Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 33 (1): 5–16, 2022
OPEN ACCESS
Available online at:
http://www.italian-journal-of-mammalogy.it
doi:10.4404/hystrix-00487-2021

Research Article

Science-based solutions to foster connectivity of wolf populations are limited by available data

Sofia Lino[1,2,*], João Carvalho[2], Eduardo Ferreira[2], Carlos Fonseca[2,3], Luís Miguel Rosalino[1,2]

[1] *cE3c - Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa,1749-016, Lisboa, Portugal*
[2] *CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal*
[3] *ForestWISE - Collaborative Laboratory for Integrated Forest & Fire Management, Quinta de Prados, 5001-801, Vila Real, Portugal*

Keywords:
barriers
Canis lupus
anthropogenic disturbance
connectivity
wolf populations

Article history:
Received: 8 Sep 2021
Accepted: 15 Jan 2022

Acknowledgements
This research was funded by FCT/MCTES (Fundação para a Ciência e a Tecnologia), through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020, by supporting CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020) and cE3c (UIDB/00329/2020) and CHANGE (LA/P/0121/2020).

S.L was supported by a Ph.D. grant (SFRH/BD/147252/2019) from FCT; J.C was supported by a research contract (CEECIND/01428/2018) from FCT. E.F. is funded by national funds (OE), through FCT in the scope of framework contract foreseen in the numbers 4, 5, and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

Abstract

European wolf populations are currently exposed to distinct sources of anthropogenic disturbance and mortality that can cause dispersal limitations and lead to isolation. The identification of factors that act as complete or partial barriers to movement, dispersal, or gene flow contribute to foster connectivity between populations. We reviewed the existing literature (N=32) on wolf population barriers to 1) identify main barriers to connectivity; 2) outline different methodologies; and 3) highlight knowledge gaps. Based on the reviewed studies that empirically tested barrier occurrence (N=14), we compiled data on wolf population structure, anthropogenic disturbance, land cover, ecological factors, geographical features, and prey availability, and tested them as predictors to explain barrier occurrence at continental scale. We report few studies directly addressing this subject for one of the most emblematic and thoroughly studied species, inhabiting one of the most modified landscapes in the world. Albeit our analysis suggested that anthropogenic features are the main drivers of barrier occurrence, we highlight that the absence of standardised data limits our understanding of this subject. Long-term, intensive monitoring programs, explicit hypothesis-driven research using empirical methodologies, and the integration of information on databases for collaborative science are needed to increase the conservation and management relevance of future scientific outcomes on this topic.

Introduction

The grey wolf (*Canis lupus* L. 1758) was historically widespread throughout the Holarctic realm, inhabiting a wide variety of biomes and habitats, from tundra and boreal forests, to open, dry steppes, Mediterranean woodlands, and deserts (Wolf and Ripple, 2017; Olson and Dinerstein, 1998). However, mainly due to direct persecution by humans, habitat destruction, and the decrease of its natural prey, wolves have been extirpated from many European regions over the past two centuries. While completely eradicated from Scandinavia and central Europe, fragmented populations remained in the Iberian and Italian peninsulas and eastern countries (Torres and Fonseca, 2016). Recent conservation efforts, such as legal protection, the recovery of wild herbivore populations, and socio-economic changes that led to an improvement in habitat quality, enabled the species to recover, and wolves progressively returned to Scandinavia, the Alps, and central Europe (e.g., Luxembourg, the Netherlands, Germany, western Poland) as a result of recolonization by individuals immigrating from the Italian and eastern populations (Schley et al., 2021; Lelieveld et al., 2016; Boitani and Linnell, 2015; Chapron et al., 2014; Fabbri et al., 2014). This recovery brought wolves into regions where they had been previously extirpated from and that are now heavily transformed by humans (Chapron et al., 2014). Wolves are currently exposed to moderate human densities (Linnell et al., 2001), and to multiple sources of anthropogenic disturbance and mortality (i.e., deforestation, urbanization, linear infrastructures) (Llaneza et al., 2012; Gula et al., 2009), which can lead to habitat fragmentation and limit dispersal (Caplat et al., 2016). It should be noted that barriers can be complete (i.e., impermeable) or partial (i.e., still allow some connectivity). Furthermore, what constitutes a barrier and whether it reduces connectivity differs among species (McRae et al., 2012). Thus, the specific mechanisms that may lead to population discontinuities need to be assessed in a species scale to guarantee adequate and effective conservation and management measures.

For European wolves, human-related factors are considered a main threat to population survival (Hindrikson et al., 2017). Wolf hunting and poaching, in particular, may hinder wolf settlement and delay or constrain the population growth (Quevedo et al., 2019; Liberg et al., 2012). Poaching is often the result of conflict with humans due to livestock depredation (Hindrikson et al., 2017), which is one of the main processes constraining wolf population recovery in many European landscapes (König et al., 2020). Human-related factors also include the construction or modification of physical and landscape elements, such as linear infrastructures, urban areas, and agricultural and agroforestry lands. Linear infrastructures, such as roads, are a clear example of a physical barrier that fragments landscapes and may restrict dispersal movements for several species (Holderegger and Di Giulio, 2010; Forman and Alexander, 1998), conditioning resource accessibility, and being a direct cause of mortality due to collisions with vehicles (Coffin, 2007; Jaeger et al., 2005). Both traffic mortality and resource inaccessibility contribute to the division of populations into smaller and more isolated ones that are less likely to receive immigrants from other areas, and thus may suffer from genetic isolation and inbreeding depression (Holderegger and Di Giulio, 2010; Jaeger et al., 2005). This is especially true for species with large movement ranges, low reproductive rates, and low natural densities, such as large carnivores (Fahrig and Rytwinski, 2009). This isolation process has already been documented for European wolves (Silva et al., 2018; Seddon et al., 2006).

*Corresponding author
Email address: sofslino@gmail.com (Sofia Lino)

*2022 Associazione Teriologica Italiana
doi:10.4404/hystrix-00487-2021

Table 1 – Hypotheses, rationale, and predictions on the variables used to test barrier effect.

Hypotheses	Rationale	Prediction	Variables	Variables' code
H1 Ecological and geographical features	Despite wolves' high mobility and dispersal ability, landscape features such as large bodies of water, rivers, mountains, and forest fragmentation may act as barriers to gene flow by decreasing connectivity between populations (Czarnomska et al., 2013; Hindrikson et al., 2013; Jędrzejewski et al., 2004). Furthermore, ecological processes such as territoriality (inter-, and intraspecific competition) may also strongly constrain functional connectivity (Rio-Maior et al., 2019; Ordiz et al., 2015).	We expect that land covers associated with a higher disturbance/less refuge conditions and related to livestock grazing (i.e., agricultural lands, mosaic landscapes) are associated with a greater barrier effect.	Presence/absence of Geographical features (i.e., rivers, mountains, large bodies of water) and Ecological factors (i.e., fragmented forests, inter- and intraspecific competition).	`Geo` `Eco`
H2 Land cover	Open and agricultural areas promote functional connectivity gaps, since: 1) they increase the risk of wolf exposure to humans due to the higher visibility; 2) the use of pastures for hunting on domestic prey (where they are often more abundant and exposed to predation) increases human-wildlife conflict; Both processes can lead to a functional barrier (Huck et al., 2010; Karlsson et al., 2007).	We expect that the presence/occurrence of ecological and geographical features to be associated with a greater barrier effect.	Land cover (5 categorical classes): Coniferous forests, Broadleaf forests, Agricultural land, Mosaic areas, Mosaic areas in alpine landscapes.	`LandCover`

