



Original research article

# Trophic overlap between wolves and free-ranging wolf × dog hybrids in the Apennine Mountains, Italy



Elena Bassi <sup>a,b</sup>, Antonio Canu <sup>a,b</sup>, Ilaria Firmo <sup>a</sup>, Luca Mattioli <sup>c</sup>,  
Massimo Scandura <sup>a,\*</sup>, Marco Apollonio <sup>a</sup>

<sup>a</sup> Department of Science for Nature and Environmental Resources, University of Sassari, Via Muroni 25, I-07100 Sassari, Italy

<sup>b</sup> C.I.R.Se.M.A.F. Piazzale delle Cascine 18, I-50144 Firenze, Italy

<sup>c</sup> Regione Toscana, Settore Attività Faunistico venatoria, Pesca Dilettantistica, Pesca in Mare, Via Testa 2, I-52100 Arezzo, Italy

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## ABSTRACT

Hybridization between wolves (*Canis lupus*) and domestic dogs (*Canis familiaris*) can represent a threat to wild populations via genetic introgression and ecological competition. Therefore understanding the ecological role of hybrids may be crucial for developing appropriate conservation strategies.

The Italian wolf population has a peculiar genetic composition due to a long-lasting geographic isolation. Nowadays, however, its genetic integrity is threatened by the spread of canine genes as a result of the hybridization with stray dogs in the wild.

The aim of the present study was to gain insights into the ecological role of free-ranging wolf–dog hybrids by investigating their winter food habits in comparison with wolves in a mountain area of Central Italy. Levels of genetic introgression from the dogs were assessed in two adjacent areas occupied by up to five different packs by analyzing non-invasive samples and carcasses collected therein with a set of uniparental and bi-parental molecular markers.

The obtained results enabled us to classify the two areas as ‘hybrid’ and ‘wolf’ areas based on their level of genetic introgression.

Trophic niche and similarity/dissimilarity analyses did not detect significant difference in the diet between the two areas: in both of them, wild boar was the main prey, followed by roe deer. Furthermore, the same age/body mass classes of the two ungulates were selected by wolves and hybrids. Our findings confirmed wolf–dog hybrids as potential competitors for wolves. Further studies on other aspects of their biology and ecology are recommended in order to better estimate the impact of hybridization on natural wolf populations.

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## 1. Introduction

Integrative hybridization is an expanding phenomenon which is compromising the genetic integrity of native populations, causing the extinction of many taxa (Allendorf and Luikart, 2007). It can have serious consequences on morphology, physiology, behavior and individual fitness, but its long term evolutionary consequences remain largely unpredictable (see Rhymer and Simberloff, 1996; Allendorf and Luikart, 2007).

\* Corresponding author. Fax: +39 079228665.

E-mail address: [scandura@uniss.it](mailto:scandura@uniss.it) (M. Scandura).

Hybrids may show a lower fitness compared to the parental taxa (outbreeding depression) due to a loss of local adaptation to environmental conditions (Allendorf and Luikart, 2007). However, in some cases, hybrids can show equal or superior fitness in new and, occasionally, even in parental habitats (Seehausen, 2004).

Wolf-like canids (genus *Canis*) evolved relatively recently and retained the potential to hybridize in nature (Wayne et al., 1997), thus giving rise to new taxa that may quickly adapt to prey community, landscape and climate changes (Randi et al., 2014). Several examples of hybridization have been reported between dogs and wild canids (e.g., *C. simensis*, Gottelli et al., 1994; *C. aureus*, Galov et al., 2015). In particular, the process of introgressive hybridization between the gray wolf and its domestic counterpart has become a growing concern for conservationists in Europe. Indeed, the spread of canine genes into wolf populations could disrupt local adaptation (Vilà and Wayne, 1999), potentially representing a serious threat to the long term survival of genetically pure wolf populations in the wild (Boitani, 2003).

Wolf–dog hybridization is of ancient origin (~10 000 years ago, Schwartz, 1997). Intentional wolf–dog crossbreeding was widely employed in order to create different wolf–dog breeds (Iljin, 1941). The risk of hybridization was often assumed to be higher next to human settlements, where stray and owned dogs are abundant (Boitani, 1983; Blanco et al., 1992), and in areas where wolves occur at low densities or are strongly persecuted (Randi et al., 2000; Andersone et al., 2002). The occurrence of an expansion phase for the wolf population was also reported to be an important factor favoring hybridization (Lorenzini et al., 2014).

In Europe, wolf–dog hybridization has been reported to occur with relatively low frequency in Scandinavia (Vilà et al., 2003) and in the Iberian Peninsula (Godinho et al., 2011), while it seems to be more widespread and frequent in Bulgaria, Latvia and Estonia (Randi et al., 2000; Hindrikson et al., 2012).

The extensive contact between wolves and dogs in Italy also resulted in wolf–dog hybridization, of which many authors have found increasing evidence (Randi and Lucchini, 2002; Verardi et al., 2006; Iacolina et al., 2010; Caniglia et al., 2013; Lorenzini et al., 2014; Randi et al., 2014).

