Contents lists available at ScienceDirect

# Mammalian Biology

Short communication

# Wolf population genetics at the south-eastern edge of their European range

Alexandros A. Karamanlidis [a,b,*,1], Sylwia D. Czarnomska [c,1], Alexander Kopatz [d,1], Lazaros Georgiadis [a], Bogumiła Jędrzejewska [c]

[a] ARCTUROS, Civil Society for the Protection and Management of Wildlife and the Natural Environment, Aetos, GR-53075, Florina, Greece
[b] Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, NO-1432, Ås, Norway
[c] Mammal Research Institute, Polish Academy of Sciences, 17-230, Białowieża, Poland
[d] NIBIO—Norwegian Institute of Bioeconomy Research, Svanhovd, 9925, Svanvik, Norway

## ARTICLE INFO

## ABSTRACT

Gray wolf populations have been recovering recently across Europe, a fact that poses serious challenges to the management of the species. We investigate the population genetics of wolves at the south-eastern edge of their European range, in Greece, and identify conservation priorities for the species in the country. During population monitoring efforts (1998–2014) 48 tissue and hair samples were collected and genotyped at 14 microsatellite loci. Eight samples were discarded from further downstream analysis because of possible dog-wolf admixture. Unlike many other edge populations, wolves in Greece showed high levels of genetic variation ($H_E = 0.73$; $H_O = 0.66$). We detected two genetic clusters of wolves but no genetic bottleneck, which suggests that the human-caused population reduction of wolves in Greece may not have been as severe as previously assumed and that enough wolves may have survived in inaccessible areas and/or neighboring countries to maintain genetic diversity. Two of the main conservation priorities identified for gray wolves in Greece are non-invasive genetic monitoring and ensuring the functional connectivity of the Natura 2000 network through the identification and protection of ecological corridors and road-less areas.

© 2016 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

Since their near extirpation in the 19th century gray wolves (*Canis lupus*) have made remarkable recoveries in several parts of Europe (Chapron et al., 2014). Innate propensities of the species (i.e., large home ranges, high mobility, high dispersal abilities) combined with legal protection, socio-ecological changes in rural areas and the increase of wild prey are causes for these recoveries, which, however, have also led to increased conflicts with humans and render wolf conservation difficult (Linnell et al., 2008).

Gray wolves in Greece constitute the south-eastern edge of the species' range in Europe; they are considered to be vulnerable and it has been estimated that approximately 600 individuals inhabit a continuous range in the country (Fig. 1). Despite partially recovering from heavy poaching and the use of poisoned baits, wolves in Greece are still threatened by human-caused mortality, limited food availability and on-going habitat destruction and fragmenta-

tion (Iliopoulos, 2009). Although belonging genetically to the wider Dinaric-Balkan population (Stronen et al., 2013), genetic structure of a population at the edge of its range, such as the wolf population in Greece, may vary at a smaller, local scale (Yannic et al., 2012) and lower genetic variation may occur after recent population recovery events (Excoffier et al., 2009). The aim of this study was to evaluate the population structure, genetic diversity and population history of wolves in Greece and identify conservation priorities for the species in the country.

We collected tissue and hair samples from 48 gray wolves during regular population monitoring efforts in Greece (1998–2014) (Fig. 1).

We genotyped each sample at 14 microsatellite loci (Table 1). PCR amplification was performed twice for each tissue sample and at least three times for hair samples following the laboratory protocol of Pilot et al. (2006) using the Multiplex PCR Kit (QIAGEN) and PCR conditions as described in the manufacturer's instructions with modifications by adding 0.1 μL BSA (Fermentas). Cycling was performed on a DNA Engine Dyad Peltier Thermal Cycler (BIO RAD) using the reaction conditions described by Czarnomska et al. (2013). We analyzed PCR products on an ABI3100 genetic analyzer

