



Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management

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For Review Only

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4 **for conservation and management**
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3 48 ABSTRACT
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The grey wolf (*Canis lupus*) is an iconic large carnivore that has increasingly been recognized as an apex predator with intrinsic value and a key ecosystem engineer. However, wolves have also long represented a primary source of human-carnivore conflict, which has led to long-term persecution of wolves, resulting in a significant decrease in their numbers, genetic diversity and gene flow between populations.

For more effective protection and management of wolf populations in Europe, robust scientific evidence is crucial. This review serves as an analytical summary of the main findings from wolf population genetic studies in Europe, covering all major studies from the “pre-genomic era” and the first insights of the “genomics era”. We analyse, summarise and discuss findings derived from analyses of three compartments of the mammalian genome with different inheritance modes: maternal (mitochondrial DNA), paternal (Y chromosome) and biparental (autosomal microsatellites and single nucleotide polymorphisms – SNPs). To describe large-scale trends and patterns of genetic variation in European wolf populations, we conducted a meta-analysis based on the results of previous microsatellite-studies and included also available new data, covering all European countries for which wolf genetic information is available (19): Norway, Sweden, Finland, Estonia, Latvia, Lithuania, Poland, Czech Republic, Slovakia, Germany, Belarus, Russia, Italy, Croatia, Bulgaria, Bosnia and Herzegovina, Greece, Spain and Portugal. We compared different indices of genetic diversity in wolf populations and found a significant spatial trend in heterozygosity across Europe from south-west (lowest genetic diversity) to north-east (highest). The range of spatial autocorrelation calculated on the basis of three characteristics of genetic diversity was 650–850 km, suggesting that the genetic diversity of a given wolf population can be influenced by populations up to 850 km away.

73 As an important outcome of this synthesis, we discuss the most pressing issues threatening
 74 wolf populations in Europe, highlight important gaps in current knowledge, suggest solutions
 75 to overcome these limitations, and provide suggestions for science-based wolf conservation
 76 and management at regional and Europe-wide scales.

79 *Key words:* *Canis lupus*, genetic variation, connectivity, large carnivores, microsatellites,
 80 mitochondrial DNA, SNP, Y chromosome.

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I. INTRODUCTION

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118 The grey wolf *Canis lupus* L. 1758 was historically widespread across Europe, but between
119 the 18th and 20th centuries was progressively eradicated from most of the continent. Once
120 viewed primarily as a threat to public safety, livestock and wild “game”, the wolf has recently
121 become recognized as an apex predator that plays a key role in ecosystems (Bruskotter *et al.*,
122 2011). As a result, conservation measures implemented since the second half of the 20th
123 century and a relaxation of control programs have led to gradual expansion of many wolf
124 populations in Europe (Musiani *et al.*, 2009; Chapron *et al.*, 2014). In addition, ongoing
125 protection of European wilderness zones, socio-economic changes, innovative laws, public
126 and political commitment, recovery of wild ungulate species and wolf dispersal ability have
127 enabled the species to recolonize many parts of its former range in Europe (Boitani, 1992;
128 Musiani *et al.*, 2009, 2010; Randi, 2011; Chapron *et al.*, 2014; Leonard, 2014; Gilroy *et al.*,
129 2015; López-Bao *et al.*, 2015) (Fig. 1). Currently, ca 12 000 wolves occupy over 800 000
130 square kilometres in 28 European countries (Chapron *et al.*, 2014), with 9 900 of these
131 animals present in 22 countries belonging to the European Union. Several remarkable
132 examples of wolf recovery in Europe have been described: (a) in Scandinavia, the current
133 population, consisting of 49 family groups and 364–598 individuals (Svensson *et al.*, 2015)
134 was founded by a few eastern (Karelian) immigrants in 1982/83 (Vilà *et al.*, 2003a); (b) the
135 Italian peninsular population, which in the 1980s occupied only the southern Apennines
136 mountain range, had by the 1990s recolonized the southwestern Alps (Lucchini *et al.*, 2002;
137 Valière *et al.*, 2003, Fabbri *et al.*, 2007, 2014), in the process growing from fewer than 100
138 individuals in the 1960s to about 1200–1700 individuals in 2009–2013 (Galaverni *et al.*,
139 2016). The latter expanding population is now coming in contact with the Dinaric-Balkan

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3 140 population in the eastern Alps (Ražen *et al.*, 2016); (c) the Northwest Iberian population has
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5 141 increased continuously since the 1970s (Kaczensky *et al.*, 2013) to between 254 and 322
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7 142 breeding packs during 1999–2003 (Álvares *et al.*, 2005); (d) the Central European Lowland
8
9 143 population has recently been established in western Poland and eastern Germany via
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11 144 recolonization primarily from northeastern Poland (Czarnomska *et al.*, 2013), with >60 packs
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13 145 established since the first reported reproduction near the German-Polish border in 2000
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15 146 (Reinhardt *et al.*, 2015).

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18 147 At the same time there are examples of European wolf populations that have recently gone
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20 148 extinct, such as the population in the Alentejo region, southern Portugal, in the 1980s-1990s
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22 149 (Álvares, 2004), or are on the verge of extinction, such as the population in Sierra Morena in
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24 150 southern Spain (López-Bao *et al.*, 2015).

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27 151 Six types of genetic markers have been used to study wolves: (1) autosomal microsatellites,
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29 152 (2) autosomal SNPs, (3) major histocompatibility complex (MHC), (4) mtDNA, (5) Y
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31 153 chromosome microsatellites and (6) Y - SNPs (Fig. 's 2, 3; note that marker types 3 and 6 are
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33 154 not shown in figures). While mitochondrial DNA (mtDNA) was initially the most frequent
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35 155 choice, autosomal microsatellites quickly gained popularity due to their superior power,
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37 156 compared with mtDNA, for identifying individual animals and for assessing population
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39 157 genetic diversity, population structuring and rates of gene flow between populations.

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42 158 Recently, the depth of population analysis has been increased further by large-scale genome-
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44 159 wide SNP data (von Holdt *et al.*, 2011; Stronen *et al.*, 2013; Pilot *et al.*, 2014a). As a general
45
46 160 rule in mammals, mtDNA is maternally inherited and cannot alone represent all historical and
47
48 161 contemporary processes acting upon populations. Nuclear data derived from biparental
49
50 162 autosomal loci and from the paternal Y chromosome are thus required to gain a more
51
52 163 complete understanding of evolutionary and contemporary population processes of wolves
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54 164 across Europe. Contrary to mtDNA and SNP data that can be combined between studies to
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3 165 cover large areas, the use of microsatellite-based investigations has an important shortcoming:
4
5 166 the data cannot be easily compared between different studies (De Groot *et al.*, 2016). Thus,
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7 167 many microsatellite data sets represent a specific country or limited region (e.g. Flagstad *et*
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9 168 *al.*, 2003; Jędrzejewski *et al.*, 2005; Fabbri *et al.*, 2007; Godinho *et al.*, 2011; Jansson *et al.*,
10
11 169 2012, 2014; Hindrikson *et al.*, 2013), with few covering wolf populations from wider areas
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13 170 (Pilot *et al.*, 2006, 2014b; Aspi *et al.*, 2009; Sastre *et al.*, 2011; Fabbri *et al.*, 2014) (Fig. 1).
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16 171 The genetic diversities of wolf populations which have suffered demographic bottlenecks and
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18 172 recoveries have also been investigated at the level of loci encoding proteins for the major
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20 173 histocompatibility complex (MHC) (Seddon & Ellegren, 2004; Arbanasić *et al.*, 2013;
21
22 174 Galaverni *et al.*, 2013; Niskanen *et al.*, 2014). In addition to the maternal and biparental
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24 175 markers, investigations of the paternal lineage (Y chromosome) have become also
25
26 176 increasingly frequent (Fig. 2), though the majority have focused primarily on wolf-dog
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28 177 hybridization (Sunqvist *et al.*, 2001; Vilà *et al.*, 2003b; Iacolina *et al.*, 2010; Godinho *et al.*,
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30 178 2011; Hindrikson *et al.*, 2012; Fabbri *et al.*, 2014; Randi *et al.*, 2014).
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33 179 Genetic diversity contributes significantly to the adaptive potential of wolf populations,
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35 180 including the ability to respond adequately to changing environmental conditions and
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37 181 anthropogenic influences, of which climate change, habitat alterations, fluctuations in prey
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39 182 base and emerging infectious diseases are perhaps the most important. In severe cases,
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41 183 diversity loss due to inbreeding depression can lead to significantly decreased fitness within
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43 184 populations (Reed & Frankham, 2003; Frankham, 2005). This makes evaluation of genetic
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45 185 diversity parameters a particularly important goal in conservation biology (Frankham, 2005;
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47 186 Allendorf *et al.*, 2013). On the other hand, the extreme dynamics of population expansion and
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49 187 re-colonization exhibited by European wolves is generating a fast-changing distribution at the
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51 188 level of the continent, reflected in the species' landscape genetics (Randi, 2011). While on
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53 189 one hand the newly established small wolf populations are passing through genetic and
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3 190 demographic bottlenecks with all the accompanying problems (Frankham, 2005; Allendorf *et*
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5 191 *al.*, 2013), establishment of gene flow between source populations is bringing new
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7 192 opportunities for long-term viability of these emerging populations. Genetic research can
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9 193 provide a deeper understanding of these processes. The approach promises knowledge of both
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11 194 local and large-scale trends in grey wolf genetic composition that is crucial for effective
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13 195 conservation and management of the species and its ecological role throughout Europe.
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21 198 **II. GENETIC PATTERNS OF EUROPEAN WOLF POPULATIONS REVEALED BY**
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23 199 **DIFFERENT MARKERS**
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28 201 The introduction of DNA-based methods in the 1980s has significantly advanced our
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30 202 understanding of wildlife populations in Europe and elsewhere. Three types of genetic marker
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32 203 system have been used: biparental (autosomal microsatellites, SNPs and MHC), maternal
33
34 204 lineage specific (mtDNA), and paternal lineage specific (Y chromosome microsatellites and
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36 205 SNPs) (Fig. 's 2 and 3). While the biparental markers are particularly well-suited for studying
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38 206 contemporary population processes, the uniparental markers, such as mtDNA and Y
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40 207 chromosome, reflect the contribution of each sex to the history of populations and can explain
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42 208 more ancient events (Vilà *et al.*, 1999; Pilot *et al.*, 2010).
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46 209 Here we provide a systematic review of the studies carried out on European wolf populations
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48 210 using markers with different inheritance modes (Appendix S1).
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3 212 **(1) Biparental markers**
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6 213 *(a) Microsatellite loci*
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8 214 Since the early 1990s, when microsatellite markers were first used to study natural
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10 215 populations (Ellegren, 1991), microsatellites have been the marker of choice for a large
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12 216 number of applications in molecular ecology and conservation genetics. Microsatellite loci,
13
14 217 also referred to as short tandem repeats (STR) or simple sequence repeats (SSR), are motifs
15
16 218 (2–12 bp) repeated adjacently in chromosomes, forming blocks with size up to 100 bp
17
18 219 (Strachan & Read, 1999). Microsatellites are abundant and randomly distributed throughout
19
20 220 mammalian genomes, and their rapid evolution and high polymorphism (Roy *et al.*, 1994)
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22 221 together with the large numbers of loci characterised in the domestic dog have made them a
23
24 222 useful tool in wolf population studies. Microsatellites have been used to analyse genetic
25
26 223 diversity, inbreeding, population structure, rates of gene flow between subpopulations,
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28 224 relatedness of individuals, demographic events and hybridization with the domestic dog.
29
30 225 Nonetheless, a major drawback of microsatellites is the limited comparability of data
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32 226 produced in different labs, requiring careful calibration to overcome the problem. Another
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34 227 drawback has been the lack of a commonly agreed set of microsatellite loci, which makes the
35
36 228 direct comparison of results generated in different studies problematic (De Groot *et al.*, 2016).
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38 229 However, with the advent of the genomic era, massively parallel array-based SNP genotyping
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40 230 and whole-genome sequencing have started to replace microsatellite analysis as the method of
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42 231 choice for many population level questions.
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48 232 Results based on microsatellite data have been published for many wolf populations in Europe
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50 233 (Appendix S1; Fig. 2). Below we summarise wolf microsatellite studies at the Europe-wide
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52 234 scale, and for each of the ten European wolf populations identified by Chapron *et al.* (2014).
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57 236 *European scale*
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3 237 Genetic differentiation among some European wolf populations has been strongly influenced
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5 238 by spatial discontinuities in the wolf range caused by historical persecution by humans.
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7 239 Lucchini *et al.* (2004) showed that the isolated Italian population is strongly differentiated
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9 240 from other European populations, with pairwise F_{ST} values ranging between 0.21 and 0.32.
10
11 241 They also found the signature of a strong, long-term population decline, suggesting that the
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13 242 Italian wolf population could have been isolated at least for several hundred generations
14
15 243 (Lucchini *et al.*, 2004). Sastre *et al.*, (2011) also revealed evidence for a dramatic strong
16
17 244 bottleneck in recent times among Iberian wolves. Significant genetic differentiation has also
18
19 245 been observed among the relatively well-connected wolf populations in central and eastern
20
21 246 Europe, for example between Baltic and Central European Lowland populations, and
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23 247 Carpathian and Dinaric-Balkan populations. Pilot *et al.* (2006) were the first to detect genetic
24
25 248 differentiation in central and east European wolves in the absence of obvious physical barriers
26
27 249 to dispersal. They suggested that ecological factors, such as climate and habitat conditions,
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29 250 and variations in wolf diet may have influenced gene flow and led to the observed genetic
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31 251 differentiation among wolf populations. This was further supported by the analysis of stable
32
33 252 isotope profiles for a subset of genotyped individuals, which provided a quantifiable proxy
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35 253 measure of individual diet and allowed the authors to assess the relationship between
36
37 254 individual foraging behavior and genotype (Pilot *et al.*, 2012). A significant correlation
38
39 255 between genetic distances and dietary differentiation was detected even when geographic
40
41 256 distance was accounted for as a co-variable, reinforcing the conclusion that dietary
42
43 257 preferences and associated habitat choice can influence the genetic structuring of wolf
44
45 258 populations (Pilot *et al.*, 2012). This general mechanism of genetic differentiation detected on
46
47 259 a large geographical scale can to some extent also drive local-scale genetic differentiation,
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49 260 and may influence the patterns of recolonisation (e.g. Czarnomska *et al.*, 2013, Leonard,
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51 261 2014).

262

263 *Scandinavian population*

264 This population consists of about 460 individuals (90% in Sweden, the rest in Norway or in
265 the border area between these countries; Svensson *et al.*, 2015). Exterminated in the 1960s
266 and naturally recolonised since the 1980s by immigrant wolves from Finland (Karelia)
267 (Wabakken *et al.*, 2001, Vilà *et al.*, 2003a), the Scandinavian population is growing and is
268 currently distributed in the central part of Sweden and southeastern Norway. The population
269 has been continuously monitored with genetic methods (Ellegren *et al.*, 1996, 1999; Flagstad
270 *et al.*, 2003; Vilà *et al.*, 2003a, b; Seddon *et al.*, 2005, 2006; Hagenblad *et al.*, 2009). It has
271 been investigated through a period of severe inbreeding depression (inbreeding coefficient F_{IS}
272 varied from 0–0.42 for wolves born in 1983–2002 in Liberg *et al.*, 2005); followed by a
273 remarkable genetic recovery thanks to a single immigrant from an eastern (Karelian?) wolf
274 population that brought new genetic material into the population (Vilà *et al.*, 2003a); and
275 through a further period of increasing inbreeding; until the recent immigration of four
276 Finnish/Russian wolves between 2008 and 2013 that rescued the population once again
277 (Åkesson *et al.*, unpublished). Particular attention has been paid to ongoing immigration from
278 neighbouring Finnish/Russian (Karelian) wolf population (Flagstad *et al.*, 2003; Vilà *et al.*,
279 2003a; Seddon *et al.*, 2006), shown to coincide with episodes of marked population increase
280 in Russian Karelia (Flagstad *et al.*, 2003), and the identification of four immigrant wolves in
281 northern Sweden in 2002–2005 from Finland (Seddon *et al.*, 2006).