Due to the past persecution, the Italian wolf population suffered from a severe bottleneck and a prolonged isolation which led to genetic erosion (Lucchini et al., 2004). Though in recent decades both its population size and range have increased, the Italian wolf is still considered vulnerable and wolf–dog hybridization is recognized as a major issue in the national action plan for wolf conservation (Genovesi, 2002).

Several studies have been conducted on wolves and dogs separately in order to better understand their behavior and role in ecological communities (Mech and Boitani, 2003; Huges and Macdonald, 2013; Gompper, 2014; Vanak et al., 2014), as well as their possible interactions (Kojola et al., 2004; Lescureux and Linnell, 2014).

Donadio and Buskirk (2006) argued that an intense competition between wolf and dog may exist in case of sympatry. In natural environments, wolves are mainly active predators of wild ungulates (Okarma, 1995; Mattioli et al., 2011; Davis et al., 2012; Bassi et al., 2012), while dogs mainly act like scavengers, but both can use such food resources as livestock and garbage (Boitani, 1983; Ovsyanikov and Poyarkov, 1996; Vanak and Gompper, 2009).

Despite the increasing number of studies focusing on genetic aspects to assess the amount of introgression in natural populations, Lescureux and Linnell (2014) warned about the lack of data on the behavior and ecology of wolf–dog hybrids under free-ranging conditions. In particular, the feeding ecology of hybrids is still unknown.

The aim of the present study was to provide preliminary information about the ecological role of hybrids in free-ranging conditions, also checking for differences in food habits with respect to sympatric wolves. In order to reach this goal, we first used molecular markers to assess the degree of introgression in packs living in two adjacent areas in the Apennine Mountains, one of which hosted individuals with anomalous morphological traits.

## 2. Materials and methods

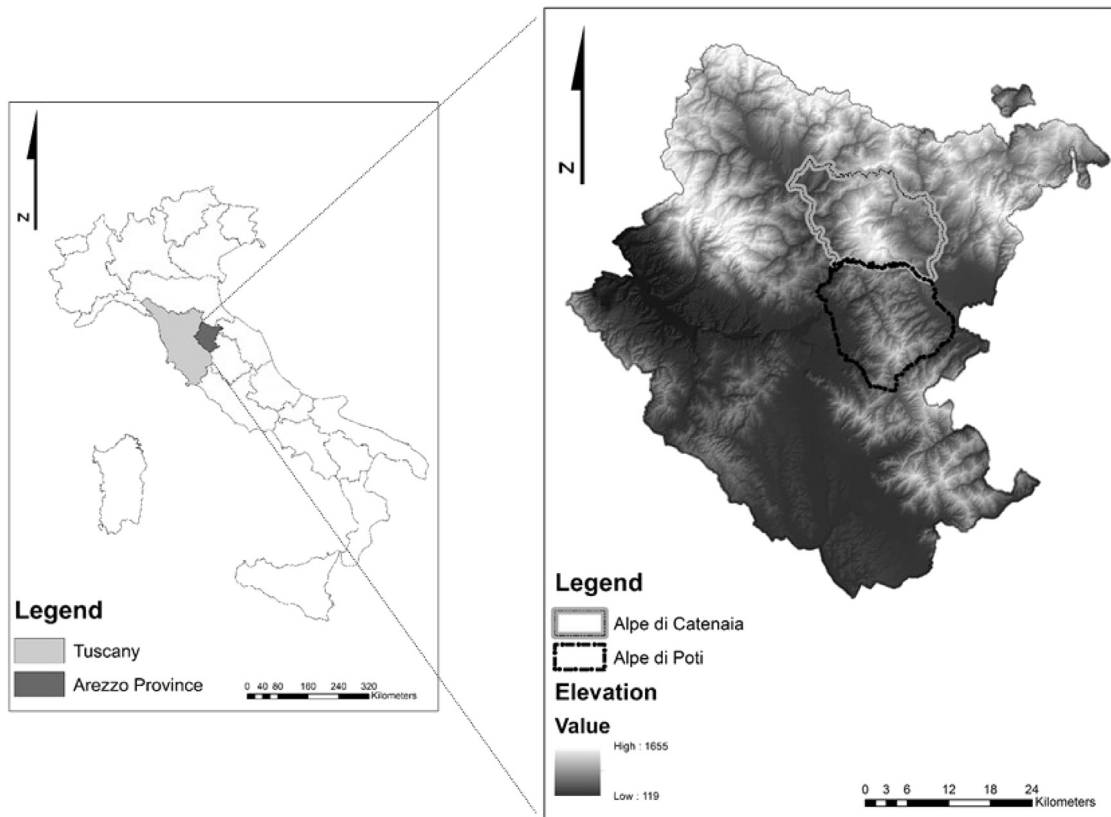
### 2.1. Study area

The study area is located in the province of Arezzo, Tuscany, Italy, on two minor massifs named Alpe di Catenaia (AC) and Alpe di Poti (AP) (Fig. 1).

