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PRIMA SCALA-MEDI

PRIMA PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

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Report on SCALA-MEDI Training

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Partners involved: All SCALA-MEDI partners

Dissemination level: PU (public)

1. Project Context and Training Objectives

1.1 The SCALA-MEDI Framework

The training activities described in this report were conducted as an integral part of the **SCALA-MEDI project** (*Improving sustainability and quality of Sheep and Chicken productions by leveraging the Adaptation potential of LocAl breeds in the MEDIterranean area*). Funded under the **PRIMA program** (Partnership for Research and Innovation in the Mediterranean Area), this initiative addresses the urgent challenges posed by climate change to Mediterranean livestock systems.

At its core, SCALA-MEDI seeks to transform animal production by focusing on the intrinsic value of local genetic resources. By tapping into the natural adaptive potential and genetic diversity of native sheep and poultry breeds, the project aims to build production systems that are not only more sustainable and productive but also resilient to a warming climate. This approach requires the seamless integration of phenotypic, genotypic, and environmental data to safeguard animal biodiversity while securing local food systems.

1.2 Mission and Training Goals

The training program was designed to align with the specific goals outlined in the project's Work Packages (WPs). Beyond mere technical instruction, these sessions served as a bridge between the project's scientific ambitions and their practical implementation, fostering a spirit of cooperation across the Mediterranean basin.

The primary objectives of the training included:

- **Technical Implementation:** Providing researchers with the tools to conduct high-level experimental and analytical tasks, particularly in the fields of advanced phenotyping and genotyping.
- **Scientific Data Mastery:** Supporting the generation, processing, and interpretation of complex datasets to ensure they directly contribute to the project's overarching scientific questions.
- **Cross-Border Knowledge Exchange:** Facilitating a meaningful transfer of expertise between partner institutions from the Northern Mediterranean (Italy and France) and the Southern Mediterranean (Algeria, Morocco, and Tunisia).

- **Capacity Building:** Fulfilling a core pillar of the PRIMA program by empowering researchers through hands-on training and direct immersion in international research activities.

1.3 A Collaborative Consortium

Reflecting the "multi-actor" approach essential to modern research, the trainings were coordinated by the Università Cattolica del Sacro Cuore (Italy). The program benefited from the diverse expertise of a high-level consortium, including INRAE (France), INRA-Morocco, the National Agronomic Institute of Tunisia (INAT), and the University of Science and Technology of Oran – USTO (Algeria).

This international framework provided a unique interdisciplinary environment, ensuring that the training was not only scientifically rigorous but also culturally and contextually relevant to the specific needs of the Mediterranean region.

Training 1

SCALA-MEDI Summer School: Genomics, Biodiversity and Breeding for Sustainable Agriculture

1. Context and Scientific Framework

Held on September 13–14, 2022, at the Università Cattolica del Sacro Cuore (UNICATT) in Piacenza, Italy, this training was organized in close collaboration with the Euro-Mediterranean Center on Climate Change (CMCC). As a cornerstone of the SCALA-MEDI project's educational mission, the summer school provided an advanced platform for exploring the intersection of modern genomics, biodiversity conservation, and climate change adaptation. The program was delivered by an international panel of distinguished experts from institutions such as UNICATT, CMCC, INRA-Morocco, CNR-IBBA, and AGRIS Sardegna, ensuring a multi-disciplinary approach to the genomic challenges facing Mediterranean livestock.

2. Educational Objectives and Curricular Scope

The overarching goal of the session was to equip participants with a robust "genomic toolkit" to address the complexities of sustainable agriculture. The curriculum was designed to achieve several high-level competencies:

- Genomic Mapping and Diversity: Participants explored current innovations in sequencing and the use of molecular markers to measure and analyze genetic diversity within and between local breeds.
- Landscape Genomics and Selection Signatures: A key focus was placed on identifying genomic regions that have been shaped by natural selection, allowing researchers to understand how animals adapt to specific Mediterranean environments.
- Predictive Analytics (GWAS and Breeding): The training demystified the principles of Genome-Wide Association Studies (GWAS) and demonstrated how genomic information can be integrated into practical breeding programs to enhance desirable traits without compromising genetic health.

