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Policy analysis

Genetic integrity of European wildcats: Variation across biomes mandates geographically tailored conservation strategies

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ABSTRACT

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Hybridisation between domestic and wild taxa can pose severe threats to wildlife conservation, and human-induced hybridisation, often linked to species' introductions and habitat degradation, may promote reproductive opportunities between species for which natural interbreeding would be highly unlikely. Using a biome-specific approach, we examine the effects of a suite of ecological drivers on the European wildcat's genetic integrity, while assessing the role played by protected areas in this process. We used genotype data from 1217 putative European wildcat samples from 13 European countries to assess the effects of landcover, disturbance and legal landscape protection on the European wildcat's genetic integrity across European biomes, through generalised linear models within a Bayesian framework. Overall, we found European wildcats to have genetic integrity levels above the wildcat-hybrid threshold (ca. 83%; threshold = 80%). However, Mediterranean and Temperate Insular biomes (i.e., Scotland) revealed lower levels, with 74% and 46% expected genetic integrity, respectively. We found that different drivers shape the level of genetic introgression across biomes, although forest integrity seems to be a common factor promoting European wildcat genetic integrity. Wildcat genetic integrity remains high, regardless of landscape legal protection, in biomes where populations appear to be healthy and show recent local range expansions. However, in biomes more susceptible to hybridisation, even protected areas show limited effectiveness in mitigating this threat. In the face of the detected patterns, we recommend that species conservation and management plans should be biome- and landscape-context-specific to ensure effective wildcat conservation, especially in the Mediterranean and Temperate Insular biomes.

1. Introduction

Global environmental changes and species' introductions/translocations (including domestic species) act as prominent forces altering habitats, species distributions and reproductive cycles, and ultimately facilitating human-mediated hybridisation (Grabenstein and Taylor, 2018; Todesco et al., 2016). The introduction of alien taxa is particularly important in fostering hybridisation, which is defined as the interbreeding of individuals from genetically distinct populations (Rhymer and Simberloff, 1996). It generates new reproductive opportunities through facilitating contact between taxonomically related species among which interbreeding was previously highly unlikely, or even impossible, due to their allopatric distributions (McFarlane and Pemberton, 2019; Todesco et al., 2016). Despite being acknowledged as an important evolutionary force, hybridisation has been shown to have deleterious effects on multiple wildlife populations and species (Allendorf et al., 2001). Of the six primary types of hybridisation proposed by Allendorf et al. (2001), types 5 (“widespread introgression”) and 6 (“complete admixture”) arise from continued processes of interbreeding and backcrossing events with parental types, leading to hybrid swarms, where populations include individuals that represent a continuum of genetic mixing (Allendorf et al., 2001; McFarlane and Pemberton, 2019). These hybridisation types raise the highest conservation concerns (Allendorf et al., 2001), and occur via secondary contact, i.e., the interbreeding of two genetically distinct species that share the same ancestor, but have undergone allopatric isolation (Lipshutz, 2018). This process can occur due to human-mediated translocations, which often lead to lower fitness of admixed individuals, hence undermining their ability to thrive (Lynch and O'Hely, 2001). Several studies have documented deleterious hybridisation effects on native wildlife fitness because of either intentional or unintentional introductions (Ottenburghs, 2021).

Aside from human-mediated secondary contact, one of the greatest global impacts of human activities on wildlife genetic diversity results from habitat conversion and change (Crispo et al., 2011). Habitat change often leads to landscape fragmentation (e.g., native habitats) or homogenisation (e.g., agroecosystems), resulting in biodiversity loss (Seehausen et al., 2008). Furthermore, fragmentation can lead to the reproductive isolation of wildlife populations (Rhymer and Simberloff, 1996; Storfer et al., 2010), with a consequent reduction in or interruption of gene flow, subsequent loss of genetic diversity (Keyghobadi, 2007), and increased risk of inbreeding depression (Johnson et al., 2010). In addition, population fragmentation can also facilitate contact between previously allopatric species, resulting in interbreeding and introgressive hybridisation (Todesco et al., 2016). In fact, habitat change was found to be the most common mechanism leading to the interbreeding and introgression of previously allopatric taxa (Ottenburghs, 2021). Although perhaps counterintuitive, habitat change may also create environmental conditions where hybrids might have a higher survival probability by, for example, creating niches where they are fitter than non-hybrids, thereby promoting a geographical and demographic expansion of the hybrid population (Todesco et al., 2016).

One of the best examples of hybridisation between wild and domestic counterparts is the well documented case between the European wildcat (*Felis silvestris* Schreber, 1777, hereafter wildcat) and the domestic cat (*Felis catus* Linnaeus, 1758). Domestic cat-wildcat hybrids have been confirmed in most regions of Europe where hybridisation was investigated (Tiesmeyer et al., 2020), supporting the claim that this could be a key factor threatening the fitness and integrity of wildcat populations. The uncertain long-term ecological and evolutionary effects of this interaction on the wildcat, alongside the difficulty in unambiguously identifying wildcats, domestic cats and their hybrids from phenotypic characters, makes the conservation of wildcat populations a priority and an issue of global concern (Daniels et al., 1998; Oliveira et al., 2008). Several studies have targeted wildcats' hybridisation processes (e.g., Oliveira et al., 2008; Say et al., 2012; Tiesmeyer et al., 2020), but most were at regional or national scales, precluding a ‘big-picture’ assessment that encompassed the entire European range of this felid.

The wildcat was once widespread in Europe, but by the early 20th century its populations exhibited a declining trend, with the occurrence of multiple local extinctions due to the loss or fragmentation of suitable habitat, and to anthropogenic persecution (Yamaguchi et al., 2015). Conversely, the domestic cat is widespread across Europe and is omnipresent throughout the wildcat's range, making encounters between both species highly probable and prevalent (Steyer et al., 2018). The wildcat range is distributed over three main European biomes: Mediterranean Forests, Woodlands and Scrubs (herewith Mediterranean); Temperate Broadleaf and Mixed Forests (herewith Temperate Broadleaf); and Temperate Conifer Forests (herewith Temperate Conifer; Olson et al., 2001) (Fig. 1). Biomes are global-scale vegetation units shaped by climate, structural and functional attributes, and can be distinguished by their unique species assemblages and ecosystems (Higgins et al., 2016). These three European biomes exhibit distinct environmental contexts (e.g., precipitation, temperature, and human-activity levels; Metzger et al., 2005) that likely cause different conservation challenges (Martinez-Fernandez et al., 2015), being thus a useful species-scale model approach to identify conservation trends and solutions.

Owing to their often well-preserved ecosystems, Protected Areas (PAs), are frequently considered the backbone for biodiversity conservation (Pringle, 2017), including genetic diversity. These conservation tools have proven to be an effective landscape management option, at least in some regions, such as North America, where approximately half of PAs supported divergent, or even higher, genetic diversity (Thompson

et al., 2021). However, where hybridizing taxa coexist, introgression should be minimized through a balance of endogenous selection and preferential selection of different environmental conditions (i.e. habitats, Moore, 1977, Barton and Hewitt, 1985, Benson et al., 2014). Alongside, populations exhibiting higher vital rates (i.e. abundance, fecundity and survival), usually hosted within PA's, tend to be more resilient to hybridization processes than peripheral ones because assortative mating (Benson et al., 2014). Therefore, quantifying a population's genetic integrity, i.e. the proportion of the genome inherited from a parental group, is expected to act as an indicator of the abilities of protected areas to conserve the genetic heritage of parental taxa. However, the role of PAs in the conservation of the wildcat's genetic integrity is still unclear and may vary according to the local landscape and environmental contexts of each particular region. This lack of information precludes an effective management of PAs when targeting wildcat conservation.

To fill these knowledge gaps, we assessed the wildcat's genetic integrity (GI) across most of its range and tested whether landscape characteristics and levels of legal protection influence the wildcat's GI within each biome. Specifically, our objectives were to (1) identify the main ecological factors influencing wildcat GI variation in each biome, and (2) identify the suitability of Europe's PA network for the conservation of genetically 'pure' wildcat populations. We hypothesised that the wildcat's GI is (H1) related to habitat quality (i.e., ability of the habitat to provide suitable conditions for individuals and populations to persist; Johnson, 2007), (H2) negatively related to levels of anthropogenic disturbance (a surrogate of domestic cat presence) within the range of conditions where wildcats are expected to occur, and (H3) positively associated with the legal landscape-protection level (Table 1).

