Abstracts of the 25th International Colloquium on Animal Cytogenetics and Genomics (*25th ICACG*), June 26-29, 2024, Naples, Italy

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Introduction

The 25th International Colloquium on Animal Cytogenetics and Genomics is dedicated to the memory of Dr. James (Jim) Womack, a pioneer in gene mapping, especially in cattle. The meeting opened with an obituary presented by Prof. Penny Riggs, a former student at Texas A&M University (TAMU) and now a professor in the same department.

The meeting was organized into 10 sessions, beginning with the General Opening Session 1, which featured three main lectures highlighting the fields of animal cytogenetics and genomics. As expected, among the 84 accepted abstracts for publication, those related to animal genomics were more prevalent than those focused solely on cytogenetics. However, several abstracts combined the two disciplines (Cytogenomics) to provide a deeper understanding of animal genomes and to better identify latent chromosome abnormalities related to fertility. Various genomic approaches were reported in several abstracts, aimed at improving the selection of animals for productive traits, disease resistance and animal biodiversity.

Given the numerous abstracts on water buffalo (river type), a specific session was dedicated to this species, which is particularly important in Eastern, South American, and Mediterranean countries. Nonetheless, research on a wide range of animal species, including domestic and non-domestic animals, non-mammalian vertebrates, and invertebrates, was also presented. Special attention was given to the posters, which were displayed throughout the meeting. Additionally, 15 of the posters, selected by the chairpersons of the poster session, are presented and discussed on the final day. Five posters received awards. All abstracts underwent peer review, and only a few required corrections or modifications. In conclusion, the colloquium featured 14 lectures (L), 27 oral communications (O), and 43 posters (P). Each presentation was numbered according to the congress program.

DR. JAMES (JIM) WOMACK OBITUARY

O1 - *Gene Mapping is Good for You!* – remembering dr. James E. Womack

Penny K Riggs,[1] and Womack Lab Former Students[1]

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A true pioneer in the field of comparative animal genomics, Prof. Jim Womack (30 March 1941 – 13 August 2023) is remembered for his remarkable career, scientific achievements, and mentorship of 50 doctoral students, and countless additional graduate students, post-doctoral scientists, and visiting scholars. Jim completed a Bachelor of Science degree at Abilene Christian College (ACU), followed by a PhD in genetics at Oregon State University. Following positions at ACU and Jackson Laboratories, he joined the faculty at Texas A&M University in 1977, remaining until his retirement as Distinguished Professor in 2018.

Womack's extensive scientific contributions included the first comparative synteny map for cattle. This publication was key for connecting chromosome maps to linkage groups, launching advances in the field that led to completion of the bovine genome sequence. Jim's research, as evidenced by more than 380 peer-reviewed publications, led to numerous recognitions and awards, including the CIBA Prize for Research in Animal Health (1993), election to the National Academy of Sciences (1999), and the Wolf Prize in Agriculture (2001). He was also recognized with awards for outstanding teaching, graduate mentorship, and service to the scientific community.

Jim thoroughly enjoyed life outside the lab and cherished his family: – his wife of 60 years, Raby Womack., children Jimmy (deceased) and Wendy, and grandsons Quaid Faltys and James Hamlin Hill. He is also survived by numerous colleagues, friends, and former students and advisees who will carry his legacy in genomics on to the next generation.

GENERAL OPENING SESSION 1

L1 - Cytogenetic Diagnostics – from Giemsa Staining to SNP Microarray and NGS

Marek Switonski

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Implementation of cytogenetic diagnostics into veterinary medicine and animal breeding has been launched in late 60-ties of XX centaury by Professor Ingemar Gustavsson, who using Giemsa staining showed a high incidence of 1/29 Robertsonian translocation in Swedish cattle. This simple approach was applied in studies on worldwide distribution of this mutation in numerous cattle breeds. Development of banding techniques in late 60-ties and early 70-ties, followed by establishing the first international G-banded standard karyotypes of several domestic animal species, was a mile stone in cytogenetic diagnostics. The use of G-banding revealed that reciprocal translocations are quite common in subfertile pigs, while C-banding was frequently applied in diagnosis of X monosomy in sterile mares. Fluorescent in situ hybridization (FISH), developed in the middle 80-ties of XX centaury, was another mile stone. Genome libraries and so-called flow karyotyping facilitated the use

of locus specific BAC probes and whole chromosome painting probes for identification of numerous chromosome aberrations. Recently a multi-hybridisation FISH approach, performed on microscopic slides with immobilized BAC probes specific for distal fragments of all chromosome arms, was successfully used for a rapid identification of reciprocal and Robertsonian translocations in AI bulls and AI boars. Classical and molecular cytogenetic techniques have a crucial limitation, which is a strict time regime regarding delivery of blood samples from a farm or clinic to the diagnostic laboratory, thus, molecular methods based on isolated DNA samples were highly demanded. Development of SNP microarrays and the next generation sequencing (NGS) is the newest mile stone in cytogenetic diagnostics. These methods were used in diagnosis of autosomal or sex chromosome aneuploidies. Molecular detection of sex chromosome aneuploidies or XX/XY leukocyte chimerism associated with freemartinism can be also performed by digital droplet PCR (ddPCR). An interesting molecular approach, recently developed, is the use of copy number variation (CNV) in pericentromeric region of bovine chromosome 29 for detection of 1/29 centric fusion carriers. Concluding, the role of molecular techniques in cytogenetic diagnostics is increasing and what is very important they allow to perform screening studies on archival DNA samples.

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L2 - Bovine Research: from Genomics to Epigenomics

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The field of bovine research is undergoing a significant transformation, driven by advances in long-read sequencing technologies. These developments have led to the construction of Telomere-to-Telomere (T2T) genomes, facilitating a thorough identification of genomic variations, including structural changes. This detailed mapping of all variants is instrumental in developing bovine pangenomes, which represent the whole genetic diversity of the species. Pangenomes consist of a core genome, present in all individuals, and accessory genomes, which are unique to certain populations, breeds, or individuals. Pangenomes will enhance the detailed investigations of intraspecific diversity and facilitate the identification of variants responsible for specific traits within genomic regions. Long-read sequencing technologies are also enhancing our ability to study gene expression and DNA methylation. In addition to being at the basis of tissue differentiation, DNA methylation is one of the epigenetic mechanisms involved in environmental adaptation, enabling organisms to adjust to local conditions and stress responses. This adaptability is vital for cattle, which face diverse environmental challenges including changes in maternal diet, heat stress, and disease.

These challenges can lead to epigenetic modifications, some of which may be heritable, thereby affecting subsequent generations. The forthcoming release of the first bovine pangenome represents a pivotal achievement, capturing the species' collective variation and signifying a new chapter in cattle genetic research. Together with new findings on the heritability of epigenetic alterations triggered by environmental stressors, the pangenome opens new perspectives in the identification of causal genetic and epigenetic variants, to understand biology and for assisted selection. The current challenge is to incorporate epigenetic insights into traditional genetic improvement programs. This presentation will shed light on the latest progress in bovine genomics and epigenomics, focusing on the development of pangenomes and the effects of epigenetic modifications on resilience and adaptability of cattle, and discussing the potential for integrating these innovative discoveries into genetic improvement strategies to improve cattle performance and welfare.

L3 - Clinical Veterinary Genetics in the Age of Whole Genome Sequencing

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Domesticated animals' genomes are a goldmine for medical research because they often have rare diseases that are very similar to those in humans, and their genomes contain selection signatures that explain, for example, breed-specific traits such as coat colour and horn status. The large SNP genotyping data available from farm animal genomic selection programmes could be readily used to map potentially associated regions in the genomes and could also help to detect chromosomal abnormalities in affected animals. Over the past decade, short-read whole genome sequencing of individual cases compared to thousands of control genomes has been used to identify causal variants for Mendelian disorders, including protein-altering single nucleotide variants, indels, as well as larger structural variants such as copy number variations, translocations or deletions predicted to cause haploinsufficiency. In the case of chromosomal abnormalities, depth of read coverage analysis allows the WGS-based detection of reciprocal translocations and other structural constitutional rearrangements beyond the resolution limit of classical cytogenetic techniques, including precise mapping of breakpoints in the genome. Although only recently introduced, long-read sequencing technologies have already demonstrated their potential also in veterinary genetic research, with the potential to greatly improve diagnostic yield, particularly for the identification of structural variants and repeat expansions. Using recent examples, this presentation will highlight the emerging potential of precision genomic diagnostics in livestock and show how these methods can contribute to the sustainable improvement of the reproductive success of livestock populations by increasing the diagnostic rate of potentially genetic losses during pregnancy, birth and rearing.

SESSION 2 - CYTOGENETICS AND GENOMICS IN ANIMAL DIAGNOSTICS

L4 - Detection and characterization of cytogenetic defects in cattle using large genotypic and phenotypic data sets generated for genomic evaluation.

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Over the past two decades, the French National Cattle Database (FNCD) has accumulated information on the life, performance and pedigree of more than one hundred million animals, as well as SNP array genotypes of 2 million individuals from various breeds generated as part of genomic selection. The exploitation of this vast amount of data offers numerous prospects in applied and basic research.
In a recent study, we developed a highly sensitive approach to detect interchromosomal rearrangements (IR) by searching for abnormal linkage disequilibrium patterns between markers from non-homologous chromosomes in large paternal half-sib families (Jourdain et al., Genome Res. 2023 Jun; 33(6)). After validation by cytogenetic analyses, we reported one Robertsonian fusion, 10 reciprocal translocations, and the first case of insertional translocation reported in cattle among 5571 normozoospermic bulls (prevalence =2.15/1000). By combining multiple sources of information, we demonstrated that most of these IRs are the result of recent *de novo* mutations due to abnormal male meiosis and that they have dramatic negative effects on several fitness related traits in the carrier sires and their carrier daughters. Finally, we performed long-read sequencing to better characterize the exact nature of seven of these IRs and to identify candidate genes whose haploinsufficiency may be health threatening.
In this talk, we will detail the main results of this study, which is to our knowledge the most comprehensive and thorough screen for interchromosomal rearrangements compatible with normal spermatogenesis in livestock species. In addition, we will also present the possibilities offered by the analysis of signal intensity and genotypes of markers located on chromosomes X and Y to identify other cytogenetic defects, with a special emphasis on heterochromosome aneuploidy and free martinism.

L5 – The Impact of Bioinformatics in Animal Cytogenetics

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The number of chromosomes of the bovine species was defined as 2n=60 in 1927 and this observation was later confirmed in several papers published from 1944 to 1957.

There is no doubt that the impetus for the development of the cytogenetics of cattle, and thus of animals of zoo-economic interest, began in 1964 when Gustavsson published the identification of the first cases of Rob1;29.
However, even though 60 years have now passed, cytogenetic approaches have not changed much. Technological advances that can be recalled are the application of banding techniques that have enabled the unambiguous recognition of chromosomes, especially for those species where most chromosomes are acrocentric, or the development of FISH that initiated the mapping of genetic factors in the pre-genomic era.
In recent decades, an evolving discipline has emerged as a result of the need to analyse the multitude of data produced by hight-througput sequencing technologies: bio-informatics.
In this presentation, the relationships between cytogenetics and bio-informatics and how the latter can be of great help to cytogenetics are presented and discussed.

O2 - Three new 65,XXY horses detected using medium-density genomic screening in the Pura Raza Español breed

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Chromosomal abnormalities are the most common cause of genetic infertility in the domestic horse. Nowadays, the age of genomics allows us to improve the accuracy of diagnostics, but also to perform large-scale screening programs. This is the case of the Pura Raza Español horse, in which all the individuals enrolled in the studbook are analyzed for chromosomal abnormalities by a genomic procedure. Hereby, we present three new cases of 65,XXY horses detected during the last 16 months. The individuals were flagged due to abnormal results in the initial STR parentage testing and then submitted to MD SNP genotyping (GGP Equine, Neogen, UK). Copy number aberration (CNA) was made per chromosome using an in-house pipeline based on the analyses of B-allele frequencies (BAF) and Log R Ratio (LRR). In addition, two of the individuals were karyotyped determining the sex-pair complements by FISH. The three cases showed ~172 ECAY-linked SNP-makers with positive amplification. In ECAX, the non-par region (SNP located >1.8Mb) showed an average BAF close to 0, depicting heterozygosity. However, in the PAR region (SNP located in the first 1.8Mb of ECAX), the three individuals showed four different peaks in BAF values, commonly associated with mosaicism. This CNA in the PAR region was produced by the presence of two ECAX and one ECAY in the same individual. In addition, no differences were observed between the results obtained from analyzing DNA obtained from blood and hair follicles, ruling out the presence of hematopoietic chimerism. Two of the three cases were confirmed as 65, XXY by FISH analysis. We demonstrated the validity of genomic screening to allow an early and accurate detection of CNA in the domestic horse.

O3 - Sperm-FISH Analysis for Validating Sexed Italian Mediterranean River Buffalo (*Bubalus bubalis*) Semen

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In both cattle and Italian Mediterranean River Buffalo breeding, sexed semen plays a vital role in economic and reproductive management. The most recent technology employed for semen sexing is laser ablation, where female sperm, containing more DNA chromosomes, are typically larger than male sperm. This technique involves passing the semen through a laser that can identify stained X chromosomes and use ablation to remove the Y chromosomes, resulting in female-sexed semen.
This study aims to assess the accuracy of this sexed semen technology through Fluorescence in Situ Hybridization of Spermatozoa (Sperm FISH). For the analysis, we utilized two different probes containing sequences complementary to those of the buffalo X and Y chromosomes from CHORI BAC libraries 240 (cattle) and validated on river buffalo metaphases. We counted 1000 spermatozoa in six bulls under the fluorescence microscope, both in the total and sexed semen fractions of each animal. The total sperm fraction showed an X signal percentage close to 50%, while sexed semen ranged between 70% and 80%. Only one sexed semen sample showed an X signal percentage identical to the total fraction, highlighting the importance of FISH analysis for sexed semen validation. Moreover, it emphasizes the need for cytogenetic methods to assess X chromosome-carrying sperm concentration, a fundamental aspect in dairy farming. Additionally, validating sexed semen is crucial as it can cost up to 10 times more than unsorted semen, underscoring the economic significance of accurate validation procedures.
This study highlights practical implications for the dairy industry and buffalo breeding, stressing the need for further research to optimize semen sexed validation methods.

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P1 - Monozygotic Origin of Three Cases of Female Dicephalic Buffalo and Bovine Calves

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Dicephaly is a rare congenital malformation observed in different mammalian species. In humans its frequency is estimated between 1:50,000 and 1:100,000 in newborns, and majority of the cases were

females. In livestock breeding dicephalic newborns cause economic losses also due to negative consequences of dystocia for a cow delivering such animal. There are several reports concerning anatomy of dicephalic bovine or buffalo calves, however, there are no reports on their molecular characteristics. The aim of the present study was DNA analysis of tissue samples isolated from both heads (ear, hair follicles or brain) of stillborn dicephalic calves (two bovine and one buffalo) to reveal their origin (mono- or dizygotic) and chromosomal sex. A panel of 12 microsatellites (BM1818, BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA227, TGLA122, TGLA126 and TGLA45), accepted by ISAG for parentage testing in cattle, was used to study both bovine cases. Moreover, PCR searching for Y-linked (*SRY* and *AMELY*) and X-linked (*AMELX*) was also performed. Genotype of the buffalo calf was analyzed at 14 microsatellite sites specific for this species (CSSM60, BMC1013, CSSM47, INRA026, CSSM19, BMO922, RM4, INRA006, CSSM42, MAF65, D5S2, CSSM38, BM1706 and CYP21), along with searching for the presence of Y-linked and X-linked genes. These analyses revealed the same genotype for all microsatellites and lack of Y-linked sequences. In conclusion our study showed that the studied calves were females and had monozygotic origin.

P2 - Assessment of the Influence of Methylation and the Position Effect on the Inactivation of Nucleolar Organizer Regions in Mares with Karyotype 64,xx,t(x;1)(xp;1p)(xq;1q).

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The nucleolar organizer regions (NORs) in the equine karyotype are located on three pairs of autosomal chromosomes 1, 28 and 31. The activity of NORs may be influenced by many factors, including age or breed, but also mechanisms such as methylation, inactivation of one of the X chromosomes and the position effect. The above-mentioned mechanisms led to the formulation of a hypothesis assuming that the translocation involving the p arm of chromosome pair 1 (where the NOR are located) and the p arm of the X chromosome will affect the activity of the NORs of the newly formed 1p:Xp structure. Therefore, the aim of the conducted research was to assess the influence of methylation and the position effect on the inactivation of NORs in mares with karyotype 64,XXt(X:1)(Xp:1p)(Xq:1q).

Cytogenetic analyses, such as lymphocyte culture, AgNOR staining and immunofluorescence with anti-5-methylcytosine antibody techniques were performed on the metaphase chromosomes of a 2-year-old Hutsul mare, carrier of a reciprocal translocation between the arms of the chromosomes no.1 and the X chromosome - karyotype 64,XXt(X:1)(Xp:1p)(Xq:1q). The control consisted of preparations from 3 Hutsul mares, 2 years old, with a normal karyotype.

Immunofluorescence technique showed that this mutation influenced the methylation pattern of p arm chromosomes no. 1, silencing the signal within the NOR, but did not affect the transcriptional activity of this region. The AgNOR staining technique used allowed for the determination of the number of active NORs, which ranged from one to six. The observed variability in the number of silvered NORs was primarily caused by the different frequency of these regions appearing on chromosomes of pairs

28 and 31. However, no differences were found in the number of active NORs on chromosome pair 1 in mares with translocation 64,XXt(X:1)(Xp:1p)(Xq:1q), which indicates the lack of influence of the effect of inactivation of one of the X chromosomes and methylation on a translocated chromosome consisting of the p arm of chromosome no.1 and the p arm of the X chromosome.