282

283 *Karelian population*

284 The Karelian wolf population is shared between Finland and Russia and consists of 220–245
285 animals (Natural Resources Institute Finland, 2015). Clear signs of genetic bottlenecks have
286 also been observed in the allele frequency distributions of this population (Jansson *et al.*,

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3 287 2014). Genetic structure and population processes, including admixture between wolves in the
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5 288 Finnish and Russian parts of the population have been investigated using microsatellites (Aspi
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7 289 *et al.*, 2006, 2009; Jansson *et al.*, 2012). Population size reduction together with the low level
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9 290 of gene flow from the Russian Karelian population (Aspi *et al.*, 2006, 2009) led the Finnish
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11 291 Karelian part of the population into a demographic and genetic crash after 2006, with a
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13 292 significant decline in observed heterozygosity and an increase in inbreeding (Jansson *et al.*,
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15 293 2012). Compared to the historical Finnish wolf population, almost 20% of microsatellite
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17 294 alleles have not been found in the modern population (Jansson *et al.*, 2014). Although the
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19 295 Karelian wolf population (including Russia) is often seen as a single large management unit, it
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21 296 may consist of smaller units (Aspi *et al.*, 2009; Jansson *et al.*, 2012).
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297

298 *Baltic population*

299 The Baltic wolf population is distributed throughout Estonia (200–260), Latvia (200–400),
30
31 300 Lithuania (300–400) and northeastern Poland (270–360), comprising 900–1400 animals in
32
33 301 total. As in other parts of Europe, the Baltic wolf population experienced near-extirpation
34
35 302 in the 1970s and 1980s (Jędrzejewski *et al.*, 2005; Baltrūnaitė *et al.*, 2013), leaving signs of
36
37 303 genetic bottlenecks in wolves from Estonia, Latvia (Hindrikson *et al.*, 2013; Plumer *et al.*, in
38
39 304 prep.) and neighbouring Russia (Sastre *et al.*, 2011). In general, the Baltic population exhibits
40
41 305 relatively high levels of heterozygosity compared with many other European wolf populations
42
43 306 (Jędrzejewski *et al.*, 2005; Baltrūnaitė *et al.*, 2013; Czarnomska *et al.*, 2013; Hindrikson *et al.*,
44
45 307 2013). Moreover, a cryptic genetic structuring has been found in the Estonian-Latvian part of
46
47 308 this population (Hindrikson *et al.*, 2013) and the authors proposed that the four genetic groups
48
49 309 identified reflect recent population bottlenecks, severe hunting pressure and immigration. The
50
51 310 Estonian population is expanding and has recently (in 2011) re-colonized the two largest
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53 311 islands of the country, Saaremaa and Hiiumaa (Plumer *et al.*, in prep.).
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313 *Central European Lowland population*

314 The Central European Lowland population is mainly shared between Poland and Germany
315 (each with > 30 packs or 150-200 animals) (Reinhardt *et al.*, 2015), with recent occurrences in
316 Denmark (Andersen *et al.*, 2015), Czech Republic and the Netherlands (Gravendeel *et al.*,
317 2013), resulting in a total of 300–400 animals ranging in approximately 24 000 km². This
318 population was formed in the late 1990s (Andersen *et al.*, 2015), when a small number of
319 wolves from northeastern Poland (Czarnomska *et al.*, 2013), recolonized the Lusatian border
320 region between Germany and Poland. While the population is steadily expanding (Kaczensky
321 *et al.*, 2013), strong founder effects have likely resulted in a genetic separation between this
322 and the Baltic founder population (Andersen *et al.*, 2015), despite its close relatedness and
323 evidence for gene flow (Czarnomska *et al.*, 2013).

324

325 *Italian peninsular population*

326 This population is situated along the Apennine Mountains and consists of approximately 321
327 wolf packs, corresponding to 1212–1711 wolves (Galaverni *et al.*, 2016). Wolves were
328 extirpated from the Alps in the 1920s, and thereafter continued to decline in peninsular Italy
329 until the 1970s, when approximately 100 individuals survived, isolated in two fragmented
330 subpopulations in the central Apennines (Lucchini *et al.*, 2004; Fabbri *et al.*, 2007).
331 Nowadays, the Italian wolf population has a nearly continuous distribution along the
332 Apennines, though three genetic subpopulations (northern Apennines, Central Apennines and
333 Southern Apennines) (Fabbri *et al.*, 2007) persist due to limited gene flow (Scandura *et al.*,
334 2011). The Italian wolf population (together with the Scandinavian population) is probably
335 one of the most extensively microsatellite-genotyped wolf populations in Europe (Dolf *et al.*,
336 2000; Fabbri *et al.*, 2007, 2014; Lucchini *et al.*, 2004; Scandura *et al.*, 2011; Randi *et al.*,

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2
3 337 2014; Caniglia *et al.*, 2014). It has been demonstrated that Italian wolves have distinct
4
5 338 microsatellite allele frequencies that are highly differentiated from other wolf populations
6
7 339 typed so far in Europe (Randi *et al.*, 2000; Randi & Lucchini, 2002), except for the Alpine
8
9 340 (Fabbri *et al.*, 2014) population and wolves in the Pyrenees in France and Catalonia in Spain
10
11 341 (Sastre, 2011).

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14
15 343 *Alpine population*

16
17 344 The Alpine wolf population comprises approximately 160 animals and is distributed in the
18
19 345 Austrian, French, Italian and Swiss Alps (at least 116 animals in France, present in a
20
21 346 minimum of 36 wolf permanent presence areas (ONCFS wolf winter survey 2014–2015),
22
23 347 57–89 animals in Italy (Galaverni *et al.*, 2016), 8 animals in Switzerland and 2–8 animals in
24
25 348 Austria). The Western Alps in Italy, Switzerland and France (Lucchini *et al.*, 2002; Valière *et*
26
27 349 *al.*, 2003; Fabbri *et al.*, 2007, 2014) have been recolonized by Italian wolves, while the
28
29 350 eastern and the central Alps are being colonized by wolves from both the Italian and Dinaric-
30
31 351 Balkan populations (Fabbri *et al.*, 2014; Ražen *et al.*, 2016). On the other hand, wolves from
32
33 352 this population have expanded south-west, recently reaching the French Massif Central and
34
35 353 the Pyrenees in 1999 and Catalonia in Spain in 2000, carrying a mtDNA haplotype unique to
36
37 354 Italian wolves (W4 in Vilà *et al.*, 1997) (Valière *et al.*, 2003; Lampreave *et al.*, 2011; Sastre,
38
39 355 2011), though without evidence of reproductive success until now.
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45 357 *Carpathian population*

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47 358 The Carpathian population inhabits a large area, including five countries, and consists of
48
49 359 ~3000 wolves (2300–2700 in Romania, 340–450 in Slovakia, 209–254 in Poland and small
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51 360 number of individuals in the Czech Republic and Hungary). The population is largely
52
53 361 continuous, though with smaller population fragments (for example in the eastern Czech
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3 362 Republic and Hungary) scattered in the border areas of the Carpathian population,
4
5 363 representing remnants of a previously wider distribution (Boitani, 2000). The Carpathian
6
7 364 Mountains represent one of the largest wolf refuge areas in Europe and are regarded as being
8
9 365 of particular importance for the long-term survival of the species in Europe because of its size
10
11 366 and potential to serve as a link between northern and southern populations (Gula *et al.*, 2009).
12
13 367 Genetic studies covering the Carpathian wolf population have largely focused on the northern
14
15 368 part of the Carpathians in Poland, Slovakia and west Ukraine (Pilot *et al.*, 2006, 2010;
16
17 369 Czarnomska *et al.*, 2013, Bakan & Paule, 2014). Both microsatellite and mtDNA data suggest
18
19 370 that the Carpathian wolves are genetically distinct from the neighbouring lowland population
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21 371 (Pilot *et al.*, 2006; Czarnomska *et al.*, 2013) and also from the Dinaric-Balkan population
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23 372 (Bakan & Paule, 2014).
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29 374 *Dinaric-Balkan population*

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31 375 The Dinaric-Balkan population consists of ~3900 wolves in eight countries: Albania
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33 376 (200–250 individuals), Bulgaria (700–800), Bosnia and Herzegovina (650), Croatia
34
35 377 (168–219), Greece (700), Former Yugoslav Republic of Macedonia (466), Serbia (750–850)
36
37 378 and Slovenia (32–43) (Chapron *et al.*, 2014). From Slovenia to northern Greece, the wolf
38
39 379 range shows substantial continuity along the Dinaric and Balkan Mountains (Musiani *et al.*,
40
41 380 2009; Gomerčić *et al.*, 2010), and Bakan & Paule (2014) also identified gene flow between
42
43 381 Serbia and Bulgaria (Fig. 1; see also Appendix S2). Of all European wolf populations, this
44
45 382 one spans the largest number of national borders, and is consequently being subject to the
46
47 383 most diverse array of monitoring and management approaches (Kaczensky *et al.*, 2013). The
48
49 384 Bulgarian (Lucchini *et al.*, 2004; Bakan & Paule 2014; Moura *et al.*, 2014; Pilot *et al.*,
50
51 385 2014b), Greek (Moura *et al.*, 2014), Serbian (Bakan & Paule, 2014), Croatian (Gomerčić *et*
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53 386 *al.*, 2010) and Slovenian (Majić-Skrbinšek, 2014) wolves have been studied with
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3 387 microsatellite markers (Appendix S1). Both Bulgarian and Croatian wolves are in the process
4
5 388 of recovering from a severe bottleneck that started in the 19th century and lasted up to the
6
7 389 1970s–1980s (Gomerčić *et al.*, 2010; Moura *et al.*, 2014). The Dinaric-Balkan wolf
8
9 390 population is a valuable source of genetic diversity for neighbouring populations, as indicated
10
11 391 by ongoing recolonization of the eastern and central Alps by Dinaric-Balkan wolves (Fabbri
12
13 392 *et al.*, 2014, Ražen *et al.*, 2016), and by the considerable level of gene flow between the
14
15 393 Caucasus and the Balkans (Bulgaria) through intermediary populations (Pilot *et al.*, 2014b).
16
17 394 The population, however, shows genetic substructuring already at relatively local scales
18
19 395 (Fabbri *et al.*, 2014), indicating the need for further research to understand the population's
20
21 396 internal genetic and demographic connectivity and delineate conservation and management
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23 397 units.
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399 *North-West Iberian population*

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32 400 The North-West Iberian population is shared by Spain and Portugal. The population
33
34 401 approximately 254 breeding packs and about 2000 individuals, of which approximately 80%
35
36 402 occur in Spain and 20% in Portugal (Álvares *et al.*, 2005; Blanco & Cortés, 2012). The
37
38 403 population is concentrated in the northwestern region of the Iberian Peninsula and in a small
39
40 404 isolated subpopulation south of river Douro in Central Portugal (Álvares, 2004; Blanco *et al.*,
41
42 405 2005). At the beginning of the 20th century, Iberian wolves were distributed throughout the
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44 406 peninsula (Rico & Torrente, 2000). However, as in other European wolf populations, in the
45
46 407 middle of the 20th century, the Iberian population disappeared from most of its former range
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48 408 and was reduced to an all-time low in the 1970s (Valverde, 1971; Grande del Brío, 1984;
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50 409 Blanco *et al.*, 1990). As a consequence of a severe demographic bottleneck in the 20th
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52 410 century, genetic studies have revealed a low effective population size ($N_E = 43.2$ to 53.8 in
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3 411 Sastre *et al.*, 2011) and indicated inbreeding ($F_{IS} = 0.153$ in Ramirez *et al.*, 2006 and $F_{IS} =$
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5 412 0.177 in Sastre *et al.*, 2011).

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9 414 *Sierra-Morena population*

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11 415 The Sierra-Morena population is isolated and critically endangered, and according to
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13 416 Andalusian government reports, consists of perhaps no more than a single pack (Blanco &
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15 417 Cortés, 2012; López-Bao *et al.*, 2015). Ferrand *et al.* (2005) conducted a microsatellite study
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17 418 (21 autosomal and 4 Y-chromosome) and mtDNA analysis, but based on a rather small
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19 419 sample size. The authors did not report evidence of hybridization, though it represents a high
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21 420 risk factor for very small populations (Leonard *et al.*, 2014).
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25 422 *(b) Single nucleotide polymorphisms (SNPs)*

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28 423 SNPs represent a widespread source of genetic variation and their abundance throughout the
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30 424 genome makes them highly suitable for population genetic analysis. Whereas earlier studies
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32 425 with microsatellites typically examined <20 markers, SNPs allow simultaneous typing of
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34 426 thousands of loci and thereby increase the statistical power to resolve population structure and
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36 427 processes (e.g. Stronen *et al.*, 2013). In comparison with microsatellites, which have rapid
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38 428 mutation rates per generation (the order of 10^{-4}), SNPs show lower mutation rates (10^{-8} – 10^{-9})
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40 429 and simpler mutation patterns that result in relatively low levels of homoplasy (Brumfield *et*
41
42 430 *al.*, 2003). Another great advantage is that SNP data are universally comparable and do not
43
44 431 require standardisation, while microsatellite data produced in different laboratories have
45
46 432 inconsistencies in allele size length that prevent their direct comparison unless meticulous
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48 433 standardisation procedures are applied. Moreover, SNPs can potentially provide a better
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50 434 means of genotyping degraded DNA compared to microsatellites (Kraus *et al.*, 2015). On the
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52 435 other hand, microsatellite markers may have some advantages over SNPs, for example in
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3 436 identifying recent events such as new barriers to gene flow or changes in population structure
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5 437 (see Stronen *et al.*, 2013). Using ten microsatellite markers, Aspi *et al.* (2009) showed that
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7 438 Finnish wolves have recently differentiated from Arkhangelsk and Karelian wolves in Russia.
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9 439 However, in a study with 67 000 SNPs (Stronen *et al.*, 2013), wolves in Finland appeared
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11 440 well-connected to populations in Russia, despite the geographic distance.
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13 441 Seddon *et al.* (2005) found that 22 out of 24 SNP loci were sufficiently variable in the
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15 442 Scandinavian population, providing the level of accuracy in individual identification
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17 443 equivalent to 12 variable microsatellites genotyped in the same population. Recently, SNPs
18
19 444 have been used in population genetics studies on a regional-scale in Polish and German
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21 445 (Czarnomska *et al.*, 2013) and Italian (Fabbri *et al.*, 2012) populations, in large-scale
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23 446 European studies (vonHoldt *et al.*, 2011; Stronen *et al.*, 2013, Pilot *et al.*, 2014a), for the
24
25 447 identification of wolf-dog hybrids (vonHoldt *et al.*, 2013; Randi *et al.*, 2014; Godinho *et al.*,
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27 448 2014) and in genetic analysis of non-invasively collected samples (Valière *et al.*, 2003; Fabbri
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29 449 *et al.*, 2012; Caniglia *et al.*, 2014; Godinho *et al.*, 2014).
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33 450 Among the large-scale studies, vonHoldt *et al.* (2011) conducted an analysis with the canine
34
35 451 SNP genotyping array (47 000 SNPs) and found that wolf populations in Italy, Spain, and
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37 452 Eastern/Northern Europe comprise distinct units. These results were later supported by a
38
39 453 study using 61 000 SNPs, where Italian, Iberian and Eastern European (including Dinaric-
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41 454 Balkan population) wolf clusters were identified (Pilot *et al.*, 2014a). The Italian and Iberian
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43 455 populations had lower heterozygosity and stronger linkage disequilibrium compared to East-
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45 456 European populations, indicating that the former have experienced long-term isolation and/or
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47 457 bottlenecks (Pilot *et al.*, 2014a). This results of this study suggested that genetic drift due to
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49 458 spatial isolation and bottlenecks is a major evolutionary force behind genetic differentiation of
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51 459 European populations. Moreover, a number of loci showing a signature of diversifying
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53 460 selection were identified, including the loci flanking the platelet-derived growth factor gene,
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3 461 which may influence differences in body size between wolf populations (Pilot *et al.*, 2014a).
4
5 462 Stronen *et al.* (2013) evaluated genetic structure in 11 countries (177 wolves, Iberian samples
6
7 463 not included) using more than 67 000 SNPs, and besides the Italian population, they found the
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9 464 Dinaric-Balkan population and certain clusters in central and northern Europe to be
10
11 465 genetically distinct. In a more recent study by Stronen *et al.* (2015), the results indicated
12
13 466 differences between northern Europe, southern Europe, the Carpathian Mountain and
14
15 467 Ukrainian Steppe population clusters for a number of SNP loci (353 candidate loci out of 67
16
17 468 000 SNPs) and neighbouring genes with known or assumed functions.
18
19 469 A major concern regarding conclusions based on SNPs is that none of the studies has included
20
21 470 all European wolf populations. The largest geographical coverage in Europe to date is
22
23 471 represented in vonHoldt *et al.* (2011) (Fig. 3), and although Stronen *et al.* (2013) used a larger
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25 472 number of samples per country, several important populations were missing from the analysis.
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27 473 To achieve the Europe-wide resolution, significantly improved sampling is required,
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29 474 especially for Alpine, Dinaric-Balkan, Baltic, Karelian (as well as from other areas of Russia),
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31 475 Scandinavian and Iberian populations. Moreover, the consolidation of data derived from
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33 476 different genotyping platforms can be a challenge. While Illumina's Canine HD chip was
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35 477 used in Stronen *et al.* (2013), vonHoldt *et al.* (2011) and Pilot *et al.* (2014a) used the
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37 478 Affymetrix Canine SNP Genome Mapping Array.
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460 (c) Major histocompatibility complex (MHC)

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462 481 Another way of investigating the genetic diversity of wolves is at the level of loci encoding
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464 482 proteins for the major histocompatibility complex (MHC). MHC is a set of cell surface
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466 483 molecules encoded by a large gene family which controls a major part of the immune system
467
468 484 in vertebrates. MHC diversity is shaped by various factors, the most prominent among them
469
470 485 being the pathogens that are a key selective force in wild animal populations (e.g. Radwan *et*

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3 486 *al.*, 2010). The high variability of MHC markers may be especially informative in studies of
4
5 487 populations that are suspected of having suffered demographic bottlenecks. European wolf
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7 488 populations have maintained relatively high levels of MHC diversity, as shown for Karelian
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9 489 (the number of MHC alleles $n=22$, Seddon & Ellegren, 2004; $n=27$, Niskanen *et al.*, 2014),
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11 490 Italian peninsular and Alpine populations ($n=23$, Galaverni *et al.*, 2013), and Dinaric-Balkan
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13 491 population ($n=31$, Arbanasić *et al.*, 2013). The only known exception is the isolated
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16 492 Scandinavian wolf population, where the MHC variation is considerably lower than in other
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18 493 populations ($n=13$, Seddon & Ellegren, 2004).
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23 495 *Comparison with other large carnivore populations in Europe, and the main challenges for*
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25 496 *future investigations*

