and animal contexts. Through an in-depth examination of the scientific and regulatory landscapes, this workshop aims to provide participants with a comprehensive understanding of these cutting-edge fields. The learning objectives encompass grasping the mechanisms of gene doping and RNA technology, discerning their potential benefits and risks, and evaluating the ethical considerations surrounding their application.

Content-wise, the presentation begins with an overview of gene doping, exploring its historical development and contemporary applications alongside emerging RNA technologies like vaccines. It then navigates through the scientific intricacies, elucidating the techniques involved and their potential impacts on performance enhancement and therapeutic interventions. The workshop also addresses regulatory frameworks governing gene doping and RNA applications, highlighting challenges in enforcement and the need for proactive measures.

Real-world examples and recent advancements are utilized to contextualize the discourse, ensuring relevance and applicability. By the end, participants will emerge equipped with a nuanced understanding of gene doping, RNA technologies, and their implications, ready to navigate these scientific complexities responsibly.

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Unraveling the genetic network of alternate gaits in Colombian Paso Horses: A multifaceted approach integrating genomics, machine learning, and objective motion analyses.

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Gait type and quality in horses constitute intricate neurological and musculoskeletal functions, with a substantial genetic contribution that remains largely unresolved. Accurately classifying horse gaits has proven challenging, especially in the context of alternate gaits in Colombian Paso Horses (CPH) (Novoa-Bravo *et al.*, 2018; Serra Bragança *et al.*, 2020). Our recent investigation into CPH gaits revealed that the differences between the trocha and Colombian trot gaits in CPHs are scarcely perceptible and cannot be attributed to the stop codon in the *DMRT3* gene (Novoa-Bravo *et al.*, 2018). To address this, we conducted an experiment to detect genomic regions associated with its alternate gaits. We assessed locomotion parameters of 225 CPHs using Inertial Motion Units (IMUs), classified the gaits using a Long-Short Term Memory (LSTM) neural network, and genotyped 85 of these horses using the 670K+ Axiom Equine Genotyping Array. GWAS detected a major QTL for gait type (trocha vs. Colombian trot, ECA16:8860175, rs1147402472, p-value=1.948955 x 10-08). Opposite haplotypes in this region accounted for 16.2% of the phenotypic variance, with trocha being associated with the most prevalent haplotype. Haplotype analysis pinpointed four candidate genes linked to variations in neurotransmission (*AT2PB2*, *LHFPL4*), development of the nervous system and sensory organs (*SRAGP3*), and cognition and learning abilities (*SETD5*). This research unveils a novel QTL associated with alternate gaits in horses beyond the *DMRT3* gene, demonstrating that the integration of machine learning, objective motion analyses, and genomics provides a promising route for unraveling the molecular mechanisms underlying complex gait traits.

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Employing Deep Neural Networks for precision phenotyping of selected kinematic traits in Arabian horses: a pilot study on heritability and the effect of inbreeding

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Lack of precise, affordable, and efficient phenotyping methods is a limiting factor in modern equine genetics. Our study addresses this challenge by employing Deep Neural Networks (DNNs) to accurately apply anatomical landmarks within video datasets for quantification of selected kinematic traits. We aim to assess the size of the genetic effect for these traits by estimating the heritability using pedigree data, and to examine the impact of inbreeding. We trained a DeepLabCut (DLC) model with 193 videos of Arabian horses from halter shows, achieving the test error of 12.93 pixels in 720p resolution. The model was subsequently applied to video recordings of 13 elite Arabian stallions and their 78 offspring (6 from each stallion), encompassing a diverse range of age and sex groups. After extracting coordinate values for key landmarks, we utilized previously developed DeepLabCut-Display extension to calculate nine key conformational and kinematic traits. Heritability of analyzed traits was low (h^2=0.18) for forelimb range of motion to moderate (h^2=0.32) for the topline angle. No effect of inbreeding was observed within the analyzed dataset.

Our preliminary findings suggest a genetic influence on kinematic traits in equines. This lays the groundwork for subsequent investigations utilizing more intricate models that encompass a broader spectrum of traits. Furthermore, expanding the sample size to include greater diversity is crucial. Consequently, our methodology holds promise for unraveling the genomics underlying equine movement.

Deep Learning Application for Predicting Endurance Horse Racing Performance via High- Density Genotyping

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In the last two decades, advancements in DNA sequencing and genotyping have transformed the collection of vast genetic data, crucial for refining animal breeding selection. Simultaneously, the rapid evolution of artificial intelligence has sparked interest in its application to genomic selection (1,2). Despite its potential, using Deep Learning to predict horse performance based on genotypes remains largely unexplored.

This study utilized data from 1072 horses to forecast three performance indices in endurance races from the genotype data using various Deep Learning methods. We experimented with different architectures: MultiLayer Perceptrons (MLP), Convolutional Neural Networks (CNN). Model hyperparameters and learning were optimized using a validation set. Our meticulous comparison of prediction accuracy, assessed through mean square root error (MSE) and correlations between actual and predicted performance indices, included six distinct MLP models and one CNN model against traditional linear methods like Lasso and Bayesian ridge regression.

The predictive models of racing distance were sorted from the most effective model to the poorest: Bayesian regression (MSE$\pm$SD=392$\pm$32) > basic MLP (785$\pm$134) > MLP with pedigree input (469$\pm$34) > MLP with chromosome architecture input (538$\pm$81) > CNN (881$\pm$598). The CNN model exhibited lower efficiency and seemed less suitable for genomic data. Among the MLP models, those incorporating pedigree data showed promise as the most effective predictive models. In addition, the chromosome architecture demonstrated improved accuracy compared to other designs.

These initial findings suggest that significantly expanding the dataset size and refining deep learning models tailored to genomic data could notably enhance predictive accuracy.

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Optimizing Mendelian and oligogenic disease discovery: tools, best practices, and framework for clinical interpretation

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Variant discovery by whole genome sequencing (WGS) is an exciting opportunity to identify causal mutations for rare diseases. However, making the leap from WGS variant identification to disease causation is not straightforward. In one study, nearly 30% of human disease-causing mutations were subsequently refuted[1]. Similarly, recently published and/or commercialized equine mutations lacking sufficient evidence supporting pathogenicity have been invalidated[2,3]. False-positive pathogenicity assignment results from poor analytical or clinical validation, or incorrect clinical interpretation. Analytical validation encompasses mapping, read alignment, and variant calling and requires workflows optimizing precision, recall, sensitivity and specificity for single nucleotide and structural variants. Clinical validity relies on optimized variant annotation, filtering, classification and prioritization. Accurate clinical interpretation requires genetic, informatic and experimental evidence connecting both gene and variant to phenotype. Currently, there are no documented best practices for mapping and alignment, variant calling, annotation, effect prediction or prioritization, nor candidate gene identification and prioritization in horses. We are creating an open-source, user-friendly, containerized pipeline to identify and prioritize putative disease-causing variants using WGS, and developing best practices to optimize variant calling, annotation and effect prediction, candidate gene identification, and candidate gene and variant prioritization. These workflows will incorporate our catalog of genetic variation and mRNA, lncRNA and miRNA tissue expression and co-expression atlases. We will present our pipeline and validated best practices for variant and candidate gene identification; and our proposed framework for clinical validation and interpretation of variants (i.e., annotation, effect prediction, prioritization) and candidate genes (i.e., tool selection and prioritization).

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Identification of the role of SEL1L in platelet function with implications for Atypical Equine Thrombasthenia in Thoroughbred horses

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Atypical Equine Thrombasthenia (AET) is a heritable platelet disorder found only in Thoroughbreds due to aberrant platelet signaling after thrombin stimulation, preventing platelets from fully activating and leading to prolonged bleeding. Despite the negative effects on horse health and racing performance, the underlying etiology of this disorder is unknown. Here we show that a missense variant in *SEL1L*, a gene not previously known to be involved in platelet function, leads to AET. To identify the underlying genetic mechanism, we performed a whole-genome association study using five affected, one equivocal, and eleven control Thoroughbreds. Filtering resulting associated variants based on expression databases, RT-PCR, and protein expression identified *SEL1L* c.1810A>G p.Ile604Val as a variant of interest. Flow cytometry and immunofluorescence studies demonstrated that SEL1L is located intracellularly in equine platelets, but not in the α-granule, and moves to the surface of the platelet upon activation with thrombin. Although the template bleeding time was normal, platelets from horses that were homozygous for the *SEL1L* variant showed defective spreading on collagen. Differentiation of human megakaryocytes revealed the presence of two SEL1L protein isoforms, p100 and p38, and both showed increased expression during megakaryopoiesis, although only p100 was delivered to mature platelets. An *Mx1-Cre*$^{+}$*;Sel1l*$^{fl/fl}$ conditional knockout mouse model and a CRISPR/Cas9 mediated genome edited *SEL1L* knockout zebrafish line demonstrated that *SEL1L* is necessary for efficient platelet or thrombocyte (fish equivalent) adhesion to exposed collagen after endothelial injury. Overall, these data support the conclusion that the *SEL1L* missense variant leads to AET in horses.

Putative Functional Genetic Variant for Equine Juvenile Spinocerebellar Ataxia in Quarter Horse Foals

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In 2020, a novel neurologic disease was observed in juvenile Quarter Horses (QHs) in North America. Twelve related QH foals developed an acute onset of ataxia between 1-4 weeks of age. We defined the clinicopathologic and histologic findings, determined the mode of inheritance, isolated an associated genomic region, and identified a putative functional variant for this disease, termed equine juvenile spinocerebellar ataxia (EJSCA). All foals had a history of acute onset of neurological deficits with no history of trauma. Neurological deficits were characterized by asymmetrical spinal ataxia, with pelvic limbs more severely affected than thoracic limbs. All foals became recumbent, necessitating euthanasia. Histological evaluation at postmortem revealed dilated myelin sheaths with digestion chambers within the spinal cord, most prominent in the dorsal spinocerebellar tracts. Pedigree analysis revealed a likely autosomal recessive mode of inheritance. Whole-genome sequencing was performed on *n*=7 affected, *n*=8 unaffected related and *n*=28 unaffected unrelated QHs and identified a 2.28 Mb region of association on chromosome 11, with 77 variants segregating with the phenotype. Nine of these variants, which were all non-coding, were not identified in any other breed based on publicly available datasets. Within this refined 82 kb region, three annotated genes (*FDXR, GRIN2C* and *TMEM104*) are expressed in nervous tissue. RNA-sequencing was performed on spinal cord tissue from 5 EJSCA-affected and 7 age-matched control foals, and *FDXR* was significantly downregulated in EJSCA-affected foals. RNA-sequencing was used to determine which of the 5 *FDXR* candidate variants were the most likely putative functional variants.

An intronic copy number variation in Syntaxin 17 determines speed of greying and melanoma incidence in Grey horses

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The Greying with age phenotype in horses involves loss of hair pigmentation whereas skin pigmentation is not reduced and a predisposition to melanoma. The causal mutation was initially reported as a duplication of a 4.6 kb intronic sequence in *Syntaxin 17* (1). The speed of greying varies considerably among Grey horses. Here we demonstrate the presence of two different *Grey* alleles, *G2* carrying two tandem copies of the duplicated sequence and *G3* carrying three. The latter is by far the most common allele, probably due to strong selection for the striking white phenotype. Our results reveal a remarkable dosage effect where the *G3* allele is associated with fast greying and high incidence of melanoma whereas *G2* is associated with slow greying and low incidence of melanoma. Epigenetic analysis, based on nanopore sequencing of genomic DNA, reveals a drastic reduction in DNA methylation in part of the duplicated sequence harboring MITF binding sites. The copy number expansion transforms a weak enhancer to a strong melanocyte-specific enhancer that underlies hair greying (*G2* and *G3*) and a drastically elevated risk of melanoma (*G3* only).

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Developing a genomic prediction model for recurrent exertional rhabdomyolysis risk in racehorses

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Recurrent exertional rhabdomyolysis (RER) affects 10% and 25% of US Thoroughbred (TB) and Standardbred (STDB) racehorses, respectively. RER is a complex genetic disorder with hundreds of alleles contributing to disease risk. We designed a custom genotype-by-sequencing assay for ~22,000 genetic variants in a cohort of 971 TB and STDB racehorses. Variants were selected using these criteria: 1) Significant allele frequency differences between cases and controls; 2) located within 100 kb of a region of interest (ROI) from Random Forest analyses; 3) located within 500 kb of a ROI from genome-wide association analysis; 4) located within a biological or positional candidate gene; 5) the predicted functional effect. Here we define and validate a genomic prediction model for RER using 1,433 RER-phenotyped TBs and STDBs genotyped for 22,688 variants. A 676 horse discovery cohort was used to create initial models with 10-fold cross validation[1] each with 500 iterations of random forest classification. Performance was evaluated with receiver operating characteristic (ROC) area under the curve (AUC) values. Using the cross-validation subset with the AUC nearest to the median, backwards elimination was used to select the optimum number of variants[2]. The best model had 206 variants and an AUC of 0.82 in the discovery cohort. Next the model was tested in an independent validation cohort of 757 horses, where the median AUC was 0.82. This represents the first genomic prediction model of a complex disease in horses. On-going work includes further model refinement and developing a commercial genetic test.

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Poster
N°

Poster Abstracts

Poster
N°

Topics1:

Equine evolution, breed development and management

Hotspots for centromere formation in horse and other Perissodactyla

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Centromeres are essential chromosomal loci which are epigenetically specified by the histone H3 variant CENP-A and typically associated with highly repetitive DNA (satellite DNA). Although satellite DNA is a common feature of mammalian centromeres, we previously demonstrated that in equids several centromeres are satellite-free and emerged recently during evolution following centromere repositioning (shifting of centromere function without chromosome rearrangements) or centric fusion (Wade et al. 2009; Piras et al. 2010, Nergadze et al. 2018, Cappelletti et al. 2022, Piras et al. 2023).