* Corresponding author at: ARCTUROS, Civil Society for the Protection and Management of Wildlife and the Natural Environment, Aetos, GR-53075, Florina, Greece.
E-mail address: akaramanlidis@gmail.com (A.A. Karamanlidis).
[1] These authors are the lead co-authors of this study.
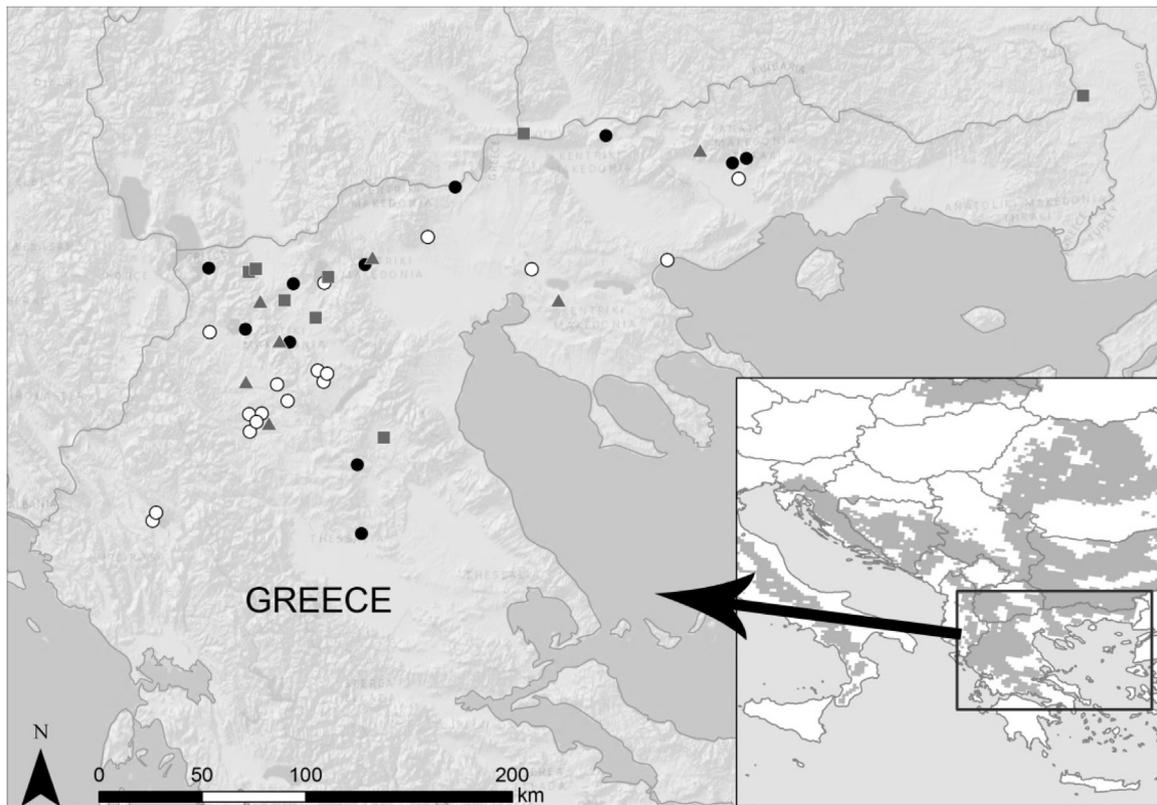
**Fig. 1.** Map of Greece indicating the locations where genetic samples from gray wolves were collected (1998–2014) and their cluster membership (black circle: Cluster 1; white circle: Cluster 2; triangle: unassigned/admixed; square: potential hybrid). The shaded areas in the inset map indicate the approximate range of wolves in Greece (Chapron et al., 2014).

and determined allele lengths using Genemarker 1.51 (SoftGenetics LLC). Following procedures described by Verardi et al. (2006) and Randi (2008) eight samples were removed from downstream analysis because of possible dog-wolf admixture.

We used MICROCHECKER v2.2.3 (Van Oosterhout et al., 2004) to test for allelic dropout, presence of null alleles and scoring errors caused by stutter peaks. To evaluate the suitability of the marker set for identifying individuals we calculated the Probability of Iden-

tity among Siblings ($P_{ID-Sib}$) (Waits et al., 2001) using Gimlet v1.3.2 (Valière, 2002).