26
27 497 Microsatellites have also been used to study brown bear (*Ursus arctos*; e.g. Taberlet *et al.*,
28
29 498 1997; Manel *et al.*, 2004; Tammela *et al.*, 2010; Kopatz *et al.*, 2012; Straka *et al.*, 2012),
30
31 499 Eurasian lynx (*Lynx lynx*; Schmidt *et al.*, 2009; Davoli *et al.*, 2013; Rueness *et al.*, 2014), and
32
33 500 wolverine (*Gulo gulo*; Dalerum *et al.*, 2007; Hedmark *et al.*, 2007) in Europe. Even though
34
35 501 microsatellites have been proven to be useful markers for studies on all large carnivores, the
36
37 502 lack of a common set of universally comparable microsatellite markers between studies has
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39 503 prohibited the analysis of microsatellite data across Europe. Therefore, Europe-wide genetic
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41 504 patterns such as differences in genetic diversity, population structure and connectivity are still
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43 505 missing for all large carnivores.
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47 506 SNP analyses that have proven to be a way forward in large-scale wolf studies are scarce for
48
49 507 other large carnivore species in Europe (Norman *et al.*, 2013), largely because of the lack of a
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51 508 domesticated analogue. Rapid advancements in high-throughput and genome-wide
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53 509 sequencing methods are likely to reduce the usage of SNP-chips in the future, depending on
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55 510 the scientific questions asked. Next generation sequencing (NGS) methods are highly
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3 511 promising since they can provide unbiased data for whole genomes, allowing a wider range of
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5 512 research questions to be addressed, in comparison with SNPs.
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7 513 However, until whole-genome sequencing becomes considerably cheaper, SNP arrays remain
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9 514 more economical for many purposes, including identification of individuals, detection of
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11 515 wolf-dog hybrids and analysis of population structure and gene flow.
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16 517 **(2) Uniparental markers**

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19 518 *(a) Maternal lineage: mitochondrial DNA*

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21 519 Maternal inheritance, lack of recombination, high mutation rate and high copy-number have
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23 520 made mitochondrial DNA an appealing molecular tool in evolutionary biology, conservation
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25 521 genetics and phylogeography for many mammal species, including canids (e.g. Savolainen *et al.*
26
27 522 *et al.*, 2004; Hailer & Leonard, 2008). Unlike nuclear DNA, non-recombining maternal mtDNA
28
29 523 has been widely used not only in phylogeographic studies, but also to study wolf
30
31 524 domestication (Vilà *et al.*, 1997; Savolainen *et al.*, 2002; Boyko *et al.*, 2009; Pang *et al.*,
32
33 525 2009; Oskarsson *et al.*, 2012), and wolf-dog hybridization in Scandinavian (Vilà *et al.*, 1997),
34
35 526 Baltic (Andersone *et al.*, 2002; Hindrikson *et al.*, 2012), Italian peninsular (Randi & Lucchini,
36
37 527 2002; Vilà *et al.*, 2003b; Verardi *et al.*, 2006; Iacolina *et al.*, 2010; Caniglia *et al.*, 2013;
38
39 528 Randi *et al.*, 2014), NW Iberian (Godinho *et al.*, 2011, 2014) and Dinaric-Balkan (Moura *et*
40
41 529 *al.*, 2014) wolf populations.
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45 530 The hypervariable control region of mtDNA has been sequenced in the majority of studies,
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47 531 either partially (e.g. Vilà & Wayne, 1999; Flagstad *et al.*, 2003; Valière *et al.*, 2003; Ramirez
48
49 532 *et al.*, 2006; Seddon *et al.*, 2006; Sastre *et al.*, 2011) or fully (Randi *et al.*, 2000; Lucchini *et*
50
51 533 *al.*, 2004; Hindrikson *et al.*, 2012). Control region sequence data has facilitated the definition
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53 534 of a set of mtDNA haplotypes that differ from dog haplotypes in the majority of European
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55 535 wolf populations. However, the separation is not complete and “dog haplotypes” have also
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3 536 been found in wolves (Vilà *et al.*, 1997, 1999; Randi *et al.*, 2000; Pilot *et al.*, 2010). One of
4
5 537 the pioneering mtDNA studies involving both wolves and dogs described ten mtDNA
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7 538 haplotypes in 13 European countries and suggested that European wolves, though restricted to
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9 539 a small fraction of their former range, had been able to preserve a relatively high degree of
10
11 540 mtDNA polymorphism (Vilà *et al.*, 1997). Pilot *et al.* (2006) found that wolf populations from
12
13 541 Eastern Europe had multiple mtDNA haplotypes that were widely distributed. In a more
14
15 542 recent large-scale study, Pilot *et al.* (2010) analysed phylogenetic relationships and
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17 543 geographical distribution of mtDNA haplotypes of 947 contemporary European wolves. The
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19 544 authors found that haplotypes representing two main haplogroups (1 and 2) overlap
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21 545 geographically, but differ significantly in frequency between populations from southwestern
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23 546 and eastern Europe (see Fig. 1 in Pilot *et al.*, 2010). Haplogroup 1 predominated in Eastern
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25 547 Europe and was fixed in the Iberian Peninsula. These populations shared a common
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27 548 haplotype, suggesting past gene flow via extinct intermediate populations from central and
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29 549 western Europe. In the Italian population, haplogroup 2 was fixed and was represented by a
30
31 550 single haplotype. The unique mtDNA control region haplotype specific to wolves in Italy has
32
33 551 neither been found in other wolf populations world-wide, nor in dogs (named as haplotype
34
35 552 W4 in Vilà *et al.*, 1997; W14 in Randi *et al.*, 2000 and W22 Pilot *et al.*, 2010). Low mtDNA
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37 553 variability in wolves has also been found in earlier studies in Iberia (Vilà *et al.*, 1999; Sastre
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39 554 *et al.*, 2011), suggesting that these peninsular wolf populations in Southern Europe have been
40
41 555 isolated for a long time and possibly have lost much of their mitochondrial diversity due to
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43 556 genetic drift, although the possible effect of historic bottlenecks on genetic diversity has not
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45 557 been tested.
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47 558 Compared with other European populations, wolves in the Dinaric-Balkan population exhibit
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49 559 higher mtDNA control region variability, and the population probably retains a significant
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51 560 proportion of the genetic diversity present in the formerly widespread and continuous
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3 561 European wolf population, as suggested from studies involving Bulgarian (Randi *et al.*, 2000;
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5 562 Moura *et al.*, 2014; Pilot *et al.*, 2014b), Croatian (Gomerčić *et al.*, 2010; Fabbri *et al.*, 2014),
6
7 563 and other populations in the Balkans (Pilot *et al.*, 2010; Djan *et al.*, 2014).
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10 564 In a study comparing the modern and historical Scandinavian population, Vilà *et al.* (2003a)
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12 565 found that the original historical gene pool did not survive the bottleneck and that the present
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14 566 gene pool is made up of new haplotypes brought by founders – immigrants from eastern
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16 567 populations (Finland and northwest Russia).
17

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19 568 The Finnish part of Karelian wolf population has experienced a significant reduction in
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21 569 mtDNA haplotype diversity: only three out of eight lineages found in the historic Karelian
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23 570 wolf population before 1920 have survived (Jansson *et al.*, 2014).
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25 571 To date, wolf mtDNA sequences have been characterised from 26 out of the 28 countries in
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27 572 which the species currently occurs. To analyse genetic relationships between different
28
29 573 mtDNA haplotypes across Europe, we found that a 609 bp mtDNA control region fragment
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31 574 provides the best balance between marker size and geographical coverage. Using a median-
32
33 575 joining approach (Bandelt *et al.*, 1999) implemented in program Network 4.510, we
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35 576 constructed a minimum spanning network based on 160 publicly available 609 bp-sequences
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37 577 covering all wolf populations in Europe and geographically close populations in West Asia.
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39 578 According to this analysis, European wolves are divided into seven haplogroups (Fig. 4), of
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41 579 which most are of mixed origin, including sequences from several different European wolf
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43 580 populations, though some are more region-specific. The largest haplogroup includes wolves
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45 581 from the Scandinavian and NE-European populations and Greece. Iberian samples were
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47 582 divided between two haplogroups, one specific haplogroup and one mixed haplogroup with
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49 583 samples from Iberia, Balkans (Bulgaria) and NE Europe. However, the representation of
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51 584 sequences across Europe is still poor and phylogenetic resolution low due to the relatively
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53 585 short mtDNA sequences (see Appendix S3 for haplotype division).
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5 587 *MtDNA studies in other large carnivore populations in Europe, and the main challenges for*
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7 588 *future mtDNA investigations*

8
9 589 MtDNA sequences have also been widely used to study other large carnivore species in
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11 590 Europe: the European lynx (Rueness *et al.*, 2014); wolverine (Zigouris *et al.*, 2013); and
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13 591 especially for brown bear (e.g. Randi *et al.*, 1994; Taberlet & Bouvet, 1994; Kohn *et al.*,
14
15 592 1995; Saarma *et al.*, 2007; Saarma & Kojola, 2007; Korsten *et al.*, 2009; Davison *et al.*,
16
17 593 2011), including analyses based on complete mitogenomes (Keis *et al.*, 2013; Hirata *et al.*,
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19 594 2013).

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21
22 595 MtDNA has been and will remain an important genetic marker to study evolutionary
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24 596 processes driven by the female lineages. One of the main drawbacks in wolf mtDNA studies
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26 597 has been the use of short sequences. The analysis of complete mitochondrial genomes of
27
28 598 brown bear by Keis *et al.* (2013) clearly demonstrated the advantage of using such data,
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30 599 which revealed spatio-temporal population processes that had not previously been detected
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32 600 using shorter mtDNA sequences. Analysis of genetic diversity and evolutionary trajectories of
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34 601 wolf maternal lineages in Europe is likely to benefit significantly in the future from
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36 602 mitogenome sequencing.

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43 604 *(b) Paternal lineage: Y chromosome*

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45 605 Studies using uniparentally inherited Y chromosome loci are scarce compared to biparental
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47 606 markers and mtDNA, primarily due to the shortage of available polymorphic loci. Paternal
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49 607 inheritance and a lack of recombination (except the pseudoautosomal regions) have made the
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51 608 Y chromosome a useful tool for studying uniquely male-inherited lineages, providing an
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53 609 essential complement to maternally inherited mtDNA and biparentally inherited microsatellite
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55 610 or SNP data. When compared with mtDNA, variation in Y-linked loci allows detection of
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3 611 contrasting patterns of male and female population processes (e.g. Bidon *et al.*, 2014). A
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5 612 limited set of paternal Y chromosome microsatellite markers have been used in wolf
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7 613 population genetics to investigate colonization patterns (Sundqvist *et al.*, 2001; Caniglia *et al.*,
8
9 614 2014; Fabbri *et al.*, 2014), population structure and kin relationships (Grewal *et al.*, 2004),
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11 615 hybridization with dogs (Vilà *et al.*, 2003b; Iacolina *et al.*, 2010; Godinho *et al.*, 2011,
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13 616 Hindrikson *et al.*, 2012; Caniglia *et al.*, 2013; Randi *et al.*, 2014) and sex-biased genetic
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15 617 diversity (Sastre *et al.*, 2011).

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18 618 As with mtDNA, Y chromosome heterogeneity is low in Scandinavian (two haplotypes in
19
20 619 Sundqvist *et al.*, 2001; Vilà *et al.*, 2003a) and Iberian (four haplotypes in Sastre *et al.*, 2011
21
22 620 and six in Godinho *et al.*, 2011) wolf populations, but significantly higher in western Russia
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24 621 (nine to ten haplotypes in Sundqvist *et al.*, 2001 and Sastre *et al.*, 2011) and the Balkan region
25
26 622 (11 haplotypes in Croatian wolves in Fabbri *et al.*, 2014). In contrast to the pattern of mtDNA
27
28 623 variation, Y chromosome variation in Italian wolves is significantly higher (four haplotypes;
29
30 624 Iacolina *et al.*, 2010; Fabbri *et al.*, 2014; Caniglia *et al.*, 2014), suggesting male-biased gene
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32 625 flow between Italy and neighbouring populations.
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38 627 *Y chromosome studies in other large carnivore populations in Europe, and the main*
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40 628 *challenges for future patrilineal investigations*

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43 629 Y chromosome investigations are rare in other large carnivores and, besides wolves, have
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45 630 only been used to investigate brown bear populations (Bidon *et al.*, 2014; Schregel *et al.*,
46
47 631 2015). The main drawback of wolf Y chromosome studies is the limited number of
48
49 632 polymorphic Y chromosome markers available. Paternal studies would greatly benefit from
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51 633 using a larger number of Y chromosome specific loci, possibly combining paternal
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53 634 microsatellite and SNP data if neither of them provides sufficient resolution on its own; see
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3 635 for example a study on canids (dingoes and dogs) by Sacks *et al.* (2013) and a human study
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5 636 by Rootsi *et al.* (2013).
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11 12 639 **III. META-ANALYSIS OF GENETIC VARIABILITY IN EUROPEAN WOLVES**

13 14 640 **(1) Materials and methods**

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17 641 Meta-analyses of genetic diversity from microsatellites are usually composed of data sets that
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19 642 vary greatly in the identity and number of markers used, and this applies to the data available
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21 643 on European wolves. This complicates the comparison of heterozygosity parameters between
22
23 644 studies. One option to overcome this limitation was presented by Skrbinšek *et al.* (2012) who
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25 645 used the reference population approach with a simple solution of scaling the genetic diversity
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27 646 of each considered population relative to the genetic diversity of a single well-studied
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29 647 population, using the reference population as a calibration ‘yardstick’. By calibrating
30
31 648 previously incompatible studies through comparisons with a reference population, they were
32
33 649 able to compare the neutral genetic diversity of brown bears from many previously studied
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35 650 populations. However, such a calibration method could not be applied to wolf studies as the
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37 651 number of overlapping loci analysed in different studies is too small (in several instances only
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39 652 three out of 16 loci were identical; Appendix S4, see also De Groot *et al.*, 2016).
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44 653 Nevertheless, as the number of microsatellite loci analysed in different studies is relatively
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46 654 large, we consider the heterozygosity parameters sufficiently robust.
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48 655 To describe general large-scale trends and patterns of genetic variation in European wolf
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50 656 populations, we analysed the results of previous microsatellite studies and included new data,
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52 657 which altogether covered nine European wolf populations in 19 countries: Russia, Norway,
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54 658 Sweden, Finland, Estonia, Latvia, Lithuania, Poland, Germany, Czech Republic, Slovakia,
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56 659 Belarus, Italy, Croatia, Bulgaria, Bosnia and Herzegovina, Greece, Spain and Portugal
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3 660 (Appendix S4; Fig. 5) (note that the Sierra-Morena population is missing due to a lack of data,
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5 661 while data for Russian populations are included). Observed and expected heterozygosities
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7 662 (H_O , H_E), inbreeding coefficient (F_{IS}), allelic richness (R_A) that uses a rarefaction on the
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9 663 minimum number of samples per populations in the study, and the number of alleles per locus
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11 664 (N_A) were extracted. If the inbreeding coefficient was presented only for subgroups, it was
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13 665 calculated for the whole population according to the sample size weighted heterozygosities as
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15 666 $F_{IS} = 1 - H_O/H_E$ (Hartl & Clark, 1997).
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17
18 667 Linear trend surface analysis was applied to each variable to determine the presence and
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20 668 direction of a gradient (Fortin & Dale, 2005), followed by a test of the spatial trend. The
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22 669 analysis calculated spatial autocorrelation (SAC) structure via variogram modelling and
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24 670 spatial weighting. We used R function *gls* with spherical SAC structure in package *nlme*
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26 671 (Pinheiro *et al.*, 2013) with rotated geographic coordinates along the gradient direction. The
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28 672 Lambert conic conformal coordinate system was used to determine the constant azimuthal
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30 673 direction of the trend over the large area analyzed. The coordinate system was rotated around
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32 674 the spatial centre of the sample points and coordinates used for testing the trend's significance
33
34 675 were measured as relative to the centre. Due to a relatively small number of data-points, we
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36 676 focused on general patterns and did not test non-linear effects, but analysed the pattern in the
37
38 677 ten European populations separately. After the trend surface analysis, the presence of residual
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40 678 spatial autocorrelation was tested using Moran's autocorrelation index (I) and the compatible
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42 679 test of significance in the R package *ape* (Paradis *et al.*, 2004).
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681 (2) Results

682 (a) Genetic variation of the European wolf populations

683 We compared four indices of genetic diversity for ten wolf populations in Europe (Table 1;
684 Fig. 5; Appendix S4). Averaged genetic diversity was lowest in the isolated populations in

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2
3 685 Iberia and Italy. The Iberian population was also characterised by the lowest allelic richness.
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5 686 The highest heterozygosity was observed in the largest population (Dinaric-Balkan, see Table
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7 687 1).
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11 689 None of the variables were correlated with the size of the distribution area of analysed
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13 690 populations. The sample size was negatively correlated with two of the genetic indices (H_E : R^2
14
15 691 = 0.32, $p = 0.025$; R_A : $R^2 = 0.31$, $p = 0.03$). However, the observed heterozygosity and
16
17 692 inbreeding coefficient had no correlation with sample size (H_O : $R^2 < 0.01$, $p = 0.71$; F_{IS} : $R^2 =$
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19 693 0.02, $p = 0.70$). Sample size itself had no trend in Europe and was not affected by spatial
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21 694 autocorrelation.
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28 696 *(b) Genetic trends in the European wolf populations*

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30 697 There was a global spatial trend of heterozygosities (H_O and H_E) in the European wolf
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32 698 population. Heterozygosity values were considerably higher towards the north-east and lower
33
34 699 in south-western populations (Table 2a; Fig. 6). The average range of connectedness of
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36 700 populations suggested that the mean size of wolf functional subunits is about 770 km, as
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38 701 indicated by the extent of significance of spatial autocorrelation on trend model residual
39
40 702 values of H_O (650 km), H_E (800 km), and F_{IS} (850 km), (Table 2b). The reliability of the
41
42 703 detected patterns was indicated by zero or near zero nugget effects of the variogram models.
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44 704 A small nugget effect indicates low variance among independent estimations (different
45
46 705 studies) in the same geographic area, and, by extension, a robust pattern in the observed
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48 706 variable, and good repeatability of measured values. Allelic richness was distributed relatively
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50 707 evenly over Europe, having only a weak signal of spatial pattern and strong nugget effect of
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52 708 the variogram.
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710 (c) *Regional patterns*

711 Various genetic diversity patterns were detected within the two large distinct regions of the
712 wolf European range – Southern Europe (Iberia, Alps, Italy, Dinaric-Balkan), and North-East
713 Europe (Russia, Karelia, Baltic, Carpathians, Central European Lowland). In the
714 Scandinavian population, only limited data were available and it was not possible to to
715 include this region in the meta-analysis of regional patterns. In southern Europe, a significant
716 gradient of H_O and H_E is directed toward the north-east (Table 3). In the largest continuous
717 population in north-east Europe, a significant west-east gradient of expected heterozygosity
718 (H_E) was observed (Table 3). The lowest values appeared in Germany and the highest in
719 Estonia and Latvia (Fig. 6). The trend model residuals of the H_E were not spatially
720 autocorrelated (Moran's $I = 0.157$, $p = 0.061$). The observed heterozygosity (H_O), inbreeding
721 coefficient (F_{IS}) and allelic richness (R_A) did not exhibit significant spatial trends in north-east
722 Europe, and were significantly autocorrelated in space. The highest genetic variability (H_O
723 and R_A) was found in Estonia and Latvia, and the lowest in Poland. Despite the relatively low
724 heterozygosity in Germany, our results do not indicate significant inbreeding.