2. Material and methods

2.1. Data collection

We used the database of Tiesmeyer et al. (2020) (n = 698), complemented with additional genetically-identified samples collected by the authors, as well as samples available at the Research Center in Biodiversity and Genetic Resources (CIBIO-InBIO, University of Porto, Portugal; n = 228) and at the Office Français de la Biodiversité (OFB, France; n = 291) (Fig. 1). The databases were combined and filtered to keep only samples putatively from wildcats, as identified by field collectors based on a suite of available potentially diagnostic parameters (e.g., coat patterns and morphological characteristics; Ragni and Possenti, 1996). Apart from putative phenotypical identification, our database contained the geographical coordinates of each samples' collection site, collection date and genetic integrity values, defined as the proportion of wildcat-typical genetic markers, over all analysed markers. The samples were taken from live-captured wildcats, fresh scat samples, hair traps

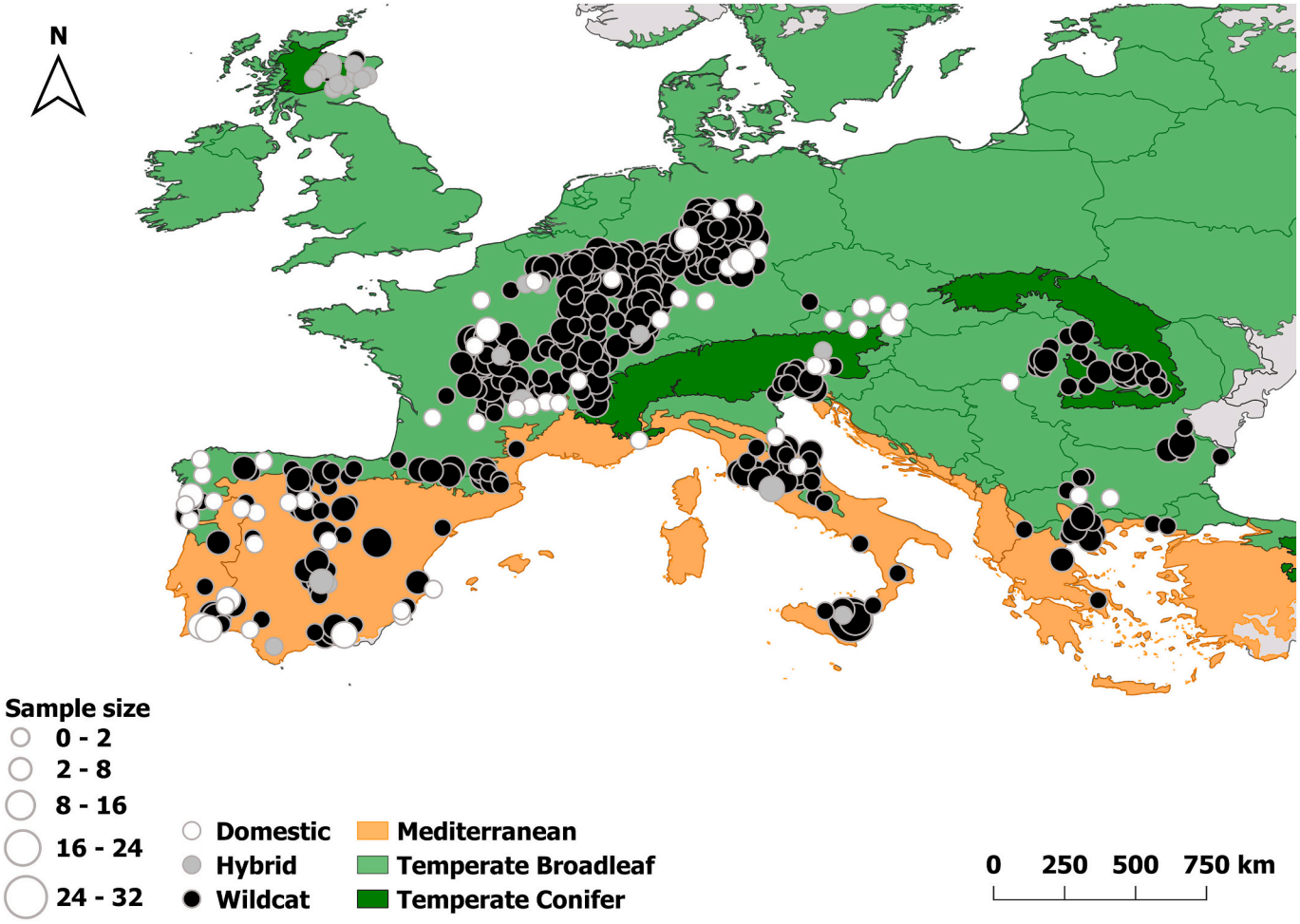

Fig. 1. Spatial distribution of the samples used in the modeling analysis of European wildcat genetic integrity. The genetic integrity thresholds are presented as wildcats (black circles), hybrids (grey circles), and domestic cats (white circles). Symbol size regards to the geographically aggregated number of samples from each genetic group. Distribution of the three main biomes within Europe (following Olson et al., 2001): Mediterranean (orange), Temperate Broadleaf (light green), and Temperate Conifer (dark green). Grey shadings show surrounding terrestrial area not covered by these biomes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Candidate covariates used in the modeling procedure to assess the drivers of variation in wildcat genetic integrity, including units and range, hypothesis reasoning, sources, and references supporting the presented reasoning.

Type	Covariate	Units [min - max]	Hypothesis reasoning	Source	References
Land Cover	Forest cove	[0.00–1.00] %	Genetic integrity increases with forest cover. This habitat is expected to be suitable for European wildcats. Therefore, by potentially encompassing denser wildcat populations, these habitats should allow suitable mate choices within the wild population, reducing hybridisation likelihood.	Global Land Cover https://lcviewer.vito.be/	Oliveira et al., 2018 Steyer et al., 2018
	Distance to Forest	[0.00–1.36] km	Genetic integrity is higher closer to forest areas and nearby forest edges. Forest edges typically host higher prey density, favoring high wildcat population densities, which can optimise suitable mate choices, and contribute to a lower hybridisation probability with domestic cats.	Global Land Cover https://lcviewer.vito.be/	Tiesmeyer et al., 2020
	Forest Landscape Integrity Index	[0.00–10.0]	Genetic integrity is higher in areas with higher forest integrity. Forests with higher landscape integrity are expected to have lower intensity of human activity. Therefore, by potentially encompassing denser wildcat populations, these habitats should allow suitable mate choices within the wild population, reducing hybridisation likelihood.	Forest Landscape Integrity Index https://www.forestintegrity.com/	Grantham et al., 2020
	Shrubland cover	[0.00–0.64] %	Genetic integrity is higher in regions with a higher cover of shrubs. This type of habitat is a key habitat in the Mediterranean region (and to Mediterranean wildcat populations) since it has a higher prey availability, and thus may contain a higher wildcat population density, leading to suitable mate choices.	Global Land Cover https://lcviewer.vito.be/	Monterroso et al., 2009 Oliveira et al., 2018 Lozano et al., 2003
	Cropland cover	[0.00–0.95] %	Genetic integrity is lower in regions dominated by agricultural fields. This type of habitat is often associated with farms and, therefore, domestic cat presence, leading to a higher likelihood of contact between wildcats and domestic cats.	Global Land Cover https://lcviewer.vito.be/	Germain et al., 2008 Lozano, 2010
Disturbance	Distance to Human settlements	[0.00–15.25] km	Genetic integrity is lower in regions closer to human settlements and activities, due to increasing abundance of the domestic cat, leading to a higher likelihood of contact between wildcats and domestic cats.	Global Land Cover https://lcviewer.vito.be/	Ferreira et al., 2011
Protection level	High protected areas Category Ia, Ib[a]	Presence/ Absence	Genetic integrity is higher in highly protected areas. These areas are expected to encompass a suite of characteristics, ranging from higher habitat quality and prey availability, low disturbance effects, absence or low abundance of domestic cats, and effective protection against threats (e.g., poaching or deforestation). The combination of all these characteristics is expected to favour sound wildcat population, with reduced interbreeding opportunities with domestic cats.	Protected Planet https://www.protectedplanet.net/	Reed and Merenlender, 2008 Jones et al., 2018
	Moderate protected areas Category II - VI[b]	Presence/ Absence	Genetic integrity is higher in moderately protected areas. These areas are expected to encompass a suite of characteristics, ranging from higher habitat quality and prey availability, moderate disturbance effects, low domestic cat presence, and effective protection against threats (e.g., poaching or deforestation). The combination of all these characteristics is expected to favour sound wildcat population, with reduced interbreeding opportunities with domestic cats.	Protected Planet https://www.protectedplanet.net/	Wierzbowska et al., 2012 Jones et al., 2018
	Not Protected areas Areas deprived of any type of protection	Presence/ Absence	Genetic integrity is lower in areas deprived of protection. These areas are expected to have unsuitable characteristics, ranging from lower habitat quality and prey availability, high disturbance effects, high domestic cat presence, and lack of effective protection against threats (e.g., poaching or deforestation). The combination of all these characteristics is expected to promote interbreeding opportunities with domestic cats.	-	Beugin et al., 2018