P3 - A Case of 78,XX/78,XY Leukocyte Chimerism in a Great Dane Dog with Disorder of Sex Development

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Disorders of sex development (DSDs) in dogs are diagnosed more and more frequently and affected animals are often of pure breed. Canine DSDs caused by sex chromosome aneuploidies (X monosomy, X trisomy and XXY complement) are rather rarely diagnosed. The most common type of DSD in dogs with a normal set of XY sex chromosomes is polygenic cryptorchidism, however, monogenic XY DSDs caused by mutations of *AMHR2*, *NR5A1* and *HSD17B3* genes were also reported. On the other hand, in chromosomal female dogs (XX) with the presence of testes or ovotestes the following DNA variants (causative or candidate) were observed: duplication of *SOX9*, CNV in 5'flanking region of *SOX9*, and SNP in *PADI6*.
The aim of this study was genetic characterisation of a Great Dane dog with malformed uro-genital system. The animal presented a female phenotype with a normal vulva, hypertrophy of the clitoris, urethral outlet at clitoral tip and the presence, laterally to the vulvar lips, of two skin folds resembling scrotum. During surgery performed to correct the malformations two small and rounded gonads were found.
Cytogenetic analyses of in vitro cultured leukocytes by fluorescent in situ hybridization (FISH) with the use of X- and Y-specific probes showed the presence of two cell lines: 78,XX (approx. 95%) and 78,XY (approx. 5%). PCR searching for *SRY* gene in DNA isolated from blood cells confirmed its presence and a normal sequence. The observed abnormalities of the uro-genital system were probably caused by placental anastomoses with a male foetus, as it was earlier reported in dogs.

P4 – Preliminary Results on Sperm Cryopreservation in Casertana Pig Breed

Chiara Luongo, Michal Andrzej Kosior, Riccardo Esposito, Federica Piscopo, Luca Masiello, Valentina Longobardi, Emanuele Danza, Giuseppe Terlizzi, Vincenzo Peretti and Bianca Gasparrini

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The *Casertana* pig is an autochthonous endangered breed, recently threatened by the finding of African swine fever in wild boars Therefore, preservation strategies including the establishment of a cryobiobank are fundamental. This work aimed to evaluate the feasibility of sperm cryopreservation in this breed. As the 1843C→T mutation in the RYR1 gene in swine is associated with higher mortality and meat loss, 100 animals were screened by PCR to select those free from this mutation. The ejaculate sperm-rich fraction was collected by gloved-hand method from 3 boars (3 ejaculates/boar), and volume and concentration were assessed. Semen was diluted with Nutrixcell Ultra (v:v, 1:1; IMV Technologies) and transported to the laboratory at 17°C. Sperm concentration was calculated by Burker chamber, and total and progressive motility were evaluated by SCA (Sperm Class Analyzer). Semen was diluted with Boarciphos A and B extender (IMV Technologies) to a final concentration of 1000 x10^6 sperm/ml, cooled at 4°C, packed into 0.5 ml straws, frozen on nitrogen vapor (15 min), and stored in liquid nitrogen. Subsequently, one dose/ejaculate was thawed to assess total and progressive motility. Among screened animals, only 2% showed the T mutation of RYR1 gene. The ejaculate volume (64.2±10.9 ml) and sperm concentration (278.7±77.2 x10^6 /ml) showed inter-individual and -ejaculate variability. Total and progressive motility were respectively 98.0±0.6% and 57.4±5.3% for ejaculated sperm and 36.1±4.7 and 6.5±0.8% for thawed sperm. In conclusion, these preliminary results demonstrated the feasibility of semen cryopreservation in the *Casertana* but further studies are needed to improve the procedure.

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P5 - Influence of Genetic Selection on *In Vitro* Embryo Yields in the Italian Mediterranean Buffalo (*Bubalus bubalis*)

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Genomic selection represents a powerful tool for accelerating genetic improvement in livestock leading to more efficient and sustainable breeding programs. However, its application in buffalo is hampered by a lack of pedigree information, data collection availability, or poor reproductive performance. Italian Mediterranean buffalo (IMB) has been selected mainly for its excellent milk quality and high cheese yield. Since 2019, the National Association of Italian Buffalo Breeders developed a selection index named IBMI to estimate the genetic response for milk yield, milk traits, composite feet and legs, mammary system, and fertility in the IMB. This work aimed to evaluate the developmental competence of buffalo oocytes collected from live donors selected by IBMI (≥130) vs a control group (CTR), i.e. animals only selected on milk records. IMBs (8/group) underwent Ovum pick-up as previously described (Petrovas G. *et al*. Animals 30;10:1997, 2020).
). Follicular and oocyte populations were recorded; good-quality oocytes were in vitro matured, fertilized, and cultured to the blastocyst stage by standard procedures (1). Animals in the CTR and IBMI groups had similar (mean±SE) number of aspirated follicles (11.5±1.2 vs 9.2±1.6; respectively). Likewise, good quality oocytes and cleavage rates were similar in CTR and IBMI

groups (30.1±0.9 vs 42.3±1.0 and 46.0 ±1.0 vs 54.9 ±1.1 %, respectively). Interestingly, embryo yields were higher (P<0.05) in IBMI compared to the CTR group (29.1±0.5 vs 15.2±0.7, respectively). These preliminary results suggest that the IBMI can be a valuable tool for the future genetic improvement of fertility traits in the IMB.

P6 - Cytogenetic Monitoring of Romanian Cattle Breeds

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The karyotype control of the Romanian cattle breeds has been developed in the frame of 7 national projects, during the last 15 years. The main objective of the cytogenetic investigation was to detect the chromosomal abnormalities responsible for the significant degradation of the reproductive activity of the cattle females at the farm level. A total number of 854 cattle females, reared in different farms from all over the country, have been karyotyped by using peripheral blood lymphocytes culture. In this study 118 cases of chromosomal instability and 1 case of leukocyte chimerism XX/XY were identified. The phenotypic effects were expressed by reproductive disturbances (repeated inseminations, lack of oestrus and loss of pregnancy), 4 cases of congenital malformations of the front and rear limbs, a case of foetal abnormality (*Schistosomus reflexus*), a case of *posterior limb malformation* and a case of *freemartin* female. For all animals with chromosomal abnormalities the SCEs test has been used and revealed a very high number of sister chromatid exchanges (9-16 SCEs/cell) with particularly presence of 2-3 interchromatidic exchanges on the same chromosome. Considering all of this, the high rate of SCEs could be related with the presence of different environmental toxic agents which can induce reproductive disturbances, foetal growth and development disorders of the carriers.

P7 - Persistent Mullerian Duct Syndrome in a Cat (38,XY): an Analysis of the *amh* and *amhr2* Candidate Genes

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Persistent Mullerian duct syndrome (PMDS) is a monogenic disorder of sex development of males (XY, *SRY*-positive) involving the presence of Mullerian ducts derivatives. In humans, it is mainly caused by mutations in the *AMHR2* (the major cause) or *AMH* genes. However, about 15% of affected patients have normal sequences for both genes. Here we describe the first case of PMDS in a phenotypically normal cat, which underwent endocrinological, histological, and genetic analyses due to unilateral cryptorchidism. Blood testosterone level was typical for males, while anti-Mullerian

hormone (AMH) level was low. Surgical removal of internal reproductive organs was followed by histological studies, which revealed inactive testicles and derivates of Mullerian ducts. Cytogenetic studies showed a normal XY sex chromosome complement, while molecular detection of Y-linked genes confirmed the presence of *SRY* and *ZFY* genes. Analysis of the coding sequences of both candidates, *AMH* and *AMHR2*, in the affected cat and control cats (n = 24) were performed using the Sanger method. In the affected cat, homozygosity was found for three missense variants in exons 1 (one SNP) and exon 5 (two SNPs) of *AMH*, but the same genotypes at these sites were also observed in one male control cat, whose sex development was not examined. Three known synonymous variants with homozygous status were found in *AMHR2*. We conclude that the DNA variants in *AMH* and *AMHR2* are not responsible for PMDS in the affected cat.

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P8 - Cytogenetic Screening of Male Buffaloes (*Bubalus bubalis*, 2n=50) Addressed to the Reproduction

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In Italy, as part of the genetic control conducted by the National Buffalo Breeder Association (ANASB), the examination of the karyotype is routinely performed on all young males intended for reproduction to screen for chromosome abnormalities (Iannuzzi A. *et al*., 2021). This practice gained prominence following the identification of a complex chromosome abnormality in a well-known bull named Magnifico and its progeny (Albarella S. *et al*., 2013). This procedure significantly enhances the value of Italian buffaloes, particularly in the context of artificial insemination both within Italy and in other countries importing semen.

The GENOBU project, with one of its objectives being the screening of young males intended for reproduction, has examined 139 individuals from 15 different farms in the provinces of Salerno and Caserta. Peripheral blood cultures were conducted to obtain normal cultures as well as cultures treated for the late incorporation of 5-BrdU. C-banding (CBA-technique) and R-banding (RBA-technique) were employed, with the latter used for constructing the karyotype. For each animal, 100 cells were examined to verify the correct diploid number, and at least two banded karyotypes were generated for each individual. All the males studied exhibited normal karyotypes, except for one seemingly normal animal that presented an abnormal X-chromosome with negative C-banding, whereas a normal X typically exhibits a prominent C-banding pattern (the largest in the buffalo karyotype). Unfortunately, further investigation involving both parents and relatives to determine the origin of this abnormality was not possible, as they had been removed from the farm by the breeder.

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P9 - Hi-C for the Detection and Characterization of Chromosomal Rearrangements in Cattle

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Recently, a new approach has been developed to detect inter-chromosomal rearrangements in cattle. This method is based on the detection of abnormal linkage disequilibrium patterns between markers located on different chromosomes in large paternal half-sib families genotyped as part of routine genomic evaluations (Jourdain et al., Genome Res. 2023 Jun; 33(6)). This method allowed to identify 12 inter-chromosomal rearrangements including, for the first time in cattle, an insertional translocation between the BTA4 and BTA8. In order to characterize this rearrangement more precisely, Hi-C experiments were carried out on a heterozygous carrier using the Arima kit. Examination of the entire Hi-C matrix revealed ectopic interactions between BTA4 and BTA8 confirming the translocation of a part of BTA4 into BTA8. The BTA4 Hi-C matrix showed an increase in interactions of regions flanking the translocated segment indicating a deletion of this segment. Analysis of the BTA8 matrix showed a decrease in chromosomal interactions compared to a normal individual at the breakpoint position confirming the insertion of BTA4 material into this region.
More unexpectedly, analysis of the BTA8 matrix revealed a characteristic butterfly pattern indicating a heterozygous inversion of a 17Mb region (breakpoints located around 11,250kb and 27,900kb). This inversion was not previously detected by the interchromosomal linkage disequilibrium analysis. Our results demonstrate the interest of the Hi-C for the detection and characterization of chromosomal rearrangements. Further analysis (capture-HiC and phasing) are planned to study the impact of this abnormality on the 3D organization and function of the genome.

P10 – Chromosome analysis in French pig and cattle populations

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For many years, chromosomal controls have been conducted on French porcine and bovine populations at the UMR GenPhySE at the Veterinary School of Toulouse. The aim of these analyses is to detect chromosomal abnormalities that could potentially cause severe reproductive problems in carrier animals or their partners. Thus, in pigs, to date, more than 52,000 purebred boars have been analyzed (2,500 analyses per year), allowing the detection of 276 original structural rearrangements (91% reciprocal translocations, 4% inversions, 1% Robertsonian translocations). Based on these results, we have been able to establish that the prevalence of balanced structural rearrangements in French porcine populations is 0.52%.

Recently, at the request of bovine selection organizations, we set up a chromosome screening (GTG banding) of young bulls before reproduction. To date, 4400 individuals have been analyzed and 2 anomalies (a tandem translocation between chromosomes BTA26 and BTA29 and a trisomy in mosaic) have been identified.

P11- Cytogenetics Complements Molecular Genetics to reveal Undetected Freemartin Heifers

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One of the most economically important traits in beef cattle is reproductive efficiency. Previously, genome-wide association studies were conducted to identify genomic regions associated with fertility status in U.S. beef cattle populations (McDaneld et al., 2012. J. Anim. Sci. 90:2142-2151). In this study, more than 21 % of the females, known to have low reproductive status, were found to also possess Y-chromosome segments. However, none of these animals were believed to be freemartins (infertile females born co-twin to a male). None of these animals had a male twin and all had normal reproductive tract phenotypes.
We conducted cytogenetic analyses from peripheral blood lymphocytes for a subset of the Y-DNA-positive female animals: heifers of Brangus, Braford, or Simbrah breeds that had not become pregnant during their first breeding season. Giemsa-stained metaphase chromosome preparations were scored for the presence of XX or XY sex chromosomes, or fluorescence in situ hybridization was conducted with X- and Y-specific probes. In all cases, we identified both XY- and XX-containing metaphase cells (range from less than 1% XY cells in one case to 96% XY cells), suggesting that these animals were unsuspected freemartins, or at least, exhibited blood chimerism. These findings demonstrate the value of cytogenetic analyses for interpretation of DNA-based diagnostic tests, and further support the value of high-throughput DNA screening as one measure for predicting the probability of reproductive success in beef operations.

P12 - Alterations in Histone Modifications in ihe Genomic Region Harbouring Genes Encoding Transcription Factors Crucial for Adipocyte Differentation in the Pig

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Adipogenesis is controlled on both transcriptional and epigenetic levels. Histone modifications have been recognized as particularly significant among the epigenetic mechanisms essential for the proper differentiation of progenitor cells into adipocytes. The aim of this study was to characterize alterations in the abundance of selected heterochromatin (H4K20me3) and euchromatin (H4K8ac) histone marks

located in genomic regions harboring genes that encode adipogenic transcription factors (*PPARG*, *GATA2*, *CEBPA*, *CEBPB*) over ten days of differentiation of porcine mesenchymal stem cells (MSCs) into adipocytes. The control involved the *GP9* and *RPL32* genes. We hypothesize that local modifications of histones may be responsible for the decompaction of chromatin domains and the changes in nuclear positioning seen for these genes. The histone modifications were examined using chromatin immunoprecipitation ChIP-qPCR. The primers span promoter regions as well as exons. Additionally, transcription factor expression levels were measured with real-time PCR, complementing ChIP-qPCR assessments. The initial findings show that histone modifications of the *PPARG* and *GATA2* genes appear to be associated with adipogenesis, exhibiting day-dependent variations. Correlations between the transcriptional profile and histone modification were seen for the *CEBPA* and *CEBPB* genes. Results from the control genes indicate parallelism in both the heterochromatin and euchromatin histone modifications, suggesting their relevance to gene expression. Our study shows that changes in histone modifications during adipogenesis depend on gene loci and time of differentiation.
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P13 - Use of Crispr/Cas9 Genome Editing to investigate the Role of the *Cebpb* Gene during *In Vitro* Adipogenesis in the Pig

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Adipogenesis, the process of fat cell formation, is controlled on both transcriptional and epigenetic levels. A number of proadipogenic and antiadipogenic transcription factors have been identified, among which C/EBP beta has been recognized as an important early transcription factor. This factor is encoded by the *CEBPB* gene. CRISPR/Cas9 is a powerful method for editing genes and regulating gene expression. The aim of the study was to introduce a deletion in the promoter region of *CEBPB* and to evaluate how this modification affects the differentiation potential of porcine mesenchymal stem cells (MSCs) into adipocytes. Single guide RNAs (sgRNAs) were designed using the CRISPOR tool and were cloned into the pX330 vector. Transfection was performed using a Nucleofector system (Lonza). The transfection efficiency was about 60%. PCR reactions were performed to screen MSC colonies with modifications. A 243-bp deletion in the region of interest was found in MSC colonies in both homozygous and heterozygous state. Sanger DNA sequencing was used to confirm the mutation sites. We are presently investigating the effects of the deletion on MSC functioning and its ability to differentiate into adipocytes. Our results provide evidence for the usefulness of CRISPR/Cas9 in modifying MSCs by regulating the gene expression of crucial transcription factors.
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P14 - Fucoxanthin induces Apoptosis through the Pi3k/Akt/Mtor Signalling Pathway in Chronic Leukemia Cells

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Chronic myeloid leukemia (CML) represents 14% of newly diagnosed cases of leukemia where the translocation of chromosomes 9 and 22 through the fusion of Abelson's tyrosine kinase 1 and breakpoint cluster region protein is the primary cause of the development of the pathology. Since the 2000s, the therapeutic use of imatinib as a tyrosine kinase inhibitor has improved the quality of life of CML patients. However, patients develop resistance that make difficult the long-term therapy (Chan et al, 2020). Therefore, the scientific development of new alternative therapeutic strategies remains a key element for scientific research.
Recently, extracts isolated from marine organisms have been discovered to possess potent anti-tumor activities. Among these, carotenoids are a subfamily of tetraterpenoids synthesized by microalgae with anti-tumor activity. Fucoxanthin (FUCO) is a carotenoid that has been reported to induce apoptosis in several tumors (Satomi et al, 1790). Apoptosis failure is generally associated to the development of various human diseases. Several apoptosis checkpoints regulate the equilibrium between cell death and cell survival and the PI3K/Akt/mTOR pathway appears to be vital in selecting cellular processing in tumor and normal cells (Satomi et al, 1790). Moreover, an increasing number of studies have demonstrated that high levels of reactive oxygen species (ROS) lead to apoptosis and necrosis, both processes implicated in cancer (Martindale et al, 2002). However, the role of ROS generation regarding the anticancer effects of FUCO remains poorly understood. In the present study, we explored the PI3K/Akt/mTOR pathway in FUCO-treated human K562 cells through the gene and proteins expression analyzed by real time PCR and western blot analysis. Moreover, cytotoxicity by MTT assay and apoptosis by flow cytometry were evaluated. Our data demonstrated that FUCO inhibited the apoptotic genes and proteins expression through the PI3k/Akt/mTOR pathway and could represent a new natural therapeutic opportunity against chronic leukemia.