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727 **IV. WOLF GENETIC VARIATION AND ITS IMPLICATIONS**728 **(1) Genetic variation in European wolf populations**

729 The meta-analysis results are in accordance with recorded population history of wolves in
730 Europe: during the period of demographic decline, larger populations survived in the Balkans
731 and Eastern Europe, while small and fragmented populations remained in the Iberian and
732 Italian peninsulas, and the species was eradicated from central Europe and Scandinavia.
733 Historically, populations in southern Europe have been isolated for long periods of time,
734 possibly for several thousands of years in the case of the Italian (Lucchini *et al.*, 2004; Fabbri

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3 735 *et al.*, 2007) and Iberian (Sastre *et al.*, 2011) populations. Population decline, long-term
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5 736 geographical isolation and a lack of gene flow into the Italian and Iberian wolf populations
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7 737 explain the low genetic diversity and divergence from other European populations, indicated
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9 738 from microsatellite (Lucchini *et al.*, 2004; Godinho *et al.* 2011; Sastre *et al.*, 2011), mtDNA
10
11 739 (Pilot *et al.*, 2010) and SNP data (vonHoldt *et al.*, 2011; Stronen *et al.*, 2013; Pilot *et al.*,
12
13 740 2014a). Long-term isolation and demographic bottlenecks within these populations have
14
15 741 resulted in rather low allelic richness ($R_A_{Iberian}=3.8$; $R_A_{Italian}=4.3$). Low allelic richness (as a
16
17 742 proxy for low overall genetic variability) may compromise the long-term survival of a
18
19 743 population, as low genetic variability can become a constraining factor when a population is
20
21 744 challenged to adapt to changing environmental conditions. The mean number of alleles per
22
23 745 locus in the NW Iberian population is somewhat higher, 4.7–6.4 (Appendix S4), though the
24
25 746 isolated population in central Portugal has a very low estimate of 3.0 alleles per locus. The
26
27 747 most effective conservation strategy would require an increase in heterozygosity through
28
29 748 elevated gene flow and population growth. The NW Iberian population has been expanding
30
31 749 naturally eastward and southward in Spain (Blanco *et al.*, 1990) but in Portugal there are still
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33 750 no signs of wolf population growth, especially in central Portugal, where the wolf may be on
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35 751 the verge of extinction (Boitani & Ciucci, 2009). On the other hand, wolves from the Alpine
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37 752 population have reached the Iberian Peninsula in the last decade, but they currently remain in
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39 753 the Pyrenees and Catalonia, with no connectivity to the NW Iberian wolf population (Valière
40
41 754 *et al.*, 2003; Lampreave *et al.*, 2011; Sastre, 2011). Despite the low levels of genetic
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43 755 variability in Italian wolves, this population has active internal gene flow between
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45 756 subpopulations, in large part directed from the Apennines to the Alps (Fabbri *et al.*, 2007).
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47 757 This population has colonized the Alps, forming a new Alpine wolf population that is now
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49 758 coming in contact with wolves of Dinaric-Balkan origin in the east (Fabbri *et al.*, 2014; Ražen
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3 759 *et al.*, 2016), which may result in natural gene flow between the Alpine and Dinaric Balkan
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5 760 populations in future.
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7 761 The relatively high heterozygosity in north-eastern populations (Fig. 6) can largely be
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9 762 explained by their demographic connectivity to the large metapopulation in western Russia,
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11 763 which has long served as an important source of immigrants. Due to gene flow between
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13 764 different countries, the Baltic population shows medium to high levels of genetic diversity
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15 765 (Jędrzejewski *et al.*, 2005; Czarnomska *et al.*, 2013; Hindrikson *et al.*, 2013), despite strong
16
17 766 hunting pressure (Jędrzejewski *et al.*, 2005; Hindrikson *et al.*, 2013) (Fig. 7). The low H_o
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19 767 (0.45–0.58) and high F_{IS} in Polish and German wolves in Central European Lowland
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21 768 population (Czarnomska *et al.*, 2013) are indications of inbreeding, but this is most likely
22
23 769 counterbalanced in a size-limited population by occasional gene flow from the Baltic
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25 770 population. It was suggested that wolves colonizing western Poland and eastern Germany
26
27 771 primarily originate from northeastern Poland (Czarnomska *et al.*, 2013). Despite the relatively
28
29 772 high levels of heterozygosity, in our meta-analysis we found signs of inbreeding in north-
30
31 773 eastern European wolf populations (Table 1). Recent inbreeding has also previously been
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33 774 found in eastern European wolf populations by Pilot *et al.* (2014a). Inbreeding may increase
34
35 775 under strong hunting pressure, which decreases population size and disrupts wolf social
36
37 776 structure (Valdmann *et al.*, 2004; Jędrzejewski *et al.*, 2005, Moura *et al.* 2014), potentially
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39 777 reducing the quality of traits that define apex predators (Ordiz *et al.*, 2013).
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47 779 **(2) Genetic trends in European wolf populations**

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49 780 We found a global spatial trend of heterozygosity with lower values in south-western
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51 781 populations and higher in northeastern (Table 2a). Such a trend is probably the result of
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53 782 several factors: recent population demographic history (hunting pressure and bottlenecks),
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55 783 connectivity (isolation in peripheral areas of the wolf distribution in Europe) and
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3 784 environmental variables. As environmental gradients in Europe have existed for a long period
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5 785 of time, they most likely have had an impact on genetic variability. For example, it is known
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7 786 that long-term human impact on wildlife forest habitats has been higher in areas where wolf
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9 787 heterozygosity parameters have low values (for example Iberia and Italy) (Kaplan *et al.*,
10
11 788 2009). The higher levels of heterozygosity in north-eastern Europe may be due to gene flow
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13 789 between northern and eastern European and Russian wolf populations (Aspi *et al.*, 2009; Pilot
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15 790 *et al.*, 2006).

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18 791 The range of spatial influence (based on analysis of three parameters of genetic
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20 792 diversity) is 650–850 km (Table 2), i.e. the genetic diversity of a wolf population in a certain
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22 793 location is influenced by populations up to 850 km distant. This is, for example, the
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24 794 approximate distance from Tartu (Estonia) to Białowieża (Poland) – indeed, it is likely that
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26 795 the gene flow extends from Estonia to northern Poland as wolves in Europe are known for their
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28 796 long-distance dispersal of 800 km and more (Wabakken *et al.*, 2007; Andersen *et al.*, 2015;
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30 797 Ražen *et al.*, 2016). Despite this, recent findings suggest that gene flow can be restricted even
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32 798 in less humanized areas, due to prey and habitat specialization (Pilot *et al.*, 2006) and human
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34 799 built obstacles (Aspi *et al.*, 2009). Radiotracking of wolves has also suggested that few
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36 800 individuals in northern Europe disperse more than 400 km (Kojola *et al.*, 2009) - half of the
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38 801 genetic distance of spatial influence found in our results. Furthermore, few of the dispersal
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40 802 events contribute to gene flow because of human caused mortality (f. e. Kojola *et al.*, 2009;
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42 803 Liberg *et al.*, 2012). In this sense, those considerations should be taken into account for
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44 804 scenarios dominated by anthropogenic landscapes to avoid significant drawbacks at smaller
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46 805 and more fragmented Europe's wolf populations (Delibes, 1990; Hindrikson *et al.*, 2013),
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48 806 particularly in southern regions (Randi, 2011).
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3 808 **V. THE MAIN THREATS ON WOLF POPULATIONS IN EUROPE**

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5 809 **(1) Europe in general**

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7 810 Common threats to almost all wolf populations in Europe are overharvesting (incl. poaching),
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9 811 low public acceptance and conflicts due to livestock depredation (Table 4), resulting most
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11 812 likely from a lack of knowledge and poor management structure, but also from livestock
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13 813 damage and deep fears of wolf attacks on humans and dogs. However, other threats, such as
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15 814 habitat destruction and large fluctuations in prey base, are also relevant to the majority of
16
17 815 populations. Thus, various human-related factors are undoubtedly the main source of threats
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19 816 to wolf populations in Europe, and the generally negative human attitude toward wolves has
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21 817 been and remains the primary threat to wolf populations. Historically, even infectious diseases
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23 818 (e.g. rabies, sarcoptic mange) have not had such a devastating impact on wolf numbers as
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25 819 negative human attitudes, resulting in severe hunting pressure (legal and illegal), which in
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27 820 many areas in Europe led to wolf eradication in the past and continues to threaten small
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29 821 endangered populations (e.g. in Sierra Morena). Large carnivores can coexist with humans if
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31 822 a favourable management policy is applied (Linnell *et al.*, 2008), but their role as apex
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33 823 predators is reduced if they don't reach ecological functionality (Estes *et al.*, 2011; Ordiz *et*
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35 824 *al.*, 2013, and references therein). Nevertheless, there is a pressing need to mitigate conflicts
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37 825 in ways that are both effective and acceptable (Sillero-Zubiri & Laurenson, 2001). Two large
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39 826 international legislation systems currently direct wolf management in Europe: "Convention on
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41 827 the Conservation of European Wildlife and Natural Habitats" (the Bern Convention), and the
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43 828 "Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna
44
45 829 and Flora" (the Habitats Directive) (Trouwborst, 2010). Although these international
46
47 830 agreements seek to standardise conservation actions across Europe, both the Bern Convention
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49 831 and the Habitats Directive have allowed some countries to make national or local
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51 832 modifications to the status of wolves under the legislation. However, the conservation actions
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3 833 taken to date have apparently not been sufficient to protect all wolf populations under threat,
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5 834 e.g. in case of wolf populations in Sierra Morena (see below).
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7 835 To handle the threats and conservation/management issues in European wolf populations in a
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9 836 systematic manner, we first identify the main gaps in current knowledge and suggest solutions
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11 837 to overcome these limitations and eventually provide suggestions for efficient science-based
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13 838 wolf conservation and management in Europe.
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18 840 **(2) Different populations in Europe**

19 841 *Scandinavian population*

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21 842 By 1966 wolves were functionally extinct on the Scandinavian Peninsula (Wabakken *et al.*,
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23 843 2001). Since their re-establishment in 1983, wolves in Scandinavia have been subject to long-
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25 844 term monitoring. Due to the very limited number of founders, major conservation issues have
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27 845 been inbreeding depression, low genetic variability and low-level gene flow with other
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29 846 populations (Vilà *et al.*, 2003a, Liberg *et al.*, 2005; Bensch *et al.*, 2006). Inbreeding has
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31 847 caused strong reductions in two fitness components: winter litter size (Liberg *et al.*, 2005) and
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33 848 recruitment of individuals to breeding (Bensch *et al.*, 2006). Poaching (Table 4; Fig. 9) has
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35 849 been another major threat, accounting for approximately half of the total mortality in Sweden
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37 850 with more than two-thirds of total poaching remaining undetected by conventional methods
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39 851 (Liberg *et al.*, 2012).
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45 852 Norway culled some wolves in 2001, claiming the population had already spread too far. In
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47 853 2010, Sweden licensed the hunting of wolves to keep the population down to 210 individuals,
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49 854 a temporary goal set by the country's parliament decree. The wolf hunt and its effect on the
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51 855 conservation and management issues has however been highly debated.
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54 856 The Swedish Society for Nature Conservation has been critical, claiming that culling is
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56 857 against EU legislation as the Swedish wolf population had not reached a healthy status. The
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3 858 issue eventually reached the European Commission (EC): the Union biodiversity legislation
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5 859 requires all member states to follow the “favourable conservation status”. As a result of the
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7 860 complaints, EC sent a reasoned opinion (in June 2015) requesting Sweden to amend its policy
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9 861 to protect the endangered wolf population in the country. This included the request for
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11 862 Sweden to bring wolf hunting into line with the EU legislation, thus guaranteeing that the
12
13 863 species reach favorable conservation status. A recent report suggests that a long-term goal for
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15 864 the Scandinavian wolf population should be 500 wolves (Kaczensky *et al.*, 2013). Based on
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17 865 another report by commissioned expert statements, the Swedish Environmental Protection
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19 866 Agency decided (in October 2015) that, given that the Scandinavian wolves is a part of a
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21 867 larger northern European population by gene flow (including minimum one effective
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23 868 immigrant per generation into the Scandinavian population), the Swedish population needs to
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25 869 consist of at least 300 wolves to be considered to have favorable conservation status.
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31 871 *Karelian population*

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34 872 The primary threat to Finnish wolves is illegal killing. The current (Finnish) Karelian
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36 873 population is not only small in size, but also significantly more inbred than before, and the
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38 874 observed heterozygosities significantly lower than among wolves born at the end of the 1990s
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40 875 (Jansson *et al.*, 2012). Additionally, gene flow between Russian Karelian and Finnish
41
42 876 populations seems to be low (Aspi *et al.*, 2009; Jansson *et al.*, 2012). In order to maintain a
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44 877 genetically healthy and viable wolf population in the long-term, the ultimate management
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46 878 goal is to facilitate gene flow between Finnish and Russian parts of Karelian population
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48 879 (Jansson *et al.*, 2014) and to decrease the hunting pressure. This goal is especially difficult to
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50 880 achieve in the reindeer husbandry area, which is very large (approximately half of Finland),
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52 881 where wolves are eliminated or driven away within days of arrival. The wolf became
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54 882 protected in Finland outside the reindeer husbandry area in 1973, but until 1995 it was listed
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3 883 as a normal game species, and the population was controlled by hunting (Bisi *et al.*, 2007).
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5 884 Following EU membership in 1995, Finland had to tighten its own legislation concerning the
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7 885 conservation status of the wolf. According to the EC Habitats Directive the wolf is listed in
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9 886 Appendix IV (strictly protected) with an exception in the Finnish reindeer herding area, where
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11 887 the wolf is listed in Appendix V (hunting is possible). The Ministry of Agriculture and
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13 888 Forestry annually grants a restricted number of licenses to kill wolves. The number of animals
14
15 889 killed per year (including animals killed in car accidents) has ranged between 5 and 27 in
16
17 890 years 2000–2005 (Bisi *et al.*, 2007). In the Management Plan of the Wolf Population Finland
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19 891 (Ministry of Agriculture and Forestry 11b/2005) from year 2005 it was recommended that
20
21 892 Finland should have at least 20 breeding pairs. However, this goal was achieved (between
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23 893 2005–2014) only in 2006 when there were 25 breeding pairs in Finland. A new management
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25 894 plan for wolves was accepted in Finland in 2015 and “population management” hunting was a
26
27 895 part of this new plan. Accordingly, the Finnish Wildlife Agency licensed the hunting of 24
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29 896 wolves in 2015 and an additional ten wolves can be killed per year based on damages or close
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31 897 encounters. The rationale of the “population management” hunting has been hotly debated in
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33 898 Finland.
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41 *Baltic population*

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43 901 Low public acceptance due to livestock depredation, especially in islands in western Estonia
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45 902 (Plumer *et al.*, in prep.), diseases and human-caused mortality, including illegal killing, are
46
47 903 the biggest threats the Baltic wolf population is facing (Table 4; Fig's. 7, 9). However, large
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49 904 infrastructure developments and fragmentation of suitable habitats by intensive forestry and
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51 905 an increase in agricultural land can also pose a significant threat. These problems are expected
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53 906 to remain, if not increase in the future, e.g. the forthcoming construction of Rail Baltic and
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55 907 new highways. Moreover, the new fence currently being built at the Estonian-Russian border
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3 908 will probably decrease gene flow between the wolf populations between the countries, though
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5 909 complete isolation is unlikely as wolves can cross Lake Peipus during wintertime. Although
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7 910 gene flow occurs between Latvia and Estonia (Hindrikson *et al.*, 2013), there is no
8
9 911 information on the extent of gene flow in the whole Baltic population. Similarly, there is a
10
11 912 lack of knowledge on gene flow with neighbouring populations. Hybridization with dogs has
12
13 913 been identified in Latvia, Estonia and northern Poland (Andersone *et al.*, 2002; Hindrikson *et*
14
15 914 *al.*, 2012; Stronen *et al.*, 2013), but not in Lithuania (Baltrūnaitė *et al.*, 2013). However, the
16
17 915 rate of introgressive hybridization has not yet been determined; if high, it can pose a treat to
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19 916 wolf long-term adaptive potential (Table 4).
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918 *Central European Lowland population*

919 In the expanding Central European Lowland population the main threats are the road
920 mortality, high human population density and illegal killing. In western Poland the loss of any
921 individual has been thought to influence the survival of the pack or interrupt colonization of
922 adjacent areas (Jędrzejewski *et al.*, 2008). Species distribution modeling has found that human
923 factors, especially road density and culling might limit the further spread of the species in
924 Germany (Fechter & Storch, 2014) (Table 4). The connectivity of the Central European
925 Lowland population with neighbouring populations is still weak and currently restricted to
926 occasional gene flow from the Baltic population and interbreeding between closely related
927 animals can occur (Kaczensky *et al.*, 2013). However, the population shows a continuous
928 increase, suggesting that the capacity limit of this poplations has not been reached yet.