H3 Prey availability	A lower wild prey abundance constrains wolf functional connectivity. It may also lead to an increased predation on livestock (Meriggi et al., 2011; Álvares and Primavera, 2004; Sidorovich et al., 2003), increasing the risk of human-wildlife conflicts. Higher livestock densities are also associated with human-related risks (Rio-Maior et al., 2019; Pimenta et al., 2018). Thus, both a low wild prey abundance and a high domestic prey abundance may act as barriers for wolf survival and population connectivity.	We expect wolves inhabiting areas of lower wild prey availability (richness and density) and higher domestic prey density are exposed to a higher risk of human-wildlife conflict and thus subjected to a greater barrier effect. Likewise, we expect wolves that mainly feed on domestic prey to be subjected to a greater barrier effect.	Wild prey species richness (# of species). Wild prey density (# individuals/km^2). Domestic prey density (# individuals/km^2). Main prey type (4 categorical classes): Large wild ungulates, Medium-sized wild ungulates, Domestic, Carrion.	`PreySp_Richness`
				`WildPrey_density`
				`DomPrey_density`
				`MainPrey`

H4 Wolf populations structure	A non-linear relationship between wolf population density and dispersal rate has been recently described (Morales-González et al., 2021). Dispersal was found to be greatest at both lower and higher wolf densities, and lowest at medium densities. Contrary to what was previously thought, lower wolf densities also seem to result in greater distances travelled (Wabakken et al., 2007), and in higher success at pairing, territory establishment, and denning (Morales-González et al., 2021). Dispersal distance and success also seems to depend on several external factors (e.g., human-induced mortality, or surrounding pack density) (Morales-González et al., 2021; Jimenez et al., 2017).	Given the most recent findings we are unable to define a clear prediction on the influence of population size on dispersal success, as many factors come into play. Nonetheless, we maintain our hypothesis that population size does have an influence on wolf dispersal and reproductive success, acting as a barrier to population connectivity, and thus will look for a pattern of response. We include different wolf populations in this analysis to search for patterns between them.	Wolf populations (7 categorical classes): Baltic, Carpathian, Dinaric-Balkan, Finnish-Karelian, Iberian, Italian, and Scandinavian. Wolf population size (# of individuals).	`Wolf_pop` `Pop_size`

H5 Anthropogenic disturbances	Linear infrastructures fragment landscapes, impact resource accessibility and are a direct cause of mortality for wolves. Also, the avoidance of human-related landscape features (e.g., settlements, open agricultural lands, windfarms) strongly constrains functional connectivity for wolf packs (Rio-Maior et al., 2019). Additionally, wolf hunting is a major driver of wolf population dynamics in Europe and may have negative consequences in pack stability in case of breeder loss (Milleret et al., 2017; Brainerd et al., 2008), being critical in areas which are the only source of immigrants for other populations (Kojola et al., 2009).	We expect areas of high anthropogenic disturbance to be associated with a greater barrier effect.	Mean human density (# people/km^2). Mean road density (km/km^2). Presence/absence of anthropogenic structures (i.e., main roads, highways, railways, windfarms, dams). Anthropogenic landscapes (i.e., agriculture, silviculture, and forestry areas; settlements and urban areas), and Wolf hunting.	`Human_density`
				`Road_density`
				`Antr_Stru`
				`Antr_Land`
				`Hunt`

H6 Combination of factors	The accumulation of obstacles may have synergistic effects, causing a barrier effect greater than the sum of the individual ones (Blanco et al., 2005).	Barrier effect is determined by a combination of variables tested separately in Hypotheses 1–5.	All above-mentioned variables.	`Geo`
				`Eco`
				`LandCover`
				`PreySp_Richness`
				`WildPrey_density`
				`DomPrey_density`
				`MainPrey`
				`Wolf_pop`
				`Pop_size`
				`Human_density`
				`Road_density`
				`Antr_Stru`
				`Antr_Land`
				`Hunt`

Some structures, such as green bridges and underpasses, may help mitigate the negative impacts of roads (Plaschke et al., 2021).

Although usually regarded as a physical obstacle to movement (Ronce, 2007), a barrier can also emerge from ecological or behavioural processes. For example, high densities of conspecifics or a competitor species (i.e., bear) were suggested to respectively increase territoriality and interspecific competition, thus affecting territory selection and spatial distribution, and, therefore, the expansion of wolf populations in Fennoscandia (Ordiz et al., 2015; Aspi et al., 2009). Other ecological processes, such as habitat and prey specialization, may influence the spatial organization of wolf populations, restricting gene flow between populations and leading to isolation by environment (Wang and Bradburd, 2014). This specialization may refer to differential selection of resources by individuals and/or populations such as natal-habitat-biased dispersal (reviewed by Davis and Stamps, 2004), or specialization in consumption of a preferred prey based on hunting success (Mech and Peterson, 2003). Prey specialization, rather than geographical distance or topographic barriers, was responsible for genetic and phenotypic differentiation between neighbouring wolf populations (Pilot et al., 2012; Musiani et al., 2007; Pilot et al., 2006). Because prey availability depends on habitat type, wolf dispersal may be habitat-biased (Geffen et al., 2004). Young individuals learn to prey on animals that share their habitat, which may result in a willingness to choose prey and habitats they are familiar with (Pilot et al., 2006) to increase their chances of survival when leaving the pack (Gese and Mech, 1991). Also, new packs usually establish their territory close to the parental one (Fuller et al., 2003), promoting the selection of similar habitats by related wolves in a natal-habitat-biased dispersal (Muñoz-Fuentes et al., 2009). Such behaviours have been described for wolves (Schweizer et al., 2016; Jędrzejewski et al., 2012; Pilot et al., 2012; Muñoz-Fuentes et al., 2009; Carmichael et al., 2007; Musiani et al., 2007; Pilot et al., 2006; Carmichael et al., 2001) and other generalist carnivores (e.g., cougar (Knopff et al., 2010), coyote (Sacks et al., 2008, 2004)).

In conclusion, barriers are not always physical obstacles, but rather dynamic features that can be affected by intrinsic ecological factors, and environmental and human-induced changes (Caplat et al., 2016). This broader definition of barriers to wildlife movement and connectivity seems to mimic more accurately the processes acting in natural landscapes. Several studies suggest that geographical, environmental, anthropogenic, and ecological factors, or a combination of them, may be acting as barriers and being responsible for the decrease in gene flow and increase in genetic structuring of European wolf populations (Szewczyk et al., 2019; Djan et al., 2014; Czarnomska et al., 2013; Hindrikson et al., 2013; Huck et al., 2011; Aspi et al., 2009; Kojola et al., 2009). Reduced gene flow can lead to small and isolated populations that are threatened by inbreeding and loss of genetic variation, decreasing the individuals' ability to respond to environmental changes (Fuller et al., 2003) and leading to a reduction in fecundity, overall fitness and, ultimately, survival (Frankham, 2005). For small and isolated populations, immigrants are of key importance to ensure genetic sustainability (Seddon et al., 2006).

