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Genetic evidence reveals extensive wolf-dog hybridisation in peninsular Italy: warnings against ineffective management

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ABSTRACT

Wolf-dog hybridisation (WDH) - a form of anthropogenic introgressive hybridisation - may have occasionally occurred since the times of domestication. More recently, however, despite the ongoing recovery of wolf populations in Europe, the high number of domestic dogs along with marginal ecological conditions may pose increased risks to their genetic integrity. Accordingly, WDH is currently recognised among the highest threats to European wolves, with potential cascading effects through socio-ecological systems. Based on high quality DNA from tissues of 748 wolves retrieved dead from 2020 to 2024, alongside 26 additional samples from 1993 to 2003, we assessed WDH in the peninsular Italian wolf population. Using 23 autosomal STR loci and 5 Y-linked loci for males, we detected a high proportion (46.7 %) of wolves admixed with dogs, 29.5 % of which recent hybrids and 17.2 % older backcross generations. While most of the original hybridisation events likely took place some 9–16 years ago, a few first-generation hybrids in our sample indicate that WDH is currently ongoing. This seriously threatens the genetic integrity not only of the Italian wolf population, but also of the neighbouring wolf populations through dispersal events. The situation we present, which has not been previously reported for any wolf population worldwide, serves as a cautionary example of the potential dynamics of WDH in human-dominated landscapes should the issue remain persistently unaddressed. They also highlight the importance of considering the genetic constituency of wolf populations when evaluating their conservation status, especially in light of the recent downgrading of their protection status across Europe.

1. Introduction

Unlike natural hybridisation, which is a source of diversity and evolutionary novelty (Abbott et al., 2016), human-induced hybridisation (i.e., anthropogenic hybridisation, Allendorf et al., 2001) has become increasingly recognised as a threat to biodiversity (Mallet, 2005). Anthropogenic hybridisation can lead to the loss of genetic integrity and coadapted gene complexes, the spread of maladaptive traits, and outbreeding depression. These effects may contribute to biodiversity loss and, in cases of widespread introgression, could result in genomic extinction through genetic swamping (Allendorf et al., 2001). The interbreeding of domestic species with their wild counterparts represents a significant example of anthropogenic hybridisation (Todesco et al., 2016). Domesticated forms have been artificially selected for a range of morphological, physiological, and behavioural traits that benefit humans, such as tameness and increased productivity. However, these traits can be disruptive if they introgress into the populations of their wild ancestors (Wilkins et al., 2014). While it has been speculated that introgressed traits from domesticated forms could benefit parental species to adapt to human-modified ecosystems (Grossen et al., 2014; Hindrikson and Tammeleht, 2025), the positive selection of introgressed traits may actually accelerate the loss of unique gene combinations that have developed through distinct evolutionary histories (Bohling, 2016).

The dog (*Canis lupus familiaris*) is the first animal domesticated from now-extinct lineages of gray wolves (*Canis lupus*), with domestication dating back approximately 40,000 years, although the exact timing is still debated (Freedman et al., 2014). While hybridisation between

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wolves and dogs (hereafter WDH) might have occurred since domestication (Frantz et al., 2016; Pilot et al., 2018), the widespread presence of dogs in human-dominated landscapes may, under certain conditions, increase the risk of WDH (Randi, 2008). Paradoxically, the recent increase in both the number and range of wolves across Europe (Di Bernardi et al., 2025) may lead to a greater chance of interbreeding with dogs, which are roughly estimated to outnumber wolves by a factor of approximately 1500 to 1 (Gompper, 2014).

Hybridisation primarily occurs between female wolves and male dogs (Godinho et al., 2011; Iacolina et al., 2010), although the reverse, male wolves mating with female dogs, may also happen, albeit rarely (Hindrikson et al., 2012). Contrary to earlier theoretical predictions (Vilà and Wayne, 1999), wolves and dogs do interbreed when given the opportunity, and their fertile offspring (F1s) can easily backcross into the wolf parental populations. Evidence supporting this has been accumulating from several European countries (Kusak et al., 2018; Pacheco et al., 2017; Salvatori et al., 2019; Tirronen and Kuznetsova, 2025). Accordingly, Euro-Asiatic wolves are, on average, significantly more introgressed than their counterparts in East Asia and North America, with Europe exhibiting the highest frequency of admixed individuals (Pilot et al., 2021). As a result, WDH is now regarded as one of the major threats to wolves in Europe (Hindrikson et al., 2017), and there are concerns that the genetic and phenotypic variations arising from dog introgression could negatively impact the behaviour and life history traits of wolves (Randi, 2008), as well as reduce stakeholders' support for wolf conservation (Stronen et al., 2025).

Timely and effective management of WDH is crucial to prevent dog introgression spreading further into European wolf populations. Accordingly, even though the Bern Convention and the Habitats Directive do not make explicit reference to WDH, both contain relevant legal obligations to address the issue (Trouwborst, 2014). Additionally, both the EU Recommendation No. 173/2014, and a guidance document on the application of the strict protection requirements outlined in the EU Habitats Directive, require European countries to address WDH through preventive and mitigation measures (Stronen et al., 2025). Failure to comply with these requirements may negatively affect assessment and conservation of wolf populations (Stronen et al., 2025).

Management of WDH, however, faces daunting technical, social, and political challenges, resulting in a lag in its implementation across Europe (Salvatori et al., 2020). Several factors contribute to the inherent complexity of WDH management, including technical difficulties in detecting introgressed individuals (Stronen et al., 2022a), inadequate population-level monitoring (Salvatori et al., 2019), a lack of consensus among scientists on management responses (Donfrancesco et al., 2019), and an unclear legislative framework at both the national and international levels (Trouwborst, 2014). Yet, demographic modeling warns that management inaction, if factors facilitating WDH continue unchecked, may result in widespread introgression and even genetic swamping in local wolf populations (Santostasi et al., 2020, 2025).

To date, no comprehensive surveys have been conducted to accurately assess and monitor the extent of dog introgression in wolves across Europe, even though the phenomenon has been reported in all nine extant European wolf populations (Salvatori et al., 2020). Nevertheless, independent genetic studies conducted in some European countries during the past decades (reviewed in Dziech, 2021) revealed low prevalence of admixture (i.e., the proportions of admixed individuals in the wolf population). A notable exception has been recently reported in some localities in central and northern Italy where intensive non-invasive genetic surveys revealed levels of dog introgression as high as 50–70 % (Salvatori et al., 2019; Santostasi et al., 2021).

Wolves in Italy faced a significant risk of extinction in the late 1960s, (Zimen and Boitani, 1975). Since then, a series of conservation measures were adopted in response to changing societal values and attitudes towards wolves, including legal protection, habitat conservation, and conflict management which contributed to a recovery of the population (Boitani, 2003; Fabbri et al., 2007). The first, field-documented evidence of wolf-dog hybrids in Italy dates the early 1970s (Boitani, 1986). In the subsequent decades, following the recovery of wolves at the national scale, there was a notable increase in the number of wolves exhibiting unusual morphological traits (Boitani and Ciucci, 1993). However, studies analysing tissue samples from wolves found dead, using both uni- and bi-parental genetic markers, did not reveal significant levels of WDH (i.e., 2–6 %) (Lorenzini et al., 2014; Lucchini et al., 2004; Randi and Lucchini, 2002; Verardi et al., 2006). It is likely that, alongside the expansion of the wolf range across Italy, WDH was indeed spreading during that period (Galaverni et al., 2017). However, those first surveys, possibly constrained by limited diagnostic markers and inadequate spatial and temporal sampling, lacked the statistical power to detect admixed individuals and their distribution at local, regional, and national scales (Ciucci, 2012). Subsequent studies, using more informative sets of genetic markers, supported this view (e.g., Caniglia et al., 2013, 2014; Randi et al., 2014). Furthermore, based on genome-wide data, Galaverni et al. (2017) confirmed that admixed individuals were spread across the peninsular Italian wolf population and that recurrent hybridisation and deep introgression had most likely been occurring since the time of population recovery.

Excluding two pilot projects co-funded by the EU Life Program (i.e., Ibriwolf, LIFE10-NAT-IT-000265; Mirco Lupo, LIFE13-NAT-IT-000728), and a one-year management project conducted by the Toscana region (G. Romeo, personal communication), no comprehensive management strategy has been implemented in Italy to prevent and mitigate WDH at the national scale. In this study, based on a particularly large number of wolves systematically collected through the national network of Istituti Zooprofilattici Sperimentali (IZSs), we investigated for the first time the current extent of WDH in peninsular Italy. In particular, we distinguished non-admixed wolves, recent hybrids (i.e., F1s, F2s, and admixed individuals of the first two generations of backcross), and introgressed individuals (i.e., third and older generations of backcrosses).

The main aims of our study were to: i) estimate the proportion of individuals with dog ancestry (i.e., prevalence) in the putative wolves sampled throughout the Italian peninsula from 2020 to 2024. As this is the first WDH survey at the scale of the peninsular wolf population, the results obtained are useful to evaluate the effectiveness of past and current WDH management in Italy; ii) assess whether recent hybrids and introgressed wolves were spatially structured or evenly distributed across regional sampling areas. Whereas the former would indicate a local occurrence of WDH, likely associated with specific ecological and anthropogenic conditions, the latter would be indicative of widely spread admixture; iii) compare estimates of WDH prevalence over time, contrasting the 2020–2024 sample with 26 additional invasive samples of putative wolves collected between 1993 and 2003. This comparison would provide additional insight into the hypothesis that hybridisation among peninsular Italian wolves peaked around the late 1990s (Galaverni et al., 2017); iv) assess whether a major genetic component of the German shepherd dog was revealed in the hybrids we detected, as a previous genome-wide assignment suggested that the main contribution to wolf-dog hybrids in Italy likely came from this breed (Galaverni et al., 2017).

