Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Effectiveness of protected areas for wildcat (*Felis silvestris silvestris*) conservation: from general hybridization patterns to local environmental drivers

Gonçalo Ferreira Machado Matias

Mestrado em Biologia da Conservação

Dissertação orientada por:
Prof. Dr. Luís Miguel Rosalino
Dr. Pedro Monterroso

2020

AGRADECIMENTOS

Em primeiro lugar quero agradecer a ambos os meus orientadores, Professor Doutor Luís Miguel Rosalino e Doutor Pedro Monterroso por todo o apoio na construção e desenvolvimento desta tese, bem como pelo apoio no decorrer do trabalho de campo e todo o material necessário para concretizar esta tese.

Um especial agradecimento ao Engenheiro José Luís Rosa, técnico superior do ICNF, por todo o apoio durante o trabalho de campo no Parque Natural de Montesinho.

Agradeço ao ICNF de Bragança, CIBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos) e CE3C (Centro de Ecologia, Evolução e Alterações Ambientais) por todo o apoio na logística para concretizar o trabalho de campo.

Por último agradeço a todos os colaboradores que providenciaram os dados necessários para concretizar o capítulo I desta dissertação.

RESUMO

A perturbação de origem antrópica pode ser uma ameaça significativa para a vida selvagem e, consequentemente, os impactos associados à presença do Homem e das suas atividades nas paisagens naturais, tais como a conversão/destruição do habitat, sobrepastoreio e introdução de espécies exóticas, podem promover reduções significativas nas áreas de distribuição das espécies, ou mesmo levar à sua extinção (Chapin et al. 2000).

Especificamente, a introdução/dispersão de taxa exóticos, intencional ou não, ou a conversão/modificação do habitat natural em agro ou silvo-ecossistemas, têm adicionado desafios à sobrevivência de espécies que já se encontram num estado de conservação débil, ao poderem promover a hibridação entre taxa selvagens e animais domésticos (Rhymer and Simberloff 1996; Allendorf et al. 2001; Todesco et al. 2016). A hibridação pode gerar a perda de diversidade genética e fitness dos indivíduos, bem como aumentar a probabilidade da extinção de espécies e/ou populações (Seehausen et al. 2008; Crispo et al. 2011; Todesco et al. 2016). A introdução de espécies invasoras na área de distribuição das nativas selvagens pode facilitar o contacto entre duas espécies que anteriormente seria improvável ou inexistente, devido à distribuição alopátrica, e consequentemente promover a hibridação. Para além disso, a conversão/modificação do habitat pode facilitar o contacto entre as espécies doméstica e selvagens, ao facilitar a incursão das primeiras em áreas naturais contíguas. Por outro lado, pode levar a um isolamento de populações silvestres (Rhymer and Simberloff 1996; Holderegger and Di Giulio 2010; Storfer et al. 2010), resultando numa redução de fluxo genético.

Um dos exemplos mais documentados de hibridação entre taxa selvagem e doméstico é o caso entre o gato-bravo europeu (*Felis silvestris silvestris* Schreber, 1777) e o gato doméstico (*Felis silvestris catus*). Desde o início do século XX, as populações de gato-bravo na Europa apresentaram uma tendência decrescente, principalmente devido à perda de habitat de qualidade, fragmentação de habitat e perseguição direta (Yamaguchi et al. 2015). Simultaneamente, houve uma expansão e generalização da distribuição do gato doméstico na Europa e, consequentemente, uma maior sobreposição com a distribuição do gato-bravo, aumentando a probabilidade de encontros entre ambas as espécies (Steyer et al. 2018). Atualmente, e com base em dados genéticos, são reconhecidas na Europa cinco principais grupos biogeográficos de gato-bravo: Península Ibérica, Itália, Escócia, Sudeste continental e Noroeste continental (Mattucci et al. 2016). Globalmente, o gato-bravo está classificado como ‘Pouco Preocupante’ segundo a lista vermelha da IUCN (Yamaguchi et al. 2015). No entanto, na Europa, existem variações regionais/nacionais nas tendências populacionais e nos graus de fragmentação das populações que, associadas a uma redução da área de distribuição da espécie, contribuíram para que, em vários países, o estatuto de ameaça seja mais elevado (ex. Vulnerável e Quase ameaçado, em Portugal e Espanha, respetivamente; Cabral et al. 2005; López-Martín et al. 2007), e que este felino tenha sido incluído nas listagem de espécies mencionada na CITES (Convenção sobre o Comércio Internacional das Espécies da Fauna e da Flora Silvestres Ameaçadas de Extinção), Diretiva das Habitats e Convenção de Berna (Nowell and Jackson 1996). Apesar disso, devido aos inúmeros esforços de conservação focados no gato-bravo, algumas populações deste felídeo apresentam uma tendência populacional positiva (Steyer et al. 2016; Nussberger et al. 2018).

Na maioria das regiões da Europa, e nos grupos biogeográficos, a hibridação entre o gato-bravo e o doméstico já foi detetada (Beaumont et al. 2001; Kitchener et al. 2005; Pierpaoli et al. 2003; Lecis et al. 2006; Oliveira et al. 2008a, b; Hertwig et al. 2009; Nussberger et al. 2014; Steyer et al. 2018), realçando

que este processo pode ser mais uma ameaça significativa para conservação do gato-bravo em toda a sua distribuição europeia. Vários fatores podem promover especificamente o contacto entre estas duas espécies de felinos, nomeadamente: o declínio de populações de gato-bravo depauperadas e que, devido a isso, a escolha do parceiro sexual é dificultada, levando a uma procura nas áreas de distribuição do gato doméstico; a expansão geográfica das populações de gato-bravo com uma tendência populacional positiva e que, por isso, passam a utilizar áreas onde o gato doméstico ocorre; e a deterioração da qualidade do habitat, com a expansão das paisagens alteradas pelo Homem, que facilita as incursões do gato doméstico nos redutos naturais habitados pelo congénere silvestre. Apesar destes processos serem frequentemente apontados como causas da hibridação entre estes dois felinos, ainda é pouco claro quais os reais fatores e mecanismos que podem contribuir para promover o contacto entre as duas espécies na Europa (Beugin et al. 2018; Oliveira et al. 2018; Steyer et al. 2018).

Para além do grau de hibridação, que nos dá uma medida da integridade genética de uma população, e por isso, do seu fitness e valor conservacionista, a abundância/densidade é uma métrica que reflete a integridade populacional da espécie numa região, e permite aferir tendências populacionais (Wright and Hubbell 1983) e viabilidade e risco de extinção das espécies (Purvis et al. 2000). A estimativa das abundâncias/densidades é, assim, um dos parâmetros fundamentais para suportar cientificamente políticas de conservação de sucesso, em particular para espécies ameaçadas (Stephens et al. 2015). Diversas abordagens metodológicas têm sido empregues para gerar estas estimativas, mas, recentemente, o uso de armadilhagem fotográfica, que permite o estudo de espécies elusivas de carnívoros (Karanth et al. 2006), possibilitou o surgimento de modelos estatísticos, baseados em dados de deteção/ocorrência com identificação individual, que geram estimativas de densidades populacionais: modelos espaciais de captura-recaptura (ECR) (Efford 2004; Borchers and Efford 2008; Royle and Young 2008; Efford et al. 2009; Royle et al. 2014). Apesar das estimativas de abundância/densidades serem cruciais para uma gestão eficaz de espécies silvestres, a verdade é que, para muitos grupos biogeográficos deste mesocarnívoro ameaçado, estes dados não estão muitas vezes disponíveis para serem usados pelos gestores de vida silvestre e do território. Um bom exemplo deste padrão é a Península Ibérica, onde as populações de gato-bravo têm vindo, globalmente, a apresentar uma tendência negativa, e estimativas de densidades populacionais baixas (Cabral et al. 2005; Lozano et al. 2007; Sobrino et al. 2009). Este panorama pouco animador, tem sido associado à perseguição humana, fragmentação/conversão do habitat e a diminuição da abundância da sua principal presa na região Mediterrânea, o coelho-bravo (*Oryctolagus cuniculus*; Gil-Sánchez et al. 1999; Lozano et al. 2003). Apesar de existirem vários trabalhos em Portugal focados no estudo da ecologia do gato-bravo (Sarmento 1996; Oliveira et al. 2008a, b; Monterroso et al. 2009; Oliveira et al. 2018), ainda existe uma falta de conhecimento sobre as tendências populacionais e densidades das populações portuguesas.

Tendo em conta este panorama geral, e por forma a preencher estas lacunas de informação, o presente estudo visa: 1) aferir quais os fatores que estão, a nível Europeu e metapopulacional, a promover a hibridação entre gato doméstico e gato-bravo e avaliar a eficácia das áreas protegidas como ferramenta para assegurar a integridade genética do gato-bravo e; 2) estimar, pela primeira vez, a densidade de uma população portuguesa de gato bravo, que habita uma das áreas protegidas do país (Parque Natural de Montesinho), e determinar os fatores ambientais que a condicionam.

No capítulo I analisámos 964 amostras biológicas, morfologicamente identificadas como gato-bravo, recolhidas em 13 países Europeus, com o objetivo de determinar quais os fatores que influenciam a integridade genética de gato-bravo e a eficácia da rede Europeia de áreas protegidas, globalmente e por grupo biogeográfico. No geral, a integridade genética é elevada. Contudo as metapopulações Ibérica e

Escocesa apresentaram valores mais baixos. A qualidade do habitat (maior proporção de floresta e matos, e menor proporção de regiões dominadas por zonas humanizadas) parece estar associada a valores elevados de integridade genética, apresentando diferentes padrões nas metapopulações. Relativamente á eficácia das áreas protegidas (APs), a rede Europeia aparenta ser eficaz na conservação da integridade genética deste felino, enquanto que as APs da Península Ibérica e as localizadas na região onde ocorre a metapopulação Noroeste de gato-bravo aparentam ser menos eficazes. Estes resultados contribuem para diagnosticar, de uma forma mais abrangente, a nível Europeu, o problema da hibridação entre o gato-bravo e doméstico, e os padrões detetados contribuem para a definição de diretrizes de gestão mais sólidas que permitam garantir a conservação do gato-bravo em toda a sua distribuição Europeia.

