Beta-casein A2 variant: is the frequency changing in Holstein cattle?

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Oocyte recovery rate is a main issue in genetic improvement since it allows to increase the number of embryos obtained from the same dam with artificial reproductive technologies thus facilitating genetic selection also through the maternal line.
The number of oocytes recovered is highly correlated with the amount of follicles recruited per follicle wave, an event regulated by various genes. In particular, *GDF9* and *BMP15* act synergistically through the regulation of several key granulosa cell enzymes that are essential for normal ovulation, fertilisation, and female reproduction. Single nucleotide polymorphisms (SNPs) in both genes have been related to an increased number of mature oocytes per cycle and to superovulation traits in different species.
The aim of this project is to look for SNPs that may be related to oocyte recovery rate from ovaries of Mediterranean Italian River buffaloes. To the purpose, we first analysed the genes *GDF9* and *BMP15*. In particular, the two exons and the intron of *GDF9* and the two exons of *BMP15* have been fully sequenced in the animals selected for this study.
GDF9 has been sequenced in 50 buffaloes: 47 females with different oocyte recovery rates, 2 hypofertile females and 1 healthy bull.
BMP15 has been sequenced in 30 buffaloes: 27 females with different oocyte recovery rates, 2 hypofertile females and 1 healthy bull.
All buffaloes were unrelated and reared in different farms of the Campania Region.
None of the genotyped animals showed SNPs in the analysed sequences of *GDF9* and *BMP15* suggesting that in Mediterranean Italian River Buffalo other genes may have a main role in oocyte recovery.

P027

Molecular traceability of food products obtained from a local goat population in Umbria region

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The safeguard of local genetic resources is important to avoid genetic erosion and to preserve their cultural and historical value. Among the different tools for breed traceability, DNA-based methods are widely recognised as the most powerful, because they can be applied at any stage of the production chain. So far, breed allocation based on molecular markers has been profitably applied for meat traceability in different animal species, including cattle and pigs, while in sheep and goat it is still difficult to implement for the high cost of genotyping relative to the economic value of the single animal, especially in minor breeds.
This work aims to develop simple screening procedures for the implementation of molecular traceability protocols along with the market and food chain for a typical genetic resource of the Umbria region: 'Capra della Valnerina'. This genetic type, also known by names that refer to the characteristic two white facial lists, is a local population reared primarily in Valnerina. For this study, blood samples were collected from 24 animals belonging to 9 different farms; 20 samples of two cosmopolitan breeds (Camosciata delle Alpi and Saanen) were included as controls. A panel of 16 SSR markers (selected from the list of recommended markers for genotyping analyses in goat breeds – FAO/ISAG) was used with the objective to select a minimum number of markers capable to reliably identify the local population from cosmopolitan ones. A Discriminant Analysis of Principal Component (DAPC) was carried out with the method implemented in the ADEGENET package within the statistical package R version 3.3.2, using 16, 10, and 7 microsatellite markers. DAPC was conducted without *a posteriori* group assignments by inferring the most likely number of genetic clusters (K) using the *find.clusters* function. The scatterplot of the first two components of the Discriminant Analysis showed that the local goat is clearly distinguishable from the cosmopolitan breeds using a minimum of 7 markers.
On the base of our results, actions directed to preserving genetic distinctiveness of 'Capra della Valnerina' can be undertaken using the molecular markers identified in this work; simple, low-cost molecular tests for traceability can be easily implemented, by using a very small SSR panel.

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P028

Beta-casein A2 variant: is the frequency changing in Holstein cattle?