By framing our findings in relation to prior investigations on both the same and other wolf populations in Europe that are threatened by anthropogenic hybridisation, we contextualise our results within a broader conservation framework. Overall, our practical scope is to raise awareness among conservationists, practitioners, administrators, and the public about the potential risks of neglecting timely and effective management of WDH, especially in human-modified landscapes recently being recolonised by wolves (Di Bernardi et al., 2025). Finally, we also discuss the implications of our findings in relation to the recent downgrading of the protection status of wolves in Europe that did not consider the genetic integrity of wolf populations (Stronen et al., 2025). While focusing on peninsular Italian wolves, the implications of our study extend beyond the wolf as a model species, as unmanaged anthropogenic introgressive hybridisation of wildlife is a threat of growing concern in increasingly human-dominated landscapes (Stronen et al., 2025).

2. Material and methods

2.1. Samples

A total of 748 tissue samples (hereafter “recent samples”) were opportunistically collected from presumed wolves found dead or rescued alive in peninsular Italy (Toscana, Umbria/Marche, Lazio, Abruzzo/Molise, Basilicata, Puglia, Campania, Calabria) between 2020 and 2024 (details in Appendix S1). Additional samples of 26 wolves dating back to the decade 1993–2003 (“old samples”) were also gathered from Abruzzo, the historical and retained core area for the wolf in Italy.

The reference wolf population database for assigning the genotypes of unknown putative wolves to their source gene pool consisted of 100 wolves collected in the Apennines between 2015 and 2017. They were selected as reference individuals after being classified as non-admixed wolves by values of individual proportional membership (q) higher than 0.990 in a Bayesian assignment procedure of autosomal STR genotypes, and by their wolf Y-chromosome haplotypes. Furthermore, the absence of any obvious morphological evidence of hybridisation with the dog was confirmed by expert zoologists and veterinarians upon inspection of carcasses or live animals used as reference (see Ciucci, 2012; Ciucci et al., 2003). Muscle or salivary swabs from 185 medium- and large-sized dogs, both purebred ($n = 58$) and mongrels ($n = 127$) (Appendix S1) were also collected for canine databasing.

2.2. Laboratory method

The laboratory method for obtaining autosomal genotypes to be statistically assigned to the respective gene pools, or to detect signals of their genetic admixture, followed a validated protocol that was described in detail in Lorenzini et al. (2022). Briefly, the DNA was isolated from approximately 15 mg of muscle or one salivary swab using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) or the Maxwell16 Instrument (Promega, Madison, WI, USA) for automated genomic DNA isolation, and quantified using the Quantus Fluorometer (Promega). Twenty-three autosomal canine STR loci (Appendix S1), selected primarily based on their capability to reliably differentiate wolf, dogs and hybrids (Lorenzini et al., 2014, 2022), were amplified for each sample to obtain individual multilocus profiles.

Male wolves were also genotyped at five STR loci on the Y chromosome (Appendix S1) and combined into haplotypes (Table S1) to evaluate the wild or domestic origin of their paternal lineage. A dominant 3-bp deletion in the K locus coding for the β-defensin CBD103 gene has been suggested as a melanistic mutation of domestic origin (Anderson et al., 2009), although it has also been reported that the deletion is not fully diagnostic of introgressed dog ancestry in the wolf from Italy (Caniglia et al., 2013). Despite this, each wolf sample was genotyped at the K locus to find for further clues of hybridisation.

2.3. Genotype assignment by Bayesian analysis

Statistical analyses to assign unknown individuals to the Italian wolf population, and to identify admixed wolf-dog genotypes were conducted using a population database consisting of 100 reference wolves and 185 reference dogs, according to a Bayesian clustering model as implemented in STRUCTURE v 2.3.4 (Falush et al., 2003). The appropriate threshold values of proportional membership (q) for assigning single multilocus genotypes to the genetic clusters inferred by STRUCTURE were derived following the results of simulation studies conducted with HYBRIDLAB (Nielsen et al., 2006) on our 285-reference wolf and dog samples. Details on simulations and statistical procedures are provided in Appendix S1 and in Lorenzini et al. (2014, 2022).

Extensive simulation work (Lorenzini et al., 2014; Godinho et al., 2015; Caniglia et al., 2020; Santostasi et al., 2021) indicates that accurately distinguishing between individual admixed generations based on STR data is unrealistic, even when utilising a high number of markers (Stronen et al., 2022b). This limitation arises from significant overlaps in their q-value ranges, with the notable exception of first-generation F1 individuals. For this reason, according to the thresholds obtained in the simulations with our reference dataset of parental classes, three diagnostic categories were defined to assign unknown genotypes: (i) non-admixed wolves, with q-values equal to or greater than 0.990; (ii) introgressed wolves (old backcrosses with minimal dog ancestry), with $0.960 < \text{q-values} < 0.990$ and/or canine Y-haplotypes; (iii) recent generation hybrids (F1s, F2s, first, and second generation backcrosses – BC1w and BC2w, respectively), with q-values equal to or lower than 0.960 (Table 1). Typically, evidence of unusual morphological traits to help identify admixture was not available, so assignment of individuals was based solely on their genotypes.

Using the R Boot package (Canty and Ripley, 2024), we tested for differences in the proportions of hybrid individuals between recent and old samples datasets using bootstrapped 95 % confidence intervals (95 % CI); H_0 (i.e., no difference) was accepted if bootstrapped 95 % CI included the value 0.

2.4. Genetic component of a dog breed in wolf-dog hybrids

Once wolf-dog admixed genotypes were identified by Bayesian assignment procedure and individual q-values were derived, we verified whether the main canine genetic component in hybrids was represented by the German shepherd dog, as hypothesised from SNP data and genome-wide ancestry reconstruction methods (Galaverni et al., 2017). For this purpose, we used our previous dataset consisting of 100 wolves and 185 dogs, with the addition of 21 new samples of German shepherd dogs, and 75 wolf-dog hybrids, selected from the sample of 219 hybrids (214 recently collected and 5 old) obtained using STRUCTURE and the entire sample set of 774 putative wolves (see Results). These 75 hybrids were selected based on their q-values being lower than 0.900. Hybrids with higher q-values ($n = 144$, ranging from 0.901 to 0.960; see Results) did not allow the canine component to be clearly evaluated and were excluded from the computations.

Initially, we performed an explorative Discriminant Analysis of

Table 1
Diagnostic classes of individual multilocus genotypes for putative Italian wolves, as defined by Bayesian analysis of 23 autosomal STR loci and 5 STR loci on the Y chromosome of males (Y-haplotype). q = individual proportional membership.

Diagnostic class	Definition	q	Dog Y-haplotype
Wolf	Individual with no or not diagnosable admixture with the dog	$q \geq 0.990$	No
Introgressed wolf	Individual with only Y-haplotype dog ancestry	$q \geq 0.990$	Yes
Introgressed wolf	Individual with minimal dog ancestry at autosomal STRs	$0.960 < q < 0.990$	No
Introgressed wolf	Individual with minimal dog ancestry at autosomal STRs and dog Y-haplotype	$0.960 < q < 0.990$	Yes
Recent hybrid	Individual recently admixed with the dog	$q \leq 0.960$	No
Recent hybrid	Individual recently admixed with the dog and dog Y-haplotype	$q \leq 0.960$	Yes

Principal Components, DAPC (Jombart et al., 2010), using the Adegenet package 2.1.6 (Jombart, 2008) for the R software 4.2.1 on the web interface, to qualitatively describe the overall genetic variability in our dataset at the population level. DAPC identifies discrete clusters of individuals, maximising variation among pre-defined groups while minimising variation within groups, without relying on any underlying genetic model. Following the STR-based results from Bigi et al. (2015), who showed that German shepherd dogs (GSD) and Czechoslovakian Wolfdogs (CLW) share the same genetic cluster and are clearly well separated from other wolflike/shepherd breeds, we entered four pre-defined groups into the computations, which corresponded to wolves, dogs, GSD + CLW, and hybrids. Secondly, to complement the multivariate analysis, STRUCTURE was implemented on the same dataset for a Bayesian assignment of individual genotypes under same settings as in previous analyses (Appendix S1).

3. Results

All individuals were genotyped at 23 autosomal STR loci. A subsample of 229 males (see below) was also genotyped at 5 STRs on the Y chromosome. Following Salvatori et al. (2019), individual multilocus genotypes showing more than 8 % missing alleles were discarded from the original dataset of 774 putative wolves. Due to high degradation of carcasses and/or low-quality of the extracted DNA, 22 samples yielded four or more missing loci in their genotypes and were removed. A total of 752 recent (n = 726, years 2020–2024) and old (n = 26, years 1993–2003) samples were eventually retained in downstream analyses. In accordance with the literature, our five Y-linked loci defined two haplotypes (Table S1) that were private to the Italian wolf, being absent in both the dog and other wolf populations across Europe (this study, Randi et al., 2014).

3.1. Analysis of recent (2020–2024) samples

Based on Y-chromosome haplotypes and threshold q-values in the wolf cluster from a 23-locus panel on autosomes, 387 individuals (53.3 %) in the entire dataset of recent samples (n = 726) were classified as non-admixed wolves ($q \geq 0.990$, sex ratio 1.10 males/female). Other 125 individuals (17.2 %) were classified as introgressed wolves, 122 of which showed $0.960 < q < 0.990$, and 3 showed wolf STRs ($q \geq 0.990$) but canine Y-haplotypes (see below and Table 1). The remaining 214 putative wolves (29.5 %) were classified as recent generation hybrids ($q \leq 0.960$).