No capítulo II usámos modelos espaciais de captura-recaptura (ECR) para determinar a densidade populacional de gato-bravo dentro de uma área protegida – Parque Natural de Montesinho – em Portugal. Identificámos 9 indivíduos com um esforço de amostragem de 3477 noites. A densidade de gato-bravo estimada foi de 0,119±0,065 gato-bravo/Km^2. Os valores estimados de densidade aumentam quando as zonas humanizadas estão mais afastadas, ou seja, quando existe menor perturbação humana e menor probabilidade de presença de gato doméstico. Os nossos resultados indicam também que o Parque Natural de Montesinho parece ter condições ambientais adequadas para a espécie, uma vez que ela ocorre em densidades semelhantes às detetadas em outras áreas protegida da Ibéria e o gato doméstico parece estar ausente da área de distribuição do gato-bravo, no P.N. Montesinho. Por estas razões, esta área protegida pode ser uma ferramenta importante na estratégia de conservação deste felino

Este estudo providencia dados importantes para a conservação desta espécie no contexto europeu, providenciando informação cientificamente válida sobre a integridade genética e populacional do gato-bravo, crucial para que gestores das áreas protegidas e decisores políticos (regionais e nacionais) possam ter informação de base para criar regulamentos, planos de gestão e políticas adequadas a proteger os recursos chave para o gato-bravo, quer a nível da Europa quer a nível de metapopulações específicas. Ambos os capítulos evidenciam o papel crucial das APs para a conservação desta espécie ameaçada, apesar dos diferentes processos ecológicos analisados (integridade genética e densidade populacional).

Palavras-chave: Gato-bravo Europeu, Integridade genética, Gestão de áreas protegidas, Densidade populacional, Conservação

ABSTRACT

The human associated disturbance can be a significant threat to wildlife and, consequently, impacts linked to human's presence/activities, such as habitat conversion/destruction, overgrazing, and introduction of invasive species can have deleterious effects on species distributions and even promote species extinction (Chapin et al. 2000).

Specifically, intentional or unintentional human-mediated range expansion of alien taxa and natural habitat conversion/change to agroforestry, have added challenges to the survival of depleted species, promoting hybridization between wild and domestic animals (Rhymer and Simberloff, 1996; Allendorf et al. 2001; Todesco et al. 2016). Hybridization may result in loss of genetic diversity, decrease of individual fitness, and increase the probability of species and/or populations extinctions (Seehausen et al. 2008; Crispo et al. 2011; Todesco et al. 2016). The introduction of invasive species in the distribution area of the native wild taxa can facilitate the contact between two species used to be unlikely or impossible, due to allopatric distribution and, consequently, promote hybridization. Moreover, the habitat conversion/change can lead to isolation of wild populations (Rhymer and Simberloff 1996; Holderegger and Di Giulio 2010; Storfer et al. 2010) with a reduction of gene flow.

One of the most documented examples of hybridization between wild and domestic taxa is the case between European wildcat (*Felis silvestris silvestris* Schreber, 1777) and the domestic cat (*Felis silvestris catus*). Since the early 20th century, the wildcat's population in Europe presented a declined trend, mainly due to suitable habitat loss, anthropogenic persecution, and habitat fragmentation (Yamaguchi et al. 2015). Simultaneously, the domestic cat become more widespread throughout Europe, consequently overlapping with wildcat's range, increasing the likelihood of encounters between the two species (Steyer et al. 2018). Presently, there are five main wildcat biogeographic groups, defined on the basis on genetic approaches (Iberian, Scottish, Italian, Southeast continental, and Northwest continental; Mattucci et al. 2016). Globally, wildcats are classified at 'Least Concern' by the IUCN Red List (Yamaguchi et al. 2015). However, in Europe, the declining populations' trend and fragmentation, and the lower distribution area of wildcat's population contribute to its classification as an endangered species in several countries (e.g. Vulnerable and Near Threatened in Portugal and Spain, respectively; Cabral et al. 2005; López-Mártin et al. 2007), and inclusion on CITES, EU Habitat and Species Directive and Bern Convention (Nowell and Jackson 1996). Despite that, mainly due to conservation efforts, there are some wildcat populations with a positive trend (Steyer et al. 2016; Nussberger et al. 2018).

In the most regions of Europe, the hybridization between these two species was detected (Beaumont et al. 2001; Kitchener et al. 2005; Pierpaoli et al. 2003; Lecis et al. 2006; Oliveira et al. 2008a, b; Hertwig et al. 2009; Nussberger et al. 2014; Steyer et al. 2018), highlighting that this could be a significant threat to wildcat conservation across his entire range. Numerous factors can promote this contact between wildcat and his domestic counterpart, such as: depleted wildcat populations with a declined trend, maybe be constrained in finding mates, thus increasing the search for a mating partner within the domestic cat distribution; wildcat range expansion, in populations with a positive trend that utilize areas where domestic cats are presence; and the deterioration of the habitat quality, increasing the likelihood of domestic cat presence throughout wildcat's habitat. Despite these processes may contribute to hybridization between these two species, it is unclear what are the real drivers and processes that promote the contact between the European wildcat and its domestic counterpart in Europe (Beugin et al. 2018; Oliveira et al. 2018; Steyer et al. 2018).

In addition to the degree of hybridization, which gives us a measure of population's genetic integrity, and therefore, its fitness and conservation value, the abundance/density is a measure that reflect the population integrity and permits to determine population's trend (Wright and Hubbell 1983) and the viability and extinction risk (Purvis et al. 2000). The abundance/density estimate is, therefore, one of the fundamental parameters to create successful conservation policies for threatened species (Stephens et al. 2015). Recently, the use of camera-trap permitted to study elusive carnivores (Karanth et al. 2006), allowed the development of statistical models based on detection/occurrence data from individual's identification, creating population's density estimate: spatial capture-recapture models (SCR) (Efford 2004; Borchers and Efford 2008; Royle and Young 2008; Efford et al. 2009; Royle et al. 2014). Despite the crucial role of these parameters for an effective management of wild taxa, there is a lack of estimates for some of wildcat's biogeographic populations. One of the best examples is the Iberian wildcat's population, which presents a decline trend and low densities (Cabral et al. 2005; Lozano et al. 2007; Sobrino et al. 2009). This negative scenario is mainly due to human persecution, habitat fragmentation/change and loss of its main prey in Mediterranean region, the European rabbit (*Oryctolagus cuniculus*; Gil-Sánchez et al. 1999; Lozano et al. 2003). Despite several studies in Portugal targeted the wildcat (Sarmento 1996; Oliveira et al. 2008a, b; Monterroso et al. 2009; Oliveira et al. 2018), studies comprehending populations densities and trends are still missing.

In order to fill these gaps, the present study aims to: 1) determine the drivers that promote hybridization between domestic cat and European wildcat, in Europe and in each metapopulations, asses the effectiveness of protected areas as a toll to ensure the wildcat's genetic integrity safeguarding; 2) estimate, for the first time, the density for a Portuguese wildcat's population, which inhabits a protected area (Montesinho Natural Park), and determine the environmental drivers that shaping this population parameter.

In Chapter I we analyzed 964 putative morphological wildcats' samples from 13 European countries aiming to determine what factors influence the wildcat genetic integrity and understand the effectiveness of European protected area's network for wildcat conservation, at European and biogeographic scale. Overall, wildcat's genetic integrity presents high values; however Iberian and Scottish metapopulation appears to have the lower values. Habitat quality (high proportion of forest and shrubland, and low proportion of regions dominated by human areas) may be linked to wildcat's genetic integrity, with difference patterns across metapopulations. Regarding to protected areas (PAs) effectiveness, European PAs seem suitable overall to conserve wildcat's genetic integrity, while Iberian and Northwest PAs suggest less suitable effectiveness. These results contribute to a wide diagnostic of the hybridization threat between the two species; therefore, the detected patterns provide solids guidelines to wildcat conservation, across its entire range

In Chapter II we used spatial capture-recapture (SCR) models to evaluate wildcat's density inside a protected area – Montesinho Natural Park – in Portugal. We were able to identify 9 individuals with a total effort of 3477 trap-nights. The wildcat's density was to 0,119±0,065 wildcat/Km2, increasing when humanized areas were further away, and consequently, in areas with lower human-disturbance and domestic cat presence. Our results indicate that Montesinho Natural Park may be suitable to assure wildcat's conservation, since this felid occurs in similar densities as those detected in other PAs in Iberian Peninsula and the domestic cat appears to be absent from wildcat's distribution inside the park. For these reasons, these PA could be an important tool in the wildcat's conservation strategy.

This study provides relevant information for wildcat's conservation in the entire range, adding scientifically valid information on the genetic and population integrity of European wildcat to the current body of knowledge. This data is crucial for PAs managers and policy makers (at regional and national scales) to create appropriate regulations, management guidelines and policies to protected key resources for this threatened species, both at European and metapopulation's level. Both chapters highlighted the pivotal role of protected areas to wildcat conservation, despite the different ecological process considered (genetic integrity and population density).

Keywords: European wildcat, Genetic integrity, Protected areas management, Population density, Conservation

CONTEXT

LIST OF FIGURES AND TABLES

FIGURES

TABLES

GENERAL INTRODUCTION

Hybridization has continuously and historically occurred in wildlife, often being a natural process (Dowling and Secor 1997). However, human mediated range expansions of alien taxa and habitat conversions that facilitate the contact of previously allopatric species, can further promote this process in the wild (Anderson and Stebbins 1954), adding an extra conservation menace to species that are already in the verge of extinction, by losing their genetic integrity. Hybridization occurs between several species of carnivores throughout the world (Williams et al. 2002; Trigo et al. 2008; Cabria et al. 2011), and one of the most notorious examples of human-mediated hybridization involves the European wildcat (*Felis silvestris silvestris*) and the domestic cat (*Felis silvestris catus*), a process that is often highlighted as one of the major threats to wildcat's survival.

The European wildcat distribution ranges from Iberian Peninsula and Scotland to South and Eastern Europe, although across several fragmentated populations (Yamaguchi et al. 2015). At a global scale, the wildcat is classified at 'Least Concern' (LC) by IUCN Red List (Yamaguchi et al. 2015). Despite that classification, the wildcats' population in Europe has a decreasing trend, mainly due to fragmentation, loss of suitable habitat and direct human persecution (Yamaguchi et al. 2015). Despite the LC's IUCN classification, this species is considered threatened in some European countries (e.g. 'Critically Endangered', 'Vulnerable', and 'Near Threatened', in Scotland, Portugal and Spain, respectively; Lozano and Malo 2012). However, in some European regions, the wildcat is currently showing natural local recolonization processes and an increasing populations trend (Steyer et al. 2016; Nussberger et al. 2018).