[a] Strict Nature Reserve, Wilderness Area.
[b] National Park, Natural Monument or Feature, Habitat/Species Management Area, Protected Landscape/ Seascape, Protected area with sustainable use of natural resources (Dudley, 2008).

and from carcasses collected opportunistically (Table S1). Samples without geographical coordinates were associated with the smallest region possible of the Nomenclature of Territorial Units for Statistics (NUTS) as an approximation to its geographical position. Samples were discarded whenever it was only possible to assign the NUTS' region to a level higher than level 3 (e.g., general districts). Of all available samples we only retained for analysis those collected between 2000 and 2020. A detailed description of the admixture analyses between wildcats and domestic cats for each database is presented in Table S2.

Despite the different molecular markers used (SNPs and microsatellites; see Table S2), both markers tend to yield equivalent estimates (López-Bao et al., 2020). A detailed description of all hybridisation analysis procedures can be found in Tiesmeyer et al. (2020), Oliveira et al. (2008), and O'Brien et al. (2009).

2.2. Statistical modeling

2.2.1. Response variable

The response variable consisted of the wildcat membership coefficient (q_{ik}), which is the proportion of each individual genome that has ancestry in the wildcat cluster, as calculated from the software STRUCTURE (Pritchard et al., 2000). In essence, the q_{ik} is the proportion of admixture sensu Allendorf et al. (2001), i.e., the proportion of alleles that comes from each of the hybridizing taxa. In our case it refers to the proportion of wildcat-typical markers over all analysed diagnostic markers. This statistic is scaled between 0 and 1, where a value of 0 corresponds to a 'pure' domestic cat sample, i.e., without any contribution of alleles typically detected in wildcats, whereas a value of 1 corresponds to a genetically 'pure' wildcat, i.e., an individual with all genetic markers typically observed in wildcat genotypes. Therefore, the response variable is the estimated genetic integrity conditional on the presence of putative wildcats, and not the probability of wildcat occurrence. We acknowledge that putative wildcats do not occur

throughout the entire species distribution range, but for the sake of this analysis we modeled GI as if they did.

2.2.2. Candidate explanatory covariates

Three types of descriptors were considered - land cover, anthropogenic disturbance and level of protection (Table 1) - as potentially important drivers of wildcat presence (Jerosch et al., 2018; Klar et al., 2008; Oliveira et al., 2018), which can be related to different ecological processes (e.g., dispersion or refuge; see Table 1). These were used as candidate covariates influencing wildcat GI, since the presence of wildcats is a paramount requirement for the occurrence of hybridisation. Furthermore, some of these covariates are also potential drivers of domestic cat presence (Ferreira et al., 2011), the second necessary condition for the occurrence of hybridisation. Another factor often associated with the probability of wildcat occurrence is prey availability (Lozano et al., 2003; Silva et al., 2013). However, we were unable to use prey availability as a candidate covariate, because data were unavailable for all the study areas and periods considered in this study.

Most of our samples were collected between 2000 and 2015 (n = 928, 76%), which is a timeframe over which land cover change has been low at the European scale (e.g., 1.2% between 2000 and 2006; Büttner et al., 2012). Therefore, we used landcover data from the 2015 version of the Global Land Cover raster, with 100 m resolution (Buchhorn et al., 2020) to characterise the landcover of the sites where samples were collected. The used landcover covariates included: i) forest cover, reclassified from all types of forest categories, ii) shrubland cover, iii) cropland cover, iv) distance to the near forest patch, and v) Forest Landscape Integrity Index (FLII) (Table 1). We calculated the proportion of forest, shrubland and cropland within a circular buffer of 5.5 km radius, i.e., 100 km^2 surface, which approximates the scale used in National Atlases for samples with accurate geographical locations (n = 1021). For samples lacking exact geographical coordinates (i.e., assigned to NUTS regions; n = 196), an approximation to habitat availability was estimated as the proportion of each respective land cover class in the NUTS region. The distance to the nearest forest patch was obtained through calculating the Euclidean linear distance from each wildcat biological sample with accurate geographical location to the nearest patch edge. FLII raster (300 m resolution) is a continuous index of forest condition as determined by the degree of anthropogenic modifications (e.g., infrastructure, agriculture, tree-cover loss) and forest connectivity. It ranges 1–10, with 10 being the highest integrity value (Grantham et al., 2020). Mean FLII values were calculated within the 100 km^2 buffer for samples with accurate locations, and within each region's area for samples lacking accurate geographical locations (i.e., linked to NUTs). The anthropogenic disturbance covariate – distance to the nearest human settlement – was assumed to be a surrogate of domestic cat abundance and anthropogenic disturbance (Klar et al., 2008). This covariate was estimated as the Euclidean linear distance from each accurate geographical location to the nearest human settlement retrieved from the Global Land Cover raster (version 2015). All distance covariates were treated as missing values for the samples linked to NUTs due to lack of accurate geographical locations.

With the aim of incorporating the effect of the level of protection in the modeling procedure, we reclassified the IUCN PA designation types, retrieved from the World Database on Protected Areas (WDPA; UNEP-WCMC and IUCN, 2019), into three levels of protection: i) No protection, ii) Moderate protection and iii) High protection (see Table 1 for detailed description). For each sample we determined whether it was collected in a highly protected, moderately protected or unprotected area. For samples without accurate geographical locations, we assigned the level of protection that represented the highest proportion of the NUT region.

All calculations were performed using the software R Studio© version 1.1.463, R version 3.5.3 (R Development Core Team, 2017), using the packages sf (Pebesma, 2018), raster (Hijmans, 2021), rworldxtra (South, 2012) under the tidyverse (Wickham et al., 2019) workflow.

2.2.3. Statistical analysis

The response variable, wildcat GI, was logit-transformed from the proportional to the natural scale, and then standardized to "z-scores" prior to model fitting to facilitate numeric convergence and to avoid dispersion bias (Shiffler, 1988).

Multicollinearity among continuous covariates was assessed through a nonparametric Spearman's correlation analysis using the `psych´ R package (Revelle and Revelle, 2015). Among correlated covariates ($|\rho| \geq 0.7$; Zuur et al., 2009), the covariate less correlated with the dependent variable was excluded from the subsequent modeling procedure. All continuous predictors were scaled to 'z-scores' to avoid data overdispersion bias and to facilitate numeric convergence and coefficient comparisons (MacKenzie et al., 2017; Shiffler, 1988). To further investigate redundancy among factorial and continuous explanatory covariates, we modeled the covariate protection level as a function of landcover covariates with a generalised linear model, both at the European and biome level. As expected, we detected significant differences in landscape composition between the protection-level categories (see Table S3). Consequently, protection-level and landscape-level covariates were precluded from being simultaneously included in the same models. Hence, the effects of these two types of covariates were evaluated separately, allowing us test two of our working hypotheses independently.

For modeling purposes our data were grouped by biomes. However, biological samples linked to the Temperate Conifer biome were split into two groups - i) Temperate conifer (i.e., Alps and Carpathian regions), and ii) Temperate conifer insular (i.e., Scotland; Temperate insular) - representing two distinct realities with different natural history, evolutionary and environmental contexts.

Generalised linear models (GLM) were the backbone of our statistical approach, which were implemented in a Bayesian framework. Because we were interested in understanding i) the expected GI of putative wildcats according to biome, ii) the effect of landscape covariates and of iii) the level of protection in shaping wildcat GI, we employed a two-step sequential modeling procedure. In the first step we performed a 'big-picture' approach whereby wildcat GI was modeled solely on the basis of biome. The second step consisted of developing biome-specific models aimed at assessing landscape characteristics related to wildcat GI. Because landscape characteristics vary significantly according to the level of landscape protection, we fitted two distinct models: i) one containing all habitat- and anthropic-related candidate covariates; and ii) another where wildcat GI was modeled solely based on the level of landscape protection.