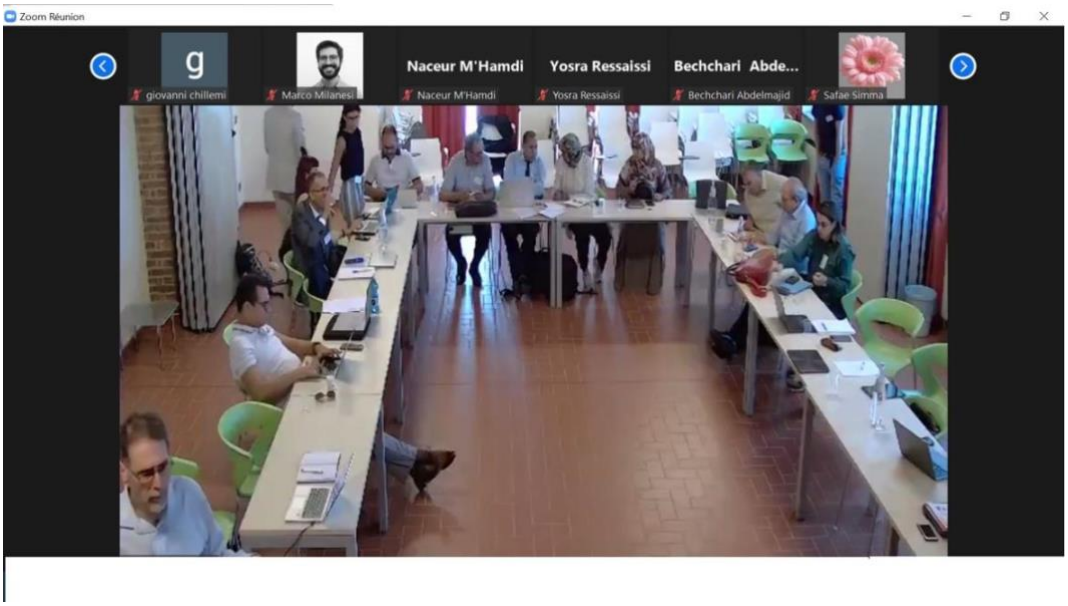
3. Implementation and Methodology

The summer school followed a logical progression from theory to application over two intensive days:

- Day 1: Foundations and Mechanisms: The initial sessions covered the state-of-the-art in genomic technologies, selection signals, and the interactions between genotypes and the environment. These theoretical lectures were grounded in Mediterranean case studies, providing real-world context to complex genetic concepts.
- Day 2: Application and Analysis: The focus shifted toward conservation strategies and the practicalities of modern breeding. Crucially, the afternoon was dedicated to hands-on practical sessions, where participants moved from the classroom to the workstation to analyze genomic datasets and interpret results under the guidance of senior trainers.

4. Outcomes and Collaborative Impact

The pedagogical approach, which blended interactive lectures with supervised data analysis, ensured that participants gained both conceptual depth and technical dexterity. By the conclusion of the school, the cohort had acquired the ability to link genomic data with the broader objectives of sustainability and conservation. Beyond the technical skills, the SCALA-MEDI Summer School served as a vital networking event, fostering long-term Euro-Mediterranean scientific collaboration. This capacity-building effort ensures that the next generation of researchers is prepared to leverage genomics to build more resilient animal production systems across the Mediterranean basin.