P15 - A mutation analysis in an XY (SRY+) cow with ovarian development

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In 2018, we published a case of a cow with 2n=60,XY karyotype, intact SRY gene and development of ovarian tissue and female phenotype (De Lorenzi L, et al. XY (SRY-positive) Ovarian Disorder of Sex Development in Cattle. Sex Dev. 2018;12(4):196-203). In order to identify a possible causative mutation, we performed an Illumina sequencing. Sequencing operations were carried out following the Illumina protocol.
We obtained 638,866,272 reads for a total of 95,624,774,649 bases. Variants were called by SNPeff, VEP and GATK and in total were identified: 5,731,661 SNP, 914,637 small In/Del, 11, 271 SVs and more than 1,000 CNV.

Following several "purification" operations, which eliminated variants already present in the various databases, variants not involving coding sequences, and variants that had little impact (e.g. silent variants), a very short list of mutations remained. None of these mutations involve genetic factors known to be involved in disorders of sexual development.
In this study, we present the results and discuss their possible responsibility in the occurrence of the observed phenotype.

SESSION 3 - ADVANCED GENOMICS AND EPIGENOMICS IN WATER BUFFALO

L6 - The Water Buffalo Genome Sequence: History, Gains and Future Perspectives

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More people in the world depend on water buffalo than any other domesticated animals. There are two main types of water buffalo, swamp buffalo that have 48 chromosomes and river buffalo which have 50 chromosomes. Having the genome sequence of water buffalo will aid the study of genetic diversity, physiology, disease, evolution, and genome function. Advances in genome sequencing technologies have made sequencing the genomes of all species accessible. The first buffalo genome sequence, of a Mediterranean buffalo, was completed in 2013 based on short read sequences, but was highly fragmented. An international consortium created a transcriptome atlas which was used for annotating this assembly and identified a panel of 94,000 SNP to explore genetic diversity and genome function.
Genome sequences produced with short reads are a patchwork of both parental chromosomes. The development of long read sequencing enabled stretches of each parental chromosome to be assembled independently. A long read-based genome sequence of the same Mediterranean buffalo was published in 2019 which was partially phased. To date the full genome sequences from 3 river buffalo and 2 swamp buffalo have been published.
We developed a method of "trio-binning" to accurately assign sequences to the parent of origin, which uses short read sequences from the parents to identify the origin of the long-read sequences from the progeny to assemble fully phased chromosome sequences. This method is now universally adopted for high quality genome assemblies in all species.
Further advances in technology make possible assembly of telomere to telomere (T2T) sequence, which include the full sequence of chromosomes including repeated, centromere and telomere sequences. A river buffalo trio, of parents and progeny, is included in the Ruminant T2T project. Current reference sequences do not reveal the extent of diversity in a species. Therefore, the next

generation reference genomes, termed "pangenomes" will include all sequence variation for a species represented as sequence graphs, rather than simple linear strings of nucleotides. These "pangenomes" are being developed for various species, including the water buffalo, which is the objective of the 1000 buffalo genome project.

L7 - Whole Genome of a Male Water Buffalo, obtained by Nanopore Technology Long-Reads Sequencing, Generates High Quality *De Novo* Assembly and Provides First Hints on Y Chromosome

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Water buffaloes are farm breeding animals used for food production in different geographic areas, including Eastern, Western and Mediterranean countries. In this context, knowledge of buffalo genome sequences is of great interest in order to better survey the subsequent quality of dairy products. To date, whole genome sequence is only available for a female Mediterranean river buffalo (2N=50; Olimpia). Therefore, we selected a male individual, of the same Campania Region (Italy), to gain further knowledge by means of Oxford Nanopore Technology sequencing. High-molecular-weight DNA was extracted and used to prepare Whole Genome Sequencing libraries after fragmentation between 35.000 and 18.000 bp. Genomic DNA was used for library preparation and sequenced on PromethION24 flow cells for about 80h. Raw files were then base-called in Super-accurate mode to obtain FastQ files using Guppy. Four runs generated over 3.8 $x10^{11}$ bases, for about 140x coverage. *Shasta* sequence assembly produced around four thousand contigs from each set, with N50 over ten million bases (for a genome size of about 2.7 Gb). Merging the four sets by *quickmerge*, resulted in high quality assembly with N50 of about 70 Mb and L50 and L90 of 14 and 45 contigs respectively. X and Y chromosome sequences were separately assembled, by filtering out reads mapping on autosomic chromosomes. Contig mapping onto the reference female sequence showed that about 99% of the autosomic genome was covered by just 61 large contigs, 8 of them covering without interruptions over 98% of the corresponding chromosome. As expected, X and Y chromosome sequences have lower depth and 30 contigs are necessary to cover 95% of the X reference. About 24 Mbases from buffalo Y chromosome cover about 70% of Y chromosome from domestic Yak. Manual joining of large contigs into scaffold produced a *de novo* sequence co-linear with 98% of the reference genome, with only 900 inconsistencies ranging from a few tens to a few thousand bases. An assembly obtained by different tool, *NECAT*, confirmed that most differences are

not due to mis-assembly, but more likely depend on inter-individual variations (i.e. our *vs* Olimpia assembly); a few derive from sequences unconnected or missing in the reference genome (Olimpia).
Acknowledgements: The study was supported by the project PON1_486 "GENOBU", Ministry of Education, Universities and Research

O4 - Genomic Structure of Mediterranean Buffalo Breed: A Variant Analysis for a New Dedicated SNP Array

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In Italy, the buffalo Mediterranean (MED) breed is reared to mainly produce the famous "Mozzarella di Bufala Campana" PDO. A better knowledge of buffalo genomic structure of the Mediterranean in comparison with other buffalo breeds is important to link it with its phenotypic characteristics. Buffalo types are river, of which MED is part of it, and swamp. Here we studied the MED variability, in comparison with other river and swamp breeds, using short (Illumina) and long reads (Oxford Nanopore - ONT) of WGS data. The UOA_WB_1 reference sequence, supplemented with Yak's Y chromosome, was used. About the former, 20 MED together with 5 "river" and 7 "swamp" males were analyzed to investigate small variants, in particular SNPs. BWA was used for the alignment, and BCFTOOLS for variant calling and filtering. Variant annotation was performed using snpEff. About the latter, 8 male MED were analyzed. Minimap2 was used to perform the alignment, and Clair3, GATK and GLnexus for the variant calling (SNPs and small InDels). Using the short-read data, almost 35 millions of biallelic SNPs were identified, after quality control. Almost 15 million, more than 12 million and less than 10 million were monomorphic for reference allele in MED, river and swamp respectively. The mean alternative allele frequency (BAF) was 0.18, 0.23 and 0.35 for MED, river and swamp respectively. The mean missingness was 0.08, 0.01 and 0.02 for MED, river and swamp respectively. The variants identified using ONT data after quality control were almost 18 million, divided into SNPs (almost 16 millions) and small InDels (almost 2 millions). Annotation analyses identified variants with "HIGH" and "MODERATE/Loss of Function" impact, having a potential functional effect on the protein.
This preliminary analysis was fundamental for the improvement of the existing buffalo SNP array. Indeed, almost 50k SNPs were selected to fill the available spot in a new version of the array, after existing variant filtering.
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O5 - Polymorphisms of *MBl2* and *LTF* Genes in Mediterranean Italian River Buffalo (MIRB) (*Bubalus bubalis*) and Association with Mastitis

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Mastitis is a challenge in genetic improvement, in fact, it causes considerable production losses, economic implications, and adverse effects on animal welfare. It is characterized by alterations in milk composition and technological properties.
The aim of this study was to search for allelic variants in Mannose-Biding Lectin 2 (*MBL2)* and Lactoferrin (*LTF*) genes and to analyze their association with mastitis susceptibility in Mediterranean Italian River Buffalo. Recent studies showed that, in buffalo a milk somatic cell count (SCC) higher than 400.000/ml combined with a differential somatic cell count (DSCC) higher than 65% could be used as an indicator of an active mastitis.
Blood and milk samples were collected approx 150 days postpartum from 90 lactating buffaloes from 5 herds. Primers to analyse *MBL2* and *LTF* (promoter, exons 5, 6, 7, 8, 10, 11, 12) genes were designed using as reference the sequences of Murrah Buffalo (NC_059179 and NC_059177). Sanger sequencing was carried out to check the genetic polymorphism in MIRB and the distribution of allelic variants between animals positive or negative to mastitis was analysed.
In total 33 and 16 SNPs were detected in *MBL2* and *LTF* gene, respectively. Haplotypes for *MBL2* and *LTF* were identified and related to mastitis positivity.
This study showed that both genes are highly polymorphic, and the results of a preliminary association analysis indicate that some variants are potentially associated with mastitis. These results should be verified on larger cohorts of females and if confirmed these variants could be recommended for the use in selection.

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O6 - Methylation Study in Mediterranean Italian River Buffalo *(Bubalus Bubalis):* Preliminary Results

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Mediterranean Italian River Buffalo (MIRB) is a livestock species with a relevant socio-economic impact in Italy. In fact, it is selected for the production of the traditional dairy product mozzarella cheese. However, in the modern dairy industry mastitis remained a major constraint with huge production, economic losses and affecting animal welfare. Epigenetics may play an important role in this disease. DNA methylation is one of the major epigenetic marks responsible for gene regulation and may impact the regulation of mammary gland health. The aim of this study was to explore DNA methylation changes related to mastitis condition in MIRB, using nanopore technology. Blood and milk samples were collected from four female buffaloes of a dairy farm located in southern Italy. Animals were classified as healthy (SCC ≤200,000 cells/mL) or mastitic (SCC >200,000 cells/mL). Barcoded libraries were created following the protocol for Rapid barcode sequencing for gDNA - barcoding (SQK-RBK004) and then sequenced using the GridION sequencer. We identified a total of 658 differentially methylated cytosine sites (DMCs), located mainly in the promoter region in both healthy control and mastitis groups. In particular, 260 DMCs were ≤1kb from the promoter region. A larger proportion of hypomethylated DMC were found mainly in intronic regions, followed by promoter regions. Hypermethylated DMC were mainly found in promoter regions, followed by exons and distal intergenic regions. In particular, COX1, ND1 and ND2 genes showed DMCs in the analysed samples. An enrichment analysis showed that these genes are involved in cellular processes, transport and immune response.

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O7 - TLR2 Polymorphisms Modelling and Italian Mediterranean Water Buffalo Brucellosis

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Brucellosis represents a serious economic problem for the livestock industry. It causes late term abortion, decreased milk production and animal culling. In Italy, brucellosis is mainly spread in the area of Caserta (Campania region), where the 43% of the Italian Mediterranean water buffalo population is concentrated.

Different studies have demonstrated that TLR2 recognizes the *Brucella abortus* surface lipoproteins and, activate the immune response by heterodimerization with TLR1 or TLR6.

This study investigates whether the SNPs on TLR2 could compromise the protein functionality, thus influencing the susceptibility of the Italian Mediterranean water buffaloes to brucellosis.

A total of 194 water buffaloes from the province of Caserta, were grouped in positive and negative to *B. abortus.* The TLR2 sequencing was carried out for the identification of SNPs; the computational investigation was for assessing the SNPs effects on protein activity and the genotyping analysis was performed for verifying the *in silico* results.

Results from the TLR2 sequencing and computational analyses (Docking, MDS, APBS) evidenced that two amino acid substitutions A125V and S345N were able to compromise TLR2 structure and

its heterodimerization process, thus influencing the immune response. The genotyping analysis revealed that Valina at 125 was correlated with resistance to brucellosis (OR = 0.22; p-value = 0.015) and the Serine at 345 was protective against *B. abortus* infection (OR = 0.50; p-value = 0.049). These results provide preliminary evidence of the association between the amino acid substitutions and resistance to brucellosis in the Mediterranean water buffalo, contributing to the animal management and welfare.

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O8 - In Silico Analysis to Predict SNPS Effects in Mediterranean Water Buffalo

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In silico analysis represents a pivotal approach within modern scientific research, using computational methods to model and analyse biological data. Through the integration of bioinformatics and statistical modelling, researchers can elucidate biological processes, from molecular interactions to ecosystem dynamics. In this study we focused our attention on Interleukin 10 (IL-10), a cytokine with anti-inflammatory properties that plays a central role in limiting host immune response to pathogens. Through *in silico* analysis, the IL-10 SNPs effects in Mediterranean water buffaloes have been investigated.

The IL-10 nucleotide sequence of buffalo, retrieved from the Bioproject PRJNA437177, has been translated into protein sequence. The 3D structure of this sequence has been obtained by homology modelling (Swiss Model tool) with the human IL-10/IL10R crystallographic structure.

To assess the effect of amino acid substitution (due to SNPs) on the binding affinity between bIL-10 and bIL-10R, the MDS analysis was performed by using GROMACS. The complex stability was evaluated by analysing the following parameters: H-bonds numbers, solvent accessible surface area, kinetic and energetic properties.

The RMSF analysis, which inspects the amino acids fluctuation, revealed that the T175M substitution, compared to control, led to a marked reduction in residues fluctuation involved in IL-10/IL-10R binding and H-bonds network, thus compromising the proper interaction with the receptor. The Solvent Accessible Surface Area (SASA) analysis has shown a reduction of attractive forces between IL-10/IL-10R when the Thr in 175 was replaced by Met.

This approach could represent a valid tool for supporting and explains the biological mechanism triggered by polymorphisms.

Acknowledgements: The study was supported by the project PON1_486 "GENOBU", Ministry of Education, Universities and Research

O9 - Third-generation Sequencing by Nanopore Technology of Unrelated 22 Male and 10 Female Water Buffaloes to Search for Haplotype Differences by SNPs Analyses

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Buffaloes are farm and breeding animals important for food production; there are two species of water buffalo: river and swamp, which have different chromosomal arrangements. Given the great interest in getting insight into the sequences of the river buffalo (2N=50) genome and, considering the few sequences available up to now, we decided to further extend our studies in this area. Therefore, thanks to previous experience with the sequencing of one male buffalo using third generation sequencing technology (Nanopore Technology, ONT), we expanded our cohort by enrolling 22 unrelated male and 10 female buffaloes from Campania Region. For each buffalo, we obtained EDTA-blood samples from which we extracted whole DNA and RNA and we prepared whole genome sequencing (WGS) libraries following the *ad-hoc* ligation protocol. In total, we prepared 32 different libraries, each starting from 1,000 ng of DNA in 48 ul of buffer. Each library was run on a PromethIon24 flowcell for about 80h. For each run, we obtained an average of about 120 GB of estimated bases, with an N50 of about 11 kb and 12 M of reads. At the end of each run for the Fast5, super-accurate basecalling was performed, resulting in the FastQ files.
The aim of the study was to understand the heterogeneity of the buffalo genome by searching for haplotype differences using SNPs analyses both on males and females. Therefore, the results will help the characterization of single individuals within the Campania buffalo species, known worldwide for its dairy production.

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P16 - Understanding Divergent Phenotypes from the Whole Genome Sequencing of the Italian Mediterranean Buffalo

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The Italian Mediterranean Buffalo (IMB) has high genetic potential for milk production, and thanks to profitability of the Mozzarella Cheese market, it has nowadays become a species of great economic interest among farmers. Indeed, there is the need to improve the qualitative and quantitative characteristics of buffalo milk and dairy products. In order to identify genomic variants associated with divergent phenotypes, 24 animals having the highest and lowest official IMB aggregate index (IBMI) were selected and sampled from 10 farms located in Campania region. IBMI ranks individuals based on production, yield and functional traits. Whole Genome Sequencing (WGS) libraries were prepared by labelling genomic DNA extracted from whole blood and paired-end sequenced on a NovaSeq 6000 instrument (Illumina), 2x150bp. An average of 122.236 Mb per sample was obtained (minimum: 83.384 Mb, maximum: 172.373 Mb). WGS data were then aligned to the water buffalo reference genome chromosomes (UOA_WB_1) with the addition of chromosome Y from the yak reference genome (BosGru3.0) using a custom pipeline based on the Nextflow workflow system. The pipeline performs all the required sequence analysis steps, including Freebayes for variant detection (SNPs, MNPs and indels). Putatively polymorphic positions were recorded in a VCF file, which can be inspected to identify differences in genes involved in the processes of interest within the divergent phenotypes under analysis. Our study suggests potential contribution of distinct sets of genes that led to divergent phenotypes in the IMB.

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P17 - Genomic Regions Associated with Foot Defect - Extremely Open Nails - in Italian Mediterranean Buffalo (IMB)

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Genome Wide Association Studies (GWAS) are tools that explore the relationship between genetic variants and specific phenotypes within a population. Genetic studies in livestock have gained importance in recent years to understand the genetics influence of various phenotypic traits such as hoof health. Identifying genetic variants associated with extremely open nails (EON) could provide valuable information for the development of selection strategies to reduce the incidence of this condition in the IMB (Italian Mediterranean buffalo). The EON defect was defined as a binary character (presence/absence). The file was composed of 548 IMB and a pedigree that included 5,783 animals. Regarding the genotypes, information from 1,770 IMB with 49,955 loci was used. SNPs (single nucleotide polymorphisms) effects were estimated by a single-step GWAS (ssGWAS), which back-solved the genomic breeding values predicted using single-step genomic BLUP (ssGBLUP) fitting a single-trait animal model. The largest genetic variance was explained by a SNPs window located on chromosome 3 (0.98%) and 2 (0.80%), respectively. These results suggest that the EON defect has a genetic component and that most of SNPs which contribute to its variability are concentrated in the same region. Overall, a total of 72 informative windows that explained at least 0.5% of additive genetic variance were identified, explaining 55,73% of the observed genetic variance

and located in the chromosome 2, 3 and 22. The identification of these regions and genes will be an important tool for breeding contributing to a better understanding of the gene expression of this defect.