The modeling approach was similar regardless of the step. We defined z_i as a logit-transformed proportional random variable, assumed to represent the true state of the GI of putative wildcat i, with $z_i = logit(GI = 1)$ denoting a pure wildcat and $z_i = logit(GI = 0)$ denoting a pure domestic cat, which was modeled as a response of site-specific covariates formulated as logit-scale parameters such that:

$$z_i = \beta 0 + \beta 1 * Cov1_i + \beta 2 * Cov2_i + \ldots + \beta n * Covn_i,$$

where z_i is the GI of individual i on the logit scale, $\beta 0$ is the baseline (intercept) logit-transformed GI and $\beta 1$, $\beta 2$, … βn are the effects of covariates $Cov1$, $Cov2$, … $Covn$ on wildcat GI.

To minimize the spatial-scale bias (i.e., difference between NUT areas and the 100 km^2 buffer areas), we introduced a weighting factor, which consisted of the surface ratio between the geographical unit each sample was associated with (i.e., NUTS polygon or buffer) and the reference buffer area of 100 km^2. Therefore, our models consisted of Bayesian-weighted GLMs, whereby each estimate of the response variable is weighted in inverse proportion to its spatial precision (Monterroso et al., 2020).

Models were fit in JAGS (Plummer, 2003), version 4.3.0., executed from R software (R Development Core Team, 2017) using jagsUI (version 1.5.1; Kellner, 2019). We ran three Markov Chain Monte Carlo

(MCMC) chains with 100,000 iterations after 50,000 iterations burn-in and thinned by 20. Prior distributions for each of our model parameters consisted of a near-flat normal distribution of mean 0 and SD of 1000 on the logit scale. We assessed model convergence from a visual inspection of chain-trace plots and from the Gelman-Rubin statistic (i.e., R-hat), where values <1.1 suggested reliable convergence (Gelman and Rubin, 1992). The relative importance of each covariate was assessed by inspecting the probability posterior distribution of corresponding coefficients. To be conservative, we considered parameters describing effects on GI as informative if the 80% credible interval of the posterior distribution did not include zero (Monterroso et al., 2020). All analyses were performed in R Studio© version 1.1.463, R version 3.5.3 (R Development Core Team, 2017). For categorisation purposes we followed Steyer et al. (2016) in defining as wildcats all individuals with GI $\geq$ 80%, as hybrids all individuals with GI $>$ 20% and $<$ 80%, and as domestic cats all those with GI $\leq$ 20%.

3. Results

3.1. General results

From a total of 1217 putative wildcat samples, 989 were categorised as wildcats. Conversely, 228 (19%) of all putative wildcat samples were categorised as hybrids (n = 72) and as domestic cats (n = 156). Most samples (n = 746) were collected from regions lacking protection level, 456 from moderately protected areas, and 14 from highly protected areas (Table S4).

Sampling covered a wide range of landscape composition conditions, and the analysis of collinearity revealed significant correlation among candidate covariates for each analysed biome (see Table S5 and Table S6).

The mean observed GI of putative wildcats across Europe was 83.3 ± 33.1% (mean ± SD), ranging between 0.1 and 99.9%]. These values varied across biomes, with the Temperate Broadleaf biome showing the highest wildcat GI (86.6 ± 30.4 [0.1–99.9%]), followed by Temperate Conifer (82.9 ± 34.9% [0.2–99.9%]), Mediterranean (76.2 ± 38.1% [0.1–99.9%]) and Temperate Insular (46.0 ± 21.2% [15.2–81.6%]) biomes.

Coherently with the observed data, our range-wide model revealed that putative wildcats from the Temperate Conifer (90.2 ± 9.00 [70.7–99.9%]) and Temperate Broadleaf biome (87.0 ± 1.30 [84.5–89.6%]) are more likely to be wildcats, while those from the Mediterranean (74.4 ± 1.50 [71.4–77.4%]) and Temperate Insular (45.5 ± 10.6 [24.7–66.1%]) are more likely to be hybrids, although Mediterranean biome estimates are closer to the wildcat-hybrid threshold (Fig. 2).

3.2. Biome-specific landscape features influencing European wildcat genetic integrity

Europe-wide patterns included a general positive effect of Forest Landscape Integrity (FLII) on putative wildcat GI (Fig. 3). The strongest, but also less precise, estimated effect of FLII ($\beta = 6.39 \pm 2.50$; CI_{80}: 2.69 / 9.38) was detected in the Temperate Conifer biome, while the weakest was in the Temperate Insular biome (β = 0.06 ± 2.37; CI_{80}: −1.23 / 6.54). Despite a tendency for having a positive effect on the GI of putative wildcats, FLII effect was largely uninformative (i.e., assuming negative and positive coefficients values in the CI80%). The same scenario was observed for the Mediterranean biome ($\beta = 0.15 \pm 0.38$; CI_{80}: −0.35/0.89). The estimated effect of FLII on the wildcat GI in the Temperate Broadleaf biome was positive and informative ($\beta = 0.79 \pm 0.18$; CI_{80}: 0.56/1.18).

All remaining covariates exhibited contrasting effects on wildcat GI across biomes. The wildcat's GI in the Temperate Broadleaf biome appears to be positively influenced by the distance to the nearest forest patch (β = 0.43 ± 0.31; CI_{80}: 0.05/1.09) and distance to human settlements (β = 2.61 ± 0.49; CI_{80}: 2.00 / 3.24), i.e., GI tends to be higher far from forest patches and human settlements (Fig. 3). For this biome shrubland was the only landscape covariate negatively influencing the wildcat's GI ($\beta = -0.83 \pm 0.21$; CI_{80}: −1.15/0.63). The effect of cropland was not possible to determine due to its multicollinearity with other competing covariates (see Table S6).

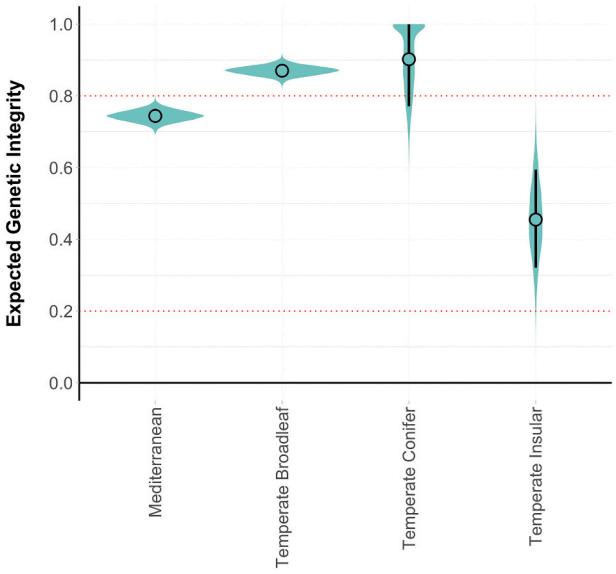

Fig. 2. Expected genetic integrity (estimate ±80% confidence interval) for each biome. Posterior density distribution (green), mean (open circles) and 80% posterior credibility e intervals (black line). Red dotted lines represent the genetic integrity thresholds between wildcats, hybrids, and domestic cats. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the Mediterranean biome wildcat GI was positively influenced by forest ($\beta = 5.67 \pm 0.62$; CI_{80}: 4.80/6.41) and cropland cover ($\beta = 1.00 \pm 0.32$; CI_{80}: 0.61/1.43). Counterintuitively, the GI appears to be negatively influenced by distance to the nearest human settlement ($\beta = -2.66 \pm 0.47$; CI_{80}: −3.26/−2.07). A tendency for a negative effect of shrubland cover was found ($\beta = -0.21 \pm 0.34$; CI_{80}: −0.60/0.24), since ca. 70% of its CI80% interval was negative, supporting a reasonable likelihood for a negative effect (Fig. 3).

In the Temperate Conifer and Temperate Insular biomes, forest, shrubland and cropland cover, and distance to the nearest forest edge were excluded from our models to avoid over-parameterisation due to small sample sizes from these biomes ($n < 20$), thus it was not possible to determine their effect on the wildcat's GI. Among the considered covariates, only landscape forest integrity in the Temperate Conifer biome revealed a well-defined positive effect on the wildcat's GI.