Three out of 214 recent hybrids (1.4 %, or 0.4 % of the overall sample) were most likely first-generation F1 crossbreds ($0.475 \leq q \leq 0.562$) and were collected in the Abruzzo, Calabria and Lazio regions. While two of them were females, the male bore a canine Y-haplotype (H6, Table S1). Three other hybrids were probably F2s and/or first-generation backcrosses BC1w ($0.660 \leq q \leq 0.719$), all from Lazio. One of them was a male carrying a dog Y-haplotype (H3). All the remaining hybrids (97.2 %) possibly were second generation backcrosses (BC2w), although we cannot exclude that BC1w could also be included. Of the 214 recent hybrids, 108 were males and 96 were females (sex ratio 1.13 males/female). For 10 individuals, sex was unknown or male Y-haplotypes were not identified. Twenty-three (21.3 %) of the 108 male hybrids that were sex-genotyped carried non-wolf Y-haplotypes, while 85 (78.7 %) carried one of the two haplotypes (H1, H13) that are private to the Italian wolf population. Thirteen recent hybrids (6.1 %) showed also one copy of the 3-bp-deletion allele at the K locus. Interestingly, two females from Marche, classified as non-admixed wolves by their STR genotypes, were homozygous for the 3-bp deletion, as the only evidence of possible past introgression from the dog. However, since the deletion is not diagnostic, it did not contribute to categorise the unknown genotypes.

Considering the 125 individuals that were classified as introgressed, 3 of them were recognised based only on their canine Y-haplotypes. Fifty-eight out of 125 were females and 64 were males (sex ratio 1.10 males/female), while the sex was unknown for 3 specimens. Fifty-five males (86.0 %) bore wolf Y-haplotypes, while 9 (14.0 %) showed the canine haplotypes H7 (n = 8) and H4 (n = 1). In our dog reference database, H7 was the most frequent Y-haplotype and was largely shared by many purebred and mongrel dogs, making this information poorly informative on the origin of the dog breed for past crossbreeding. By contrast, H4 was only found in the Czechoslovakian Wolfdog breed. The 3-bp-deletion allele at the K locus was present in single copy only in one introgressed female.

To investigate whether dog introgression into the wolf gene pool could be traced exclusively through Y-haplotypes, an explorative subsample of 60 males classified as non-admixed wolves by their autosomal STR genotypes were also analysed at the Y-chromosome STR loci. Fifty-seven (95.0 %) of them carried wolf Y-haplotypes, while three showed a dog haplotype (H7) and were finally diagnosed as introgressed wolves (see above) based on their paternal lineage, despite showing wolf STR genotypes.

3.2. Geographical distribution of samples

The samples classified as recent hybrids, introgressed wolves and non-admixed wolves from the entire dataset were geographically distributed according to the regions where they were collected. Their distribution (Fig. 1, Table 2) suggested that the proportion of recent hybrids was higher in the western regions (Toscana, Lazio, Campania, Calabria), ranging from 27.2 to 42.8 %, compared to the eastern regions (Abruzzo/Molise, Umbria/Marche, Basilicata, Puglia), where the proportion ranged from 15.8 to 25.8 %. Conversely, the reverse seems to apply for the introgressed wolves, which showed the lowest proportions in the western regions of Campania, Lazio and Toscana, with percentages of 11.5, 12.2 and 16.8, respectively.

3.3. Analysis of old (1993–2003) samples

Seventeen (65.4 %) out of 26 putative wolf samples collected in the historical Apennine core range (Abruzzo) during 1993–2003 were assigned to the wolf population (Table S2). Four were introgressed wolves, and 5 were recent hybrids. Two individuals were classified as introgressed wolves by the presence of canine Y-haplotypes (H3, H7), but not by their autosomal STR genotypes. Two hybrids, one female and one male, were most likely first-generation F1 crossbreds (q-values in the wolf cluster = 0.407 and 0.587, respectively), with the male showing also a canine Y-haplotype (H6). Three female hybrids were probably BC1w and/or BC2w ($0.667 \leq q \leq 0.937$).

The proportion of recent hybrids in the dataset of old samples (5/26 = 0.192) (Table S2) was lower than the proportion in the dataset of recent samples (214/726 = 0.295), even though this difference, based on 10^4 bootstrap re-samplings, was not significant (95 % CI = −0.2840-0.073). By contrast, the proportion of first-generation F1 crossbreds dating back to the decade 1993–2003 (2/26 = 0.077), which represented 40 % of all recent hybrids, was significantly higher than the proportion obtained from the samples collected during 2020–2024 (3/726 = 0.004), which represented 1.4 % of all recent hybrids from peninsular Italy (bootstrapped 95 % CI of the difference between the two datasets = 0.065–0.105).

3.4. Contribution of the German shepherd to wolf-dog hybrids

The overall genetic variability was qualitatively described using DAPC, where four pre-defined groups were entered into the computations, which corresponded to wolves, dogs, GSD + CLW, and hybrids. The cross-validation procedure suggested that 10 principal components (PC) were retained in the analysis as a proxy for optimal description of the original data, with the first two PCs best summarising most of the allelic diversity (Fig. 2). The first PC axis clearly distinguished the dog

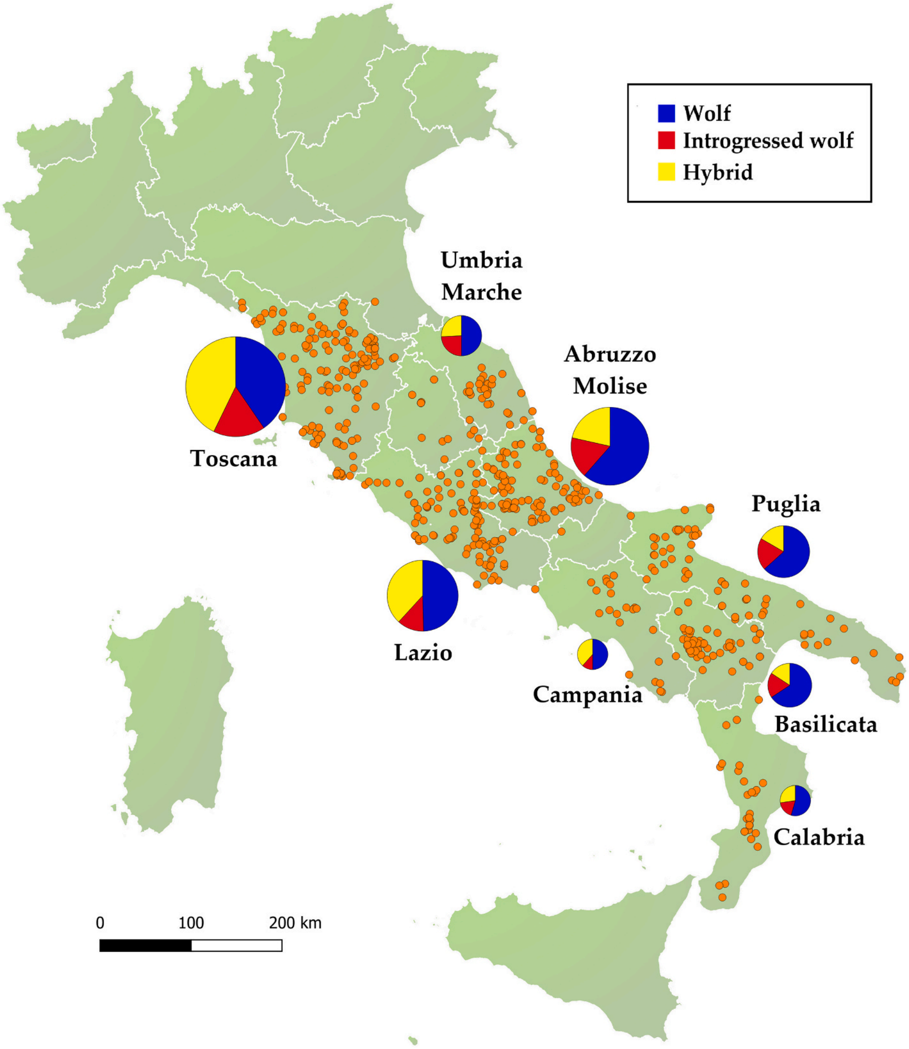

Fig. 1. Spatial distribution of 726 samples analysed during 2020–2024 in peninsular Italy. Pie charts show the proportions of non-admixed wolves (Wolf), introgressed wolves (Introgressed wolf) and recent hybrids (Hybrid) on a regional scale (see also Table 2 for details). The size of circles represents sample size in each region. Samples from Umbria and Marche were pooled, as were those from Abruzzo and Molise.

Table 2
Diagnostic classes and distribution across regions of 726 putative wolves collected from peninsular Italy in the period 2020–2024. In brackets the percentage for introgressed wolves and recent hybrids.

	Toscana	Umbria/Marche	Lazio	Abruzzo/Molise	Campania	Puglia	Basilicata	Calabria
Wolf	70	35	61	83	13	57	50	18
Introgressed wolf	29 (16.8 %)	17 (24.3 %)	15 (12.2 %)	23 (17.0 %)	3 (11.5 %)	18 (20.0 %)	14 (18.4 %)	6 (18.2 %)
Recent hybrid	74 (42.8 %)	18 (25.8 %)	47 (38.2 %)	29 (21.5 %)	10 (38.5 %)	15 (16.7 %)	12 (15.8 %)	9 (27.2 %)
	173	**70**	**123**	**135**	**26**	**90**	**76**	**33**

population(s) from both wolves and hybrids, the latter expectedly partially overlapping with wolves. The second PC axis separated GSD + CLW from the other dogs, with limited overlapping between the two clusters.

Based on the same dataset used in DAPC, we ran STRUCTURE setting $K = 4$ as the best-fitted number of meaningful genetic clusters inferred from our dataset according to the Puechmaille's method (Table 3, Fig. 3). In line with the DAPC, German shepherd dogs ($n = 21$) and Czechoslovakian Wolfdogs ($n = 8$) shared the same genetic cluster (III, average proportional membership $Q = 0.948$) with individual q-values ranging from 0.867 to 0.993. Conversely, both the other purebred dogs and mongrels of the database ($n = 177$) fell mainly into two alternative clusters (I, IV, summing up to $Q = 0.928$), which would also suggest some kind of population substructuring. Wolves were assigned to a single private cluster (II) with average $Q = 0.993$ (range of q-values 0.977–0.996). Hybrids shared only a small part of their ancestry with the dog clusters (Q-values = 0.043, 0.028 and 0.054 in clusters I, III and IV, respectively), while most of their ancestry was in common with the wolf cluster ($Q = 0.874$), as also clearly shown by the DAPC scatterplot. This supports our previous findings that most hybrids were not first-generation crossbreds.