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P18 - Exploring the Genomic Inbreeding Level in Italian Mediterranean Buffalo Using Whole Genome Sequencing Data

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The water buffalo (*Bubalus bubalis*) is a domesticated species mainly farmed for milk production, with a European population of about 500,000 heads, of which ~ 88% are raised in Italy. Despite its economic importance, little research based on SNP array regarding the genetic variability and the level of genomic inbreeding in the Italian Mediterranean Buffalo (IBM) breed has been carried out. To efficiently explore the unique characteristics of this breed we sequenced 24 individuals belonging to 10 different farms from Caserta (Italy), focusing first on autozygosity estimates to infer whole genome selection signatures and possible candidate genes related to milk production. QC was performed with PLINK v.1.9, keeping only variants on autosomes and removing SNPs with call rate lower than 0.95 and MAF<0.01, with a final dataset of 16,680,804 SNPs. The detection of ROHs was performed with the R package detect RUNS, by applying the method "consecutive" and the following parameters settings: the minimum number of consecutive SNPs included was 100; the minimum length of ROH was 500 kb; a maximum gap of 100 kb; a maximum of five SNPs with missing genotypes and up to three heterozygous genotypes were allowed for the ROH to be called. Overall, our findings described genome-wide ROH patterns and identified potential selection hotspots containing genes which can be a target for future studies aimed to improve the performance of this breed.

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P19 - High-Throughput *De Novo* Sequencing of Laser Microdissected Y Chromosome in the Mediterranean River Buffalo (2n=50,XY)

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The sequencing and correct assembly of the Y chromosome sequences is still a challenge in mammals. In fact, apart from the pseudoautosomal regions (PARs), the Y chromosome lacks a homologous counterpart in the X chromosome. Therefore, in males, the use of genomic DNA as template for NGS allows isolating the specific Y sequences only as a difference from the X sequences with many potential gaps and assembly errors for the contemporary presence of X and Y. To overcome this problem, we present a high-throughput sequencing approach based on the direct isolation of Y-chromosome by laser microdissection in the Mediterranean river buffalo.
Peripheral blood lymphocytes from 10 buffalo bulls were cultured *in vitro* for normal cultures. Fixed lymphocytes were spread on a polyethylene naphthalate membrane (PEN) which was attached to a 24x60 glass slide and treated for GTG-banding. Ten copies of Y-chromosome from each bull were laser microdissected and collected in individual PCR tubes for DOP-PCR amplification and labelling. FISH confirmed the specific hybridization of each Y-probe on lymphocyte metaphases before NGS. Library preparation was performed with NEB Next Ultra II DNA Library Prep Kit for Illumina. High-throughput sequencing was performed by Illumina technology with NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles). Raw data were processed by TrimGalore (v0.6.7) and *de novo* assembly accomplished by SPAdes genome assembler v3.15.5. Gene sequences were predicted by AUGUSTUS (v3.5.0).
We generated about 40 Gb (90×) Illumina short reads. Total assembly length was 2,260,027 bp with average GC% of 48.11%. A total of 861 genes were predicted and 807 of them have a hit to reference, 210 are uncharacterized and around 30 without description. Total microsatellites identified were 552. Variant calling was conducted using the GATK4 pipeline, specifically employing the HaplotypeCaller tool for each sample separately and also in multi sample version. Total amount of variants across all samples was 25100.
Our approach yielded valuable insights into the genomic characteristics of the Y chromosome and our results represent a milestone for the river buffalo.

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P20 – A New Web Tool for Rapid Evaluation of Mammalian Genome Assemblies

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Quality assessment and multiple assembly comparison are essential steps while assembling new genomes. Many tools for evaluating assemblies typically provide synthetic parameters representing assembly quality or overall features, while others provide long detailed files where it is not always easy to identify and visualize the regions of correspondence and difference among different chromosome assemblies. A typical example is Quast, very effective in finding small as well as large similarities, which has to rely on Icarus to graphically display the mapped similarities.
Here we present a new web tool which uses Quast output to quickly identify and display similarities and differences between the compared assemblies both as text and graphic modes.
The program uses a combination of PHP and R scripts to setup a web accessible tool which takes as input one or more alignment results obtained by Quast or other similar tsv files, in order to preprocess them and produce the many reports, summary tables and plots.
The presented tool uses information about the alignment blocks, their start and end, in reference and query coordinates, together with additional annotations to represent the main alignment regions at the chromosomal or sub chromosomal scale, highlighting similarities and colinearity between compared sequences, points of inconsistency, discontinuities, repeated regions and interruption in the assembled sequences. It provides a summary of genome coverage chromosome by chromosome and graphical alignment representations which highlight alignment blocks in detail. The program was developed while assembling water buffalo genome and found very handy and informative while evaluating the assembled genome sequence.

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SESSION 4 – COMPARATIVE CYTOGENETICS AND GENOMICS

L8 - Satellite DNA and RNA: advances and challenges

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Satellite DNA sequences found in (peri)centromeric regions of chromosomes play a crucial role in the composition of the genomes. These sequences display a wide range of characteristics, including

nucleotide composition, complexity, and abundance. Various families of satDNA have been identified and studied over time, each showcasing unique organization and localization patterns in several genomes. Recent advancements in satellite DNA analysis have significantly improved our understanding of these sequences. Despite this progress, the exact function and significance of satellite DNA within the genome remain unclear, posing a major challenge in deciphering their biological importance. Comprehensive genomic studies utilizing multiple techniques have proven to be successful in unravelling the mysteries of satDNA biology and offer promising avenues for future research in this field.

O10 - The Nature and Organization of Repetitive DNA in the Genome of Goat, *Capra hircus*

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Goats are hardy and productive animals with high economic value to smallholders for meat, fibre and milk. Using whole-genome survey sequencing and published genome assemblies, we are characterizing the nature and diversity of the nuclear and mitochondrial repetitive DNA sequences in reference telomere-to-telomere genome assemblies, unprocessed sequence reads, and on chromosomes of goats and relatives in the Bovidae taxonomic family. End-to-end assemblies of goat and sheep chromosomal sequences, combined with k-mer and graph-based clustering of high-throughput short reads identifies the major repetitive DNA families. We analysed WGS of goats from Kurdistan (Iraqi Meriz goats) and the breeds Jintang Black and Saanen, exploiting graph-based clustering (RepeatExplorer) to identify endogenous retroviruses (ERVs), LINEs, any other transposable elements, telomeric, rDNA and satellite sequences. We used BLAST NCBI, and ClustalW alignments to analyse putative satellite results and compare them with other related species. A novel 22bp satellite at the end of many chromosomes, and the well-known 800bp satellite I was characterized in different chromosomes in the *Capra hircus* breed Saanen dairy goat assembly, along with telomeric sequences. A detailed search of chromosome 11 identified all ERVs (LTR retrotransposons) and LINEs (non-LTR retrotransposons). Using fluorescent in situ hybridization, we localized satellite I repeats, telomeric (TTAGGG) repeats, and the telomeric and subtelomeric 22bp repeats on sheep and goat somatic chromosomes. We annotated 5.8S, 18S and 28S (45S) goat sequences from the Iraqi Meriz goat and comparison with other bovids, as well assembling 5S rDNA

monomers and complete arrays. Repetitive sequences have been difficult to analyze using short-read assembly tools, but the use of long reads assembly methods and read-clustering is allowing us to characterize the nature and variation of these rapidly evolving components of the genome, contributing to chromosome evolution and the critical structural variations found between breeds as well as species.

O11 - In Pursuit of High-Resolution Chromosome Banding: Brief Review in Memory of Mogens Rønne (*6 November 1941 – 29 March 2018*)

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The development of chromosome banding techniques enabled recognizable landmarks across standardized karyotypes and set the stage for modern molecular cytogenetic methods. As a young scientist, Mogens Rønne focused much of his work on optimizing chromosome preparation, banding techniques, and attempting to understanding the molecular nature of chromosome structure.
Mogens was born in Rønne, Denmark, and spent much of his early life in Copenhagen until entering the Danish military at age 17. After military service, he studied at the University of Copenhagen Institute of Genetics. In 1976, he joined the University of Odense where he became a prolific and well-respected cytogeneticist. Mogens was a prolific author and, over the span of his career, developed and published detailed methods for producing high resolution G and R banding. He became most well-known for his detailed and elongated R band karyotypes which were used as the R band karyotype standards for numerous domestic animal species; these remain in use today. He was particularly interested in the molecular nature of chromosome structure that underlies banding and breakage at fragile sites. The techniques he developed made chromosome banding an art form to which others aspired.
Mogens was devoted to his family – survived by Elisabeth his wife of 52 years, daughter Annette My, son Mark, and grandson Thomas. Mogens was also generous, hardworking, and constantly learning, no matter what challenges life brought. He is remembered fondly by family, friends, and colleagues.

O12 - Chromosomal Inversions and their Potential Impact on Evolution of Mosquito *Aedes aegypti*

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Chromosomal inversions play a crucial role in evolution and have been found to regulate epidemiologically significant traits in malaria mosquitoes. However, they have not been characterized in *Aedes aegypti*, the primary vector of arboviruses, due to the poor structure of its polytene chromosomes. The Hi-C proximity ligation approach was used to identify chromosomal inversions in 25 strains of *Ae. aegypti* obtained from its worldwide distribution and in one strain of *Ae. mascarensis*. The study identified 21 multi-megabase polymorphic inversions ranging in size from 5 to 55 Mbp. Inversions were more abundant in African than in non-African strains, 15 versus 3 inversions, with the highest number of them observed in West Africa. All inversions were grouped into two geographic clusters of either African or non-African origin, suggesting their association with *Ae. aegypti* subspecies. Inversions were unevenly distributed along chromosomal arms, with the highest number found in the 1q and 3p arms homologous to the inversion-rich 2R chromosomal arm in the malaria vector *An. gambiae*. Direct comparison of inversions between the species revealed significant overlap in their genomic locations. This finding may explain the parallel evolution of the two species under similar environmental conditions. Some of the inversions colocalized with chemoreceptor genes and quantitative trait loci associated with pathogen infection, suggesting their potential role in host preference and disease transmission. Our study revealed a large pool of structural variation in the *Ae. aegypti* genome potentially involved in mosquito adaptation to humans, interactions with pathogens and provide new insights into mosquito genome evolution.

P21 - Comparative Analysis of HSAT1 Satellite DNA in Primates

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The classical human satellite DNAs (Hsat1-3) represent a significant amount of the human genome and are present in the chromosomes pericentromeric constitutive heterochromatin regions. SatDNAs have long been described as dynamic sequences in the genomes displaying a singular evolutionary path. HSat1 constitutes the most AT-rich region of human genome and can be distinguished as two different sequences – Hsat1A and Hsat1B - with distinct sequence and location on the human genome. While Hsat1A is made of a 42bp monomer forming arrays located in the pericentromeric region of human chromosome 1, 3, 4 and in the acrocentric chromosomes (13, 14, 15, 21 and 22), HSat1B is composed of a 2.5Kb repeat predominantly located in the human Y chromosome and also present in chromosome 22. Previous studies have identified HSat1B in other primate genomes, although for HSat1A there is no information regarding its presence in other primates. Recent advances in sequencing technologies increased the number of available genomes, representing a novel opportunity for comparative and evolutionary genomic studies.

In the present work we have used cytogenetic and bioinformatic analysis of available sequencing data to achieve information regarding the presence and chromosome location of HSat1A and HSat1B in different species of primates. HSat1B was isolated in primate genomes using PCR, followed by cloning, sequencing and physical mapping by fluorescent *in situ* hybridization (FISH). We performed the quantification of HSat1A (SAR – Dfam: DF000001062.4) and HSat1B (GenBank: X00470.1) arrays in chromosomes from five species: human (*Homo sapiens)*, chimpanzee (*Pan troglodytes)*, gorilla (*Gorilla gorilla)*, rhesus monkey (*Macaca mulatta)* and gibbon (*Nomascus leucogenys)* using available genome assembly data.
Our analysis revealed that Hsat1A is shared between human, chimpanzee and gorilla, being present in autosomes and in the Y-chromosome. Regarding Hsta1B, we performed its isolation by PCR in the genome of gibbon, showing for the first time its presence in this species. Besides, genome assembly data confirmed its location in gibbon chromosomes as well as in chromosomes from chimpanzee, gorilla and rhesus monkey. This study places HSat1A in the ancestral from gorilla, chimpanzee and humans at about 8 mya. On the other hand, Hsat1B revealed to be in primates common ancestral at around 25 mya, showing it has been on primate genomes since longer time and has accompanied this species karyotype evolution. This study constitutes the first approach to the comparative study oh Hsat1 satellite DNA among primates.

P22 - Using of Molecular Painting Probes Specific for Domestic Cat Chromosomes to Visualise Selected Chromosomes of the Pantherinae Members

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The karyotype of representatives of the Felids is characterized by high conservatism. In most cats, the karyotype is 2n=38 and the chromosomes have similar morphology.
The domestic cat is used in research on wild felines as a model animal. This is related to research material availability. The present study used the ZOO-FISH technique to visualize sex and selected autosomal chromosomes in the Bengal tiger (*Panthera tigris tigris*, ♂), Amur tiger (*Panthera tigris altaica*, ♀), Leopard (*Panthera pardus*, ♀) and African lion (*Panthera leo* ,♂). The blood samples were collected for the purpose of preventive examinations. The ZOO-FISH was performed using painting probes specific for cat sex chromosomes and chromosomes FCA A1, FCA B1, FCA C1, FCA F1, obtained by laser microdissection.
Signals specific for chromosomes B1, C1 and F1 have been observed in all examined animals confirming the homology of these chromosomes. The signal specific for chromosome A1 has not been observed in Bengal tiger. In male individuals of the Bengal tiger and the African lion, a signal

specific to the feline Y chromosome was observed. The X chromosome signal was observed in all examined individuals except the Bengal tiger, which is due to a different banding pattern. The use of the ZOO-FISH technique with domestic cat probes is a great tool for analysing the karyotype of representatives of the Pantherinae subfamily. This fact is an important application aspect in the context of karyotype analysis of these animals.

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P23 - Inference of Maternal Inheritance in Three Sicilian Donkeys through MtDNA Analysis

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Donkeys have significantly impacted human society but in the 20th century, they suffered a demographic decline globally. This study aims to explore the current genetic variation of mitochondrial DNA (mtDNA) in three Sicilian donkey populations. D-Loop region was sequenced (479 bp) in Ragusano (n=32), Pantesco (n=23) and Grigio-Siciliano (n=26) donkeys. Nucleotide diversity (π) was low in Pantesco (0.0008±0.0003) as expected, and higher in Grigio Siciliano (0.0220±0.003) and Ragusano (0.0250±0.002). Grigio-Siciliano exhibited the highest haplotype diversity (hd) (0.871±0.056) with 13 haplotypes, followed by Ragusano with 12 haplotypes (hd 0.859±0.048), while Pantesco had only one. The network based on the Median-joining algorithm on mtDNA D-loop sequences (238bp) from Sicilian and other Mediterranean domestic donkeys, as well as Ethiopian, Somali, and Nubian wild asses, distinctly identified three macro-haplo-groups. Somali wild asses exclusively represented Group 1. Most of Sicilian donkeys (67%), including all Pantesco and several Grigio-Siciliano, fell into Group 2, sharing the H5 haplotype with other Italian breeds. Group 3 included Ethiopian and Nubian wild donkeys, asses from the Balkans and some Ragusano and Grigio Siciliano and the H4 haplotype mainly represents it. Pantesco confirmed its history of isolation and the consequent genetic bottleneck. At the same time, the distribution of Grigio-Siciliano and Ragusano individuals across two groups suggests a wider phylogenetic maternal inheritance which appears to be mediated by donkeys from Balkans. These outcomes are useful to trace the evolutionary history of Sicilian donkeys and support their conservation.

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SESSION 5 – MUTAGENESIS AND BIOMONITORING USING CYTOGENETIC AND GENOMIC TESTS

L9 – Cytogenetic and Genomic Biomarkers: A Glimpse into the Latest Advances

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Over the past three decades, animal cytogenetics has witnessed remarkable progress, establishing cytogenetic assays as essential tools for monitoring livestock health. These techniques serve as crucial biomarkers, revealing DNA damage caused by exposure to pollutants or drugs. Genotoxicity assays, akin to toxicology indicators, are invaluable for assessing potential hazards in the diet and environment of livestock, although they do not directly inform on fertility or genetic quality.
As the field advances, there is a notable shift from traditional cytogenetics to genomic biomarkers. Genomic techniques, particularly those analysing DNA methylation patterns, offer a more comprehensive view of cellular states and environmental interactions. Initially focused on human cancers, the advent of animal genome and epigenome data has propelled research into veterinary epigenetics. DNA biomarkers provide nuanced insights into animal health and environmental exposures.
This generational transition from cytogenetics to genomics is characterized by significant improvements in both time and cost efficiency. Genomic assays, once prohibitively expensive and time-consuming, have become more accessible and faster due to advancements in sequencing technologies and computational analysis. This has allowed for broader and more detailed investigations into the genetic and epigenetic factors affecting livestock health.
This lecture delineates the advancements in genotoxicity assays and genomic biomarkers, emphasizing their potential in holistic animal welfare monitoring. By integrating these biomarkers, veterinary medicine can achieve more robust and context-dependent assessments of animal health, addressing a significant unmet need in the field. The generational leap from cytogenetic to genomic methodologies represents a transformative shift, offering deeper insights and more efficient solutions for the ongoing challenge of livestock health monitoring.