3.3. Biome-specific landscape protection level influencing European wildcat genetic integrity

We found that wildcat GI tends to be high across the Temperate Broadleaf biome regardless of the level of landscape protection (Fig. 4). In this biome putative wildcats are predicted to have high genetic integrity ($GI \geq 83.0$) even in landscapes with moderate or lacking legal environmental protection. However, some degree of hybridisation may also be found in highly protected areas (Fig. 4; CI_{80}: 78.1/99.9).

Landscape protection revealed a positive effect on wildcat GI in the Temperate Conifer biome, at least for moderately protected landscapes, where putative wildcats are expected to have a GI of 97.3 ± 4.9 (CI_{80}: 91.0/99.9). This estimate is much higher than the estimate for unprotected landscapes ($\widehat{GI} = 76.9 \pm 13.4$; CI_{80}: 60.2 / 98.7), despite the high uncertainty associated with the estimate for this level of protection

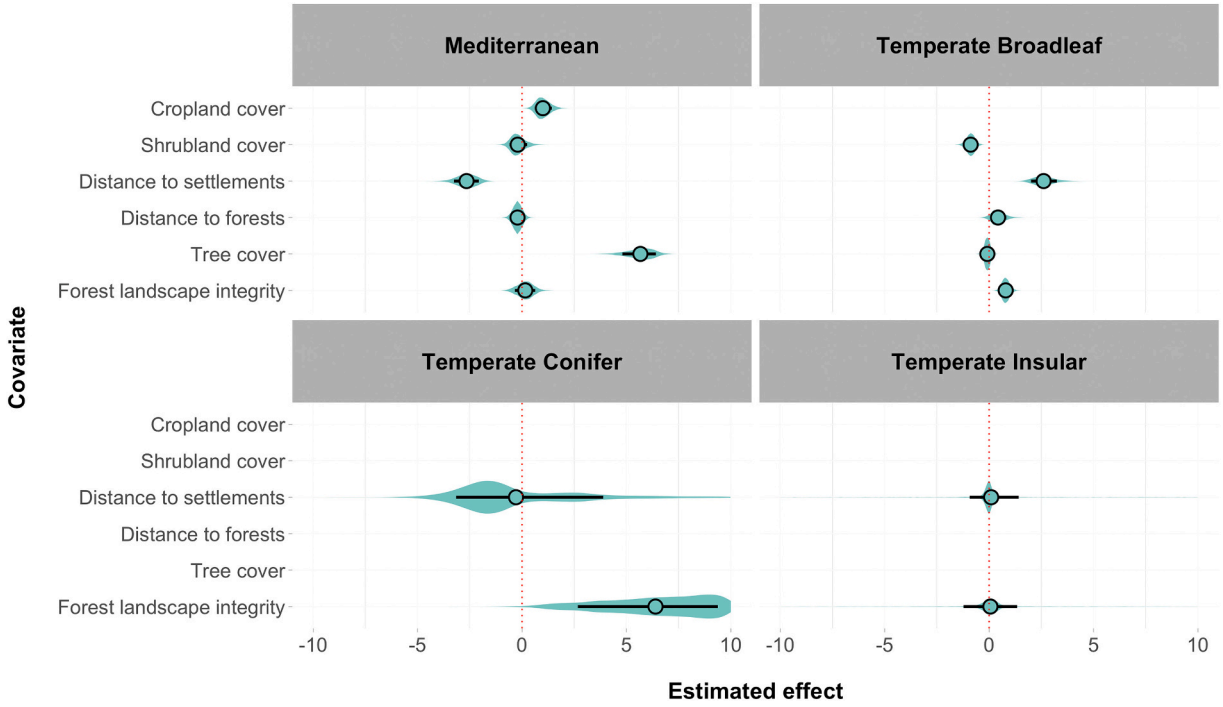

Fig. 3. Posterior distributions of untransformed covariate effects on wildcat genetic integrity for each biome, with respective mean (open circles) and 80% posterior credibility intervals (black line).

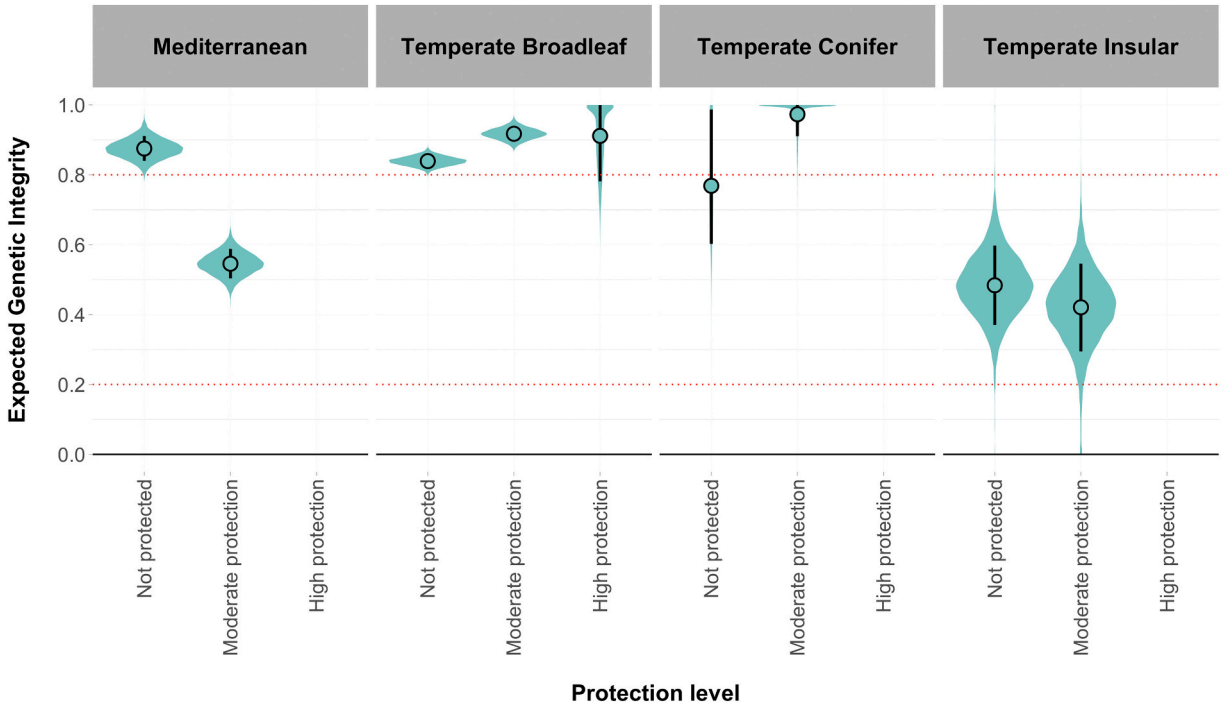

Fig. 4. Biome-specific expected genetic integrity of putative European wildcats according to landscape protection level. Posterior density distribution for each protection level category, with respective mean (open circles) and 80% posterior credibility interval (black line). Red dotted lines represent GI thresholds between wildcats, hybrids, and domestic cats. Protection level categories: Not protected – areas deprived of any type of protection; moderate protection – includes areas with IUCN categories II to VI; high protection – includes areas with IUCN categories Ia and Ib. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4). The absence of samples from highly protected landscapes in this biome precluded us from assessing the effect of this category on wildcat GI.

The Temperate Insular biome revealed the most concerning scenario. Despite the low number of samples from unprotected ($n = 8$) and moderately protected ($n = 7$) areas, both revealed low estimates of GI for putative wildcats ($\widehat{GI} = 42.1 \pm 10.6$, CI_{80}: 29.5 / 54.6; and $\widehat{GI} = 48.4 \pm 9.5$, CI_{80}: 37.0 / 59.8).

We found a contrasting scenario in the Mediterranean biome, where putative wildcats in moderately protected areas are expected to have higher admixture rates $\widehat{GI} = 54.6 \pm 3.3$ (CI_{80}: 50.3 / 58.8), when compared to those found in areas without landscape protection $\widehat{GI} = 87.5 \pm 2.8$ (CI_{80}: 84.0 / 91.1). Having only one sample from a highly protected landscape (Table S4) also precluded us from assessing the effect of this category on wildcat GI in this biome.