Training 2

Practical on cryopreservation – INRAE

1. Introduction

From May 22nd to May 26th, 2023, a specialized intensive training program was conducted at the Laboratory of Reproductive Physiology and Behavior (PRC), located within the INRAE Research Center in Nouzilly, France. This session, titled *"Advanced Training on Rooster Semen Preservation and Preparation of Primordial Germ Cells in Poultry,"* represents a vital technical pillar of the SCALA-MEDI project. By gathering researchers and technical experts specialized in cryobiology and germ cell technologies, the program aimed to bridge the gap between avian reproductive theory and the practical requirements of genetic resource conservation. The overarching mission was to empower participants with the biotechnological tools necessary to safeguard poultry biodiversity, ensuring that the unique genetic traits of Mediterranean local breeds can be preserved *ex situ* and utilized in sustainable breeding programs.

1. Objectives and Technical Scope

The pedagogical framework of the week was designed to transition from fundamental biological principles to high-level laboratory mastery. The specific objectives were twofold:

- **Semen Cryoconservation:** Participants were trained in the full workflow of avian germplasm management—ranging from the initial collection and rigorous evaluation of rooster semen to the application of complex freezing and storage protocols. This is essential for maintaining "biological insurance" for avian lines that are at risk of extinction or are central to climate-resilient breeding.
- **Germ Cell Technologies:** A significant focus was placed on Primordial Germ Cells **(PGCs)**, the embryonic precursors to gametes. Participants mastered the delicate protocols for isolating and handling PGCs from chicken embryos. Because traditional embryo cryopreservation is not feasible in poultry, PGC technology offers the most promising avenue for regenerating entire avian lines from cryobanked material.

2. Training Methodology and Implementation

The training utilized a highly interactive, "hands-on" methodology, alternating between theoretical lectures and supervised laboratory demonstrations. Over the five-day period, the curriculum was divided into:

- **Theoretical Foundations:** Detailed explorations of avian reproductive physiology, the biophysics of semen biology during the freezing process, and the developmental pathways of germ cells.
- **Practical Laboratory Immersion:** Extensive technical work conducted under controlled experimental conditions. Participants performed hands-on exercises in semen quality assessment and the intricate preparation of PGCs, ensuring they acquired the technical "dexterity" required for high-precision laboratory work.

3. Synthesis of Acquired Expertise

By the conclusion of the program, participants had successfully integrated complex biotechnological concepts with practical skills. The primary outcomes included a specialized expertise in handling and preparing primordial germ cells and a refined mastery of semen conservation techniques. More importantly, the training provided a deeper understanding of how these reproductive technologies serve as the backbone of genetic resource management. By securing these skills, the SCALA-MEDI consortium strengthens its capacity to preserve the biodiversity of the Mediterranean poultry sector, ensuring that local breeds remain a viable and productive resource in the face of environmental change.





Training 3

Analysis of the Genomic Revolution in Livestock Selection

1. Introduction

The landscape of bovine genetic improvement underwent a monumental shift in 2010 with the introduction of genomic selection. This training session, held on June 7, 2023, at ESAM Mateur and led by Professor Michèle Tixier-Boichard (INRAE, GABI unit), explored the transition from traditional pedigree-based methods to the use of high-resolution molecular markers. By leveraging INRAE's extensive expertise, the session illustrated how genomics has fundamentally restructured the management of animal populations.

A central theme of the training was the complexity of Genotype-by-Environment (GxE) interactions. There is often a disconnect between a "superior" animal in a controlled, high-input environment (SELENV) and its actual performance in a real-world Mediterranean production setting (PRODENV).

- The Scientific Challenge: High performance in optimized conditions does not always translate to resilience in the field, where climate and nutritional stresses are prevalent.
- The Genomic Solution: Molecular data allows researchers to identify specific alleles associated with adaptation. By breeding for these "resilience markers," we can ensure that selected lines are specifically tailored to thrive in their intended production environments.

Genomic selection is evolving beyond mere productivity; it is becoming a holistic tool for **sustainable population management**.