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930 *Italian populations (includes both Italian peninsular and Alpine populations)*

931 The current wolf population expansion on the Italian peninsula is increasing conflicts with
932 humans, especially in areas where free-grazing on open pastures is widespread (Meriggi *et al.*,

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3 933 2011; Milanesi *et al.*, 2012). Both Italian peninsular and Alpine populations face threats that
4
5 934 are mainly related to low public acceptance, poor management structure, lack of knowledge,
6
7 935 persecution, accidental mortality, etc. (Table 4; Fig. 9). Of these, illegal killing through
8
9 936 poisoning remains the most important cause of mortality (Marucco *et al.*, 2009; Marucco &
10
11 937 McIntyre, 2010). Hybridization with dogs in areas of the central Apennines has also become a
12
13 938 serious concern (Randi, 2008). The genetic diversity of these populations is one of the lowest
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15 939 in Europe (see Table 1) but there are signs of improved connectivity with other European
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17 940 populations: on one hand the Alpine population is incorporating animals from the Dinaric-
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19 941 Balkan population (Fabbri *et al.*, 2014; Ražen *et al.*, 2016); on the other hand, wolves from
20
21 942 the Alpine population have expanded south-west, recently reaching the French Massif
22
23 943 Central, the Pyrenees and Catalonia in Spain (Valière *et al.*, 2003; Lampreave *et al.*, 2011;
24
25 944 Sastre, 2011) (see also Fig. 1). In general, administrative fragmentation and the obvious
26
27 945 absence of any national authority responsible for wolf management can be considered as
28
29 946 important threats that need to be urgently addressed through a renewed effort by the Ministry
30
31 947 of Environment, the key agency coordinating the regional governments in implementing
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33 948 national and EU laws.
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35 949

36 950 *Carpathian population*

37
38 951 In Poland, Slovakia and Romania the main problems are connected to livestock depredation
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40 952 (Kaczensky *et al.*, 2013) (Table 4; Fig. 9). For example in Slovakia where depredation on
41
42 953 livestock is commonplace, the current overlap of wolf habitats with sheep farming is ~90%
43
44 954 (Rigg, 2004). In some areas of the Carpathian population range, overhunting and poaching are
45
46 955 the main threats (Kaczensky *et al.*, 2013) (Fig's. 7, 9). Nevertheless, the population range and
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48 956 wolf numbers have increased in Slovakia despite hunting over the last 70 years: for example,
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50 957 during the last 20 years the population range has increased by 10% (=1264 km²) (L. Paule
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3 958 pers. comm). Similarly, there is a general lack of data on gene flow and impact of wolf
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5 959 hunting in Ukraine on the number of wolves in neighbouring Poland, Slovakia and Romania.
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10 961 *Dinaric-Balkan population*

11 962 In general, low acceptance (for example in Bulgaria, Slovenia, Bosnia and Herzegovina and
12
13 963 The Former Yugoslav Republic of Macedonia) due to overharvesting of wild ungulate
14
15 964 populations and therefore conflicts with hunters (mainly Greece, Bulgaria) or farmers
16
17 965 (livestock conflicts in Bulgaria, Slovenia) are common causes for human persecution
18
19 966 (Kaczensky *et al.*, 2013) (Table 4; Fig. 7, 9). In several countries (Serbia, Bosnia and
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21 967 Herzegovina, Bulgaria and The Former Yugoslav Republic of Macedonia) the main threats
22
23 968 are the limited knowledge of wolf ecology and population trends and poor management
24
25 969 structure (Kaczensky *et al.*, 2013) (Table 4; Fig. 9). The population appears to be more or less
26
27 970 continuous throughout the Dinaric-Balkan range and is one of the genetically most
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29 971 heterogeneous in Europe (Table 1), having connections with the Alpine (Fabbri *et al.*, 2014)
30
31 972 population (Fig. 1). In general, there is a need to clarify the distribution and populational sub-
32
33 973 structuring within this large population. In some countries such as Albania, Greece and
34
35 974 Southern Croatia (Dalmatia), hybridization with dogs might pose a potential risk (Kaczensky
36
37 975 *et al.*, 2013; Stronen *et al.*, 2013; Majić-Skrbinšek, 2014). In Bulgaria, a recent genetic study
38
39 976 found hybridization of wolves with domestic dogs and possibly also with golden jackals
40
41 977 (Moura *et al.*, 2014), while in Greece, an animal with dog ancestry was identified (Stronen *et*
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43 978 *al.*, 2013).
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52 980 *NW Iberian population*

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54 981 This Wolf population is considered by the IUCN as “Near Threatened (NT)” because of the
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56 982 fragmentation in management regimes, the lack of a population level management plan and
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3 983 the occurrence of largely unpredictable events (human reactions against wolves) that may
4
5 984 threaten the population at the local level (IUCN, 2007). In fact, the lack of coordination
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7 985 between authorities in the two countries and within the various autonomous regions of Spain,
8
9 986 together with the separation between science and management, are considered critical issues
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11 987 for the NW Iberian population (Kaczensky *et al.*, 2013). Additionally, there is no genetic
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13 988 assesment considered for management planning (but see Godinho *et al.*, 2014).

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15
16 989 The lack of updated population information due to non-standardized census methods and the
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18 990 unreliable breeding wolf pack estimations in several regions are a major source of concern
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20 991 given the fact that wolves are exposed to hunting or to regional administration culls, except in
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22 992 Portugal, where they are fully protected (Kaczensky *et al.*, 2013).

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24
25 993 An important threat is the low acceptance of the species by rural people due to wolf damage
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27 994 to livestock, leading to high rates of illegal killings, both in Spain and in Portugal (Blanco *et*
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29 995 *al.*, 1990; Álvares 2004; Blanco & Cortés, 2009; Table 4). Other threats include human-
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31 996 related disturbance and loss of habitat quality (non natural fires, infrastructure development
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33 997 and lack of wild prey, particularly in Portugal; Santos *et al.*, 2007).

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35
36 998 In addition, hybridization with dogs is another possible threat in some areas, depending on
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38 999 wolf distribution and human perturbation (i.e. Leonard *et al.*, 2014): in a recent genetic survey
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40 1000 of the Iberian population, 4% of the sampled individuals were hybrids (Godinho *et al.*, 2011).
41
42 1001 On the other hand, genetic heterozygosity (Table 1) is the lowest in Europe and connection
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44 1002 with other wolf populations is non-existent, as indicated by the high inbreeding coefficient
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46 1003 ($F_{IS}=0.142$).

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51 1005 *Sierra Morena population*

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54 1006 The population is located in Sierra Morena, southern Spain (Andalusia and Castilla-La
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56 1007 Mancha Autonomous Regions), is isolated and critically endangered despite nearly 30 years
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3 1008 of legal protection. The population was estimated to contain 6-10 packs in 1988 (Blanco *et*
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5 1009 *al.*, 1990). However, in the following years the breeding population has reduced drastically,
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7 1010 perhaps to one pack, according to the Andalusian regional government wolf monitoring
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9 1011 program (see also López-Bao *et al.*, 2015). Proable causes are illegal killing to reduce
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11 1012 competition for game species and avoid damage to livestock. Unless effective measures are
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13 1013 implemented, the Sierra-Morena wolf population will be the first to become extinct in Europe
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15 1014 during the 21st century (López-Bao *et al.*, 2015).
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24 1017 **VI. SIGNIFICANT GAPS IN KNOWLEDGE AND POSSIBLE SOLUTIONS**

25 1018 Although numerous wolf population genetic and other studies have been published, several
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27 1019 significant gaps can be highlighted:
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32 1021 *Gap 1.* There is a lack of Europe-wide genetic studies covering all European wolf
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34 1022 populations. In their recent publication, Chapron *et al.*, (2014) divided wolves in Europe into
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36 1023 ten populations, based largely on wolf distribution data. However, for an accurate definition
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38 1024 of management units, such information should be coupled with a deeper understanding of
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40 1025 wolf dispersal (gene flow) and population genetic structure. Knowledge about levels of gene
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42 1026 flow within and between different wolf populations in Europe, and with neighbouring
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44 1027 populations in West Asia and countries out of EU (e.g. in Caucasus, Russia, Belarus, Ukraine
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46 1028 and Albania) is limited.

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49 1029 *Solution:* Europe-wide population genetic project, also engaging researchers from West Asia
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51 1030 and non-EU countries.
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3 1032 *Gap 2.* Sampling schemes are not always adequate in terms of sample numbers and
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5 1033 geographical coverage. Moreover, analyses are often based on dead individuals (that are not
6
7 1034 part of the population any longer); ideally, one should be able to obtain a real-time picture of
8
9 1035 a wolf population and track the fate of animals for a longer period of time to understand
10
11 1036 ongoing population processes, at least in problem areas (see Godinho *et al.*, 2014). To this
12
13 1037 end, non-invasive sampling (e.g. based on scats) is highly appropriate.

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16 1038 *Solution:* Develop unified sampling protocols and encourage the use of non-invasive sampling
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18 1039 methods.

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23 1041 *Gap 3.* There is a lack of common methods and sets of genetic markers that are universally
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25 1042 comparable between studies.

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27 1043 *Solution:* The rapidly developing field of genomics holds great promise for wolf population
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29 1044 analysis. However, it is not yet clear which methods will be most appropriate to adopt in
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31 1045 terms of data quality and cost.

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34 1046 For analysis of the maternal lineage, the focus in the future should be on sequencing complete
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36 1047 mitochondrial genomes, which has already demonstrated its advantages for example in brown
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38 1048 bears (e.g. Keis *et al.*, 2013). For the paternal lineage, there is an urgent need to develop a
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40 1049 panel consisting of a large number of polymorphic Y chromosome specific loci (SNPs,
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42 1050 microsatellites). For the analysis of autosomal biparental markers, there are three main
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44 1051 options: (1) to use low-coverage whole-genome sequencing; (2) to use SNP-chips; or (3) to
45
46 1052 use NGS-based microsatellite genotyping. For population analysis, the second and third
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48 1053 options are currently more economical, but the advantages of whole-genome sequencing are
49
50 1054 apparent: it provides more comprehensive data, enabling coverage of autosomes, the
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52 1055 mitogenome and the Y chromosome. The main problem associated with whole-genome
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54 1056 sequencing is its cost. If only individual identification is required, e.g. for cost-effective and
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3 1057 long-term genetic monitoring of wolves across Europe (preferably based on non-invasive
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5 1058 samples), then nanofluidic SNP genotyping technology based on 96 SNP loci (Kraus *et al.*,
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7 1059 2015) and the commercially available multiplex kit for 19 microsatellite loci are perhaps the
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9 1060 best options available at the moment, but the latter requires standardization to compare data
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11 1061 produced by different groups. The standardization has recently been highlighted also by De
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13 1062 Groot *et al.* (2016).

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18 1064 *Gap 4.* Lack of Europe-wide genetic studies to analyse hybridization between wolves and
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20 1065 dogs and the level of introgression of dog genes into wolf populations.

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22 1066 *Solution:* to develop a Europe-wide hybridization project. All three types of parental markers
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24 1067 should be used to evaluate the level of hybridization and introgression, and also their
25
26 1068 directionality.

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31 1070 *Gap 5.* Limited knowledge of wolf depredation on livestock. Since public attitudes and
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33 1071 management measures are largely dependent on rates of wolf depredation, it is necessary to
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35 1072 have accurate measures of depredation rates. As livestock can be killed not only by wolves,
36
37 1073 but also by domestic dogs and other predators, genetic methods should be used to identify the
38
39 1074 involvement of wolves and other predator species in livestock depredation (Sundqvist *et al.*,
40
41 1075 2008; Echegaray & Vilà 2010; Caniglia *et al.*, 2013; Milanesi *et al.*, 2015; Plumer *et al.*, in
42
43 1076 prep.). The impact of certain management actions on livestock predation should also be
44
45 1077 studied, as it has recently been shown that culling actually increases attacks on livestock in
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47 1078 North America (Wielgus & Peebles, 2014).

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49 1079 *Solution:* establish a unified genetic methodology to analyse the proportion of livestock killed
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51 1080 by wolves, and a unified management reporting system.

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6 1083 **VII. SUGGESTIONS FOR SCIENCE-BASED WOLF CONSERVATION AND**
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8 1084 **MANAGEMENT IN EUROPE**
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10 1085 For the long-term survival of European wolves and to achieve favourable conservation status
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12 1086 (mandatory by EU rules), there is a need to increase the overall population size and favour
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14 1087 wolf dispersal and connectivity between and within populations. It is therefore important to
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16 1088 evaluate the effective size of entire meta-population to establish scientifically based
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18 1089 demographic and genetic targets.

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21 1090 There are several outstanding issues to be solved in order to warrant the most efficient
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23 1091 science-based wolf conservation and management (Table 5, Table 6; Fig. 8).
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28 1093 1) Wolf populations should ideally be managed according to biological units, i.e. a
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30 1094 population should include areas connected with moderate to high gene flow. Further
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32 1095 genetic analysis covering all wolf populations in Europe will be necessary to define
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34 1096 the exact number and spatial distribution of populations.

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37 1097 2) A European Union Wolf Scientific Committee (EU-WSC) involving scientists from
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39 1098 all EU countries containing wild wolf populations should be established to guarantee
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41 1099 evidence-based scientific decision making. Representatives of government officials,
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43 1100 major stakeholders and scientists from neighbouring wolf-countries should also be
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45 1101 invited to take part when necessary.

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47
48 1102 3) For better implementation of EU legislation and strengthening the evidence-based
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50 1103 scientific decision making, we suggest establishing a European Union Reference
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52 1104 Laboratory of Wolf Studies (EURL-Wolf) (Table 5; Fig. 8). The aim of EURL-Wolf
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54 1105 is to coordinate a network of national reference laboratories, train laboratory staff and
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56 1106 provide reference methods and services to countries without a national reference
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3 1107 laboratory. It is important to note that the priority to conduct scientific research would
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5 1108 remain with national laboratories, whereas the role of the reference laboratory would
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7 1109 be to help develop and adapt reference methods, organise services to those countries
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9 1110 without a reference laboratory, and coordinate Europe-wide data analysis and data
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11 1111 sharing. Thus, EURL-Wolf would support the creation of a well performing network
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13 1112 of laboratories throughout the European Union, strengthen science-based decision
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15 1113 making in wolf conservation and management in the EU, stimulate innovation through
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17 1114 developing and adapting new methods, tools and standards, and share its know-how
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19 1115 with the Member States, the scientific community and international partners.
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25 1117 Under EURL-Wolf, several dedicated reference laboratories could be established, responsible
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27 1118 for various scientific analyses that are necessary to provide adequate information on wolf
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29 1119 populations across Europe. Two such dedicated reference laboratories are perhaps most
30
31 1120 urgently required: (a) wolf population genetics (EURL-Wolf-Gen), and (b) wolf diet and
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33 1121 pathogens (EURL-Wolf-DP).

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36 1122 EURL-Wolf-Gen would coordinate genetic research, provide standardization and regularly
37
38 1123 analyse samples from different countries in Europe (and beyond) for various wolf-monitoring
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40 1124 and scientific purposes. A platform for direct exchange of genetic and other data should be
41
42 1125 established to facilitate effective information exchange, while guaranteeing intellectual
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44 1126 property rights.

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47 1127 EURL-Wolf-DP would coordinate research, provide standardization and regularly analyse
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49 1128 samples connected with analysis of wolf food habits and pathogens across Europe. Studies on
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51 1129 food habits provide essential data for the wolf prey-base in different regions in Europe (e.g.
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53 1130 Valdmann *et al.*, 2005; Zlatanova *et al.*, 2014), the ratio of wild prey/livestock in wolf diet,
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55 1131 etc. Knowledge on wolf food habits is crucial for reducing conflicts with various stakeholders
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3 1132 and envisaging appropriate conservation-management plans. Although a large number of local
4
5 1133 studies have been performed, the overall level of knowledge about wolf diet is poor.
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7 1134 Wolf pathogens should also be studied on a regular basis to understand their role in wolf
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9 1135 mortality and potential transmission of pathogens between wolves and free-ranging dogs and
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11 1136 from them to humans. Wolves are well known to transmit rabies, but they can also transmit
12
13 1137 other hazardous zoonotic pathogens (parasites, viruses, etc.), for example tapeworms
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15 1138 *Echinococcus granulosus* and *E. multilocularis* (e.g. Moks *et al.*, 2006; Marcinkute *et al.*,
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17 1139 2015) that are the cause of life-threatening diseases: cystic and alveolar echinococcosis,
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19 1140 respectively.
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23 1141 4) Using questionnaires, regular European-wide studies should be initiated to
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25 1142 investigate public attitudes. Based on these and other available data, significant effort
26
27 1143 should be made to improve the knowledge of problems related to wolves and their
28
29 1144 mitigation. A “European Union Wolf Web-page” should be established to provide up-to-
30
31 1145 date information on wolves in Europe (scientific results in popular format, changes in
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33 1146 legislation, population data, etc.).
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36 1147 5) A tradition of biannual European wolf conferences should be established. Such
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38 1148 conferences would serve as a main meeting place for wolf experts and other interested
39
40 1149 parties to present new results and discuss and share ideas to improve wolf research,
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42 1150 protection, management, public awareness, etc.
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47 48 49 50 1153 **XIII. CONCLUSIONS**

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52 1154 (1) Ongoing protection of European wilderness zones, socio-economic changes and recovery
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54 1155 of wild ungulates has enabled wolves to recolonize many parts of its former range in Europe.
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56 1156 Currently, ca 12 000 wolves occupy over 800 000 square kilometres in 28 European countries
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3 1157 with 9 900 of these animals present in 22 countries belonging to the European Union. Several
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5 1158 remarkable examples of wolf recovery in Europe have been described, e.g. in Scandinavia and
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7 1159 Italy. At the same time there are examples of populations that have recently gone extinct, such
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9 1160 as in the Alentejo region (Portugal), or are on the verge of extinction, such as in Sierra
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11 1161 Morena (Spain).