4. Discussion

Our results revealed a common range-wide pattern corroborating our initial hypothesis (H1): wildcat genetic integrity tends to be maximised in areas with a higher forest landscape integrity across European biomes. Well-conserved native forests (e.g., low degrees of anthropogenic modification and high forest connectivity) have been previously described as a key preference for wildcat habitats (Oliveira et al., 2018; Gil-Sánchez et al., 2020), providing suitable feeding resources and breeding conditions. Therefore, these landscapes are expected to provide favorable conditions for hosting robust wildcat populations, which enhance opportunities for intraspecific reproduction (Klar et al., 2008; Oliveira et al., 2018) and thus reduce the probability of hybridisation. We also identified biome-specific and landscape-protection drivers adding to this general response, which reflect different ecological and landscape contexts influencing wildcat – domestic cat interactions.

4.1. The Temperate Broadleaf & Mixed Forests biome

The highest values of observed GI for the wildcat were detected in the Temperate Broadleaf biome. Despite the high expected GI values (87.0 ± 1.3) for the mean landscape conditions of this biome, the wildcat's GI is expected to be mostly maximised in landscapes dominated by intact forests (see above), but also distant from human settlements and with low shrubland cover. The positive effect of distance to human settlements on wildcat' GI corroborates our H2. The home ranges of domestic cats tend to occur mostly near human settlements (Ferreira et al., 2011; Pisanu et al., 2020). However, they engage in foraging and exploratory bouts into wild habitats (e.g., Say and Pontier, 2004), reaching distances than can vary from 2.5 km to 6.8 km from these settlements (Germain et al., 2008; Ferreira et al., 2011). These bouts may promote encounters between wildcats and domestic cats, and thus increase hybridisation probability near human settlements (Germain et al., 2008). Our results indicate that putative wildcat samples collected >1.5 km from the nearest human settlement exhibit an expected GI >90%. In contrast, our results suggest that wildcat GI is expected to decrease with increasing shrubland cover. When structured into a mosaic system (e.g., shrublands and traditional farmlands), these landscapes favour abundance and diversity of small mammals (Benedek and Sîrbu, 2018), which are wildcat's main prey in this biome (Lozano et al., 2006). However, extensive shrubland-dominated landscapes tend to have an opposite effect, with usually a limited abundance of the wildcat's main prey (Silva et al., 2013). In such landscapes, reduced prey availability may only support wildcat populations at low population densities, thus allowing a greater opportunity for domestic cats to occupy this habitat, especially if human settlements are present. Therefore, under Hubb's Desperation Hypothesis (Hubbs, 1955), populations at low population density may suffer from restricted mate choice, promoting mating with heterospecifics and consequently leading to hybridisation (Bohling and Waits, 2015).

The distance to the nearest forest edge revealed only a marginal positive effect on the wildcat's GI (Fig. 3). In fact, the expected GI for putative wildcats for the average landscape conditions of this biome is >95%, regardless of the distance to the nearest forest. However, most of our samples (n = 89%) were collected closer than 500 m from forests. Forest patches of varying dimensions tend to be omnipresent throughout rural and semi-natural landscapes of this region of Europe (Palmero-Iniesta et al., 2020), leading to this unintentional sampling bias. Such a limitation precludes us from fully capturing the effect of this covariate on wildcat GI at a more biologically relevant scale. Regardless, the tendency for a slight positive influence of distance to forests may be a statistical artefact, because forest quality could already be partly captured in our models by the forest integrity covariate. We suggest that forest patches alone, irrespective of their intrinsic characteristics (e.g., tree composition and density, growth stage), may only play a small role in shaping the wildcat's genetic integrity (Klar et al., 2008; Oliveira et al., 2018).

Previous studies have revealed contradictory estimates of wildcat-domestic cat hybridisation for this biome, e.g., 8%–10% in Bulgaria (Spassov et al., 1997), 3.5% in Germany (Steyer et al., 2018) or 7–22% in the Franco-Swiss Jura region (Nussberger et al., 2018). This variability suggests that despite the generally encouraging scenario we found for wildcats in the Temperate Broadleaf region of Europe, sub-regional variations in GI may occur, likely due to local-scale demographic histories and distinct threats acting on the integrity of some wildcat populations across this biome (Steyer et al., 2016; Nussberger et al., 2018). Therefore, coordinated and spatially-structured monitoring approaches over the diversity of scenarios found in the Temperate Broadleaf biome are of utmost importance to identify possible differences in the observed pattern, detect tipping points, and properly assess the GI of wildcats within this biome.

4.2. The Mediterranean Forests, Woodlands & Scrub biome

Our results revealed that the GI of putative wildcats in the Mediterranean biome is near the wildcat-hybrid threshold (GI = 74%). Previous regional-level studies have reported admixture estimates of 15% in Italy (Mattucci et al., 2013) and 14% in Iberia (Oliveira et al., 2008). However, both target regions have important parts of their territory in the Temperate Broadleaf Forests biome (e.g., northern Iberia or large stretches of the Italian Apennine Mountain chain), from which an important number of samples originated (Mattucci et al., 2013; Oliveira et al., 2008). While admixture rates and genetic integrity values cannot be directly compared, our results depict a more concerning scenario than previously thought. Furthermore, by stratifying estimates by biome, we were able to investigate patterns occurring through ecologically-coherent spatial units. Therefore, the large sample size collected over a broad biome-stratified geographical range likely provides a more thorough, realistic and coherent description of the Mediterranean biome scenario than formerly assessed.

Our models show that the wildcat's GI in the Mediterranean biomes is enhanced by the amount of cropland and forest cover, and by proximity to human settlements. Mosaic-structured landscapes, where areas with natural dense cover are interspersed with open areas (e.g., scrub-pasturelands), are known to benefit wildcat populations (Lozano et al., 2003; Monterroso et al., 2009) and to maximise the abundance of European rabbits (*Oryctolagus cuniculus*), this felid's staple prey in the Mediterranean region of Iberia (Lozano et al., 2006). In addition, agroecosystems (a common Mediterranean system often also structured in a landscape mosaic) usually support high abundances of rodents (Jareño et al., 2015), which are the main prey for wildcats when European rabbit availability is limited (Lozano et al., 2006), and are the staple prey for this felid in eastern Mediterranean regions (Apostolico et al., 2016; Can et al., 2011). Therefore, such landscapes should provide rewarding reproductive and foraging options by increased prey availability, while also providing adequate breeding conditions (Lozano et al., 2003; Migli et al., 2021), thereby favoring healthy wildcat populations and limiting hybridisation risks.

Counterintuitively, we found that wildcat GI tends to decrease with increasing distance from human settlements. We expected wildcat GI to be lower near to human settlements due to increased contact probability with domestic cats. However, our results refute this reasoning for the Mediterranean biome. We propose several potential factors that could

have acted in isolation or synergistically to drive this observed pattern: i) low population densities and historical reductions of wildcats typically found in this biome (Gil-Sánchez et al., 2020; Matias et al., 2021) likely led to high permeability to domestic cats and consequent widespread introgression even in more remote areas (Oliveira et al., 2018); ii) higher prey abundance at the interface of agricultural fields and natural habitats (Delibes-Mateos et al., 2009; Jareño et al., 2015), exhibiting higher carrying capacity for wildcats than prey-poor remote areas; iii) high levels of landscape anthropogenisation in the Mediterranean region such that few remote areas remain (or were not sufficiently sampled) that are capable of hosting healthy wildcat populations; iv) the common presence of isolated country houses far from human settlements in the remaining remote and unpopulated areas (e.g., Sierra Morena and Toledo Mts., in Spain), where game or livestock keepers inhabit, usually intermittently, and where semi-feral domestic cats are kept for rodent control; and v) classification bias whereby collectors might be more confident that samples, either phenotypic or non-invasive, collected in more remote habitats belong to wildcats, while tending to be more conservative in assessing samples from less likely habitats, such as areas close to human settlements. Such biases could lead to our database including a higher number of hybrid cat samples from areas assumed to be used exclusively by wildcats (i.e., distant from human settlements; Kilshaw et al., 2016). Regardless, the observed scenario across the Mediterranean biome reflects remnants of disturbed demographic histories (Lozano et al., 2007; Gil-Sánchez et al., 2020; Gerngross et al., submitted; Matias et al., 2021), leading to the hybridisation of parts of former wildcat-occupied areas by domestic cats (e.g., Sarmento et al., 2009), and where differences between geographically isolated subregions (e.g., basal levels of genetic integrity) may play a role in the introgression process.