The family Equidae belongs to the order Perissodactyla together with Tapiridae and Rhinocerotidae. While equid karyotypes underwent rapid evolution, the karyotypes of tapirs and rhinoceros remained quite stable and similar to the hypothetical ancestral karyotype. The only exception is the Malayan tapir (*Tapirus indicus*) whose karyotype was restructured through a series of fusions.

We identified by ChIP-seq with an anti-CENP-A antibody two satellite-free centromeres on chromosome 4 (TIN4) and chromosome 15 (TIN15) of the Malayan tapir. These centromeres emerged from centromere repositioning. TIN4 and horse chromosome 11 (ECA11) are colinear and carry a satellite-free centromere at orthologous positions. Similarly, TIN15 is orthologous to donkey chromosome 8 (EAS8) and the position of the satellite-free centromeres is conserved in the two species. Given the evolutionary distance between the Tapiridae and Equidae families, these results suggest the presence of hotspots for neocentromere formation in the genomes of Perissodactlya. In addition, these findings prove that the exceptional plasticity of centromeres is present in other Perissodactyla besides equids.

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Reference quality genomes for Egyptian Arabian and Shire horses and future applications

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The development of the pangenome for the horse requires the utilization of multiple reference quality genomes. Previous studies focused on the assembly of a reference quality genome for a single breed, the Thoroughbred. Given the need to be better capture the genetic diversity of the horse, in addition to the Thoroughbred, our objective is to assemble reference quality genomes for the Shire and Egyptian Arabian breeds. DNA was collected and extracted from the Egyptian Arabian sire, Shire mare, and their F1 offspring. Short read whole genome sequencing from the sire and mare and HiFi and HiC reads from the F1 were utilized to scaffold and assemble the two genomes. In addition, IsoSeq data from the F1 was generated from eight tissues: chorioallantois, dorsal skin, hoof skin, endometrial cup, gonad, lung, kidney, endothelium. Our results indicate we have two high reference quality genomes with scaffolding N50 lengths of 81,541,174 and 83,120,118 for the Egyptian Arabian and Shire, respectively. This is an improvement from the current EquCab3.0 Thoroughbred reference. In addition, we mapped IsoSeq data from the F1 hybrid to both parental haplotypes. Here we noted preliminary results on the mapping quality which differed between the two parents. Future efforts include implementing 5methC data from the HiFi reads. This is an exciting opportunity to conduct parent of origin allele specific expression which will improve the assembly of the maternal and paternal chromosomes.

Whole Genome Sequencing of 171 Arabian horses provides insights into the population structure and contribution to global equine breeds

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The Arabian horse, long celebrated for its grace and endurance, has significantly influenced equine breeds globally. While genetic and single nucleotide polymorphism (SNP) array-based genomic data have provided important insights into the population structure of the Arabian breed, the broader potential of whole genome sequences (WGS) remains under-explored. Here, we present a large-scale WGS-based joint population genomics analysis of 533 horse samples, combining publicly available data with 171 horses from multiple Arabian strains sequenced for this study. Altogether, the dataset represents several breeds from the Middle East (n=186), Central Asia (n=15), East Asia (n=72), Europe (n=196), and North America (n=64). Population structure analysis showed that Arabian horses have markedly influenced European and American warmblood breeds. Admixture analysis revealed a shared genetic component between Arabians and Central and East Asian breeds, including Akhal-Teke, Kazakh, and Mongolian. Principal Component Analysis (PCA) distinguished horse breeds based on geographic and genetic ancestry, with Arabians, Thoroughbreds, Asian, and European breeds forming distinct clusters. In tracing the maternal lineage of Arabian horses, our combined mitochondrial-based phylogenetic and pedigree analyses linked the majority of our Arabian samples to 16 foundational maternal great-granddams from the 19th century. Furthermore, window-based genomic comparisons between Arabian horses and other breeds revealed distinct genomic differentiation (Fst) in regions associated with sensory functions, particularly olfactory and auditory processing, indicative of selective pressures. This research highlights the Arabian horse's genetic legacy in global equine breeds and underscores the importance of WGS in understanding breed evolution.

Runs of homozygosity and genomic inbreeding in the German riding horse population

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In Germany, most of the studbooks for riding horses have introduced routine genome-wide SNP genotyping of all registered foals. Together with respective SNP genotype data generated in the context of projects on implementing genomic evaluations and breeders' support through analyses of genetic characteristics, genomic information on over 68,000 German Warmblood horses is stored in the central equine SNP database. Because genetic diversity is an important topic not only for native breeds and small populations, but also for the European Warmblood riding horse with its international and mostly liberal breeding policies, the aim of this study was to determine key parameters needed for assessing genomic inbreeding and development of genetic diversity. 70K+ SNP genotypes of 64,318 German Warmblood horses were used to determine runs of homozygosity (ROH) using PLINK software with its function '--homozyg' (Purcell et al. 2007). Information on 57,286 quality-controlled SNPs entered the analyses performed with a range of lengths of DNA stretches considered as ROH: 0.5 Mb, 1 Mb, 1.5 Mb, 2 Mb, 3 Mb, 4 Mb, 5 Mb, 6 Mb and 10 Mb. The minimum number of SNPs within one ROH was set to 25; no heterozygotes were allowed. For each individual and each scenario, genomic inbreeding based on ROH was calculated according to McQuillan et al. (2008): F_{ROH} = total length of all ROH / autosomal genome length covered by SNPs. Mean F_{ROH} ranged between 0.9 and 7.9%, with higher values for the scenarios with shorter ROH lengths (highest with 0.5Mb). Considering SNP density across autosomes, results obtained with 2 Mb, indicating a mean F_{ROH} of 6.9%, should best reflect the genomic inbreeding in the German Warmblood population. Low frequency of long ROH of 10 Mb in the population, with only 69.4% of the horses (N = 44,640) having at least one of them (mean F_{ROH} = 0.9%), imply smaller role of recent than more historical inbreeding. Further analyses on the genomic population structure, also separated by breed, and on possible signatures of selection based on ROH are planned. Results are supposed to serve as basis of new tools for support of mating planning and improved breed management, increasing visibility of the benefits of large-scale availability of equine genomic data.

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Skim sequencing: a high-throughput technique for Thoroughbred horses genotyping

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Recent advances in next-generation sequencing (NGS) approaches have made high-throughput genotyping more practical and affordable. Over the past ten years, many genotyping methods have been developed (Adhikari et al., 2022). Skim, or low pass sequencing is a low- coverage and low-cost method that can play a large role in the future of animal breeding and genetic investigations. In this study, we downsampled 20X WGS to emulate skim sequencing data to identify a large number of polymorphic markers, study genetic diversity, and assess de novo SNP discovery at a low cost on almost two hundred North American Thoroughbred horses. Here we obtained fastq files with 20x coverage from the Illumina platform. All samples were downsampled to decrease the coverage to between 10X and 1X. For evaluation of the impact of the sequence depth, different ranges of the minimum read depths were applied. The generated SNP sets were then compared with the genotype data derived from 20X coverage and the GATK Best Practice Pipeline to assess the genotype accuracy (Malmberg et al., 2018). The total number of samples used in the current study is ideal for producing an accurate SNP profile, however further data analysis is essential to evaluate the utility and accuracy of this approach for the North American Thoroughbred.

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Genetic Diversity and Inbreeding in the Australian Heritage Brumby

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Locally referred to as “brumbies”, Australia contains the largest global population of feral horses, estimated at over 1,000,000 individuals. Brumbies are considered an invasive species in Australia, where they reportedly contribute to a range of unfavourable ecological events, such as soil compaction and degradation of native species. To minimize the impacts of brumbies on the environment, Australia’s current national response centres on managing population size, primarily via population reduction. Current program goals and methods to reduce the population fail to account for the genetic viability of the remaining brumbies, both those remaining in the wild, and the fostered, passively trapped brumbies. Current non-random culling and the associated subsequent population bottlenecks present significant welfare concerns for the post-cull population and future generations of brumbies. As such, the current study investigates genetic diversity and genomic inbreeding levels in a population of brumbies that reside in Australia’s Snowy Mountains. Hair and blood samples from passively trapped brumbies (n=275) were collected from 2020-2023. Genomic DNA was extracted from these samples and genotyped on either the 670K Axiom Equine Genotyping Array (n=96) or the Illumina 80K Infinium Array (n=179). Preliminary analyses (n=70 horses) indicate that inbreeding coefficients vary greatly across trapping locations within the Snowy Mountains and place the mean inbreeding coefficient at 0.21. Final results from the complete data will be presented at the workshop. Results aim to fill a critical knowledge gap in the brumby conservation/eradication debate in Australia and are expected to provide quality information for future national feral horse management strategies.

Centromere protein B and chromosome stability in equids

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Centromeres are nucleoprotein structures essential for chromosome segregation during cell division. Their function is not determined by the underlying DNA sequence, but epigenetically specified by the histone H3 variant CENP-A. CENP-B is the only centromeric protein binding a specific DNA sequence (CENP-B-box), which is typically contained in the major centromeric satellite repeat. Although CENP-B and its binding site are conserved among taxa its role remains elusive.
Given the coexistence of satellite-free and satellite-based centromeres, the genus *Equus* is a powerful model system to study the function of centromeric proteins. Here, we demonstrated that the numerous satellite-free centromeres that we previously identified in horse, domestic donkey, Grevy's and Burchell's zebra (Wade et al. 2009; Piras et al. 2010, Nergadze et al. 2018, Cappelletti et al. 2022) do not contain any CENP-B-box and are not bound by the CENP-B protein. Surprisingly, the major CENP-A binding horse satellite (Cerutti et al. 2016), does not contain any CENP-B-box which is instead present in a previously undescribed repeat. In horse, CENP-B binds only a fraction of primary constrictions while in donkey and Burchell's zebra, although a functional protein is expressed, no CENP-B binding could be detected at centromeres. In Grevy's zebra, CENP-B binds several non-centromeric sites corresponding to ancestral inactivated centromeres. Our results suggest that the uncoupling between CENP-B and centromeres is responsible for the evolutionary instability of equid centromeres. A model for the evolution of satellite repeats and their role in centromere stability will be presented.

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Title: The genomic history of modern horse breeding

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Horses have played a pivotal role in human history, providing means of fast transportation and military support throughout the last 4,200 years. The past genetic diversity of horses has been mapped with extensive genome time-series across Eurasia, but its legacy to the modern world remains largely unknown. A large-scale genomic characterization of non-industrial breeds, populations of village and/or feral horses, and specific geographic is still especially lacking. In this study, we have considerably extended the characterization of the genetic diversity present in modern horse populations, to map patterns of variation, inbreeding and admixture across the world. We have also significantly expanded the number of ancient horse genomes available for the time period covering the last 2,000 years to investigate the main genetic sources of modern diversity. Our work not only provides comprehensive genomic resources for the horse, but also advances our understanding of the breeding practices that have shaped patterns of diversity present in modern populations.

Opportunities and Barriers in cross studbook transition from Microsatellites to Single Nucleotide Polymorphism

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The equine industry in Ireland is in the process of transitioning parentage verification of sport horses from microsatellite (MS) markers to single nucleotide polymorphisms (SNP). Previous research has investigated the benefits of SNP genotyping. However, limited knowledge exists regarding the applied transition process from MS to SNP. Some studbooks have supported their transition by imputing MS markers from SNP genotypes (Nolte et al., 2022). However, this is not always practical or feasible for studbooks with limited genetic resources. In Ireland, a different approach was taken whereby a reference library of SNP genotypes from approx. 8,500 mares and stallions was created with funding from the Irish Department of Agriculture Food and the Marine. The reference library has been utilised for parentage verification of foals born in 2023 however, MS testing is still widely used where genotypes for parents are unavailable. Learnings from the first year of implementation include that a reference library smaller than the collective foal crop is not adequate, particularly for cross bred populations. The referencing exercise should be extended or avenues such as SNP-SNP imputation explored to facilitate the development of internal processes and minimise the generational gap between progeny and parents. Studbooks should seek collaborative funding avenues across studbooks with similar breeding programmes for SNP marker sharing, minimising the re-sampling of horses existing on a studbook database. Studbooks should clearly indicate the testing platform on which individual animals have been genotyped/verified, facilitating breeders and studbooks to determine if re-sampling or SNP marker sharing is appropriate.

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Optical mapping and functional analysis of a complex X-autosome rearrangement in a Thoroughbred mare

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Complex structural X chromosome abnormalities are rare in mammals but provide unique opportunity to evaluate X chromosome content and functional status with the phenotypic effects. We recently described the first equine case of a complex unbalanced X-autosome rearrangement in a healthy but small (134 cm) Thoroughbred mare with a karyotype 2n = 63,X,der(X;16) (Mendoza et al. 2020). The derivative chromosome (der) was large, dicentric and comprised of normal Xp, a palindromic duplication of Xq12q21 to which chromosome 16 was attached, and the distal Xq22q29 was lost. Here we used Bionano optical genome mapping (OGM) to determine sequence features of the rearrangement sites and immunostaining to determine the functional status of the normal X and der. OGM detected only Xq22q29 deletion but not the Xq12q21duplication or X;16 translocation – most likely because EquCab3-X assembly is incomplete, missing complex regions of X. Functional analysis by BrdU incorporation in fibroblast cultures during early and late S-phase, followed by immunostaining with BrdU antibodies, revealed that the normal X was early replicating and active, and the rearranged X was late replicating and undergoing X inactivation (XCI).
This was confirmed by immunostaining with anti-5-methylcytosine antibody showing more methylation in der than normal X. These analyses also showed that while the rearranged X was inactivated, XCI had not spread into the chromosome 16 portion. Finally, we used antibody for CENPA - a protein that defines functionally active centromeres, to show that the dicentric derivative chromosome retained active X centromere and silenced chromosome 16 centromere.