The purpose of our study is to review the existing literature to identify the main drivers of barriers to wolf connectivity in Europe. We defined "driver of barrier" as any factor related to movement resistance, or responsible for limited dispersal or diminished gene flow, inferred in the published literature. We also aim to outline the different methodologies used, as well as highlight potential knowledge gaps. In the absence of a systematic pan-European evaluation of the drivers of restricted wolf movement and gene flow, conservation plans at the continental scale may be constrained or limited in terms of effectiveness. Thus, we investigate the drivers that may be promoting the existence of barriers to wolf movements at the European scale using studies that empirically tested the impact of putative barriers. For this analysis we proposed a hypothesis-driven framework (Tab. 1) to explain the occurrence of barriers, linked to different mechanistic biotic and abiotic processes: 1) ecological factors and geographical features; 2) land cover; 3) prey availability; 4) wolf population structure; 5) anthropogenic disturbances; and 6) a combination of all these processes. To the best of our knowledge, this is the first study to propose an investigation of factors that may contribute to barriers at the continental level (but see Geffen et al., 2004). Most studies focus on identifying barriers in a specific population, reporting its effects at the local scale. Our approach is novel because we compiled published data from different populations at a continental scale and considered factors often not included at this level due to their difficult accessibility (e.g., prey availability, but see Pilot et al., 2012), which limits a deeper and broader analysis of barriers. This is a holistic approach to connectivity limitations of a large carnivore population that is currently expanding and recolonizing former territories. In this setting, the identification of barriers and their main drivers will allow conservationists and policymakers to draw strategies to mitigate their impact, to ensure connectivity between populations and anticipate negative human-carnivore interactions (i.e., persecution, killing) that could imperil the recovery of wolf populations. Additionally, identifying methodological limitations and knowledge gaps allows for better-informed decisions in future research.

Materials and methods

Literature Search

We reviewed published papers that identify barriers to wolf connectivity in Europe. We used Publish or Perish software (Harzing, 2007) to search the Web of Science, Scopus, and Google Scholar databases, using combinations of search terms related to wolves and barriers in the European continent. Our structured search included the terms "wolf*" OR "*Canis lupus*" AND "barriers" AND "dispersal" OR "connectivity" OR "genetic structure" OR "gene flow barriers" OR "landscape genetics" OR "population structure" OR "genetic structure" AND "Europe", and returned 1235 studies. After excluding duplicated entries, we rejected unsuitable entries (i.e., mistargeted, non-peer reviewed articles, articles not in English, reports, theses, and reviews). A total of 137 studies were deemed appropriate, but this number decreased after title and abstract screening. We supplemented our literature search by screening the references lists of selected studies. After testing eligibility of the full texts, we returned a total of 32 studies (Supplementary Material, Figure S5). Due to the diversity of methodologies applied in these studies, we followed an approach similar to Zeller et al. (2012), originally designed to review parametrization of resistance surfaces to movement, to describe our reviewed papers based on their 1) biological input data (animal's detection data, movement data, or genetic data), and 2) methodology used to derive barriers (expert-opinion-based, or empirical methods). We then summarise the main drivers of barriers identified by empirical methods.

Biological input data

Detection data

Detection data are defined by single point locations of unknown individuals (Zeller et al., 2012). These data can be obtained using several approaches, such as: camera-trapping (Kusak et al., 2009), howling sessions (Kusak et al., 2005), track signs surveys (Louvrier et al., 2018), or census data that comprise a range of different and complementary methods (Grilo et al., 2019; Huck et al., 2011; Rodríguez-Freire and

Table 2 – Factors identified through empirical methods as drivers of barriers to wolf dispersal and connectivity in Europe, number of studies in which they were tested, and number of studies (with %) in which they were identified as barriers. Linear infrastructures (i.e., paved roads, highways, dams), human presence and disturbance (i.e., settlements and urban areas, cultivated lands, areas with presence of livestock, windfarms), geographical features (i.e., rivers, mountains, large bodies of water), wolf hunting (i.e., legal hunting and poaching), and ecological processes (i.e., forest fragmentation, territoriality).

Factors	# studies tested	# studies identified as barrier
Linear infrastructures	9	3 (33 %)
Human presence and disturbance	7	4 (57 %)
Geographical features	7	4 (57 %)
Wolf hunting	3	3 (100 %)
Ecological processes	2	1 (50 %)

Crecente-Maseda, 2007; Jędrzejewski et al., 2004). Such biological information can be used in habitat ecology studies (e.g., habitat suitability models, resource-selection functions, maximum entropy, environmental niche factor analysis) that may then be applied to resistance surfaces analyses, and to identify population discontinuities or landscapes that are highly impermeable to movement.

Movement data

We divided movement data into two types: relocation and pathway data (Zeller et al., 2012). Both types of movement data consist in two or more sequential locations of an individual, the difference lying on the interval of time between each data collection. If the interval is sufficiently short for the movement to be treated as a sequential, it is considered as pathway, if not it is relocation (Zeller et al., 2012). Kusak et al. (2009) argued that 6-hour intervals between GPS fixes may be too wide for fast-moving animals, such as wolves. Merrill and Mech (2003) also found that some wolf activity peaks may not be detectable via GPS telemetry if the interval is larger than 3 hours. Thus, for the detected studies using movement data, we considered those using intervals of more than 3 hours between locations to be relocation data, and those of less than 3 hours to be pathway.

Genetic data

Genetic data consist of genetic samples collected at multiple locations (Zeller et al., 2012). It can be used to estimate the genetic structure of a population and measure the genetic distance (or relatedness) between individuals or populations, thus allowing to estimate gene flow rate and dispersal behaviour (Jones and Wang, 2012). According to Zeller et al. (2012), one downside of using genetic data to infer barriers to connectivity through gene flow estimates is that individual movement resistance is not measured directly. Another is that there may be a lag time before a new barrier signature is detectable and another after it is removed (Landguth et al., 2010). Nonetheless, for populations of highly mobile species, such as wolves, the signal of a barrier is lost within 15 generations (Landguth et al., 2010). A clear advantage of this type of data is that, in contrast to movement data, it reflects not only successful movements (i.e., those resulting in successful breeding) of mating individuals over time, but also the presence of individuals dispersing from a genetically different population (e.g., first generation migrants), being useful to infer barriers to connectivity.

Methodological approaches

We considered two broad types of methods representative of our dataset: expert opinion, and empirical methods.

Expert opinion

Throughout our systematic review we found a recurring pattern that led us to create a category for when expert opinion was applied to identify barriers. When the occurrence or impact of a barrier was not derived directly from biological data and/or empirical approaches but deduced by the authors from literature or their knowledge of the targeted ecological systems, we considered them as expert-opinion-based. Some studies relied on expert opinion entirely, while others resorted to a combination of expert opinion and empirical methods. For instance, through an analysis of molecular variance and Bayesian clustering methods, Aspi et al. (2009) found genetic differentiation and low levels of gene flow between Russian and Finnish wolves and hypothesised that physical obstacles, wolf hunting, and territorial behaviour could be the reason behind it.

Empirical methods

Zeller et al. (2012) divided empirical methods in two categories (one-stage and two-stage) but considered it a matter of degree and not a dichotomic character. For ease of comparison with expert opinion we pooled empirical methods in a single category. When using empirical methods, the identification of barriers is based on empirical inference of barrier occurrence or impact. Different empirical methods can make use of different biological data types and be applied into distinct types of analyses. Barrier occurrence, its impact, or permeability can be assessed in movement ecology studies by calculating crossing frequencies or successful dispersal (i.e., settling, pairing, or denning; Gese and Mech, 1991) across a potential barrier. In a more analytical approach, there are mainly two distinct methods for understanding connectivity across a landscape: one stemming from landscape ecology that mostly uses statistical models (e.g., habitat selection models, resource selection functions, maximum entropy modelling) and one from population genetics (i.e., landscape genetics; Shafer et al., 2012). Habitat models, resource-selection indexes, and environmental niche factor analysis can be inversed to derive cost values for landscape features, to conduct resistance surfaces and least-cost path analyses (LCPs; Wang et al., 2008). Deriving cost values is a critical step for estimating landscape resistance, and according to Beier et al. (2008) should be preferably based on movement data, genetic distances, or rates of interpatch movement; if these are not available, second order data could be used, such as occurrence data, density, or fitness. When inferred from actual locations and used to derive resistance surfaces, habitat models show meaningful biological interpretation and can be good predictors of gene flow (Shafer et al., 2012). Empirical data provides a better basis than literature review or expert opinion (Beier et al., 2008), but due to a limited availability of data on animal movements most studies rely on the latter (Milanesi et al., 2017b). In the absence of movement data, movement paths (either connecting individuals or populations) can be identified through resistance surfaces (Milanesi et al., 2017b; Huck et al., 2011). Comparison of LCPs and suitable habitat patches give insights into habitat characteristics that might hinder or enable dispersal (Huck et al., 2011), and distances along LCPs can be related to genetic distances of individuals or populations (Milanesi et al., 2017b). In a landscape genetics approach researchers look for correlations between genetic distances and landscape or environmental features to find discontinuities and understand how landscape features influence movement of genes between individuals or populations (Landguth et al., 2010; Manel et al., 2003). Given that the spatial location of individuals or populations and a resistance surface for the study area are known, it is possible to visualize landscape connectivity and identify possible barriers (Rodríguez-Freire and Crecente-Maseda, 2007). In conclusion, there is a wide variety of methodological approaches that can be applied to identify barriers, which may be as simple as calculating barrier crossing frequencies or as advanced as landscape genetics.

