

**CONSERVATION GENETICS OF THE EUROPEAN WILDCAT  
(*FELIS SILVESTRIS SILVESTRIS*)**

**GENETICA DELLA CONSERVAZIONE DEL GATTO SELVATICO  
(*FELIS SILVESTRIS SILVESTRIS*)**

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**Abstract.** Introgression of domestic cat genes and habitat fragmentation are the main conservation threats for the European wildcat (*Felis silvestris silvestris*). Highly informative molecular markers combined with advanced statistical approaches were recently used to improve our knowledge on the evolution of the European wildcat populations in Europe and their conservation status. Here we showed some of the results obtained.

**Riassunto.** L'introgressione di geni di gatto domestico e la frammentazione dell'habitat sono le principali minacce per la conservazione del gatto selvatico europeo (*Felis silvestris silvestris*). Alcuni marcatori molecolari altamente informativi, combinati con approcci statistici avanzati, sono stati recentemente utilizzati per migliorare le nostre conoscenze sull'evoluzione delle popolazioni di gatto selvatico europeo in Europa e sul loro stato di conservazione. Il lavoro riporta alcuni dei risultati ottenuti.

The European wildcat *Felis silvestris silvestris* is a medium-sized carnivore widely spread across Europe (PIERPAOLI *et al.* 2003; O'BRIEN *et al.* 2008; OLIVEIRA *et al.* 2008a,b; HERTWIG *et al.* 2009; MATTUCCI *et al.* 2016) in fragmented populations both at regional and local scales (MATTUCCI *et al.* 2016) resulting from hundreds of years of intense anthropogenic persecution and from the loss of suitable habitat (STAHL & ARTOIS 1995; YAMAGUCHI *et al.* 2015). Although there is recent evidence of increasing population sizes and natural recolonization of the species' historic range in at least some regions (STEYER *et al.* 2016; NUSSBERGER *et al.* 2018; VELLI *et al.* 2015; GAVAGNIN *et al.* 2018), the European wildcat is legally protected in Europe (EC 2015) both under the Bern Convention and the European Habitats Directive, and is listed as "least concern" in the IUCN red list.

Main conservation threats include habitat loss and fragmentation, road mortality, persecution and crossbreeding with free-ranging domestic cats (KLAR *et al.* 2008, 2009; LOZANO & MALO 2012; YAMAGUCHI *et al.* 2015).

The domesticated form originally derived from *Felis silvestris libyca* populations inhabiting the Near East/North Africa which gradually spread around the world following the human-mediated dispersal routes (DRISCOLL *et al.* 2007; OTTONI *et al.* 2017).

The widespread diffusion of stray or feral cat populations, often living in much higher density than

wildcats (SUNQUIST & SUNQUIST 2002) and the full fertility of their hybrid offspring (RAGNI 1993) likely promoted reproductive interaction between the two subspecies, increasing the risk of introgression of domestic alleles into the wildcat genome (RANDI *et al.* 2001; YAMAGUCHI *et al.* 2015).

Even if recent studies documented cases of beneficial introgression of domestic mutations in wild populations (COULSON *et al.* 2011; GROSSEN *et al.* 2014), hybridization between free-ranging domestic animals and their wild conspecifics may spread artificially-selected maladaptive variants causing fitness declines, outbreeding depression and gradual alterations of locally adapted gene complexes, thus increasing the risk of extinction of wild populations or entire species (RANDI 2008; RHYMER & SIMBERLOFF 1996; TUREK *et al.* 2013; TODESCO *et al.* 2016).

Putative wild  $\times$  domestic cat hybrids were detected in most regions where hybridization was investigated, but the degree of introgression varied considerably (PIERPAOLI *et al.* 2003; LECIS *et al.* 2006; OLIVEIRA *et al.* 2008a,b; RANDI 2008; SAY *et al.* 2012; NUSSBERGER *et al.* 2014b, 2018; STEYER *et al.* 2018; BEUGIN *et al.* 2020; TIESMEYER *et al.* 2020), leading to a complete hybrid swarm in wild-living cats in Scotland (BEAUMONT *et al.* 2001).

Such geographical heterogeneity in admixture levels might be explained by different environmental conditions and ecological barriers (GIL-SÁNCHEZ *et al.* 2015), or by the choice of markers and sampling design (STEYER *et al.* 2018)

In absence of strong ecological barriers,

hybridization can potentially threaten some of the extant wildcat populations.