Domestic cats originally derived from the African wildcat (*Felis lybica*) and evolved in the Near East/North Africa. Its near global distribution resulted from a human-mediated dispersal process (Driscoll et al. 2007; Ottoni et al. 2017), and this species is now sympatric with the wild conspecific throughout Europe. This geographical pattern enhances the tangible risk of encounters between the two *Felis* species, which seems to increase the likelihood of hybridization (Steyer et al. 2018). Several studies detected admixture between the European wildcat and its domestic counterpart in most regions of Europe (Randi et al. 2001; Beaumont et al. 2001; Driscoll et al. 2007; Pierpaoli et al. 2003; Lecis et al. 2006; Oliveira et al. 2008a, b; O'Brien et al. 2009; Hertwig et al. 2009; Nussberger et al. 2014, 2018; Steyer et al. 2018), with the lowest proportion of hybrids in Western and Southeast Europe and the higher in Scotland (Tiesmeyer et al. 2020). Although several studies evaluated the presence and admixture rate between these two subspecies, it is still unclear what drivers promote the hybridization across Europe (Beugin et al. 2018; Steyer et al. 2018). Such information is pivotal for any Species Conservation Plan, and therefore it should be a priority to define the areas where wildcat's genetic integrity is higher, as well as to identify the drivers that might be contributing to decrease this species genetic uniqueness. Furthermore, in many European regions, Protected Areas are important conservation tools used to guarantee the preservation of natural heritages. They may be used to provide the necessary resources and protection from anthropic influence to threatened species, such as the European wildcat, ensuring their protection and long-term survival (Reed and Merenlender 2008; Le Roux et al. 2015).

In addition to the degree of hybridization, which gives us a measure of the genetic integrity of a population, and, therefore, of its fitness and conservation value, abundance/density is a metric that reflects the population's structure, and is the baseline to assess populations trends (Wright and Hubbell 1983), viability and extinction risk (Purvis et al. 2000). Furthermore, a fundamental requirement to determine a species threat status, and to understand and evaluate the efficacy of management efforts, is to accurately assess its populations' densities (Wright and Hubbell 1983; Stephens et al. 2015). Since protected areas often support higher habitat quality areas, with lower human disturbance, it is presumable that they may harbor larger and healthy wildcat populations (Jones et al. 2018). In Portugal, the European wildcat is listed as "Vulnerable" in the Portuguese Red list (Cabral et al. 2005), mainly due to population decline, reduction on range and occupancy area, and hybridization with its domestic counterpart (Cabral et al. 2005). These threats have led to a suspected decline of 30% of the wildcat population in the last 24 years, further highlighting the importance of assessing population trends, and understanding the drivers shaping it, through appropriate monitoring programs. Despite the existence of several studies targeting the European wildcat in Portugal (Sarmento 1996; Sarmento et al. 2006; Oliveira et al. 2008a, b; Monterroso et al. 2009; Duarte et al. 2012), most do not provide any reliable estimate of this species population density or abundance. Therefore, a severe gap remains regarding the estimation of population density of this small felid in the country, which will deter the establishment of an adequate and effective management and conservation plan focused on this threatened cat.

To fill these information gaps, we implemented the present study that aimed to: i) assess the wildcat genetic integrity patterns throughout Europe, ii) determine the drivers of genetic integrity patterns, iii) investigate how density varies in a Portuguese Protected area, and iv) identify which factors may be determining the assessed density gradient. Therefore, we tested the following hypothesis: H1 - the European wildcat's genetic integrity is related to habitat quality and landscape's legal protection level; H2 - the European wildcat's density at Montesinho Natural Park will be consistent with the estimates for Iberian Peninsula and shaped by the presence of humans and of forests.

Chapter I

Wildcat genetic integrity throughout Europe: The role of Protected Areas and habitat quality

Matias, G.[1], **Rosalino, LM.**[1], **Alves, PC.**[2,3,4], **Monterroso, P.**[2]

[1] cE3c - Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal
[2] CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quinta 7, Vairão 3385-661 Portugal
[3] Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, 4099-002 Porto, Portugal
[4] Wildlife Biology Program, Department of Ecosystem and Conservation Sciences, University of Montana, Missoula, 59812 Montana, USA

Abstract

Hybridization between domestic animals and wild taxa can pose severe threats to wildlife conservation. This human-induced hybridization is often linked to the introduction of alien species and habitat conversion, which may promote reproductive opportunities between species for which interbreeding is naturally highly unlikely. The hybridization between the European wildcat (*Felis silvestris silvestris)* and the domestic cat (*Felis silvestris catus*) is one of the best examples of that process. We analyzed 964 putative morphological European wildcats' samples, from 13 Europeans countries (and five metapopulations), using a set of 96 ancestry-informative SNP's to assess their genetic integrity, and determine the protected areas' (PAs) role in protecting that integrity. We identified 147 domestic cats, 59 hybrids and 758 pure wildcat's trough Europe. Overall, European wildcats have high levels of genetic integrity (*ca.* 87%), while Iberian and Scottish metapopulation revealed lower observed levels of 63% and 49%, respectively. We found that habitat quality (e.g. forest cover) is linked to European wildcat genetic integrity, at a broad scale, although different patterns and drivers shape the genetic integrity found across the five metapopulations. At the European scale, PAs network seemed suitable to conserve wildcats' genetic integrity, as revealed by higher integrity values found from putative wildcats across all protection levels. However, we found a consistent and concomitant increase of genetic integrity and protection level in Iberian PAs', supporting that Iberian wildcat's genetic integrity, and therefore, its conservation, is strongly linked to landscape protection. Regionally low values of wildcat's genetic integrity, associated to a lower effectiveness of protection areas to assure the preservation of healthier wildcat's populations, presents a potential risk for long-term survival of this felid. In face of the detected patterns, we recommend that species conservation and management plans should be metapopulation and landscape context specific to ensure the wildcat conservation, especially in the regions with depauperated wildcat populations.

Keywords: European wildcat, Genetic integrity, Metapopulation, Protected areas management, Conservation

1.Introduction

Hybridization is a natural process, defined as the interbreeding between two genetically different species (Abbott 1992), and has been detected in several wild animal and plant species (Dowling and Secor 1997); (Mallet 2008). Hybridization has long been associated to anthropogenic actions (Anderson and Stebbins 1954) and entails several evolutionary implications, such as the loss of genetic diversity, decrease of individual fitness, and ultimately acts as a driving force steering species and/or populations towards extinction (Seehausen et al. 2008; Crispo et al. 2011; Todesco et al. 2016). Hybridization is often promoted by intentional and unintentional human-mediated range expansions of alien taxa and habitat changes (Rhymer and Simberloff 1996; Allendorf et al. 2001; Todesco et al. 2016). Amongst these, the intentional or unintentional introduction of alien taxa is particularly prolific in fostering hybridization as it can generate reproductive opportunities through facilitating contact between two taxonomically close species, among which interbreeding used to be highly unlikely, or even impossible, due to allopatric distributions. Geographic isolation is, therefore, the major factor limiting hybridization among allopatric species, assuring species' genetic integrity (GI) (Mooney and Cleland 2001; Vellend et al. 2007; Todesco et al. 2016). Hybridization via secondary contact is defined as the reproduction between two genetically distinct species that share the same ancestor and that have undergone allopatric isolation (Lipshutz 2018). This type of hybridization can occur due to human-mediated translocations, and has several evolutionary impacts in native species, especially by leading to lower fitness of admixed individuals (Lynch and O'Hely 2001). Several studies have documented deleterious hybridization effects on native wildlife as a result of human-associated introductions. For example, the Sika deer (*Cervus nippon*) was introduced from Japan into Scotland in the 80's. This species hybridized with the native Red deer (*Cervus elaphus*), leading to introgression in the native population, which induces a high risk for the genetic diversity weakening of the native species (Senn and Pemberton 2009).

Wildlife fitness and survival is strongly impacted not only by human-mediated introduction of alien species. One of the greatest impacts of human activities on wildlife genetic diversity results from habitat conversion and change (Crispo et al. 2011). Habitat conversion/change processes often lead to landscape fragmentation or homogenization, resulting in biodiversity loss (Seehausen et al. 2008). Furthermore, fragmentation can lead to the isolation of wild populations (Rhymer and Simberloff 1996; Holderegger and Di Giulio 2010; Storfer et al. 2010), with a consequent reduction or interruption of gene flow, and a subsequent loss of genetic diversity (Keyghobadi 2007). Alongside, fragmentation processes can also facilitate the contact of previously allopatric species, whose distribution change may facilitate interbreeding and hybridization (Todesco et al. 2016). For example two savanna guenon allotaxa, *Cercopithecus aethiops pygerythrus* and *C. aethiops tantalus*, started hybridizing in Uganda when the natural habitat was converted into agricultural land and native vegetation was reduced to fragmented patches (Chapman and Chapman 1996). After these landscape changes, previously segregated species started to share the same native patches. Although counterintuitively, habitat alteration may also create conditions where hybrids have a higher survival probability by, for example, creating niches where they are fitter than non-hybrids, promoting a geographic and demographic expansion of the hybrid population (Todesco et al. 2016).

One of the best examples of hybridization between wild and domestic carnivore counterparts is the well documented case between European wildcat (*Felis silvestris silvestris* Schreber, 1777) and the domestic cat (*Felis silvestris catus*). The wildcat was once widespread in Europe, but by the early 20th century, the European wildcat population exhibited a declining trend, with the occurrence of local extinctions across its range due to the loss of suitable habitat, fragmentation, and anthropogenic persecution (Yamaguchi et al. 2015). At the same time, the domestic cat began to spread all Europe being omnipresent throughout the wild counterpart's range, making encounters between both sub-species highly probable and widespread (Steyer et al. 2018). Currently, the European wildcat range is subdivided in five main biogeographic groups (hereafter named "metapopulations") - the Iberian, Scottish, Italian, Southeast Continental and Northwest Continental (see Mattucci et al. 2016) - based on their genetic similarity. Although, these groups may accommodate ecological and/or behavioral differences, these aspects are still yet to be investigated.