Table 3 – Input biological data and methodological approaches used in the 32 reviewed studies. Sum of number of studies is larger than 32 as more than one input data type was used in some studies.

Input biological data	Methodological approach	# studies
Detection	Expert opinion	7
	Empirical approach	2
Movement	Expert opinion	3
	Empirical approach	10
Genetic	Expert opinion	10
	Empirical approach	4

Drivers of wolf barriers

Data collection

In this analysis, we used only the data from studies that empirically tested the presence of barriers (N=14, 44% of the total studies; see results). We used the data compiled and summarised in Tab. 2 (factors identified as drivers of barriers) as baseline information for our analysis. We categorized each study as "Yes" (`Y`) or "No" (`N`), depending on whether they had found a particular factor responsible for creating barriers to connectivity. This was our response variable "`Barrier_effect`" (binary, `Y/N`). Then, we compiled information on each identified factor in the reviewed studies and others known to influence wolf occurrence and distribution (e.g., land cover, prey availability, road density; Roder et al., 2020; Llaneza et al., 2012; Eggermann et al., 2011; Huck et al., 2010). In total we selected 14 predictors to assess

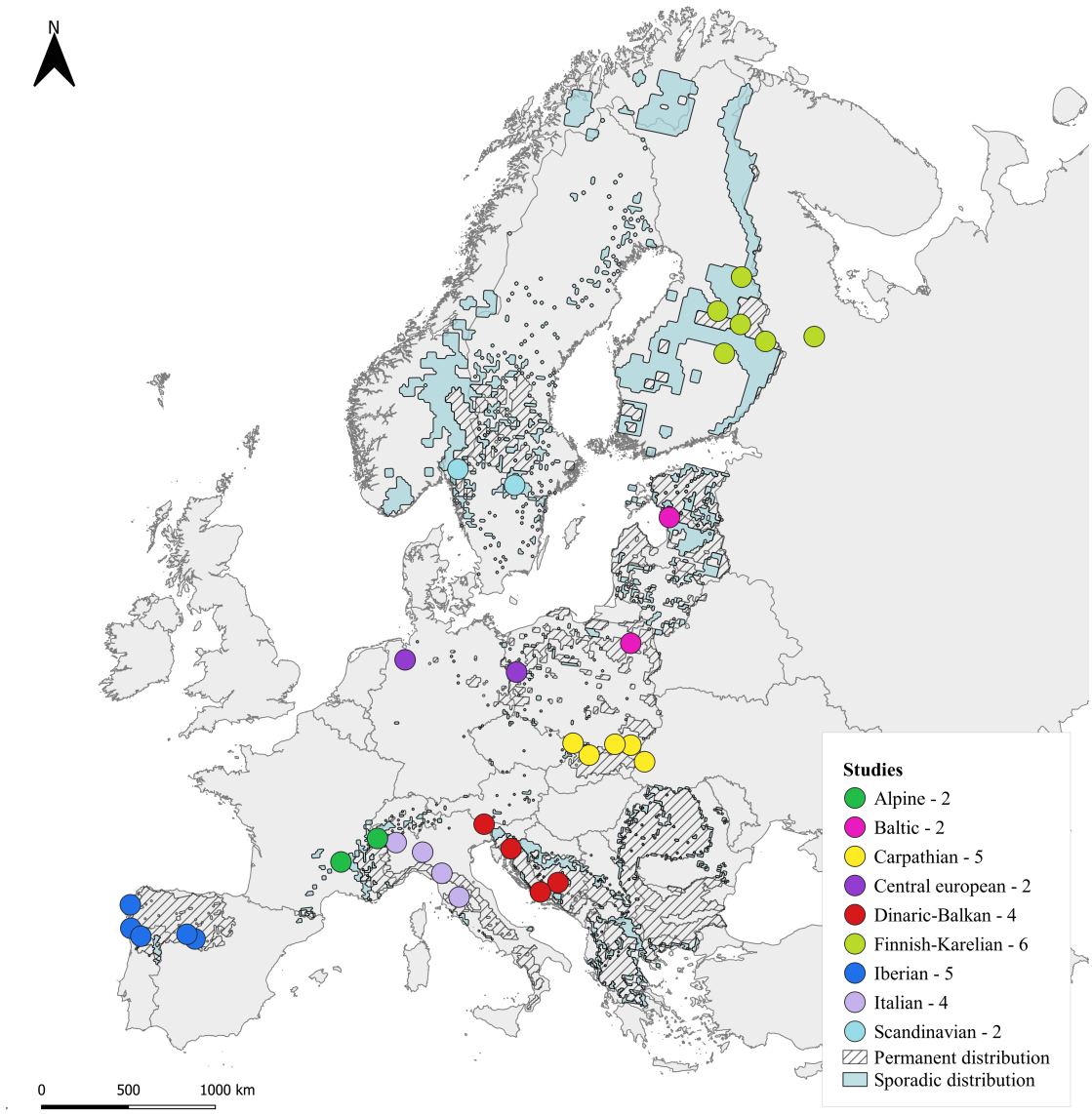

Figure 1 – Map of the study area depicting regions of permanent and sporadic wolf distribution (updated from Hulva et al. (2017); data from Russian Karelia, Belarus and Ukraine are missing), and the distribution and number of studies analysed per wolf population.

their role as drivers of barriers. These predictors were grouped into five hypotheses formulated to assess which underlying processes resulting in population discontinuities had more support from the collected data (Tab. 1). Thus, we hypothesised that barriers were mostly linked to: H1 – Presence/absence of geographical features (rivers, mountains, large bodies of water) and ecological factors (fragmented forests, territoriality); H2 – Predominant land cover; H3 – Prey availability (main prey type, wild prey species richness, wild prey density, and domestic prey density); H4 – Wolf population structure (population group and population size); and H5 – Anthropogenic disturbances [mean human density, mean road density, presence/absence of anthropogenic structures (e.g., main roads, highways), of anthropogenic landscapes (agricultural and forestry areas, settlements and urban areas), and wolf hunting] (Supplementary Material, Tab. S3). When information was missing in the original study (e.g., lack of information on prey availability) we retrieved it from other published accounts to complete our dataset (see Supplementary Material, Tab. S4). Land cover was categorized into five classes (European Environment Agency, 2006): coniferous forests (`CONIF`), broadleaf forests (`BROAD`), agricultural land (`AGRIC`), mosaic areas (`MOSAIC`), and mosaic areas in alpine landscapes (`MOSALP`). Main prey type was selected according to its contribution to wolf diet in Europe (Newsome.ea.2016) and categorized as four classes, namely large wild ungulates (`LWU`; 240–460 kg), medium-sized wild ungulates (`MWU`; 20–130 kg), Domestic ungulates, and Carrion (Supplementary Material, Tab. S3). We did not consider medium-sized mammals (e.g., beaver, badger), small mammals, rodents, or birds as these were found to contribute minimally to wolf diet in Europe (Newsome et al., 2016). To test for differences between metapopulations we categorized wolf populations in seven groups, corresponding to each study's subject: Baltic, Carpathian, Dinaric-Balkan, Finnish-Karelian, Iberian, Italian, and Scandinavian (Chapron et al., 2014). All variables were numerical except for wolf populations, predominant land cover, and main prey type, which were nominal. Geographical features (`Geo`), ecological factors (`Eco`), anthropogenic structures (`Antr_Stru`), anthropogenic landscapes (`Antr_Land`), and wolf hunting (`Hunt`) were binary with `0` coding for absence and `1` for presence of these features.