We tested for population structure using the Bayesian assignment algorithm STRUCTURE 2.3.4 (Pritchard et al., 2000). For the analyses a maximum of $K = 15$ was set and population admixture and correlated allele frequencies were assumed. We performed a total of ten independent runs for each $K$ value; for each run we set the burn-in period to 100,000 Markov Chain Monte Carlo iterations, followed by a sampling of 1,000,000 iterations. We used

**Table 1**

Genetic diversity indices for wolves in Greece, including the number of alleles per locus ($A$), allelic richness ($A_R$), expected ($H_E$), observed ($H_O$) heterozygosity and individual inbreeding coefficient relative to the subpopulation ($F_{IS}$). Significant $F_{IS}$ values are marked by *; loci deviating from Hardy-Weinberg Equilibrium are in bold.

| | Wolves in Greece | | | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | All samples | | | | Cluster 1 | | | | | Cluster 2 | | | | |
| Locus | $A$ | $H_E$ | $H_O$ | $F_{IS}$ | $A$ | $A_R$ | $H_E$ | $H_O$ | $F_{IS}$ | $A$ | $A_R$ | $H_E$ | $H_O$ | $F_{IS}$ |
| FH2088 | 5 | 0.70 | 0.68 | 0.032 | 4 | 3.9 | 0.69 | 0.67 | 0.035 | 4 | 3.5 | 0.67 | 0.59 | 0.128 |
| FH2010 | 4 | 0.38 | 0.42 | −0.110 | 4 | 3.3 | 0.37 | 0.43 | −0.156* | 4 | 3.4 | 0.41 | 0.41 | −0.018 |
| FH2054 | 8 | 0.74 | 0.80 | −0.070 | 5 | 4.9 | 0.76 | 0.80 | −0.060 | 8 | 6.6 | 0.80 | 0.88 | −0.109 |
| FH2096 | 3 | 0.65 | 0.73 | −0.127 | 3 | 2.9 | 0.44 | 0.47 | −0.059 | 3 | 3.0 | 0.68 | 0.82 | −0.214 |
| FH2079 | 2 | 0.51 | 0.33 | 0.345 | 2 | 2.0 | 0.37 | 0.22 | 0.407 | 2 | 2.0 | 0.45 | 0.36 | 0.217 |
| FH2140 | 8 | 0.81 | 0.76 | 0.065 | – | – | – | – | –[a] | – | – | – | – | –[a] |
| VWF | 7 | 0.71 | 0.78 | −0.088 | 6 | 5.4 | 0.75 | 0.80 | −0.063 | 7 | 6.0 | 0.70 | 0.76 | −0.098 |
| FH2001 | 6 | 0.78 | 0.74 | 0.047 | 5 | 4.9 | 0.75 | 0.64 | 0.152 | 5 | 4.7 | 0.74 | 0.76 | −0.030 |
| C213 | 13 | 0.85 | 0.68 | **0.212*** | 7 | 6.7 | 0.87 | 0.80 | 0.079 | 10 | 7.2 | 0.80 | 0.59 | 0.268 |
| C250 | 9 | 0.84 | 0.88 | −0.044 | 6 | 5.3 | 0.76 | 0.93 | −0.244* | 8 | 7.0 | 0.86 | 0.82 | 0.045 |
| C253 | 6 | 0.76 | 0.68 | **0.110** | 6 | 5.9 | 0.84 | 0.73 | 0.125 | 5 | 4.8 | 0.72 | 0.71 | 0.023 |
| C466 | 9 | 0.76 | 0.68 | **0.114** | 4 | 3.6 | 0.65 | 0.53 | 0.188 | 9 | 6.8 | 0.83 | 0.82 | 0.002 |
| C642 | 12 | 0.92 | 0.5 | **0.462*** | – | – | – | – | –[a] | – | – | – | – | –[a] |
| AHT130 | 7 | 0.75 | 0.65 | 0.139 | 4 | 3.8 | 0.64 | 0.57 | 0.115 | 6 | 5.3 | 0.80 | 0.77 | 0.041 |
| Mean | 7.1 | 0.73 | 0.66 | **0.088*** | 4.7 | 4.4 | 0.66 | 0.63 | 0.038 | 5.9 | 5.0 | 0.71 | 0.69 | 0.019 |
| SD | 3.1 | 0.14 | 0.15 | | 1.4 | | 0.17 | 0.20 | | 2.5 | | 0.14 | 0.17 | |

