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Supplementary material

Questioning assumptions

While making their case for the Baltic and CE wolf populations to be considered as a single management unit, Gula et al. (2020) make a series of assumptions prior to concluding that there is no genetic structure among Baltic and CE wolves. In the main text we show that the premises on which the reasoning is built are questionable. However, these assumptions are also worth a closer look, as Gula et al.'s reasoning hinges on their validity.

Gula et al. assume that a) a lack of detection of genetic structure implies genetic homogeneity, b) genetic homogeneity is proof of genetic connectivity and c) genetic connectivity means demographic connectivity and therefore populations should be managed as a single unit.

Firstly, failure to detect genetic structure may represent a type 2 statistical error: not finding a pattern that is truly present. Bayesian clustering approaches typically used in population genetics (e.g. STRUCTURE) group genotypes such that within-group imbalance (Hardy-Weinberg disequilibrium and linkage disequilibrium among alleles across loci) is minimized. When sampling depth (no. of individuals and no. of genetic loci) within true groups is insufficient (which depends on the degree of genetic structure), however, the sampling error within groups easily exceeds the among-group variance, and true groups are not recovered (see Frantz et al. 2012 for an example with incremental sample sizes). Failure to reject the null hypothesis (no structure) should not be confused with accepting the null hypothesis, especially in the absence of a power analysis.

Secondly, a lack of genetic structure (observed genetic homogeneity) can be proof of ample

genetic connectivity close to panmixia, but only under very restrictive assumptions, including migration-drift equilibrium (Whitlock and McCauley 1999). Because the recovery of the CE wolf population is extremely recent and in full development, there is a certain and strong violation of these assumptions. Therefore, it is impossible to conclude from the data reviewed in Gula et al. (2020) that the Baltic and CE wolf populations can be considered an evolutionary homogenous unit. Moreover, Szewczyk et al. (2019) actually show that there is genetic structure between the Baltic and CE wolf populations.

Finally, gene flow and the resulting equilibrium genetic structure among populations is driven by the absolute number of migrants, which is the product of the population size N and the migration rate m (Wright 1951). High gene flow may still result from low migration rates, when N is large. As an example, a comprehensive genome-wide analysis in Atlantic herring *Clupea harengus* shows neutral genetic homogeneity across the tenfold salinity dilution from the North Sea, through the Baltic Sea up to the Bothnian Gulf, despite very strong directional selection, clear adaptive differentiation and demographic independence of various populations (Lamichhaney et al. 2012). In contrast to genetic connectivity, demographic connectivity depends directly on the migration rate (Begon et al. 1996), as it implies that emigration and immigration directly impact population dynamics. Low migration rates that hardly affect population demography (independent population dynamics) can still result in high gene flow and shallow genetic structure. Hence, absence of measurable genetic structure is no proof of demographic connectivity, let alone continuity (Lowe and Allendorf 2010).

Detailed discussion of similarities and differences between the recent studies addressing wolf genetic structure in central Europe

The genetic structure of wolves recolonizing central Europe was studied by Czarnomska et al. (2013), Hulva et al. (2018) and Szewczyk et al. (2019). They all performed analyses of the mtDNA control region and autosomal microsatellites. However, they had different foci and thus differed markedly in sampling design.

Czarnomska et al. (2013) focused on determining the genetic structure of wolves within Poland and identifying the source population of the newly established subpopulation in western Poland (although several samples from eastern Germany were also included). They analyzed samples gathered during multiannual intense monitoring (2001-2009), resulting in very dense sampling especially in north-eastern Poland (e.g. 124 analyzed samples and 45 identified individuals in the Biebrza River Valley region, then inhabited by only 3-4 wolf family groups according to MRI PAS 2010). On the other hand, only 57 individuals were identified west of the Vistula River, representing all 10 wolf family groups scattered across a very large (>100 000 km²) area at that time. Both microsatellites and mitochondrial haplotype frequencies suggested clear structuring

between the lowlands and the Carpathian Mountains. Moderate genetic differentiation was detected between certain north-eastern lowland regions, sometimes separated by less than 50 km. Additionally, spatial analyses indicated further division between the Roztocze region in the southeast, and northern Poland. However, western Poland and eastern Germany (region 12 in Czarnomska et al. (2013)) seemed to be very weakly differentiated from certain north-eastern regions (Napiwoda-Ramuki and Pisz forests – regions 10 and 11). On the other hand, region 12 had the highest number of private alleles, suggesting immigration from areas not sampled by Czarnomska et al. (2013).

The study of Hulva et al. (2018), using population and landscape genetic tools, focused mainly on the Carpathian wolf population, where relevant information was missing at that time, using a model area in the western Carpathians (Slovakia), as well as the expanding wolf population in central Europe outside its well established range (Czech Republic). To interpret distributional records in the Czech Republic and to ascertain potential admixture between the Carpathian and lowland populations, Hulva et al. (2018) included >40 individuals from the Polish lowlands, but only 4 of these were from the easternmost Polish regions. For the whole dataset, the Evanno method (Evanno et al. 2005) applied to STRUCTURE results showed the highest support for K=2 population clusters, mirroring strong diversification between wolves in the Carpathians and the European lowlands. Hierarchical STRUCTURE results for higher values of K showed further substructure in the Carpathians and a homogenous cluster in the European lowlands. A separate analysis for the European lowlands was not performed.