Data analysis

Prior to model fitting, we standardised the continuous variables to allow an easier comparison of effects sizes (Zuur et al., 2007), and checked the predictors for collinearity using Variance Inflation Factors (VIF; Zuur et al., 2007), repeating this step for each individual model. We assumed no multicollinearity between explanatory variables when all predictors presented VIF values smaller than 5 (Zuur et al., 2007). Due to our small sample size we found issues of separation (i.e., one or more explanatory variables perfectly predict the outcome; Zorn, 2005). To solve this we tested for the existence of complete or quasi-complete

Table 4 – Model selection results with the best model per hypothesis selected using backward selection. Degrees of freedom (df), AICc value and ΔAICc. Best overall model (i.e., with the lowest AICc value and a ΔAICc=0) in bold. Variable's acronyms are defined in Tab. 1.

Hypotheses	Model	df	AICc	ΔAICc
	Null	2	22.500	10.995
	Full	18	61.643	50.138
H1	`Eco` `Geo`	6	28.153	16.648

H3	`PreySp_density`
	`DomPrey_density`
	`WildPrey_density`
		8	32.051	20.546

H4	`Pop_size`	4	22.515	11.010
H5	`Human_density` `Antr_Stru`	**6**	**11.505**	**0.000**

separation in our dataset to find which parameters were responsible for infinite maximum likelihood estimates, using the `brglm2` package (Kosmidis, 2018) and "detect separation" method for `glm` function in R (Konis, 2007). We then applied `brglmFit` method from the same package to fit the model via mean bias reduction, using a quasi-Fisher scoring algorithm. We fitted a logistic regression (Zuur et al., 2007) to model the influence of our explanatory variables in predicting a barrier effect, using the response variable N (no effect) as the reference level. We used backward selection (Zuur et al., 2007) to select the most parsimonious variables' combination in each hypothesis. Variables included in all five models whose CI 90% coefficients did not include the zero, were used to test a sixth hypothesis, which assumes a combination of factors linked to distinct mechanisms in predicting barrier effect (Tab. 1). Model ability to represent data variability was assessed by the Akaike Information Criteria corrected for small sample sizes (AICc; Burnham and Anderson, 2002), and models with ΔAICc<2 were identified as the best overall models (Burnham and Anderson, 2002). We assessed the most supported model's predictive performance using the area under the curve (AUC), estimated from the receiver operating characteristic (ROC) curve (Hanley and McNeil, 1982). AUC values between 0.7 and 0.9 indicate that the model can accurately predict the presence of barriers (Hanley and McNeil, 1982). The analysis was performed in R version 4.0.0 (R Development Core Team, 2020) using the `glm` function in the `nnet` package for model fitting (Venables and Ripley, 2002) and `roc` function in the `pROC` package (Robin et al., 2011).

Results

Systematic review

We identified 32 studies, published between 2003 and 2019, that fulfilled our search criteria. Although we managed to identify studies that targeted all European wolf populations, knowledge is sparse and the literature is biased, with half of the studies focusing on three populations: the Carpathian (N=5), Finnish-Karelian (N=6) and Iberian (N=5) (Fig.1). The Italian and the Dinaric-Balkan populations were represented in four studies each, whereas the Alpine, Baltic, Central European, and Scandinavian were analysed in only two. The reviewed studies were all site-specific and differed greatly in terms of objectives, input data type, sample size, and methodology. As several papers used more than one biological input data type (Fig.2), the sum of cases in which they were employed is larger than the total number of studies (Tab. 3).

Biological input data

Detection data were used in nine studies and included both presence-only data (Louvrier et al., 2018; Kusak et al., 2009; Rodríguez-Freire and Crecente-Maseda, 2007) and presence–absence data (Grilo et al., 2019; Ronnenberg et al., 2017; Ordiz et al., 2015; Huck et al., 2011; Jędrzejewski et al., 2005, 2004). Most presence–absence data contained observations assumed to represent true absences, but some considered pseudo-absences (Grilo et al., 2019; Ronnenberg et al., 2017).

Movement data were the second most common type of data, used in 13 studies. When the focus of a study is to estimate individual movement resistance, relocation data should be preferred over detection data, and pathway data preferred over both, for being the most informative. Despite the advantages of pathway data over other types of data, these were only used in four studies (Rio-Maior et al., 2019; Gurarie et al., 2011; Blanco et al., 2005; Kusak et al., 2005), while relocation was used in nine (Ražen et al., 2016; Ciucci et al., 2009; Gula et al., 2009; Kojola et al., 2009; Kusak et al., 2009; Blanco and Cortés, 2007; Wabakken et al., 2007; Kojola et al., 2006; Blanco et al., 2005). Global positioning system (GPS) and very high frequency radiotelemetry (VHF) collars were employed in five and four studies, respectively, while a combination of both was used in four. The smallest number of wolves collared was one (Ražen et al., 2016; Ciucci et al., 2009; Gula et al., 2009) and the largest was 82 (Kojola et al., 2009).

Genetic data were the most common data type, used in 14 studies, and collected from different sources, such as wolf carcasses (Szewczyk et al., 2019; Hulva et al., 2018; Djan et al., 2014; Hindrikson et al., 2013; Jansson et al., 2012; Scandura et al., 2011; Gula et al., 2009; Fabbri et al., 2007; Valière et al., 2003), pelts (Aspi et al., 2009), prey carcasses (Szewczyk et al., 2019; Milanesi et al., 2018), or scats (Hulva et al., 2018; Ordiz et al., 2015; Czarnomska et al., 2013; Scandura et al., 2011; Valière et al., 2003).

Methodological approaches

Barriers to wolf connectivity were deduced through expert opinion in 18 studies and identified by empirical methods in 14 (Fig. 3). From the 18 studies that relied on expert opinion, nine analysed wolf habitat use, selection, or suitability, but did not integrate their results in a resistance surface or least-cost path, nor attempted to evaluate dispersal success or crossing frequencies of presumed barriers (Grilo et al., 2019; Louvrier et al., 2018; Ronnenberg et al., 2017; Ordiz et al., 2015; Gurarie et al., 2011; Jędrzejewski et al., 2005; Kusak et al., 2005; Jędrzejewski et al., 2004; Valière et al., 2003). Eight other studies analysed wolf genetic structure through Bayesian clustering algorithms and analyses of molecular variance, but relied on expert opinion and literature to explain their findings, hypothesising barrier occurrence (Szewczyk et al., 2019; Djan et al., 2014; Czarnomska et al., 2013; Jansson et al., 2012; Scandura et al., 2011; Aspi et al., 2009; Fabbri et al., 2007; Aspi et al., 2006). One study assigned suitability values for each landscape feature based on expert opinion (Rodríguez-Freire and Crecente-Maseda, 2007), which can be subjective and error prone (Milanesi et al., 2017b). Regarding the empirical approaches employed (N=14), more than half of the studies (N=8) analysed crossing frequencies or dispersal success (Ražen et al., 2016; Ciucci et al., 2009; Kojola et al., 2009; Kusak et al., 2009; Blanco and Cortés, 2007; Wabakken et al., 2007; Kojola et al., 2006; Blanco et al., 2005), while the others (N=6) used landscape genetics (Hulva et al., 2018; Milanesi et al., 2018; Hindrikson et al., 2013; Gula et al., 2009), least-cost paths derived from habitat suitability models using detection data (Huck et al., 2011) and resource selection functions based on movement data (GPS locations; Rio-Maior et al., 2019).