Reference:

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Genome-wide association study for Roan coat color in Belgian draft horses

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The Belgian draft horse (BTP), an emblematic breed renowned for its strength and gentle temperament, is originated from Belgium and was imported in America. The European population has evolved differently compared to its American counterpart, especially in terms of coat colors. In Belgium, the roan coat color became and is still predominant. This color is characterized by a mixture of colored and white hairs evenly distributed throughout the body. The aim of this study was to identify genomic regions associated with this color in BTP. High-density SNP genotypes (670K) were available for 299 BTP horses and were divided into two groups: 203 horses with a roan coat color: bay roan, blue roan or red roan) and 96 horses without a roan coat color: bay, black or chestnut. A quality control was performed on the 299 horses and the 31 autosomes using PLINK and consisted of: (1) removal of horses with a call-rate ≤0.95, outlying heterozygosity and (2) removal of SNPs with a call-rate ≤0.95, minor allele frequency ≤0.05 and Hardy-Weinberg equilibrium ≤0.0001 in non-roan horses. After quality control, 292 individuals (198 roan and 94 non-roan horses) and 352,981 SNPs were retained. A genome-wide association analysis accounting for genetic relationships between individuals was performed using EMMAX. After Bonferroni correction, we identified 261 genome-wide significant SNPs located on chromosome 3 (ECA3). The top SNPs were located in the *KIT* gene supporting results from previous studies in other horse breeds (Grilz-Seger et al., 2020; Voß et al., 2020; Marklund et al., 1999).

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Variations in the genomic landscape associated to the onset of inbreeding depression in fertility parameters of Pura Raza Español horses.

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Inbreeding depression is a known genetic effect that decreases fertility in animals. Nowadays, the age of genomics allows us to analyze this phenomenon at the chromosome level, gathering much more precise conclusions. In mares, we demonstrated the negative effect of a genomic inbreeding increase (FROH) in phenotypic values of reproductive efficiency [1]. Hereby we analyzed the same effect but analysing using a gBLUP approach at chromomose level. Phenotypes of four reproductive traits (Average_foaling_number (AFN), interval (AFI) and productive life (APL) and age_at_first_foaling (AFF) and genotypic data (~60K SNPs per individual) were analyzed. We first determined individual FROH values per chromosome using DetectRuns[2]. Those values were employed to estimate the inbreeding effect using a multivariate gBLUP approach including coat colour, herd size, herd of origin and age at last foaling^2 as fixed factors and the FROH (whole or per each chromosome) as covariate. All the calculations were made using BLUPF90+[3] program suite. The effect of was estimated as the solution for the FROH covariate. The average FROH was 0.07, ranging from 0.066 (ECA26) to 0.112 (ECA2). The increase in FROH delayed AFF (1.14), extended AFI (2.15), and reduced AFN (-0.42) and APL (-1.60). However, results per chromosome were highly variable, showing some ECA a more significant effect than whole_FROH in some cases. However, some ECA´s showed a non-significant effect, whereas in a few cases and traits, increased FROH showed the the opposite opposite to the observed in whole FROH. To conclude, a chromosome-level evaluation of FROH could improve the detection of inbreeding depression in fertility traits in horses.

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Genome structure survey of Caspian horse breeds by microsatellite markers

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During the last few decades, the number and the effective population size (Ne) of Caspian horse breeds has declined drastically, resulting in the classification of them into the critically endangered category. The present study was undertaken with the objective of evaluating all aspects of genetic diversity, and population structure of Caspian horses on a large scale using 17 microsatellite markers data. For this purpose, we use a large set of the microsatellite genotyping results (from 1982 to 2022) from 971 Caspian horses from the Equestrian Federation of Iran (114), Animal Science Research Institute of Iran (ASRI) (387), Caspian Conservation Society (CCS) (110), and registry authorities from the United States (275), Sweden (25), Norway (35), and UK (25), typed at 17 microsatellites. The number of alleles observed for each locus was 4 (HTG7) to 16 (ASB17) alleles with an average of 10.11. The average expected heterozygosity in the Caspian population of Iran was (0.79 ± 0.04) and the Caspian population of foreign countries (0.73 ± 0.13). The highest polymorphism in the populations was 1.80 ± 0.22 for the Caspian of Iran and 1.51 ± 0.45 for the Caspian of foreign countries. Bottleneck studies revealed that no recent bottleneck problem has taken place in both the population. This study contributes to our knowledge of the genetic diversity of the Caspian horse population and it can be concluded that the above results are a guide for further implementation of effective programs for the conservation of the Caspian horse breeds.

Survey of population structure and linkage disequilibrium pattern of Iranian native horse breeds by SNP markers

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This research was carried out with the aim of investigating the structure and genetic distances, inbreeding, linkage disequilibrium (LD), and understanding the relationships between native Iranian horse breeds. For this purpose, 167 horses from Arabian Asil (24), Caspian (22), Dareshouri (15), Kurdish (66), and Turkmen (40) breeds were used. The samples were genotyped using Illumina 70k SNP equine bead chip. Data quality control was applied and loci with MAF < 2%, Mind < 5%, HWE ($<10^{-6}$), and genotype call rate < 1% were removed. Finally, 159 horses with 45,270 SNP loci were reminded for genetic structure analysis of samples. Clustering analysis results showed that in K=5 Caspian and Kurdish breeds were placed in a common cluster, and Turkmen, Dareshouri, and Arabian Asil breeds were also located in distinct clusters. The LD decay among all breeds showed a significant decrease below 400 bp. Caspian and Dareshouri breeds experienced the lowest and highest LD and the highest and lowest effective population size, respectively. The inbreeding value based on the runs of homozygosity (ROH) were highest and lowest for Arabian Asil and Caspian breeds, respectively. The results of this study showed that the Iranian native horse breeds were well identified by SNP markers, which can be used for stud base foundations of breeds with more accuracy.

Evaluating signatures of selection associated with equine adaptation to wetlands

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Brazil is home to diverse, locally adapted, equine types. Over five centuries of selection helped shape unique horses specially adapted to hot and humid wetlands, located in the Northern region (Baixadeiro, Puruca and Marajoara) and the Midwest (Pantaneiro). By leveraging publicly available datasets (Petersen et al., 2013; Nogueira et al., 2021) combined with a dataset of 23 Campolina horses genotyped using the GGP Equine (Illumina Inc.) we investigated the association of four Brazilian wetland-adapted breeds (N=141, Baixadeiro, Marajoara, Pantaneiro and Puruca) in comparison to 31 breeds of world distribution (N=727) and 6 Brazilian autochthonous breeds (N=93), non-adapted to wetland environments. After merging the datasets and filtering for genotyping rate, 36,566 SNPs remained with a genotyping rate of 0.998 for 961 individuals. Underlying kinship was evaluated with Plink2.0 to assure no 1st degree relationship between samples. We calculated *FST* (Weir, 1996) using GCTA (Yang et al., 2011) comparing between wetland-adapted and non-adapted populations. Four SNPs with *FST* ≥ 0.45 denoting strong divergence between populations targeted 125 kb upstream/downstream regions on ECA3, ECA18, ECA19 and ECA20, based on approximate LD values for the horse. While investigation of Tajima's D and π approaches are ongoing, *FST* regions included the *Protein Phosphatase 3 Catalytic Subunit Alpha* (*PPP3CA*), required for normal differentiation and survival of keratinocytes, providing interesting wetland-adaptation candidates. As climate change will increase both temperatures and flooding, especially in tropical regions, understanding genomic factors leading to equine adaptation may be important for improving horse welfare in the future.

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Comparative genetic analysis of equine populations by parallel genotyping for Short Tandem Repeats (STRs) and High Density SNP array (670K HD)

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Polymorphic Short Tandem Repeats (STRs) have been instrumental for accurate and efficient identification of individual horses, parentage verification, determining the genetic structure and relatedness of breeds, evaluating the status of endangered populations, and proposing genetics-based measures for management, protection, and conservation.
Importantly, STR genotyping data is available for many diverse horse breeds and populations, including indigenous, rare and/or endangered ones. Since the discovery of millions of single nucleotide polymorphisms (SNPs) in the horse genome and the development of three generations of equine SNP chips (50K, 74K, and 670K High Density, HD), genetic analysis of main commercial breeds has shifted towards this platform. The latter provides more comprehensive genomic data but is also more expensive and bioinformatics dense. As a result, most journals prefer SNP-based population studies and consider STR data outdated, despite the wealth of data and low cost. This is prohibiting for small groups that work with limited funding on rare breeds or feral horses, essentially reducing our knowledge of these populations. Here we obtain detailed population genetic information for four historic European draft breeds (Swedish Ardennes, Estonian draft, Lithuanian draft, Haflinger), 24 horses each, by parallel genotyping the same individuals for STRs and 670K HD. We have completed STR genotyping analysis and will start SNP data analysis shortly. We anticipate STR and SNP data will give similar results for basic population genetic parameters and prove that STR genotyping remains a valid and valuable approach for genetic evaluation of equine populations.

The Arabian horse population from Poland. An excellent example of cooperation between science and practice.

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The Arabian horse is the second most influential breed worldwide, where breeding selection related to socioeconomic, historical or even esthetical purposes created separated subpopulations. Previous international cooperation, concerning analysis of genetic diversity, clearly showed segregation into subgroups and the Polish population was one of them (Cosgrove et al., 2020).
Over 200 years of meticulous breeding work in State Studs, consisting in detailed phenotypic assessment and improvement of desired traits, has developed a consolidated population that is excellent for research purposes. In addition, breeding horses have full pedigree and phenotypic documentation, including biometric values, results of performance tests on race tracks and endurance competitions, full veterinary documentation, detailed reproductive documentation for mares and stallions. Additionally, photographic and film documentation are also available.
For almost 10 years, our team has used the NGS methods to support and improve breeding decisions. One of the first findings was identification an association of *SLC16A1* gene with improvement of racing performance (Ropka-Molik et al 2019). More recently, we used RNA seq data to explore pathways and genes determined endurance ability during competitions held at 120 km (Myćka et al. 2023), providing further support for regions under selection pressure in Arabian horses. To improve the association analyses and facilitate the search for rare SNPs, we create a tool for filtration of genetic variants in Arabian horse based on WGS of 120 healthy individuals from Poland, Syria and USA populations (Szmatoła PAG 2024).
The latest research cooperation with State Stud resulted in implementation of Infinium Equine iSelect microarray to deeper genetic evaluation. 576 Arabian horses were included in study. First we improved filtration tool on microarray results, then the general genetic population structure analysis was performed, according to genomic inbreeding and ROH patterns, revealing certain and exclusive regions for the breed. Based on phenotypic data, we also conducting a number of association studies to reveal genomic regions involved in coat color, virtiligo, and conformation traits.

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Comparative Genomic Characterisation of the Rare Native Irish Kerry Bog Pony Breed

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The Kerry Bog Pony is an endangered native Irish equine breed. The breed represents an important aspect to Ireland's cultural heritage, where the Kerry Bog Pony served as a working pony throughout recent generations. Advancement of mechanical transport methods and road networks realised a drastic decline in Kerry Bog Pony numbers, to as little as 20 animals in the mid-1990s. To better understand this breed's current genetic status, our study utilised genome-wide single nucleotide polymorphism data to establish an across breed comparative snapshot of key genomic characterisation parameters within an actively breeding cohort of 192 Kerry Bog Pony animals. Comparatively high genetic diversity (Ho = 0.30), low inbreeding (F = -0.009) and reduced runs of homozygosity (ROH) counts (1.87 counts less than average) provided evidence for a highly variable gene pool within the Kerry Bog Pony. Breed relationship and population structure analysis provided insights into core drivers behind high gene pool variability, where observed gene flow events with Welsh A and Shetland pony breeds aligned with recent derogation-based breed management attempts. Furthermore, similar breed origin inferences between the Kerry Bog, Icelandic and Shetland pony breeds were noted within a population cluster emergence pattern at K=3. Little evidence of genetic drift was detected between founder sire cross and non-founder sire cross groups, and outcrossing was apparent across both groups; leaving the Kerry Bog Pony critically exposed to further genetic erosion.

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Poster
N°

Topics 2:

Functional genomics, epigenetics and reproduction

Imputation of ungenotyped horses from the genotypes of their progeny

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Sport horse studbooks worldwide are currently in the process of transitioning parentage verification from microsatellite markers to single nucleotide polymorphisms (SNP). There are several barriers to this transition, one of which is the lack of SNP data on older animals from which to verify parentage against. Therefore, the objective of this study was to assess the ability to impute the SNP genotype of a stallion by utilizing the genotypes of its progeny, with or without the inclusion of the genotype of the progeny's other parent in the reference population. Genotype information from 56,705 SNPs was available on 8,367 horses. A total of 48 stallions had genotype data on >10 progeny and the genotypes of these stallions were used as a test population. Imputation was undertaken using FImpute3 combining a family- and population-based imputation approach. Several different scenarios were assessed where the ability to impute the genotype of a stallion was quantified where genotype data were available on half sibling progeny. Using genomic information from 5 progeny the average genotype concordance of the imputed sire genotype compared to the actual sire's genotype was 92%. The average genotype concordance rate increased to 94% when the genotypes of 12 progeny were included in the reference population. The inclusion of the genotypes of the dams of the progeny increased the average genotype concordance rate using only 5 progeny from 92% to 96%. The average genotype concordance rate of the sire increased to 99% when 12 progeny and dams were included in the reference population.