O13 - Effect of Dietary *Hermetia illucens* Oil on Bovine Genome Stability: A Sister Chromatid Exchange (SCE) Study

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Hermetia illucens (HI) oil, extracted from black soldier fly larvae, represents a sustainable alternative to traditional fat sources in bovine feeding. However, its impact on bovine genome stability was never investigated. Sister chromatid exchange (SCE) is a biological indicator of genomic instability, useful to assess the effect of genotoxic agents. This study aimed to evaluate the effect of HI oil inclusion in the feed ration on bovine genome stability by analyzing SCE *in vitro*.

Twenty-six Valdostana Red Pied cows fed mixed hay *ad libitum* were divided into two balanced groups that received isonitrogenous and isoenergetic concentrates containing a conventional lipid source, palm oil (control group), or HI oil at 3% as fed (case group). Peripheral blood lymphocytes were cultured *in vitro* for conventional (normal cultures) and 5'-bromodeoxyuridine (BrdU) incorporation, the latter added 26 h before harvesting at final concentration of 10 µg/ml to obtain preparations for the SCE test. Slides obtained from both normal cultures were karyotyped by GTG banding, whereas the BrdU-treated cultures were stained for 10 min with acridine orange (0.01% in buffer phosphate), washed with distilled water, and mounted in P-buffer. Three time points were analysed, zero time (T0 - no HI oil inclusion), 30 days (T1), and 50 days (T2) after the start of the experimental feeding.

Cows were all karyologically normal (2n=60,XX). A total of 2882 metaphases were counted for the SCE test. Statistical analysis using the Student's t-test showed no significant differences in SCE frequency between the control and case groups at T0 (6.84 ± 0.15 *vs* 6.72 ± 0.14; p=0.53) and T1 (6.28 ± 0.12 *vs* 6.27 ± 0.12; p=0.89). However, after 50 days (T2) a significant reduction in SCE frequency was observed in the case compared to the control group (5.73 ± 0.11 *vs* 6.29 ± 0.12; p=0.002).

In conclusions, results suggest that the tested dietary HI oil inclusion level does not have a negative effect on bovine genome stability *in vitro*. The reduction in SCE frequency in the case group appears as a putative protective effect of the HI feed inclusion on genome stability although further studies and other genotoxic tests need to confirm this trend.

O14 - A Screening Methodology of the Cell Nuclei Based on Functional Status of the Chromatin in *Ziphius cavirostris*

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Utilizing methods capable of detecting changes in chromatin states as a means to screen cells based on their functional state offers a valuable approach for assessing the effects of pollutants such as PFAS on cetacean species' cells and evaluating the status of environmental living conditions. In this study, a myogenic cell line obtained from *Ziphius cavirostris* were examined using a computed set of morphometric parameters to analyze cell nuclear morphology. Additionally, cytogenetic analyses were performed to determine the diploid number, distribution of constitutive heterochromatin (HC), and telomeric regions.

Cells were stained with Hoechst 33342 to measure nuclear morphometric parameters through high-throughput screening analysis, combining various parameters such as nuclear length, inverse aspect ratio (Inv/AR), and nuclear intensity. This methodology was employed to assess the cytotoxic effects induced by PFAS in cetacean cells. Cytogenetic techniques, including CBA-banding and FISH-mapping with PNA-Telomeric probes, were applied to metaphase chromosomes obtained from myogenic cells. Our model facilitated the grouping and quantification of nuclei into three populations and six groups: the normal population (nuclei in G0 phase, synthesis phase, and mitosis phase), the large population (senescent nuclei), and the small population (nuclei fragmentation). By plotting frequency distributions of Hoechst intensity, we generated cell cycle profiles of nuclei. The combination of cell nuclei counting with cell cycle analysis allowed us to determine the dose-response effect to PFAS. Cytogenetic results confirmed that Ziphius c. has 2n=42, although several cells exhibited polyploidy, predominantly tetraploidy. CBA-banding revealed large blocks of HC in various chromosomes and chromosome arms. All chromosomes exhibited positive FITC-signals on all telomeric regions.
This method provides an objective semi-quantitative tool for screening different nuclear phenotypes depending on the functional status of the chromatin. Cytogenetic analyses revealed characteristic CBA-banding patterns and telomeric regions.

O15 - Investigating the Impact of Short-Term Environmental Stress on Telomere Length in Goats

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Rapid environmental changes present novel challenges for animals, notably thermal stress, which triggers the hypothalamic-pituitary-adrenal axis, elevating stress hormone levels. Prolonged stress can disrupt the body's metabolic equilibrium, leading to increased oxidative damage and impacting vertebrate telomeres composed of repeats (TTAGGG), commonly associated with stressful conditions.
To investigate the effects of short-term environmental stress on goats, ten multiparous individuals with free access to water and permanent pasture were selected for the study. Milk samples were collected throughout the lactation period to assess telomere length, providing insights into the potential impacts of stress on cellular aging and health. In addition to milk sampling, comprehensive meteorological data, including temperature, dew point, humidity, wind speed, and precipitation, were meticulously recorded from the Italian Meteorological Centre. These data sets enabled a detailed analysis of the relationship between environmental parameters and telomere length in goats.
Telomere length, analysed via qPCR, was found to be negatively correlated with both dew point ($r = -0.676$, $p<0.05$) and relative humidity ($r = -0.769$, $p<0.01$), underscoring the significant influence of environmental factors on cellular aging processes in goats.
These findings offer valuable insights for farm management to minimize stress conditions impacting both animal health and production aspects.

In conclusion, the study highlights the importance of understanding the impact of environmental stress on telomere length in goats and suggests that managing environmental factors such as dew point and relative humidity could potentially mitigate the negative effects of stress on animal health and productivity

P24 - Comparative Analysis of Mulberry Leaf-Based Diet vs Conventional Diet on Sister Chromatid Exchange (SCE) in Rabbits

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This study investigates the impact of mulberry leaf meal (MLM)-based diet compared to a conventional diet on sister chromatid exchange (SCE) in rabbits. Mulberry leaves are renowned for their rich nutrient content and potential health benefits. Rabbits are an ideal model for studying dietary effects due to their sensitivity to nutritional changes. The Sister Chromatid Exchange assay is used as a sensitive marker for genotoxicity, assessing the frequency of SCEs indicative of DNA damage.
A total of 140 Hycole x Grimaud crossbred rabbits (42 days old) were allotted to two dietary treatments (5 cages/group, 14 rabbits/cage). Animals received the same post-weaning feed (42-63 days), but two different finishing feeds (64-90 days): 1) control diet (C), containing 85% commercial feed, 8% alfalfa meal and 7% barley meal, and 2) experimental diet containing 85% commercial feed, 10% MLM, 4% barley meal and 1% soybean meal. A total of 30 rabbits (15/diet) were slaughtered at 90 days of age. Peripheral blood was individually collected. Lymphocyte cells were cultured *in vitro* for conventional (normal cultures) and 5'-bromodeoxyuridine (BrdU) incorporation, the latter added 24 h before harvesting at final concentration of 10 μg/ml to obtain preparations for the SCE test. Staining was performed for 10 min with acridine orange (0.01% in buffer phosphate) then slides were mounted in P-buffer.
Comparative evaluation of SCE frequencies between the two dietary groups provides insights into potential genotoxic effects associated with each diet. Therefore, 891 metaphases were analysed and 5685 SCEs counted. Statistical analysis using the independent Student's t-test showed no significant differences ($p=0.703$) in SCE frequency between the control (6.42 ± 0.15) and case group (6.34 ± 0.13) at the end of the test. These findings suggest that the mulberry leaf-based diet does not induce greater genotoxicity compared to the conventional diet. The results of this study contribute to understanding the potential health implications of incorporating mulberry leaves into animal diets. In fact, mulberry leaves are rich in nutrients, antioxidants, and bioactive compounds, potentially improving nutrition, supporting digestion, providing antioxidant protection, aiding weight management, and contributing to disease prevention.

SESSION 6 – CYTOGENETICS AND GENOMICS OF NON-MAMMALIAN VERTEBRATES

L10 - Billfish in the Mediterranean Sea: Genetic and Tagging Approach to Study Large Pelagic Fish

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Billfishes (swordfish, sailfish, spearfishes and marlins) are large highly migratory predators that represent important fishery resources in many countries. For Mediterranean species other than the widely studied swordfish (*Xiphias gladius*), there is little and fragmentary information on the spearfishes. A genetic and tagging approach has been applied to improve the knowledge on these animals. Genomic DNA (gDNA) extraction (from muscle tissues of Atlantic and Mediterranean fish), amplification of two mitochondrial genetic markers (COI and D-loop) by polymerase chain reaction (PCR) protocols, and subsequent sequencing of the amplified fragments were applied. Pop-up satellite tags were used to follow animals in key study areas of Mediterranean Sea. Tags were programmed for 240-d deployments in which they recorded temperature, pressure (depth) and light measurements at 2-min resolution when the tag was in data collection mode. The analysed sequences, consisting of a total of ≃1,103 bp in length of which 619 bp belonging to the COI and ≃500 for CR, were all compared with other sequences deposited in GenBank. All specimens genetically identified in Mediterranean Sea correspond to the taxonomy as correspond to the taxonomy as Mediterranean spearfish (*Tetrapturus belone*). The genetic analysis, performed using the COI region (highly conservative) and the D-loop region, CR (highly variable) as markers, allowed us to construct neighbour-joining (NJ) trees, allowing us to affirm that the individuals examined can be divided into two clades: a Mediterranean one, attributable to *Tetrapturus belone* and one from the Atlantic, identified as *Kajikia albida*, with bootstrap values (BS) greater than 98%. Tagged individuals in Straits of Messina and Sardinia remained within the Mediterranean, confirming the results of genetic analysis.

O16 – Telomere Length Dynamics and Expression of Telomerase Transcriptase (TERT) Gene in Diploid and Triploid Rainbow Trout (*Oncorhynchus mykiss*) Females

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Telomeres are nucleoprotein complexes at the ends of eukaryotic chromosomes. Telomeres protect chromosomes from end-to-end fusions and degradation, guarantee their complete replication and allow DNA repair machinery distinguish natural chromosomal ends from the ends that appear in the course of breakage events. Telomeres shorten with every cell division however the process of telomere loss may be compensated by telomerase, an enzyme whose catalytic protein subunit (TERT, telomerase reverse transcriptase) adds telomeric DNA repeats to the end of telomeres using as a template an integral RNA component (TERC). In the present study, changes in the length of telomeric DNA, as well as expression of the *TERT* gene in diploid (2n) and triploid (3n) rainbow trout (*Oncorhynchus mykiss*) females were investigated. The additional set of chromosomes causes cytogenetic incompatibility that in triploid rainbow trout females (XXX) results in the reproductive sterility; the ovaries in the triploids are underdeveloped and contains a few usually aneuploid oocytes. Triploid females do not invest an energy in gametogenesis and continue to grow whereas normal diploid specimens suffer from declines in growth and survival during sexual maturation. Despite genetic and physiological differences, triploid and diploid trout exhibited similar pattern of the telomere dynamics. Telomere length in the embryos, larvae and one-year old juveniles did not change significantly. In the second year after hatching, sub-adults exhibited substantially shortened telomeres while significant increase of the telomere length was reported in the three-year old adults. Larger differences between diploids and triploids were observed when expression of *TERT* gene was analysed. Increased expression was observed in all the somatic tissues sampled from the triploid specimens compared to diploid individuals. In turn, in the ovaries of triploid females, expression of the *TERT* was significantly downregulated compared to properly developed gonads of diploid females. The ovaries of triploid rainbow trout were strongly reduced and contained few oocytes and the low number of reproductive cells, which are usually characterised by telomerase activity, probably contributed to the low expression of the TERT gene observed in sterile ovaries.

O17 - Oocyte Nuclear RNA Profiles Along Centromere and Subtelomere Regions of the Telomere-to-Telomere Chicken Genome Assembly

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Here we aimed to characterise the transcriptional activity at centromere and subtelomere regions on all chicken chromosomes, including sex and dot chromosomes, during the diplotene stage of oogenesis. To do this, we examined the oocyte nuclear and cytoplasmic RNA-seq data aligned to the complete telomere-to-telomere assembly of the chicken genome (doi: 10.1101/2024.02.05.577752). The Cen1, Cen2, Cen3, Cen4, Cen7, Cen8 and Cen11 repeat arrays on GGA1-4, 7-8 and 11 were found to be transcriptionally silent in oocyte nuclear and cytoplasmic RNA fractions. Next, we confirmed our previous data on the appearance of transcripts of the non-tandemly-repetitive centromeres on GGA5, GGAZ and GGA27 chromosomes in a lampbrush configuration. In the centromere regions of chicken acrocentric chromosomes, the 41-bp repeats CNM and PO41 form higher-order repeats. According to oocyte nuclear RNA-seq data, most CNM repeat clusters at centromere regions are transcriptionally silent. At the same time, the CNM arrays at sub-telomeres,

including those adjacent to the centromeres, are actively transcribed. The subtelomere regions of the chicken chromosomes show a similar structural organisation and a similar transcriptional profile. We found, that active transcription of telomere-derived RNAs, including telomeric repeat-containing RNA (TERRA) and subtelomere repeat-containing RNA, is initiated in opposite directions from long terminal repeats (LTRs) located within the telomere-adjacent CenTE-5k sequence, suggesting a role for active retrotransposon promoters in the regulation of telomere non-coding RNA synthesis.

The research was carried out using the equipment at the Genomics Core Facility (Skoltech) and the Molecular and Cellular Technologies Resource Centre (St Petersburg State University).

O18- Mechanisms of Programmed DNA Elimination in Songbirds

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Genome stability is a crucial feature of eukaryotic organisms. Nevertheless, some organisms can selectively eliminate certain parts of their genetic material during ontogenesis. In songbirds, germline-restricted chromosome (GRC) represents a fascinating example of inheritance through the female germline, being eliminated from somatic cells of both sexes and during meiosis in males. Although the elimination of GRCs during spermatogenesis has attracted considerable attention and has been intensively investigated, the GRC removal from somatic cells remains drastically understudied. Here we used cytogenetic approaches and GRC-specific probe to investigate the mechanisms of GRC elimination from somatic cells in *Loncura domestica*. We detected GRC elimination from somatic cells in embryos from freshly laid eggs and in embryos from unlaid eggs. In eggs incubated for more than 30h, we did not observe GRCs in the somatic cells of embryos. Similarly to male meiosis, the GRCs eliminated from the somatic cells nucleus formed a micronucleus in the cytoplasm of somatic cells. We applied immunostaining of histone modifications against H3K9me3 and H3S10p to evaluate the possible accumulation of heterochromatin in GRC micronuclei. We did not observe accumulation of any of these modifications in GRC micronuclei in somatic cells, although such histone modifications are highly enriched in GRC micronuclei and in male germ cells. Thus, GRCs are eliminated from somatic cells during early embryonic development and, after their removal from the nuclei, they are sequestered to micronuclei similar to those in male germ cells. However, the epigenetic modifications of GRCs are different in somatic and male germ cells.

P25 - Chromosomal Abnormalities in the Germline Cells of the *Pelophylax Grafi* Hybridogenetic Tadpoles

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Several reproductive strategies are observed in hybrid vertebrates, and one of them is hybridogenesis. It relies on F1 hybrids selectively passing on the unrecombined genome of one of the parental species to the next generation. The gametes contain only one of the parental genomes that result from selective genome elimination and endoreplication of the remaining one. The next-generation hybrids are formed by combining such gametes with those provided by the other parental species. *Pelophylax grafi*, a hybridogenetic hybrid whose genome consists of 13 chromosomes of *Pelophylax perezi* and 13 of *Pelophylax ridibundus* origin, exemplifies this unique phenomenon.
By applying comparative genomic hybridization (CGH), we aimed to unravel the possible course of genome elimination in *P. grafi* hybrid tadpoles. Our findings strongly indicate the elimination of the *perezi* chromosomes and a one-time endoreplication of the *ridibundus* chromosomes in the hybrid germline cells. Furthermore, we discovered a spectrum of chromosomal aberrations and interphase abnormalities, likely resulting from abnormal mitoses. One of the most common aberrations was aneuploidy, which in the majority of the cases could be interpreted as a typical result of chromosome elimination. Others included shattered and pulverized chromosomes, anaphase/telophase bridges, premature sister chromatid separations, and stretched centromeres.
Taken together, these findings underscore the complexity of hybridogenesis and the unpredictable outcomes of chromosomal dynamics during the formation of hybrid organisms. Our study not only contributes to the understanding of hybrid evolution and speciation mechanisms but also highlights the importance of integrating cytogenetic approaches to elucidate the genetic complexity of hybrid organisms.

