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Original Investigation

Genetic structure of expanding wolf (*Canis lupus*) populations in Italy and Croatia, and the early steps of the recolonization of the Eastern Alps

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ABSTRACT

After centuries of range contraction and demographic declines wolves are now expanding in Europe, colonizing regions from where they have been absent for centuries. Wolf colonizing the western Alps originate by the expansion of the Italian population. Vagrant wolves of Italian and Dinaric-Balkan origins have been recently observed in the Eastern Alps. In this study we compared the genetic structure of wolf populations in Italy and Croatia, aiming to identify the sources of the ongoing recolonization of the Eastern Alps. DNA samples, extracted from 282 Italian and 152 Croatian wolves, were genotyped at 12 autosomal microsatellites (STR), four Y-linked STR and at the hypervariable part of the mitochondrial DNA control-region (mtDNA CR1). Wolves in Croatia and Italy underwent recent demographic bottlenecks, but they differ in genetic diversity and population structure. Wolves in Croatia were more variable at STR loci ($N_A = 7.4$, $H_O = 0.66$, $H_E = 0.72$; $n = 152$) than wolves in Italy ($N_A = 5.3$, $H_O = 0.57$, $H_E = 0.58$; $n = 282$). We found four mitochondrial DNA (mtDNA CR1) and 11 Y-STR haplotypes in Croatian wolves, but only one mtDNA CR1 and three Y-STR haplotypes in Italy. Wolves in Croatia were subdivided into three genetically distinct subpopulations (in Dalmatia, Gorski kotar and Lika regions), while Italian wolves were not sub-structured. Assignment testing shows that the eastern and central Alps are recolonized by wolves dispersing from both the Italian and Dinaric populations. The recolonization of the Alps will predictably continue in the future and the new population will be genetically admixed and very variable with greater opportunities for local adaptations and survival.

Introduction

During the last few decades, populations of ungulates and carnivores, which declined in the past because of Pleistocene climate changes (Sommer and Benecke 2005; Sommer and Nadachowski 2006; Hofreiter and Stewart 2009) or anthropogenic pressures (Rosser and Sue 2002), began to expand, recolonizing parts of their historical ranges. Expanding carnivore populations often met and admix in contact zones (Hailer and Leonard 2008). The outcomes of hybridization are variable, depending on the extent of genetic divergence and origin of the parental populations. Hybridization and subsequent introgression might disrupt coadapted gene pools, threatening the integrity of local populations or entire species and leading to genetic extinctions (Allendorf et al. 2001). In other cases, admixture zones might generate novel gene arrays that are filtered by natural selection, eventually increasing the fitness of admixing populations (Arnold and Martin 2009; Kays et al. 2010). Moreover, gene flow might help rescuing genetically depleted and isolated inbred populations (Tallmon et al. 2004).

Wolves (*Canis lupus*) in Europe strongly declined in the last centuries due to human persecution and decline of their natural prey (Breitenmoser 1998). Isolated populations survived in Iberia and southern Italy, while larger wolf populations remained in Eastern Europe, particularly in the Carpathian and Dinaric-Balkan mountain ranges (Linnell et al. 2008). Wolves in Italy and Croatia shared partially similar demographic histories. Wolves in Italy were isolated south of the Alps at least for one century, but probably for the past few thousands years (Lucchini et al. 2004), and are genetically differentiated from all the other wolf populations in the world (vonHoldt et al. 2010; their Fig. 3). At demographic bottleneck in the ‘70s wolves in Italy survived split in two fragmented subpopulations of less than 100 individuals,

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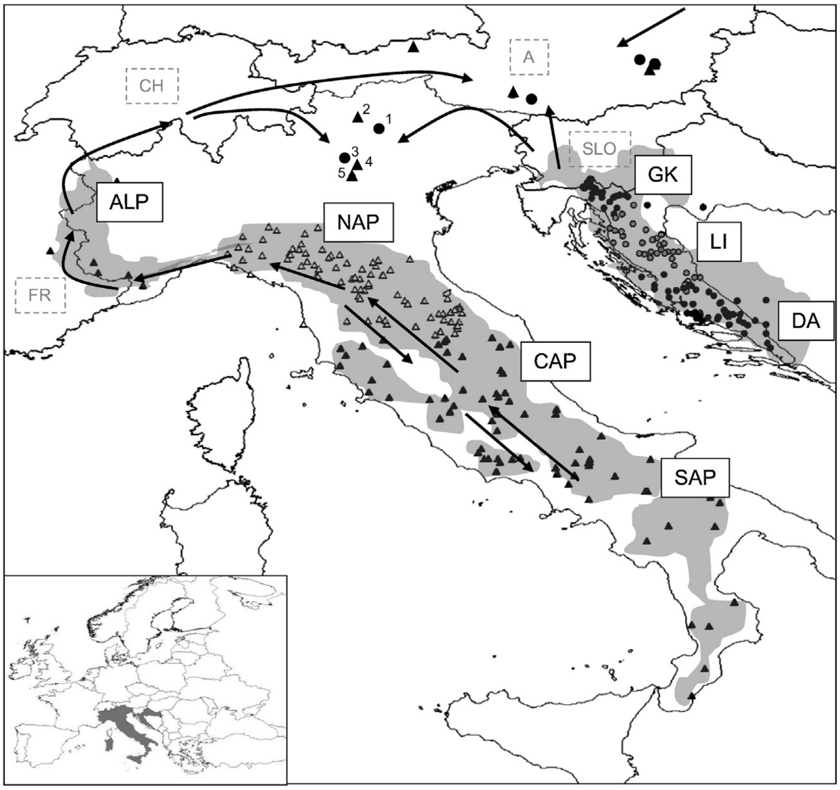