The two ridges cover an area of 458 km<sup>2</sup> (AP 234 km<sup>2</sup>; AC 224 km<sup>2</sup>). AC includes a protected area of 27 km<sup>2</sup> where hunting is banned. Altitude ranges from 300 to 1414 m a.s.l. in AC and between 200 and 990 m a.s.l. in AP. In both areas, vegetation cover is mainly composed of mixed deciduous hardwoods. The climate is temperate and seasonal with hot and dry summers, and cold and wet winters.

The Province of Arezzo is inhabited by a wolf population which has been continuously monitored since 1998 by direct observations, wolf-howling surveys, snow-tracking, non-invasive genetics (see Scandura et al., 2011; Bassi et al., 2015) and, more recently, by camera trapping. The presence of 1–2 resident wolf packs has been recorded in the AC area ever since. Conversely, the AP area has been inhabited by wild canids (wolves and hybrids) only since 2004, being frequented by a maximum of three packs. Hence, the comparative data on the two areas of the present study refer to the period 2004–2013.

In both areas, wild ungulates are mainly represented by roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*); red deer (*Cervus elaphus*) has been occasionally observed in AC. Among lagomorphs, only the brown hare (*Lepus europaeus*) is present. A number of farms, mostly raising sheep (*Ovis aries*), constitute a potential additional source of prey for wolves and hybrids.



**Fig. 1.** Study areas Alpe di Catenaiia and Alpe di Poti in the Province of Arezzo (right) and position of this province in Italy (left).

## 2.2. Hybridization assessment

### 2.2.1. Sampling and genotyping

Scats and hairs were collected in AC and AP, as well as in the rest of the province, along trails chosen on the basis of documented wolf presence. We also opportunistically sampled tissues from carcasses recovered in the province and nearby areas. For each carcass, a close morphological examination was carried out in order to detect any anomalous phenotypic trait (such as melanism, spur in hind-legs, abnormal coat color patterns) possibly due to the introgression of domestic genes.

Since the start of genetic monitoring, a total of 1148 samples were analyzed, including 1109 invasive and non-invasive wolf (or presumed wolf) samples from the Province of Arezzo and 49 samples from other provinces in Tuscany (i.e., scats, hairs and tissues). DNA was extracted from scats following Gerloff et al. (1995). The GenElute Mammalian DNA miniprep Kit (Sigma-Aldrich, St Louis, Missouri) and the InstaGene Matrix (Bio-Rad, Hercules, CA, USA) were employed for extracting DNA from tissues and hairs, respectively. As for laboratory analyses, we followed the recommendations provided by Budowle et al. (2005) for animal DNA forensics.

Samples were PCR-amplified and genotyped at 12 unlinked autosomal microsatellites: six dinucleotides (CPH2, CPH4, CPH5, CPH8, CPH12; Fredholm and Wintero, 1995; C09.250 Ostrander et al., 1993) and six tetranucleotides (FH2004, FH2079, FH2088, FH2096, FH2132 and FH2137; Francisco et al., 1996). PCR conditions were optimized for each primer pair and are available upon request. Non-invasive samples were genotyped by NGB Genetics Srl (Bologna, Italy) by using a multiple-tube protocol as implemented by Lucchini et al. (2002). Consensus genotypes were reconstructed by using GIMLET 1.3.3 (Valière, 2002). Gender of non-invasively sampled individuals and non-sexed wolf carcasses was determined by employing the Amelogenin marker as in Randi et al. (2014). The genotyping led to the identification of more than 200 different putative wolf genotypes, corresponding to different individuals on the basis of the probability of identity for sibs ( $P_{(ID)sib}$ , Waits et al., 2001).

In addition, for each individual, a portion of the hypervariable domain of the mtDNA control region 1 (CR1) was amplified and sequenced following Vilà et al. (1999). According to Iacolina et al. (2010), males were analyzed at two Y-chromosome microsatellite loci (MS34A and MS34B), and alleles at the two linked loci were combined to construct haplotypes.

All the aforementioned analyses were also carried out for 39 dogs (either owned or stray dogs kept in kennels) sampled in the same areas.