P26 - Visualisation of Chromatin Domains Revealed by Single Oocyte Nucleus Hi-C on Chicken Lampbrush Chromosomes

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Lampbrush chromosomes are a form of nuclear chromatin that appears at the diplotene stage of oogenesis in most vertebrate species as a result of hypertranscriptional activity. Lampbrush chromosomes are giant in size and structured into distinct chromatin domains: fully decondensed transcriptionally hyperactive lateral loops and compact transcriptionally inactive chromomeres. Recently, we developed a single nucleus Hi-C (scHi-C) protocol for chicken growing oocytes (Gridina et al., 2022) and obtained data on chromatin contact density along chicken lampbrush chromosomes. Here we compared chromatin domains of different contact density with the cytological lampbrush chromatin domains.
Genomic coordinates of the distinct chromatin domains were predicted by visual analysis of scHi-C data in Juicebox software. For FISH visualisation of the exact domains, we selected BAC clones that overlapped with the predicted chromatin domains from the chicken BAC library CHORI-261. 27

BAC clone-based tricolour DNA-probes were mapped within three genomic regions (on GGA1, GGA4 and GGA13) on lampbrush chromosome preparations. Mapping of the selected genomic regions onto lampbrush chromosomes revealed the correspondence of high contact density scHi-C domains with chromomeres and cross-like patterns with transcription loops. Probes that match the transcription loops hybridise with the nascent RNA transcripts. We also found that small (~250 kb) scHi-C high contact density domains within cross-like patterns represent compact chromatin knots on the axes of lateral loops between adjacent transcription units. Possible mechanisms for segregating lampbrush chromatin into individual chromomeres are outlined.
The study was conducted using equipment at the "Molecular and Cellular Technologies" Research Resource Centre (St Petersburg State University).

P27 - Expression of Telomerase Reverse-Transcriptase (TERT) Gene in The Rainbow Trout (*Oncorhynchus mykiss*) Eggs and Development of Gynogenetic Doubled Haploids

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Telomerase is a ribonucleoprotein enzyme that plays a key role in maintenance of the telomere length and integrity. Telomerase consists of telomerase reverse transcriptase (TERT), telomerase RNA component (TERC) and dyskerin (DKC1). The activity of telomerase is controlled by transcriptional and post-transcriptional regulations of the TERT gene. Expression of the TERT gene and activity of telomerase have been reported in the somatic tissues and gonads in fish irrespective of their age and size. Though, little is known about TERT expression in the fish eggs. In the present research, TERT expression was confirmed in the rainbow trout ovulated eggs before and after activation with non-irradiated and UV-irradiated sperm. Eggs originating from eight females had high and comparable quality expressed by similar hatching rates. However, survival of gynogenetic Doubled Haploids (DHs) developing in eggs activated with UV-irradiated sperm and further exposed to the High Hydrostatic Pressure (HHP) shock varied between females at the hatching stage from 2.1 ± 0.4% to 40.5 ± 2.2%. In turn, increased expression of TERT was observed in eggs originating from two females and gametes from only one of them showed improved competence for gynogenesis (27.3 ± 1.9%). On the other hand, eggs from the female with the highest survival after gynogenetic activation were characterized by the lowest TERT expression. Telomerase in the fish eggs may compensate erosion of the telomeres during early embryonic however characterized by rapid cleavages, its upregulation does not assure increased ability of the rainbow trout eggs for the gynogenetic development.

P28 - The Hybrid Quest: What May Affect Gametogenesis in Water Frog Hybrids of *Pelophylax Esculentus* complex?

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Hybridization may lead to the emergence of asexual hybrids. Such hybrids modify their gametogenesis, causing hemiclonal genome propagation. However, irregularities during asexual gametogenic pathways were frequently found in asexual lineages. While these irregularities expand the diversity of gametogenic pathways in asexual organisms, their causes and consequences remain unclear. To reveal them, we studied water frog hybrids from *Pelophylax esculentus* complex, known for asexual reproduction via hybridogenesis. During hybridogenesis, hybrids typically produce gametes carrying only one parental species genome, either *P. ridibundus* or *P. lessonae*. Using fluorescent *in situ* hybridization with species-specific probes, we analyzed spermatocytes and spermatids from 47 hybrid males collected across four locations in Eastern Ukraine. Notably, seven hybrids exclusively carried *P. lessonae* genome, while 15 had only *P. ridibundus* genome, suggesting the premeiotic elimination of corresponding parental genomes. Conversely, 17 hybrids showed both *P. lessonae* and *P. ridibundus* spermatocytes simultaneously (amphigameticity), indicating the elimination of one or both parental genomes from distinct gonocyte populations. Comparative analysis of gametogenesis among hybrids from different localities revealed variability of gametogenic pathways, implying independent origins for these hybrids in each locality. Furthermore, we observed six hybrids with abnormal pairing between *P. ridibundus* and *P. lessonae* chromosomes. Through comparative genome hybridization, we identified extensive rearrangements between *P. ridibundus* and *P. lessonae* chromosomes in such hybrids. Moreover, in two hybrids with aberrant chromosome pairing we detected unstained chromosomal segments possibly indicating genome introgression from unknown species. These findings underscore the impact of genomic rearrangements and introgressions on hybridogenetic reproduction, contributing to inviable gametes.

SESSION 7 - ADVANCED IN ANIMAL GENOMICS AND EPIGENOMICS

L11 - Capturing Additional Genetic and Epigenetic Variance by Third Generation Sequencing

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Genetic variation plays a pivotal role across various genomic related domains such as selective breeding, biodiversity preservation, and conservation efforts. Selective breeding stands out as a primary catalyst for enhancing livestock productivity. Notably, it has spearheaded a remarkable 192%

augmentation in the genetic merit of Holstein and Jersey cattle since 2009, effectively doubling efficiency per unit of live weight.
The advent of the sequencing era has significantly propelled the identification of novel variants within animal genomes, mainly single nucleotide polymorphisms (SNPs), insertions/deletions (indels), and microsatellites. Nevertheless, conventional sequencing techniques may overlook certain genomic variations. Recently, third-generation sequencing methodologies have emerged a promising tools for uncovering other types of variants, particularly methylation patterns and structural variation.
This research used two distinct datasets. The first dataset, comprising data from 32 animals, was employed to evaluate the applicability of epigenotyping by low pass sequencing (EpiGLows) to detect methylation in the Bos Taurus genome. The second data set used sequencing data from 21 animals, with a coverage >10x, to detect structural variants across various *Bos taurus* breeds.
The latest nanopore chemistry (Kit14) yielded a modal base calling accuracy of 99.55%, marking a substantial improvement over previous kits. The analysis yielded over four million reliably methylated sites, even at shallow sequencing depths, primarily distributed within distal intergenic (87%) and promoter (5%) regions.
Regarding structural variants, a considerable number was identified, with insertions and deletions emerging as predominant types. Furthermore, the prevalence of structural variation exhibited breed-specific disparities, underscoring its role in shaping genetic diversity within *Bos taurus* populations. Each breed showcased a unique profile of structural variants, with the Wagyu breed demonstrating a notably higher count compared to the reference genome. Approximately 1,000 variants were shared across all breeds, with over 75% of structural variants localized within intergenic regions.
This study underscores the utility of the latest nanopore chemistry in elucidating novel epigenetic and structural variants, thereby enriching genetic and genomic research aimed at uncovering additional layers of genetic variation.

L12 - Advancing Dairy Farming with Genomics: Improving Profit, Sustainability, and Cow Health

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Genomic selection is the most transformative innovation in dairy genetics since artificial insemination. It revolutionizes dairy cattle breeding by enhancing selection capabilities, thereby improving health, fertility, and production efficiency in dairy cattle. Looking to the future, genomic predictions will become increasingly important as they enable us to leverage the costly phenotypes associated with feed efficiency and methane production in Holsteins worldwide. Over the past decade, a comprehensive range of genomic predictions targeting economically important health and wellness traits in dairy cattle has been developed and established, with extensive documentation of the methods and validation studies in scientific research. Additionally, these traits have been integrated into economic selection indexes, empowering producers to make more profitable decisions. Accurate estimates of trait heritability and genetic variation are essential for effectively utilizing these new health and wellness traits in a selection index. A newly released Zoetis study report evaluated the effectiveness of a genomic economic selection index in predicting the observed lifetime profit of US Holstein cattle. The study found that genomic selection indexes incorporating wellness traits

accurately predicted significant differences in observed lifetime profit for Holstein cattle. With a discount rate of 10.5%, each 1-point increase in the genomic selection index corresponded to an additional $2.36 in lifetime profit. Furthermore, animals in the top genomic group exhibited 55% less metritis, 33% less mastitis, and 42% less lameness compared to those in the lowest genomic group. This study shows that in well-managed herds when implementing comprehensive genomic selection indexes, genetic progress can result in demonstrable profit. Notably, the genetic improvement achieved using genomic indexes has also been linked to a reduction in cows' methane intensity. As dairy cows become healthier and more productive, efficiency improves via the dilution of maintenance effect, and both resource use and enteric methane emissions are reduced per unit of milk. In a recent Zoetis study analyzing genomic data from 13,000 cows across 11 dairy herds and 9 years of on-farm records, the top 25% of cows with superior genetics demonstrated 8% lower enteric methane emissions, 43% less antibiotic usage over their lifetimes, and required 5% less feed for maintenance compared to the bottom 25% group. These reductions were accomplished while the superior cows also produced 34% more milk and generated an average of $ 869 more in lifetime profit per cow compared to those in the inferior genetics group. Therefore, healthy and efficient cows can be both profitable and make significant contributions to long-term sustainability outcomes. Moreover, the proven value and accuracy of genomic predictions have driven the adoption of various selection, breeding, and management strategies. These include heifer culling and optimizing replacement inventories, strategic allocation of semen, implementation of embryo programs, and adjustments in herd management based on analysis of observed response to genetic potential. This genomic information plays a crucial role in guiding precision decisions related to selection, breeding, and management. These genomic-assisted decisions aspire to redefine the decision-making process enhancing effectiveness, efficiency, and sustainability in animal care.

O19 - Hypertranscription on the Lateral Loops of Chicken Lampbrush Chromosomes is Aimed to Produce Maternal RNA for Thousands of Genes

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All vertebrates, except placental and marsupial mammals, have a hypertranscriptional type of oogenesis, accompanied by an extremely high rate of RNA synthesis. High transcriptional output leads to transformation of the chromosomes into a lampbrush configuration. The set of genes transcribed on the lateral loops of avian lampbrush chromosomes remained unknown. Here, we have systematically characterised the transcriptome in the nuclei and cytoplasm of chicken oocytes at the lampbrush chromosome stage of oogenesis. In chicken oocyte nucleus we detected transcripts for ~60% of protein-coding genes, ~40% of long non-coding RNA genes and ~15% of miRNAs. Transcription on the majority of lateral loops is initiated at gene promoters; transcript boundaries coincide with gene boundaries and the transcribed strand corresponds to the orientation of a gene. The set of transcribed genes is associated with essential cellular processes including transcription, mRNA catabolism, piRNA metabolism, DNA repair, RNA splicing, protein synthesis, DNA

replication, cell cycle progression and mitochondria functions. Transcription of the expressed set of genes is positively controlled by KDM5B demethylase and the associated histone modification H3K4me3. For a number of genes we directly visualised nascent transcripts on the lateral loops of the lampbrush chromosomes by RNA-FISH. We found that the nuclear RNA-seq profile predicted the chromomere-loop pattern of lampbrush chromatin domains. Thus in avian oocytes, hypertranscription on the lateral loops of giant lampbrush chromosomes is aimed to produce large amounts of maternal RNAs for thousands of genes.

The research was supported by the Russian Science Foundation (grant #19-74-20075) and was performed using the equipment of the Genomics Core Facility (Skoltech) and Resource Center "Molecular and Cell Technologies" (Saint-Petersburg State University).

O20 - Functional Annotation of Regulatory Elements and its Application on Identification Genetic Variants Associated with Complex Traits in farm animals

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Human and mouse Encyclopedia of DNA Elements (ENCODE) projects have made great impacts on understanding genetic control of complex traits and human diseases. International Functional Annotation of Animal Genomes (FAANG) consortium was initiated in 2015 with the aim to enhance genome-based biology in fundamental and applied research and improve our prediction of economically important traits in farm animals. In order to functionally annotate regulatory elements in the genome, FAANG core assays including RNA-seq, ATAC-seq, ChIP-seq on four histone modification marks were used to generate a landscape of regulatory elements such as enhancers, promoters, silencers across tissues in chicken, pig and cattle. A ChromHMM model was trained using epigenomic FAANG core assays and fifteen chromatin states were predicted. We integrated the annotation of regulatory elements across tissues with genome-wide association studies (GWAS) summary statistics from different complex traits. A significant enrichment was found in tissue-specific regulatory elements, in which the tissues are biologically relevant to complex traits. Further integrative analysis with RNA-seq across tissues and Hi-C data pinpointed potential causative variants for complex traits. Further annotation of regulatory elements in different developmental stages and physiological cues are warranted in order to improve animal production and health.

P29 - Chicken Oocyte Piwi-Interacting RNSs and their Predicted Interspersed Repeat Targets

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Post-transcriptional processing of the repeat transcripts may lead to the appearance of small regulatory RNAs such as endogenous small interfering RNAs (endo-siRNA) and PIWI-interacting RNAs (piRNA). We sequenced small RNA libraries from chicken lampbrush stage oocytes and

classified them according to known types of small non-coding RNAs. In our nuclear and cytoplasmic small RNA samples from chicken lampbrush stage oocytes, there are three major peaks in the length distribution of reads – at ~22 nt, at ~27 nt and at ~33 nt. More than half of the peak at ~22 nt corresponds to miRNA, as can be seen from the length distribution of reads after miRNA filtration; the rest of the small RNAs from the ~22 nt peak could represent endo-siRNA, while the peak at ~27 nt corresponds to piRNA. Predicted piRNAs from the ~27 nt peak were the most abundant RNAs in the cytoplasmic small RNA samples. A substantial proportion of the predicted piRNAs with the highest abundance in the oocyte cytoplasm correspond to known chicken piRNAs from the piRBase database. We then analysed which repeats are targeted by the predicted piRNAs in the chicken GGswu1 genome assembly. The most abundant cytoplasmic and nuclear piRNAs target the CR1 (chicken LINE) retrotransposable element, the ERVL LTR element and the ERVK LTR element; small number of piRNAs target the TcMar-Mariner DNA transposon and almost no piRNAs target SINE elements. Predicted piRNAs in chicken enucleated oocytes characterised in this study may be involved in the inactivation of retrotransposable elements during early embryogenesis.

The research was supported by the Russian Science Foundation (grant #19-74-20075) and was performed using the equipment of the Genomics Core Facility (Skoltech).

P30 - Expression Profile of MiRNAs is Altered in the Undescended Testicles of Unilateral Cryptorchid Dogs

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Cryptorchidism, a major disorder of sex development (DSD) in dogs, has a complex polygenic background. We recently showed that the expression of numerous protein-coding genes in undescended testes is upregulated or downregulated and partially associated with altered epigenetic mechanisms: DNA methylation and acetylation of histone H3 (Stachowiak et al., 2024). Following this, we investigated the RNAseq global expression of short non-coding RNAs (ncRNAs) in undescended (UD) and descended (D) testes of nine unilateral cryptorchid dogs and eight control (C) testes. Comparison of UD and D testes revealed 224 differentially expressed miRNA (DEGs), with 118 (8 upregulated, 110 downregulated) meeting these criteria: FDR<0.05 and -1.5<logFC>1.5. Comparison of UD and C testes indicated 200 DEGs while 102 (9 upregulated and 93 downregulated) met the same criteria. Five miRNAs were validated by ddPCR in larger cohorts (26 UD, 26 D, 25 C). For miRNA DEGs, using the TargetScan database we sought potential target sequences in 3'UTR of mRNA DEGs. We selected the top ten upregulated miRNAs (with the highest FDR) to compare their expression with top ten downregulated mRNAs reported by Stachowiak et al. (2024). The same approach was applied for top ten downregulated miRNAs versus top ten upregulated mRNAs. Two comparisons were performed: UD versus D and UD versus C. The first revealed four downregulated miRNAs (cfa-miR-8831, cfa-miR-449a, cfa-miR-34b, cfa-miR-449b) with target sequences in 3'UTR of the upregulated *PDGFRA*, and downregulated cfa-miR-15b, which has its target sequence

in 3'UTR of the upregulated *G0S2*. The same relationships were observed in the second comparison. We conclude that altered expression of miRNAs also contributes to the altered expression of protein coding genes in undescended testes.
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P31 - Transcriptome Analysis of Muscle Tissue Around the Navel in Pigs with Umbilical Hernia

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Umbilical hernia (UH) is a serious problem in pig production, affecting animal welfare and causing economic losses. The condition is caused by genetic variants, by altered gene expression in the muscle or connective tissue near the site of hernia, and also by environmental factors. The aim of this study was to identify genes with differential expression profiles in the muscle tissue near the navel of pigs with UH and to compared these with healthy animals. We also sought altered DNA methylation and DNA variants in the regulatory regions of these genes. Muscle tissue dissected from near the navel underwent RNA-seq analysis for fifteen UH and fifteen control animals, and 234 differentially expressed genes (DEGs) were identified. Next, eleven genes were selected for validation by qPCR performed on larger groups (34 UH and 34 controls). Eight genes (*SIM1*, *PITX1*, *HOXA7*, *METTL21C*, *PVALB*, *ALX1*, *EYA2*, *TBX1*) showed statistically significant changes in expression with the same direction of change as in RNA-seq analysis. For these genes, DNA methylation analysis performed by pyrosequencing revealed significant CpG methylation changes in seven genes (*SIM1*, *PITX1*, *HOXA7*, *METTL21C*, *ALX1*, *EYA2*, *TBX1*). Sanger sequencing identified one significant SNP (rs330073569) located in the 5'-flanking region of the *METTL21C* gene, in potential regulatory sequences for transcription factors. These results indicate that altered gene expression is a possible mechanism of hernia occurrence, and that these alterations may be caused by epigenetic factors such as DNA methylation.
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P32 - Alterations of the Copy Number of the Mitochondrial Genome in Equine Sarcoid Tissue

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Mitochondria are the major source of energy in cells, which makes them critical organelles. Different mitochondrial dysfunctions were reported in cancer development. Cumulative evidence has indicated

that the variation in mitochondrial DNA copy number (mtDNA-CN) is one of them. mtDNA-CN reflects mitochondrial activity, and its alterations were linked to metabolic reprogramming. Therefore, mtDNA-CN emerged as a critical factor associated with the progression of various cancer types. However, such data are not available for equine sarcoids, which are the most common skin tumors in this species. Thus, in the present study, we aimed to assess the number of mtDNA copies in 15 healthy tissue (control) and 30 sarcoid samples and their aberrations. To this end, we applied the qPCR method, using primers specific for two mitochondrial genes, namely *ND1* (NADH dehydrogenase subunit 1) and *ND6* (NADH dehydrogenase subunit 6). As a result, 72 and 51 copies were determined on average for the sarcoid samples on the basis of *ND1* and *ND6* results, respectively. For the control samples, the average number of copies was equal to 117 (*ND1* results) and 113 (*ND6* results). The obtained results indicated a -1.69 (*ND1*) and -2.18 (*ND6*) fold decrease of mtDNA copies in the sarcoid samples with reference to the control. In conclusion, we report preliminary data indicating mitochondrial dysfunction in the course of sarcoid neoplastic transformation manifested via mtDNA-CN deregulation. Understanding the nuanced interplay between mitochondrial dysfunction and cancer holds potential for the development of targeted therapeutic interventions. The obtained results constitute a good starting point for further research to thoroughly characterize this phenomenon in equine sarcoids and determine its usefulness in diagnostics and treatment strategies.

