Supplementary Material

**Suppl. Inf 1.** Compilation and summary of the frequently cited articles regarding wolf - dog hybrids identification based on microsatellites.

Andersone et al. (2002) identified 12 hybrids (considering individual as a hybrid if qw < 0.9) among 31 wolves, using mtDNA and 16 autosomal microsatellites. Nine of them were probably close relatives (a litter of seven pups and their parents). Therefore it would be unreliable to infer the extent of admixture in the population based on a case in which the majority of hybrids may come from a single hybridization event.

In 2003 Vilà et al. identified a wolf-dog hybrid in a Scandinavian wolf population using maternal, paternal and biparental genetic markers. Two samples from 1999 were compared with wolf samples collected after 1980 in different countries (Finland, Russia, Estonia and Latvia) and 44 dog samples (Vilà et al.2003).

Iacolina et al. (2010) performed genetic analyses with nine autosomal and four Y-chromosome microsatellites on 104 wolf and 30 dog samples. Wolf samples were collected during the monitoring project in Northern Apennines between 1998 - 2009. The results imply the possible introgression of dog DNA into the grey wolf population in the region. Based on a Bayesian assignment in STRUCTURE, four individuals were identified as possible hybrids, while FCA confirmed admixed origin of 6 animals (Iacolina et al. 2010).

Hindrikson et al. (2012) conducted genetic analysis on eight putative hybrids, 74 wolf-like individuals and 21 dogs using mtDNA, 11 autosomal microsatellites and seven Y-chromosome microsatellites. Hybrid identification was conducted in STRUCTURE (Pritchard et al. 2001), NEWHYBRIDS (Anderson & Thompson 2002) and GENETIX (Belkhir et al. 2004). Bayesian clustering implemented in STRUCTURE yielded more reliable results than in NEWHYBRIDS. All the putative hybrids were classified as admixed individuals, and two further individuals were identified as possible hybrids. FCA (factorial correspondence analysis) based on autosomal microsatellites and the minimum spanning network calculated with Network with an MJ (median-joining) approach (Bandelt et al. 1999), also confirmed the admixed origin of the putative hybrids. Worth emphasizing is the fact that two of the hybrids had an mtDNA haplotype typical of dogs, indicating male wolf x female dog hybridization. The other 6 admixed individuals belonged to the same wolf pack and might be a result of a single hybridization event (Hindrikson et al. 2012).

Caniglia et al. (2013) studied a specific wolf population (n = 16), with the morphological traits suggesting hybridization. They analysed 13 autosomal microsatellites, the mtDNA control region, four linked Y-chromosome microsatellites and two melanistic mutations. The authors performed genotyping on 40 wolf reference samples and 40 randomly selected dog samples. The research also covered three hybrids and 16 other individuals of unknown origin. 16 genotypes were obtained from the target population. The methodology involved Bayesian clustering in STRUCTURE, the genotypes were simulated in HYBRIDLAB (Nielsen et al. z2006) to assess the power of assignment and to define a reliable threshold, and NEWHYBRIDS was used to assign individuals to particular classes. All the reference wolves, individuals of uncertain origin and those from the target population had the W14 mtDNA haplotype, specific to Italian wolves. Hence, this does not indicate introgression from the maternal lineage. All the males from the target population also had a Y haplotype not detected in the sampled dogs. The specific dog allele responsible for the black coat was found in 17 reference dogs, eight individuals of unknown origin and nine animals from the target population, and all but two of these individuals had black fur. An FCA plot differentiated the reference wolves and dogs and assigned three hybrids, 10 individuals of unknown origin and all the animals from the target population as intermediate (closer to the wolf cluster). Furthermore, STRUCTURE analysis indicated an admixed origin of the hybrids and 15 of the 16 individuals of unknown origin. The animals from the target population were assigned to the wolf cluster, albeit with a lower threshold than the reference wolves (0.69 – 0.94/0.66 – 0.85, depending on the program settings, whereas for the reference wolves qi > 0.95). The credible intervals were narrower for the reference wolves than for the individuals from the target population. The probable explanation is that the wolves from the target populations were hybrids after a few generations. In addition, NEWHYBRIDS identified all the members of the target pack as backcrosses. Summarizing, the analysis confirmed the admixed origin of the target population and the majority of wolves of unknown origin, i.e. 34 or 35 hybrids. However, the individuals from the target population were related and must have originated from mating between siblings (Caniglia et al. 2013).

Caniglia et al. (2014) conducted a research, which did not focus on WDH but the number of hybrids was defined using STRUCTURE and HYBRIDLAB anyway. 518 genotypes were used in the genetic analysis, 414 of which belonged to wolves. Using simulated genotypes in HYBRIDLAB, the threshold was established at qw > 0.95. Consequently, 16 individuals of admixed origin were defined as hybrids (0.05 < q < 0.95), a result resembling those obtained in most other studies, i.e. ca 4% of all wolf-like genotypes (Caniglia et al. 2014).