### 2.2.2. Admixture analyses

The first step to assess the degree of admixture of wolves sampled in the AC and AP areas was the creation of a reference dataset of supposedly pure Italian wolves and dogs. As regards wolves, we considered all the different individuals successfully genotyped at  $\geq 10$  loci, except those sampled in AC and AP. Then, to be conservative, we removed each animal showing a sign of possible introgression from the dataset. Therefore, each individual having a canine haplotype in Y-chromosome or not showing the typical Italian wolf haplotype w14 at the CR1 (Randi et al., 2014) was discarded. Additionally, genotypes were removed whenever associated to any anomalous phenotypic trait (on carcasses). A total of 37 wolves were retained. Their microsatellite genotypes, along with those of the 39 dogs, were included in a first dataset with the aim of identifying and removing any additional individual showing introgression at autosomal microsatellites. For this purpose, we performed a first analysis by employing the Bayesian clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000), with the following settings in the prior: admixture model, uncorrelated allele frequencies among populations, and no population information. Ten independent runs were carried out for a number of genetic clusters varying from  $K = 1$  to  $K = 10$ , with 250 000 iterations following a burn-in period of 250 000 iterations. The most likely value of  $K$  was determined according to the method developed by Evanno et al. (2005) in order to verify the prevailing partition into two main groups (dogs and wolves). As expected, our results indicated  $K = 2$  as the most likely partition, thus identifying a dog and a wolf cluster. We considered the  $Q$ -values of the run with highest posterior probability and removed all the individuals showing less than 97.5% of membership to a single cluster. In so doing, we obtained the reference populations of pure wolves ( $n = 34$ ) and dogs ( $n = 37$ ) showing no evidence of introgression at any marker. A second dataset was then created, including the reference populations and 47 different genotypes from the AC ( $n = 24$ ) and AP ( $n = 23$ ) areas sampled between winters 2004/05 and 2012/13. Subsequently, STRUCTURE was run 10 times, with  $K$  fixed at 2, with 250 000 iterations and 250 000 burn-ins, admixture model, uncorrelated allele frequencies and the option “update  $p$  from pop flag only” activated. In this way, the estimated allele frequencies of the wolf and dog reference clusters were not affected by the allele frequencies of the other samples to be classified. The degree of admixture of individuals inhabiting the AC and AP areas was assessed as the individual membership to the two clusters inferred ( $Q_{\text{WOLF}}$  for wolves and  $Q_{\text{DOG}}$  for dogs, respectively) by considering the run with the highest posterior probability.

### 2.3. Prey abundance assessment

Densities of wild boar and roe deer within the study area were estimated by the Provincial Administration of Arezzo with drive censuses every May (described in Mattioli et al., 2004). Population surveys took place each spring in both the protected and non-protected portions of the study area: between 13 and 19 (AC) and between 16 and 19 (AP) forest blocks were sampled each year.

Animal density within each forest block surveyed was estimated as described in Davis et al. (2012). Post-birth abundance of roe deer was estimated on the basis of their spring counts, percentage of adult females in the population and female fertility. The percentage of adult females in the population was estimated by direct observations during the drive censuses, while female fertility was estimated by counting the fetuses found in females shot by hunters. Wild boar population structure was derived from direct observations, while the sex ratio in the population and fertility parameters were obtained through an analysis of fetuses found in the uteri of the females shot in regular hunts.

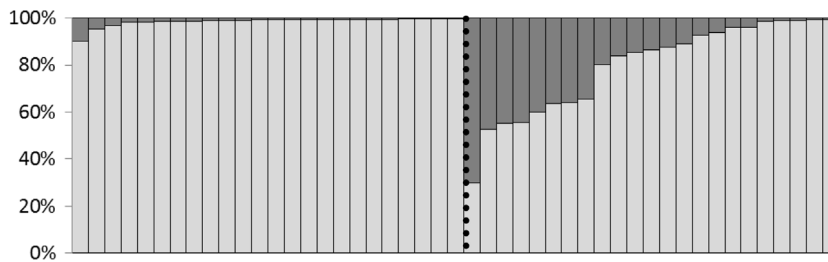
### 2.4. Diet assessment and trophic niche analysis

From 2004/2005 to 2012/2013, a total of 515 and 339 winter scats were collected in AC and AP, respectively. In order to have a comparable sample size between the two areas, we randomly created and used a subsample of 339 winter scats for AC.

The diet compositions were determined by means of scat analysis. Scats were washed in a sieve of 0.5 mm and the macroscopic prey remains (e.g., hairs and bones), fruit and grasses found in every scat were dried at 68 °C for 24 h. Prey categories included wild boar, roe deer, hare, small rodents and livestock (goats, sheep and cattle). Prey remains were identified by comparison with a reference collection of mammal hairs, bones and teeth. When possible, we identified the prey species and age or body mass class (only for the ungulates). This identification was based on the macroscopic characteristics of hairs and bones following Mattioli et al. (2011). Boar remains were assigned to one of the following three body mass classes: newborn piglet (<10 kg), piglet (10–35 kg) and adult (>35 kg). Roe deer remains were classified into two classes: juvenile (<12 months) and adult (>12 months) (for the methodology, see Mattioli et al., 2011). Blind tests were conducted in order to verify the operators' skills to discriminate among species and age/sex classes: 50 bags with different ungulates fur and bones were randomly assigned to each operator (with further 25 random samples specifically aimed at identifying wild boar body mass classes). Only the operators who correctly identified all the test samples were selected to analyze the scat samples collected.

In order to estimate the contribution of each species to the diet, we calculated the average percentage volume (AV%) and the relative biomass value for each food item. The relative biomass was calculated for each species by using the relative volume values and applying the biomass models of Weaver (1993) and Ciucci et al. (2001).

The trophic niche breadth and overlap between hybrids and wolves were evaluated by using, respectively, Levins' index (1968) and Pianka's index (1973) applied to the volume of food categories in their diet.



**Fig. 2.** Estimated proportions of membership to the two clusters inferred (dog: dark gray; wolf: light gray) by STRUCTURE analysis, performed on 24 putative wolves sampled in the Alpe di Catenaiia (AC, on the left of the dotted line) and 23 wolves/hybrids in the Alpe di Poti (AP, on the right). Each individual is represented by a vertical bar.