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P33 - First insights into genome organization and expression signature of equine somatic and tumor piRNAs

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PIWI-interacting RNAs (piRNAs) belong to the class of small non-coding RNAs. They were first considered to be specific to germ cells. However, recent evidence has revealed their expression in somatic tissues and engagement in the regulation of myriads of biological processes, including those associated with neoplastic transformation. Although tissue-specific piRNA repertoire has been intensively investigated since then, the state of knowledge for livestock species is very limited, especially for piRNAs of non-germinal origin. Therefore, in this study, we aimed to elucidate piRNA expression signature and genome organization in the horse skin as well as determine the influence of sarcoid formation, which is the most common equine skin tumor, on the piRNA profile. To this end, we performed an *in silico* analysis to predict piRNA cluster localization on the basis of NGS data for 11 horses (GSE87901; PRJEB27174). Then, we characterized the piRNA expression profiles of healthy skin and sarcoid tumors and revealed their aberrations in the sarcoid samples, identifying 33 sequences with altered levels. They included previously known germinal piRNAs (e.g. piR-eca-1415182, piR-eca-3625002) and novel sequences. Two significantly upregulated and one significantly downregulated piRNAs were validated with the RT-qPCR method. Functional analysis

indicated the enrichment of processes crucial for cell functioning and engaged in tumorigenesis in the sarcoid tissue. In conclusion, we report new data on equine piRNA genome organization and expression signatures associated with sarcoid oncogenesis. To the best of our knowledge, the obtained results demonstrate the first somatic and tumor tissue piRNA data, not only in equids, but also in livestock animals. They shed light on the potential significance of piRNAs in sarcoid cell functioning providing candidates for further studies.

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P34 - Illustration of a Deep Learning Approach to Identify Key Genomic Sequence Features of 3D Genome Organisation

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Biology and genomics are not immune to the revolution of artificial intelligence (AI), and particularly the recent successes of deep learning. The intrinsic characteristics of genomics, including the massive production of heterogeneous data, make it a prime candidate for deep learning applications. Numerous applications of this approach have been proposed in recent years. What is particularly appealing is the ability to predict molecular signatures of genome function (chromosome accessibility, 3D genome organization) from the genomic sequence alone, thereby mimicking the functioning of a cell or an organism that must orchestrate its functions by using the information provided by the genome. In addition, this predictive capability, based solely on the genomic sequence, virtually enables predictions from a modified genomic sequence, thereby allowing the study, *in silico*, of the potential impact of mutations. We will show how a deep learning model trained in human (Zhou *et al.*, Nat Genet 54:725, 2022) can be used, by taking as input the bovine genome reference assembly, to predict the 3D organization in bovine. We demonstrate the potential of the model by performing an *in silico* mutational screen that confirmed that the model recovered the crucial role of CTCF binding sites for the formation of TAD borders. We will also show how this model can be used to identify sequence features potentially involved in the formation of A/B compartments.

P35 - Effect of Cannabidiol on Sarcoid Cell's MMP-2 Level

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Equine sarcoids are tumors that affect the skin and sometimes the underlying tissues of horses. These tumors are caused by the bovine papillomavirus (BPV) and can be locally invasive and aggressive, very often ulcerating. Research indicates that infection with papillomavirus and the expression of viral genes in infected hosts cause an increase in the expression of matrix metalloproteinase (MMP) genes, responsible for the degradation of collagen and other extracellular matrix (ECM) proteins. Recent studies have also confirmed abnormal expression of MMP genes in equine sarcoid tissues, including overexpression of the MMP-2 gene. Cannabidiol (CBD) is a nonpsychotropic derivative of cannabis. Previous work showed that CBD can inhibit tumor formation and propagation in different types of cancer. Therefore, this study aimed to determine the effect of CBD on MMP-2 secretion by sarcoid cells. The experiment was carried out on three sarcoid primary cell lines of the third and fourth passage to assess the effect of different doses of CBD (20, 6.75, and 0.67 µM) and different incubation times (6h, 24h, and 48h). The concentration of MMP-2 protein was determined in the collected medium using the ELISA method. Our preliminary results showed that higher CBD concentrations (20 µM) significantly decreased MMP-2 level in all incubation times. This effect was not observed for the other doses (6.75 and 0.67 µM), where protein concentration change was observed where protein concentration change was observed irregularly. Our data show that CBD may have a different effect on the level of MMP-2 in equine sarcoid cells depending on dose and suggest that CBD may decrease MMP-2 production at higher concentrations. Thus, we hypothesize that, by reducing the level of MMP-2, cannabidiol could potentially contribute to reducing the severity of equine sarcoids and improving treatment outcomes.

P36 - Evaluation of Pirna's Potential as Epigenetic Regulators in Equine Sarcoids

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PIWI-interacting RNAs (piRNAs) are classified as small non-coding RNAs and are generated by a unique biosynthesis process. They are involved in the regulation of important biological processes via different mechanisms, such as post-transcriptional silencing and epigenetic modifications. The latter ones include the induction of the methylation of CpG sites effectuated via the recruitment of DNA methyltransferases (DNMTs), which leads to further alterations in transcription efficiency. In our previous study, we identified disruptions in the piRNA profile in sarcoid tumor, which is reported to be the most common skin neoplasm in equids. Taking into account these results and the involvement of piRNAs in carcinogenesis, the aim of this study was to investigate the engagement of deregulated sarcoid piRNAs in its oncogenesis via the modifications of DNA methylation patterns. To this end, in this pilot experiment, we transfected three equine sarcoid primary cell lines with three most significant piRNA sequences selected on the basis of the previous results. Next, we determined global DNA methylation levels with the MethylFlash Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric) (EpigenTek). The obtained results showed changes in the average percentage of methylated cytosines in sarcoid samples with reference to the control at the level of 0.50% for piR-1,

0.38% for piR-2 and 0.60% for piR-3. Our data reveal that piRNA sequences may act as epigenetic regulators and modify DNA methylation patterns in equine sarcoid cells in the course of neoplastic transformation. Further research is warranted to elucidate the details of the mechanism and assess its suitability to be a target in sarcoid treatment strategies.
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P37 - Whole Genome Sequencing of two Cats (38,XX; *SRY*-Negative) with Disorders of Sex Development

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Disorders of sex development (DSD) in animals with female sex chromosomes (XX, *SRY*-negative), manifesting as the presence of testes or ovotestes, have been diagnosed in several domestic species, including goats, dogs, pigs, and horses. The disorder's molecular background has been fully elucidated only in goats. Our aim was to find causative DNA variants in two cats with testicular XX DSD (*SRY*-negative) using whole-genome sequencing (WGS). We first analyzed the genome sequence of the affected cat (case 1) and parents, yielding identification of 16,854,631 DNA variants meeting our initial quality criteria. We hypothesized that this disorder is caused by a recessive mutation, and thus we searched for homozygous variants in the DSD cat, which were heterozygous in the parents. Altogether 653,795 such variants, harbored by 9337 genes, were found. Among them, 5311 variants (with homozygous status) occurred in 75 candidate genes of 217 analyzed, which were a priori selected given knowledge of the molecular background of human DSDs (Cools et al., Nature Reviews. Endocrinology, 2018). We found homozygous missense substitutions in seven genes (*SF3A2, AMH, ORC1, DOCK8, PRKAR1A, SOX10* and *TMEM186*) in case 1. Second, we analyzed the WGS of another XX DSD cat (case 2), finding that 2869 identified variants (773 with homozygous status) in case 2 were shared with case 1. Interestingly, a homozygous missense substitution in *TMEM186* was noted. This polymorphic site also occurs within the intron of an overlapping *PMM2* candidate for DSD, located on the alternative DNA strand. The potential role of identified variants needs further investigations.
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P38 - Characterization of Heterozygosity-Rich Regions (HRR) in two Sicilian Local Cattle Breeds

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Cinisara and Modicana, are Sicilian cattle breeds well-adapted to harsh environments. Genomic analysis of 26 Cinisara and 38 Modicana cattle, using the BovineHD BeadChip, focused on genomic heterozygosity-rich regions (HRR) which may disclose balancing-selection adaptive mechanisms. Data quality control was done using PLINK 1.9 and HRR were investigated by the detectRuns R package. The top 0.1% of SNPs (breed recurrence ≥20%) were considered to form the HRR islands. The gene and QTL annotations and enrichment analysis were conducted using the GALLO R package. The results reported 198 HRR in Cinisara and 293 in Modicana cattle, averaging 7.62 ± 2.68 and 7.71 ± 2.29 HRR per animal, respectively. The longest HRR were found on BTA15 (532 kb) for Cinisara and BTA21 (519 kb) for Modicana. Cinisara had eight HRR islands harbouring 265 markers, while Modicana had ten islands with 488 markers. A hotspot on BTA5 shared by 45% of all animals included QTLs associated with milk and meat production traits. In Cinisara, 53.33% of annotation was linked to meat and carcass traits, while Modicana's QTLs (97.44%) related to milk production. Significant QTLs associated with productive life length, subcutaneous fat thickness, and milk casein percentage, were found on BTA22 (Cinisara) and BTA6 (Modicana). These results could help to characterize these local genetic resources, disclose adaptation-related traits, and support their conservation.

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P39 - Genotyping of Exon 1 of *Mbl*2 Gene in Comisana Sheep Breed and Haplotype Association with Mastitis

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Udder health is crucial for dairy animals and has a direct impact on the profitability of the dairy industry. For this reason, in the last years genetic improvement plans are taking into account also parameters that may reflect animal resiliency to mastitis. The identification of gene variants

associated to resistance to develop mastitis represent an actual target of genetic association studies. To this purpose genetic analyses are needed to identify genes associated with udder health in dairy animals. The Mannose Binding Lectin 2 (*MBL2*) gene is one of the genes associated with immunity, in fact, its protein binds to various sugars expressed on pathogen membrane and several studies on different species have related polymorphisms of the *MBL2* gene with somatic cell score (SCS), milk quality and the susceptibility to *Brucella abortus*.

The aim of the present study was to investigate the *MBL2* gene variants and its association with mastitis and milk quality traits in Comisana sheep. To this end, 66 lactating ewes, with and without mastitis, were enrolled. The characterization of exon 1 revealed three alleles: A1, C and D. The A1 variant, probably derived from the A allele found in other studies, was the most common in the analyzed population. The A1C genotype was found exclusively in healthy animals, suggesting a possible association between this genotype and the absence of mastitis. This association could explain the lower somatic cell count observed in animals with the A1C genotype compared to those with the A1A1 and DD genotypes.

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SESSION 8 – CYTOGENETIC AND GENOMIC CHARACTERIZATION OF ANIMAL BIODIVERSITY

L13 - Invasive Marine Species: Transforming a Threat into a Resource

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In shallow subtidal Mediterranean marine ecosystems, the invasion of the green algae *Caulerpa cylindracea* poses a significant threat to the natural patterns of marine biodiversity. This invasion leads to the heavy dominance of invasive algae in infralittoral habitats, which in turn affects the structure of benthic assemblages by altering the relative importance of endemic species and modifying habitat complexity.

However, a still too neglected aspect is how this invasion can impact the feeding behaviour of endemic fauna and subsequently influence ecosystem functioning through shifts in trophic resources. Our study focused on the white seabream *Diplodus sargus*, a widely distributed Mediterranean fish, by analysing stomach contents from populations sampled in locations with varying degrees of *C. cylindracea* substrate colonization. Our results offer new insights into the feeding behaviour of a species of economic and ecological importance and how it is altered in response to algal invasion. Based on several studies, we demonstrated that, due to still not clarified mechanisms that determines changes in the feeding behaviour of the white sea bream, a conspicuous quantity of *C. cylindracea* is entering in its diet. Our research contributes to a broader context that focuses on the effects of invasive species metabolites at the population and ecosystem levels. By shedding light on the mechanisms

underlying potential changes in ecosystem functioning due to shifts in secondary metabolites resulting from biological invasion, our studies aim to enhance our understanding of the impacts of invasive species on marine ecosystems.

O21 - The Highly Conserved Karyotype of the *Myotis* Genus (*Chiroptera, Vespertillionidae*): What is Making the Difference?

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The order Chiroptera is one of the most numerous and diverse mammalian groups, with more than 1400 recognized species distributed worldwide. Amongst these, the Vespertilionidae family and particularly, the *Myotis* genus, can be found in highly variable environmental niches, making them ideal for studying chromosome evolution and understand species adaptation. Comparative chromosome maps allow to analyse the dynamics of karyotype and genome evolution, as well as to clarify systematics and taxonomic questions. In this study, human paint probes were used to obtain comparative maps for the Vespertilionidae *Myotis daubentonii* (MDA) and *Myotis blythii* (MBL). Biological samples were collected by specialized technicians from natural populations following official licenses issued by the Portuguese Authority for Nature Conservation. *In vitro* cell culture from wing tissue, chromosome preparations, conventional cytogenetics, paints preparation and Comparative chromosome painting (CCP) were done according to routine procedures. G- and C-banding showed that the karyotypes of MDA and MBL, as the other *Myotis* studied on this respect, are highly conserved, consisting of 44 chromosomes: 21 pairs of autosomes, which included 4 metacentric pairs, 14 medium-sized acrocentric pairs, and 3 smaller acrocentric pairs. In both species, the X chromosome is metacentric, and the Y, acrocentric. CCP data reinforced these observations, revealing, at the first sight, highly conserved karyotypes, presenting the syntenic associations observed in the putative ancestral karyotype. But although the big picture showed great similarity, some minor intrachromosomal differences between species were detected, which may well be explained by the repetitive fraction of these genomes, that we believe are the main drivers for the striking differences between species. Further studies on other vespertilionids using both CCP together with the analysis of repeats are needed to better understand the impact of these sequences as "architects" of evolution and possibly, drivers of adaptation. Molecular cytogenetics is, undoubtedly, a valuable tool for detecting hidden and intrachromosomal alterations between genomes, as well as for understanding the global genome structure, particularly in species where there is limited DNA sequencing data available.

O22 – Origin of the Current Wild Boar Population of Campania Region (Italy) and SNPs Identification in Rela Gene

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Italian wild boar populations diverge from the original Mediterranean strains (*Sus scrofa meridionalis* and *Sus scrofa minor*), with exceptions in limited areas (central southern Italy and Sardinia) due to the introduction of wild boar from other countries. The population size and the absence of health control measures render them susceptible to becoming carriers of significant diseases. Presently, African Swine Fever (ASF) poses a serious threat to biodiversity and to the survival of native swine breeds. This study aims to identify the wild boar strains contributing to the current population in the Campania region and to ascertain if there exist polymorphisms within their genomes that could confer resistance to the ASF virus. To this purpose Mitochondrial DNA control region (mtDNA-CR) analysis and polymorphism analysis on the *RelA* gene were conducted on twenty adult wild boars harvested during the official hunting season in the province of Avellino. mtDNA-CR is commonly used as a molecular marker for studies in population genetics and phylogenetics. The *RelA* gene encodes a p65 kD protein, a critical subunit of the NF-kB transcription factor, known for its pivotal role in regulating both innate and adaptive immunity. Polymorphisms on the *RelA* gene, have been recently associated to resistance to ASF virus infection in domestic pigs. The analyses of mtDNA-CR sequences revealed that the majority of animals in this study corresponded to haplotypes originating from northeastern Europe and Asian pig breeds. Moreover, some polymorphisms have been observed in *RelA* gene.

