



Short communication

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

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ARTICLE INFO

Article history:

Received 2 March 2016

Accepted 24 June 2016

Handled by Frank E. Zachos

Available online 25 June 2016

Keywords:

Conservation

Greece

Canis lupus

Wildlife management

Population monitoring

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.

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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 μ L 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

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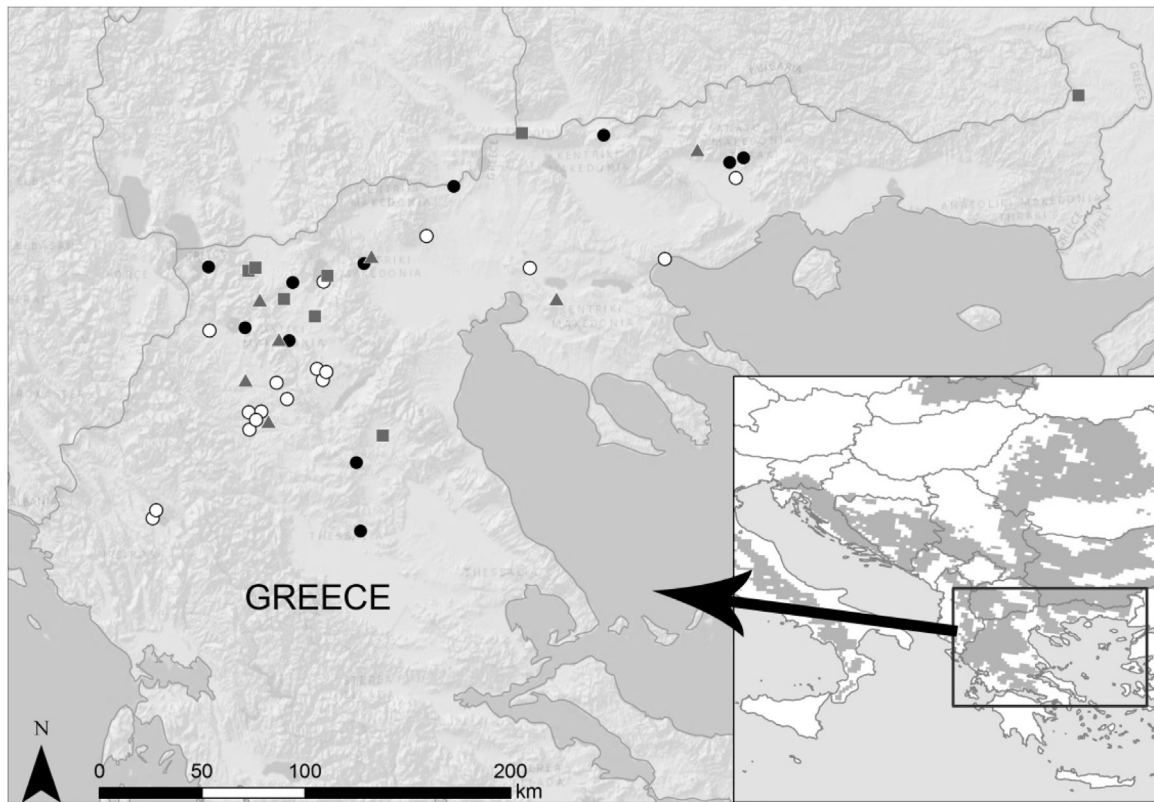


Fig. 1. Map of Greece indicating the locations where genetic samples from gray wolves were collected (1998–2014) and their cluster membership (black circle: Cluster 1; white circle: Cluster 2; triangle: unassigned/admixed; square: potential hybrid). The shaded areas in the inset map indicate the approximate 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 Ident-

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).
* $P < 0.05$.

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