Although wildcat populations across Europe have declined (Nowell and Jackson 1996; Driscoll et al. 2007; Yamaguchi et al. 2015), there are recent evidences suggesting an increasing trend of some metapopulations (e.g. Northwest and Southeast Continental) (Steyer et al. 2016; Nussberger et al. 2018). European wildcats are globally classified as "Least Concern" in the IUCN Red List (Yamaguchi et al. 2015). However, localized declining populations, population fragmentation and reduced extent of occupied area led to its classification under unfavorable conservation status in many countries (e.g. Portugal, Spain and Scotland; Cabral et al. 2005; Lozano and Malo 2012; Kitchener et al. 2005). Moreover, the difficulty in making an unambiguous identification of wildcats, domestics and hybrids by phenotypic characteristics (Daniels et al. 1998; Daniels et al. 2001) makes population assessment particularly challenging and, consequently, limits the use of traditional sampling techniques to assess the hybridization ecology (e.g. coat pattern). The misidentification of hybrid or domestic cat as a European wildcat can lead to severe effects for the conservation of this species (e.g. overestimates of population density and range).

Domestic cat-wildcat hybrids have been confirmed in most regions of Europe where hybridization was investigated (Beaumont et al. 2001; Kitchener et al. 2005; Pierpaoli et al. 2003; Lecis et al. 2006; Oliveira et al. 2008a, b; Hertwig et al. 2009; Nussberger et al. 2014; Steyer et al. 2018), supporting the claim that this could be a key factor threatening wildcat populations' trend and integrity. The uncertain long-term ecological and evolutionary effects of this interaction on the wildcat, makes its conservation a priority and an issue of global concern (Daniels et al. 1998). Although with reasonable uncertainty, previous studies have shown that introgressive hybridization (i.e. gene flow between an interspecific hybrid with one of its parent, by repeated backcrossing; Anderson 1953) can reduce the fitness of wild populations, as well as eliminate adaptations by introducing maladaptive genes (Rhymer and Simberloff 1996), hence undermining their ability to thrive (Lynch and O'Hely 2001). Given its importance for the conservation and management of wildlife populations, hybridization is one of the processes currently at the heart of ecological and conservation biology research (Sutherland et al. 2006), and it can be influenced by biotic, abiotic or human-induced disturbance factors.

The degree of documented introgression in European wildcats varies across and within regions in Europe: 3%-11% in Italy (Pierpaoli et al. 2003); 8%-10% in Bulgaria (Spassov et al. 1997); 3.5%-43% in Germany (Hertwig et al. 2009, Steyer et al. 2016); 14% in In the Iberian Peninsula (Oliveira et al. 2008a, b); 21%-29% in Switzerland -Jura Mountains (Nussberger et al. 2014); 29% in France (Say et al. 2012); 25%-31% in Hungary (Lecis et al. 2006) and 80% in Scotland (Beaumont et al. 2001; Pierpaoli et al. 2003). Although several studies focused in assessing the degree of hybridization between wildcat and domestic cat, most were at regional or national scales, precluding a big-picture assessment that encompassed the entire European range of this felid. Additionally, none of these studies explored the factors leading to the observed admixture levels.

Numerous factors may enhance hybridization rates between these two cat forms. For example, the "desperation hypothesis" (Hubbs 1955) postulates that hybridization can be promoted by restricted mate choices related to depleted populations, in areas where the two species occur, but one is scarce. The likelihood of wildcat hybridization at the European context has been proposed to be related with two processes: i) at the wildcat range expansion front in increasing populations - on the outskirts of the expansion area, populations are expected to be scarcer and extending towards suboptimal habitats, likely exhibiting higher permeability to domestic cats; and ii) at declining wildcat populations - where the scarcity of the wild form makes the population permeable to domestic cat incursions. Despite the proposal of these scenarios of high-likelihood of hybridization, a factual quantification of the causes contributing to the hybridization process likelihood between these two sub-species across Europe remains underexplored (Beugin et al. 2018; Oliveira et al. 2018; Steyer et al. 2018). Moreover, despite the tools used to conserve biodiversity – e.g. Protected areas - their role on European wildcat's GI and conservation it is still uncertain, thus this inadequacy of information may be a significant challenge to their effectively management.

To fill this gap, our study aims to: i) identify the main factors influencing European wildcat Genetic Integrity (i.e. probability to be genetically 'pure' wildcat; GI) across its entire distribution range and for each metapopulation, and ii) identify the suitability of Europe's protected area network for the conservation of genetically 'pure' European wildcat populations. To fulfil our objectives, we formulated and tested several hypothesis (see in detail in Table 1.1), briefly resume as: i) European wildcat's GI is related to habitat quality (e.g. GI increase whit higher forest cover and shrubland and decrease with croplands and human build-up cover), and ii) European wildcat's GI is positively associated with the landscape legal protection level (e.g. GI increase with the increment of legal landscape protection level).

2.Material and methods

2.1 Data collection

We used the database from Tiesmeyer et al. (2020), complemented with additional genetically-identified samples available at the Research Center in Biodiversity and Genetic Resources, University of Porto (CIBIO-UP). Both databases were combined and filtered to keep only samples putatively from European 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, Kitchener et al. 2005). Apart from the putative identification, our database contained the geographical coordinates of samples collection, collection date and value from genetic integrity, defined as the proportion of wildcat-exclusive Single Nucleotid Polymorphisms (SNPs) over all diagnostic SNPs analyzed. Biological samples were taken from live captured wildcats, fresh scat samples, hair traps and from carcasses opportunistically collected. These biological samples were grouped as: i) phenotypic wildcats, i.e. samples collected from live captured wildcats or carcasses that exhibited phenotypic traits compatible with wildcats, ii) scats, i.e. fecal samples collected from areas with suitable wildcat habitats, and that exhibited circumstantial and morphological characteristics compatible with European wildcats; or and iii) hair, i.e. samples of hair collected from animals as defined in 'i)' or from hair traps specifically designed for retrieving wildcat hair and deployed in areas with potential wildcat presence (Table 1.1, Appendix). Samples without geographic coordinates were associated with the smaller region possible of the Nomenclature of Territorial Units for Statistics (NUTS) as an approximation to its geographical position. Samples were discarded whenever the NUTS' region to each the sample was assigned to was higher than level 3 (e.g. general districts).

Available samples were collected between 1974 and 2016, from 13 European countries (Figure 1.1), comprising a total of 964 samples, of which 711 contained exact coordinates and 253 were linked to NUTS' units. The level of admixture between wildcats and domestic cats was quantified using 96 ancestry informative SNPs (Nussberger et al. 2013). Furthermore, SNPs including recombinant (autosomal) and non-recombinant markers (mitochondrial and Y-chromosome markers), were selected to identify wildcat, domestic cat, and their hybrids (Nussberger et al. 2013), and analyzed using Bayesian statistical tools. Since samples were collected across several European countries, through a relatively large time frame and analyzed in multiple laboratories, the laboratory procedures for DNA extraction and analysis varied slightly. To overcome this limitation, whenever samples had several GI values extracted from different loci number (e.g. 12,13 or 38 loci), we selected the value with the higher number of loci analyzed. A detailed description of all hybridization analyses procedures can be found in Tiesmeyer et al. (2020).

2.2 Statistical Modeling

2.2.1 Response variable

The response variable consisted of the proportion of diagnostic wildcat SNPs over all SNPs analyzed, for each cat sample, scaled between 0 and 1. The 0 value corresponds to a 'pure' domestic cat sample , i.e. without any contribution of exclusive wildcat genes over those analyzed, whereas the 1 value corresponds to a genetically 'pure' wildcat, i.e. an individual will all markers with exclusive wildcat genotype. The response variable is therefore the expected genetic integrity, conditional on the presence of putative European wildcats, and not the probability of European wildcat occurrence. We are aware that putative European wildcats do not occupy the entire wildcat's distribution range, but for the sake of this analysis we modeled GI as if putative European wildcats were widespread throughout the species' distribution range.

We developed two independent analysis approaches: i) accommodating the entire sample set, and ii) metapopulation-specific models (i.e. Iberian, Italian, Scottish, Northwestern Continental and Southeastern Continental), considering the geographic units defined by Mattucci et al. (2016). These modeling approaches are described in detail below.

2.2.2 Selection of the explanatory covariates

Three types of descriptors were considered - land cover, disturbance and level of protection (Table 1.1) - as potentially important drivers of European wildcat presence (Lozano et al. 2003, Klar et al. 2008, Monterroso et al. 2009, Silva et al. 2013b, Kilshaw et al. 2016; Jerosch et al. 2018, Oliveira et al. 2018), and that can be related to different ecological processes (e.g. dispersion, refuge; see Table 1.1). They were used as candidate covariates influencing the likelihood of European wildcat genetic integrity, since the presence of European wildcats is a paramount assumption for the occurrence of hybridization. Furthermore, some of these covariates are also potential drivers of domestic cats' presence (Ferreira et al. 2011; Horn et al. 2011), the second necessary condition for the occurrence of hybridization. One of the factors that is often associated with European wildcats' occurrence is prey availability (Lozano et al. 2003; Monterroso et al. 2009; Ferreira et al. 2011; Lozano 2010; Silva et al. 2013a). However, we were unable to use prey availability as a candidate covariate due to data unavailability for all the study areas and periods considered in this study.