Additionally, we performed Chi-Square tests to check for any significant difference in the diet composition between the two areas. Then, we compared presence–absence data for prey categories by using non-metric multidimensional scaling based on a Bray–Curtis (BC) dissimilarity matrix; the value obtained from this analysis ranged from 0 (identical diet composition) to 1 (totally different composition). The analysis was replicated by using both methods of diet quantification (AV% and relative biomass) and considering wild boar and roe deer as macro-categories in the first place, and according to their body mass/age classes at a second stage.

Subsequently, the analysis of similarities (ANOSIM) and the similarity percentage analysis (SIMPER) were carried out in order to understand, respectively, whether the use of food items differed between the two areas and which prey category contributed the most to the similarity observed (Clarke, 1993).

Finally, we calculated the Manly's selectivity index (Manly et al., 1972) to determine the preference of wolves and hybrids for the two main prey species (i.e., wild boar and roe deer) for each winter season ( $n = 7$ ; only for winters in which we had  $>20$  scats for each area). In so doing, we used the estimated density values obtained by drive census on roe deer and wild boar, as described by Davis et al. (2012). All the analyses were performed in R 3.1.2 (<http://cran.r-project.org/>).

### 3. Results

#### 3.1. Genetic analysis

Out of 47 different individuals genotyped in the two areas in the period 2004–2013, no AC individual was classified as hybrid by using a threshold of 90% membership to the wolf cluster (i.e.,  $Q_{WOLF} < 90\%$ ), whereas as many as 14 out of 23 AP individuals showed an admixed ancestry (Fig. 2). By elevating the threshold to  $Q_{WOLF} < 95\%$ , these values increased to 1 AC and 16 AP individuals.  $Q_{WOLF}$  averaged 0.985 in AC and 0.797 in AP. The difference among  $Q_{WOLF}$  values in the two areas was highly significant (Wilcoxon rank sum test:  $W = 488$ ,  $p < 0.001$ ). Uniparental markers did not reveal any striking difference between AC and AP: 100% of animals in both areas carried the Italian wolf haplotype w14. A canine Y-chromosome haplotype (i.e., H3 in Iacolina et al., 2010) occurred in males of both areas with a similar frequency (0.10 in AC and 0.125 in AP).

Nevertheless, canine Y-haplotypes were associated with  $Q_{WOLF} > 0.99$  in AC and with  $Q_{WOLF} < 0.50$  in AP, possibly indicating a past introgression in the former area and a more recent hybridization in the latter.

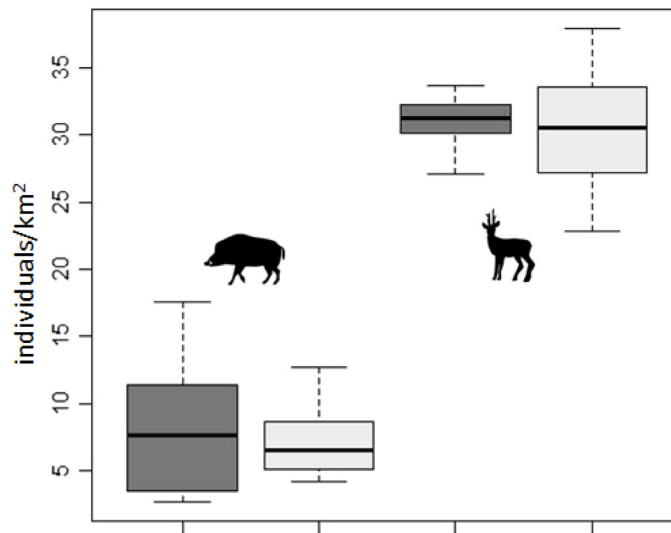
In the light of these genetic results, for the sake of simplicity, below we will refer to AP as 'hybrid' area and to AC as 'wolf' area.

#### 3.2. Prey abundance

No relevant difference in the density of the two main prey species was observed between the two areas (Fig. 3). Roe deer was the most abundant prey species in both areas, with an average density of 30.9 individuals/km<sup>2</sup> in AC and 30.6 individuals/km<sup>2</sup> in AP (range of post-reproductive densities: AC = 27.1–33.7; AP = 22.9–37.9 deer/km<sup>2</sup>). During the study period, the roe deer population resulted to be relatively stable in the study areas. Conversely, wild boar was the less abundant prey species and its population showed large fluctuations, with average densities of 8.4 and 7.2 boars/km<sup>2</sup> in AC and AP, respectively (range: AC = 2.7–17.5 boars/km<sup>2</sup>; AP = 4.2–12.7 boars/km<sup>2</sup>).

#### 3.3. Diet analysis

We identified 8 food categories for the AP area and 11 for the AC area (Table 1). Wolves and wolf–dog hybrids from the two neighboring areas had a very similar diet composition. Wild ungulates were the main category consumed in both areas (in total AV% = 95.00% and biomass% = 98.73% in AP and AV% = 92.23% and biomass% = 94.41% in AC) among them, wild boar represented the main item, followed by roe deer. Hares, small mammals, livestock and vegetables can be considered accessory food items, amounting, in total, to 5.00% (AV%) for the AP area and 7.77% (AV%) for the AC area. Levins index



**Fig. 3.** Boxplot of unguulate densities estimated between 2004 and 2013. The number of wild boar (left) and roe deer (right) per km<sup>2</sup> is reported on the Y axis. Density values of individuals in Alpe di Catenaia (AC, wolf area) and Alpe di Poti (AP, hybrid area) are represented by dark gray and light gray boxes, respectively.