4.3. The Temperate Conifer Forest biome

The highest expected wildcat GI was estimated for this biome (90%), although with high variability in our estimates. These results are consistent with previously reported admixture levels, namely 15% in Slovenia and Croatia (Urzi et al., 2021) and 6% in the eastern Italian Alps (Mattucci et al., 2013). The low number of analysed samples from this biome prevented a detailed analysis of all landscape features related to GI. However, and despite the expected high GI values, our results suggest that the conservation of forest habitats with low anthropogenic disturbance and high forest connectivity (i.e., high forest integrity) is key to safeguarding this felid's GI, as these were the most influential drivers of GI variation (Fig. 3). As found for other continental biomes, the preservation of well-conserved forests provides suitable feeding resources and breeding conditions for this felid (Klar et al., 2008; Gil-Sánchez et al., 2015; Oliveira et al., 2018) and may limit levels of admixture in this biome. In fact, a similar pattern has been described in France where admixture levels were low in a forest-dominated landscape, arguably due to the continuity of natural forested habitats with low anthropogenic disturbance (Beugin et al., 2020).

4.4. The Temperate Conifer Insular biome

The Temperate Insular biome encompassed the most hybridised population – Scotland – with low levels of expected GI (ca. 46%). This result is consistent with previous studies from that region where it is recognised that the wild-living population resembles a “hybrid swarm” – a continuing process of repeated backcrossing and mating between hybrids (McFarlane and Pemberton, 2019; Senn et al., 2019). The critical conservation scenario of the Scottish wildcat population derives from a long history of isolation and considerable change in the land-management practices, including high levels of persecution combined with low forest cover and prey population densities, over the last two centuries (Hobbs, 2009), with the remaining wild population now estimated to be 115–200 individuals (Kilshaw et al., 2015). Such a widespread and continuous hybridisation process likely led to a scenario whereby habitat and other environmental features are no longer relevant in explaining the spatial structure of this felid's GI.

4.5. Effectiveness of European protected areas network

Our results show that regardless of the baseline high levels of genetic integrity, wildcat GI is still positively associated with level of legal landscape protection across temperate continental European biomes, corroborating our initial hypothesis (H3). However, our models expose a more concerning scenario for the Mediterranean and Temperate Insular biomes.

Wildcat GI tends to be high across the Temperate broadleaf biome regardless of the level of landscape protection. This result may be linked to the reported low admixture rate across this biome (e.g., 3.9% in Germany; Steyer et al., 2016), which is in line with our expected high GI (87%). Moreover, recent range expansions of wildcats in this biome (Nussberger et al., 2018; Say et al., 2012), alongside various conservation projects that strive to connect suitable patches of wildcat habitat (i.e., steppingstones and corridors; Klar et al., 2012), could contribute to reducing interactions of wildcat populations with domestic cats, while decreasing anthropogenic disturbance, domestic cat presence, and, consequently, admixture likelihood. Despite the low number of samples, a similar pattern was found for the Temperate conifer biome, suggesting that the wildcat's GI increases with legal landscape protection. Areas without legal protection are typically characterised by widespread human presence and anthropogenic activities. Therefore, higher abundances of domestic cats linked to humans (Reed and Merenlender, 2008), coupled with increased anthropic disturbance (Jones et al., 2018) and lower habitat quality (Geldmann et al., 2014), result in a higher propensity for interbreeding events and for genetic admixture between the wildcats and domestic cats in these areas. Nevertheless, our results revealed that unprotected landscapes could still provide suitable conditions for wildcat populations with high genetic integrity (maximum GI of 99%) in the Temperate conifer biome, which could be related to forest regrowth in areas abandoned by humans (e.g., Italian Alps; Tasser et al., 2007), or to recent forest gain across extensive areas lacking legal protection (e.g., Romanian Carpathians; Kucsicsa and Bălteanu, 2020).

The Temperate Insular biome revealed the most critical scenario in terms of PA-network effectiveness regarding the conservation of wildcat GI. However, this pattern simply reflects the highly concerning scenario for the entire population (inhabiting protected or non-protected areas), which exhibits widespread low genetic integrity. This result is consistent with the pattern reported in a survey targeting the six priority conservation areas for wildcat conservation in Scotland (Littlewood et al., 2014), which revealed that the ratio of unneutered hybrids to wildcats in those areas was 6:1 (Breitenmoser et al., 2019). Howard-McCombe et al. (2021) also suggested that wildcats are exclusively found in the UK captive population, further supporting a critical scenario for the wild-living population in Scotland.

The Mediterranean biome revealed an unexpectedly concerning scenario. We detected higher-than-expected GI values in landscapes deprived of legal protection compared with those in moderately protected areas. Our findings are consistent with a regional-level study carried out in this biome, where Gil-Sánchez et al. (2020) found that key areas for the conservation of healthy wildcat populations (i.e., highest population densities) were located outside protected areas. We hypothesise that land-use restrictions across many Mediterranean protected areas could alter prey availability for wildcats. Traditional agricultural practices in the Mediterranean region promote landscape heterogeneity, with multiple different niches, providing suitable conditions for many of the preferred prey of wildcats, such as European rabbits, rodents or even birds and reptiles, some of which are associated with resources linked to agricultural activities (Rosalino et al., 2009; Leal et al., 2011; Kazes et al., 2020). This heterogeneity allows wildcat populations to better cope with the seasonality of the Mediterranean

environment (Mediterraneity sensu Virgós, 2002), allowing them to fulfill their energetic requirements throughout the year, and be more resilient and reduce the probability of admixture with domestic cats. In contrast, in protected areas, protection can lead to a high density of vegetation, favoring high abundance of wild ungulates, that negatively affect small mammals and European rabbits (Muñoz et al., 2009). Despite being more "natural", such landscapes provide fewer and less diversified resources and may be unsuitable for key prey species. This can lead to less dense wildcat populations (Ferreras et al., 2021) that, together with the presence of domestic cats in PAs (Sarmento et al., 2009), can increase the probability of hybridisation. This reduced effectiveness of PAs to conserve the wildcat's GI in Mediterranean areas, in contrast to the pattern detected for northern biomes, highlights once more the need to regionally adapt the wildcat's conservation action plans to the environmental and socio-cultural contexts of different European landscapes.

European Protected Areas, together with forest landscapes of high integrity, are strongholds for the high genetic integrity of wildcats across most of this felid's range. Nevertheless, while Protected Areas tend to have a low human footprint, they are still subject to a multitude of anthropogenic activities (e.g., hunting, grazing, forestry, tourism; Cretois et al., 2021), which may alter ecological, distributional and demographic processes of wildlife populations, ultimately compromising conservation efforts (Sarmento and Berger, 2017; Grantham et al., 2020). For the specific case of wildcats, such changes could include reducing their fitness and facilitating interactions with domestic cats. Therefore, ensuring the ecological integrity of PAs should be a pivotal goal in a wider wildcat conservation strategy. Furthermore, our results highlight that this strategy may not suffice in Mediterranean regions. Strict landscape conservation actions should be complemented with strategies fostering traditional farmland with High Nature Value (HNV), which plays a central role in preserving wildlife and habitats (Lomba et al., 2020) and appears to be linked to high wildcat genetic integrity within the Mediterranean biome. However, we caution that extensive and uncontrolled presence of domestic cats in semi-natural landscapes in moderately protected area (IUCN's levels II-VI) could act as ecological traps, generating hybridisation opportunities between wildcats and domestic cats.