Most interestingly, the lowest average dog ancestry of the hybrid class fell just within cluster III (Q-value = 0.028), suggesting that, on average, the main genetic component of hybrids is not shared with either German shepherd dogs or Czechoslovakian Wolfdogs. The only exception is an individual from Abruzzo collected in 2001, with q-value = 0.518 in cluster III, which could be a crossbred with a German shepherd dog. Furthermore, this hybrid showed the same Y haplotype

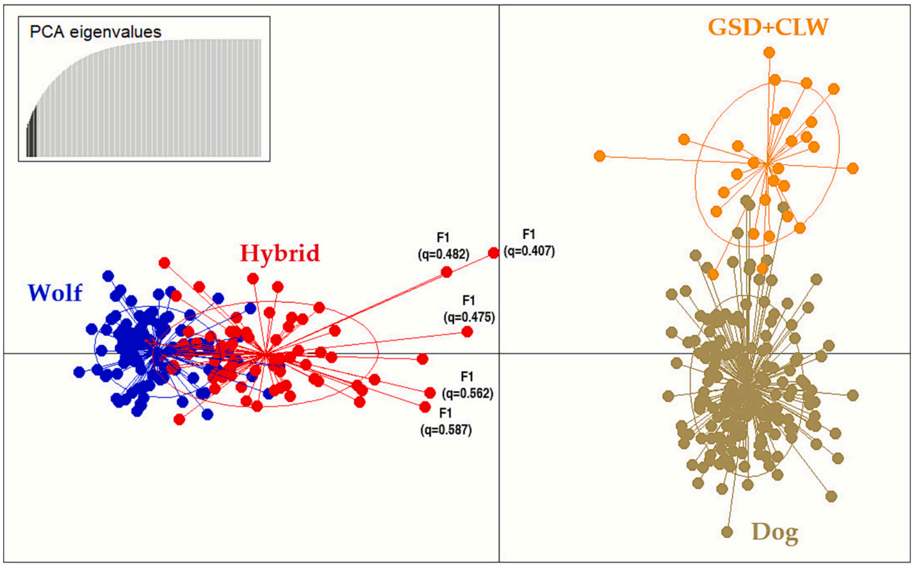

Fig. 2. DAPC scatterplot based on 23 STR loci describing the genetic variability in the dataset of 100 wolves (Wolf), 75 wolf-dog hybrids (Hybrid), 177 mongrels and purebred dogs (Dog), 29 German shepherd dogs and Czechoslovakian Wolfdogs (GSD + CLW), represented by first (X-axis) and second (Y-axis) discriminant functions. The graph features single individuals as dots and groups as 95 % inertia ellipses. In the top-left insert the proportion of variability that is explained by the first 10 principal eigenvalues. First generation hybrids (F1) obtained from the Bayesian assignment analysis are also indicated with their respective individual proportion of membership values (q) to the wolf population.

Table 3

Average probability of membership (Q) for reference wolves, dogs, German shepherd dogs + Czechoslovakian Wolfdogs (GSD + CLW), and hybrids (with q-values <0.900) in the genetic clusters inferred by STRUCTURE for K = 4. Ranges for probability of membership of relevant clusters are in parentheses. * When a presumed F1 wolf x German shepherd hybrid was removed from the analysis, then the Q-value of the hybrid class in cluster III decreased to 0.022, with individual values 0.001–0.154 (see text for details). N = sample size.

	N	Cluster I	Cluster II	Cluster III	Cluster IV
Wolf	100	0.002	**0.993** (0.977–0.996)	0.002	0.002
Dog	177	**0.494**	0.004	0.069	**0.434**
GSD + CLW	29	0.024	0.004	**0.948** (0.867–0.993)	0.024
Hybrid	75	0.043 (0.002–0.451)	**0.874** (0.407–0.992)	0.028 (0.001–0.518)*	0.054 (0.003–0.393)

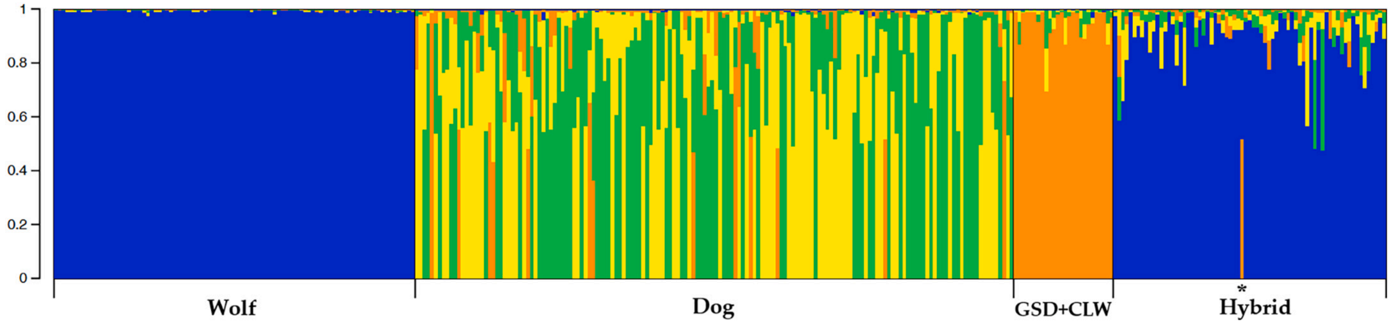

Fig. 3. Bar plotting from STRUCTURE assuming four genetic clusters (K = 4). Each vertical bar represents one individual and the length of the different coloured sections is proportional to membership (q-values) in the inferred genetic clusters (I-IV, see Table 3). The star indicates a possible F1 between a wolf ($q_{wolf} = 0.407$) and German shepherd dog ($q_{GSD+CLW} = 0.518$). Dataset as in Fig. 2. The bar plot was drawn using STRUCTURE PLOT (Ramasamy et al., 2014).

(H6) that was also present in all German shepherd males ($n = 9$) of our database, but different from those of the Czechoslovakian Wolfdog males (H4, $n = 4$). If this individual was excluded from the analysis, the Q-value of hybrids in cluster III predictably dropped only slightly (0.022 vs 0.028), but the range for individual probabilities of membership narrowed considerably (0.001–0.518 vs 0.001–0.154).

4. Discussion

We detected a remarkably high proportion (46.7 %) of wolf-dog hybrids in our sample of wolves retrieved dead throughout peninsular Italy from 2020 to 2024. Admixed wolves comprised 29.5 % recent hybrids, up to the first- and second-generation backcrosses, and 17.2 % introgressed individuals from older backcross generations. Although admixed wolves were widely distributed across peninsular Italy, the western regions appeared to share a higher proportion than the eastern regions. Along a temporal scale, accounting for the difference in sample sizes between the 1993–2003 and the 2020–2024 datasets, we did not detect any differences in the proportion of admixed wolves between the two sampling periods, even though the former dataset shared a higher proportion of F1s compared to the most recent sample. Finally, we failed to find evidence supporting a previous hypothesis that the German shepherd breed contributed the most to admixture in Italian wolves (Galaverni et al., 2017). The situation we hereby report for peninsular Italian wolves presents extraordinary practical conservation challenges. As it follows decades of ineffective WDH management, it serves as a cautionary example to timely prevent similar situations in other wolf populations.

4.1. Caveats

Our classification of 'recent generation hybrids' (i.e., with q-values $\leq$0.960) conforms with the approach described by Caniglia et al. (2020) and reflects the operative definition of a wolf-dog hybrid recently proposed for conservation purposes (Stronen et al., 2025). This category comprises admixed individuals whose dog genetic component is substantial (i.e., $\geq$12.5 %) and that have been therefore regarded of management concern (Caniglia et al., 2020; Stronen et al., 2022a). A critical issue when addressing the estimation of any population parameter, such as the prevalence of admixed individuals, deals with the representativeness of the sample used to draw inference at the population level (Ciucci et al., 2007). Because the retrieval of wolves found dead represents a form of opportunistic sampling, we cannot exclude that some sources of bias might affect our estimate of admixture prevalence. However, due to the dense inter-regional network of IZSs and their alertness, the systematic carcass retrieval system that contributed to our dataset was particularly effective. It allowed us to analyse for the first time a conspicuous set of invasive samples from peninsular Italy, comprising about one third of the estimated living wolf population in 2020–2021 (2127–2844 wolves; Gervasi et al., 2024). In addition, by referring to a period of 5 years (i.e., slightly more than one wolf generation time; Mech et al., 2016), our sample embraces a biologically meaningful time laps, thus preventing estimation issues associated with a multi-generational temporal framework. Furthermore, whereas most WDH assessments typically involve non-invasive samples (e.g., Godinho et al., 2015; Jarausch et al., 2023; Pacheco et al., 2017), our dataset is based on invasive samples that typically yield higher DNA quality. Therefore, we could use a relatively higher number of genetic markers to detect wolf-dog hybrids, thereby yielding higher statistical power while avoiding the technical issues typically associated with non-invasive samples (e.g., Godinho et al., 2015). Nevertheless, we caution that our findings are dependent on the assumption that the probabilities of a wolf to die, as well as to be successively found and retrieved through the IZS network, are comparable between admixed and non-admixed wolves. Although further investigations are needed to compare survival rates between these two forms, in the unique study we are aware of that tackled the issue, apparent survival of admixed wolves was either equal or higher than that of non-admixed wolves, depending on the model used to estimate population size (Santostasi et al., 2021). According to this study, and in the worst scenario of higher survivorship of admixed wolves, our sample would be expected to underestimate and not overestimate the prevalence of admixed individuals in the population.