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16 1163 (2) A Europe-wide meta-analysis was conducted based on the results of available and new
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18 1164 microsatellite- data. As a result, the range of spatial autocorrelation was 650–850 km,
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20 1165 suggesting that the genetic diversity of a given wolf population can be influenced by
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22 1166 populations up to 850 km away.

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27 1168 (3) As an important outcome of this synthesis, we have discussed the most pressing issues
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29 1169 threatening wolf populations in Europe, highlighted important gaps in current knowledge,
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31 1170 suggested solutions to overcome these limitations, and provided suggestions for science-based
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33 1171 wolf conservation and management at regional and Europe-wide scales. Among these the
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35 1172 most significant are: 1) wolf populations should ideally be managed according to biological
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37 1173 units, which requires additional genetic analysis covering all wolf populations in Europe to
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39 1174 define the exact number and spatial distribution of populations; 2) to establish a European
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41 1175 Union Wolf Scientific Committee; 3) to establish a European Union Reference Laboratory of
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43 1176 Wolf Studies.

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25 1193 Competitiveness.
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35 1196 **X. REFERENCES**

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1753 **XI. SUPPORTING INFORMATION**

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46 1754 Additional supporting information may be found in the online version of this article.
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48 1755 **Appendix S1.** Different genetic markers used in analyses of the ten European wolf
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50 1756 populations.
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52 1757 **Appendix S2.** Confirmed dispersal between and inside wolf populations in Europe.
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54 1758 **Appendix S3.** MtDNA haplotypes.
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56 1759 **Appendix S4.** Wolf populations in Europe included in the meta-analysis.
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Figure Legends

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Figure 1. Wolf distribution and directions of gene flow in Europe. Green indicates wolf permanent occurrence, and dark grey sporadic occurrence (modified from Chapron *et al.*, 2014). Wolf occurrence in Russia, Ukraine and Belarus is not marked on the map. Confirmed dispersal between and inside wolf populations is indicated by red arrows (see also Appendix S1 for additional information and references).

Figure 2. Different genetic markers used in studies of European wolf populations (according to Table S1). Yellow: autosomal microsatellites (biparental); Orange: mitochondrial DNA (maternal); Red: Y-chromosome microsatellites (paternal).

Figure 3. Wolf autosomal SNP genotyping studies in Europe. Countries marked as (1) Red represents sampling locations from vonHoldt *et al.* (2011; 47 000 SNPs); (2) Orange represents sampling locations from Stronen *et al.* (2013; 67 000 SNPs); (3) Yellow represents sampling locations from Pilot *et al.* (2014a; 61 000 SNPs).

Figure 4. Median joining network of mtDNA control region sequences (609 bp) of 160 wolves from Europe and adjacent populations. Filled circles represent median vectors (haplotypes not sampled or extinct). Additional data for haplotypes are in Appendix S3.

Figure 5. The geographic location of wolf microsatellite studies included in the meta-analysis. Colours represent populations according to Chapron *et al.*, (2014). The Sierra-Morena population is missing due to lack of data, while data for Russian populations are included. The numbers in circles represent ID's according to Appendix S4.

Figure 6. Spatial trends of observed (H_O) and expected (H_E) heterozygosities in European wolf populations. Significant spatial trends were observed along the slope direction for both H_O and H_E (see Table 2 for trend model parameters). The arrow represents the direction of a gradient (x-axis of the graphs). The numbers correspond to populations according to

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3 1792 Appendix S4. Colour codes on the maps (left) correspond to the level of heterozygosity,
4 1793 whereas colours on graphs (right) correspond to populations according to Fig. 5.

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8 1795 **Figure 7.** The levels of legal hunting pressure in European wolf populations. Red – high
9 1796 hunting pressure (>35% of population size); Orange – medium hunting pressure (10-35%);
10 1797 Yellow – low hunting pressure (<10%; including countries where wolves are protected).
11 1798 Note that in Italy and Portugal, where wolf hunting is illegal, the level of hunting pressure
12 1799 comes from poaching that is estimated to remove ca 20% and <10% of total wolf
13 1800 population per year, respectively. For other countries only legal hunting pressure is
14 1801 illustrated on the map.

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21 1803 **Figure 8.** Science-based wolf conservation and management in Europe, coordinated by an
22 1804 international scientific committee and two reference laboratories. Above are six major
23 1805 Europe-wide scientific focus areas to promote effective wolf conservation and management
24 1806 in Europe. * Projects that include genetic analysis. See also Tables 5, 6.

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29 1808 **Figure 9.** Threats to wolves in Europe. Threat points are calculated according to Table 4:
30 1809 “-” – 0 points; “?” – 0 points; “+/-” – 1 point; “y” – 2 points). Yellow: 1–6 points; Orange:
31 1810 7–12 points; Red: 13 – ... points. Grey cells indicate sporadic occurrence (from Chapron *et*
32 1811 *al.*, 2014). Wolf occurrence in Russia, Ukraine and Belarus is not marked on the map.

Table 1. Mean values \pm standard deviation for observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and allelic richness (R_A) on the largest four European wolf populations. *includes Baltic, Scandinavian, Finnish and Russian wolves.

Region	Sample size (N)	H_O	H_E	F_{IS}	R_A
Northern and eastern Europe*	869	0.606 ± 0.095	0.677 ± 0.055	0.123 ± 0.111	6.08 ± 1.25
South-Europe, including:	2448				
Dinaric-Balkan	338	0.658 ± 0.033	0.700 ± 0.027	0.062 ± 0.018	6.10 ± 0.59
Italy (Italian peninsular and Alpine populations)	1622	0.551 ± 0.061	0.568 ± 0.048	0.032 ± 0.036	4.34 ± 0.81
NW Iberia	488	0.526 ± 0.018	0.621 ± 0.021	0.142 ± 0.035	3.76 ± 2.50
Total		0.590 ± 0.085	0.648 ± 0.067	0.098 ± 0.097	5.44 ± 1.53

Table 2. Spatial trends (a) and local autocorrelation pattern (b) of four genetic diversity indices. A spherical variogram model was used. The variogram nugget is measured as relative to maximal modelled semivariance.

Index	a. Spatial trend				b. Local pattern			
	Azimuth of gradient	R^2	$df_{\text{residuals}}$	$P_{\text{sp. corrected}}$	Variogram nugget	Variogram range, km	Moran's I of trend residuals	P_{SAC}
H_O	68°	0.23	54	0.011	0.00	650	0.50	< 0.001
H_E	67°	0.32	56	0.025	0.17	800	0.40	< 0.001
F_{IS}	117°	< 0.01	53	0.695	0.00	850	0.55	< 0.001
R_A	98°	0.31	46	0.032	0.50	2835	0.19	0.037

Table 3. Spatial trends (a) and local autocorrelation patterns (b) of four diversity indices in two large wolf population clusters. A spherical variogram model was used. The variogram nugget is measured as relative to maximal modelled semivariance.

Index	a. Spatial trend				b. Local pattern			
	Azimuth of gradient	R^2	df _{residuals}	P _{sp. corrected}	Variogram nugget	Variogram range, km	Moran's I of trend residuals	P _{SAC}
South Europe (NW Iberia, Alpine, Italian peninsula, Dinaric-Balkan)								
H_O	28°	0.28	24	0.037	0.33	1200	0.51	0.001
H_E	13°	0.14	25	0.031	0.14	1100	0.69	< 0.001
F_{IS}	88°	0.07	24	0.909	0.47	1600	0.32	0.034
R_A	40°	0.37	17	0.089	0.33	3307	0.32	0.034
North-East Europe (Russia, Karelia, Baltic, Carpathians, Central European Lowland)								
H_O	19°	0.38	26	0.225	0.07	800	0.60	< 0.001
H_E	99°	0.34	27	0.016	0.55	750	0.16	0.088
F_{IS}	148°	0.25	27	0.091	0.33	500	0.35	< 0.001
R_A	19°	0.01	25	0.843	0.35	640	0.32	< 0.001

Table 4. The most relevant threats to different wolf populations in Europe. “y” – threat considered important in the population; “-“ – threat not considered important in the population; “+/-“ – threat considered important in some parts of the population; “?” – no information;

	Alpine	Baltic	Carpa- thians	Central- European Lowlands	Dinaric- Balkan	Italian Peninsula	Karelian	NW Iberia	Scandinavian	Sierra Morena
Overharvest and poaching	y	y	-	Y	y	y	y	y	+/-	y
Low public acceptance	y	y	y	+/-	y	y	y	-	y	y
Habitat destruction	-	+/-	-	y	+/-	-	-	y	-	+/-
Barriers to gene flow	-	+/-	-	+/-	+/-	-	+/-	-	y	y
Poor management	-	-	-	-	y	-	y	y	-	y
Poor scientific knowledge	-	+/-	+/-	-	+/-	y	-	y	-	y
Inbreeding	-	+/-	-	+/-	y	-	+/-	y	y	y
Conflicts due to livestock depredation	y	y	y	y	y	y	y	y	y	-
Hybridization with dogs	y	+/-	+/-	+/-	+/-	y	-	y	-	y
Prey overharvest	-	-	-	-	+/-	-	-	-	-	-
Diseases	-	y	-	+/-	?	-	-	y	+/-	?

Table 5. Priorities and tasks for conservation and management planning of European wolf populations.

Priorities	Tasks	Subtasks
<p>A. Establishment of EU Wolf Scientific Committee: a panel of wolf experts that meets on a regular basis</p>	<p>1) Consulting officials and managers in EU 2) Coordinating Europe-wide scientific projects on wolf population genetics, wolf-dog hybridization, livestock depredation, food habits, pathogens and public attitudes 3) Raising public awareness</p>	<p>a) Organise yearly panel meetings b) Organise biannual wolf conferences c) Create and maintain European Wolf Webpage d) Publish scientific papers, annual reports and popular science papers</p>
<p>B. Establishment of EU Wolf Reference Laboratory (EU-WRL). Establishment of dedicated reference laboratories under EU-WRL: 1) on population genetics (EU-WRL-Gen); 2) on diet and pathogens (EU-WRL-DP)</p>	<p>1) Conducting Europe-wide scientific analyses a) EU-WRL-Gen: on population genetics, wolf-dog hybridization, livestock depredation b) EU-WRL-DP: food habits and pathogens 2) Harmonization of methods 3) Data storage 4) Publishing results</p>	<p>a) Establish unified scientific protocols b) Establish databanks to store various wolf data c) Publish scientific papers, annual reports and popular science papers</p>

Table 6. Tasks for scientific research and conservation-management planning of European wolf populations to be coordinated at the European level by the EU Wolf Scientific Committee and EU Wolf Reference Laboratory.

Task	Subtasks	Methods
1) Development of harmonized research protocols	Development of protocols for non-invasive sampling, genetic analysis, morphological analysis	SNP and whole-genome analysis. Standardized microsatellite multiplexing, high-throughput NGS-based microsatellite genotyping
2) Monitoring gene flow between different wolf populations at the European scale	Performing genetic analyses	a) SNP-chips; b) low-coverage genome sequencing; c) nanofluidic SNP genotyping; d) NGS-based microsatellite genotyping e) complete mitogenome sequencing f) Y chromosome microsatellite typing
3) Monitoring wolf-dog hybridization at the European scale	Estimating the level of introgressive hybridization between wolves and other canids (dogs and jackals)	SNP-chips; low-coverage genome sequencing; multiplexing microsatellites; NGS-based microsatellite genotyping
4) Monitoring wolf depredation on livestock	Genetic identification of predator species (proportion of wolves and other species in depredation)	SNP-chips (with mtDNA, autosomal and Y-markers); microsatellites
5) Monitoring wolf food habits and pathogens	Genetic and morphological identification of dietary items and pathogens	Metabarcoding, traditional identification
6) Evaluating public attitudes	Development of unified questionnaires	Questionnaires

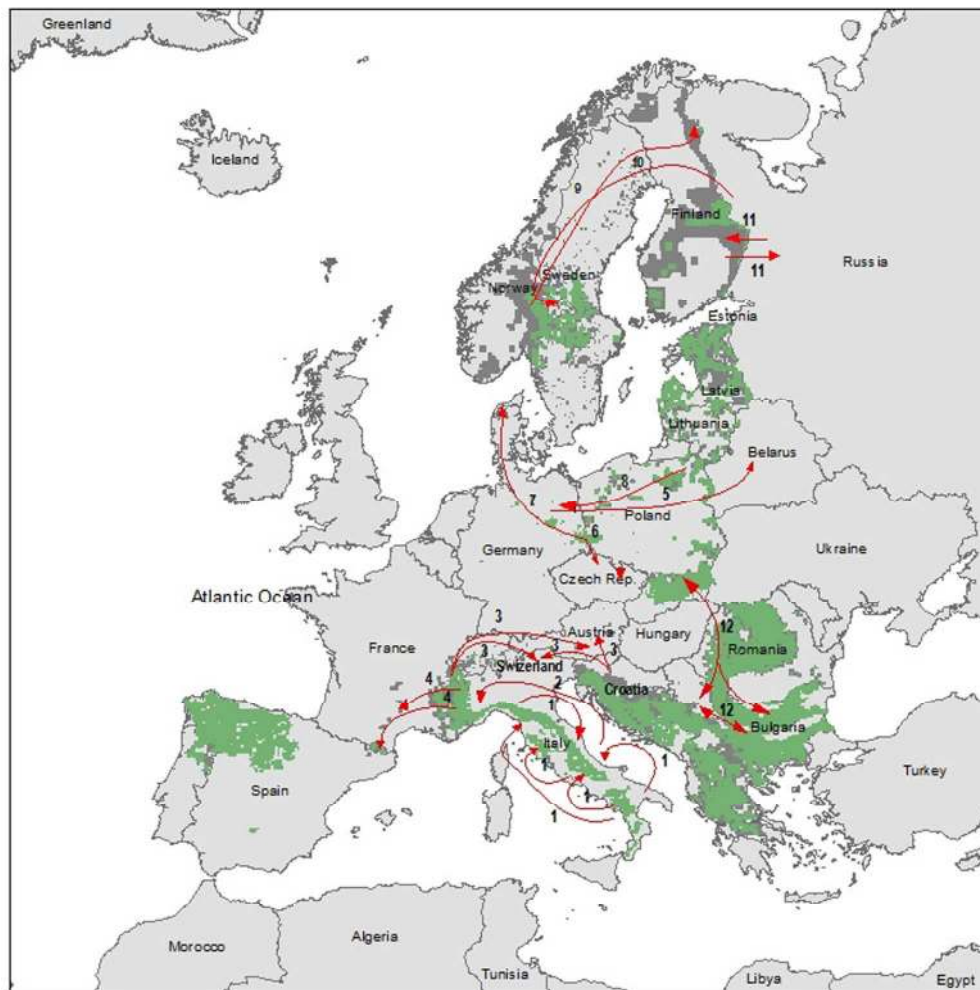


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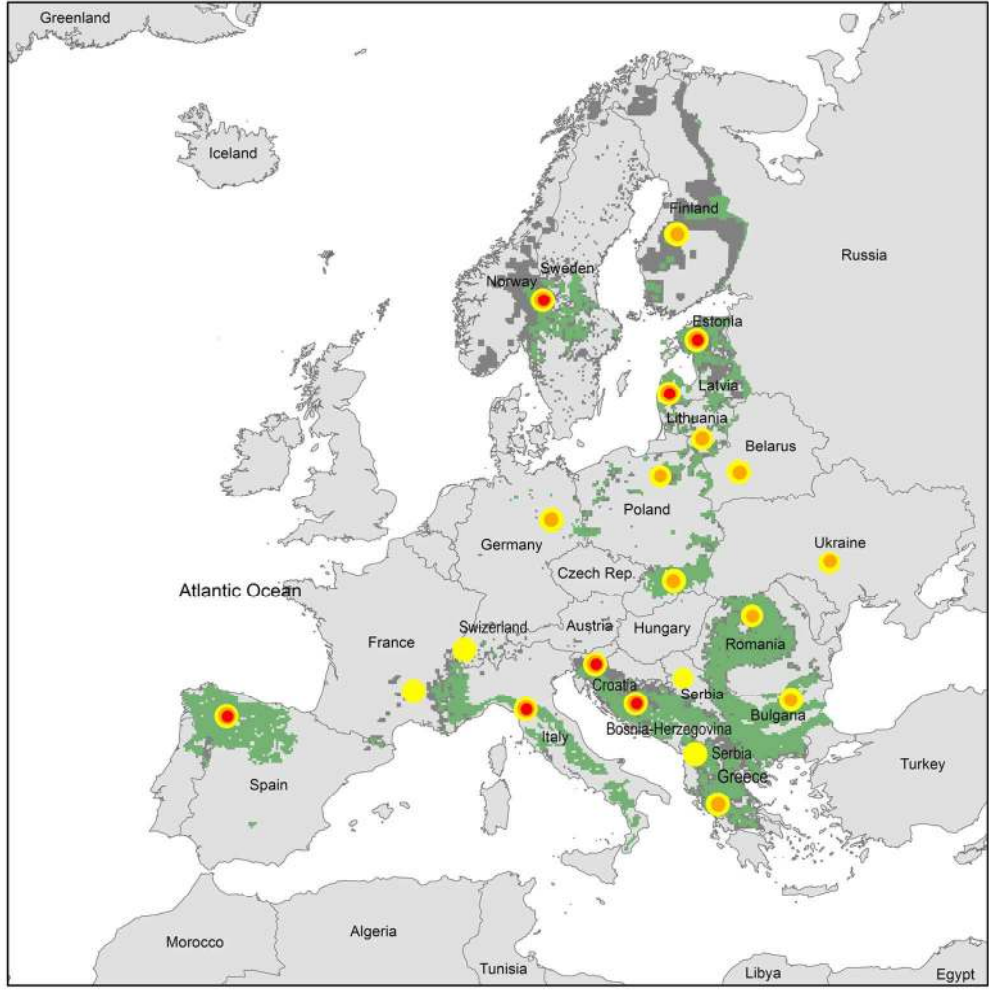