Table 5 – Best model (Hypothesis 5; see Tab. 4)) coefficients (Coef), standard errors (SE), significance value [p (>|z|)], and 90 % confidence intervals (CI 90 %) of the variables included in the best model explaining the occurrence of barriers to wolf connectivity. Variable's acronyms are defined in Tab. 1.

				CI 90 %	
Predictors	**Coef**	**SE**	***p*(>|z|)**	**5 %**	**95 %**
Intercept	1.953	1.665	0.241	−0.786	4.692
`Human_density`	7.467	4.222	0.077	0.523	14.412
`Antr_Stru`	−7.285	3.420	0.033	−12.911	−1.658

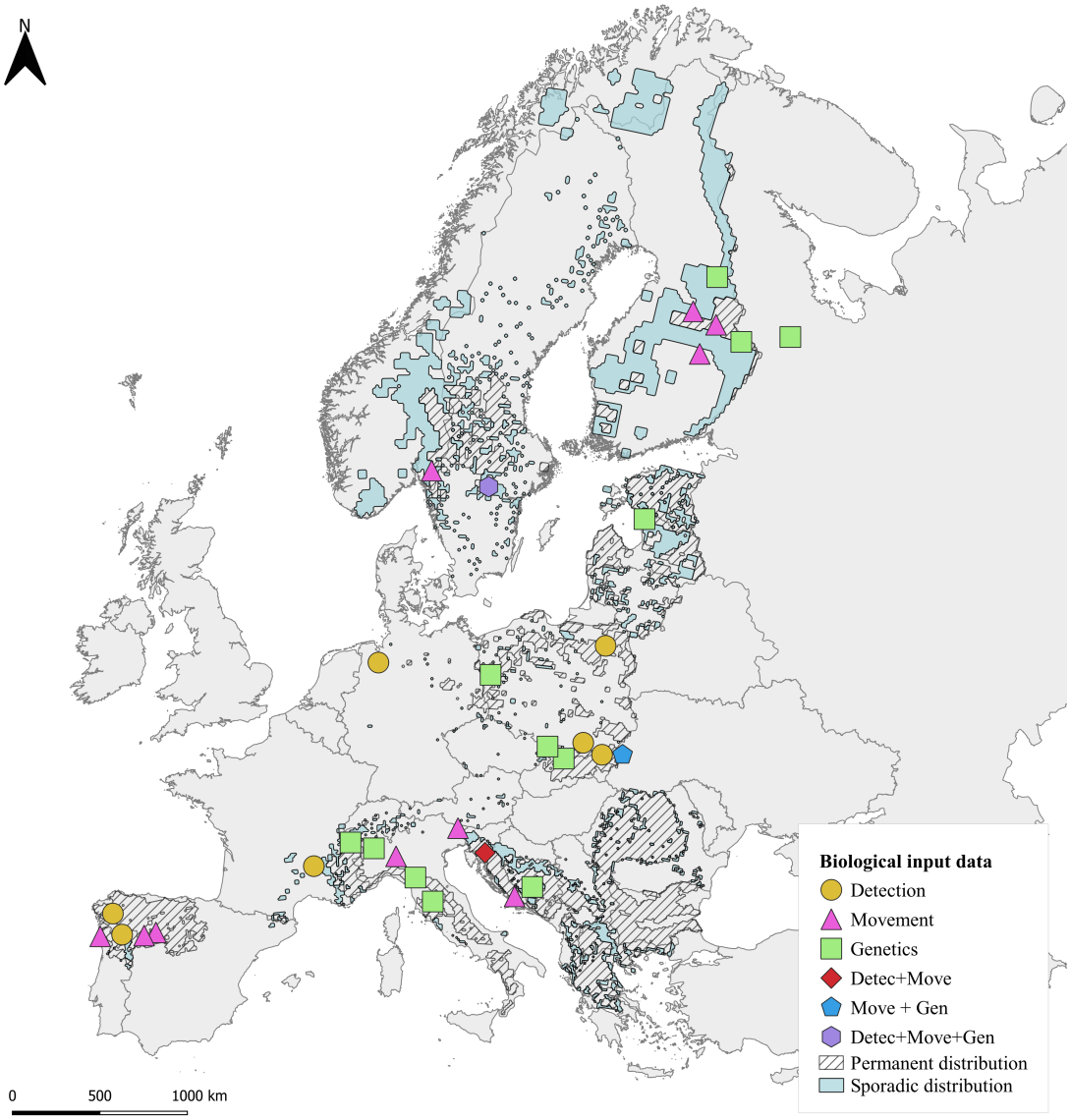

Figure 2 – Map of the study area depicting the distribution of studies per their biological input data type (i.e., detection, movement, genetic data, or combinations of them) and regions of permanent and sporadic wolf distribution (updated from Hulva et al. (2017); data from Russian Karelia, Belarus and Ukraine are missing). Abbreviations: Detec=Detection data; Move=Movement data; Gen=Genetic data.

Factors identified as drivers of barriers to connectivity

The main drivers of barriers to wolf connectivity that were identified through empirical approaches are summarised in Tab. 2.

Linear infrastructures were the most common drivers of barriers tested in our reviewed studies. They were analysed in nine studies, using different types of data [e.g., road density (Huck et al., 2011; Gula et al., 2009), presence or proportion of paved roads (Rio-Maior et al., 2019), highways (Hulva et al., 2018; Ražen et al., 2016; Ciucci et al., 2009; Kojola et al., 2009; Kusak et al., 2009; Blanco and Cortés, 2007; Kojola et al., 2006; Blanco et al., 2005), and dams (Ražen et al., 2016)]. The possible negative impact of these structures on wolf connectivity varied among studies, but was mitigated by the presence of crossing structures, such as bridges or underpasses, which were reported to be used by wolves in several cases (44%; (Ražen et al., 2016; Ciucci et al., 2009; Kusak et al., 2009; Blanco et al., 2005)).

Human presence and disturbance were the second most assessed factors. They were analysed in seven studies using different types of data such as human density (Gula et al., 2009), land cover data (Rio-Maior et al., 2019; Hindrikson et al., 2013; Huck et al., 2011; Gula et al., 2009), and indicators of human-related risks (e.g., presence or proportion of settlements and urban areas, cultivated areas, areas with presence of livestock, windfarms; Rio-Maior et al., 2019; Ražen et al., 2016; Ciucci et al., 2009; Blanco et al., 2005). In most cases (57%), their role in creating barriers to wolf connectivity was confirmed.