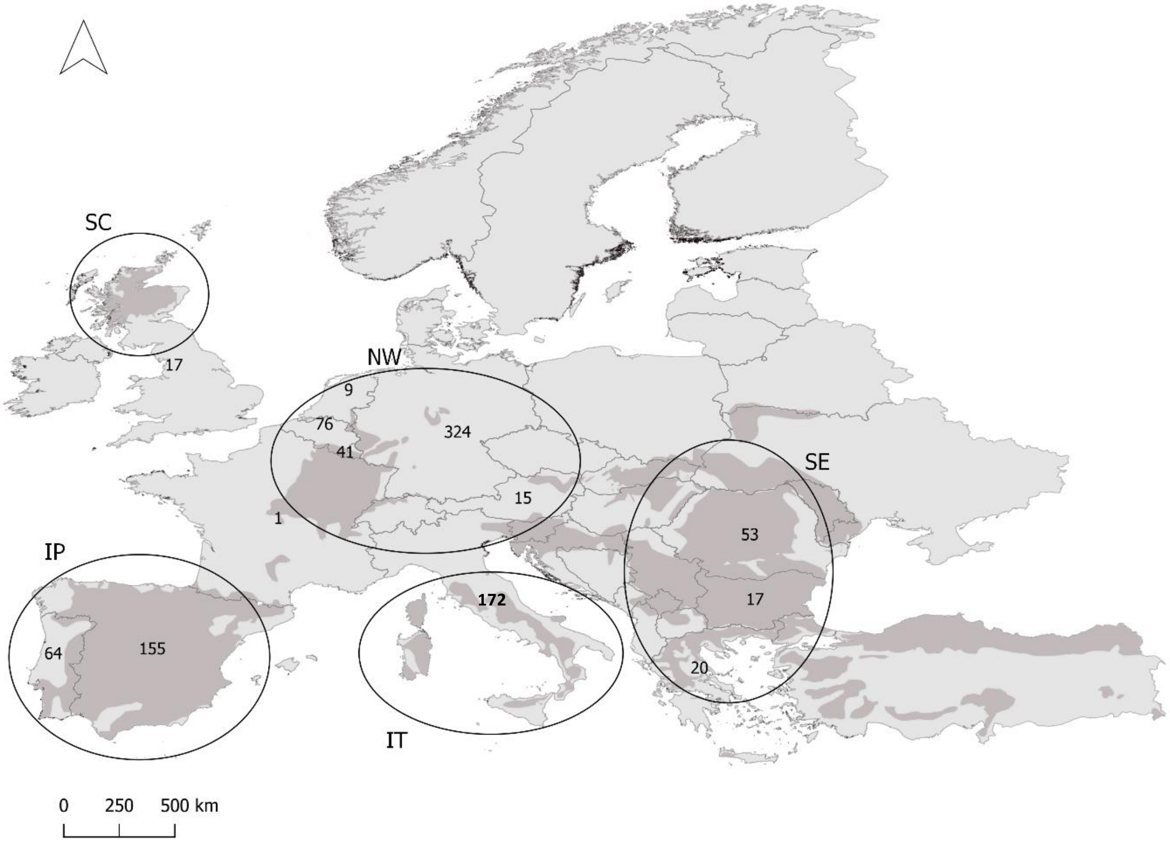

Figure 1.1. Countries from where the samples used in the analysis on European wildcat genetic integrity were collected, and respective sample size. European wildcat (*Felis silvestris silvestris*) distribution is highlighted in gray (Yamaguchi et al. 2015). The five European wildcat metapopulations (following Mattucci et al. 2016) are delimited by circles: IP (Iberian Peninsula- Portugal and Spain), SC (Scottish- Scotland), NW (Northwestern Continental - Austria, Belgium, France, Germany, Luxembourg and Netherland), IT (Italian- Italy and Sicily) and SE (South East Southeastern Continental - Bulgaria, Greece and Romania).

Table 1.1. Candidate covariates used in the modelling procedure to assess the drivers of wildcat's genetic integrity, with the corresponding acronym, units and range, hypothesis reasoning, source, and references supporting the presented reasoning.

Type	**Covariate (Acronym)**	**Units [min - max]**	**Hypothesis reasoning**	**Source**	**References**
Land Cover	Forest cover **(Frst)**	[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 hybridization likelihood.	Global Land Cover https://lcviewer.vito.be/	Oliveira et al. 2018 Steyer et al. 2018
	Forest ^{2} **(Frst)^2**	[0.00 - 1.00] %	Despite benefiting from forest cover, edge habitats and meadows are important feeding grounds. Therefore, local maxima of optimum forest cover could benefit sound wildcat populations, leading to higher resilience against introgression of domestic genes. Here we tested the quadratic relation between forest cover and genetic integrity.	Global Land Cover https://lcviewer.vito.be/	Oliveira et al. 2018 Steyer et al. 2018
	Distance to Forest **(D_Frst)**	[0.00 - 1.36] Km	Genetic integrity is higher closer to forest areas. The edge of the populations is expected to have lower densities when compared to core areas (i.e. inside or at the edge of forest habitats), which can reduce suitable mate choices, and contribute to a higher hybridization probability with domestic cat.	Global Land Cover https://lcviewer.vito.be/	Tiesmeyer et al. 2020 Dittmer and Allee 1931
	Shrubland cover **(Shrb)**	[0.00 - 0.64] %	Genetic integrity is higher in regions with higher cover of shrubs. This type of habitat is a key habitat in the Mediterranean region (and to wildcat's Mediterranean populations) since it has a higher prey availability, 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
	Shrubland ^{2} **(Shrb)^2**	[0.00 - 0.64] %	Genetic integrity is lower in regions with a homogeneous shrubland matrix. As for forest habitats, a homogeneous matrix of shrubs does not provide good hunting opportunities to wildcats as it should not host high abundance of this felid's preferred prey. Therefore, wildcat populations are not expected to reach higher densities in landscapes over encroached by shrubs, leading to unsuitable mate choices. Here we tested the quadratic relation.	Global Land Cover https://lcviewer.vito.be/	Monterroso et al. 2009 Oliveira et al. 2018
	Distance to Shrubs **(D_Shrb)**	[0.00 - 52.87] Km	Genetic integrity is higher near shrublands. This habitat provides access to shelter and predictably higher prey availability, thus could contain a higher wildcat population density, leading to suitable mate choices.	Global Land Cover https://lcviewer.vito.be/	Oliveira et al. 2018
	Cropland cover **(Crops)**	[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 wildcat and his domestic counterpart.	Global Land Cover https://lcviewer.vito.be/	Germain et al. 2008 Lozano 2010
	Cropland ^{2} **(Crops)^2**	[0.00 - 0.95] %	Genetic integrity is lower in regions with a homogeneous cropland matrix. This type of matrix is often associated with farms and, therefore, domestic cat	Global Land Cover https://lcviewer.vito.be/	Germain et al. 2008

			presence, leading to a higher likelihood of contact between wildcat and his domestic counterpart. Here we tested the quadratic relation.		
	Distance to Cropland **(D_crops)**	[0.00 - 4.60] Km	Genetic integrity is lower in regions closer to crops, as a result of the increasing probability of domestic cat presence, leading to a higher likelihood of contact between wildcat and the domestic counterpart.	Global Land Cover https://lcviewer.vito.be/	Ferreira et al. 2011
Disturbance	Human buildup areas cover **(Urb)**	[0.00 - 0.75] %	Genetic integrity is lower in regions dominated by human buildup, due to an increasing presence of the domestic cat, leading to a higher likelihood of contact between wildcat and the domestic counterpart and to frequent backcrosses with the domestic populations.	Global Land Cover https://lcviewer.vito.be/	Germain et al. 2008 Ferreira et al. 2011
	Distance to Human buildup areas **(D_urb)**	[0.00 - 15.25] Km	Genetic integrity is lower in regions closer to humanized areas, due to an increasing presence of the domestic cat, leading to a higher likelihood of contact between wildcat and the domestic counterpart.	Global Land Cover https://lcviewer.vito.be/	Ferreira et al. 2011
Level of protection	Strickly protected areas **(StriclyProtected)** **Category Ia, Ib***	Presence/Absence	Genetic integrity is higher in strict 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 wildlife-threatening actions (e.g. poaching or deforestation). The combination of all these characteristics is expected to favor sound wildcat population, with reduced interbreeding opportunities with domestic cats.	Protected Planet https://www.protectedplanet.net/	Reed and Merenlender 2008 Le Roux et al. 2015 Jones et al. 2018
	Moderately protected areas **(ModeratlyProtected)** **Category II, III, IV, V****	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 wildlife-threatening actions (e.g. poaching or deforestation). The combination of all these characteristics is expected to favor sound wildcat population, with reduced interbreeding opportunities with domestic cats.	Protected Planet https://www.protectedplanet.net/	Wierzbowska et al. 2012 Jones et al. 2018
	Poorly protected areas **(PoorlyProtected)** **Category VI*****	Presence/Absence	Genetic integrity is lower in areas with limited protection. These areas are expected to encompass characteristics that may negatively affect the wildcat, ranging from development of recreation small-scale tourism, provide natural products to communities, low restrict rules against human and domestic cat presence. The combination of all these characteristics is expected to promote the interbreeding opportunities with domestic cats.	Protected Planet https://www.protectedplanet.net/	Reed and Merenlender 2008 Wierzbowska et al. 2012 Jones et al. 2018
	Not Protected areas **(NotProtected)** **Areas deprived of any type of protection**	Presence/Absence	Genetic integrity is lower in areas deprived of protection. These areas are expected to not include suitable characteristics, ranging from higher habitat quality and prey availability, moderate disturbance effects, low domestic cat presence, and effective protection against wildlife-threatening actions (e.g. poaching or deforestation). The combination of all these characteristics is expected to promote the interbreeding opportunities with domestic cats.	–	Beugin et al. 2018

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

Landcover data were obtained from the 2015 version of the Global Land Cover raster, with 100 m resolution (Buchhorn et al. 2020). Despite encompassing a large temporal range, most samples were collected between 2000 to 2012 (n= 520, 54%), and land cover change has been minimal throughout most of the period under analysis (i.e. *ca*. 1% land cover changed between 1990 and 2006, Maucha and Pataki 2011). The original data from landcover rasters were reclassified into four important and relevant covariates: forest, shrubland, cropland and human buildup area (Table 1.1). 'Forest' cover was calculated as the sum of all types of forest categories represented in the Global Land Cover raster (e.g. Open Broadleaf forest, Closed Mixed forest, Evergreen needled-leaved forest, etc.).

The protected areas' data were obtained from the World Database on Protected Areas (WDPA; IUCN, UNEP-WCMC (2019). With the aim to incorporate the landcover protection status in the modeling procedure, we reclassified the IUCN protected areas designation types into 4 levels of protection (Table 1.1): i) Not protected, ii) Poorly protected, iii) Moderately protected, iv) Strictly protected.

For samples with accurate geographical location (n= 711), we estimated the proportion of each land cover within a buffer of approximately 100 Km^2 (5.5km radius, approximately the scale used in National Atlas. Distance-based covariates were estimated through the estimation of the linear Euclidean distance to the nearest landscape feature edge. For samples deprived of geographical coordinates (i.e. assigned to NUTS regions; n= 253), an approximation to habitat availability was estimated as the proportion of each respective land cover class on the regions' area. We were not able to calculate any distance-based covariates for this subset of samples.

All calculations were performed using the software's R Studio© version 1.1.463, R version 3.5.3 (R Development Core Team 2017), and QGIS 3.4.10 vector tools (QGIS Development Team 2016).

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 (Shiffler 1988).