A cell atlas of the complete equine airway

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Performance limiting conditions affecting the equine respiratory tract, including asthma, exercise induced pulmonary haemorrhage (EIPH) and laryngeal neuropathy are known to be heritable, with several studies identifying genomic regions associated with these diseases (Gerber 2020; Blott et al. 2019; Boyko et al. 2014). The conditions are complex, with mechanisms that are difficult to unravel. Transcriptomic and expression quantitative loci (eQTL) studies offer the potential to better understand the control of gene expression and gene regulatory networks. Studies based on single cell RNA sequencing are highly informative and have already been used to study equine broncheolar lavage fluid and nasal epithelial gene expression (Riihimäki et al. 2023; Lee et al. 2023). The link between different equine airway tissues and cell type transcriptomes is currently unknown and a complete airway cell atlas, based on single cell RNA sequencing, would enable researchers to understand how gene expression varies across the different cell types and tissues. We propose a round table discussion to facilitate the design and development of an open access equine airway cell atlas. The session will begin with a review of the current state of research on equine airway disease, followed by facilitated discussion to establish an appropriate experimental design for an airway cell atlas. Participants in the discussion will be able to guide the design process, evaluate the strengths and weaknesses of techniques and methods that could be used to generate the atlas, propose experiments that should be included and debate the best data dissemination methods.

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Shallow whole genome sequencing demonstrates euploidy in equine *in vitro* produced blastocysts

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Early pregnancy loss rates are higher for *in vitro* produced (IVP) equine embryos compared to *in vivo* derived ones, especially for slow developing embryos reaching the blastocyst stage more than eight days after ICSI (D8). Pregnancy loss in humans has been mainly attributed to the high incidence of aneuploidy in early development. Yet, in horses, little is known on aneuploidy. Here, we developed shallow whole genome sequencing to determine aneuploidy in equine D7-10 IVP blastocysts. Embryos were produced following De Coster et al., 2023. Briefly, slaughterhouse derived oocytes were matured in groups in TCM199 with 10% FBS, 9.4 µg/mL FSH and 1.88 µg/mL LH for 30h in 5% CO_2 in air. Following piezo assisted ICSI, embryos were cultured in DMEM/F-12 with 10% FBS in 5% CO_2, 5% O_2 and 90% N_2 until blastocyst formation. Blastocysts were treated with acid tyrode to remove the zona pellucida, washed in PBS, snap frozen and stored at -20°C. Shallow whole genome sequencing was performed as described by Popovic et al., 2018. Briefly, after whole genome amplification of individual embryos, DNA sequencing was performed (Hiseq3000), followed by CNV detection (WisecondorX, Vivar software). All 15 blastocysts (6 D7, 4 D8, 1 D9 and 4 D10) were euploid. This coincides with our previous SNP array analysis revealing 12/14 blastocysts to be euploid, while 5/6 arrested embryos were aneuploid. Overall, *in vitro* embryo production in the horse appears to give rise to a high rate of euploidy in the resulting blastocysts, irrespective of the day of blastocyst formation.

Reference:

Popovic et al (2018) PMID: 29796631 De Coster et al (2023) PMID: 37913607

Functional Impact of Putatively Identified Introgressed Alleles within the Horse Genome

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Introgression is the transfer of genetic material between two different species via repeated back crossing of the interspecific hybrid and one of the parent species. These are important events to understand in the study of molecular evolutionary relationships between species as introgression is nature's way to fast track evolution by spreading pre-adapted alleles. Previous studies have found 3,199 genes with signals of introgression, likely hundreds of thousands to millions of years ago from a non-caballine equid that are present in current horse populations. Our hypothesis is that the introgressed alleles provide an increase of fitness via functional differences in molecular phenotypes. Preliminary results utilizing the Equine-FAANG database have yielded differences in ChIP-seq signals based on the haplotype(s) of the individual, some of which appear to be introgressed. However, more individuals are needed to prove a statistically significant difference between the molecular phenotypes (ChIP-seq and RNA-seq) of horses homozygous for the reference and either the heterozygous horses or the ones homozygous for the putatively identified introgressed allele. Due to the current reproductive barrier between caballines and non- caballines, studying this gene flow between species will allow us to use introgression as a model for gene editing. Introgression within horses, specifically the well-known thoroughbred, will help us piece together evolutionary relationships as the shared alleles allow for rapid adaptation to their new environment by changing gene function.

Single-nucleus RNA sequencing of the equine brainstem

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The emergence of single-nucleus RNA sequencing (snRNAseq) has given greater insight into the physiology of healthy and diseased cell sub-populations by defining the transcriptomes of individual cells within tissues. In human medicine, the technique has provided further understanding of many neurological disorders including Multiple Sclerosis (Schirmer et al, 2019), Alzheimer's disease (Zhou et al, 2020) and Amyotrophic Lateral Sclerosis (Liu et al, 2020). Despite the success of snRNAseq in human medicine, the technique has yet to be utilised in horses, whose brain cell populations and their transcriptome remain undefined. Nuclei were isolated in duplicates from snap-frozen brainstems of 5 Warmblood adult geldings using an optimised mouse brain protocol (10x Genomics) and captured using a 10x Chromium Next Gem Single Cell 3' kit. Data were aligned using Cell Ranger and analysed using Seurat and Bioconductor R packages with results from both pipelines compared. Clusters were identified using marker genes outlined from published studies in other species to define distinct cell populations within the equine brainstem. Populations of astrocytes, neurons, microglia, mature and precursor oligodendrocytes and endothelial cell types were identified by differential expression of cell specific genes, with two distinct subpopulations of neurons. All cell groups were identifiable using both the Seurat and Bioconductor packages, although there was slight variability in differentially expressed genes. This study shows that snRNAseq technologies can be adapted and utilised in equine studies and establishes a neurological cell population standard for use in further research.

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Characterising differences in chromatin accessibility and gene expression between two equine muscles with different primary functions

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Mature equine muscle is comprised of different ratios of three fibre types: Type 1 (Slow twitch-oxidative), 2A (Fast twitch-oxidative) and 2X (Fast twitch-glycolytic). The proportions of these fibre types varies according to the muscles' functions[1].
In the current study, we are comparing two different muscles, sacrocaudalis dorsalis medialis (SCDM) and semimembranosus (SM) from Warmbloods and Thoroughbreds using ATAC-Seq[2]. The SCDM is a primarily oxidative muscle, while the SM is a primarily glycolytic muscle. Therefore, using these muscles we expect to generate a baseline profile of differences in gene regulation between oxidative and glycolytic muscles in horse. This profile will be informed by both ATAC-Seq and confirmed by RNA-Seq data of the same muscles.
Samples from the SCDM and SM of four Warmbloods and four Thoroughbreds were collected and nuclei extracted using a dounce homogeniser and cryopreserved in sucrose and 10% DMSO. ATAC-Seq libraries were prepared using an ATAC-Seq kit from Active Motif that we optimised for use in equine muscles. Initial quality control and low pass sequencing (3 million reads/sample) results indicated efficient optimisation of the ATAC-Seq protocol and differences in the number of peaks called in SCDM and SM. We are currently analysing high depth ATAC-Seq data (200 million reads/sample, 150bp pair-end reads) to assess more accurately the differences between the two muscle types. We will use RNA-Seq data to investigate how these differences in chromatin accessibility affect gene expression. The results of this work will inform future equine genetic studies related to muscle disease.

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A genomic approach to the origin of the horse mane

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The horse mane is an iconic trait that has long symbolized beauty and grace. The now- extinct ancestor of the horse (*i.e.,* the Tarpan *Equus ferus*) was seemingly short-maned, as depicted in hundreds of cave paintings made by horse hunters in the late Paleolithic period. Therefore, we can hypothesize that a long mane that falls on the neck and forehead was the product of selective breeding as a result of domestication, as the vast majorty, if not all, horse breeds have growing non-erect manes. We have investigated the genetic mechanisms underlying mane length and structure using the Icelandic horse as a model. In an attempt to identify genes involved in the variation in the growth of mane hair, we calculated heritability estimates and performed GWAS, RNA-seq, and Gene Ontology (GO) analyses. Variance components of mane length were estimated for 367 phenotyped horses using 373,662 markers on 31 autosomes. The additive (genomic) heritability was estimated at 0.83 (SE=0.16), indicating a strong genetic influence on the variation in mane length. A GWAS revealed a single region comprising 12 SNPs associated with mane type. To identify other loci involved, RNA-seq data were analyzed from skin biopsies from neck and forelock regions and revealed several differentially expressed genes. GO analysis of these genes indicated the steroid hormone pathways to be implicated in hair length differences. These results have the potential to impact studies on hair health and growth in humans, as humans are one of the few mammal species with indefinite hair growth on the head.

Development of a pipeline for automated analysis of single cell RNA sequencing data from equine peripheral blood mononuclear cells

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Insect bite hypersensitivity (IBH) is an allergic dermatitis caused by bites of insects of the genus Culicoides. Icelandic horses exported from their Culicoides-free homeland, Iceland, to Culicoides-rich environments in Europe/USA have a high incidence of IBH. To gain deeper insights into the disease and explore preventive allergen immunotherapy, we conducted a three-year trial involving immunized Icelandic horses exported to Switzerland. Peripheral blood mononuclear cells from horses that developed allergy or remained healthy were restimulated with the vaccine allergens and sequenced using Illumina single-cell RNA (scRNA) sequencing. To address the challenges associated with scRNA-seq data a Snakemake pipeline has been developed. This pipeline enables fully automated analysis of scRNA-seq data from quality filtering to clustering of cells based on transcript expression. It also includes a module for automated annotation of cell clusters identified using PBMC scRNA-seq from human and horse studies. The pipeline identifies five major cell types. We would present at the conference the summary of the analysis and results of sub-clusters of the major cell types. The pipeline will soon be made available publicly.

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Differential DNA methylation across ten tissue types of Thoroughbred stallions within the Equine FAANG initiative

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In order to extend the knolwedge about epigenetics for a variety of horse FAANG tissues, we have determined tissue specific differentially methylated CpG sites (tDMS) in adipose, hoof lamina, left ventricle of heart, liver, longissimus dorsi muscle, lung, testis, cartilage, sesamoid bone and third metacarpal bone of two Thoroughbred stallions. Targeted genome bisulfite sequencing focused on CpG rich regions. Reduced Representation Bisulfite Sequencing (RRBS) libraries were sequenced using 150 cycles of pair-end NGS (25M of passing filter reads in each direction). RRBS approach revealed 12237 tDMS across 10 tissues, including 909 tDMS affected by SNPs. The obtained methylome clusters from 10 tissues were concordant with their embryonic lineage. Several thousand tDMS were discovered in each tissue. A disproportionatly large number of tDMS characterized the testis where 2154 of the tDMS associated genes were found. From all determined tDMS, 915 resided transcriptional start sites. Over representation analysis of predicted transcription factor binding motifs covering identified tDMS showed a group of transcriptional factors enriched in regulation of ossification. These included EGR2, HAND2, HIF1A, MGP, SMAD5, TFAP2A and TWIST2, which contained 158 putative binding sites in 117 coding loci, covering CpG sites hypomethylated in sesamoid bone versus all other tissues. These data illustrate how RRBS can be used to find potential sites of epigenetic gene regulation in the part of the genome and also reveal possible biological relevance of differential DNA methylation especially with regard to sesamoid.

RRBS experiment was supported by project No. 501-181-721 of the National Research Istitute of Animal Production.

In Silico Analysis of Genomic Vitamin D Response Elements in the Horse Suggests Unique Mechanisms Behind Equine Response to Vitamin D

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Vitamin D is one of the major regulators of the immune system. Despite its importance, little is known about D3 in the horse since endogenous production of (25(OH)D3) is absent. However, evidence suggests that horses use D3 to support immune function, with mares clearing endometritis inflammation 60% faster with intrauterine D3. To elucidate the equine genomic vitamin D response, we combined RNAseq data from the previous study, with an *in silico* search of EquCab3.0 for the vitamin D response element as defined by JASPAR v 10 (Jaspar.elixir.no; *Homo sapiens* – MA0074.1; *Mus musculus* – MA0693.1). In total, 160 putative vitamin D response elements (VDRE) were identified, with 102 genes <25 kb. We evaluated the correlation between these 102 genes with their change in expression following intrauterine infusion of D3. Surprisingly, these genes did not change more (12/102; 11.8%) than the overall genome (2806/30374; 9.2%). Lastly, genes with known VDRE in humans (n = 24) were evaluated for 1) the proximity of the nearest VDRE and 2) significant change in expression following vitamin D infusion. Averaged distance to the nearest VDRE was >5 million bp, with the closest at 517kb. However, despite the lack of proximal VDREs, these genes showed increased regulation by vitamin D than random genes (9/24, 37.5%; $P < 0.05$). These data suggest that horses have vitamin D response mechanisms; however, these mechanisms differ from humans and mice. Horses may have a differing sequence for their VDRE or may utilize non-genomic mechanisms to elicit changes despite showing similar immunological responses.

Exploring the genomic component of equine sex development and reproduction

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Normal development of gonads and sex characteristics are complex genetically regulated processes that form the foundation for vertebrate reproduction. Disorders of sex development (DSDs) are clinically heterogeneous conditions affecting sex determination, sexual differentiation, gonadal development, and reproduction. Many cases and forms of DSDs have been described in horses, though knowledge about the genomics of equine DSDs is sparse and no causative genes or risk factors are known for most. Here we initiated systematic research on the genomics of three select, recurrently observed, chromosomally normal equine DSDs: 64,XX mares with X-monosomy-like gonadal dysgenesis; 64,XY *SRY*- positive female-like horses, and 64,XX intersex horses. We refined clinical phenotypes by hormonal profiling and used short-read (Illumina, n=88) and long-read (PacBio HiFi, n=18) whole genome sequencing to discover candidate genomic regions and variants within the three phenotypic groups. Alignment of short-reads to EquCab3 in combination with over 1100 other horses is ongoing to discover rare or unique variants present only in cases.
These in combination with long-read data will be aligned with the telomere-to-telomere reference based on a F1 female horse-donkey hybrid and our equine pangenome facilitates the discovery of rare structural variants and haplotypes. While genomic analysis is hypothesis free, special attention is paid to known master sex development genes like *SOX9* and its regulatory regions. The findings will contribute to the development of improved molecular diagnostics of horse DSDs and advance the knowledge about the genetic regulation of sex development in horses and other mammals.