[a] Marker excluded (see Results and Discussion).

* $P < 0.05$.
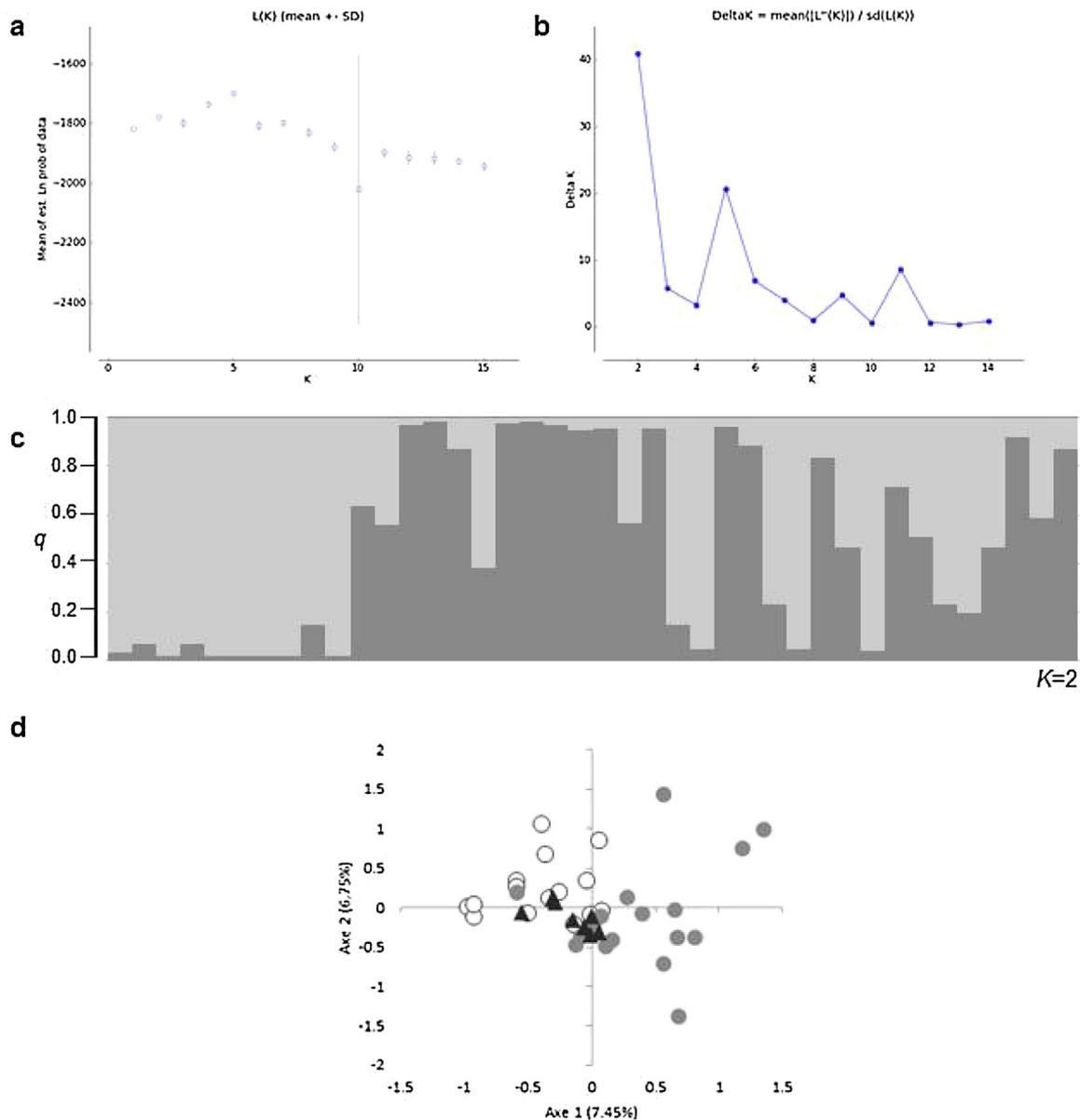
**Fig. 2.** (a) Results of the Bayesian clustering analysis of Greek wolves ($N = 40$) with STRUCTURE; (b) Rate of log-likelihood values ($\Delta K$) using Evanno's ad-hoc approach; (c) Assignment probabilities (i.e., individual $q$-matrix and bar plots) of each wolf for $K = 2$; (d) Factorial correspondence plot for wolves in Greece for $K = 2$ (Cluster 1: white circles; Cluster 2: gray circles; ambiguously assigned genotypes with a membership value of $q < 0.7$: dark triangles).