Geographical features (i.e., large bodies of water, rivers, mountains) were tested in seven studies and confirmed as drivers of barriers in four of them. Ice-free bays (due to natural ice melting) were responsible for hindering dispersal and impeding contact between populations (Kojola et al., 2009; Wabakken et al., 2007), while a large gulf and mountainous terrains were found to limit gene flow between subpopulations (Milanesi et al., 2018; Hindrikson et al., 2013). In three other cases, geographical features were not a barrier, as wolves were reported to have crossed rivers through man-made structures such as dams, or shallow areas (Ražen et al., 2016; Blanco and Cortés, 2007; Blanco et al., 2005).

Wolf hunting was addressed and identified as a barrier to wolf connectivity in three studies (Kojola et al., 2009; Wabakken et al., 2007; Kojola et al., 2006), in Fennoscandian areas of reindeer husbandry where wolf hunting is legal. All tracked wolves that dispersed into these areas were killed before they succeeded in reproducing.

Ecological processes were assessed in two studies. In one of them, the effect of forest fragmentation (measured with fragmentation indexes; Jaeger, 2000) on wolf dispersal was analysed and was not considered a barrier (Gula et al., 2009). On the other, intraspecific competition between disperser and resident wolves, measured by the presence

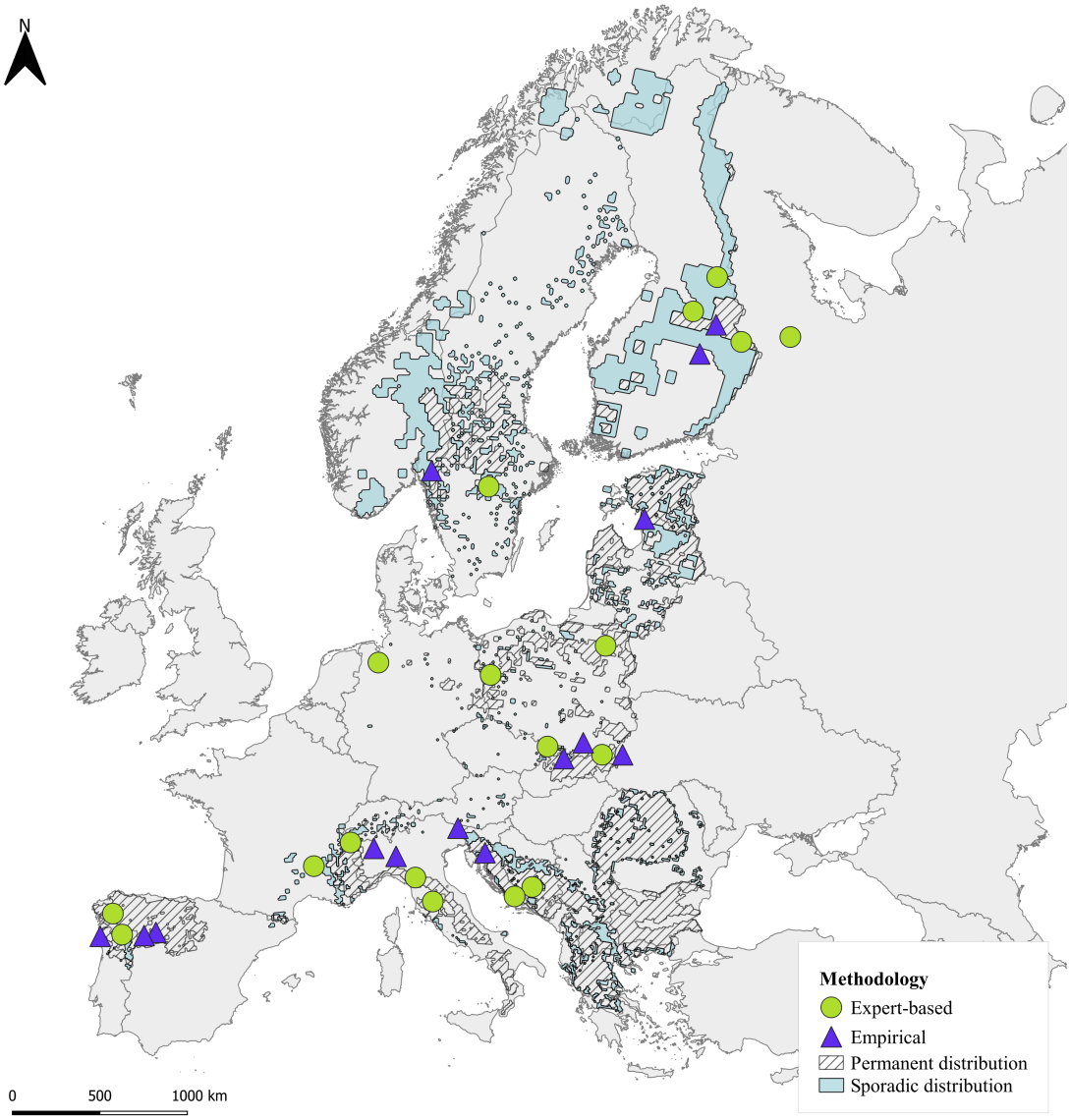

Figure 3 – Map of the study area depicting the distribution of studies per their methodological approach (i.e., expert-opinion-based, or empirical) and regions of permanent and sporadic wolf distribution (updated from Hulva et al. (2017); data from Russian Karelia, Belarus and Ukraine are missing).

of home sites of different packs, was identified as a barrier (Rio-Maior et al., 2019).

Drivers of wolf barriers

Three candidate predictors (`Wolf_pop`, `MainPrey`, `LandCover`) displayed high collinearity (i.e., VIF⩾5) before and after regression and were removed from the modelling procedure, leaving eleven predictors to be used for model building (Tab. 1). Our second hypothesis (Land Cover) was excluded due to the predictor's high collinearity. The backward selection procedure (Zuur et al., 2007) resulted in four models to be analysed (one per hypothesis), consisting in: H1 – Ecological and geographical features: 2 variables; H3 – Prey availability: 3 variables; H4 – Wolf populations structure: 1 variable; H5 – Anthropogenic disturbances: 2 variables (Tab. 4). As only `Human_density` and `Antr_Stru` presented a CI 90% that did not include the 0, our sixth hypothesis (combination of factors) was redundant and thus excluded from the analysis.

The hypothesis best supported by our model selection procedure (i.e., the only to present a model with ΔAIC<2) was Hypothesis 5 – The occurrence of barriers is influenced by anthropogenic disturbances (Tab. 4). This included two variables with differing influence: higher values of mean human density and the absence of anthropogenic structures were related to a higher probability of barrier occurrence (Tab. 5). The model presented a perfect predicting capacity, with a AUC value of 1.00 (Hanley and McNeil, 1982).