Genetic regulation of placentation in mares – preliminary results.

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Implantation in mares is a critical event of early development and its' regulation remains poorly understood in the mare. In this study, we aimed to identify cells and genes which may be involved in the control of implantation by comparing characteristics of immune and non-immune cells' populations present in the endometrium before and after implantation. Endometrial biopsies were taken from pregnant endometrium at day 33 (n=3) and at day 42 (n=4), and following tissue lysis, 170 290 isolated cells were processed using Chromium10x Genomics scRNAseq. Following QC, data for 107 378 cells was analyzed with Seurat R package v5.0. Clusters were annotated using published markers.
Initial cluster analysis revealed 11 immune and 16 non-immune cell clusters which were further analyzed separately. A total of 76 180 non-immune cells divided into 19 clusters, annotated as epithelium, ciliated epithelium, glandular epithelium, stromal fibroblasts and endothelium. A total of 31 198 immune cells resolved in 25 clusters including those annotated as $CD3^+$, $CD3^-$ $CD7^+$ (NK cell) and $CD49A^+$ (tissue resident) lymphocytes, antigen presenting cells, monocytes and B cells. A second pipeline allowed us to compare day 33 and 42 cell populations within clusters, that resulted through the measurement of frequencies in the discovery of an influx of novel cells ($CD49^+$ lymphocytes) temporally associated with embryo implantation, but also allowed us to determine specific genes changing expression within clusters which may control this process at the immune level as *LY49F* or be involved in development of the placenta and epithelial mesenchymal transition such as *FGF7 andLCN2.*

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Poster
N°

Topics 3:

Genomics of performances and welfare

Exploring genetic diversity and identifying key contributors in the Thoroughbred horse population for development of an imputation reference panel.

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Imputation of SNP array genotypes to whole genome sequence (WGS) level has been successful for animal populations to increase the range of SNP variation classes, including low frequency alleles. The effective population size of Thoroughbred horses is small (Hill *et al* 2021), therefore it is expected that a relatively small number of WGS will be sufficient to capture common alleles contributing to population-wide genetic variation. However, recently Thoroughbred WGS has revealed an unexpectedly high number of rare variants in coding regions, making them promising candidates for performance traits (Tozaki *et al* 2021).

Careful selection of animals for reference panels is important when dealing with rare variants. Several strategies including pedigree and/or genetic information may be employed. We used a SNP genotype archive for 16,000 Thoroughbreds to systematically examine genetic diversity and identify the most informative individuals for a reference panel.

First we employed visual methods (principal component analysis) on a set of n=230 stallions (McGivney *et a*l 2020) and a projection method to place 16,000 horses within the core genetic diversity space. Next we used the key contributors algorithm (Neuditschko *et al* 2017), which identified 421 individuals. Post-processing based on the genetic relatedness matrix and the genetic contribution scores (GCS) refined the set to n=100 with mean GCS=0.9.

The WGS obtained in this project will enable imputation of a large repository of SNP genotypes, which will dramatically improve genomic prediction of health and performance traits to inform genome-enabled breeding and management programmes.

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Causative gene identification strategy in thoroughbred horses using a whole-genome variant database

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Genome-wide association studies (GWAS) using single nucleotide polymorphism (SNP) arrays is a common method used to identify trait-related genes, because it allows comprehensive analysis without setting a null hypothesis. However, fine mapping after GWAS may require next-generation sequencing (NGS) analyses. We have sequenced the whole genomes of 101 thoroughbred horses, calculated the allele frequencies of 12 million SNPs and 1.56 million insertion-deletion variants (INDELs), and created a whole-genome variant database with allele frequency information (Tozaki et al., 2021; 2023). In this study, we introduce a strategy for identifying trait-related genes based on the candidate gene approach using the whole-genome variant database. The identification strategy included the following steps: 1) an information search for trait-related genes in humans and mice; 2) the identification of horse orthologs of the searched genes; 3) a search for nonsynonymous substitution SNPs or INDELs from the whole-genome variant database; 4) an association analysis of phenotypes and the searched SNPs. As an example, we attempted to identify genes associated with horse behavior. We identified 15 candidate orthologous genes from previous studies in humans and mice, and 55 nonsynonymous substitutions from the whole-genome variant database. Among these, we conducted association analyses with horse behavioral data for eight targeted SNPs with a high allele frequency (minor allele frequency > 0.3) in the population, and found a relationship with three genes (SNPs) involved in the serotonin nervous system. The database created in this study will contribute to the identification of other trait-related genes in thoroughbreds.

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Single-step genome-wide association study of factors for evaluated and linearly scored traits in Swedish Warmblood horses

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Swedish Warmblood horses (SWB) are bred for show jumping and/or dressage with young horse test scores as indicator traits. This study aimed to investigate possible candidate genes/regions for young horse test traits. Factor analysis was done for eight evaluated traits together with height at withers for 20,814 SWB, and for 50 linearly scored traits with height at withers for 4,782 (of the 20,814) SWB, using the Psych package in R (Nazari-Ghadikolaei, et. al. 2023). Among those horses, 380 were genotyped using a 670K SNP array. All genotyped horses had evaluated trait scores and 379 were linearly scored. A single-step (ss) GWAS was done using the BLUPF90 family of programs for four factors for evaluated traits and 13 factors for linearly scored traits. Significant top SNPs associated with three factors related to size were located on chromosome 3 within or nearby the well-known *LCORL/NCAPG* region. Significant SNPs were also detected for two factors for evaluated traits representing conformation and jumping, and four factors for linearly scored traits related to body length, neck conformation, walk, and trot (hindlegs), respectively. Among nearby genes, *Calcium/Calmodulin-Dependent Protein Kinase Type 1D (CAMK1D)* for the factor for linearly scored traits related to neck conformation, and *GLI Family Zinc Finger 2 (GLI2)* for the factor for evaluated jumping traits, were most promising. For these, top associated SNPs were detected within the genes, and their known functions may be related to the phenotypes. In conclusion, ssGWAS is beneficial to detect plausible candidate genes/regions for desired traits in warmblood horses.

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Heritability of blood parameters and their relationship with longevity in jumping competition

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In order to find early selection criteria to improve the longevity of show jumping horses in competition, a specific protocol was constructed. Before entering competition, young horses were measured for many traits. These horses were offspring of two groups of sires selected as having the highest and lowest estimated breeding values for functional longevity in jumping competition, as calculated from progeny. Functional longevity was defined as the time spent in competition corrected for the level of performance. The dataset included 952 horses (mainly French Saddlebred) and 77 blood parameters. Heritability was estimated using a mixed model including the effect of age, sex, place and date of collection, weight and animal random additive value with 10,280 horses in pedigree. Heritability of blood parameters was generally moderate to high: 21 values were higher than 0.5 and 39 were between 0.2 and 0.5. The most heritable traits were hematology and enzyme traits: mean corpuscular volume (0.90) and mean corpuscular hemoglobin concentration (0.92) but also traits as liver isozyme (0.72) or total alkaline phosphatase (0.68), small lymphocytes (0.67), superoxide dismutase (0.60). Principal Component Analysis reveals the low correlations between traits: 32 variables were required to achieve 90% of the variance explained. However, correlation patterns between variables in the same function group revealed functional relationships. Logistic regression to predict group of sires according to longevity revealed, in order of appearance, the effect of mean corpuscular hemoglobin concentration, leukocytes, liver isozyme of alkaline phosphatase, $\alpha 2$-globulin, monocytes, $\alpha 1$-globulin and aspartate transaminase. Biological explanations are underway.

4,200 years of selection for key horse phenotypes

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The domestication of the horse transformed human history, reshaping long-range mobility, warfare, and fostering a remarkable diversity of equine phenotypes. Yet, the timing, locations, and the strength of selection driving these traits remains unclear. Ancient DNA time series provide considerable statistical power to measure the timing and strength of selection by providing explicit temporal allelic frequency trajectories. In this study, we leveraged the availability of extensive genome time-series and a new population model summarizing the last 50,000 years of demographic changes to reconstruct the selective history that accompanied the emergence of key phenotypic traits in horses. Our work confirms strong patterns of selection at two loci, underpinning the emergence of modern domestic bloodlines. Functional validation further confirms mobility, and selection related to locomotion phenotypes, as the crucial driver of DOM2 horse domestication. Remarkably, our work also reveals that, size apart, the genetic variation underlying most traits of key relevance for the modern industry were only selected in the 80-120 generations. As such, those traits of interest in modern domestic bloodlines do not reflect the traits that were of importance to human societies even 640-960 years ago.

Keywords: Horse, Traits, Selection, Demography

Analysis of Equine Genomic Regions Comprising Candidate Genes Associated with Ehlers-Danlos Syndrome in Humans

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Hypermobile people may early in life be inflexible due to osteoarthritis. Yet, there is no knowledge about the situation in horses. Very animated gaits may lead to reduced orthopaedic health of the tendon and ligament structures. Equine suspensory ligaments together with digital flexor tendons are prone to injury, and high-level dressage horses have been found to be overrepresented with suspensory ligament desmitis (Murray *et al* 2016). Though there is a demand for increased mobility in dressage horses, concerns are raised regarding hypermobility (Ablondi et al., 2022).
We previously identified putative signatures of selection in SWB horses (Ablondi *et al* 2019), comprising genes associated with joint laxity and hypermobility syndromes (Giunta *et al* 2008). We have now further analyzed these findings in 379 horses, and 5,707 SNPs located within 18 genes associated with Ehlers-Danlos Syndrome (EDS) in humans ±500kb. We investigated genetic association between markers, and performance traits available from young horse tests applying a linear mixed regression model, adjusting for event and sex. Five SNPs on ECA6 were putatively associated with the conformation trait “sickle hock”. The top SNP is located within the gene *calsyntenin-3* (*CLSTN3*) (p=0.0424; FDR=0.0253), a regulator of energy and bone homeostasis (Kim *et al.* 2020), 29,3Mb upstream of *complement component 1R* (*C1R*), known to be associated with periodontal EDS (pEDS). One SNP within the *Spi-1 proto-oncogene* (*SPI1*)/*SLC39A13* gene complex on ECA12, showed significant association with large or small hooves (p=0.0108; FDR=0.0109). In humans, *SLC39A13* is associated with spondyloplastic EDS (spEDS). Our findings are now further validated.

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Combining novel motion technology and genotype data in three Italian horse native breeds.

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The loss of biodiversity is a concrete issue across species, including equines. In Italy, over than 90% of local breeds are endangered due to shifting breeding goals. This study aims to give new opportunities for the maintenance of the endangered breeds, by investigating the genetic basis of new phenotypes, seeking markers that could be used to enhance the breeding program, facilitating the transition from working to riding purposes. A total of 101 horses from three Italian local breeds were studied: Bardigiano (73), Murgese (20), and Tolfetano (8). A sensor (EquiSense motion S), containing an accelerometer, and a gyroscope was used to collect gait-related traits, allowing for the simultaneous and objective measurement of 16 phenotypes per horse.
Genotyping was conducted using a medium-density Illumina SNPchip. Quality control and population structure analysis via principal component analysis (PCA), were carried out using PLINK v 1.9. A subsequent Genome-wide Association Study (GWAS) with PCA components 1 and 2 as covariates, alongside significant biometric measurements, revealed six SNPs reaching Bonferroni significance (P=5.88). SNPs exceeding Logarithm of Odds (LOD) 5, given the limited sample size, were considered suggestive. Nineteen SNPs were identified, and within a ±250kb window, the first scan discovered over 90 protein coding genes associated with 7 traits.
In conclusion, gait traits are highly polygenic and challenging to study but thanks to sensor, it is possible to study them in horses. The markers found could potentially be utilized in future selection for more rideable horses, supporting these endangered breeds in meeting evolving market demands.

French genomic evaluation of show jumping in sport horses

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In France, estimated breeding values (EBV) of show jumping horses are based on performances in official competitions. Genomic data were first introduced in 2023. Performance data have been collected since 1985 and included 347,137 horses, Genotypes were obtained from 3 different beadchips from 54602 SNPs (Illumina Equine 50) to 670,806 SNPs (Affymetrix Axiom Equine) on 3,658 horses available from previous research including 2,241 males. After quality control, all genotypes were imputed to 375,687 SNPs. Two traits were considered: annual sum of points earned in each event according to rank and technical difficulty, and rankings considered as the result of the expression of an underlying unobservable performance. The model included fixed effects of a combination of age, year and sex, random effect of genetic additive value (relationship matrix included 617,903 horses) and permanent environment effects to consider the repeatability inside one year for ranking and between years for ranking and the annual points. A Single Step GBLUP model was applied using BLUPf90 software which combines all the information (genotypes, performance and pedigrees). Heritability was 0.31 for annual sum of points and 0.13 for ranking. For the population of 57,921 horses with performance in 2023, correlations between GEBV and EBV ranged from 0.934 for genotyped horses to 0.992 for non-genotyped horses. For sires, correlation was around 0.99. Currently, the equine industry is working on a project based on a 60,000 SNP array developed by the French research to be used routinely for parentage control and breeding evaluation.

