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Book of Abstracts

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analysis of NW with 6.24 ± 0.09 in BS and 6.47 ± 0.15 in NP sows pointed to significant effects ($p < .05$) of PAR, SF, MF, BR*NSH and BR*MF. Accordingly, differences between the breeds could be ascribed to the higher number of controlled farrowing in population of NP *vs* BS with the best results in LS within a group from 4 to 10 sows per herd.

P003

A methodology for the parentage diagnosis of the Italian Brown breed

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Parentage is a measure of the genetic similarities between two related individuals: it is defined as the possession of genes identical by descent. Since the amount of common genes determines phenotypical similarities, e.g. morphological and production-related features, an accurate parentage test is crucial in the selection process. The advent of genomic analyses has paved the use of SNPs information to accurately investigate parentage. However, the current transition from traditional to genomic selection needs methods able to combine different sources of genetic information. In particular, since genomic information is not available for all animals, there is the practical and economic need to cross-examine parentage of genotyped offspring and parents with microsatellites information only. Two steps are necessary to overcome this issue: firstly, to assign microsatellite information from SNPs data of a genotyped animal and secondly, to use microsatellites for the lineage verification.
The objective of this study was to design a method capable to analyse microsatellites data that validates pedigree information. The data was provided by National Brown Cattle Breeders' Association (ANARB) and was made up of 49,828 cattle with microsatellite information from SNP data, 37,262 cattle with official microsatellite data and a pedigree database with 2,399,305 cattle.
The first stage was to create an algorithm that cross-examined 12 microsatellites per animal along with the microsatellites from the presumed parents, to check the correctness of the pedigree.
The procedure was developed by using the software R, which has permitted to deal with large databases. The conditions used for the parentage diagnosis followed the ISAG protocol and the ICAR guidelines. The accuracy of the method was checked by comparing the results obtained by microsatellite analysis with the official parentage data for the 37,262 animals where official analyses were available. The comparison of the results between the proposed method and the available official verifications led to an accuracy of 96.2%. Consequently, the procedure has allowed more than 12,000 new parentage verifications and the correction of 600 pedigree information.
This procedure is useful for direct verification, without further external laboratory testing, of parentage compatibility when different sources of information are already available: SNPs and microsatellites.

Acknowledgements

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P004

Biodiversity in tench populations of Sicily

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Previous studies on mtDNA revealed the existence of genetic variability in Italian tench populations. With this study we extended the analysis to tench populations of Sicily, with the aim of verifying if the long geographical isolation affected their genetic differentiation.
A total of 72 fin samples were collected from six wild tench populations living in rivers (Alcantara, ALC; Irminio, IRM; Prainito, PRA; Sant'Elia, SEL) or water basins (Cesarò, CES; Santa Ninfa, NIN) located in different areas of Sicily. The PCR-RFLP technique was used to analyse the variability at the same four mtDNA segments considered in the previous studies (ND1, ND6, cyt*b* and D-loop) to make it possible the comparison of all the Italian populations studied so far. MEGA and Arlequin softwares were used for data analysis.
Three haplotypes (H1, H3, H5, according to the nomenclature used in the previous studies) were found in the Sicilian populations: H1 was present in all the populations (frequency: 0.4 ÷ 1.0); H3 was observed in ALC, CES, IRM and PRA, while H5 was found only in PRA. Therefore H1 was confirmed as the most widespread haplotype in tench; H3, exclusive of Italian populations, had been also reported in tench of the Central Italy, suggesting a common phylogenetic history; surprisingly, tench living in Prainito river share the H5 haplotype with fish from Valagola lake (Autonomous Province of Trento, Northern Italy). The more ancestral origin of H3 with respect

to H5 could explain in part its wider distribution. All the haplotypes found belong to the haplogroup A, indicating that the analysed populations derive from the same maternal lineage emerged after expansion from isolated glacial refugia, according to phylogeographic structure common to many freshwater fish. As for the genetic variability, estimated by the haplotype (H) and nucleotide (π) diversity, NIN and SEL were monomorphic, while IRM, PRA and ALC showed a considerable level of variability (H: $0.60 \div 0.67$; π: $0.029 \div 0.037$). In general, the Sicilian populations exhibit a higher haplotype diversity compared to the other Italian populations studied until now (mean H value: 0.36 *vs* 0.26), while show similar values for the mean π value (0.018 *vs* 0.020). Stocking from outside Sicily could have contributed to the high variability of some Sicilian populations.

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P005

Lipofection conditions of CHO-K1 cells with the use of two transposon systems

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This study aimed to elaborate CHO-K1 cells transfection conditions with the use of two transposon systems: piggyBac and Tol2 by lipofection method. Firstly, we optimized transfection conditions for Tol2 transposons. We compared effect of cells density (1×10^5 cells/ml and 5×10^5 cells/ml), amount of lipofectant Xtreme HP DNA (1-8µl) and plasmid constructions: pCMV-Tol2 with pL-miniTol2-OVA5IFN, pCMV-Tol2 with pL-miniTol2-OVA5Egfp and pL-OG-OVAIFNEnh-Egfp (control vector) on viability of cells. To select transfected cells we used G418 at concentration 400µg/ml, which was determined based on antibiotic kill curve. The viability of cells was the marker of transfection efficiency and was examined with the use of flow cytometry FACS Aria after iodide propidium (PI) staining. Secondly, using the most effective conditions of lipofection, we transfected cells with plasmids piggyBac transposons: pCMV-sPBo (Transposagen) with pLPB-NeoOVA5fEIFN and pCMV-sPBo with pLPB-NeoOVA5fEegfp. Significant differences in the viability of transfected CHO-K1 cells were calculated with a three-factor analysis of variance followed by Bonferroni and Games-Howell tests.

The highest transfection efficiency was obtained for concentration 1×10^5 cells/ml. In comparison to control (32%), the highest lipofection efficiency for Tol2 transposon vectors was achieved for cells transfected with 0.5µg pCMV-Tol2 with 1µg pL-miniTol2-OVA5IFN and 0.5µg pCMV-Tol2 with pL-miniTol2-OVA5Egfp and 1µl Xtreme HP DNA (62.0 and 60.1%, respectively). The same conditions of lipofection used to transfect the cells for pCMV-sPBo with pLPB-NeoOVA5fEIFN and pCMV-sPBo with pLPB-NeoOVA5fEegfp resulted in cells viability 76.8 and 77.9%, respectively. The percentage of live cells after transfection with the use of control vector at density 1×10^5 cells/ml and 5×10^5 cells/ml was 67.3% and 36.7%, respectively. The EGFP expression after transfection for 1×10^5 cells/ml and 5×10^5 cells/ml was 32.3 and 11.2%, respectively. These elaborated transfection conditions will be used to transfect chicken primordial germ cells (PGCs) in the next step of our project.

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P006

Genetic variability detected at the (c-type) milk lysozyme encoding gene in donkey

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Lysozyme is known to be a natural antimicrobial agent since it catalyses the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. It inhibites the development of many pathogens bacteria, thus making the milk somewhat selective in regards to the milk bacteria content. Three major distinct types of lysozymes have been identified: chicken-type (c-type), invertebrate-type (i-type), and goose-type (g-type). In particular, there are at least 4 non-stomach lysozyme genes in ruminants (i.e., mammary gland, kidney, trachea, intestinal). Lysozymes in ruminants and equine milk are