Therefore the accurate detection of hybrids and the quantification of introgression in hybridizing populations are needed for developing appropriate wildcat conservation plans and correctly allocate resources for their application (NUSSBERGER *et al.* 2013; OLIVEIRA *et al.* 2015; GIL-SÁNCHEZ *et al.* 2020).

Recently, hybridization has been more reliably assessed using molecular markers, mainly small panels of hypervariable microsatellites (short tandem repeats, STRs) and short mitochondrial DNA (mtDNA) sequences. However, the resolution of hybridization is limited due to the relatively low availability, repeatability between laboratories and technical capacity to analyze microsatellite markers (BEAUMONT *et al.* 2001; DANIELS *et al.* 2001; RANDI *et al.* 2001; LECIS *et al.* 2006; OLIVEIRA *et al.* 2008a,b; HERTWIG *et al.* 2009; O'BRIEN *et al.* 2009; SAY *et al.* 2012; DEVILLARD *et al.* 2014; STEYER *et al.* 2016). Consequently, standardized and more powerful panels of molecular markers are required to lower the risk of underestimating the prevalence of introgressive hybridization in natural wildcat populations.

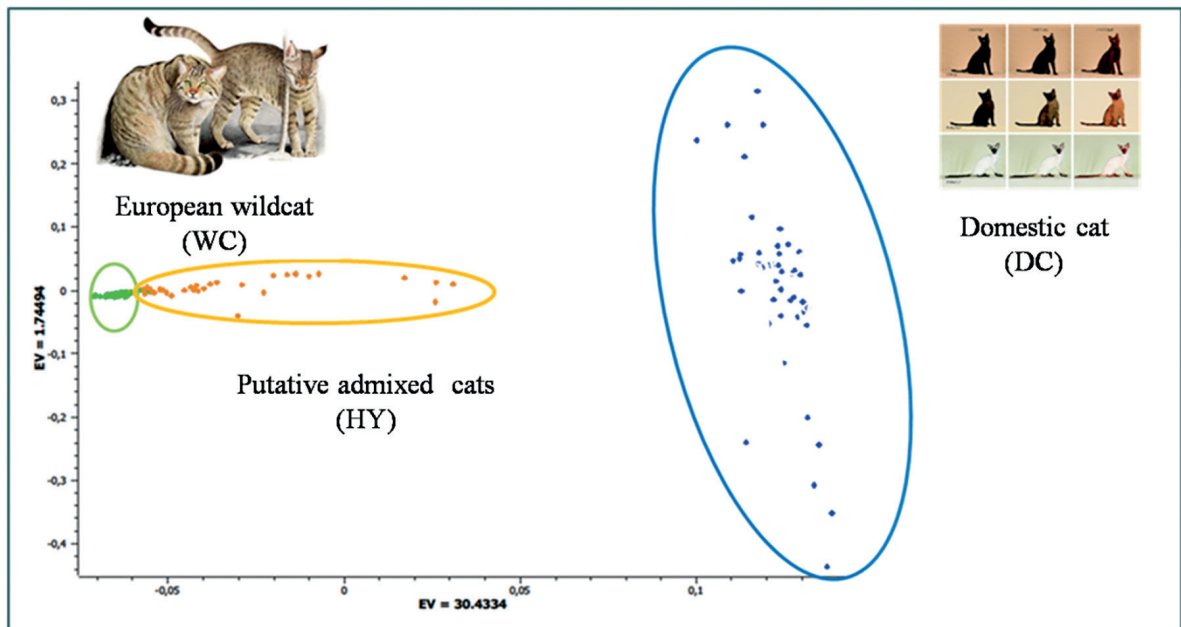
Over the last decades, next-generation sequencing technologies offered the possibility to assemble extensive and cost-effective panels of ancestry-informative markers (AIMs) such as Single Nucleotide Polymorphisms (SNPs), which represent the most widespread source of genome-wide variation (DAVEY *et al.* 2011).

SNPs have been shown to be highly accurate and sensitive in identifying hybrid individuals between wildcats and domestic cats, irrespective of origin and available reference database, and quality of biological samples (OLIVEIRA *et al.* 2015; STEYER *et al.* 2018; MATTUCCI *et al.* 2019; TIESMEYER *et al.* 2020; VON THADEN *et al.* 2020).