Szewczyk et al. (2019) focused on genetic diversity and structure in the recently recolonized parts of central Europe, mostly western Poland. As references, DNA samples from wolves inhabiting the main forests in eastern Poland and the western Carpathians were included, as well as samples gathered in Lithuania, Belarus, Ukraine (Chernobyl zone), Slovakia and the eastern Czech Republic. Interestingly, Szewczyk et al. (2019) detected a very strong east-west structuring across the Polish lowlands, as the clustering algorithms implemented in STRUCTURE first separated the western cluster (with the Carpathian and most of the Baltic wolves merged into one group at K=2). The Evanno method (Evanno et al. 2005) applied to STRUCTURE results indicated that the best result was K=3 (clear distinction of the western, eastern lowland and Carpathian genetic groups), followed by K=5 that suggested further subdivision within the Baltic population into north-eastern, northern and south-eastern (Roztocze) clusters. Spatial GENELAND clustering and multivariate DAPC analyses generally supported the STRUCTURE results, although the northern cluster identified in STRUCTURE was not recognized in GENELAND, while DAPC results suggested that this may be an intermediary cluster between the western and north-eastern clusters, in line with gene flow estimates calculated in the BAYESASS software. However, all methods congruently

separated the CE wolves (western cluster) from the core Baltic population (north-eastern cluster = Lithuania, Belarus and north easternmost Poland).

We argue that the differences between the clustering solutions described above resulted mostly from sampling design. Bayesian clustering algorithms implemented in the STRUCTURE software have been shown to be vulnerable to the presence of closely related individuals in analysed datasets (Rodriguez-Ramilo and Wang 2012) and unequal sample sizes (Kalinowski 2011, Puechmaille 2016). Moreover, STRUCTURE has a tendency to merge genetically distinct populations into one cluster if they are undersampled, while on the other hand sometimes identifying multiple artificial clusters within oversampled populations (Puechmaille 2016). Additionally, it has been shown that relying solely on the ΔK statistic (Evanno et al. 2005), the most popular method of determining the “true” number of genetic clusters, can lead to underestimating genetic population structure (Janes et al. 2017). This is in line with the recommendations of the software creators, who advocated reporting results for the whole range of tested K-values and when possible performing hierarchical analyses (Pritchard et al. 2000).

The ΔK statistic indicated highest support for K=2 both in Czarnomska et al.’s (2013) and Hulva et al.’s (2018) STRUCTURE results, but log-likelihood values suggested further substructure. The detected subdivisions reflect the different aims and subsequent sampling priorities of each study: Czarnomska et al. reported differentiation within the eastern lowlands (described above) and a homogenous Carpathian cluster, while Hulva et al. found clear substructure across the Carpathian Mountains. Szewczyk et al. (2019), who had relatively equal sampling across the CE, Baltic and Carpathian populations, found highest support for K=3. Moreover, Szewczyk et al. 1) balanced the number of analyzed individuals per pack, reducing relatedness bias and 2) included reference individuals from Lithuania and Belarus, which were found to be more differentiated from the CE population than wolves inhabiting eastern Poland. Thus, the results of Szewczyk et al. (2019) better describe the Baltic-CE structuring, while the studies of Czarnomska et al. (2013) and Hulva et al. (2018) present more complete pictures of the fine-scale substructures in the eastern Polish lowlands and the western Carpathians, respectively.

Table A1. Sources of information on permanent wolf presence used to prepare distribution maps in Fig. 1

Country	Source	Comments
Austria	Chapron et al. 2014, unpublished data (S. Smith)	Alpine and Dinaric-Balkan part of wolf population not shown in Fig. 1

Belarus	Boitani and Ciucci 2009	approximate, may not include distribution gaps
Belgium	Schockert et al. 2020, unpublished data (J. Mergeay)	
Czech Republic	< www.mzp.cz/C1257458002F0DC7/cz/news_200225-pocet-vcich-smecek-se-za-rok-zvysil-do-Ceska-zasahuje-osmnact-vcich-teritorii/\$FILE/CZ_wolves_2018-19.pdf >	based on 2018/2019 data
Denmark	Sunde and Olsen 2018, < www.ulveatlas.dk/ >	based on 2020 data
Germany	www.dbb-wolf.de/Wolfsvorkommen/territorien/karte-der-territorien .	based on 2018 data
Hungary	< www.termesztvedelem.hu/_user/browser/File/V4_BP_2014_05_27/HU_V4%20Large%20carnivores%20in%20Hungary%202014.pdf >, Lanszki et al. 2012	
Latvia	Chapron et al. 2014	
Lithuania	Chapron et al. 2014, < www.lcie.org/Large-carnivores/Wolf- >, Szewczyk et al. 2019	
Netherlands	Klees et al. 2019 < www.bijl2.nl/wp-content/uploads/2020/02/Monitoringplan-Wolf-2019.pdf >	
Poland	Szewczyk et al. 2019, Gula et al. 2020, the authors (S. Nowak, R. Mysłajek, M. Szewczyk) unpublished data	
Romania	Chapron et al. 2014, < www.lcie.org/Large-carnivores/Wolf- >	
Russia	Boitani and Ciucci 2009	approximate, may not include distribution gaps
Slovakia	Chapron et al. 2014, , < www.lcie.org/Large-carnivores/Wolf- >, Hulva et al. 2018	
Ukraine	Boitani and Ciucci 2009	approximate, may not include distribution gaps

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