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Since the detection of bovine β-lactoglobulin genetic variants in 1957, genetic variation has been identified and characterised in all milk proteins. The effects of several variants as well as of

casein cluster haplotypes on production traits have also been investigated, but their use in selection schemes was rarely exploited. In the late 1990s, after epidemiological evidence in New Zealand indicated that beta-casein CSN2*A1 milk consumption incremented mortality from ischaemic heart disease, a company, A2 Corporation, started marketing milk produced only by CSN2*A2A2 individuals. Bioactive peptide with opioid properties β-casomorphin-7 (BCM7), one of the several peptides released during milk protein digestion, was suspected to be the risk factor in this human disease. Furthermore, BCM7 has been suggested to be involved in sudden infant death syndrome, neurological disorders (e.g. autism and schizophrenia) and milk allergy. CSN2*A2 and CSN2*A3 differ from CSN2*A1, CSN2*B and CSN2*C for the substitution of a His with a Pro at position 67 in the mature protein. The presence of His67 determines the enzymatic cleavage, which releases BCM7 in the three variants. An European Food Safety Authority report (EFSA) could not establish a cause-effect relationship BCM7 or related peptides and the aetiology of the abovementioned diseases. More recent publications pointed at possible intolerances and gastrointestinal effects of BCM7, associating CSN2*A1A1 milk consumption to delayed intestinal transit, looser stool consistency, and intestinal inflammation, and even if the number of studies is still limited, CSN2*A2A2 milk is now marketed in various countries. We analysed data from Illumina beadchip of 214210 (including directly genotyped and imputed SNPs) Holstein cattle bred in different countries born between 1952 and 2017 and found that the CSN2*A2 frequency remained substantially unchanged in the 1980s (44.75%) and in the 1990s (45.78%), but it increased substantially in the 2000s (50.46%) and it is still on the rise (53.94% in the 2010s and 54.21% in this decade). A similar trend was also observed in the 51871 Italian Holsteins analysed: 38.92% in the 1990s, 49.03% in the 2000s, 53.07% in the 2010s and 55.72% in individuals born since 2010. These data indicate an ongoing selection of bulls and cows carrying only the CSN2*A2 variant, both in Italy and worldwide.

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P029

Phenotypic traits correlation for hygienic behaviour in *Apis mellifera ligustica*

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Beekeeping is a farming activity with huge economic importance beneficial effects on rural development and ecological balance. Thanks to national beekeeping register, a total of over 50,000 beekeepers and over 1.1 millions of hives were recorded in 2017; it was estimated that the beekeeping sector is worth over 2 billion euros. However currently, more and more bees are dying, because of the extensive pesticides use and numerous diseases.
Honey bee (*Apis mellifera*) hygienic behaviour is performed by single bees in a colony and it is a specific mechanism of resistance against a number of important pathogens, including *Ascosphaera apis* (which induces chalkbrood disease), *Paenibacillus larvae* (which causes American Foulbrood) and the parasitic mite *Varroa destructor*. The hygienic activity usually involves uncapping behaviour of the diseased or damaged brood cells and removing behaviour that involves the removal of the pupae from the damaged cells.
The aim of the study was to characterise and pick out *Apis mellifera* colonies according to their hygienic behaviour first and then to their propolis and honey productions, and to their grooming behaviour. We evaluated a total of 50 colonies, among the most productive and strong ones on almost 900 colonies. Colony's strength was assessed by counting the number of frames with brood and frames covered by bees within the hives. The hygienic behaviour was carried out according to the liquid nitrogen-killed brood test (LNKB): a small portion of capped brood was frozen using liquid nitrogen and then returned to the colony. Initially, we selected frame to be tested, looking at frame with the best brood pattern. Twenty-four hours and forty-eight hours later the freeze-killed portion was checked to see how much dead brood the colony removed. The normal distribution of traits under investigation was verified by Shapiro–Wilk test. Pearson correlation was performed to explore the relationship among the different traits. All the statistical analyses were conducted in R environment. Overall hygienic behaviour highlighted suggestive trends with productive traits (i.e.: propolis yield). In particular, 18 colonies showed a relevant hygienic behaviour associated with a higher attitude in grooming behaviour. Further studies are needed to investigate the biological bases of our finding.

P030

Multivariate factor analysis of milk fatty acids profile for GWAS analysis in Comisana sheep breed

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