**Table 1**

Diet composition for the two areas (AP: Alpe di Poti; AC: Alpe di Catenaia). Results are reported as average volume percentage (AV%) and biomass percentage for both areas.

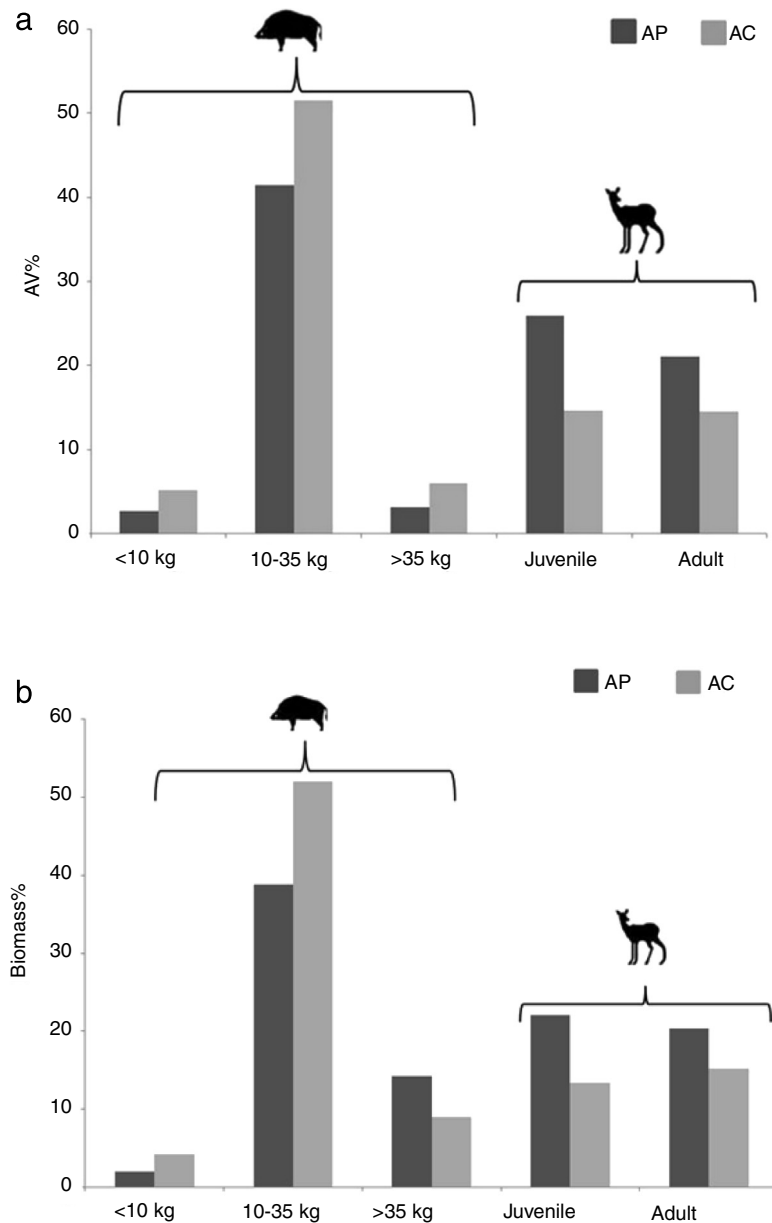
Food item	AP (hybrid)		AC (wolf)	
	AV%	Biomass %	AV%	Biomass %
Wild boar	47.23	55.09	62.76	65.34
Roe deer	47.08	42.49	29.24	28.65
Cervids	0.68	1.15	0.23	0.42
Wild ungulates total	95.00	98.73	92.23	94.41
Hare	0.00	0.00	1.77	1.34
Small mammals	0.15	0.10	0.46	0.33
Sheep	0.15	0.22	1.23	1.95
Goat	0.00	0.00	0.31	0.40
Cow	0.00	0.00	0.31	0.66
Livestock	0.61	0.94	0.54	0.90
Vegetables	3.56	–	2.31	–
Other	0.53	–	0.85	–
Other categories total	5.00	1.27	7.77	5.59

indicated a specialization on a maximum of two main items in both areas, i.e., wild boar and roe deer (Levins index: AP = 2.24, AC = 2.08 (AV%); AP = 2.06, AC = 1.96 (biomass%)). Pianka's index revealed an almost complete overlap for both methods of diet quantification (OAV% = 0.94, Obiom% = 0.97). Chi-square tests confirmed that there was no significant difference in diet composition between AC and AP ( $\chi^2_{AV\%} = 11.54, p > 0.05$  and  $\chi^2_{biom\%} = 7.8, p > 0.05$ ).