4.2. Wolf-dog hybridisation in peninsular Italy

The extent of admixture we detected in peninsular Italian wolves is strikingly higher compared to the estimates obtained in previous decades, ranging from 2 % to 6 % (Lorenzini et al., 2014; Lucchini et al., 2004; Randi and Lucchini, 2002; Verardi et al., 2006). It is also higher than the estimate more recently (2020−2021) obtained as a side product of a nation-wide survey based on non-invasive genetic sampling (Gervasi et al., 2024). Here, 22.5 % of admixture was reported for peninsular Italian wolves, comprising 9.6 % recent hybrids and 12.9 % introgressed individuals ($n = 622$ single genotypes). Recalculating these figures by excluding dogs, originally included in the computation of prevalence, yields an admixture of 27.3 %, comprising 11.7 % recent hybrids and 15.6 % introgressed individuals ($n = 513$ single genotypes; recalculated from Aragno et al., 2022, and Gervasi et al., 2024, Supporting Information). Compared to this last estimate, ours is much higher, especially considering the proportion of recent hybrids (29.5 % vs 11.7 %). Although the two approaches are not strictly comparable due to differences in sample types, sampling techniques, and the statistical power to detect hybrids, both sets of data clearly indicate that the peninsular Italian wolf population is highly admixed with dogs, to a much higher extent than previously thought. These findings suggest that, contrary to earlier theoretical predictions (Vilà and Wayne, 1999), interbreeding between wolves and dogs does indeed occur under certain anthropogenic conditions.

Paradoxically, wolves that were rescued from demographic extinction in Italy in the early 1970s due to effective conservation campaigns (Boitani, 2003) are now at high risk of genomic extinction, despite longstanding warnings about WDH being a threat to conservation (Boitani, 1984; Boitani and Ciucci, 1993; Ciucci and Boitani, 1998; Boitani, 2003). Nevertheless, the fact that about 53 % of the wolves we sampled were non-admixed indicates that, while the peninsular Italian wolf population shows widespread introgression (i.e., type 5 anthropogenic hybridisation sensu Allendorf et al., 2001), complete admixture (i.e., genomic swamping) has not occurred yet, at least based on current genetic and genomic evidence from this and previous research (Galaverni et al., 2017; Caniglia et al., 2020; Stronen et al., 2022b; Battilani et al., 2025). This, however, should not be interpreted as evidence that behavioural or ecological barriers are effectively preventing introgression from developing into genomic swamping. On the one hand, it should be recognised that non-admixed wolves may comprise an unknown extent of introgressed individuals of older generations of backcross that evade genetic detection; on the other hand, as introgressive hybridisation continues, the ratio of non-admixed to admixed wolves is likely to decline, increasingly reducing the capacity of the wolf population to dilute the introgressed dog variants.

In practical conservation terms, we believe that any reactive management response, such as removal or neutralisation and release (Donfrancesco et al., 2019), would currently be largely impractical under these conditions and would raise significant social and ethical opposition. Focusing on preventive and proactive interventions seems to be the only viable solution left for the peninsular Italian wolf population, with the goal of minimising any further increase in WDH and preventing complete admixture.

4.3. Geographical distribution of hybrids

The admixed wolves identified in our study were widely distributed across peninsular Italian regions, although their prevalence appeared to be higher in the western areas. However, due to the nature of our dataset, the locations from which the dead wolves have been retrieved cannot be considered representative of their natal area, or of the areas where the hybridisation events originated. Wolves and likely wolf-dog hybrids possess significant dispersal capabilities, especially at the scale of peninsular Italy (Fabbri et al., 2007; Ciucci et al., 2009). Nevertheless, our findings suggest that the western regions such as Lazio, Toscana, and Campania may serve as hotspots for recent and still ongoing WDH. Indeed, when compared to the eastern regions, such as Puglia and Umbria/Marche, they show the highest numbers of recent hybrids and, conversely, the lowest numbers of older generation backcrosses. In the absence of data on the relative densities of wolves and dogs, as well as on the conditions facilitating their affiliative behaviour, we can only speculate that the observed regional differences may be attributed to anthropogenic factors. These can include degraded ecological conditions, high levels of illegal wolf killing, and the high abundance of free-ranging dogs. Further investigations on this issue are needed to foster a mechanistic understanding of the onset of WDH, based on which predictive models of WDH likelihood could be developed with the practical aim to inform and prioritise preventive management at the landscape scale.

4.4. *Timing of WDH in peninsular Italian wolves*

The high proportion of non-first-generation hybrids that we detected supports the notion that the peninsular Italian wolf population is not facing an early stage of WDH. This is also indicated by the DAPC scatterplot and by the fact that most admixed wolves' ancestry fell within the wolf cluster. In fact, we detected a few F1s, whereas the majority (97.2 %) of admixed individuals were most likely second-generation backcrosses to wolves. Based on a wolf generation length of 3–4 years (Mech et al., 2016; Skoglund et al., 2011), this means that most of the original hybridisation events that we detected occurred some 9–16 years before sampling (i.e., in 2004–2015) or earlier. Based on samples collected throughout peninsular Italy during 1992–2015 and genome-wide data, Galaverni et al. (2017) suggested that, even though a few cases of WDH might have occurred both in the 1940s and in the 1970s, the phenomenon surged in the 1980s and peaked in the late 1990s, likely due to a marked expansion of wolves into increasingly human-dominated areas. Indeed, based on our findings, the number of F1s in Abruzzo (central Italy) appeared to be higher during 1993–2003, when they represented 40 % of all recent hybrids, than across peninsular Italy in 2020–2024, when they represented 1.4 % of all recent hybrids. Therefore, a higher proportion of F1s in the older samples might have captured a peak in original hybridisation events during the late 1990s, as hypothesised by Galaverni et al. (2017). However, although we controlled for sample size effects, the quite small sample size for the older dataset ($n = 26$) suggests caution in interpreting these results. Nevertheless, the occurrence of F1s and other recent hybrids in our more recent dataset indicates that original hybridisation events are still taking place, though likely at a reduced frequency compared to the recent past. In turn, this suggests that the factors and conditions facilitating interbreeding between wolves and dogs are still operating.

4.5. *Genetic contribution of the German shepherd to wolf-dog hybrids*

Our findings did not support the hypothesis that the German shepherd, as the most widespread breed in recent decades, constitutes most of the dog ancestry in Italian admixed wolves (Galaverni et al., 2017). Except for one admixed individual in our dataset, we failed to identify the German shepherd's gene pool as the primary dog component in admixed wolves (F1s included). However, our data do not allow us to draw conclusions about the contributions of specific dog breeds, as our canine database was constructed as a mix of canine variability, encompassing both purebred and mix-breed dogs, without substructuring into separate groups. It is reasonable to hypothesise that there is likely no single breed more prone to hybridisation; rather, it may be a group of dog breeds—or canine types—that are the most prevalent locally (i.e., village dogs).

4.6. *Implications for other European wolf populations*

The recent dynamics, extent, and spread of WDH that we detected in peninsular Italian wolves not only deeply affects their conservation status but also represents a serious risk for the genetic integrity of the neighbouring wolf populations. Wolves are capable of long-distance dispersal through which also geographically disjunct populations at the European scale can become genetically connected (Ražen et al., 2016; Morales-González et al., 2022; Konec et al., 2024). In particular, the Alpine population, recolonised in the early 1990s by wolves dispersing from the northern Apennines, is connected to these through a migration rate of about 1.25–2.5 wolves/generation (Fabbri et al., 2007). Nonetheless, long-term non-invasive genetic monitoring of the Alpine wolf population has until now revealed only limited introgression (Dufresnes et al., 2019). This was further confirmed by a non-invasive genetic survey conducted in the Italian Alps in 2020–2021 that estimated about 134 wolf packs, of which only two of admixed, recent origin (Marucco et al., 2023). The limited extent of dog admixture in Alpine wolves may be indicative of either a lower tendency for long-distance dispersal of admixed wolves, or their lower efficacy in establishing their own pack or integrating into existing ones.

Similarly to the Alps, also other European wolf populations show low or very low proportion of admixed individuals (Dinaric-Balkan: Moura et al., 2014; Scandinavian: Stenøien et al., 2021; Karelian: Smeds et al., 2021; but see Tirronen and Kuznetsova, 2025). However, several technical and sampling issues may undermine the reliability and comparability of WDH estimates across wolf populations and, as a results, we still have a limited understanding of the prevalence of wolf-dog hybrids and their dynamics across Europe (Salvatori et al., 2020; Stronen et al., 2025). In any event, accurate and continuous assessment of WDH should be a fundamental component of the conservation strategy of European wolf populations, in order to ensure timely management responses when and where needed. As effectively illustrated by the uncontrolled spread of WDH in peninsular Italy in the past decades, in the absence of effective management it takes a few wolf generations for WDH to increase to unmanageable levels.

4.7. *Conservation implications*

The situation we hereby report bears relevant implications for conservation management. First of all, many experts agree that wolf-dog hybrids should be viewed as a serious threat to wolf conservation (Donfrancesco et al., 2019). In addition to the often-mentioned risks of loss of genetic identity, outbreeding depression, and loss of coadapted gene variants (e.g., Allendorf et al., 2001), the expected differences in physiology and behaviour in admixed wolves, though difficult to detect, can profoundly impact wolves' life history traits, population dynamics, and ecology, including interactions with humans (Ciucci et al., 2025). Preliminary data suggest that reproductive physiology and social behaviour may differ in admixed wolf packs (e.g., Crispino et al., 2021), and that even small genetic variants of dog origin could have relevant behavioural effects in wolves (e.g., Pilot et al., 2018).