Discussion

A recent change in the conservation context enabled wolf expansion back into regions of its historical range that are now heavily transformed by humans (Cimatti et al., 2021; Chapron et al., 2014). The main challenge for large carnivore conservation in human-shaped landscapes is ensuring the stability of the expanding populations while promoting the recovery of the ones declining or isolated. The identification of barriers to connectivity, and their drivers, is thus crucial to overcome these challenges. Here, we outline different data types and methods used for detecting barrier occurrence in European wolf populations. We found several approaches which greatly differ in both input data (i.e., detection, movement, genetic) and complexity of methodological analyses (i.e., crossing frequencies, dispersal success, least-cost and resistance surfaces, landscape genetics). We found that most of the scientific literature did not aim to identify or describe barriers to wolf connectivity, dispersal, or gene flow. Most studies focused on analysing distinct ecological patterns but addressed population connectivity issues as part of the discussion of their findings, whilst relying on expert opinion. However, this was not due to the lack of data or low data quality, but to the specificities of each study. The use of genetic data in

wolf studies is growing as methods become more accurate and affordable (Linnell et al., 2013). Genetic data were the most common data type used (in 43% of studies) but are less often applied in approaches based on explicit hypotheses testing, such as landscape genetics (29%). Movement data was the second most used data type (40% of studies), mainly to analyse crossing frequencies and dispersal patterns and success. Although being a useful baseline to habitat ecology studies and to derive resistance surfaces, detection was only used in 28% of studies. We found knowledge gaps concerning some populations, mainly the Alpine and Central European, which did not present any empirical-based study, but also the Scandinavian and Baltic, which are each represented by one empirical study. Regarding the Alpine and Central European populations, the identification of barriers would be crucial to assess their potential of expansion and connectivity to other populations since both suffer from a strong founder effect ((Szewczyk et al., 2019; Fabbri et al., 2007). Even for other well-studied populations (e.g., Finnish-Karelian, Carpathian, Iberian) there is a lack of standardised empirical approaches for assessing barriers. Nevertheless, we were able to identify key drivers of barriers for seven of the nine European wolf populations (Chapron et al., 2014). The fact that more than half of the systematically reviewed studies did not identify barriers through empirical approaches challenges a correct and rigorous identification of its drivers and the search for a general pattern for European wolf populations. These studies, however, should not be dismissed as they may contribute to drive future research questions on this topic. Despite the challenges, barriers linked to human presence and disturbance, and geographical features were widely reported for different populations. Wolf hunting was depicted as a factor that strongly hinders the dispersal, settlement, and gene flow of Finnish-Karelian and Scandinavian wolf populations. Poaching (i.e., illegal killing) is estimated to be the main cause of wolf deaths in Scandinavia, but almost 70% of events are undetected (cryptic poaching; (Liberg et al., 2012)). Thus, many killing events are not being reported and their role in contributing to a barrier effect may still be underestimated in these and other wolf populations. The thawing of the gulf between Sweden and Finland was also identified as a barrier to wolf dispersal and connectivity. Both populations, but especially the Scandinavian, depend on immigrant wolves to maintain their genetic diversity. Current wolf killing levels together with ongoing and future climate change might have a negative effect on the immigration rate between these populations (Kojola et al., 2009). The existence of barriers to connectivity between populations is one of the main obstacles to wolf recovery in European landscapes (Hindrikson et al., 2017), but no study has yet analysed barriers in this broad context. Although acknowledging that the site-specificity and the scarcity of high-quality data at a continental scale make our approach a challenging endeavour, we argue that it is crucial to systematise the available information to allow a more generalised perspective of what may be limiting wolf range expansion and connectivity in Europe. Guided by this conservation and management need, and recognising sample size limitations, we identified what seems to be a more general pattern in the underlying mechanisms leading to connectivity barriers.

The most supported hypothesis to explain the occurrence of barriers to wolf connectivity included two predictors related to anthropogenic disturbance. Higher mean human density and the absence of anthropogenic structures were found to be associated with a higher probability of the occurrence of barriers. Anthropogenic barriers are a common pattern for many large carnivores. For instance, in the definition of the Jaguar Corridor Initiative, which aims to promote jaguar conservation through the establishment of ecological corridor between jaguar's Conservation Units in Central and South America (Rabinowitz and Zeller, 2010), it is clear that anthropogenic features are promoters of jaguar's mortality risk, and therefore may act as a barrier to individual movement and gene flow (Zeller et al., 2013). In a recent review of the impacts of human dominated landscapes on bears, Morales-González et al. (2020) highlighted that human density, structures, and activities constrain bear presence and, consequently, movements. Some of those have been clearly identified as barriers for specific populations, such as transportation infrastructure for the Cantabrian (Northern Spain) bear population (Mateo-Sánchez et al., 2014). Our results are also in accordance with those of Hindrikson et al. (2017), who reported human-related factors as the main source of threats to European wolves, and with Cimatti et al. (2021), who found that a decrease in human population density together with land cover changes likely contributed to the recent recovery of wolf and other European carnivore populations. However, our results also contradicted our expectation of a barrier effect related to the presence of anthropogenic structures. These findings probably reflect our inability to account for passages and corridors that allow wolves to disperse, and which were described in several studies (Ražen et al., 2016; Ciucci et al., 2009; Kusak et al., 2009; Blanco et al., 2005). Wolves were often found crossing roads and motorways, using bridges, or under- and overpasses, displaying a high behavioural plasticity in adapting to linear infrastructures. Our results may suggest that the existing crossing structures, which we could not account for in the analysis, are effective in allowing connectivity between wolf packs and populations.

Conclusions

Our review summarised the main factors contributing as barriers to wolf population connectivity in Europe. We report very few studies directly addressing and empirically testing this topic, for one of the most emblematic and thoroughly studied species, inhabiting one of the most modified landscapes in the world (Venter et al., 2016). We found knowledge gaps, but also a lack of a standardised methodology to evaluate the occurrence and impacts of barriers through empirical approaches. The establishment of a network of intensive, long-term monitoring programs, working at broader geographical scales, together with explicit hypothesis-driven research using standardised empirical methodologies, could help addressing the lack of data and knowledge on this topic. The integration of wolf-related research into online international platforms such as the EUROMAMMALS database, from which the species is still currently lacking, can help promote the access to standardised data and knowledge sharing among researchers.

Our analysis of drivers of barriers highlighted the role of anthropogenic factors in the establishment of barriers to wolf population connectivity. Although wolves have developed ways of coping with anthropogenic disturbance (Cimatti et al., 2021; Cretois et al., 2021), their individual movements and dispersal are still conditioned by the increasing human densities within their range (Wolf and Ripple, 2017), which may lead to less connected populations. As the European human population is expected to continue increasing in the near future (Giannakouris, 2008) wolves will be in a closer contact with humans and, consequently, exposed to higher and more frequent human-wildlife conflicts (Milanesi et al., 2017a). Reducing the impact of an increase in human density requires coordinated efforts between European countries to ensure the continued existence of transnational safeguards corridors and crossing structures to allow wolf movements. On the other hand, the ongoing land abandonment process is expected to continue in European farmlands (Verburg and Overmars, 2009). The associated decreasing human pressure (as the expected increase in human density is mostly linked to urban areas) and land cover changes will probably benefit large carnivore populations (Cimatti et al., 2021; Boitani and Linnell, 2015; Navarro and Pereira., 2012). However, as wolf populations recover and expand and the buffer zones between wolves and people are reduced, the fear of "losing the landscape" becomes real for many local communities (Ghosal et al., 2015). In their understanding, wolf protection is being preferred over production by both the public opinion and policymaking, and thus fear losing their traditional way of life and land management to wolf conservation (Pettersson et al., 2021). This fear might fuel negative attitudes towards wolves. The lack of effective conservation policies together with negative attitudes towards wolves increase wolf extinction risk (König et al., 2020). Thus, policymakers should implement protective legislation in countries or regions where it is still lacking, and increase sanctions for wolf killing where it is already an illegal activity. Regional governments should implement coexistence programs which consider the interests of different stakeholders, so that trust is built between the parts and the effectiveness of

these programs is ensured. In the long-term, environmental education and awareness campaigns are envisaged as an important step towards human coexistence with wolves, in a land-sharing approach.

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Associate Editor: M. Festa-Bianchet

Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 PRISMA checklist.

Table S2 Reviewed papers regarding their biological input data and methodological approaches used to identify barriers, with mention of the original sample size (no. of sampled and/or collared individuals, or no. of observations) and summary of each approach.

Table S3 Predictor variables used in the analysis of drivers of barriers to wolf connectivity.

Table S4 Data sources consulted for each predictor, per study (n=14).

Figure S5 PRISMA flow chart showing the exclusion process, References.