Shape and Gaits 2.0: Preliminary results on new QTL suggestively associated with objective gait quality parameters in Franches-Montagnes horses

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Gait quality is an important, but subjective selection criterion of sports horses. The aim was to use objective gait quality parameters (stride length and frequency, maximal limb protraction and retraction angles, suspension duration) as phenotypes in genome-wide association studies (GWAS). We measured 220 Franches-Montagnes (FM) horses in hand at the walk and trot on a hard surface in straight line of approximately 30 meters using the gait analysis tool kit EquiMoves® (seven inertial measurement units placed on the poll, withers, pelvis and the four limbs). Speed was measured with Freelap®. All FM horses were genotyped with the Affymetrix 670K SNP array, resulting in 394'062 SNPs after QC (MAF < 0.05, HWE < 0.0001). We defined categorical cofactors for sex (male/female), age (3-4, 5-8, 9-16, >16), Froude number (Fr = $speed \div \sqrt{g \times wither's\ height}$), shoeing, who placed the sensors and led the horse. Two suggestive QTL were identified at walk: for maximal hind limb retraction angle on ECA2 (96,038,485-98,833,836 bp, $p = 9.56 \times 10^{-7}$) containing the gene *PCDH10*, and for mean stride length on ECA12 (32,816,253-32,855,923 bp, $p = 5.96 \times 10^{-7}$) in the gene *ANO1*. At trot, one suggestive QTL ($p = 7.34 \times 10^{-6}$) was found for suspension duration on ECA11 (52,191,552-52,151,882 bp) near the gene *NTN1,* which mutations are causal for congenital mirror movements in humans. Hence, we believe that *NTN1* polymorphisms might affect intra-limb coordination of the diagonal limb movement at trot. Further analyses need to be conducted including additional genotyped horses to confirm our preliminary results.

Heritability of performance traits in North American Standardbred racehorses estimated from SNP genotyping data

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Standardbred racehorse breeders work to produce faster horses with long careers, thus increasing earning potential. Previous data have shown that some diseases (e.g. recurrent exertional rhabdomyolysis) occur more frequently in elite performers. Suggesting selection for performance, may inadvertently increase disease risk. Genomic prediction models for performance and disease would allow breeders to simultaneously breed for performance and against disease. The first step is to identify moderate-to-highly heritable performance traits that are likely to be influenced by selection.

We estimated heritability of 13 performance traits in North American Standardbred racehorses. Single nucleotide polymorphisms (SNP) array genotypes from 294 Standardbreds were imputed to ~2 million SNPs. Performance data was obtained from the United States Trotting Association, and included earnings, career length, and race times. Heritability was estimated using restricted maximum likelihood analysis in GCTA with and without a LDAK-adjusted kinship matrix including gender and gait as covariates.

Heritability estimates ranged from 0.000001(±0.28) to 0.51(±0.30), comparable to reports in other breeds. Mile times, years raced, and number of starts and wins had moderate heritability estimates (i.e., ≥ 0.3, range: 0.30-0.51), suggesting amenability to genomic prediction. Genetic architecture is being estimated for each trait.

We are currently adding data from an additional 1,500 North American Standardbreds and will present the heritability and genetic architecture for more than 30 performance traits in this large cohort. Understanding heritability and genetic architecture is a crucial step towards building genomic prediction models for performance in Standardbred racehorses.

Strategy to detect gene doping in blood collected in lithium-heparin tubes in the horse athlete.

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Doping control laboratories must anticipate the emergence of the gene doping threat by developing new detection methods based on molecular biology techniques in order to be able to reveal gene transfer (i.e. transgene administration) and/or genome editing. Generally, doping control analyses for horseraces are conducted on biological samples where blood is mostly collected in lithium heparin tubes (LiHep). Therefore, the development of molecular biology methods based on blood or plasma collected in LiHep tubes constitute an analytical challenge as heparin is a well-known potent inhibitor for polymerase enzymes used in PCR reactions. The consequences of the use of this type of biological material are usually a drastic loss in sensitivity or the absence of signal.
To face this issue, a sample preparation optimization was achieved to make possible the transgenes and plasmids detection by qPCR from plasma collected in LiHep tubes. To this purpose, additional purification steps were included in the extraction process to reduce PCR inhibition. huIGF1, huEPO and huGH transgenes, pCMV, poly-adénylation signal sequence and ITR from AAV were detected with both simplex and multiplex methods. The detection performances were compared between extracts obtained from EDTA plasma and LiHep plasma with and without inhibitors removal. The results obtained, highlighted that as expected the highest sensitivity was obtained from EDTA plasma and that heparin precipitation steps allowed to partially recover signal from the LiHep plasma enabling the gene doping control from this type of biological material.

Poster
N°

Topics 4:

Genomics of equine diseases

Transcriptomic integrated approach of horse bovine papillomavirus-related sarcoids

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Sarcoids are the most common skin tumors in horses representing up to 90% (35-90%) of skin neoplasms. Often associated with Bovine Papillomavirus (BPVs) infections, sarcoids can occur as single or multiple lesions. Some forms represent an extremely invalidating pathology characterized by high incidence, resistance to therapy and frequent recurrence. The aim of this study was to better understand the host-pathogen interaction by applying transcriptomics on the tumor microenvironment.
Transcription (both mRNA and small RNA fractions) was analyzed via high-throughput Illumina sequencing comparing two groups: 12 sarcoids, resulted positive to BPV1 infection, and 12 portion of healthy skin, from subjects tested negative to the viral DNA. Differentially expressed miRNAs and genes in sarcoids vs controls were identified and miRNA target mRNAs retrieved. Moreover, transcription data were screened for chimeric transcripts (CTs) and isoforms. On these genes a Gene Ontology (GO) enrichment analysis was carried out.
A total of 2415 up-regulated and 3620 down-regulated genes along with 66 up-regulated and 40 down-regulated miRNAs were found. Enriched biological processes for differentially expressed genes were cytokine and chemokine production, growth factors and collage binding, cell adhesion and development, tissue morphogenesis and inflammatory and immune response; while for miRNA targets, transcriptional modulations, cytoskeleton organization processes, DNA and RNA packaging and transport and host-viral interaction were found. CTs and isoforms can contribute to migration, invasion, and transformation in cancer.
Our data identified a great transcription discrepancy between sarcoid lesions and healthy skin with an overall enrichment for processes related to infection and cellular transformation.

Utilizing empirical and simulated equine whole genome sequencing data to create recommendations for variant discovery in the horse

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Rapid and accurate disease-causing variant discovery requires standardized, well-validated workflows, however, no studies have evaluated equine-specific recommendations for the initial variant calling and filtration. Using empirical data from 152 equine genomes and data from 90 simulated equine genomes we assessed performance differences between GATK HaplotypeCaller and BCFTools mpileup; and hard filtering and variant quality score recalibration (VQSR). Simulated genomes were modeled after empirical equine genomes and preselected genetic variants were inserted into simulated reads to create a truth set of single nucleotide polymorphisms (SNPs) and insertion-deletion (indels). The truth set was used to calculate precision and recall of the variant callers and accuracy of filtration methods. SNP recall and precision was marginally different between callers. Indel recall was 9.4% (5.2%-17%) lower, and precision was 8.5% (6.5%-10.4%) lower for BCFtools. VQSR decreased accuracy 2% and identified 198,000 more false negative SNPs. In empirical data, 18.6% of variants were discordant between callers and 16% were discordant between filtration methods. Differences between concordant and discordant variants in empirical data was largest in annotation values and variant distribution across the genome. Specific regions of the genome showed above average numbers of misidentified (simulated) or discordant (empirical) variants. These preliminary results support GATK HaplotypeCaller with hard filtering for variant discovery in the horse. These recommendations will be incorporated into a disease-causing variant discovery pipeline that will include automated variant effect prediction and variant prioritization to create a tool to enhance disease-causing variant discovery in the horse.

Administration and detection of a gene doping multi-target AAV gene doping vector in horses using multiple matrices and molecular techniques.

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Gene doping, defined as 'the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance', is prohibited in horseracing and equestrian sports.
To enhance the transgene detection screen currently employed at LGC, a custom adeno-associated virus serotype 8 (rAAV8) vector was designed to include 20 PCR binding sites for next-generation sequencing (NGS), and 9 binding sites for quantitative PCR (qPCR) for genes that are suspected to give performance enhancement such as *VEGFA.*[1] The vector was injected via an intramuscular route into 2 Thoroughbred horses, and multiple matrices were collected at defined timepoints. DNA was analysed using 3 detection methods: qPCR, digital PCR (dPCR), and NGS.
Overall, there was a good correlation across the different detection methods employed, although dPCR was less sensitive. Vector was detected in all matrices, although detection in urine was poor. High concentrations of vector were detected at early timepoints in plasma, which rapidly dropped at 24 hrs to trace levels by 96 hrs post-administration. Clearance of rAAV8 in plasma followed a similar timeline to the previous AAV6-GFP study,[2] giving confidence to the accuracy of both projects. Whole blood followed a similar clearance profile to plasma, although trace levels were detectable for a longer timeframe (216 hr). Vector was detected in dried blood spots at lower levels, but the detection timeline was similar to whole blood. Detection in hair root bulbs in one horse was seen up to a month post-administration, which was unexpected and opens new avenues for future gene doping testing.

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X-chromosome association study for microphthalmia in Warmblood horses

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Microphthalmia, a developmental eye disorder, has been increasingly reported in the Warmblood horse population. In this condition, an underdevelopment of one or both eyes is seen which results in small and abnormal eye(s) resulting in an impaired vision. It affects a majority of females and predominantly the left eye. As microphthalmia is more prevalent in females than in males, we performed an association study on X chromosome to identify X-linked regions associated with this disorder. Since 2021, we collected DNA on 52 affected horses (42 females and 10 males, 37 left-sided, 11 right-sided and 4 bilateral) and genotyped them on a 70K SNP array. Sixty unaffected and unrelated Warmblood horses were used as controls. We performed quality control on the X chromosome using PLINK as following: (1) individuals with a call-rate ≤0.95, outlying heterozygosity and (2) SNPs with a call-rate ≤0.95, minor allele frequency ≤0.05 and Hardy-Weinberg equilibrium ≤0.0001 in female controls. After quality control, around 2 300 SNPs and 109 individuals (49 cases and 60 controls) were retained. We performed several X-chromosome association studies under two different assumptions: (1) escape X-inactivation or (2) random X-inactivation, using PLINK. After Bonferroni correction, two suggestive SNPs were identified for microphthalmia in Warmblood horses. This X-linked association study adds another layer to the unraveling of the genomic background of microphthalmia in Warmblood horses.

Improving Candidate Gene Prioritization Standards in the Horse

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Identifying and prioritizing relevant candidate genes are critical steps towards disease variant discovery. However, current methods return thousands of potential candidate genes with marginal priority agreement between programs, results depend on user-defined input, and programs become outdated and are replaced frequently. Lack of validation, and unknown translatability from human data and phenotypes, pose further challenges when these programs are applied to animal disease. To overcome these limitations, we propose ranking genes by combining output across two or more programs. Using forty known Mendelian disease genes in horses and dogs, we evaluated six widely-used programs with different approaches to candidate gene prioritization including text-mining (OMIM, OpenTargets); phenotype searching (Phenolyzer, PhenoPred); and genomic data analysis (Endeavour, GeneTIER). The known causative gene was identified by at least one program for 16/20 equine and 18/20 canine diseases. Identification success ranged from 30% to 90%, with the gene rank varying between programs. On average the implicated gene ranked in the top 35th percentile with a wide range of 1st to 97th percentiles across the forty diseases (raw rank of 1 (highest) to 2,150 (lowest)). Using iterative combinations of three programs, the known causative gene was ranked in the top 500 >60% of the time. A hierarchical workflow combining prioritization methods is under construction and will be integrated into our Mendelian and oligogenic disease discovery pipeline. This work will also guide best practice recommendations for candidate gene prioritization in the horse and other domestic animals.

Exon-specific expression of *RYR1* occurs in Thoroughbreds susceptible to recurrent exertional rhabdomyolysis and is mitigated by treatment with dantrolene

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Recurrent exertional rhabdomyolysis (RER) is a complex moderately heritable (0.40) disease causing intermittent exercise-induced muscle degeneration under specific dietary/environmental conditions. Abnormal regulation of Ca^{2+} flux across the sarcoplasmic reticulum in susceptible horses is the purported etiology for RER. Dantrolene, which decreases Ca^{2+} release through the pore of the Ca^{2+} release channel (RYR1), mitigates rhabdomyolysis. Previously, we evaluated differential expression (DE) of genes and proteins in gluteal muscle of RER-susceptible mares, RER-susceptible mares treated with dantrolene and control mares all housed in the same race-training environment (Aldrich et al., 2020). Calcium regulatory pathways were enriched in RER-susceptible versus control mares. *RYR1* was both a downregulated gene and an upregulated protein in RER-susceptible mares. Here we performed differential exon analysis on RNA-sequencing data and found differential exon expression (DE) of *RYR1* in RER-susceptible mares compared to controls. *RYR1* showed DE of exons 1-90 in RER-susceptible mares relative to controls within which are exons encoding amino acids controlling isoform-specific as well as exons impacting the open probability of the Ca^{2+} pore. In contrast, RER-dantrolene treated mares showed no DE of exons 1-86, relative to controls. However, RER-susceptible mares treated with dantrolene did have DE of exons 87-103, encoding the sarcoplasmic reticulum luminal Ca^{2+} pore and its gating. DE results were confirmed by qRT-PCR. DE of *RYR1* regions controlling the open probability for Ca^{2+} flux through *RYR1* in untreated, but not dantrolene treated RER-susceptible mares, suggests that this DE region of *RYR1* could impact the pathogenesis of RER.