In particular, the recently released Illumina Infinium iSelect 63k DNA cat array containing 62,897 variants that are mostly polymorphic within the domestic cats (GANDOLFI *et al.* 2018) offered a suitable molecular tool to further investigate the ancestry of European wildcat populations in conservation and monitoring projects (GANDOLFI *et al.* 2018).

Recent studies showed how the employment of thousands of markers might help to unveil previously undetectable backcrosses (older than two–three generations in the past), estimate the timing from the admixture events (HOHENLOHE *et al.* 2013; GALAVERNI *et al.* 2017), and allow researchers to better understand the dynamics and consequences of anthropogenic hybridization, helping to face specific management and conservation issues (MCFARLANE & PEMBERTON 2019).

A wide sampling ( $n = 182$ ) of European wildcats, domestic cats and known or putative admixed cats from a large part of the European wildcat home range distribution ( $n = 10$  European countries) was genotyped with the Illumina Infinium iSelect 63k DNA and analysed by applying



**Fig. 1.** - PC1 versus PC2 results from an exploratory principal component analysis (PCA) computed in SVS on the 57k SNP panel set (after quality pruning procedures) and including domestic cats (blue dots; DC), putatively admixed wildcats (orange dots; HY) and European wildcats (green dots; WC). The two axes are not to scale, in order to better distinguish individuals along PC2.

multivariate, Bayesian and gene-search analysis tools (MATTUCCI *et al.* 2019).

Results of a preliminary genomic screening, based on pairwise  $F_{ST}$  values, multivariate (Fig.1) and assignment tools, showed that wild and domestic cats remain highly differentiated and well-distinguished.

On average, 17% of the analyzed putative admixed wildcats show genomic domestic ancestry which likely originated from hybridization events occurring from 6 to 22 generations before sampling (MATTUCCI *et al.* 2019). The most ancient admixture traces were detected in individuals which had been misclassified as pure in previous microsatellite-based analyses (MATTUCCI *et al.* 2016), confirming the deeper diagnostic power of genomic data in detecting past backcrossing.

The availability of efficient AIMs widely distributed across the entire genome can allow us to identify introgressed linkage blocks hosting candidate genes that may be associated with functional traits that are still unknown (TWYFORD & ENNOS 2012; MCFARLANE & PEMBERTON 2019), further help to unravel historical and contemporary admixture (PAYSEUR & RIESEBERG 2016) by analyzing the distribution of haplotype block lengths (PALAMARA *et al.* 2012; MCFARLANE & PEMBERTON 2019).

Approximately more than 600 coding genes with an excess of wild or domestic ancestry were identified in the admixed wildcats. These genes were significantly enriched for Gene Ontology categories mainly related to social behavior, functional and metabolic adaptive processes (wild-like genes), involved in cognition and neural crest development (domestic-like genes), or associated with immune system functions and lipid metabolism (parental-like genes; MATTUCCI *et al.* 2019)

However, such genes provide just a preliminary insight on the inheritance patterns of domestic and wild ancestry block. Hence, in the future all these data will need to be integrated with information on fitness, survival and breeding rates of the admixed individuals, in order to better understand the adaptive patterns of wild-living admixed individuals.

Genomic ancestry analyses could be reliably applied to unravel dynamics, direction and consequences of anthropogenic hybridization (MCFARLANE & PEMBERTON 2019), helping to face specific management and conservation issues.

However, to design more efficient conservation plans in European wildcats and other hybridizing populations, knowledge of population dynamics, species' distribution, and natural/anthropogenic causes of genetic structure are further needed (BURGMAN *et al.* 1993; LANCIA *et al.* 1994).

Past climate changes, historical evolutionary events and, eventually, more recent anthropogenic pressures shaped the genetic diversity within and among populations (HEWITT 1999) in Europe. In addition, the fragmentation of populations due to habitats loss and alterations, may have eroded the neutral and adaptive genetic diversity, reducing the effective population size and the inter-population connectivity (JOHANSSON *et al.* 2007), and increasing the risk of genetic bottleneck.

Extant populations of European wildcat are fragmented across the continent.