Genetix 4.05.2 (Belkhir et al., 1996–2004) for a Factorial Correspondence Analysis (FCA) to visualize the relative similarity among wolf genotypes and potential genetic substructure. Also, Isolation-by-Distance (IBD) among pairs of all wolves was calculated using SPAGeDI 1.5 (Hardy and Vekemans, 2002) with the kinship coefficient by Loiselle et al. (1995).

In order to evaluate the genetic diversity of wolves in Greece, we analyzed the indices for genetic variation for the population as a whole, but also for each genetic cluster, which consisted of genotypes with a membership coefficient $q \geq 0.7$ as assigned by STRUCTURE. We investigated for inbreeding due to deviations of each locus from Hardy-Weinberg Equilibrium (HWE) assumptions by calculating $F_{IS}$ and performed tests for Linkage Disequilibrium (LD) between pairs of loci with Genetix 4.05.2 (Belkhir et al., 1996–2004). We tested for deviations from HWE for each locus with Fisher's method (Rousset and Raymond, 1995) using Genepop v4.0 (Rousset, 2008), with unbiased $P$ values by a Markov chain method of 1,000 burn-in iterations, 500 batches and 1,000 iterations per batch. We calculated the number of alleles, expected and observed heterozygosity and pairwise $F_{ST}$ with Arlequin v3.5.1.2 (Excoffier and Lischer, 2010). Allelic richness ($A_R$) for each genetic group was calculated with the program HP-Rare (Kalinowski, 2005).

To evaluate whether the population had undergone a genetic bottleneck, we tested the data for heterozygote excess deviating from the mutation-drift equilibrium ($H_O > H_E$) using Bottleneck 1.2.02 (Piry et al., 1999). The two-phase mutation model using 95% single step mutations to estimate the expected heterozygosities (20,000 iterations) was applied and the significance of the differences between observed and expected heterozygosities was tested using the Wilcoxon test.

A total of 40 wolf genotypes were analyzed and all tests for allelic dropout, null alleles or possible scoring errors due to stutter peaks were negative. The accumulated, more conservative $P_{ID\text{-}Sib}$ was 4.96e-06, lower than 0.01, the value recommended if the data are to be used for population size estimation (Waits et al., 2001).

Individual genotype assignment by STRUCTURE did not show clear population subdivision (Fig. 2a); we therefore applied the approach of Evanno et al. (2005) [as implemented in STRUCTURE HARVESTER (Earl and vonHoldt, 2012)] which showed $K = 2$ clusters with the highest likelihood for $\Delta K$ (Fig. 2b and c). Admixture might be indicated by lower membership coefficients ($q$) or point to possible migrants. Eight wolves were not clearly assigned to either of the two clusters, while 32 individuals (80%) were assigned with a membership coefficient $q > 0.7$. Clustering into two groups was also supported by the FCA-analysis and grouping of genotypes per cluster was visible while the ambiguously assigned, admixed genotypes grouped between the two clusters (Fig. 2d). Differentiation between the two clusters can be described as moderate (Balloux and Lugon-Moulin, 2002), with a pairwise $F_{ST} = 0.059$ ($P < 0.001$). We detected also a negative relationship between kinship and spatial distance between pairs of wolves (b = $-1.00e-04$, $P < 0.05$), suggesting IBD.