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CELLEQUS: an MHC haplo-bank of equine mesenchymal stem cells (MSCs) from homozygous donors

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Allogeneic cell therapy presents advantages over autologous therapy, but immune recognition of foreign cells can compromise safety and effectiveness. In equine mesenchymal stem cells (MSCs), such recognition is related to the matching or mismatching for the major histocompatibility complex (MHC) between donor and recipient, and to the immune profile of MSCs. To improve allogeneic MSC treatments in horses, our goal was to create CELLEQUS: a bank of equine MSCs derived from homozygous donors for the most frequent MHC-haplotypes, focusing on the most common breeds in Spain. This bank will facilitate the use of MHC-matched MSCs with a well-characterized immune profile. First, we used a panel of 10 intra-MHC microsatellites (Sadeghi et al., 2018) to study MHC diversity in Purebred Spanish, Purebred Arabian, Hispano-Arabian and Lusitano horses, and determine the most common MHC-haplotypes. Second, we searched homozygous horses for the most frequent MHC-haplotypes and recruited them as bone marrow MSC donors. Third, MSCs (n=9) underwent characterization through immunophenotyping (CD90, CD105, CD44, CD45, CD11, MHC-I, MHC-II; flow cytometry) and gene expression (MHC-I, MHC-II, IL6, COX2; RT-qPCR). Importantly, donors with the same haplotype can exhibit very different immune profiles, so not all homozygous horses would be equally suitable as donors. So far, no correlation has been observed between the MHC-haplotype and the expression levels (gene or surface) of MHC-I and MHC-II. CELLEQUS Bank arises from applying knowledge on the equine genome to improve equine health, by providing critical foundational resources for the advancement of veterinary regenerative medicine, spanning research and clinical application.

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Investigating the effects of Galectin 1 expression levels and its association with fracture risk in Thoroughbred horses.

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Bone over loading fractures are the leading cause of euthanasia in racehorses with 60 horses per year suffering a fatal fracture in the UK (Parkin, Clegg et al. 2004).
Fibroblasts isolated from Thoroughbreds and samples at high and low genetic risk of fracture were identified using a polygenic risk score. These were used to generate induced pluripotent stem cells (iPSCs) and were differentiated into osteoblasts (Baird, Lindsay et al. 2018). RNA sequencing identified 112 differentially expressed genes, 48 of which had no previously known links to bone.
This work aims to determine if these genes have novel osteogenic associations. 16 genes were initially investigated. Galectin-1 (*LGALS1*) was expressed at lower levels in high-risk samples and is robustly expressed in equine bone tissue and Saos2 cells. *LGALS1* was therefore selected for follow-up assays.
Overexpression of *LGALS1* in Saos2 cells resulted in few changes in alkaline phosphatase (ALP) activity, and osteoblast and osteocyte associated gene expression, however there was decreased mineralisation and a significant decrease in cell viability.
Knockdown of *LGALS1* in Saos2 cells also resulted in a significant decrease in cell viability but also led to a change in cell morphology, a significant increase in matrix mineralisation, ALP activity and favoured an increased expression of osteoblast and osteocyte associated genes.
These changes in gene expression, cell proliferation and osteogenic differentiation associated with *LGALS1* modulation suggest that the lower *LGALS1* expression in osteoblasts derived from high-risk horses may contribute to an increased risk of bone over loading fractures in Thoroughbred racehorses.

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Integration of genome-wide association analysis and tissue-specific transcriptomics to prioritize functional and predictive variants for equine osteochondrosis

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Osteochondrosis (OC) is a developmental orthopedic disease that affects up to 40-60% of foals across breeds and frequently requires surgical intervention to prevent long-term adverse effects on joint health, making OC a significant economic burden to the equine industry as well as a significant health and welfare issue. Genetic factors contribute to disease risk, but specific genes/variants underlying OC risk are unknown. In this work, we have taken an integrated approach to prioritize functional and predictive genetic variants for OC risk.

615 Standardbred horses (258 cases with hock OC, 357 controls without joint pathology) were included in the GWAS population. Genotyping was performed on the Affymetrix 670k equine SNP array and subsequently imputed to whole-genome sequence density. RNAseq was performed on articular cartilage and subchondral bone from 6 healthy neonates, 6 healthy adults, and 10 hock OC fragments. Differential gene analysis was performed among all groups. Differentially expressed genes within GWAS regions of interest were considered biological candidates for disease risk. SNPs falling within candidate genes were identified using whole-genome sequencing from 18 OC-phenotyped Standardbreds. Random forest (RF) classification analysis was used on the imputed genome-wide genotyping data to identify predictive SNPs. In total, 1566 biological candidates and the top 4434 predictive candidates were selected for inclusion in a custom genotype-by-sequencing panel. 1920 horses will be genotyped using this custom assay to identify which SNPs are most highly associated with disease status. Biological candidates can then be investigated mechanistically, while predictive variants can be used to construct a predictive assay.

Genomic approaches for understanding the genetics of catastrophic fracture in Thoroughbred horses

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Catastrophic bone fractures are the main cause of euthanasia on the racecourse in the UK. Bone fracture is a complex condition caused by environmental and genetic factors. To understand the genetic risk of fracture we developed a genome-wide polygenic risk score (PRS) and applied it to 44 Thoroughbred cell samples. The PRS allowed us to generate equine-induced pluripotent stem cells (iPSCs) (Baird et al., 2018) based on their relative risk. We differentiated iPSCs from the highest and lowest genetic risk into osteoblast-like cells and applied RNA-sequencing (RNA-seq). RNA-seq analysis revealed 112 differentially expressed genes (DEGs) between high and low risk samples. Gene ontology analysis associated the DEGs with numerous biological processes such as adhesion, development, morphogenesis and differentiation. Additionally, the DEGs were associated with ECM (extracellular matrix), collagen chain trimerization and ERK (extracellular signal-regulated kinase) pathways among others. To identify genetic variants significantly associated with fracture risk we performed Whole Genome Sequencing (WGS) on 7 cases and 7 controls. Variants were filtered based on their segregation between cases and controls, consequence (missense or upstream), region of interest in the genome (Blott et al. 2014 or DEGs), and minor allele frequency (MAF) across breeds. 474 variants were selected for genotyping using the Agena Bioscience MassARRAY platform. Identification of causative genetic variants would provide a significant step forward in developing a DNA test to identify and manage high-risk horses.

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Decoding the Genetic Basis of Myotonic Dystrophy in Horses: Insights from In-Depth Genomic Exploration

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Myotonic Dystrophy (MD) is a hereditary neuromuscular disorder characterized by stiffness, abnormal muscle relaxation, and muscle atrophy or hypertrophy. Afflicted muscles exhibit prolonged contracture and display complex repetitive discharges in electromyography (EMG). In humans, MD is linked to genetic mutations disrupting RNA processing. The veterinary literature on MD in horses is limited and the genetic origin remains elusive. Analysing whole genome sequencing (WGS) data from our MD dataset, we aim to elucidate contributing genes and molecular mechanisms for potential non-invasive diagnostics and therapeutic interventions.

Due to the condition's rarity, only a limited number of horses were available for analysis. Five MD horses and seven age, sex, and breed-matched controls underwent low-pass whole-genome sequencing (4X coverage). Fst values identified six chromosome regions with elevated values, conserved across multiple window sizes. Notably, the chromosome 3 peak contains the candidate gene *THEGL* (Testicular Haploid Expressed Gene Protein-Like), linked to Facioscapulohumeral Muscular Dystrophy 1 in humans. Chromosome 6 peak contains *SSPN* (Sarcospan), linked to human Muscular Dystrophies.

SNP analysis revealed a splice variant in *THEGL*, possibly causing intron retention or exon skipping, and a missense mutation in *SSPN* (cysteine to arginine). Higher-depth sequencing (15X) was conducted to confirm Fst regions and mutations. Results confirmed a polygenic trait and eight new regions in the genome are investigated. This study aims to unveil novel molecular mechanisms in equine MD. Additionally, findings may offer insights into human MD, affecting approximately 1 in 8000 individuals.

Genetic susceptibility to sarcoid in Arab horses: associations with MHC but not with NKC genomic regions

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Although the Major Histocompatibility Complex (MHC) has been repeatedly associated with susceptibility to equine sarcoid, a disease associated with bovine papillomavirus (BPV1) infection, the role of the MHC in the mechanisms of the disease is not fully understood (Semik-Gurgul, 2021). The objectives of our work were to analyze associations between polymorphic markers of the MHC genomic subregions and of the Natural Killer Cell complex (NKC) and the presence of sarcoids in Arab horses. Microsatellite loci located in the MHC class I, II and III subregions and two MHC class II genes (*DRA, DQA1*), along with a set of NKC microsatelllite markers, were genotyped (Horecky et al., 2018). Fourteen microsatellites of the standard parentage kit, located outside the MHC and NKC regions, were also tested. The presence of BPV1 in cutaneous swabs was confirmed in all horses. Standard chi-square and Fisher tests with Bonferroni corrections were used for association analyses. No associations with sarcoid were found for non-MHC, non-NKC microsatellite loci, MHC class I and/or class III microsatellites, or for NKC-linked microsatellite loci. Significant associations were found between the presence of sarcoids and MHC class II-linked microsatellites *CZM004, COR114, TKY3324,* and *COR112.* The expressed genes *DRA* and *DQA1* showed no associations. The results suggest that the MHC class II region could play a role in immune responses to sarcoid. Ongoing long-read Nanopore sequencing of the region encompassing the marker *COR112* should provide more information on this part of the MHC region containing no expressed genes.

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In-depth analysis of the equine genome related to disorders of sexual development using the CGH microarray technique

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Since the first horse genome was sequenced, impressive progress has been made in the field of equine genomics, including the discoveries of mutations responsible for single-gene disorders and the genetic basis of many complex diseases (Raudsepp et al. 2019). Despite these advances, the genomics of equine reproductive traits have not been intensively studied, even though reproduction is crucial for horse breeders and the survival of wild populations. Both numerical and morphological chromosomal abnormalities can have a direct impact on the efficiency of reproduction, and can lead to embryonic death, perinatal death and reduced fertility of carriers (Bugno-Poniewierska and Raudsepp, 2021). Advanced cytogenetic techniques, such as fluorescence in situ hybridization (FISH) and array-based comparative genomic hybridization (aCGH), provide greater diagnostic precision for chromosomal abnormalities. The aim of this study was to conduct an in-depth analysis of the karyotype of horses with sexual development disorders, using aCGH. The study included 3 horses with the following karyotypes: 63X/64XX/65,XXdel(Y)(q?), 64,XX/65,XXY/66,XXYY and 64,XX/64,XY. All horses showed signs of intersex development, such as a male body shape and behavior typical of a stallion, remnants of a vulva and vagina in the perineal area under the tail, with a formation resembling an enlarged clitoris or glans penis, and small testicles located in the abdominal cavity. The aCGH analysis showed amplification of genes related to reproductive function on the X chromosome: STS, AR, AMELX, MAMLD1, MIR18B, MIR767, MIR672, MIR98, MIR1468, NR0B1, and PLAC1. Aberrations on chromosome 7, embracing microRNA genes associated with infertility in animals and humans: MIR23A, MIR27A, and MIR24-1, were also identified. These results expand our knowledge of the genetic factors that determine and influence the fertility of horse populations.
The study was financed from funds of the project BIOSTRATEG, the decision number BIOSTRATEG2/297267/14/NCBR/2016.

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Using SNP genotyping as a complementary technique to detect chromosomal abnormalities in Pura Raza Español horses

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Chromosomal abnromalities are a well-know cause of reproductive failure in the domestic horse. Most of the cases are related to failures in the sex chromosomes, produces non-visibles abnormal phenotypes in which the individals appears to be normal but infertiles. However, some cases are associated with morphological abnormalities in the reproductive tract. Horses of breed Pura Raza Español horses are screened by the the Breeders association (ANCCE) by combining the results of routinely parentage STR analysis and potential chromosomal abnormalities. All of them are submitted to SNP genotiping to refine and confirm the presence of such chromosomal abnormalities. Hereby we report results of 8 new cases including 63,X mares, 64,XYdsd mares and 65,XXY males detected during the last 12 months. Individuals were genotyped using the Equine GGPV5 genotyping array (Neogen, UK). Raw data, including BAF (B allele frequency) and LRR (Log R ratio) patterns from presumtive affected horses was analyzed using the methodology described by our group. In all the cases, we were able to determine the presence of ECAX monosomy (63,X, n=3), sex reversal mares (64,XYdsd, n=3) and sex par trysomy (65,XXY, n=2). Mares with ECAX monosomy were characterized by no BAF heterozigosity and reduce LRR average values in the PAR region of ECAX, whereas sex reversal mares showed male BAF and LRR patterns in the same chromosome. Finally, 65,XXY horses showed a trysomic BAF and LRR pattern in par region of ECAX and female patterns in the rest of the chromosome. All the cases were also analyzed using FISH karyotiping, showing a coïncidence rate of 100%. This study highlights the reliability of the use of SNP genotyping to determine the presencxe of chromosomal abnormalities in the horse by genomic methods.

A comparison of commonly used variant annotators for quantifying the genetic burden in the horse.