Multicollinearity among continuous covariates was assessed through a nonparametric Spearman's correlation analysis using the *psych* R package (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 dispersion bias and facilitate model numeric convergence and coefficients comparisons (Shiffler 1988; MacKenzie et al. 2005).

To further investigate redundancy among factorial and continuous explanatory covariates, we modelled the covariate protection level as a function of land cover covariates with a generalized linear model procedure, both at the Europe and metapopulation-level scales. We detected significant differences in land cover proportions between the protection level categories (see Table 1.2, Appendix). Consequently, protection level and land cover covariates were precluded from being simultaneously included in the same modeling approaches. Hence, the effects of these two types of covariates were evaluated separately, allowing us test two of our working hypothesis groups.

Generalized linear models (GLM) were the backbone of our statistical approach, which followed a sequential stepwise procedure to capture the best fitting model set to our response variable. In a first step, we tested two model families – fixed-effects only model (GLM) and linear mixed-effects (GLMM) – as full fixed-effects models, i.e. including all fixed-effects covariates (land cover and disturbance). GLMMs combine the properties of linear mixed models (which incorporate random effects) and generalized linear models (Bolker et al. 2009), and are the best tool for analyzing data that encompasses groups of non-independent samples, that may include several sources of random variability or that show a non-normal data structure (Bolker et al. 2009). We tested the effect of three potential random factors: i) 'biome'; ii) 'ecoregion', both to accommodate potential noise inherent to the characteristics of these factors (e.g. extent of protection Dinerstein et al. 2017); and iii) 'metapopulation', is related to the five biogeographic groups (e.g. Iberian Peninsula, Italy, Scottish, Northwest Continental and Southeast Continental; see Mattucci et al. 2016 for details), which entail significant genetic differentiation and hence potential ecological and/or behavioral differences. This covariate allows to incorporate the metapopulation geographic structure and minimize the effect inherent to these groups (Mattucci et al. 2016). This approach was employed twice: i) with the full dataset, but excluding the distance-based covariates, which were not available for a subset of our samples (see dataset description above), and ii) with the subset of samples with detailed geographic location, and including the entire set of covariates.

Since the full dataset included samples whose location was only linked to NUTS regions, and thus was not possible to determine the detailed geographic location, we did not include distance-base covariates in models containing all the data. Thus, to minimize the data scale bias (i.e. difference between NUT area and the buffer used ca. 100km^2), we introduced a weight factor, which consisted in the proportion of the NUTS' area compared to the buffer area. Thus, a higher weight value corresponded to a sample with a larger area, and consequently, a smaller weight in the modeling analysis.

Models were ranked based on the Akaike's Information Criterion, corrected for small sample sizes (AICc) and on Akaike model weights (Burnham and Anderson 2002). The ΔAICc is a measure of each model performance relative to the best model (i.e. the one with the lowest AICc; Burnham and Anderson 2002) and represents the difference between the AICc of the model and the lowest AICc obtained. Akaike weights are used to indicate the support that a given model is the best among the set of candidate models (Mazerolle 2006). As a measure of goodness-of-fit (GoF), we calculated the pseudo-R^2 (value to assess the predictive capacity of the logistic regression model; Veall and Zimmermann 1996). The dataset (full or subset with geographic detail) that produced full fixed effects models with the highest goodness-of-fit (pseudo-R^2) was retained for subsequent analysis. The best model family and random effect to retain was selected based on AIC, whereby the full fixed-effects model with the highest support was chosen for subsequent analyses. We compared the pseudo-R^2 for each model from the two methods and selected the type of model (i.e. with or without distances covariates) that encompass the higher pseudo-R^2. When the pseudo-R^2 did not differ from the two methods, we used the model that comprise the higher number of samples analyzed.

We then generated a set of models including all fixed landscape covariate combinations using the *MuMIn* package (Bartoń 2019), with the constraint that correlated covariates could not be included in the same model and a maximum 1:10 ratio between the number of estimated parameters (covariate coefficients) and sample size, to avoid model overparameterization (Burnham and Anderson 2002). Six model sets were generated: one fit to the data from the entire European wildcat range, and one fit to the data from each biogeographic region (metapopulation). Models with a $\Delta AICc < 7$ were considered models as having substantial support and were considered as the top models (Burnham and Anderson 2002). Whenever more than one model reached a $\Delta AICc < 7$, model-averaged coefficients were calculated using the 'model.avg' function of the package *MuMin* (Bartoń 2019) to get the best estimates of covariates' effects from the top-ranked model set. We then used the model-averaged coefficients to predict European wildcat GI across Europe and for each metapopulation_using the 'modavgPred' function of the package *AICcmodavg* (Mazerolle 2017). As an additional measure of each covariate's effect on the European wildcat's genetic integrity, we calculated its relative importance as the sum of Akaike weights (Σωi) of all models that included that covariate over the total Akaike weights of the considered model set (Arnold 2010). Relative importance near 1 indicates a high support for a covariate to be highly influential in explaining the response variable variability, whereas relative importance near 0 indicates little support (Burnham and Anderson 2002).

The relative importance of protected areas in safeguarding the GI of European wildcats was assessed following an analogous procedure to that described above for landscape covariates, but using protection level as the sole categorical covariate potentially explaining the variability of the response variable. Predicted European wildcat's GI across protection levels was estimated using a parametric bootstrap procedure with 500 iterations using the 'bootmer' function of the package *lme4* (Bates et al. 2007), and from which the mean value and the 95% confidence interval of the predicted probability were estimated. Differences between predicted GI amongst protection levels for the entire Europe and metapopulation-level datasets, was examined through pairwise contrasts using ANOVA and Tukey post-hoc for multiple comparisons (Maxwell 1980) using the 'glht' function of the package *multcomp* (Hothorn et al. 2007). Analysis were performed in R Studio© version 1.1.463, R version 3.5.3 (R Development Core Team 2017).

3. Results

3.1 General Results

We gathered 964 samples at the European level, from which 758 were from wildcat (GI ≥ 80%), 59 were from hybrids (GI > 20% and < 80%) and 147 were from domestic cats (GI ≤ 20%) (Steyer et al. 2016). Most samples (N=429) were collected from regions deprived of protection level, 216 from poorly protected areas, 302 moderately protected and 17 from strictly protected areas (Table 1.3, Appendix). These values differed across metapopulation (Table 1.3, Appendix). Sampling covered a wide range of landscape composition conditions with regard to the potentially relevant covariates for wildcat's genetic integrity. The average sampled landscape was composed by 54.4 ± 24.9% [0.2 – 100.0] forest, 29.1 ± 23.2% [0.0 – 95.0] crops, 4.5 ± 23.2% [0.0 – 75.4] human buildup and 3.8 ± 8.1% [0.0 – 64.0] shrubs (Table 1.4, Appendix), with values differing across metapopulation (Table 1.4, Appendix).

The mean observed GI of putative wildcats across Europe was 80.8 ± 35.4%, ranging between [1.0 – 99.9 %]. These values varied across, and within, biogeographic regions. The Italian metapopulation showed the highest average value 90.6 ± 20.7% [1.0 -99.8 %], followed by the SE continental with a mean observed GI of 88.3 ± 27.4% [0.2 - 99.9%], and the NW continental with 85.6 ± 32.2% [0.1-99.9%]. The Iberian revealed the second lowest GI average value of 62.5 ± 45.6% [0.1 – 99.9 %], while the Scottish metapopulation showed the lowest observed GI of 48.9 ± 21.8% [15.2 – 81.6%] (Figure 1.2).

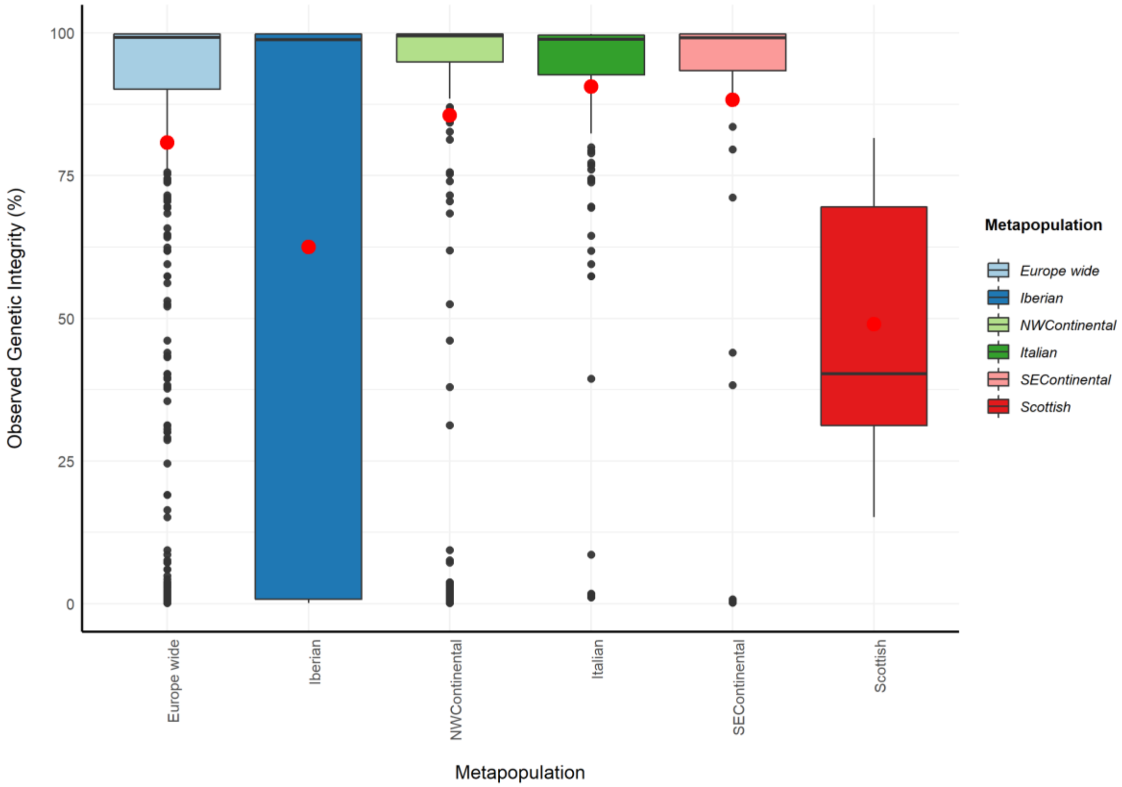

Figure 1.2. Observed genetic integrity for Europe and each metapopulation, with the respective 95 % confidence intervals. The mean observed genetic integrity is presented at red circle.