Fig. 2
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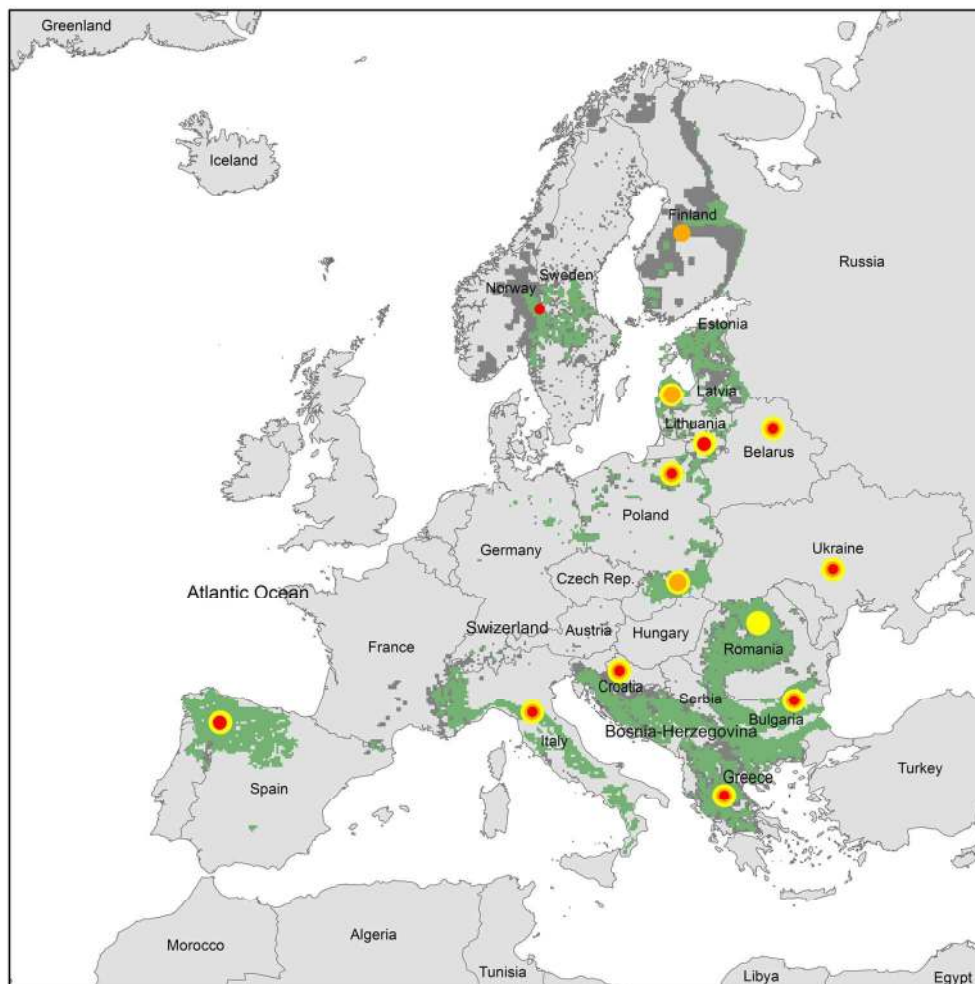


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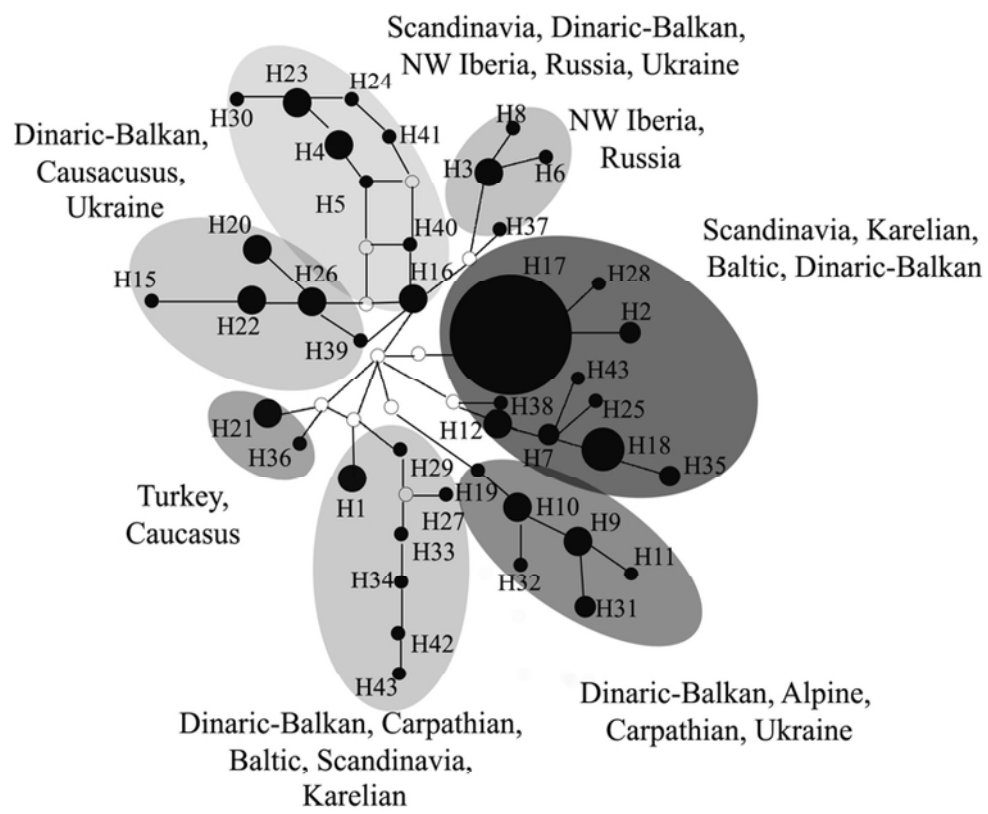
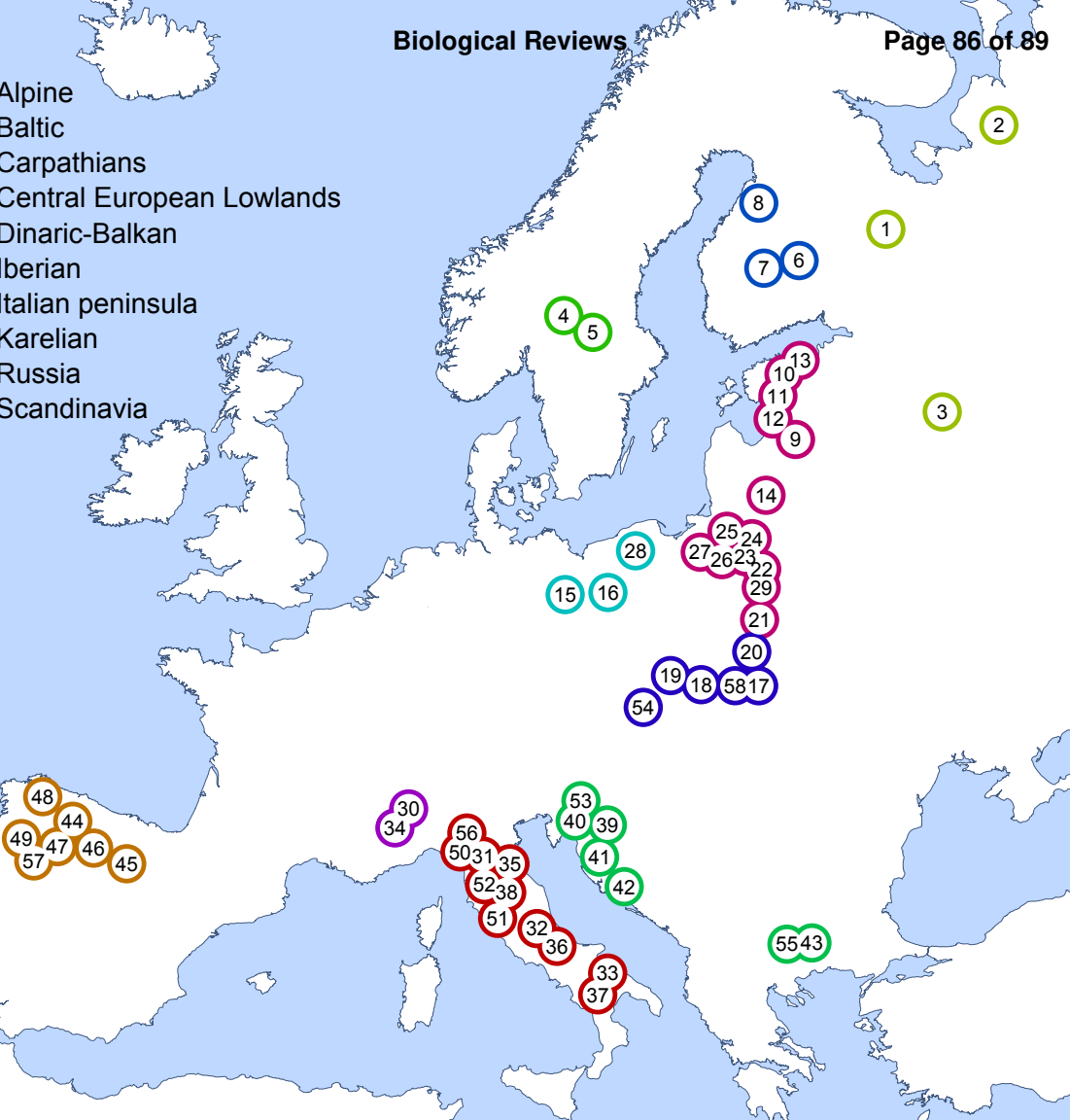


