

This study highlights that *S. boulardii* is a valid probiotic, useful for improving intestinal eubiosis conditions and the well-being of healthy dogs in breeding conditions.

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### Runs of Homozygosity in 21 Italian dog populations

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The Italian peninsula is characterized by a high level of genetic diversity in all its indigenous domesticated animals, with the dog species being no exception to this. We characterized the length, abundance, distribution and evolution of the Run Of Homozygosity (ROHs) and genomic inbreeding (FROH) of 379 dogs of 21 Italian indigenous breeds and populations. Our aim is to provide insight in the homozygosity and inbreeding of the Italian canine populations. We estimated ROHs on a high density (230k) Dog SNP chip using the PLINK software considering ROH of length  $\geq 1$  Mb, in sliding window of 50 SNP with no heterozygous allowed, and a maximum gap of 0.1 Mb between markers. We calculated FROH as the proportion of the autosomal genome covered by ROHs. Lastly, to better describe the ROH distribution in the different populations, we subdivided them in five length classes: 1–2, 2–4, 4–8, 8–16, >16 Mb.

We observed a great variability in FROH values within and across the populations considered. For instance, the mean population FROH goes from the very high values of Lupo Italiano breed (0.40) and Neapolitan Mastiff (0.35) to a minimum of 0.05 in the Oropa shepherd dog, while the population with the wider range of internal variation is the Bergamasco shepherd dog (from 0.03 to 0.40). The ROHs length classes are well balanced in almost all breeds, with a prevalence of ROHs belonging to the classes 2–4 and 4–8 Mb. We also found a very low presence of extremely long ROHs (>16), apart from the Cirneco dell'Etna and the Mannara dog. Characterizing ROHs distribution and evolution could be a useful genomic tool to better understand today's structure and the past history of small dog populations. This could be applied to monitor and safeguard the conservation of the Italian canine diversity. Using ROHs information, breeders' associations could evaluate more efficiently the evolution of homozygosity level, the inbreeding within and across breeds and discover regions that host gene under selection for positive as well as negative traits. At the same

time, single breeders could improve the management of reproduction with a more accurate choice of the animals mating.

#### Acknowledgements

The authors are grateful to breeders, Prof. M. Polli, Prof. D. Bigi, Prof. R. Cocco for biological samples and Dr. E. A. Ostrander for genotypes.

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### Fonni's dog: genetic variability and relationship with other breeds

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The Fonni's dog is an ancient Sardinian breed of livestock and property guardian dogs, known for its longevity, loyalty, and strong protective instinct that even made it suitable for use as war dog in the past. Despite not being officially recognised as such, different genomic studies have proven the Fonni's dog to be a full-fledged breed, a remarkable achievement considering the selection based only on behaviour. Fonni's dogs, in fact, show a considerable variability in morphology and physical appearance: the double layered coat is defined as coarse 'goat' hair with a woolly undercoat, but shorthaired dogs are admitted too; coat colours range from black to ash to honey; a natural lack of tail can be present. Nevertheless, this breed keeps some peculiar features, like the typical 'monkey face', with amber frontal eyes and an intense and unfriendly expression. This study aimed to assess the genomic background of Fonni's dogs. We sampled and genotyped 30 Fonni's dogs and 380 subjects of other 25 Italian and foreign breeds of shepherd dogs, hunting dogs, sighthounds and molossers using the Canine 230K SNPChip. All the animals have been photographed, underwent to morphological evaluation, and were judged to be eligible for registration as Fonni's dogs. A quality check was performed on genomic data with standard thresholds. The genetic background of the breeds was evaluated with ADMIXTURE 1.3 and the individuals' probability to be assigned to each cluster (Q-score) was calculated.

Quality check left 379 dogs (30 Fonni's dogs) and 120,853 SNPs. Mean  $\pm$  standard deviation of Fonni's dogs' Q-score for their own cluster was  $62 \pm 22\%$ , ranging from 19% to 100%. Particularly, it was  $\leq 25\%$  in 1 dog,  $>25\text{--}50\%$  in 10 dogs,  $>50\text{--}75\%$  in 11 dogs, and  $>75\%$  in 8 dogs. The most represented secondary breeds