Forest and Cropland covariates were significantly correlated (*p* = -0.76) for all considered datasets. Consequently, the covariate Cropland was discarded from subsequent analyses since it had the lowest correlation with the response variable (Table 1.5, Appendix; Zuur et al. 2009). The comparison between full fixed-effects models fitted to the full and to the geographically detailed datasets revealed that, only for the Iberian Metapopulation the later performed better. Thus, despite encompassing a 54.8% smaller dataset, the geographically detailed produced models with a better GoF for this metapopulation ($pseudo\text{-}R^2_{\mathrm{full}}$ = 0.179 *vs.* $pseudo\text{-}R^2_{subset}$ = 0.343; Table 1.6, Appendix). For all the other datasets models performed better using the full datasets (Table 1.6, Appendix)

The first-step modeling procedure showed that the generalized linear mixed model with 'ecoregion' as random effect was the best model family for the Europe-wide and Iberian metapopulation models. The linear model was the best model explaining wildcat's GI for all remaining metapopulations (Table 1.7, Appendix).

3.2 Land Cover drivers influencing European wildcat genetic integrity

We produced 18 candidate models potentially describing wildcat's GI in Europe, of which four were considered as best models (Table 1.8, Appendix). These included the linear and quadratic effect of forest, and the linear effects of shrubs (land cover) and of human buildup (disturbance) as informative covariates. Forest cover showed a positive effect on GI, while human buildup areas seem to have a negative effect, both being the most informative covariates and revealing the highest relative importance, with a $\Sigma\omega i = 1.00$ and 95% confidence intervals not overlapping the zero (Table 1.2, Figure 1.3). The best models revealed a goodness-of-fit (pseudo-R^2) of 0.175 and the predicted baseline GI for the average sampled landscape composition of 0.94 ± 0.44 with a confidence interval 95% (CI 95; [0.86, 0.97]) (Figure 1.4).

From 144 models, for the Iberian metapopulation, accounting for all covariate's combinations, 20 were considered best models and comprised the covariates forest, forest^2, shrubs, shrubs^2, distance to forest, human buildup and croplands (Table 1.8, Appendix). However, only forest^2, urban and distance to forest revealed a 95% confidence interval that did not included the zero, with forest^2 showing a positive effect, and human buildup area and distance to forest a negative effect on wildcat's GI (Table 1.2, Figure 1.3). These covariates, along with forest area, reveal the higher relative importance: forest^2 ($\Sigma\omega i = 1.00$), forest ($\Sigma\omega i = 1.00$), urban ($\Sigma\omega i = 0.98$), and distance to forest ($\Sigma\omega i = 0.94$; Table 1.2). This model achieved a pseudo-R^2 of 0.343 and supported a high variability in baseline genetic integrity, which a mean estimate of 0.18 ± 2.02 (CI 95; [0.00, 0.92]) for the average sampled landscape composition (Figure 1.4).

We generated 18 models for the Italian metapopulation, of which 12 were considered as best models (Table 1.8, Appendix). Forest, forest^2, human buildup, shrubs, and shrubs^2 were included in the top-ranked model set, hence were considered as informative in explaining the wildcat GI for Italian metapopulation (Table 1.2). Only forest^2 had a 95% confidence interval that did not included the zero, exhibiting a well-defined negative effect as supported by a $\Sigma\omega i = 0.86$ (Table 1.2, Figure 1.3). Forest, human buildup, and shrubs revealed high values of relative importance, with $\Sigma\omega i = 0.97$, $\Sigma\omega i = 0.64$, and $\Sigma\omega i = 0.54$, respectively (Table 1.2), which suggests an imprecise but informative effect of these covariates. This model revealed a pseudo-R^2 of 0.08 and a baseline GI of 0.99 ± 0.25 (CI95; [0.98, 0.99]) for the average sampled landscape composition (Figure 1.4).

We produced nine models for the Scottish metapopulation, all with relative support, including the null model (Table 1.8, Appendix). Those models included the covariates forest, forest^2, shrubs, shrubs^2, and human buildup. However, all covariates' 95% confidence intervals overlapped zero, suggesting their negligible effect on wildcat GI across the sampling conditions from where Scottish samples were obtained (Table 1.2). Moreover, the proportion of shrubs cover was only covariate revealing a relatively high Akaike weight ($\Sigma\omega i = 0.50$; Table 1.2, Figure 1.3). This model revealed a pseudo-R^2 of 0.157 and suggests a baseline GI of 0.49 ± 0.27 (CI95; [0.35, 0.63]) for the average sampled landscape composition (Figure 1.4).

From 18 models produced for the Southeastern continental metapopulation, all had relative support. This model set included the forest, forest^2, human buildup, shrubs, and shrubs^2 covariates (Table 1.8, Appendix), but forest cover was the only that did not include the zero in the 95% confidence interval, revealing a positive effect. Coherently, this covariate revealed the highest relative importance ($\Sigma\omega i = 0.78$), followed by shrubs ($\Sigma\omega i = 0.53$), and urban ($\Sigma\omega i = 0.51$; Table 1.2, Figure 1.3). This model revealed a pseudo-R^2 of 0.05 and a baseline GI of 0.98 ± 0.39 (CI95; [0.96, 0.99]) for the average sampled landscape composition (Figure 1.4).

Finally, we produced 18 models for the Northwestern continental metapopulation, ten of which were considered best models and included the covariates forest, forest^2, shrubs, shrubs^2, and human buildup (Table 1.8, Appendix). The only covariate exhibiting a robust effect was the proportion of human areas, with a negative influence on wildcat's GI. This variable presented a $\Sigma\omega i$ of 0.99, supporting its high relative importance (Table 1.2, Figure 1.3). Although informative, forest cover revealed a moderated importance

(Σωi = 0.56). This model revealed a pseudo-R^2 of 0.02 and a baseline GI of 0.98 ± 0.22 (CI95; [0.87, 0.99]) for the average sampled landscape composition (Figure 1.4).

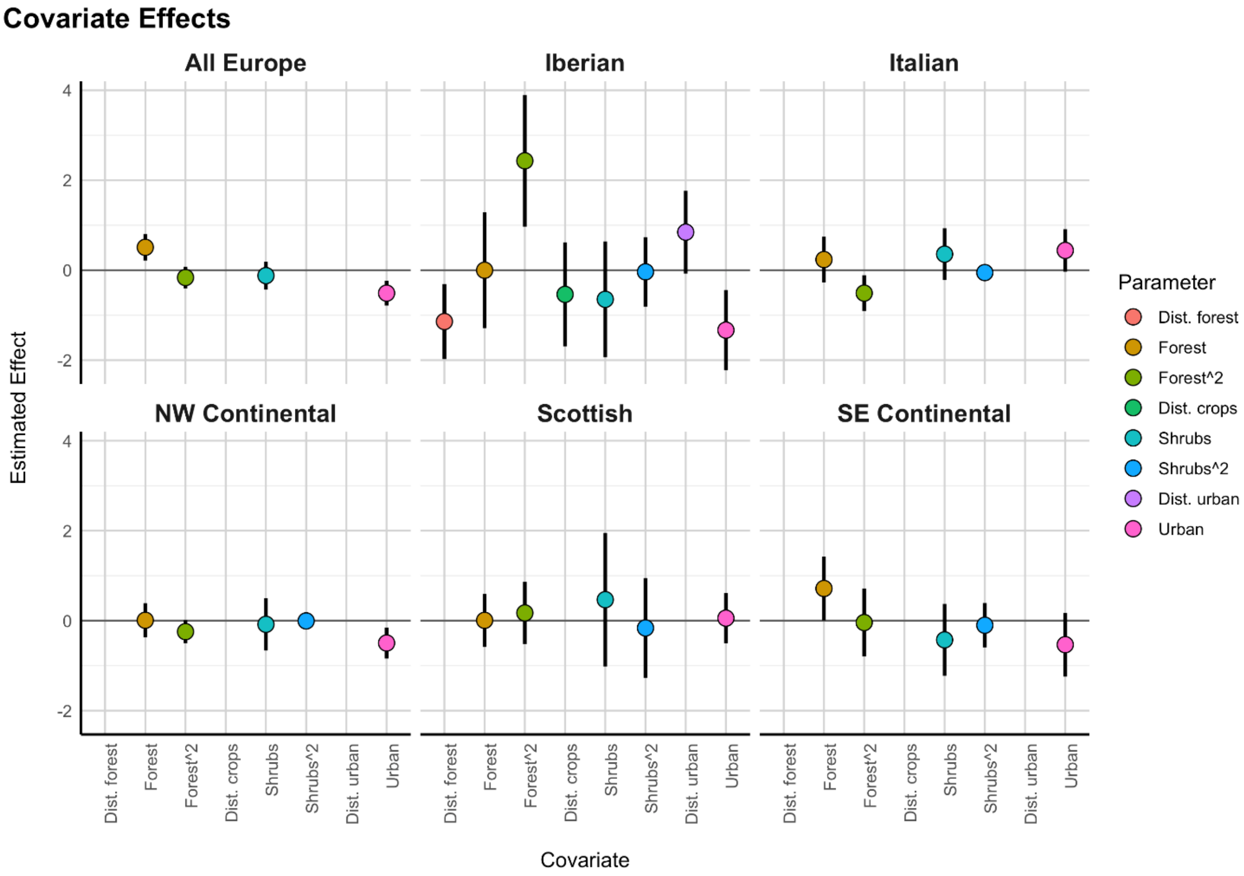

Figure 1.3. Untransformed model-averaged covariate effects (mean ± 95% confidence intervals) in wildcats' genetic integrity for Europe-wide and metapopulation-specific models. Colors represent each covariate, and black line represents the 95% confidence intervals (variables acronyms are described in Table 1.1).