Overall, significant ($P < 0.01$) LD after sequential Bonferroni correction was found in 9 out of 91 marker pairs (9.9%). When analyzing per cluster, 6 out of 91 marker pairs (6.6%) showed significant LD. The pairs of loci with LD were not consistently found across clusters. Further, markers FH2140 and C642 showed low variation due to missing amplification and those two markers were removed from further analyses per cluster. Greek wolves showed high genetic variation with mean expected and observed heterozygosity being $H_E = 0.73$ and $H_O = 0.66$ (Table 1). The average number of alleles per locus was 7.1. Allelic richness of Cluster one was 4.4, and 5.0 for Cluster two. Four loci (C213, C253, C466 and C642) showed deviation from HWE within the total population ($P < 0.01$). Loci C213 and C642 deviated due to deficit of heterozygotes, while for loci C253 and C466 the heterozygote deficit and excess tests were not significant. Loci C213 and C642 also showed significant, elevated values of $F_{IS}$. The whole sample from Greece deviated from HWE including a significant and elevated value for the overall $F_{IS}$ (Table 1). This deviation seemed to be caused by the Wahlund effect. Indeed, when analyzing the clusters separately, neither clusters deviated from HWE and markers C250 and FH2010 in Cluster 1 did not show significant $F_{IS}$ values. Cluster 2 showed slightly higher values for heterozygosity and number of alleles compared to Cluster 1 (Table 1).

No recent genetic bottleneck was detected (Wilcoxon test $P > 0.76$).

Our study provides information on basic genetic parameters of a wolf population at the south-eastern edge of its European range. Empirical research indicates that genetic diversity is often lower and divergence higher in peripheral relative to core populations (Arnaud-Haond et al., 2006). Reduced genetic diversity may be expected due to genetic drift caused by geographical isolation and low mutation rates (Hoffmann and Blows, 1994) and has been observed in the recently-established wolf population in the Alps (Fabbri et al., 2007). This however is not the case with wolves in Greece which have high levels of genetic variation, similar to those found elsewhere in the Dinaric-Balkan population (Moura et al., 2014), indicating potential connectivity and gene flow with other populations further north. The levels of genetic diversity of gray wolves in Greece are consistent with the documented demographic recovery in the country (Iliopoulos, 2009) and expectations from European-wide phylogeographic studies of the species. As Balkan wolves were never completely eradicated and survived a period of severe human persecutions in the 18th and 19th century in isolated populations (Breitenmoser, 1998), they are expected to carry a significant portion of the genetic diversity previously found in the large and continuous European wolf population of the Last Glacial Maximum (Pilot et al., 2010).

The two genetic clusters identified are not geographically or temporally distinct and show a low level of differentiation. Interpreting results by the program Structure of populations under the influence of IBD can be challenging and should be done with caution (Frantz et al., 2009), however, the detected IBD pattern could not fully explain the current genetic structure. It may be, that the clusters represent two, older populations or lineages which occur now in the same region, and have merged or are in the process of mixing. Although the results of the different genetic analyses appear consistent, the occurrence of two genetic clusters within Greece is challenging to interpret, as there is no obvious explanation.

Our sampling was representative of the current distribution of wolves in Greece (Fig. 1). Despite the previous population decline in the country, we detected no genetic bottleneck. This may indicate that the reduction of wolves in Greece may have not been as severe as previously assumed and that enough wolves may have survived in areas and/or neighboring countries to maintain genetic diversity. However, simulation studies have shown that the statistical power of genetic bottleneck detection decreases with low sample size (Zachariah Peery et al., 2012). Therefore it may also be possible that our sample size was not large enough to detect a genetic bottleneck. Alternatively, it may be a combination of low sample size and limited impact of the past bottleneck. We suggest that future analyses should incorporate historical material to further investigate the population's demographic history.