Moura et al. (2014) identified potential hybrids in Bulgaria examining the genetic structuring of wolves in the country using 14 microsatellite loci. Based on tissue samples, 10 wolf-like individuals were identified as admixed with dogs (~10% of the samples).

Karamanlidis et al. (2016) studied wolf genetic structure and, using STRUCTURE, identified eight individuals which were possibly admixed, i.e. ~17% of all the individuals examined. According to the authors, sampling was representative for the wolf distribution in Greece. It suggests a much higher proportion of admixed individuals than in the other regions of Europe (Karamanlidis et al. 2016). However, the study was characterised by a small number of samples (48 individuals), which were collected during the long period of time (16 years). Further analyses would be recommended in order to investigate the situation.

Hulva et al. 2018 conducted analyses of 250 Carpathian wolves in STRUCTURE. No signs of F1hybrids were identified (however some minor admixtures might be noted on the figures presented in the supplementary). Nevertheless, hybrid identification wasn’t the main aim of the study and less admixed individuals resulting from older hybridization events might be present among the sampled animals.

Bassi et al. (2017) investigated the presence of wolf-dog hybrids in Arezzo province (Italy) and the ecological niche they thrive in. Based on 1148 samples, they identified more than 200 putative wolf genotypes. The analysis also included 39 genotypes obtained from dogs. Hybrids were identified from two areas in Arezzo. The methodology involved genotyping for 12 unlinked microsatellites, two Y-chromosomal microsatellites and the mtDNA control region (CR). In order to create reference datasets, all the wolf samples collected outside the target area and showing any signs of possible introgression (dog Y-chromosome/mtDNA CR haplotype, morphological traits) were excluded. Therefore, only 37 putative pure wolf genotypes remained, which were then analysed using Bayesian clustering implemented in STRUCTURE. Individuals with qw < 0.975 were removed from the analysis. Ultimately, 34 pure wolves were left in the first (reference) dataset. The second dataset consisted of the reference dataset (No. 1) and 47 other distinct genotypes obtained from samples collected in the target area. The analysis indicated the presence of 14 admixed individuals for threshold qw < 0.9 and 17 for threshold qw <0.95, i.e. ~30% and ~36% respectively (Bassi et al. 2017). This study was conducted in two relatively small, neighbouring areas, so the results cannot be extrapolated to the whole country, however the proportion of admixed individuals is remarkably high, especially in contrast with the other wolf populations (e.g. Randi & Lucchini 2002).

In 2017 Torres et al. conducted analyses based on wolf scats collected in territory of 3 wolf packs. Dog DNA was obtained from tissue samples. The samples were genotyped for mtDNA and 24 autosomal microsatellites. The mtDNA haplotypes were compared using MEGA 6 software (Tamura et al. 2013) and the phylogenetic tree was created using the NJ algorithm. As reference samples for principal coordinate analysis (PCoA) and assignment tests, which were performed with GenAlEx software (Peakall & Smouse 2012), STRUCTURE and NEWHYBRIDS, the authors used wolf and dog genotypes from Godinho’s (2011) study. 51 of the 93 samples belonged to the Iberian wolf (*Canis lupus signatus*) (identification based on mtDNA) and comprised 21 distinct genotypes. All shared the same mtDNA haplotype W1, which had previously been identified in Iberian wolves (Vilà et al. 1997). The genetic analysis indicated the presence of just one hybrid individual (Torres et al. 2017).

Dufresnes et al. (2019) provided knowledge regarding the presence of wolf - dog hybrids in Switzerland and neighbouring areas. Based on 11 STRs, mtDNA control region and 1 Y - chromosome marker, 2 admixed individuals from 117 putative wolf genotypes were identified together with 5 others, for which probability of belonging to backcrosses was higher than 0.1. Taking them into account it would constitute ~6% of all the sampled individuals. Nevertheless, estimates for the two admixed individuals were considered unreliable because of a significant amount of missing data (more than 15%) (Dufresnes et al. 2019). What is important to note, the identification of putative wolves was based on scats mtDNA, so individuals resulting from the rare male dog female - wolf hybridization could be overlooked.

Korablev et al. (2021) studied the wolf population in Central Russia. During analyses of genetic diversity and structuring based on 11 autosomal microsatellites, 3 individuals from 101 were defined as admixed. The authors used Bayesian clustering implemented in STRUCTURE for hybrid identification, although the power of markers for differentiation between dogs and wolves was low (Korablev et al. 2021).

Santostasi et al. (2021) conducted research using a capture - recapture framework and two different approaches: minimizing type 1 and type 2 errors. Based on the samples from 39 wolf individuals collected during a single breeding period in a relatively small area of Appennino Tosco–Emiliano National Park in Italy. Reference populations were based on the samples obtained from previous studies, consisting of 190 pure wolves and 89 dogs. Main aim of the study was to improve the hybrid identification by minimizing type 2 errors. The authors provided information of a new statistical approach with more reliable hybrid identification and reported a high proportion of admixed individuals in the studied population (43.6% - 70%, depending on the scenario) (Santostasi et al. 2021).

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