Second, the extent of admixture with dogs we reported in peninsular Italian wolves provides as a stark example of the dangers associated with neglecting WDH management when needed. Our findings offer a cautionary instance, indicating that similar dynamics of WDH may arise in other expanding wolf populations if management obligations are consistently unattended. While some may view the postponement of management for such a complex issue as a politically convenient short-term tactic, this approach only postpones resolution and exacerbates existing challenges in the long term. In Italy, the lack of management responses since the first evidence of WDH has characterised prevailing management attitudes at the local, regional, and national scales, both by governmental and non-governmental bodies. Consequently, no serious media campaign has ever been launched, and awareness of this phenomenon has been very limited among specialists, journalists, and the public. The negligence regarding the management of WDH may stem from the untested assumption that interbreeding between wolves and dogs is a rare occurrence (Vilà and Wayne, 1999). However, also when the situation became more apparent in Italy—both at the local (e.g., Salvatori et al., 2019; Santostasi et al., 2021) and national (e.g., Caniglia et al., 2020; Galaverni et al., 2017; Gervasi et al., 2024) levels—no management response has been implemented, thereby contributing to the uncontrolled increase and spread of this phenomenon (Santostasi et al., 2020, 2025). In addition to ineffective management, several other factors may facilitate WDH in anthropogenic landscapes. These include a relatively high number of free-ranging dogs (Lorenzini et al., 2014), and a high and persistent human-caused mortality that increases the likelihood of pack disruption (Rutledge et al., 2010), which in turn may encourage affiliative encounters between female wolves and male dogs (Bohling and Waits, 2015). Additionally, the large availability of anthropogenic food subsidies (Ciucci et al., 2020) may nutritionally support female wolves that breed with dogs during pregnancy and lactation, boosting survival of their admixed offspring. In particular, the disruption of the social cohesion of wolf packs due to human-caused mortality could be a relatively common yet underreported phenomenon in human-dominated environments. Although wolves in Italy have long been strictly protected by law, human-caused mortality remains widespread, including substantial poaching (Lovari et al., 2007; Boitani and Ciucci, 2009; Musto et al., 2021). Evidence is this study, that was made possible by the large number of dead wolves retrieved by the IZS network which, in relation to the estimated peninsular wolf population in 2021 (Gervasi et al., 2024), corresponds to a minimum known mortality rate of approximately 6 % per year.

Third, our findings have relevant implications also for the other European populations in view of the recent downlisting of the protection status of wolves by the Bern Convention and the Habitats Directive (European Parliament 2025). Unfortunately, this pronouncement did not consider the genetic constituency -including WDH- of European wolf populations. Ignoring this aspect when assessing their conservation status increases the risk of overestimating the abundance of non-admixed individuals. This oversight, in turn, could lead to two negative consequences: incorrectly assessing their Favorable Conservation Status (FCS; Linnell and Boitani, 2025), and delaying necessary management responses to mitigate the risk of WDH (Stronen et al., 2025). For instance, based on our findings, a less strict protection status for wolves in peninsular Italy is not warranted and highly impractical, as non-admixed wolves, recent hybrids, and introgressed individuals do not form distinct populations and often live in mixed packs (Crispino et al., 2021; Salvatori et al., 2019; Santostasi et al., 2021). While this greatly confounds the assessment of FCS, harvesting wolves could further deteriorate their genetic constituency, while increasing the likelihood of WDH spreading further (Moura et al., 2014; Tirronen and Kuznetsova, 2025). In addition, selectively harvesting admixed wolves from the distance is impractical as morphological cues (e.g., coat colour) are not deemed reliable indicators of admixture (Lorenzini et al., 2014; Stronen et al., 2025).

Although seemingly counterintuitive, we reinforce the assertion that that wolf-dog hybrids, where detected, should be allowed the same legal protection status of wolves to prevent legal loopholes (Peltola and Heikkilä, 2018; Sonne et al., 2019), and their management placed under the responsibility of governmental agencies (Trouwborst, 2014). In this context, it appears critical to enhance our efforts to detect wolf-dog hybrids and systematically assess their occurrence in European wolf populations through reliable and comparable genetic procedures (Stronen et al., 2025). The goal should be to maintain European wolf populations void of WDH or within limits that allow practical management interventions, while preventing WDH spreading to the levels we hereby report for peninsular Italy. At the same time, it is critical to foster an international debate within the scientific community, as well as among stakeholders and the public, as to which are the most effective and socially acceptable means to address the remarkable difficulties and challenges that WDH is posing to wolf conservation.

CRediT authorship contribution statement

Rita Lorenzini: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Antonella Pizzarelli:** Methodology. **Lorenzo Attili:** Writing – review & editing, Methodology, Data curation. **Massimo Biagetti:** Methodology. **Carla Sebastiani:** Methodology. **Paolo Ciucci:** Writing – review & editing, Writing – original draft, Conceptualization.

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Declaration of competing interest

The authors have no relevant financial or no-financial interests to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2025.111615.

Data availability

The multilocus genotype datasets used in this study are available on reasonable request from R.L.

References

Abbott, R.J., Barton, N.H., Good, J.M., 2016. Genomics of hybridization and its evolutionary consequences. Mol. Ecol. 25, 2325–2332. https://doi.org/10.1111/mec.13685.

Allendorf, F.W., Leary, R.F., Spruell, P., Wenburg, J.K., 2001. The problems with hybrids: setting conservation guidelines. Trends Ecol. Evol. 16, 613–622.

Anderson, T.M., vonHoldt, B.M., Candille, S.I., Musiani, M., Greco, C., Stahler, D.R., Smith, D.W., Padhukasahasram, B., Randi, E., Leonard, J.A., Bustamante, C.D., Ostrander, E.A., Tang, H., Wayne, R.K., Barsh, G.S., 2009. Molecular and evolutionary history of melanism in North American gray wolves. Science 323, 1339–1343. https://doi.org/10.1126/science.1165448.

Aragno, P., Salvatori, V., Caniglia, R., De Angelis, D., Fabbri, E., Gervasi, V., La Morgia, V., Marucco, F., Mucci, N., Velli, E., 2022. La popolazione di lupo nelle regioni dell'Italia peninsulare 2020/2021. Relazione Tec. Realiz. Nell'ambito Della Conv. ISPRA-Minist. Della Transizione Ecol. "Attività Monit. Naz. Nell'ambito Piano Azione Lupo" ISPRA.

Battilani, D., Gargiulo, R., Caniglia, R., Fabbri, E., Madrigal, J.R., Fontsere, C., Ciucani, M.M., Gopalakrishnan, S., Girardi, M., Fracasso, I., Mastroiaco, M., Ciucci, P., Vernesi, C., 2025. Beyond population size: whole-genome data reveal bottleneck legacies in the peninsular Italian wolf. J. Hered. 116, 10–23. https://doi.org/10.1093/jhered/esae041.

Bigi, D., Marelli, S.P., Randi, E., Polli, M., 2015. Genetic characterization of four native Italian shepherd dog breeds and analysis of their relationship to cosmopolitan dog breeds using microsatellite markers. Animal 9, 1921–1928.

Bohling, J.H., 2016. Strategies to address the conservation threats posed by hybridization and genetic introgression. Biol. Conserv. 203, 321–327.

Bohling, J.H., Waits, L.P., 2015. Factors influencing red wolf–coyote hybridization in eastern North Carolina, USA. Biol. Conserv. 184, 108–116. https://doi.org/10.1016/j.biocon.2015.01.013.

Boitani, L., 1984. Genetic considerations on wolf conservation in Italy. Bolletino Zool. 51, 367–373. https://doi.org/10.1080/11250008409439476.

Boitani, L., 1986. Dalla parte del lupo. I libri di Airone. G Mondadori E Assoc. Spa Milano.

Boitani, L., 2003. Wolf conservation and recovery. In: Wolves: Behaviour, Ecology and Conservation. University of Chicago Press, pp. 317–340.

Boitani, L., Ciucci, P., 1993. Wolves in Italy: critical issues for their conservation. In: Wolf in Europe: Status and Perspectives. Wildbiologische Gesel, pp. 75–90.

Boitani, L., Ciucci, P., 2009. Wolf conservation across Europe: species conservation without boundaries. In: Musiani, M., Boitani, L., Paquet, P.C. (Eds.), A New Era for Wolves and People: Wolf Recovery, Human Attitudes and Policy. The University of Calgary Press, Calgary, Alberta, Canada, pp. 15–39. ISBN 978-1-55238-270-7.

Caniglia, R., Fabbri, E., Greco, C., Galaverni, M., Manghi, L., Boitani, L., Sforzi, A., Randi, E., 2013. Black coats in an admixed wolf × dog pack is melanism an indicator of hybridization in wolves? Eur. J. Wildl. Res. 59, 543–555. https://doi.org/10.1007/s10344-013-0703-1.

Caniglia, R., Fabbri, E., Galaverni, M., Milanesi, P., Randi, E., 2014. Noninvasive sampling and genetic variability, pack structure, and dynamics in an expanding wolf population. J. Mammal. 95, 41–59.

Caniglia, R., Galaverni, M., Velli, E., Mattucci, F., Canu, A., Apollonio, M., Mucci, N., Scandura, M., Fabbri, E., 2020. A standardized approach to empirically define reliable assignment thresholds and appropriate management categories in deeply introgressed populations. Sci. Rep. 10, 2862. https://doi.org/10.1038/s41598-020-59521-2.

Canty, A., Ripley, B.D., 2024. boot: Bootstrap R (S-Plus) Functions. Funct. R Package Version 13-31.

Ciucci, P., 2012. Ibridazione con il cane come minaccia per la conservazione del lupo: status delle conoscenze e criteri per l'identificazione degli ibridi. Tech. Rep. Action A2 LIFE IBRIWOLF Proj. LIFE10NATIT265 Sapienza Univ. Rome Ital..

Ciucci, P., Boitani, L., 1998. Il Lupo. Elementi di biologia, gestione e ricerca. Istituto Nazionale della Fauna Selvatica "Alessandro Ghigi", Technical Reports no. 23. Ozzano Emilia, Italy. (In Italian).

Ciucci, P., Lucchini, V., Boitani, L., Randi, E., 2003. Dewclaws in wolves as evidence of admixed ancestry with dogs. Can. J. Zool. 81, 2077–2081. https://doi.org/10.1139/z03-183.

Ciucci, P., Chapron, G., Guberti, V., Boitani, L., 2007. Estimation of mortality parameters from (biased) samples at death: are we getting the basics right in wildlife field studies? A response to Lovari et al. (2007). J. Zool. 273, 125–127.

Ciucci, P., Reggioni, W., Maiorano, L., Boitani, L., 2009. Long-distance dispersal of a rescued wolf from the Northern Apennines to the Western Alps. J. Wildl. Manag. 73, 1300–1306. https://doi.org/10.2193/2008-510.