With regard to the fecal content represented by wild ungulates only (Fig. 4(a) and (b)), body mass and age classes of wild boars and roe deer appeared to be used in similar proportions in the two areas. As for wild boar, the most frequently used class was piglets (AV% = 41.49% in AP and AV% = 51.56% in AC), followed by adults (AV% = 3.11% in AP and AV% = 6.03% in AC) and newborn piglets (AV% = 2.63% in AP and AV% = 5.17% in AC). As regards roe deer, the most frequently used age class was represented by juveniles (AV% = 25.96% in AP and AV% = 14.67% in AC), followed by adults (AV% = 21.12% in AP and AV% = 14.57% in AC). Even in this case, we did not detect significant difference between the two areas either in the use of wild boar ( $\chi^2_{AV\%} = 0.66, p > 0.05$ , and  $\chi^2_{biom\%} = 3.04, p > 0.05$ ) or in the use of roe deer ( $\chi^2_{AV\%} = 0.17, p > 0.05$ , and  $\chi^2_{biom\%} = 0.17, p > 0.05$ ).

The results from the BC dissimilarity matrix indicated very low dissimilarity between the two areas: the BC values obtained were 0.20 (AV%) and 0.14 (biomass%) when the two main prey species were used as categories, and remained low when these were split into body mass/age classes: 0.20 (AV%) and 0.19 (biomass%).

The analysis of similarity showed no difference in the use of wild boar and roe deer in the two areas ( $p > 0.1$ ), while a difference in the use of accessory items ( $R = 1, p = 0.01$ ) was detected, likely due to a higher use of livestock in AC



**Fig. 4.** Use of different body mass and age classes of the two main prey species by packs in the two areas (AC, Alpe di Catenaiia, wolf area; AP, Alpe di Poti, hybrid area), reported in terms of either AV% (a) or relative biomass (b). Wild boars are divided into three body mass classes (< 10 kg, 10–35 kg and > 35 kg), and roe deer into two age classes (juvenile and adult).

(cumulative AV%: = 0.76% in AP and 2.38% in AC). The similarity observed seemed to be explained mainly by a similar use of wild boar (SIMPER results: 0.58% of the overall similarity,  $p = 0.01$ ), while the use of roe deer could not account for it ( $p > 0.1$ ).

Manly's selectivity index, calculated for each winter season, highlighted a strong positive selection for wild boar in both areas, with values of  $\alpha$  ranging between 0.89 (year 2005/2006) and 0.96 (2010/2011) for AC and between 0.78 (2005/2006) and 0.92 (2006/2007) for AP.

#### 4. Discussion

In a recent review on the interactions between wolves and dogs, [Lescureux and Linnell \(2014\)](#) called attention to the lack of data on the behavior and ecology of wolf–dog hybrids under free-ranging conditions, which undermines any discussion on the ecological impact of hybridization. This represents a big limitation in such areas as Italy, where the increased number

of diagnosed hybrids is seen with raising concern (Lorenzini et al., 2014; Randi et al., 2014). Accordingly, in the present study, we detected evident signs of introgression from the dog in the resident wolves inhabiting a mountain area of Central Italy.

During the study period, we detected strikingly different levels of introgression in two adjacent areas: individuals sampled in AP were highly introgressed as a consequence of presumably recent hybridization events, while those inhabiting AC only showed little sign of a seemingly past introgression. As expected considering the known asymmetry in the hybridization process (male dog  $\times$  female wolf, Hindrikson et al., 2012), introgression from the dog into the local wolf population appeared only in autosomal and patrilineal molecular markers.

Our results showed that, in similar ecological conditions, wolf–dog hybrids have the same food preferences as wolves, with the tendency to feed mainly on wild ungulates. As suggested by all our statistical analyses on scat content data, independently from the degree of canine introgression, packs inhabiting AC and AP areas consumed the same prey items, relying in a similar way on the different age and body mass classes.

In both wolf and hybrid areas, individuals selected wild boar as prey species, preferring piglets between 10 and 35 kg; though roe deer was the most abundant and available species, it represented a second choice, with juveniles being the class selected.

Is this trophic behavior transmitted by the wolf parental line? Several studies have been conducted on dog and wolf food habits, separately.

Even though some studies have shown the capability of free-ranging dogs to kill and feed on wildlife (Kuuk and Snell, 1981; Campos et al., 2007) and their ability to prey large-sized mammals (Boitani, 1995; Butler and du Toit, 2002), the majority of research highlighted the tendency of dogs to rely mainly on such anthropogenic food as livestock and garbage (Butler and du Toit, 2002; Butler et al., 2004; Vanak and Gompper, 2009; Echegaray and Vilà, 2010).