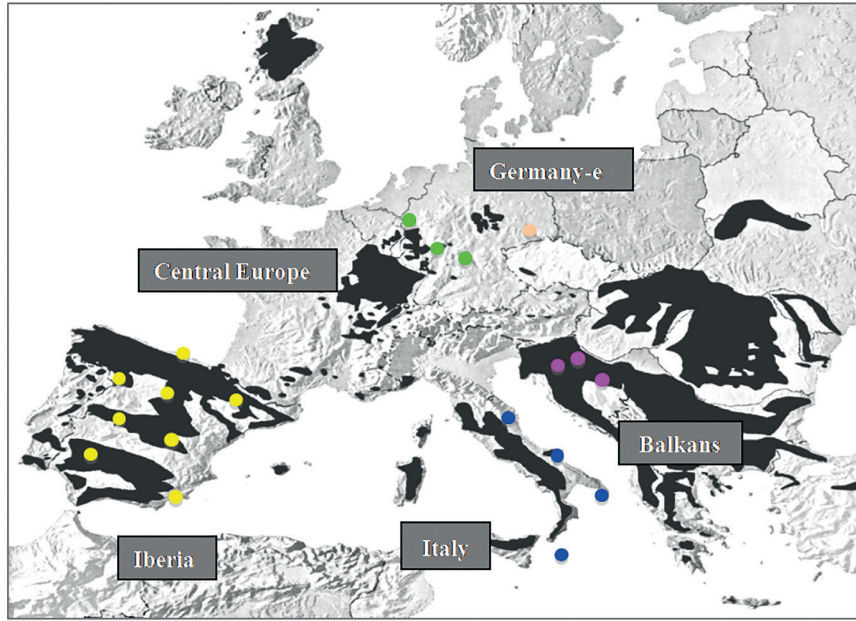
A comprehensive range-wide study of their population structure ( $n = 668$  from 15 European countries) was designed aiming at quantifying their genetic variability, reconstructing patterns of population clustering and fragmentation, and obtaining estimates of population divergence times (MATTUCCI *et al.* 2016).

Results of Model-based structure analyses and non-model multivariate clustering using a panel of 31 domestic cat-derived microsatellites concordantly identified five main biogeographic clusters, respectively: the Iberian Peninsula, central Europe, central Germany, Italian Peninsula and the island of Sicily, and the Dinaric Alps (north-eastern Italy and northern Balkan regions), Fig. 2A-B. Approximate Bayesian Computation simulations suggested a model of late Pleistocene–early Holocene population splittings (from c. 60k to 10k years ago), contemporary to the last Ice Age climatic changes. At a smaller geographic scale, wildcats in peninsular Italy are differentiated into three genetic groups coherently distributed in Sicily, peninsular Italy and the Alps, following a model of LGM isolation and genetic diversification into Mediterranean glacial refuges (HEWITT 1999; Fig. 3A-B).

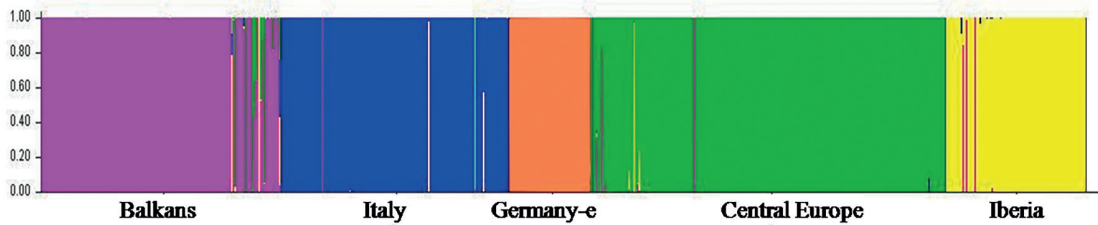
A more recent differentiation have been described in the Maremma area of the central peninsular Italy, where the population might have experienced periods of isolation and local adaptation to a peculiar Mediterranean-type habitat (MATTUCCI *et al.* 2013; Fig. 3C). The studied populations of the European wildcat have maintained relative high levels of genetic diversity, nevertheless some isolated small patches within these groups might have been exposed to the deleterious consequences of genetic drift and inbreeding (MATTUCCI *et al.* 2016).