Fig. 1. Approximate distribution range (shaded areas) and sampling locations of wolves in Croatia (dots) and Italy (triangles). The arrows indicate the main directions of wolf expansion and colonization of the Alps. The biggest filled black symbols indicate solitary wolves in dispersion in the Alps (triangles: wolves of Italian origin; dots: wolves of Balkan-Dinaric origin). ALP: Alps; NAP: Northern Apennines; CAP: Central Apennines; SAP: Southern Apennines; GK: Gorski kotar; LI: Lika; DA: Dalmatia; FR: France; CH: Switzerland; A: Austria; SLO: Slovenia.

confined to remote mountain areas of the central and southern Apennines (Boitani 2003). Thereafter, beginning from the ‘80s, wolves expanded rapidly recolonizing the Apennines and moving towards the Italian and French Western Alps where they established a stable transborder population (Fabbri et al. 2007; Marucco et al. 2012). Now the Western Alpine and Italian Apennine wolf population might number *c.* 800–1000 individuals (Linnell and Boitani 2012).

Wolves were widely distributed across all Croatia until the end of the 19th century (Frković and Huber 1992). Hunting and eradication programmes in the 20th century wiped them out from most of the country. Wolves declined to 30–50 individuals in the 1980s, confined to Croatian mountains in the Gorski kotar and Lika regions. This population always remained and still is connected with the neighbouring small Dinaric wolf population in Slovenia (Adamič 1992), and with a larger one (about 400 individuals) in Bosnia and Herzegovina (Kusak 2010). By the early 1990th, wolves in Croatia started to grow to a current estimated size of 175–240 individuals (Kusak and Huber 2010a). During the last 20 years wolves spread from the Gorski kotar and Lika regions to neighbouring Dalmatia, Banija and Učka regions, reconstructing a single larger and continuous population (Kusak and Huber 2010b).

The central and eastern sectors of the Alps are currently explored by young male wolves in dispersion, which did not yet establish stable reproductive packs (Fig. 1) (Boitani 2000; Weber and Fattebert 2008). Wolves of Italian and eastern European origins have been recurrently detected in Switzerland, Austria and in the eastern Italian Alps (Salvatori and Linnell 2005). A GPS-collared male, named “Slavc”, born in Slovenia in 2010 left the Dinaric population in 2011 reaching first Trentino and then the Veneto regions in Italy in 2012 (Written by Krofel: http://www.volkovi.si/en/blog/233-gibanje-z-ovratnico-opremljenih-volkov-v-juliju-2012; LIFE project: SloWolf http://www.volkovi.si/). Slavc met a first female of Italian origin (named V1302F in this study) that was soon found poisoned in August 2012 (as reported by the local press). In the same area another female of Italian origin (TN2F), travelling with Slavc, was genetically identified from scat samples collected along snow tracks in Autumn 2012. The first successful reproduction between this two individuals (Slavc and TN2F) was reported in August 2013 by the local press (http://www.larena.it/stories/Home/550274__il_primo_branco_di_lupidelle_alpi_orientali/ SloWolf: http://www.volkovi.si/en/archive/284-slavc-in-julija-imata-mladie) and confirmed by the managers of the Regional Natural park of Lessinia (*pers. comm.*). Wolf recolonization is characterized by early and sporadic episodes of long-distance dispersal, followed by settings of stable reproductive packs (Valière et al. 2003). Habitat suitability analyses suggest that wolf expansion in the Alps might continue in the future (Marucco and McIntire 2010). Consequently, wolves of Italian and Dinaric-Balkan origins have chances to continue to breed in the Alps, generating new genotypes and increasing genetic diversity through gene flow, with novel opportunities for local adaptations and evolution (Hedrick and Fredrickson 2010).

This study was planned to describe the genetic structure of the expanding Dinaric and Italian wolf populations at the very early stages of their expansion towards the central and eastern Alps and before the beginning of their predicted admixture process. Aims of the study are to: (1) estimate and compare the genetic variability of the two populations; (2) describe the genetic differentiation between them; (3) to explore local population structuring; and (4) identify the origin of wolves colonizing the central and eastern Italian Alps. These results are being used to build a baseline population genetic dataset, which will help to monitor the current and predict the future dynamics of the wolf populations in the Alps.