O23 - The Importance of Cell Banking to Species Chromosome Characterization and Conservation

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Cryobanks represent stocks of viable somatic cells or gametes that ensure long-term integrity at the molecular level and preservation of genetic variability of species or populations for various purposes. The LIB Biobank at Museum Koenig, member of GGBN (Global Genome Biodiversity Network), recently started an ambitious initiative for archiving viable cells and tissues – fundamental to better characterization of the biodiversity and a powerful tool for future conservation strategies. To date, we have cryopreserved viable cells and/or tissues for 208 species represented by birds (44.2 %), mammals (33.2%), reptiles (7.2%), fishes (6.3%) and amphibians (4.8%). In addition to storing viable material, cytogenetic analyses are carried out on selected samples, contributing to the characterization of the species in the cell bank by including new chromosome data. Here we demonstrate that

conventional cytogenetics are still important, and we describe new karyotypes as well as banding patterns of three mammals, three birds, and one fish species. Karyotypes were obtained from cells, and time of colchicine and hypotonic exposure were adapted for each taxonomic group. Giemsa staining, CTG and GTG-banding were performed according to routine cytogenetic methods. Cytogenetic results showed (i) 2n=48 for the broom hare (*Lepus castroviejoi*); (ii) 2n=65 for the Linnaeus' two-toed sloth (*Choloepus didactylus*); (iii) 2n=48 for the spiny mouse (*Neacomys rosalindae*); (iv) 2n=80 for the bleeding-heart dove (*Gallicolumba luzonica*); (v) 2n=82 for Ural owl (*Strix uralensis*); (vi) 2n=76 for Socorro dove (*Zenaida* aff. *graysoni*); and (vii) 2n=42 for Evers ricefish (*Oryzias eversi*). The karyotypes of the bleeding-heart dove and ricefish as well as a new karyomorph for the spiny mouse are being described for the first time. The latter possess a heteromorphic pair possibly due to a pericentric inversion. To our knowledge, this is also the first time that CTG-banding has been performed in the broom hare, spiny mouse, Socorro dove, and rice fish. We reiterate that classical cytogenetic studies remain essential to understand the evolution and genome of species since basic information such as chromosome number and banding pattern are still absent for many taxa and the importance of establishing primary cell cultures to access karyotype information with good quality.

O24 - Cytogenetics on Snakes (*Serpentes: Squamata*) in a Phylogenetic Context

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Despite the homogeneous morphology, snakes achieved great adaptive radiation into over 4000 species, reflecting in chromosomal variability of constitution, morphology, and molecular organization. To investigate snake chromosome's structure and evolution, cytogenetic studies were conducted on *Boa constrictor amarali*, *Bothrops jararaca*, *B. insularis*, *B. fonsecai*, *B. moojeni*, *Chironius bicarinatus*, *C. flavolineatus*, *Oxyrhopus guibei*, *Philodryas patagoniensis*, *P. olfersii*, *Spilotes pullatus*, and *Naja kaouthia*. Metaphases were obtained from leukocytes incubated with 0,1% colchicine, hypotonized in 0,0075M KCl and fixated in Carnoy. Giemsa staining, Ag-NOR and C-banding were proceeded following routine techniques, and telomere FISH was performed per adapted Dako instructions. Cytogenetic data were plotted onto the most recent phylogeny to infer the hypothetic direction of rearrangements. Most species exhibited 2n=36, except *O. guibei* (2n=44), *Naja kaouthia* (2n=38) and *C. flavolineatus* (2n=34) – an undescribed diploid number was found in the latter species. Henophidia representative, *Boa constrictor*, exhibited homomorphic XX/XY, contrasting with the ZZ/ZW found in caenophidians. Ag-NOR in *P. olfersii* and *P. patagoniensis* corroborated translocations in NOR-carrying micro and macrochromosomes. FISH and C-banding revealed centromeric heterochromatin in *Boa constrictor* XX/XY, unlike heterochromatic and repetitive content accumulation on W chromosome in some Caenophidia, including a large heterochromatic block and repetitive telomeric sequences in *N. kaouthia*. FISH also unveiled different interstitial signs by comparing the island *B. insularis* to the continental *B. jararaca*. These results highlight important variations in diploid number, morphology and chromosome architecture,

including sex chromosomes evolution complexity, evincing rearrangements throughout snakes karyotypic evolution and diversification.

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P40 - Identification of Species-Specific Indels in *Bubalus bubalis*, *Bos taurus*, *Capra hircus*, and *Ovis aries*

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InDels are the second most common type of variation across eukaryotes genomes. Several studies have shown that InDels are the major cause of evolutionary changes so contributing significantly to intra-interspecific divergence. The aim of this study was to identify specie-specific short-InDels for *Bubalus bubalis*, *Bos taurus*, *Capra hircus*, and *Ovis aries*. For this purpose, genomic sequences of all *Artiodactyla* and *Perissodactyla* species available in GeneBank were aligned for candidate genes associated to milk and meat quali-quantitative traits. The *in-silico* investigation has evidenced that *Ovis aries* and *Bubalus bubalis* are characterized by a 14bp deletion at 5'UTR of αs2-casein encoding gene (*CSN1S2*, KT283354.1:g.643-644delAGAAATCAAATCTT) and by a deletion of an heptamer at exon 10 (3'-UTR) of *PRLR* gene encoding for Prolactin Receptor (MF461277.1:g.12162-12163delCACTACC), respectively. Likewise, the 5'UTR of αs1-casein encoding gene (*CSN1S1*) of *Capra hircus* is characterized by a 28bp sequence (KC951931.1:g.1989-2016insTGTACAATGCCATTAATATATTGTACAA). In particular, the first 20 nucleotides are absent in *Bubalus bubalis* and *Bos taurus* sequences, while the last 7bp are constitutively deleted in *Ovis aries*. Finally, it was evidenced that *Bos taurus* is characterized by the deletion of 16bp (AB076403.1:g.1207-1208delGAGTAGGTTATGGCTT) at intron 1 of myostatin gene (*MSTN*). To verify the specificity of these genetic markers, four allele-specific-PCR protocols were developed. The genotyping of a preliminary panel of 400 samples (100 each species, belonging to different breeds) seems to confirm the *in-silico* analyses. These markers may become a new tool to carry out phylogenetic studies or to set up PCR methods to verify the animal origin of the components of a product.

P41 - Identification of a donkey *CSN1S2*I allele resulting from a non-constitutive splicing event

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The αs2-casein is a phosphoproteins secreted in the milk of most mammals, and it is the most hydrophilic of all caseins. Contrary to ruminants, in donkey two different encoding genes (*CSN1S2*I and *CSN1S2*II) have been identified. The first, spanning over a cDNA fragment of 1016 nt, is made of 19 exons and it encodes for the αs2-I protein of 221 amino acids. Nonetheless, while ruminants have been extensively studied in this regard, detailed characterization of the variability at this *locus* in donkeys has been lacking so far. Therefore, this study aimed to identify and analyse the variability of the *CSN1S2*I *locus* in Ragusana and Amiatina donkeys reared in Italy. For this purpose, transcripts and genomic DNAs of 8 subjects for each breed were sequenced. The sequences comparison revealed a transition G>A at the splice acceptor site of exon 17 that results in a skipping of the first 15 nt of this exon encoding for the peptide 176NKINQ180 and the recognition of an in-frame cryptic splicing acceptor site: arAACAAAATCAACCAG. The comparison of the sequences available in GeneBank showed that this peptide is constitutively spliced in all species belonging to *Perissodactyla* order in contrast to what is observed in species belonging to the *Cetartiodactyla* and *Carnivora* orders. Furthermore, the contemporary presence of the canonical and cryptic acceptor site of the 17th exon is observed only for species belonging to the sub-order *Ruminantia*. It is interesting to note that the 176NKINQ180 sequence is a perfect duplication of the pentapeptide encoded by the first 15 nucleotides of exon 12 (92NKINQ96) that is a trait of two major IgE-binding epitopes of the bovine □s2-CN. Therefore, the absence of duplication could be related to the demonstrated low allergenic properties of donkey's milk. The transition G>A alters a *Xba*I restriction site. Thus, a PCR-RFLP protocol was set up for a quick genotyping of 105 Ragusana and 14 Amiatina donkeys. Out of the total investigated population the G allele has a frequency of 0.7563 with no evidence of departure from the Hardy-Weinberg equilibrium. Results indicate that donkey, similar to buffalo at the same *locus*, has a *CSN1S2*I allele resulting from a non-constitutive splicing event.

P42 - Genomic Investigation on Casertana and Commercial Italian Pig Breeds

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The Casertana pig (C) is a breed native to Southern Italy, prized for its exceptionally fine meat and high nutritional value. Preserving and understanding its genetic makeup is crucial for both production and reproductive purposes. In this preliminary study, we conducted genetic screening on 33 Casertana pigs and 31 hybrid pigs, including Duroc-Large White (DLW), Pietrain-Large White (PLW), Pietrain, and Duroc-Landrace breeds. We employed two genomic approaches: RAPD-PCR (Random Amplification of Polymorphic DNA) and mtDNA (mitochondrial DNA) analysis.

RAPD-PCR was performed using 14 different primer pairs to generate unique fingerprints for each breed. While we obtained a high number of unique and repeatable bands, no specific differences were identified between Casertana pigs and the hybrid pigs.
mtDNA, known for its high interspecific variability and suitability in determining population origins, was analyzed using four primer pairs: MT1-2-3 (Gvozdanovic et al., 2018; Karabasanavar et al., 2014) and DISPI. The amplified products yielded four different amplicons (487, 712, 734, and 700 bp) without intra-breed differences. MT3 showed two bands, a common 712 bp band across all samples and a 500 bp band specific to DLW hybrids and some Casertana samples. Additionally, for some Casertana samples, the 712 bp band split into two separate bands.
These preliminary results suggest that mtDNA, especially when coupled with sequencing analyses, could be valuable in determining specific characteristics of the investigated breeds.

SESSION 9 – CYTOGENETICS AND GENOMICS OF INVERTEBRATES

L14 - Comparative Cytogenetics of the Order *Hymenoptera* (*Insecta*)

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Hymenoptera are an extremely speciose, taxonomically complicated and economically important group of insects, with arrhenotoky and haplodiploidy representing the main ancestral genetic features of their life cycle. This order harbors more than 150,000 described species, although karyotypes of just about 2,000 its members are studied up to now. For these insects, chromosome numbers of n = 1 to 60 are known; in addition, B chromosomes were detected in some species.
As in other animals, metacentrics and submetacentrics predominate within chromosome sets of many Hymenoptera with lower n values, whereas subtelocentrics and acrocentrics often prevail within karyotypes with higher chromosome numbers. Although differential chromosome staining and other cytogenetic techniques often reveal important features of karyotype evolution, morphometric analysis of routinely stained chromosomes can also detect chromosomal rearrangements as well as distinguish between karyotypes of different members of this order. Blocks of pericentromeric heterochromatin are characteristic of most Hymenoptera, although chromosomes of certain species can carry fully heterochromatic arms. Hymenopteran karyotypes usually harbor one or two nucleolus organizing regions (NORs). Deletions/duplications of the constitutive heterochromatin, fusions/fissions, translocations, inversions, polyploidy and aneuploidy are the most frequent chromosomal rearrangements found in this order.
Current genomic research on Hymenoptera has twofold implications for the cytogenetic studies of this group. First, genome sequencing is essential for creating robust phylogenies, which, in turn, are indispensable for studying karyotype evolution in this order. On the other hand, an advent of molecular techniques greatly enhanced resolution of the cytogenetic study of Hymenoptera. Specifically, use of base-specific fluorochromes visualized chromosomal segments enriched either with AT or GC base pairs. Fluorescence *in situ* hybridization (FISH), including chromosome painting,

combined with microdissection, proved its importance for studying chromosomal rearrangements within Hymenoptera. A few so-called supergenes have been recently revealed in certain species using both cytogenetic and bioinformatic approaches. The same tools were used to study hymenopteran telomeric repeats. Although the canonical insect telomeric motif TTAGG predominates in the basal clades of Hymenoptera and is therefore ancestral for this group, multiple substitutions and reappearances of this repeat occurred there, thus making Hymenoptera the most diverse animal order in terms of its telomeric motifs.

O25 - Dynamics of the 3d Genome Architecture in the Malaria Mosquito Development

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Functional interactions between regulatory elements and gene promoters play a role in regulating gene transcription during cellular differentiation and response to external stimuli. However, the 3D aspect of gene regulation has not been investigated in insects that transmit human diseases. Here, we examined the dynamic aspects of 3D genome architecture during mosquito individual development. We performed genome-wide chromatin conformation capture (Hi-C) on embryonic, larval, and adult stages of mosquito development, as well as on body parts of adult females and males, including heads, antennas, proboscises, maxillary palps, thoraxes, and gonads of *Anopheles coluzzii*. Comparison of Hi-C maps obtained from adult and embryonic tissues identified long-range chromatin interactions, particularly on the 3R arm, that occur at specific stages or in certain body parts during mosquito development. Some giant loops are specific to the soma, as they are absent in ovaries or testes but present in the thoraxes and heads of adult mosquitoes. Additionally, heads have stronger contacts, as well as additional giant loops that are absent in thoraxes, suggesting their possible function in the nervous system. The eyes/brain samples contained the majority of giant chromatin loops, while fewer loops were found in the antennas and even fewer in the maxillary palps. Interestingly, genes located at the loop anchors have roles in cell-cell signaling, sensory perception, neuron differentiation, signal transduction, and response to stimulus. The dynamic nature of the chromatin interactions in different organs suggests their functional significance for the development and function of the nervous system in malaria mosquitoes.

O26 - Telomere Length as biomarker of wellness in honey bees *(Apis mellifera, L.)*

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Honey bees (*Apis mellifera*, L.) play an essential role in global agriculture as key pollinators of many crops, being essential for food production and ecosystem stability. However, these crucial pollinators face numerous challenges, including habitat loss, pesticide exposure, pathogens, and climate change, leading to alarming rates of worldwide colony losses.

In recent years, molecular biomarkers have emerged as important tools in modern agriculture, facilitating the monitoring of animal health and providing objective assessments of well-being. Within the realm of honeybees, genomic biomarkers hold promise owing to their ability to integrate multifaceted, context-dependent information.

In this effort, telomere length emerges as a promising genomic biomarker, as observed in other species such as mammals. Telomeres, repetitive DNA sequences situated at chromosome ends, play a central role in safeguarding genetic material from damage and have been implicated in processes related to health, aging, and stress in mammalian models. While the telomere sequence is evolutionarily conserved across species, the development of species-specific protocols and primer designs is imperative for accurate analysis. Currently, standardized protocols for measuring telomere length in honeybees are lacking. Therefore, our study attempt to establish a qPCR protocol for assessing telomere length in bees, serving as a biomarker for their well-being.

Quantitative real-time PCR (qPCR) enables precise quantification of telomere length relative to an internal reference gene (GAPDH), ensuring stable measurements across diverse environmental conditions. Implementation of this novel protocol will facilitate the evaluation of telomere length dynamics in honeybees under varying conditions, thereby providing a valuable tool for assessing the health and well-being of these indispensable pollinators.

Keywords: telomere length, qPCR, animal wellness, pollinators, genomic biomarker

O27 - **Oriental Fruit Fly Invasion in Southern Italy: Understanding Genetic Variability, Spread and Potential Threats to Agriculture**

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In 2018, monitoring in Southern Italy, identified the first Italian and European instances of *Bactrocera dorsalis* (Diptera: Tephritidae), the Oriental fruit fly. Intensified monitoring in 2019 found more specimens, but no detections were made in 2020-2021. However, in 2022-2023, a significant resurgence in captures led to renewed monitoring efforts. This invasion was studied with three main objectives: a) determine whether the invasions originate from a single or multiple species within the *B. dorsalis* complex; b) assess haplotype variability; c) analyze the species' dispersal patterns.

Activities involved the weekly inspection of traps in fields and the following genetic analysis on collected samples (COI barcoding). Capture data revealed an expanding diffusion area during summer and fall, with fewer captures and reduced infested areas in colder months, suggesting a core infestation area of *B. dorsalis* in the monitored region. Genetic analysis identified several new haplotypes in addition to previously identified ones. Notably, in 2022, two previously unknown high-

frequency haplotypes were discovered, and in 2023, most detected haplotypes seemed new, although the overall genetic diversity remained moderate.
The findings indicate that a single species with high dispersal capabilities is driving the invasion with multiple introductions of new individuals. However, not all introductions result in active infestations, and the discontinuity in haplotype findings could indicate a struggling overwintering.
Ongoing studies aim to monitor haplotype changes over time and space to identify those capable of adapting locally. Furthermore, assessing long-term dispersal and diffusion will help estimate the potential threats this invasive species poses to European agriculture.

P43 - An Overview of DNA Methylation in Mytilus Galloprovincialis Through Nanopore Technology

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Mollusks represent the second-largest animal phylum and the class *Bivalvia* includes marine invertebrates with high significant ecological and commercial value, like the Mediterranean mussel *Mytilus galloprovincialis*. This species is a cosmopolitan bivalve widely distributed in the Mediterranean Sea, with a genome size of 1.4 Gb and 14 chromosomes.
The role of DNA methylation is widely explored in vertebrates. However, many aspects of this phenomenon remain poorly investigated and unclear in invertebrates and even more in mollusks. This is the first study performed by Nanopore technology, that offers an overview of DNA methylation in *Mytilus galloprovincialis*, to gain a deeper understanding of the complexity of pigenetic mechanisms in this invertebrate species.
A sample of mussel was collected during the summer of 2023 from a commercial shellfish farm. After DNA extraction, libraries were created following the protocol for Ligation sequencing for gDNA (SQK-LSK110) and then sequenced using the MinION sequencer. The generated raw Nanopore data (fast5 files) were basecalled using Dorado basecaller v0.5.3 and methylation was called using a high accuracy model. To extract methylation information, we used modbam2bed v0.10.0. The reads were indexed and then aligned to reference genome of *Mytilus galloprovincialis* (MytGallo_primary_0.1). Data was imported into R software for analysis.
We calculated the whole genome average methylation frequency that was approximately 18 %. Interestingly this value is lower than what is typically observed in mammals (~70–80%). This study highlights the potential of Nanopore Sequencing as a useful tool to describe and characterize the methylome of *Mytilus galloprovincialis*.