- Broadening Selection Criteria: Modern programs are now prioritizing traits linked to animal welfare, disease resistance, and environmental resilience.
- Precision Inbreeding Management: Molecular tools allow for a more nuanced control of genetic relationships, enabling selection while minimizing the loss of rare alleles.
- Mass Customization: The long-term vision is the 100% genotyping of herds, transforming genomic data into a routine management tool for every individual animal.

Conclusion

The insights gained from this session highlight that genomic selection is far more than a technical upgrade; it represents a total restructuring of the livestock industry. By providing the flexibility to meet both economic demands and the pressing challenges of climate change, genomics stands as a cornerstone for the future of sustainable Mediterranean animal production.




Training 4

Practicals at the ESAM Chicken and Sheep Experimental Station

1. Introduction

Beyond genetic and epigenetic frameworks, the advancement of livestock populations is fundamentally dependent on the precision and scale of phenotyping. To address this, a specialized training session was conducted on June 7–8, 2023, at the ESAM experimental station in Mateur, led by the project partner Nature 4. This module focused on the integration of connected sensors for the real-time behavioral and physiological monitoring of animals, bridging the gap between theoretical data and field application. The program was structured to provide a comprehensive transition from conceptual foundations to hands-on practice, beginning with a theoretical overview followed by a practical pilot study using an AnimalCollar system on sheep to track environmental interactions. The second day extended this application to avian models, where participants conducted practical trials on chickens to evaluate the efficacy of internal sensors. By mastering these digital tools, the training emphasized the role of high-throughput phenotyping in capturing the complex traits necessary for building more resilient and adaptive production systems within the SCALA-MEDI project.

2. Outcomes: Strategic Insights into Sensor Performance and Breeding Integration

The practical implementation of these monitoring technologies provided critical insights into the robustness and field-applicability of digital phenotyping under real-world experimental conditions. A primary technical finding was the significant impact of technological performance relative to sensor placement; specifically, the choice of the RFID microchip's implantation site was found to be a decisive factor in signal stability and the precision of internal thermal readings. This highlights that the successful deployment of "Internet of Things" (IoT) tools in livestock requires not just advanced hardware, but also a biologically optimized protocol to ensure data integrity. From a broader perspective, these findings have profound applications in animal breeding, as they provide the high-throughput, objective phenotyping data necessary to refine genomic selection models. By capturing granular responses to environmental shifts, these sensors enable researchers to identify "resilient" phenotypes, individuals that maintain physiological stability despite heat stress, thereby

providing the essential data layers needed to develop climate-resilient lineages within the SCALA-MEDI framework.

4. Conclusion: Synchronizing Technology and Biology for Sustainable Livestock

The integration of sensor-based technologies, often referred to as the Internet of Things (IoT), represents a transformative expansion of the technological toolbox available to modern breeders and geneticists. Rather than replacing traditional methods, these digital tools act as a sophisticated bridge that connects real-time biological reality with genomic theory. By providing a continuous stream of data on animal health, physiological states, and behavioral patterns, IoT devices offer an unprecedented window into the lived experience of the animal within its environment.

This real-time transparency is essential for deciphering the complex Genotype-by-Environment (GxE) interactions that define the SCALA-MEDI project. When we can measure exactly how an animal responds to a heatwave or a change in forage quality, we move closer to identifying the specific genetic backgrounds that confer true resilience. Ultimately, the synergy between high-throughput phenotyping and genomic selection supports the creation of livestock systems that are not only more productive but are also ethically aligned with animal welfare standards and ecologically adapted to the evolving Mediterranean climate.



Training 5

Study of Epigenetic Mechanisms and Sequencing Data Analysis

1. Introduction to Epigenetics: Deciphering the "Software" of Heredity

Epigenetics represents one of the most compelling frontiers in modern biology, focusing on the study of heritable and stable changes in gene expression that occur without altering the underlying DNA sequence. While genomics provides the "hardware" or the blueprint of an organism,

epigenetics serves as the biological "software," determining how that blueprint is read and executed. This field is fundamentally crucial because it provides the molecular explanation for how cells with an identical genome can differentiate into vast, specialized types—such as neurons or muscle cells—and, perhaps more importantly for the SCALA-MEDI project, how external environmental factors can leave a lasting imprint on an animal's phenotype. The primary objective of this module was to demystify these complex regulatory mechanisms and to master the specialized sequencing and bioinformatics workflows used to track these changes in livestock populations.