Hence, the use of powerful computational tools and hypervariable molecular markers proved to be efficient for assessing species' phylogeographic pattern which might be used to improve our understanding, thus helping to identify the most appropriate conservation issues (HICKERSON *et al.* 2010).

A



B



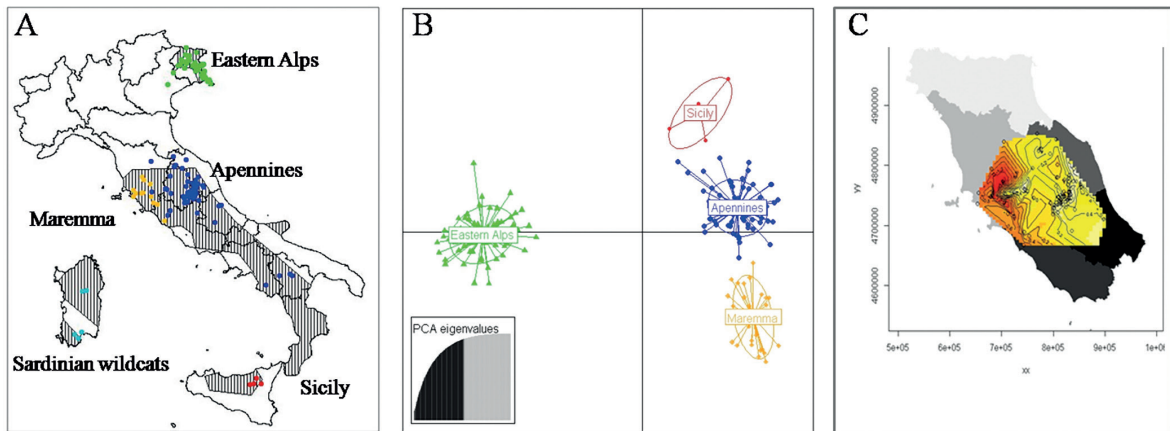
**Fig. 2** - A) Approximate distributions of five European wildcat (*F. s. silvestris*) biogeographic groups identified through multivariate and Bayesian cluster analyses across Europe with 31 autosomal microsatellite loci. The grey areas represent the European wildcats distributions (obtained from © IUCN-International Union for Conservation of Nature- Red List 2015 *Felis silvestris* Downloaded on 09 May 2019; YAMAGUCHI *et al.* 2015). B) Patterns of hierarchical splitting of European wildcat populations assuming  $K = 5$ , the “admixture” and the “F” models in STRUCTURE; PRITCHARD & WEN 2003). Each cat genotype is represented by a vertical bar split in  $K$  colored sections, according to its relative assignment to the  $K$  genetic clusters.

Long-term monitoring programs and legal protection of this endangered species should focus on saving the local functional wildcats (see also DANIELS & CORBETT 2003), for instance, protecting the environmental conditions that favor pure wildcats, guaranteeing natural ecological corridor to interconnect fragmented habitats, mapping the distribution of non-introgressed natural populations, providing information on the health status of wild-living individuals (through the analysis of genes related to illness, immune response, reproductive patterns or adaptation to specific ecological pressure, ALLENDORF *et al.* 2010), and identifying appropriate evolutionary and management units (ESU and MU; FUNK *et al.* 2012) where apply appropriate local management practices.

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**Fig. 3** - A) European wildcats (*F. s. silvestris*) biogeographic distribution identified through Bayesian cluster analyses across Italy with 35 autosomal microsatellite loci: Eastern Alps (in green), Maremma (areas in Tuscany and Lazio Maremma in the western Italian peninsula, in orange), Apennines (areas in Marche, Umbria, Abruzzo, Campania, and Basilicata regions, in blue), and Sicily (in red). The gray areas indicate the approximate wildcat distribution ranges in Italy. African wildcats living in Sardinia (*F. s. lybica*) are further showed in light blue. B) Scatterplot of a Discriminant Analysis of Principal Component (DAPC) obtained with ADEGENET showing genetic distinctions among the four European wildcat subpopulations identified in Italy: The proportion of genetic diversity described by each Principal Component (PCA eigenvalues) is showed in the barplots (see the insert). C) Spatial Principal Component Analysis (sPCA, obtained with ADEGENET) of European wildcats sampled in central Apennines. Individual genotype scores are interpolated and represented by circles. Contour lines quantified the degree of genetic differentiation among individuals.

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