Effective wolf conservation in Greece is still hampered by the lack of reliable scientific information on the status of the species, the lack of a Management Action Plan and the poor enforcement of the existing legal framework. This is the first genetic study of wolves in Greece, which allows the identification of basic research and management priorities for the species consistent with those of the EU (Linnell and Boitani, 2012). Given the current financial crisis in Greece and the cost-efficiency of the method (Stenglein et al., 2010), we suggest that non-invasive genetic methods should become an integral part of the national wolf conservation strategy (Linnell et al., 2008) and the basic research tool for monitoring population structure and dynamics and genetic diversity. As dog-wolf admixture is relatively common in Greece (i.e., 17% of our samples showed traces of possible dog-wolf admixture) genetic monitoring should be used also to closely monitor hybridization between these two taxa (Randi, 2011).

Considering the fact that the recent construction of large highways is restricting wolf movement and gene flow in Greece (Karamanlidis et al., 2016), management efforts should be prioritized to ensure the functional connectivity of the existing Natura 2000 network and its ecological coherence (Habitats Directive 92/43/EU, article 10) through the identification and protection of (a) ecological corridors according to the Greek Biodiversity law (L.3937/2011, article 5) and the EU Biodiversity Strategy in the framework of supporting green infrastructures and their ecosystem services (European Commission, 2011) and (b) road-less areas (IENE, 2014; Votsi et al., 2016) as areas with a low human impact.

Wolves have been recovering recently in Greece. We believe that non-invasive genetic monitoring and ensuring unhindered gene flow are two of the main research and management priorities for gray wolves in the country and that if they are consequently pursued they will significantly contribute towards safeguarding the future of the species in Greece.

## References

Arnaud-Haond, S., Teixeira, S., Massa, S.I., Billot, C., Saenger, P., Coupland, G., Duarte, C.M., Serrão, E.A., 2006. Genetic structure at range edge: low diversity and high inbreeding in Southeast Asian mangrove (*Avicennia marina*) populations. Mol. Ecol. 15, 3515–3525.

Balloux, F., Lugon-Moulin, N., 2002. The estimation of population differentiation with microsatellite markers. Mol. Ecol. 11, 155–165.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 1996–2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000. Université de Montpellier II, Montpellier, France.

Breitenmoser, U., 1998. Large predators in the Alps: the fall and rise of man's competitors. Biol. Conserv. 83, 279–289.

Chapron, G., et al., 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346, 1517–1519.

Czarnomska, S.D., et al., 2013. Concordant mitochondrial and microsatellite DNA structuring between Polish lowland and Carpathian Mountain wolves. Conserv. Genet. 14, 573–588.

Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Res. 4, 359–361.

European Commission, 2011. Our life insurance, our natural capital: an EU biodiversity strategy to 2020. Communication from the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions, Brussels.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.

Excoffier, L., Foll, M., Petit, R.J., 2009. Genetic consequences of range expansions. Annu. Rev. Ecol. Evol. Syst. 40, 481–501.

Fabbri, E., et al., 2007. From the Apennines to the Alps: colonization genetics of the naturally expanding Italian wolf (*Canis lupus*) population. Mol. Ecol. 16, 1661–1671.

Frantz, A.C., Cellina, S., Krier, A., Schley, L., Burke, T., 2009. Using spatial Bayesian methods to determine the genetic structure of a continously distributed population: clusters and isolation by distance. J. Appl. Ecol. 46, 493–505.

Hardy, O.J., Vekemans, X., 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2, 618–620.

Hoffmann, A.A., Blows, M.W., 1994. Species borders—ecological and evolutionary perspectives. Trends Ecol. Evol. 9, 223–227.