2. Materials and Methods: The Epigenetic Workflow and Application

The technical training provided a comprehensive immersion into the entire epigenetic workflow, bridging the gap between raw biological samples and actionable scientific insights. This process was divided into two critical phases: the wet-lab preparation of genetic libraries and the dry-lab bioinformatic processing required to decode environmental signatures.

The first stage of the workflow focused on the precise isolation and chemical modification of nucleic acids to "freeze" the epigenetic state of the cell at the time of sampling.

Nucleic Acid Isolation: Utilizing high-precision commercial kits, we practiced the extraction of total DNA and RNA, ensuring the purity required for sensitive downstream applications.

The Bisulfite Conversion Principle: To distinguish between methylated and unmethylated cytosines, we utilized Bisulfite Sequencing (BS-seq). In this chemical process, sodium bisulfite converts unmethylated cytosines into uracils (which become thymines during PCR), while methylated cytosines remain protected and unchanged.

Strategic Sequencing Approaches: We compared WGBS (Whole Genome Bisulfite Sequencing), which provides exhaustive coverage of the entire methylome, against RRBS (Reduced Representation Bisulfite Sequencing). The latter targets CpG-dense regions, offering a cost-effective yet statistically powerful alternative for large-scale population studies.

Technological Platforms: The training covered the industry-standard Illumina (NovaSeq/MiSeq) sequencing-by-synthesis method, alongside emerging long-read technologies like Oxford Nanopore and PacBio, which are increasingly vital for mapping complex structural epigenetic variations.

Processing the resulting "Fastq" raw data required a specialized bioinformatic pipeline, executed on the Galaxy platform to ensure reproducibility and transparency:

- Quality Control (FastQC): Initial validation of raw reads to ensure sequencing integrity. Trimming: Using automated tools to strip sequencing adapters and eliminate low-quality bases that could bias the results.
- Specialized Mapping (Bismark): Unlike standard genomic alignment, we utilized Bismark, a dedicated tool designed to align bisulfite-converted DNA by accounting for the C-to-T transitions created during chemical treatment.
- Methylation Calling (MethylDackel): The final step involved extracting precise methylation percentages for every CpG position across the genome, transforming raw reads into a digital map of epigenetic regulation.

The practical application of these techniques revealed how epigenetic diversity serves as a silent driver of animal health and productivity:

- Inter-Breed Diversity: By comparing the CpG methylation landscapes of Angus (*Bos taurus*) and Nellore (*Bos indicus*) cattle, we observed how distinct evolutionary histories have left unique "epimarks" on their respective genomes.
- Biomarkers for Reproduction: The training explored the role of miRNAs in sperm as non-invasive biomarkers. Identifying these small RNA molecules allows for a more nuanced prediction of bull fertility than traditional morphology alone.
- Environmental Adaptation: Perhaps most relevant to the Mediterranean context, we analyzed how DNA methylation facilitates rapid adaptation. These mechanisms allow animals to respond to local stressors—such as fluctuating feed quality or heat—by modulating gene expression without the need for long-term genetic mutations, a key pillar of the resilience sought by the SCALA-MEDI project.

Conclusion

The completion of this training module has provided a high-level mastery of both the fundamental concepts of the epigenome and the sophisticated technological ecosystems required for its analysis. Far from being a mere academic exercise, the study of epigenetics offers a powerful lens through which we can view the future of the livestock sector. By deciphering how external environmental pressures, ranging from nutritional shifts to thermal stress, leave a molecular "signature" on the

genome, we gain the ability to predict and improve animal health and productivity with unprecedented precision. These methodologies open vital perspectives for the SCALA-MEDI project, allowing us to move beyond static genetic selection toward a dynamic understanding of gene-environment interactions. Ultimately, this expertise empowers researchers and breeders to develop sustainable management strategies that respect the innate adaptive capacity of local Mediterranean breeds while optimizing their performance in a changing climate.