Wolves feed mainly on wild ungulates, with a small percentage of their diet made up by accessory items according to their opportunistic behavior (Okarma, 1995; Gazzola et al., 2005; Lanski et al., 2011; Nowak et al., 2011; Davis et al., 2012; Bassi et al., 2012). The prey species used and their size vary from place to place according to many factors: environmental features (e.g., climate and terrain), prey (e.g., species, availability, vulnerability, density, social behavior) and predators (e.g., experience, pack size, tradition, individual preference) (Huggard, 1993; Mech and Peterson, 2003). All these factors made wolves able to specialize on some prey species (usually 1–2) in different regions (Jędrzejewski et al., 2012). In Southern Europe, when present at sufficient densities, wild ungulates may represent the main trophic resources (Meriggi and Lovari, 1996; Barja, 2009). In the Italian peninsula, a wolf specialization on wild ungulates was observed, with wild boar and roe deer usually representing the main items in the diet (Meriggi et al., 1996, 2011; Capitani et al., 2004; Marucco et al., 2008; Mattioli et al., 2004, 2011; Milanesi et al., 2012).

Very few studies focused on the competition between wolf and dogs and, in fact, they showed contrasting patterns. Jhala (1993) observed a competition between dogs and Indian wolves (*Canis lupus pallipes*) for blackbuck fawns (*Antelope cervicapra*), while Echegaray and Vilà (2010) observed a higher consumption of livestock by free-ranging dogs than by wolves in Northern Spain. Anyway, all the studies conducted on wolf and dog food habits pointed out that both predators have an opportunistic feeding behavior (Meriggi and Lovari, 1996; Capitani et al., 2004; Campos et al., 2007; Vanak and Gompper, 2009).

According to our results, hybrids clearly share the same food habits with local wolves.

This result can be easily interpreted. The majority of studies have reported hybridization between female wolves and male dogs, while crosses between female dogs and male wolves are very rare (e.g., Hindrikson et al., 2012). This sexual asymmetry in hybridization has been confirmed by several genetic studies (Randi et al., 2000; Vilà et al., 2003; Verardi et al., 2006; Randi, 2008). A male dog usually neither assist the female in pup rearing and care (Boitani, 1995; Vilà and Wayne, 1999) nor forms long-term bonds with her; therefore, in case of mating between a male dog and a she-wolf, pups are likely to be reared only by the mother, which then transmits them her habits.

It has been observed that learning, traditions and individual preferences are involved in determining prey species preferences, and packs in a natural environment usually maintain long traditions of hunting routes and habits (Haber, 1996). Due to their sociality, wolves show a considerable potential for cultural transmission (Boitani, 2003) and this enables them to gain a thorough knowledge of the prey in their territory as well as to develop habits which increase their hunting efficiency (Mech and Peterson, 2003)[p]. This can explain why wolf–dog hybrids in our study area showed a wolf-like behavior. Hybrid offspring may learn from the wolf mother the feeding habits which are more likely to be advantageous in the local environment.

Also the body size could play a role in prey selection and food habits. But no difference in body size was suggested by the inspection of fresh carcasses recovered in the two study areas (data not shown).

Given the seemingly high trophic overlap, the presence of hybrids may in the long run represent an ecological (and not only genetic) threat to the local wolf population.

Indeed, a wolf-like trophic behavior of hybrids may represent one of the factors facilitating the spread of introgressed individuals among the Italian wolf population. By behaving like wolves, in fact, hybrids can perfectly adapt to the natural environment, find wolf mates and form new introgressed packs (this seems to be the case for the AP packs, data not shown). This might also explain the high frequency of backcrosses among the hybrids identified in Italy (Randi et al., 2014) and is confirmed even by our genetic results, which show signatures of past hybridization events. Yet diluted by backcrossing, the frequency of dog genes in the wolf population could be maintained by high ongoing hybridization rates or by hybrid–hybrid matings, which are more likely to occur if the phenomenon is widespread (as it seems to be in some areas of Central Italy).



Several studies demonstrated that natural predation may regulate herbivore abundance (e.g., [Hairston and Hairston, 1993](#); [Eberhardt, 1997](#)). Both wolves and hybrids turned out to rely mainly on wild ungulates, likely contributing to regulate their populations in our study area.

Given that changes in behavior in the dog compared to its wild ancestor have a genetic basis ([Saetre et al., 2006](#)), a possible consequence of hybridization is a less elusive behavior, leading to a higher frequentation of human-modified landscapes and a higher impact on livestock. Interestingly, instead, our data did not highlight a higher occurrence of livestock in the fecal contents of hybrids. This can also be due to a very limited consumption of this food item overall, due to the common adoption of prevention measures (nocturnal housing and guarding dogs) all year round. This is confirmed by published dietary data for very close areas, where the occurrence of livestock in wolf diet was very limited ([Capitani et al. 2004](#); [Mattioli et al., 2004, 2011](#)). Unfortunately our data covers only half of the year (November–April) and we cannot know if the same preferences are maintained also during the warm season. However, the aforementioned studies did not show any appreciable difference in livestock use between winter and summer periods.

To date, no study has investigated the actual competition between wolf and wolf–dog hybrids yet. Though based on a limited number of packs (due to the low frequency of hybrid packs in the population), the present study represents the first contribution on the feeding ecology of free-ranging hybrids suggesting them as potential competitors for wolves. Further studies on other aspects of the ecology and socio-biology of wolf–dog hybrids (e.g., reproductive behavior, sociality, territoriality) are necessary in order to better assess the impact of hybridization on natural wolf populations. Further knowledge of wolf–dog hybrids may be crucial for the conservation of the Italian wolf population.

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