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Whole genome sequencing (WGS) projects characterizing genetic variation within a population have improved understanding of genetic diversity. A surprising finding has been the higher-than-expected number of variants computationally predicted to be deleterious in healthy individuals, termed the genetic burden. An important challenge to quantifying the genetic burden is differences in predicted annotation for the same variant by different variant annotators. Using two commonly used variant annotators (Ensembl-VEP and SnpEff) we compared variant annotation calls and quantified the genetic burden in 605 horses representing 48 breeds. We identified 25,994 genetic burden variants (18,795 SNPs and 6,969 indels) or variants called high impact by both annotators or high by one annotator and moderate by the other. Of these, 25,500 variants were called high impact by both Ensembl-VEP and SnpEff, 58 variants were called high by Ensembl-VEP and moderate by SnpEff, and 336 variants were called moderate by Ensembl-VEP and high by SnpEff. The median (interquartile range) genetic burden per horse was 741 (621-840). The median (interquartile range) allele frequency of each genetic burden variant was 0.16% (0.08-0.33%) and this was significantly lower than the median (interquartile range) allele frequency for non-genetic burden variants of 2.00% (0.25-10.30%), $p < 0.0001$, 95% confidence interval 1.75-1.92%.
Overall, the agreement between Ensembl-VEP and SnpEff was excellent and the genetic burden in horses is higher than the genetic burden reported in humans. Work is ongoing to add ANNOVAR into this analysis to evaluate three of the most used variant annotators.

Integrative genomics of Thoroughbred horse recurrent laryngeal neuropathy

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Understanding the molecular underpinnings of equine physiology in response to exercise training and recurrent laryngeal neuropathy (RLN) is pivotal for optimizing athletic performance and managing health conditions. This study investigated differential gene expression (DGE) between untrained and trained horses, considering the presence or absence of RLN, to delineate the genetic signatures associated with training adaptation and RLN pathogenesis.
Utilizing RNA-seq data, DGE analysis was conducted on Thoroughbred skeletal muscle samples obtained from untrained and trained horses, stratified based on RLN status. Initial findings revealed distinct gene expression patterns in trained versus untrained horses, highlighting transcriptional alterations correlated with exercise adaptation. In addition to the DGE analysis, pathway analysis using multiple pathway databases (KEGG, DAVID, Reactome) and network analysis using the STRING database was used to determine the key genes that RLN related signatures interact with. This generated six gene sets for RLN and non-RLN cohorts. These were then integrated with two RLN GWAS data sets by employing a new genomic integration tool—gwinteR. Using GWAS summary statistics, this methodology enabled detection of newly prioritised SNPs within and in proximity to key structural genes, revealing possible novel gene targets for RLN therapy.
In conclusion, this study elucidates the intricate genetic landscape characterizing training-induced adaptations in thoroughbreds and unveils potential molecular signatures associated with RLN. These findings not only enhance the understanding of equine exercise physiology but also offer valuable insights into the molecular mechanisms underlying RLN, potentially paving the way for targeted interventions and management strategies in athletic and clinical settings.

Breed-specific SNP and genomic regions associated with equine recurrent exertional rhabdomyolysis susceptibility overlapping with up- and down-regulatory histone modifications

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Recurrent exertional rhabdomyolysis (RER) is a myopathy characterised by episodes of exercise-induced myofibre necrosis, muscle stiffness and fasciculations, with extreme cases resulting in kidney failure or death. There is a genetic component attributed to RER[1-3], but specific causative genes and mutations have not been identified. Due to the disease's metabolic nature, we hypothesised that regulatory regions of the genome may be implicated[4]. We aimed to identify genetic markers for RER in Thoroughbreds (TB), Warmbloods (WB) and Connemara ponies (CP). Since many of these were located in non-coding regions we compared their locationwith peaks in the publicly-available equine ChIP-seq data obtained from the FAANG portal[5]

33 CP (17 cases, 16 controls) and 94 WB (50 cases, 44 controls) were genotyped using a 670k genotyping array. GWAS, regional heritability mapping (RHM) and FST analyses were then run both across and within breeds. Various window sizes around significant and suggestive markers from these analyses, and 26 previously-identified US TB RER markers[2] re-mapped to EquCab3.0, were compared to locations of histone markers identified in longissimus dorsi samples from two horses in the FAANG data using bedtools.

No ChIP-seq peaks directly overlapped TB QTLs, but 6 in CPs and 2 in WBs overlapped with significant and suggestive significant SNPs for RER susceptibility. Within 10 kb of TB QTLs there were significantly fewer H3K27me3 and more H3K4me3 peaks than expected, whilst WB RHM regions contained significantly more H3K27me3 peaks and fewer H3K4me1 peaks than expected. No Bonferroni-corrected significant differences were identified in CP alone.

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Whole-Genome Sequence Study of Megaesophagus in Friesian Horses

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Megaesophagus is a hereditary disease common in Friesian horses. It involves dilation of the esophagus, most often in the thoracic region. Megaesophagus causes reduced esophageal motility and esophageal obstructions or choke, which can be as severe as fatal. Whole-genome sequencing using Illumina Short-Read method was performed on 19 Friesian horses, including 7 affected and 12 unaffected sires (4), dams (6) and full-sibs (2). Following the hypothesis of a single recessive variant responsible for Megaesophagus, initial analyses found no variants common as homozygous non-reference (Equcab 3.0) among affected horses, while absent in unaffected horses. Then the variant files from each affected individual were compared separately to another dataset of whole-genome sequence variants in 532 horses of 40 breeds. On average, 120,361 private homozygous variants in each individual were found, meaning not present as homozygous in any of the 532 control horses. Ensembl Variant Effect Predictor was used to identify potential effects of private variants and to prioritize based on their impact on gene function. Several candidate genes were identified, and functional genomic analyses including RNA-sequencing are underway to determine whether altered expression is associated with megaesophagus.

Review: Long read sequencing applied to equine genomics to improve selection for exercise ability and health

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Our research team aims to provide the equine industry with genomic methods to produce high-performing and healthy horses. Long-read technology is exciting because it overcomes the limitations of short-read sequencing, enabling a chromosomal-scale exploration of genome variability and complexity. Numerous specific questions can benefit from long-read sequencing, to name a few: telomere to telomere genome assembly, identification of structural variations, transcriptome analysis, resolution of repeat regions, improved epigenetic profiling, and environmental and metagenomic studies.
In this context, we developed original long-read sequencing protocols: i) mitochondrial genome sequencing based on the selective elimination of nuclear DNA (linear) using an exonuclease treatment and on the specific amplification of circular mitochondrial DNA using a multiple displacement amplification step; ii) direct mRNA sequencing from total RNA; iii) equine influenza virus genome sequencing based on the specific amplification by RT-PCR of the eight segments that constitute its RNA-based genome.
We will briefly report the use of these methods in three independent projects:
- **Identifying mitochondrial DNA variants** in endurance horses to discover genotypes associated with high or poor performance. We sequenced mtDNA followed by GATK SNP calling and GWAS analysis on a cohort of 434 horses (Arabian 83.4% and Anglo-Arabian 9.6%) from 232 different sires with an average family size of 1.8 offspring per sire. We identified 1268 SNPs; among them, 15 SNPs were significantly associated with the performance traits in endurance (raw $p<0.01$) [1; 2].
In addition, we sequenced the mitochondrial genome of Twilight (Acc#3729), and we determined the correct identities of six undetermined bases (N) in the published MH586816 sequence, enabling the improvement of the equine mitochondrial reference genome.
- **A transcriptomic analysis to characterize the differential gene expression in melanoma**, a common skin tumour associated with the STX17 mutation in grey horses [3]. We performed direct long- read RNA sequencing on nine melanoma biopsies compared to nine normal skin. We identified 333 genes differentially expressed in melanoma versus normal skin (corrected $p<0.05$).
- **A genetic characterization of circulating equine influenza H3N8 viruses**. Flu is highly contagious and spreads worldwide with international competitions and exportations. To improve the monitoring and management of future outbreaks, obtaining complete genome sequences of the virus is essential to follow the emerging variants of the virus. [4]
In conclusion, long-read sequencing spans various genetic applications, from basic research to clinical diagnostics. It will continue to drive advances in our understanding of equine genetics and genomics. We will present in detail the protocols developed and the significant results obtained.

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A genome-wide association of variable sensitivity to multimodal analgesia in horses

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Equine analgesia has been known to elicit a range of side effects, varying in severity and prevalence and typically escalating with higher dosage rates. Multimodal analgesia, or combining different drugs, has been seen to reduce commonly documented side effects; however, large variations still exist across individuals. Recent investigations have begun to explore the CYP450 enzyme family and the melanocortin-1 receptor's role in opioid analgesia (Knych et al. 2019, Bacon et al. 2023). This study adopts a genome wide approach to identify candidate regions involved in analgesic sensitivity, with the goal of advancing precision medicine in equine pain management and reducing the incidence of adverse side effects. A subset (n=47) of the Pioneer 100 horses from the University of California Davis were administered both hydromorphone and detomidine prior to cerebrospinal fluid (CSF) centesis. Pain responses were scored (1-3) as the measure of analgesic sensitivity and combined with whole genome sequence data. Genome wide association was conducted using linear regression, with breed and dosage rates of administered drugs included as covariates in final models. SNPs on chromosomes 12, 20 and 27 reached Bonferroni significance ($P<5x10^{-8}$) threshold in association with analgesic sensitivity. Significant SNPs on chromosome 27 were within the LOC100630731 gene, though there is little functional information available for this gene. No other significantly associated SNPs were within known genes. This study served to identify locations warranting further interrogation to better understand and subsequently manage variable analgesic sensitivity in horses.

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Antisense oligonucleotides directed to the mitochondrial long non-coding eca-ASncmtRNA inhibit equine skin tumor cell proliferation.

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Introduction

ASncmtRNAs are mitochondrial long non-coding RNAs, previously identified in humans and mice (1), showing differential expression between tumor and normal cells. Antisense oligonucleotides (ASOs) targeting of ASncmtRNAs induces proliferative arrest and apoptosis without affecting normal cells (2, 3). To translate this technology to equine cancer, we screen ASO candidates in two equine tumor cell culture models with different etiology: Melanoma, associated with an STX17 gene mutation, highly prevalent in gray horses, and sarcoid tumors caused by bovine papillomavirus infection (4, 5).

Material and methods

We designed *in silico* targeting the eca-ASncmtRNA. A melanoma cell line (6) and sarcoid biopsy cultures were transfected with ASO candidates and controls. Cell viability assays and cell counts were used to perform screenings followed by flow cytometric assays. Full phosphonothioate (PS) and locked nucleic acids (LNA) oligo chemistries were evaluated.

Results

ASO candidates reduced cell viability and induced 65% death in melanoma cells at 500nM, which was further confirmed by Flow cytometry. ASO-08 repeated transfection increases the anti-proliferative effect (65 to 89%). PS only ASOs are more effective compared with LNA chemistries. In sarcoid cell cultures, similar effects are detected at 250nM, inducing no significant changes in cell viability in control myoblasts.

Conclusion

ASOs directed to the eca-ASncmtRNA induce selective antiproliferative effects in two tumor cell models phenocopying the effects of ASncmtRNAs targeting in humans and mice. Further research will be required to test oligonucleotide therapy in horse skin tumor cases.

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Effect of pasture vs stable management on gastric and fecal microbiota of healthy horses

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Equine gastrointestinal microbial communities are complex, susceptible to husbandry changes, and variable across the gastrointestinal tract. Microbiota of native equine gastric fluid have not been described. The objective was to evaluate the effect of a change from pasture to stable management on the gastric fluid and fecal microbiota of healthy horses. Horses were maintained on pasture for 6 weeks, stabled for 5 weeks, and then returned to pasture. A consistent forage diet was provided throughout the study. Gastric fluid and feces were collected weekly during 3 weeks of pasture turnout and 5 weeks of stabling, then once 6 weeks after returning to pasture. Samples were collected via a sterile double-tube technique without introduction of additional fluid. Full length 16S ribosomal DNA sequencing and microbial profiling analysis was performed. A total of 770 taxa were identified in gastric and 5,284 taxa in fecal samples. Alpha diversity (Chao1, Shannon, Simpson) was significantly different between sample types ($p<0.001$), but not housing location ($p>0.3$). PERMANOVA of Bray diversity estimates identified a significant effect of housing and horse on both gastric ($p=0.005$, $p=0.009$) and fecal ($p=0.001$, $p=0.001$) microbiota. When horses were stabled, relative proportions of lactobacillaceae increased and streptococcacae decreased in gastric samples, while firmicutes increased and bacteriodota decreased in feces. However, there was no significant week-to-week variation during pasture or stable for gastric ($p=0.9$) or fecal ($p=0.09$) microbiota. Overall, findings support the maintenance of stable gastric and fecal microbial populations under each management condition, with differences in Beta but not Alpha diversity between conditions.

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Evidence of FBN1:p.(Ala882Val) as Likely Causal for Congenital Bilateral Ectopia Lentis in a Horse

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Although a number of inherited ocular disorders have been extensively studied in horses, few reports of equine ectopia lentis exist and no genetic investigations have been performed. Here we examined a 3-day old Oldenburg x Thoroughbred colt due to concerns over bilateral ocular anomalies and hypothesized a either a recessive or a *de novo* mutation was the genetic cause. Examination revealed bilateral microphakia and spherophakia with medioventral lens subluxation. Histopathology of the globes was consistent with ectopia lentis. Whole genome sequencing of the affected foal was conducted and forty-six candidate genes were screened for SNPs and small INDELS. Testing both hypotheseis, eighty-two variants were detected, of which 69 were present in publicly available data from 504 horses. Of the thirteen remaining variants, two variants were found in 3' UTRs (of *ADAMTS17* and *OAF*), ten were intronic, and only one was a coding variant located in *fibrillin-1 (FBN1)* (FBN1:p.(Ala882Val)). This variant was also predicted to be deleterious to protein function.
The affected foal was confirmed by Sanger Sequencing to be heterozygous for this variant and his dam and five paternal half-siblings, all clinically unaffected, were homozygous for the reference allele. Additionally, this same substitution is reported to be pathogenic causing Marfan syndrome in humans with a dominant mode of inheritance, of which ectopia lentis is a common feature. These findings support a *de novo* hypothesis for the variant in FBN1:p.(Ala882Val) as likely causal to ectopia lentis in this foal.