Conflicting management policies for the Arabian wolf in the Negev Desert: is this justified?

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Supplementary Information 1

DNA extraction and typing

We used a guanidinium thiocianate and silica protocol (Reed et al., 1997) to extract DNA from c. 100 mg of the outer layer of scats. To assign species to each scat we amplified two diagnostic mtDNA segments: a 350 bp fragment of the mtDNA control region I, and a 500 bp fragment of cytochrome b. The fragments were amplified via polymerase chain reaction (PCR). We used primers Thr-L 15926 and DL-H 16340 for the control region I (Vila et al., 1999) and L15513 and H15915 for cytochrome b (Wayne et al., 1997). Negative control tubes (no template DNA) were set in every PCR run to monitor for contamination. Sequences were obtained with an ABI PRISM 3100 automatic sequencer and compared to available sequences available online using the Basic Local Alignment Search Tool (BLAST).

To identify individuals we amplified autosomal microsatellite loci that have been developed for dogs and shown in previous studies to be polymorphic in wolves (Carmichael et al., 2007; Lucchini et al., 2002; Randi et al., 2002; Scandura et al., 2006). Ten primers were used: CXX140, CXX250, CXX251, CXX618, CXX733, and CXX758 (Ostrander et al., 1993; 1995), FH2010, FH2079, FH2137 and FH2175 (Francisco et al., 1996). One primer of each pair was fluorescently labeled and PCR products were analysed by capillary electrophoresis on an ABI PRISM 3100 automatic sequencer. We determined allele sizes using *GeneMarker v. 1.6* (SofetGenetics, LLC, State College, USA)

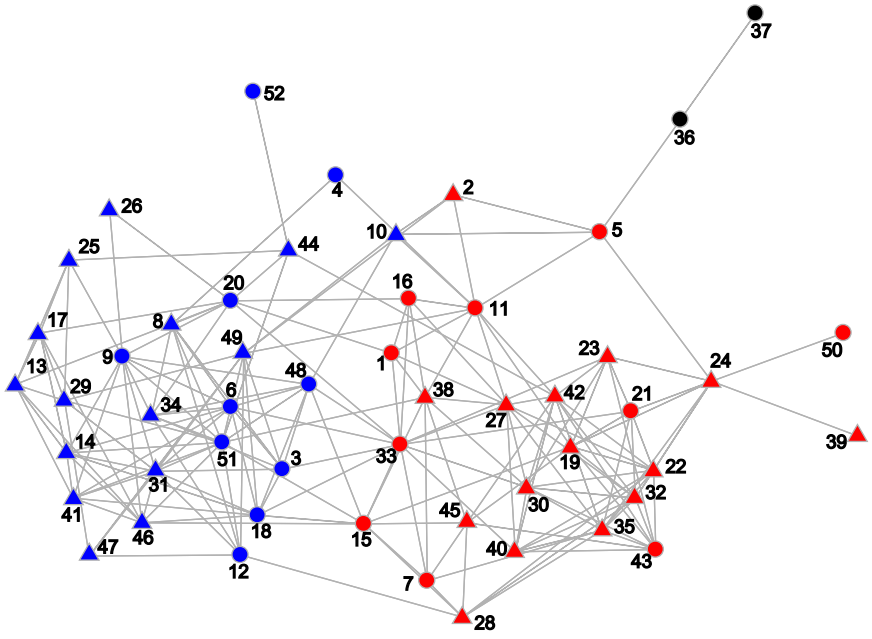
Because DNA extracted from faecal samples is generally low in both quality and quantity there is a risk of genotyping errors such as allelic dropout or false alleles (Taberlet et al., 1996). To reduce the effect of such errors we used a modified version of the multiple-tubs approach suggested by Taberlet et al. (1996). All samples were amplified at least twice. We considered a sample to be heterozygote if each allele was scored at least twice; a sample was considered homozygote if three independent replicates showed the same single allele. When an uncertain genotype appeared we performed additional replicates, up to six replicates per sample per locus. If after six replicates a sample could not be reliably typed, we considered these alleles as missing data. Identification of unique genotypes, error rates and probability of identity were calculated using *GIMLET v. 1.3.3* (Valiere, 2002).

Relatedness network analysis

To estimate genealogical relatedness we used *ML-RELATE*, which calculates maximum likelihood estimates of relatedness (Kalinowski et al., 2006). This evaluates the coefficient of relatedness (r) for all individual pairwise combinations. For two individuals with no genetic relatedness $r \approx 0$, whereas for full siblings or parent–offspring $r \approx 0.5$. These r-values were used to construct a relatedness network of the sampled wolves. Social networks constitute a graphical representation of the relationship between individuals or groups. In the network we created each node represents an individual wolf and the edges connecting the nodes are weighted by the genetic relatedness between the individuals. To reduce the influence of weak edges on the structure of the network we percolated all the edges below a threshold value (Rozenfeld et al., 2008). Our threshold value was the maximum r-value that allowed the network to connect all individuals; a higher threshold point would result in a network that contains unconnected clusters. Next we used an algorithm developed by Girvan & Newman (2002) to find communities within a network. This algorithm is based on finding and removing edges with the highest 'betweenness centrality' (Freeman, 1977). To identify the quality of a particular division of a network we used the modularity measure Q_m (Newman & Girvan, 2004), which ranges from 0 (i.e. community structure is no different than random) to 1 (networks with strong community structure). We selected the optimal number of communities by using the highest Q_m value.

We created a relatedness network using the coefficient of relatedness (r) between every pair of individuals. The percolation point was set at 0.18; lower values of relatedness were excluded and are not represented in the network. The Girvan-Newman algorithm revealed two main communities in the network, each containing 25 individuals, and an additional community that contains only two individuals (modularity $Q_m = 0.4$; Fig. S1). For each community we counted the number of individuals sampled from the Eilat district and the South district, and used these frequencies to correlate sample location and genetic community. For both communities nine wolves were sampled in the Eilat district and 16 in the South district. These frequencies showed no significant relationship between genetic structure and INPA districts ($\chi^2_1 = 0$, $P = 1$).

  doi:10.1017/S0030605311001797



Supplementary Fig. S1 Network of genetic relatedness among all 52 unique haplotypes identified. Network community is denoted by colour and INPA district by symbol (triangle is the South district and circle is the Eilat district).