Ciucci, P., Mancinelli, S., Boitani, L., Gallo, O., Grottoli, L., 2020. Anthropogenic food subsidies hinder the ecological role of wolves: insights for conservation of apex predators in human-modified landscapes. Glob. Ecol. Conserv. 21, e00841. https://doi.org/10.1016/j.gecco.2019.e00841.

Ciucci, P., Sand, H., Åkesson, M., Kusak, J., 2025. Wolf Canis lupus Linnaeus, 1758. In: Hackländer, K., Zachos, F.E. (Eds.), Handbook of the Mammals of Europe, Handbook of the Mammals of Europe. Springer International Publishing, Cham, pp. 1–62. https://doi.org/10.1007/978-3-319-65038-8_118-2.

Crispino, F., Costanzo, M., Lucia, A., Gervasio, G., 2021. Early and double breeding in a pack of hybrid wolves in Calabria (Southern Italy). Biodivers J 12, 379–384.

Di Bernardi, C., Chapron, G., Kaczensky, P., Álvares, F., Andrén, H., Balys, V., Blanco, J. C., Chiriac, S., Ćirović, D., Drouet-Hoguet, N., 2025. Continuing recovery of wolves in Europe. PLoS Sustain. Transform. 4, e0000158.

Donfrancesco, V., Ciucci, P., Salvatori, V., Benson, D., Andersen, L.W., Bassi, E., Blanco, J.C., Boitani, L., Caniglia, R., Canu, A., 2019. Unravelling the scientific debate on how to address wolf-dog hybridization in Europe. Front. Ecol. Evol. 7, 175. https://doi.org/10.3389/fevo.2019.00175.

Dufresnes, C., Remollino, N., Stoffel, C., Manz, R., Weber, J.-M., Fumagalli, L., 2019. Two decades of non-invasive genetic monitoring of the grey wolves recolonizing the Alps support very limited dog introgression. Sci. Rep. 9, 148.

Dziech, A., 2021. Identification of wolf-dog hybrids in Europe–an overview of genetic studies. Front. Ecol. Evol. 9, 760160.

Fabbri, E., Miquel, C., Lucchini, V., Santini, A., Caniglia, R., Duchamp, C., Weber, J., Lequette, B., Marucco, F., Boitani, L., Fumagalli, L., Taberlet, P., Randi, E., 2007. From the Apennines to the Alps: colonization genetics of the naturally expanding Italian wolf (*Canis lupus*) population. Mol. Ecol. 16, 1661–1671. https://doi.org/10.1111/j.1365-294x.2007.03262.x.

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.

Frantz, L.A.F., Mullin, V.E., Pionnier-Capitan, M., Lebrasseur, O., Ollivier, M., Perri, A., Linderholm, A., Mattiangeli, V., Teasdale, M.D., Dimopoulos, E.A., Tresset, A., Duffraisse, M., McCormick, F., Bartosiewicz, L., Gál, E., Nyerges, É.A., Sablin, M.V., Bréhard, S., Mashkour, M., Bălăşescu, A., Gillet, B., Hughes, S., Chassaing, O., Hitte, C., Vigne, J.-D., Dobney, K., Hänni, C., Bradley, D.G., Larson, G., 2016. Genomic and archaeological evidence suggest a dual origin of domestic dogs. Science 352, 1228–1231. https://doi.org/10.1126/science.aaf3161.

Freedman, A.H., Gronau, I., Schweizer, R.M., Ortega-Del Vecchyo, D., Han, E., Silva, P. M., Galaverni, M., Fan, Z., Marx, P., Lorente-Galdos, B., Beale, H., Ramirez, O., Hormozdiari, F., Alkan, C., Vilà, C., Squire, K., Geffen, E., Kusak, J., Boyko, A.R., Parker, H.G., Lee, C., Tadigotla, V., Siepel, A., Bustamante, C.D., Harkins, T.T., Nelson, S.F., Ostrander, E.A., Marques-Bonet, T., Wayne, R.K., Novembre, J., 2014. Genome sequencing highlights the dynamic early history of dogs. PLoS Genet. 10, e1004016. https://doi.org/10.1371/journal.pgen.1004016.

Galaverni, M., Caniglia, R., Pagani, L., Fabbri, E., Boattini, A., Randi, E., 2017. Disentangling timing of admixture, patterns of introgression, and phenotypic indicators in a hybridizing wolf population. Mol. Biol. Evol. 34, 2324–2339.

Gervasi, V., Aragno, P., Salvatori, V., Caniglia, R., De Angelis, D., Fabbri, E., La Morgia, V., Marucco, F., Velli, E., Genovesi, P., 2024. Estimating distribution and abundance of wide-ranging species with integrated spatial models: opportunities revealed by the first wolf assessment in south-central Italy. Ecol. Evol. 14. https://doi.org/10.1002/ece3.11285.

Godinho, R., Llaneza, L., Blanco, J.C., Lopes, S., Álvares, F., García, E.J., Palacios, V., Cortés, Y., Talegón, J., Ferrand, N., 2011. Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Mol. Ecol. 20, 5154–5166. https://doi.org/10.1111/j.1365-294X.2011.05345.x.

Godinho, R., López-Bao, J.V., Castro, D., Llaneza, L., Lopes, S., Silva, P., Ferrand, N., 2015. Real-time assessment of hybridization between wolves and dogs: combining noninvasive samples with ancestry informative markers. Mol. Ecol. Resour. 15, 317–328. https://doi.org/10.1111/1755-0998.12313.

Gompper, M.E., 2014. Free-ranging Dogs and Wildlife Conservation. Oxford University Press, USA.

Grossen, C., Keller, L., Biebach, I., Consortium, I.G.G, Croll, D., 2014. Introgression from domestic goat generated variation at the major histocompatibility complex of Alpine ibex. PLoS Genet. 10, e1004438.

Hindrikson, M., Tammeleht, E., 2025. Introgression—friend or foe? Mol. Ecol. 34, e17810.

Hindrikson, M., Männil, P., Ozolins, J., Krzywinski, A., Saarma, U., 2012. Bucking the trend in wolf-dog hybridization: first evidence from Europe of hybridization between female dogs and male wolves. PLoS One 7, e46465. https://doi.org/10.1371/journal.pone.0046465.

Hindrikson, M., Remm, J., Pilot, M., Godinho, R., Stronen, A.V., Baltrūnaité, L., Czarnomska, S.D., Leonard, J.A., Randi, E., Nowak, C., Åkesson, M., López-Bao, J.V., Álvares, F., Llaneza, L., Echegaray, J., Vilà, C., Ozolins, J., Rungis, D., Aspi, J., Paule, L., Skrbinšek, T., Saarma, U., 2017. Wolf population genetics inEurope: a systematic review, meta-analysis and suggestions for conservation and management. Biol. Rev. 92, 1601–1629. https://doi.org/10.1111/brv.12298.

Iacolina, L., Scandura, M., Gazzola, A., Cappai, N., Capitani, C., Mattioli, L., Vercillo, F., Apollonio, M., 2010. Y-chromosome microsatellite variation in Italian wolves: a contribution to the study of wolf-dog hybridization patterns. Mamm. Biol. 75, 341–347. https://doi.org/10.1016/j.mambio.2010.02.004.

Jarausch, A., von Thaden, A., Sin, T., Corradini, A., Pop, M.I., Chiriac, S., Gazzola, A., Nowak, C., 2023. Assessment of genetic diversity, population structure and wolf-dog hybridisation in the Eastern Romanian Carpathian wolf population. Sci. Rep. 13, 22574.

Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11, 1–15.

Konec, M., Collet, S., Nowak, C., Skrbinšek, T., Stronen, A.V., 2024. Dispersal of a wolf with complex ancestry from the Dinaric-Alpine region to Germany demonstrates the value of transboundary monitoring networks. Conserv. Sci. Pract. 6 (9), e13181.

Kusak, J., Fabbri, E., Galov, A., Gomerčić, T., Arbanasić, H., Caniglia, R., Galaverni, M., Reljić, S., Huber, D., Randi, E., 2018. Wolf-dog hybridization in Croatia. Vet. Arh. 88. https://doi.org/10.24099/vet.arhiv.170314.

Linnell, J.D.C., Boitani, L., 2025. Developing methodology for setting Favourable Reference Values for large carnivores in Europe. In: Report to the European Commission under contract N° 09.0201/2023/907799/SER/ENV.D.3 "Support for Coexistence with Large Carnivores. Task B.3 – Assessment of large carnivores' conservation status". https://brage.nina.no/nina-xmlui/bitstream/handle/11250/3175288/LinnellDevelopingFRV%20REPORT%20FINAL2025diamant.pdf?sequence=2&isAllowed=y. (Accessed 8 August 2025).

Lorenzini, R., Fanelli, R., Grifoni, G., Scholl, F., Fico, R., 2014. Wolf–dog crossbreeding: "Smelling" a hybrid may not be easy. Mamm. Biol. 79, 149–156. https://doi.org/10.1016/j.mambio.2013.07.080.

Lorenzini, R., Attili, L., Tancredi, C., Fanelli, R., Garofalo, L., 2022. A validated molecular protocol to differentiate pure wolves, dogs and wolf x dog hybrids through a panel of multiplexed canine STR markers. Diversity 14, 511. https://doi.org/10.3390/d14070511.

Lovari, S., Sforzi, A., Scala, C., Fico, R., 2007. Mortality parameters of the wolf in Italy: does the wolf keep himself from the door? J. Zool. 272, 117–124. https://doi.org/10.1111/j.1469-7998.2006.00260.x.

Lucchini, V., Galov, A., Randi, E., 2004. Evidence of genetic distinction and long-term population decline in wolves (*Canis lupus*) in the Italian Apennines. Mol. Ecol. 13, 523–536. https://doi.org/10.1046/j.1365-294x.2004.02077.x.

Mallet, J., 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20, 229–237.