IENE, 2014. Malmö declaration protect remaining roadless areas. Call for a European Strategy to protect roadless areas. In: IENE 2014 International Conference on Ecology and Transportation Life for a Greener Transport Infrastructure, Malmö, Sweden.

Iliopoulos, G., 2009. *Canis lupus* (Linnaeus, 1758). In: Legakis, A., Maragou, P. (Eds.), Red Data Book of the Threatened Animal Species of Greece. Hellenic Zoological Society, Athens, pp. 389–390.

Kalinowski, S.T., 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol. Ecol. Notes 5, 187–189.

Karamanlidis, A.A., De Gabriel Hernando, M., Georgiadis, L., Kusak, J., 2016. Activity, movement, home range and habitat use of an adult gray wolf in a Mediterranean landscape of northern Greece. Mammalia, http://dx.doi.org/10.1515/mammalia-2015-0091.

Linnell, J.D.C., Boitani, L., 2012. Building biological realism into wolf management policy: the development of the population approach in Europe. Hystrix Ital. J. Mammal. 23, 80–91.

Linnell, J., Salvatori, V., Boitani, L., 2008. Guidelines for Population Level Management Plans for Large Carnivores in Europe. pp. 1–78.

Loiselle, B.A., Sork, V.L., Nason, J., Graham, C., 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubicaceae). Am. J. Bot. 82, 1420–1425.

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

Pilot, M., Jedrzejewski, W., Branicki, W., Sidorovich, V.E., Jedrzejewska, B., Stachura, K., Funk, S.M., 2006. Ecological factors influence population genetic structure of European grey wolves. Mol. Ecol. 15, 4533–4553.

Pilot, M., Branicki, W., Jędrzejewski, W., Goszczyński, J., Jędrzejewska, B., Dykyy, I., Shkvyrya, M., Tsingarska, E., 2010. Phylogeographic history of grey wolves in Europe. BMC Evol. Biol. 10, 104.

Piry, S., Luikart, G.L., Cornuet, J.-M., 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J. Hered. 90, 502–503.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure multilocus genotype data. Genetics 155, 945–959.

Randi, E., 2008. Detecting hybridization between wild species and their domesticated relatives. Mol. Ecol. 17, 285–293.

Randi, E., 2011. Genetics and conservation of wolves *Canis lupus* in Europe. Mamm. Rev. 41, 99–111.

Rousset, F., Raymond, M., 1995. Testing heterozygote excess and deficiency. Genetics 140, 1413–1419.

Rousset, F., 2008. GENEPOP '007: a complete reimplementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.

Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., Mack, C.M., 2010. Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. J. Wildl. Manage. 74, 1050–1058.

Stronen, A.V., et al., 2013. North-south differentiation and a region of high diversity in European wolves (*Canis lupus*). PLoS One 8, e76454.

Valière, N., 2002. GIMLET: a computer program for analysing genetic individual identification data. Mol. Ecol. Notes 2, 377–379.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.

Verardi, A., Lucchini, V., Randi, E., 2006. Detecting introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis pupus*) by admixture linkage disequilibrium analysis. Mol. Ecol. 15, 2845–2855.

Votsi, N.E.P., Zomeni, M.S., Pantis, J.D., 2016. Evaluating the effectiveness of Natura 2000 Network for wolf conservation: a case-study in Greece. Environ. Manage. 57, 257–270.

Waits, L.P., Luikart, G., Taberlet, P., 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Mol. Ecol. 10, 249–256.

Yannic, G., Basset, P., Buchi, L., Hausser, J., Broquet, T., 2012. Scale-specific sex-biased dispersal in the Valais shrew unveiled by genetic variation on the Y-chromosome, autosomes, and mitochondrial DNA. Evolution 66, 1737–1750.

Zachariah Peery, M., Kirby, R., Reid, B.N., Stoelting, R., Doucet-Beer, E., Robinson, S., Vásquez-Carillo, C., Pauli, J.N., Palsbøll, P.J., 2012. Reliability of genetic bottleneck tests for detecting recent population declines. Mol. Ecol. 21, 3403–3418.