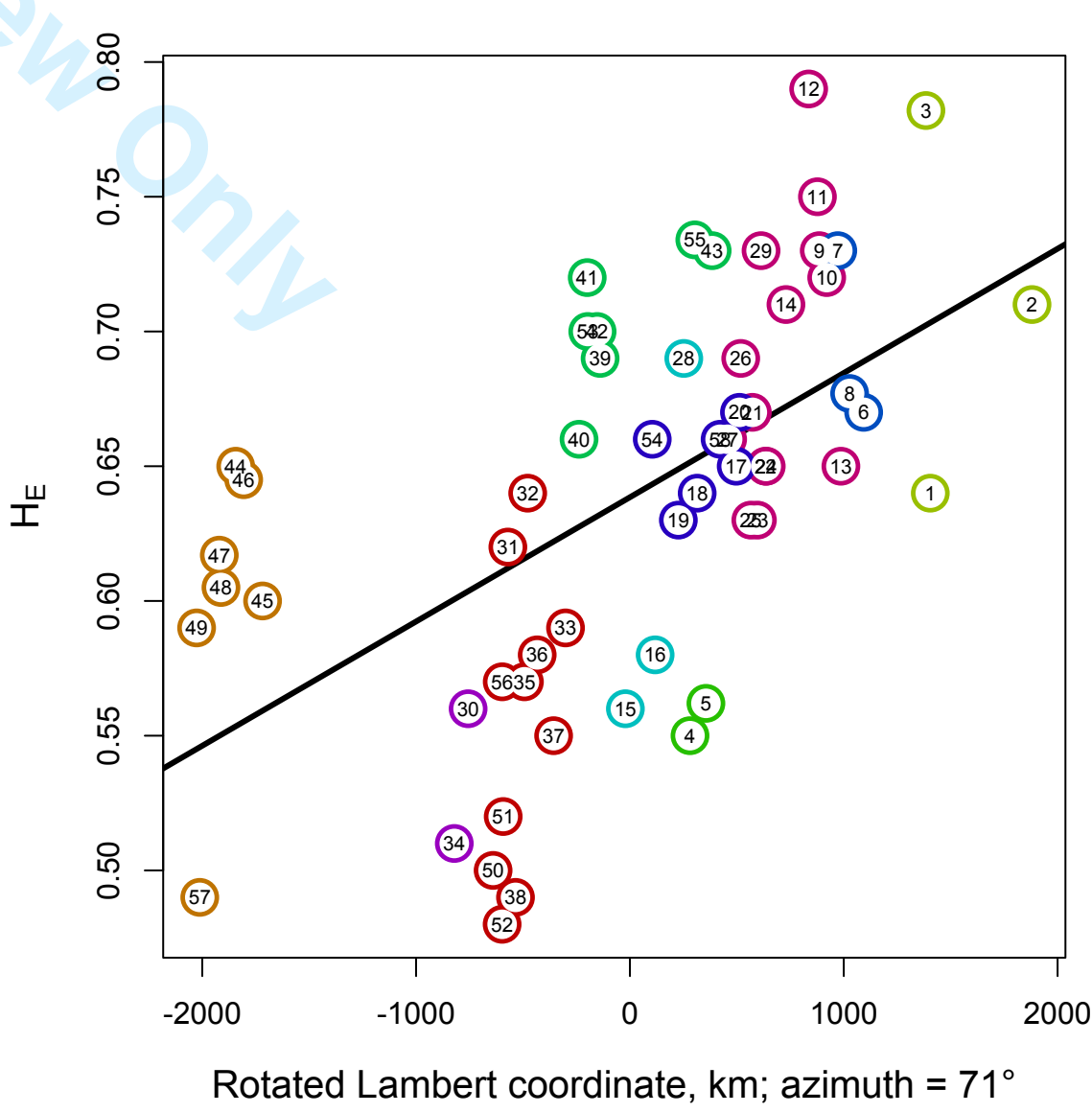
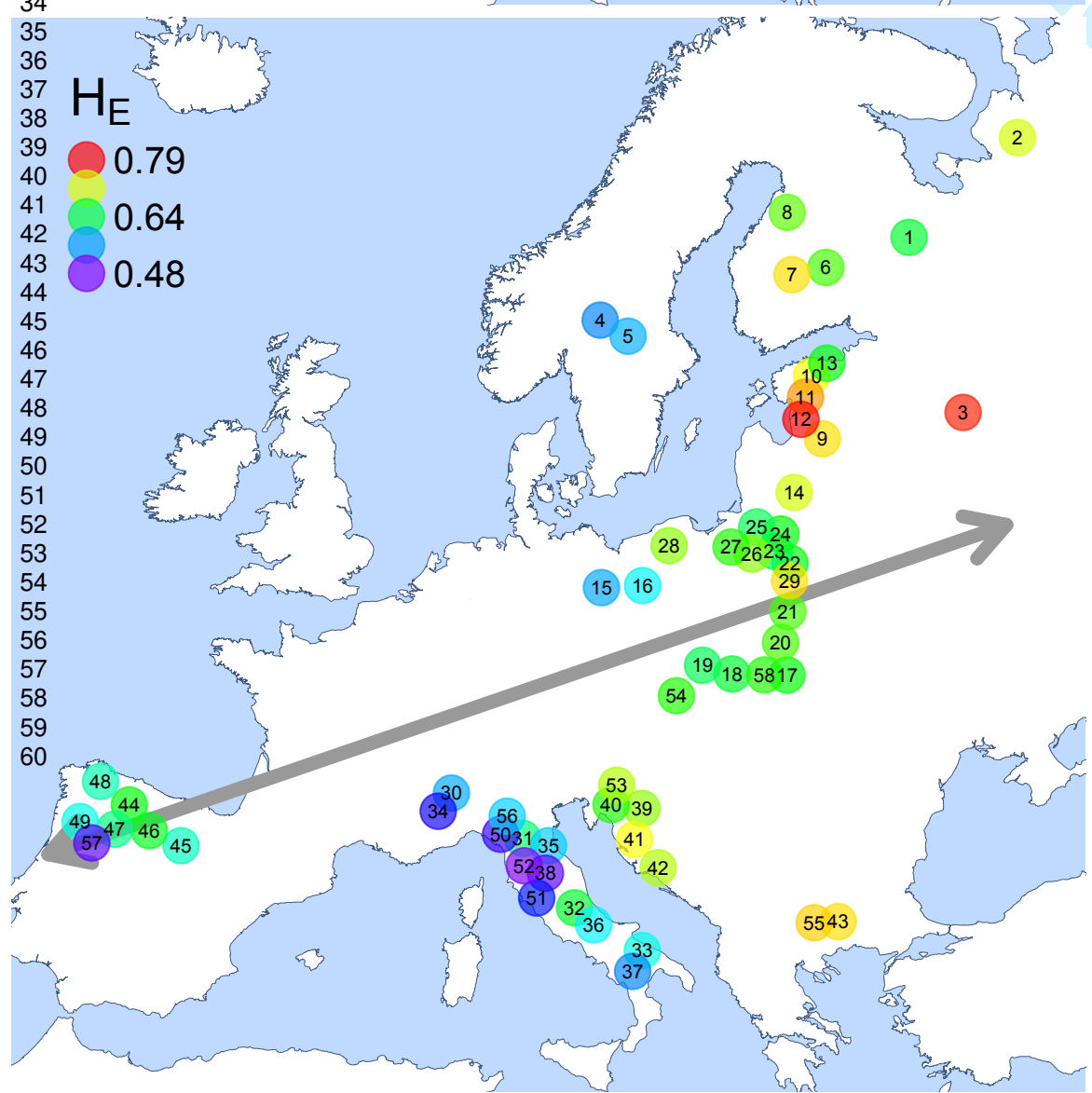
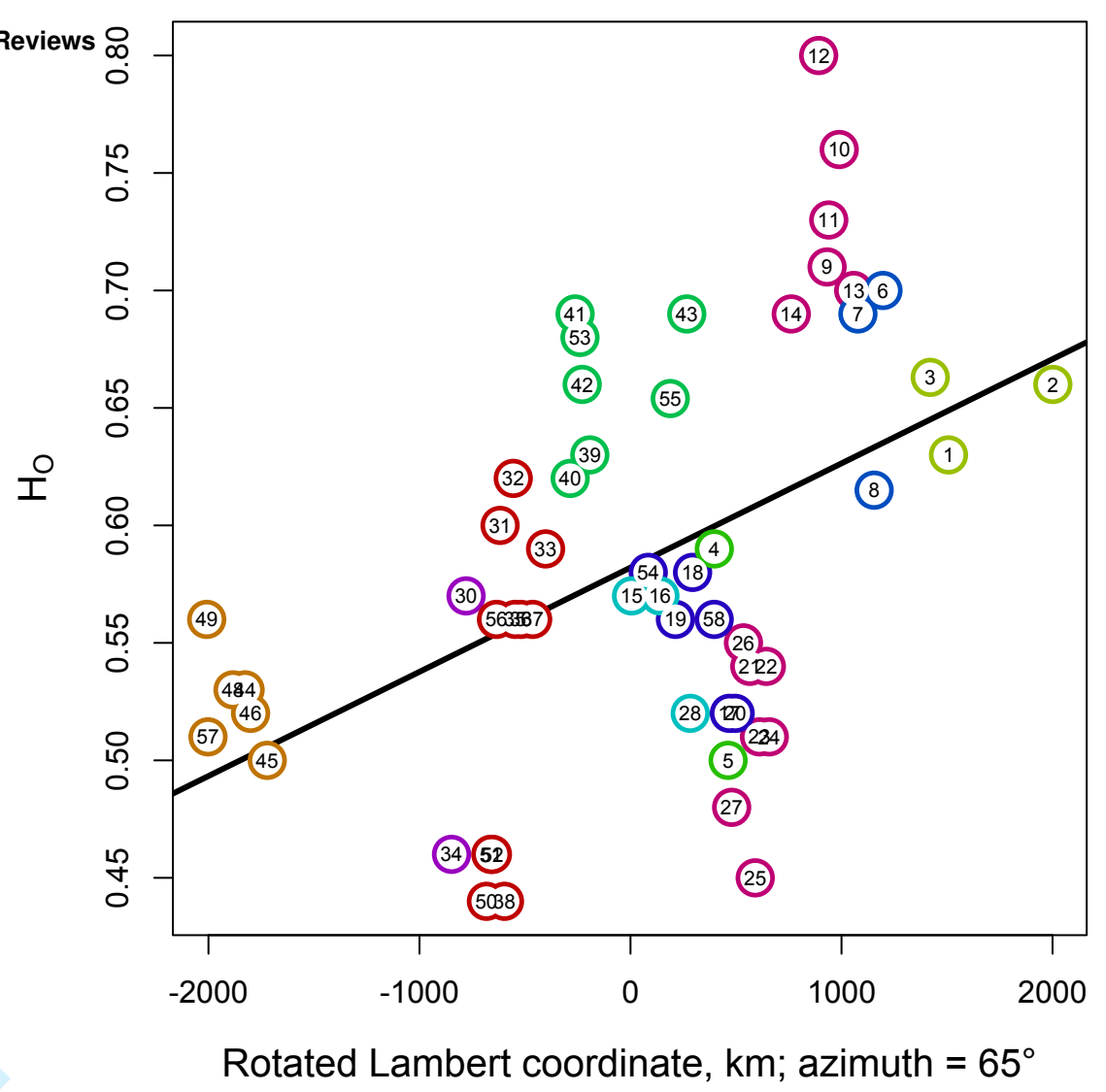
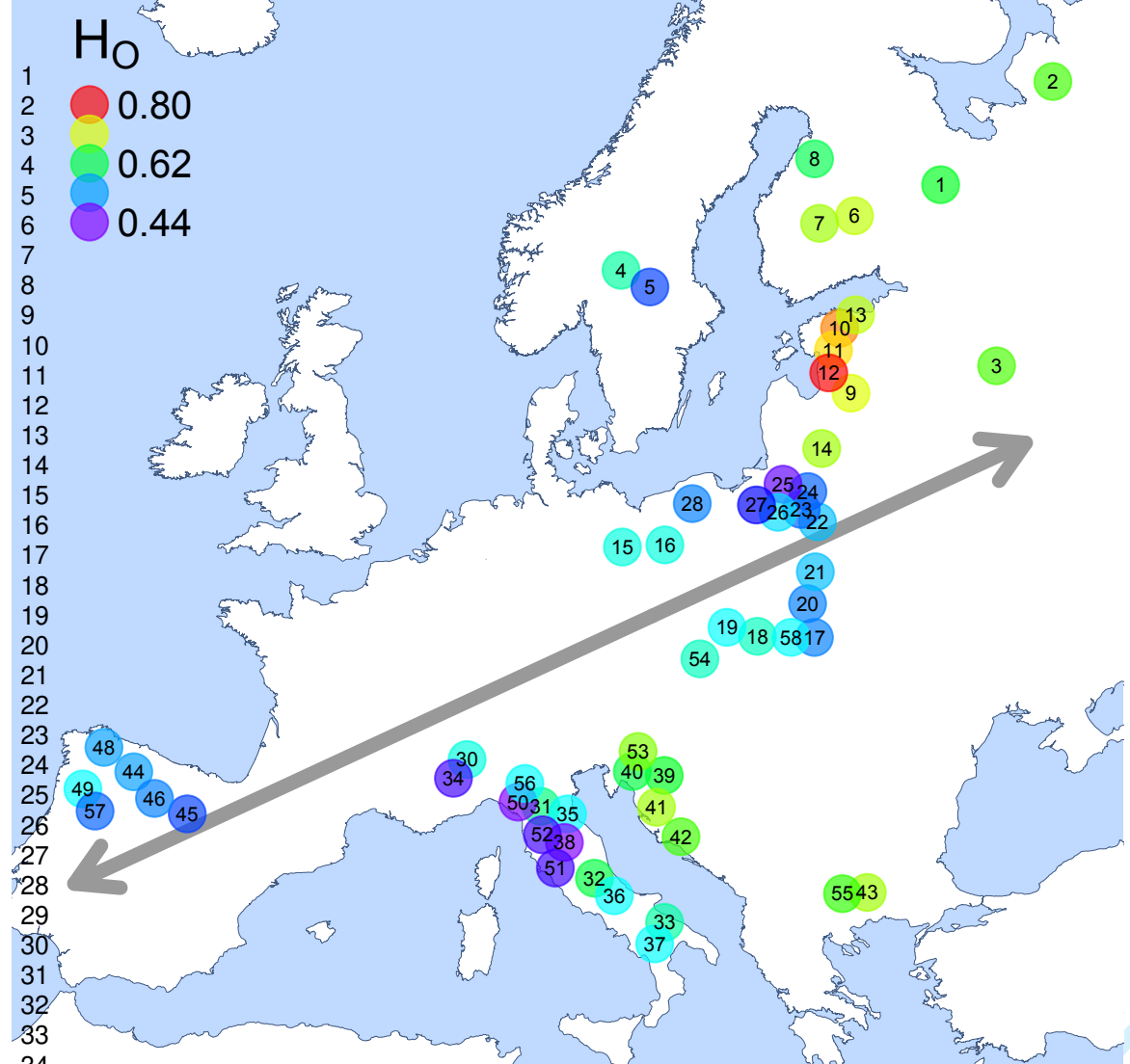
Fig. 4
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- ③ Central European Lowlands
- ④ Dinaric-Balkan
- ⑤ Iberian
- ⑦ Italian peninsula
- ⑧ Karelian
- ⑨ Russia
- ⑩ Scandinavia

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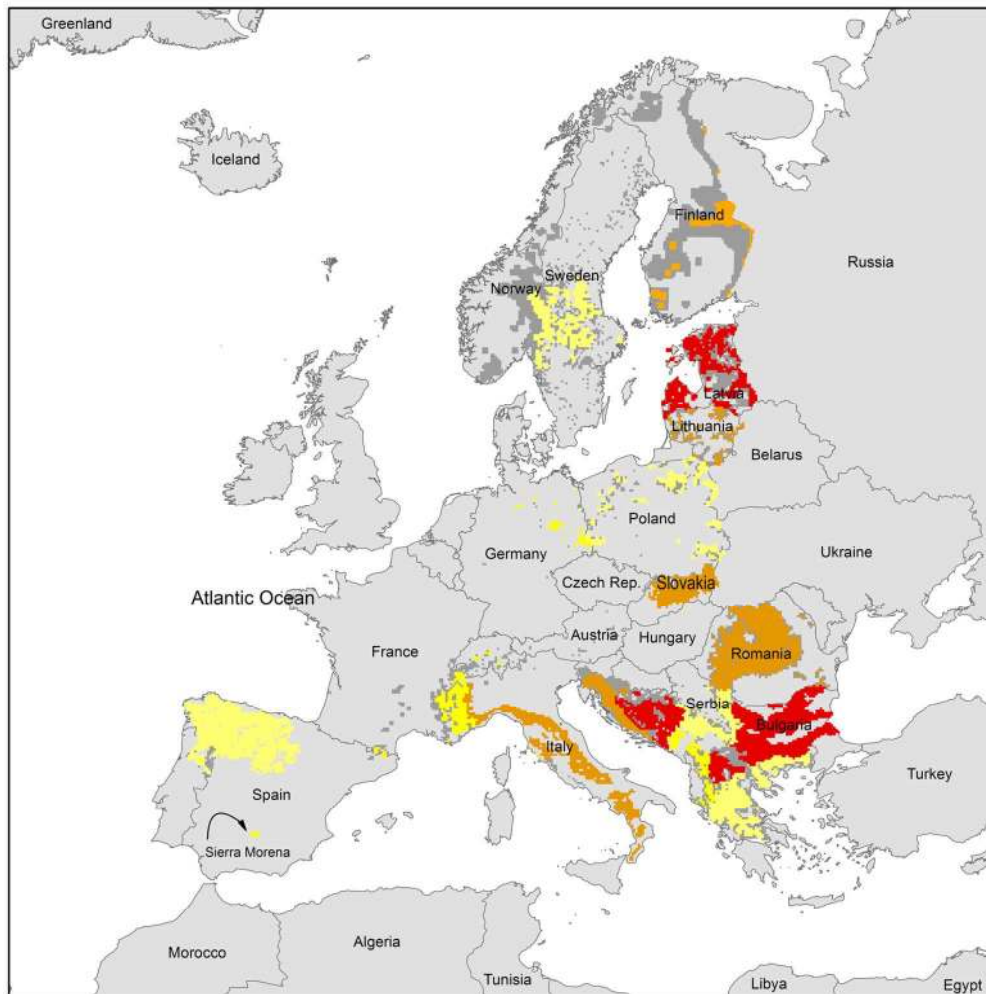


Fig. 7
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**SCIENCE-BASED WOLF
CONSERVATION AND MANAGEMENT IN EUROPE**

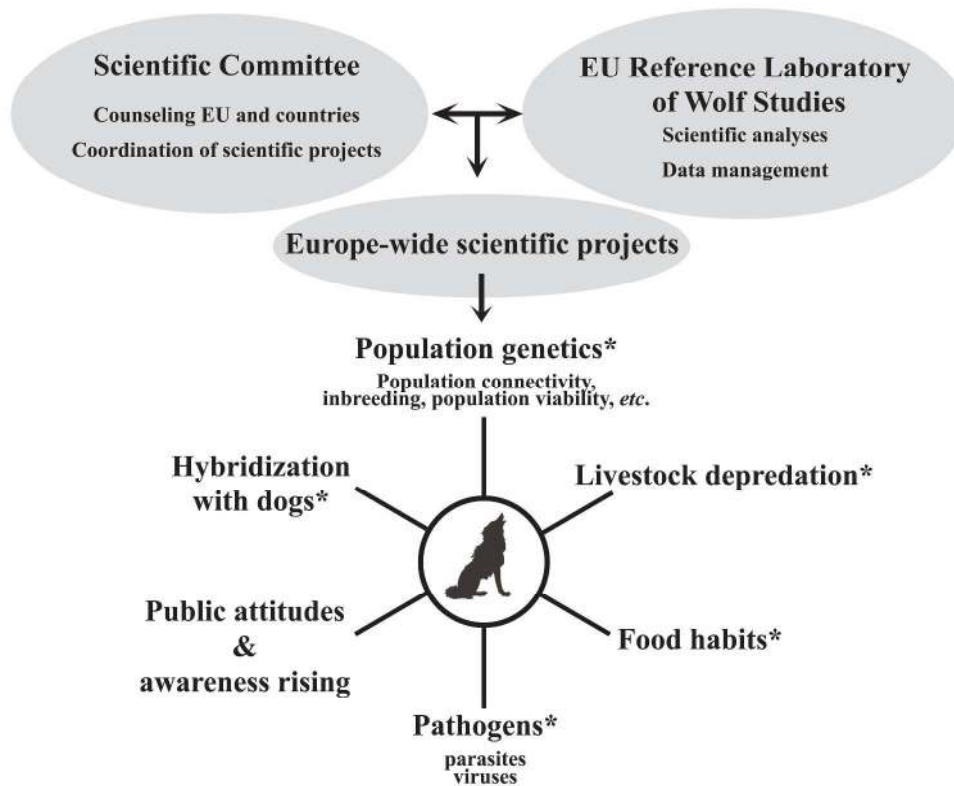


Fig. 8
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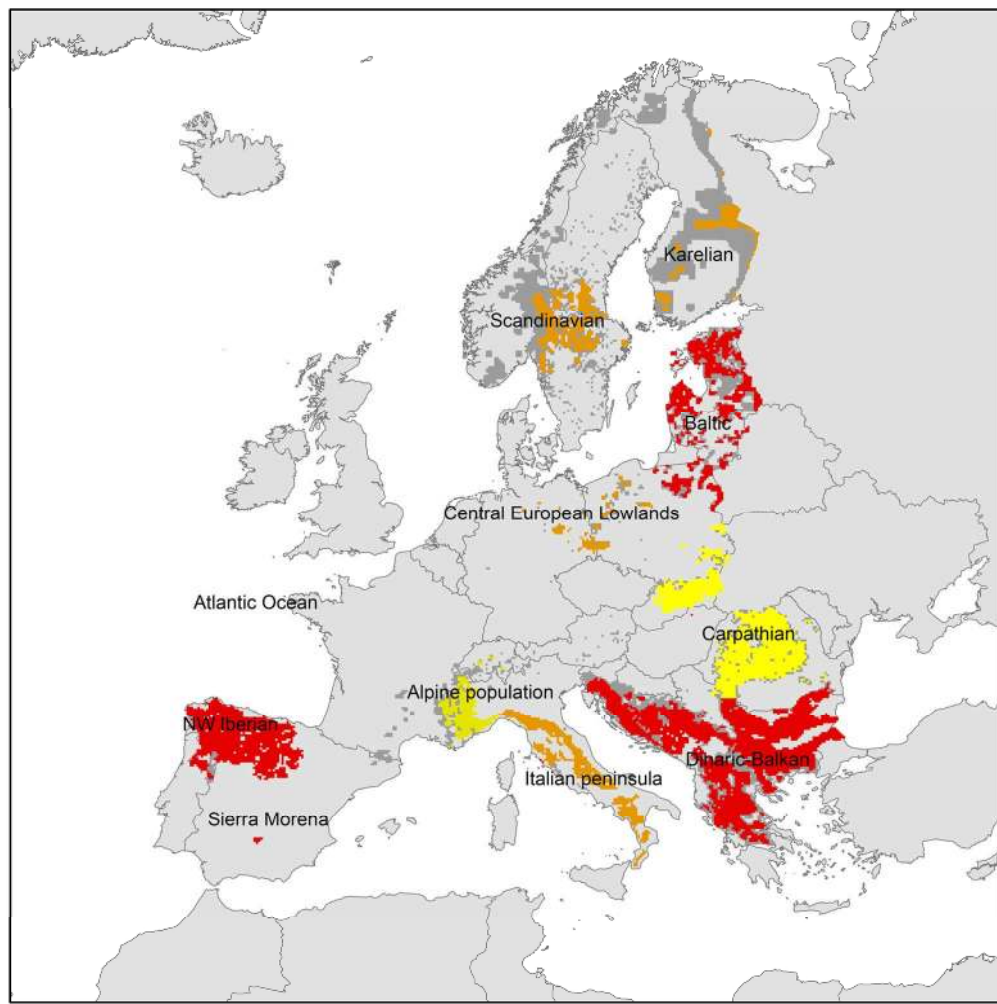


Fig. 9
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