Marucco, F., Boiani, M.V., Dupont, P., Milleret, C., Avanzinelli, E., Pilgrim, K., Schwartz, M.K., Von Hardenberg, A., Perrone, D.S., Friard, O.P., Menzano, A., Bisi, F., Fattori, U., Tomasella, M., Calderola, S., Carolfi, S., Ferrari, P., Chioso, C., Truc, F., Bombieri, G., Pedrotti, L., Righetti, D., Acutis, P.L., Guglielmo, F., Hauffe, H.C., Rossi, C., Caniglia, R., Aragno, P., La Morgia, V., Genovesi, P., Bischof, R., 2023. A multidisciplinary approach to estimating wolf population size for long-term conservation. Conserv. Biol. 37. https://doi.org/10.1111/cobi.14132.

Mech, L.D., Barber-Meyer, S.M., Erb, J., 2016. Wolf (*Canis lupus*) generation time and proportion of current breeding females by age. PLoS One 11, e0156682. https://doi.org/10.1371/journal.pone.0156682.

Morales-González, A., Fernández-Gil, A., Quevedo, M., Revilla, E., 2022. Patterns and determinants of dispersal in grey wolves (*Canis lupus*). Biol. Rev. 97 (2), 466–480.

Moura, A.E., Tsingarska, E., Dąbrowski, M.J., Czarnomska, S.D., Jędrzejewska, B., Pilot, M., 2014. Unregulated hunting and genetic recovery from a severe population decline: the cautionary case of Bulgarian wolves. Conserv. Genet. 15 (2), 405–417.

Musto, C., Cerri, J., Galaverni, M., Caniglia, R., Fabbri, E., Apollonio, M., Mucci, N., Bonilauri, P., Maioli, G., Fontana, M.C., 2021. Men and wolves: anthropogenic causes are an important driver of wolf mortality in human-dominated landscapes in Italy. Glob. Ecol. Conserv. 32, e01892. https://doi.org/10.1016/j.gecco.2021.e01892.

Nielsen, E.E., Bach, L.A., Kotlicki, P., 2006. HYBRIDLAB (version 1.0): a program for generating simulated hybrids from population samples. Mol. Ecol. Notes 6, 971–973. https://doi.org/10.1111/j.1471-8286.2006.01433.x.

Pacheco, C., López-Bao, J.V., García, E.J., Lema, F.J., Llaneza, L., Palacios, V., Godinho, R., 2017. Spatial assessment of wolf-dog hybridization in a single breeding period. Sci. Rep. 7, 42475.

Peltola, T., Heikkilä, J., 2018. Outlaws or protected? DNA, hybrids, and biopolitics in a Finnish wolf-poaching case. Soc. Anim. 26, 197–216.

Pilot, M., Greco, C., vonHoldt, B.M., Randi, E., Jędrzejewski, W., Sidorovich, V.E., Konopiński, M.K., Ostrander, E.A., Wayne, R.K., 2018. Widespread, long-term admixture between grey wolves and domestic dogs across Eurasia and its implications for the conservation status of hybrids. Evol. Appl. 11, 662–680. https://doi.org/10.1111/eva.12595.

Pilot, M., Moura, A.E., Okhlopkov, I.M., Mamaev, N.V., Manaseryan, N.H., Hayrapetyan, V., Kopaliani, N., Tsingarska, E., Alagaili, A.N., Mohammed, O.B., Ostrander, E.A., Bogdanowicz, W., 2021. Human-modified canids in human-modified landscapes: the evolutionary consequences of hybridization for grey wolves and free-ranging domestic dogs. Evol. Appl. 14, 2433–2456. https://doi.org/10.1111/eva.13257.

Ramasamy, R.K., Ramasamy, S., Bindroo, B.B., Naik, V.G., 2014. STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. SpringerPlus 3, 1–3.

Randi, E., 2008. Detecting hybridization between wild species and their domesticated relatives. Mol. Ecol. 17, 285–293. https://doi.org/10.1111/j.1365-294x.2007.03417.x.

Randi, E., Lucchini, V., 2002. Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conserv. Genet. 3, 29–43. https://doi.org/10.1023/a:1014229610646.

Randi, E., Hulva, P., Fabbri, E., Galaverni, M., Galov, A., Kusak, J., Bigi, D., Bolfíková, B.Č., Smetanova, M., Caniglia, R., 2014. Multilocus detection of wolf x dog hybridization in Italy, and guidelines for marker selection. PLoS One 9, e86409.

Ražen, N., Brugnoli, A., Castagna, C., Groff, C., Kaczensky, P., Kljun, F., Potočnik, H., 2016. Long-distance dispersal connects Dinaric-Balkan and Alpine grey wolf (*Canis lupus*) populations. Eur. J. Wildl. Res. 62, 137–142.

Rutledge, L.Y., Patterson, B.R., Mills, K.J., Loveless, K.M., Murray, D.L., White, B.N., 2010. Protection from harvesting restores the natural social structure of eastern wolf packs. Biol. Conserv. 143, 332–339.

Salvatori, V., Godinho, R., Braschi, C., Boitani, L., Ciucci, P., 2019. High levels of recent wolf × dog introgressive hybridization in agricultural landscapes of central Italy. Eur. J. Wildl. Res. 65, 73. https://doi.org/10.1007/s10344-019-1313-3.

Salvatori, V., Donfrancesco, V., Trouwborst, A., Boitani, L., Linnell, J.D., Alvares, F., Åkesson, M., Balys, V., Blanco, J.C., Chiriac, S., 2020. European agreements for nature conservation need to explicitly address wolf-dog hybridisation. Biol. Conserv. 248, 108525.

Santostasi, N.L., Ciucci, P., Bearzi, G., Bonizzoni, S., Gimenez, O., 2020. Assessing the dynamics of hybridization through a matrix modelling approach. Ecol. Model. 431, 109120.

Santostasi, N.L., Gimenez, O., Caniglia, R., Fabbri, E., Molinari, L., Reggioni, W., Ciucci, P., 2021. Estimating admixture at the population scale: taking imperfect detectability and uncertainty in hybrid classification seriously. J. Wildl. Manag. 85, 1031–1046. https://doi.org/10.1002/jwmg.22038.

Santostasi, N.L., Bauduin, S., Grente, O., Gimenez, O., Ciucci, P., 2025. Simulating the efficacy of wolf–dog hybridization management with individual-based modeling. Conserv. Biol. 39, e14312. https://doi.org/10.1111/cobi.14312.

Skoglund, P., Gotherstrom, A., Jakobsson, M., 2011. Estimation of population divergence times from non-overlapping genomic sequences: examples from dogs and wolves. Mol. Biol. Evol. 28, 1505–1517. https://doi.org/10.1093/molbev/msq342.

Smeds, L., Aspi, J., Berglund, J., Kojola, I., Tirronen, K., Ellegren, H., 2021. Whole-genome analyses provide no evidence for dog introgression in Fennoscandian wolf populations. Evol. Appl. 14, 721–734. https://doi.org/10.1111/eva.13151.

Sonne, C., Hansen, H.P., Alstrup, A.K.O., Olsen, K., Jensen, T.H., Haugaard, L., Sunde, P., 2019. Discussion: illegal kills of protected wolves call for public reasoning. Sci. Total Environ. 665, 617–619. https://doi.org/10.1016/j.scitotenv.2019.01.432.

Stenøien, H.K., Sun, X., Martin, M.D., Scharff-Olsen, C.H., Alonso, G.H., Martins, N.F.G., Lanigan, L., Ciucani, M.M., Sinding, M.-H.S., Gopalakrishnan, S., Gilbert, M.T.P., 2021. Genetisk opphav til den norsksvenske ulvestammen (*Canis lupus lupus*) – NTNU Vitenskapsmuseet naturhistorisk rapport 2021–11, pp. 1–53.

Stronen, A.V., Aspi, J., Caniglia, R., Fabbri, E., Galaverni, M., Godinho, R., Kvist, L., Mattucci, F., Nowak, C., von Thaden, A., 2022a. Wolf-dog admixture highlights the need for methodological standards and multidisciplinary cooperation for effective governance of wild x domestic hybrids. Biol. Conserv. 266, 109467.

Stronen, A.V., Mattucci, F., Fabbri, E., Galaverni, M., Cocchiararo, B., Nowak, C., Godinho, R., Ruiz-González, A., Kusak, J., Skrbinšek, T., 2022b. A reduced SNP panel to trace gene flow across southern European wolf populations and detect hybridization with other *Canis* taxa. Sci. Rep. 12, 4195.

Stronen, A.V., Birkenhain, C.S., Caniglia, R., Djan, M., Fabbri, E., Fumagalli, L., Galaverni, M., Godinho, R., Lobo, D., Mattucci, F., 2025. European conservation policy needs a clear definition of 'wolf-dog hybrid.'. Biol. Conserv. 308, 111254.

Tirronen, K.F., Kuznetsova, A.S., 2025. Wolf-dog hybrids in the eastern fringe Northern European wolf population on the background of increased hunting pressure. Polar Biol. 48 (1), 1.

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., 2016. Hybridization and extinction. Evol. Appl. 892–908.

Trouwborst, A., 2014. Exploring the legal status of wolf-dog hybrids and other dubious animals: international and EU law and the wildlife conservation problem of hybridization with domestic and alien species. Rev. Eur. Comp. Int. Environ. Law 23, 111–124. https://doi.org/10.1111/reel.12052.

Verardi, A., Lucchini, V., Randi, E., 2006. Detecting introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. Mol. Ecol. 15, 2845–2855. https://doi.org/10.1111/j.1365-294x.2006.02995.x.

Vilà, C., Wayne, R.K., 1999. Hybridization between wolves and dogs. Conserv. Biol. 13, 195–198. https://doi.org/10.1046/j.1523-1739.1999.97425.x.

Wilkins, A.S., Wrangham, R.W., Fitch, W.T., 2014. The "domestication syndrome" in mammals: a unified explanation based on neural crest cell behavior and genetics. Genetics 197, 795–808.

Zimen, E., Boitani, L., 1975. Number and Distribution of Wolves in Italy.