Training 6

New breeding tools for local breeds (A. Stella)

1. Introduction and Objectives Led by Alessandra Stella in Paris in 02 July 2025, this training module serves as a strategic extension of the SCALA-MEDI project's mission to modernize the management of local genetic resources. The primary objective was to introduce and master innovative breeding tools specifically designed for local Mediterranean sheep and poultry breeds. Recognizing that traditional selection methods often overlook the unique challenges of small populations, this session focused on integrating advanced digital tools and algorithms to balance genetic progress with the essential preservation of biodiversity.

2. Technical Scope and Methodologies The curriculum explored cutting-edge software applications for genomic data analysis, shifting the focus toward "resilience-based" selection criteria. The training emphasized several high-level competencies:

- **Precision Inbreeding Management:** Utilizing molecular tools to refine mating strategies, ensuring selection for productivity while strictly minimizing the loss of rare alleles in local populations.
- **Bridging IoT and Selection:** Learning to transform raw data from connected sensors such as physiological and behavioral metrics into actionable breeding values for high-throughput phenotyping.
- **Optimizing Local Adaptation:** Developing customized selection models that account for Genotype-by-Environment (GxE) interactions, ensuring that selected lineages are tailored to thrive in the specific stress-prone environments of the Mediterranean.

3. Synthesis and Collaborative Impact By mastering these new breeding tools, the SCALA-MEDI consortium strengthens its ability to bridge the gap between advanced genomic research and practical field implementation. This module reinforces the project's vision that the future of sustainable animal production relies on "mass customization" and precision breeding. Ultimately, these tools empower researchers to develop livestock systems that are economically viable, ethically aligned with animal welfare, and ecologically resilient to the evolving challenges of climate change.

Training 7

Methods for Phenomics (Jouy-en-Josas, 02 July 2025) (D. Laloé)

1. Introduction

Complementing the genomic and epigenetic modules of the SCALA-MEDI project, this training session focused on the rapidly evolving field of **phenomics**, the systematic study of the physical and biochemical traits of an organism. As animal production faces increasing pressure from climate change, the ability to accurately and efficiently measure how animals interact with their environment has become a critical bottleneck for genetic improvement. This session was designed to provide researchers with a comprehensive understanding of high-throughput phenotyping and its role in building more resilient Mediterranean livestock systems.

2. Objectives

The primary goal of the training was to transition from traditional, manual data collection to automated, digital phenotyping. The curriculum focused on several key areas of expertise:

- **Digital Sensor Integration:** Mastering the use of "Internet of Things" (IoT) devices, such as connected collars and internal RFID microchips, for real-time monitoring of physiological and behavioral patterns.
- **Real-Time Stress Monitoring:** Utilizing high-frequency data to capture granular responses to environmental stressors, such as heatwaves or changes in forage quality, which are essential for identifying "resilient" phenotypes.

- **Data Integrity and Protocol Optimization:** Understanding the biological requirements for sensor deployment, including the critical impact of implantation sites on signal stability and thermal reading precision.
- **Genotype-by-Environment (GxE) Analysis:** Using phenomic data layers to bridge the gap between genomic theory and biological reality, allowing for more accurate selection models.

3. Conclusion

The mastery of phenomics methods represents a transformative expansion of the SCALA-MEDI technological toolbox. By providing a continuous stream of objective data on animal health and adaptability, these methods allow breeders to move beyond static observations toward a dynamic understanding of animal resilience. Ultimately, these phenomic tools ensure that selection programs are not only more productive but also ethically aligned with animal welfare standards and ecologically adapted to the evolving Mediterranean climate.