5. Conclusions and management recommendations

Our findings provide evidence that, for the conservation of the wildcat as for most wide-ranging species, the golden rule should be that one size does not fit all. Diverging patterns across the wildcat's distribution support the need for biome-specific conservation management actions. Wildcats in continental Temperate biomes retain high levels of genetic integrity, even in anthropogenically disturbed landscapes. However, more peripheral regions, such as the Mediterranean and Temperate Insular biomes, display a more concerning scenario. We suggest that the integrity of the wildcat genotype is likely maintained by assortative mating and by ecological affinities among individuals within this parental type, as supported by evidence of spatial segregation and different habitat selection patterns by wildcats and domestic cats, where intermediate habitat conditions are selected by hybrids (Germain et al., 2008; Gil-Sánchez et al., 2015; Kilshaw et al., 2016; Beutel et al., 2017). However, coherent with the hybrid-superiority hypothesis (Moore, 1977), which proposes that hybrids may be more fit than parental phenotypes in specific environments, disruptions in ecosystem structure (e.g. depletion of prey base or habitat change) could lead to a decrease in relative suitability for wildcats, favoring hybrid's or domestic parental type's fitness. While spatial segregation between the wild and domestic counterparts may be strongly maintained by sex-biased behavioral patterns leading females to occupy the most well-preserved and least disturbed sections of the landscape (Beugin et al., 2016; Oliveira et al., 2018), clear and stable spatial overlap is not a prerequisite for hybridization to occur (Germain et al., 2008). Furthermore, domestic cats have repeatedly demonstrated an exceptional ability to colonize areas of previous wildcat domain upon the latter's decline, even within protected areas (e.g. Sarmento et al., 2009; Soto and Palomares, 2014). Therefore, decreases in a wildcat population's vital rates leading to a breakdown of its demographic structure could facilitate interbreeding events, the maintenance of hybrids in the landscape and, ultimately, steer the population towards complete admixture via continued fusion of the hybridizing taxa through introgression (Moore, 1977). A parallel situation regards to the hybridization of grey wolves (*Canis lupus*) and domestic dogs (*Canis familiaris*), which occurs extensively across Europe, and is facilitated by wolf expansion into anthropogenic landscapes where densities of free-ranging dogs are higher, and by locally high levels of anthropogenic wolf mortality that disrupt the social structure and cohesion of wolf packs, leading to higher chances of wolf-dog encounters and to interspecific mating (Salvatori et al., 2020).

Our results reveal distinct predominant stages of wildcat genetic integrity across European biomes: apparently robust source wildcat populations across the Temperate Continental biomes might tip the scale towards the maintenance of their genetic integrity; the Temperate Insular biome (Scotland) appears to be at the end of the wildcat-domestic cat admixture process; while the Mediterranean biome holds an intermediate scenario, where an apparent decline and fragmentation of wildcat populations is leading to a breakdown of ecological barriers shielding them from extensive hybridization with domestic cats. In a broad sense, protected areas aim at securing natural species, habitats, and ecological and evolutionary processes (Dudley, 2008). Coherently, the Natura 2000 Network was designed and implemented to complement the existing PA system, with the goal of protecting vulnerable habitats and species across their natural range in Europe, and of ensuring that they are restored to, or maintained at, a favorable conservation status (Wegefelt, 2008). However, our results suggest that the degradation of Mediterranean wildcat's genetic integrity might potentially be accelerated by management policies of lower-level protected areas that may be too accessible to pet and feral cats. The pervasiveness of humans across Mediterranean landscapes deems domestic cats an omnipresent threat (Sarmento et al., 2009; Ferreira et al., 2011; Gil-Sánchez et al., 2015; Soto and Palomares, 2014; Gil-Sánchez et al., 2020), particularly fostered by a strategy aimed at integrating cultural values and traditional natural resource management systems with nature conservation at lower-level protected landscapes (i.e. IUCN levels II-VI; Dudley, 2008). As for the wolf-dog hybridization case, because the wildcat-domestic cat hybridization phenomenon involves a domestic animal that has profound links to people, its management is particularly challenging from a legal, public opinion and scientific standpoints (Donfrancesco et al., 2019). Additionally, considering the transnational nature of these species' ranges and of the hybridization process, their management requires extensive work and cooperation with local communities, but also coordinated efforts between countries (Salvatori et al., 2020). Wildcats are protected under the annex II of the Bern convention, which states that each signatory country should take appropriate and necessary legislative and administrative measures to ensure the special protection. This, in theory, should mandate an effective management of hybridisation with domestic cats. However, while the conservation threat posed by wolf-dog hybridisation is acknowledged by the Bern Convention Standing Committee (BCSC), the equivalent case for wildcats continues to be ignored, procrastinating directed conservation actions.

Our results support the need to re-erect the ecological walls that minimize interbreeding between wildcats and domestic cats across the Mediterranean biome, and call for the reappraisal of the role of lower-level protected landscapes to conserve European wildcats, at least across the European Mediterranean biome. We argue that recovering wildcat's genetic heritage would require the combination of a long and a short term strategy: i) at the long term, an approach aimed at revitalizing/recovering remnant wildcat populations by securing undisturbed breeding habitats, recovering their prey base (particularly European

rabbits), and by reducing anthropogenic mortality (Bastianelli et al., 2021); and ii) at the short, taking actions directly limiting wildcats from finding in domestic cats reproductive alternatives, e.g. through Trap–Neuter–Return of feral or free-roaming domestic cats, particularly in landscapes where wildcats are expected to occur (Meredith et al., 2018). However, the scarcity of studies quantitatively assessing the effectiveness of alternative management actions to mitigate anthropogenic hybridization between wild and domestic carnivores deems the definition of consensual management approaches particularly challenging (Donfrancesco et al., 2019).

The impacts of domestic carnivore (i.e. cats and dogs) presence in natural landscapes are multifold, and extend beyond the spillover of domestic genes into their wild counterparts (this study, Salvatori et al., 2020), towards disease transmission and predation of wild prey (Hughes and Macdonald, 2013; Széles et al., 2018). Therefore, their role should be carefully considered in protected areas' management plans so that nature conservation goals are more effectively met. While our results support high genetic integrity of wildcats broadly across Temperate continental biomes, continuous monitoring and research programs targeting key wildcat populations is strongly advised to allow early detection of hybridization problems arising, and to contribute for a deeper understanding of the complex ecological and demographic dynamics driving hybridization. European wildcats in the Temperate Insular biome appear to have surpassed a threshold allowing for self-recovery of their genetic distinctiveness (Senn et al., 2019), leaving little choice but to engage in ex situ breeding and reintroduction if this species is to be recovered in Scottish landscapes.

The boundaries between ‘pure’ and ‘hybrid’ individuals are, in essence, artificial and arbitrarily defined based on the relative contributions of each of the parental groups' genetic material (Donfrancesco et al., 2019; Salvatori et al., 2020). However, this deems identification of admixed individuals based on pelage or other external morphologic characteristics particularly challenging, and may promote a cryptic maintenance of domestic genes within the wild counterpart's populations, with potential prejudice to the fitness of their individuals (Lorenzini et al., 2014; Senn et al., 2019). Therefore, we propose that a quantitative (rather than qualitative) monitoring of genetic integrity, i.e. the proportion of the genome inherited from a parental group, could act as a good indicator of the abilities of protected areas to conserve the genetic heritage of parental taxa.

We note that the processes leading to hybridisation and introgression take generations to build up and are highly dependent on the demography of the concerned species. These factors could not be addressed here, but our findings provide a steppingstone for understanding the roles of ecological and landscape protection on the wildcat's genetic integrity. Both landscape structure and protection level patterns highlight that the wildcat faces distinct conservation challenges across the biomes within its range due to specific environmental and anthropic contexts, all of which should be carefully considered when defining wildcat regional management and conservation plans. However, these should share a common underlying strategy that fosters prey abundance, promoting high population densities, and that efficiently enforces legal landscape protection.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Gonçalo Matias: Formal analysis, Investigation, Data curation, Writing – original draft. **Luís Miguel Rosalino:** Conceptualization, Writing – review & editing, Supervision. **Paulo Célio Alves:** Methodology, Writing – review & editing. **Annika Tiesmeyer:** Investigation, Data curation, Writing – review & editing. **Carsten Nowak:** Methodology, Writing – review & editing, Funding acquisition. **Luana Ramos:** Investigation, Data curation. **Katharina Steyer:** Investigation, Writing – review & editing. **Christos Astaras:** Investigation, Writing – review & editing. **Mareike Brix:** Investigation, Writing – review & editing. **Csaba Domokos:** Investigation, Writing – review & editing. **René Janssen:** Investigation, Writing – review & editing. **Andrew C. Kitchener:** Investigation, Writing – review & editing. **Xavier Mestdagh:** Investigation, Writing – review & editing. **Lionel L'Hoste:** Investigation, Writing – review & editing. **Nicolas Titeux:** Investigation, Writing – review & editing. **Despina Migli:** Investigation, Writing – review & editing. **Dionisios Youlatos:** Investigation, Writing – review & editing. **Markus Pfenninger:** Investigation, Writing – review & editing. **Sébastian Devillard:** Investigation, Data curation, Writing – review & editing, Funding acquisition. **Sandrine Ruette:** Investigation, Data curation, Writing – review & editing, Funding acquisition. **Stefano Anile:** Investigation, Writing – review & editing. **Pablo Ferreras:** Investigation, Writing – review & editing, Funding acquisition. **Francisco Díaz-Ruiz:** Investigation, Writing – review & editing. **Pedro Monterroso:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2022.109518.

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