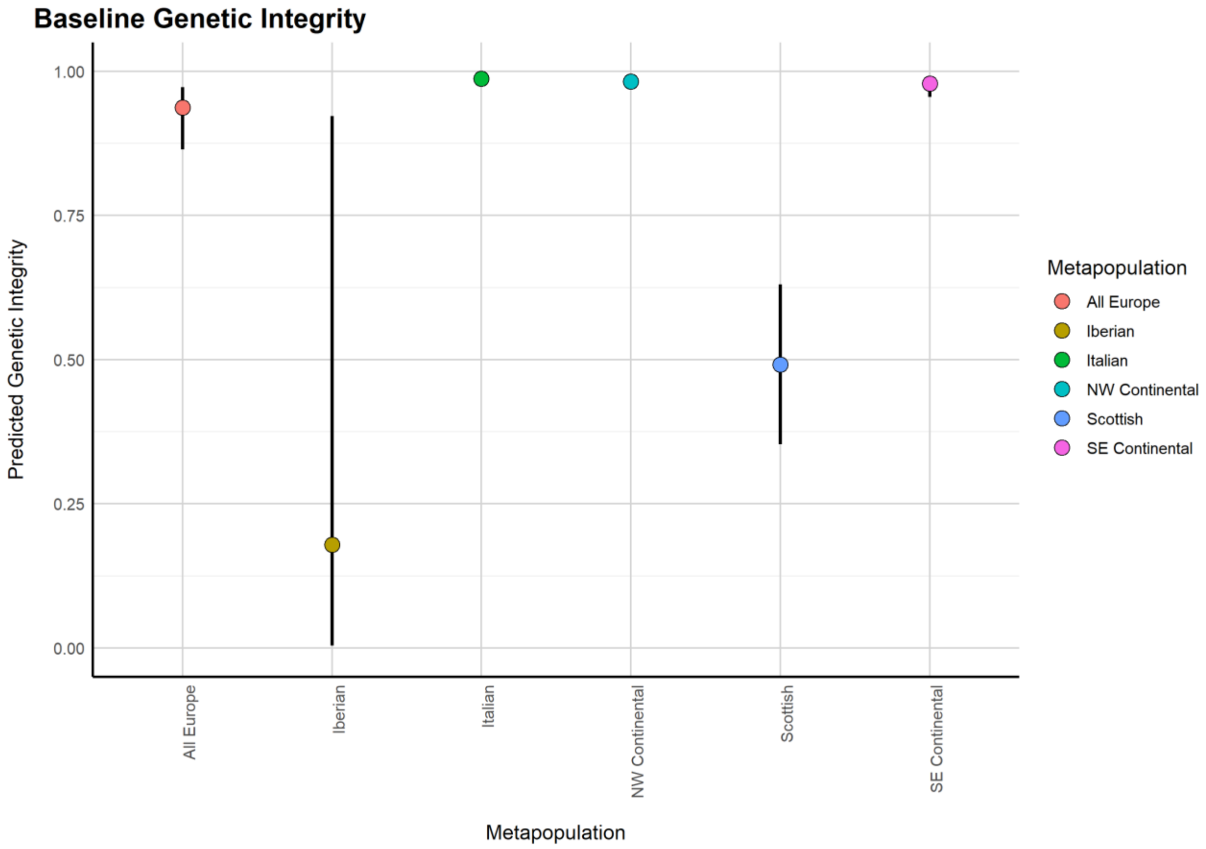

Figure 1.4. Model-averaged baseline predicted genetic integrity (estimate ± 95% confidence interval) for each metapopulation and for the entire Europe, for each respective average sampling conditions. Metapopulations' countries: Iberian – Portugal and Spain; Italian – Italy and Sicily; NW Continental – Austria, Belgium, France, Germany, Luxembourg and Netherland; Scottish – Scotland; SE Continental – Bulgaria, Greece and Romania.

Table 1.2. Covariates included in the top-ranked model set explaining the wildcats' genetic integrity for each metapopulation and for the entire Europe, with respective model-averaged untransformed coefficients, standard error (SE), and 95% confidence interval (CI 95%) and relative importance (sum of Akaike weights; Σωi) (variables acronyms are described in Table 1.1). Landscape covariates with a CI 95% that did not include the zero are presented in bold.

	Europe	Σωi	Iberian*	Σωi	Italian	Σωi	Scottish	Σωi	SE Continental	Σωi	NW Continental	Σωi
(Intercept)	2.71 ± 0.44 [1.85 / 3.57]		-1.52 ± 2.02 [-5.52 / 2.48]		4.35 ± 0.26 [3.85 / 4.85]		-0.04 ± 0.27 [-0.6 / 0.53]		3.85 ± 0.39 [3.07 / 4.63]		4.02 ± 0.22 [3.59 / 4.46]	0.56
Forest	**0.51 ± 0.15 [0.21 / 0.80]**	1.00	0.00 ± 0.65 [-1.29 / 1.29]	1.00	0.24 ± 0.26 [-0.27 / 0.75]	0.97	0.47 ± 0.70 [-1.02 / 1.95]	0.17	**0.71 ± 0.36 [0.01 / 1.42]**	0.78	0.01 ± 0.19 [-0.37 / 0.39]	0.99
Urban	**-0.51 ± 0.14 [-0.79 / -0.23]**	1.00	**-1.33 ± 0.45 [-2.22 / -0.44]**	0.98	0.44 ± 0.24 [-0.03 / 0.91]	0.64	0.01 ± 0.28 [-0.58 / 0.6]	0.15	-0.53 ± 0.36 [-1.24 / 0.18]	0.51	**-0.50 ± 0.18 [-0.84 / -0.15]**	0.38
Forest^2	-0.16 ± 0.12 [-0.41 / 0.08]	0.21	**2.43 ± 0.45 [0.97 / 3.89]**	1.00	**-0.51 ± 0.20 [-0.91 / -0.11]**	0.86	0.06 ± 0.26 [-0.51 / 0.62]	0.02	-0.43 ± 0.40 [-1.23 / 0.37]	0.19	-0.24 ± 0.13 [-0.5 / 0.01]	0.36
Shrubs	-0.12 ± 0.16 [-0.43 / 0.19]	0.16	-0.65 ± 0.65 [-1.94 / 0.64]	0.53	0.36 ± 0.29 [-0.22 / 0.93]	0.54	-0.16 ± 0.52 [-1.27 / 0.95]	0.50	-0.04 ± 0.38 [-0.80 / 0.71]	0.53	-0.08 ± 0.30 [-0.67 / 0.5]	0.09
Shrubs^2	-	-	-0.04 ± 0.39 [-0.91 / 0.74]	0.12	-0.05 ± 0.07 [-0.19 / 0.09]	0.18	0.17 ± 0.32 [-0.52 / 0.86]	0.06	-0.10 ± 0.25 [-0.60 / 0.39]	0.14	-0.01 ± 0.08 [-0.15 / 0.14]	-
D_crops	-	-	-0.54 ± 0.58 [-1.69 / 0.62]	0.40	-	-	-	-	-	-	-	-
D_forest	-	-	**-1.14 ± 0.42 [-1.97 / -0.31]**	0.94	-	-	-	-	-	-	-	-
D_urban	-	-	0.85 ± 0.46 [-0.07 / 1.77]	0.66	-	-	-	-	-	-	-	-

	Europe	Iberian	Italian	SE Continental	NW Continental
(Intercept)	3.19 ± 0.48 [2.24 / 4.13]	0.33 ± 1.21 [-2.04 / 2.71]	4.86 ± 0.58 [3.71 / 6.01]	4.01 ± 0.84 [2.35 / 5.67]	1.43 ± 1.85 [-2.21 / 5.08]
Not Protected	-1.22 ± 0.35 [-1.90 /-0.54]	-1.08 ± 1.08 [-3.21 / 1.06]	-0.66 ± 0.58 [-1.80 / 0.48]	-1.18 ± 0.77 [-2.71 / 0.36]	-0.88 ± 0.50 [-1.86 / 0.10]
Moderately Protected	-0.50 ± 0.33 [-1.14 / 0.14]	1.32 ± 0.92 [-0.49 / 3.13]	-1.77 ± 0.37 [-2.51 / -1.04]	-0.70 ± 1.46 [-3.60 / 2.20]	-0.45 ± 0.55 [-1.52 / 0.63]
Strictly Protected	0.71 ± 0.98 [-1.22 / 2.64]	4.00 ± 2.23 [-0.39 / 8.39]	-1.64 ± 1.16 [-3.92 / 0.65]	-	-1.81 ± 1.81 [-5.36 / 1.74]

*Samples collected within the Iberian metapopulation include only data with exact geographical coordinates. Thus, we could analyze the influence of the distances to specific land covers on wildcat's genetic integrity.

3.3 Effectiveness of European protected areas' network

For the European scale, the post hoc Tukey's test revealed significant difference between unprotected areas and areas with poor level of protection (GLMM Tukey's: z = -3.512, P = 0.002). Although, no differences were detected for any of the other pairs, GI tends to be higher in strictly protected areas: $GI_{unProt} = 0.87\ [0.71 - 0.95]$ vs. $GI_{poorProt} = 0.96\ [0.88 - 0.99]$ vs. $GI_{modProt} = 0.93\ [0.83 - 0.97]$ vs. $GI_{strictProt} = 0.98\ [0.70 - 0.99]$ (Figure 1.5).

Despite the low precision in our parameter estimates, the level of protection revealed a well-established relationship with European wildcat' GI for the Iberian metapopulation. Our results support that the wildcat GI increases with increasing protection level: $GI_{strictProt} = 0.99\ [0.46 - 0.99] > GI_{modProt} = 0.84\ [0.26 - 0.99] > GI_{poorProt} = 0.58\ [0.09 - 0.95] > GI_{unProt} = 0.32\ [0.03 - 0.83]$ (Figure 1.5). Furthermore, we detected significant differences between the different protection levels (GLMM ANOVA: F = 2.845, P = 0.041).

The Italian and SE continental metapopulation revealed that the European wildcat's GI is not directly influenced by the level of protection, since the average GI has high values regardless of legal protection level ($GI_{strictProt} = 0.96\ [0.13 - 0.99]$, $GI_{modProt} = 0.96\ [0.84 - 0.99]$, $GI_{poorProt} = 0.99\ [0.97 - 0.99]$, and $GI_{unProt} = 0.96\ [0.94 - 0.99]$, for Italian, and $GI_{modProt} = 0.97\ [0.70 - 0.99]$, $GI_{poorProt} = 0.99\ [0.93 - 0.99]$, $GI_{unProt} = 0.95\ [0.77 - 0.99]$, for SE continental; Figure 1.5). However, the post hoc Tukey's test revealed significant difference between moderately protected areas and areas with poor level of protection (GLMM Tukey's: z = -4.782, P < 0.001).

Despite the uncertainty associated with the effect of protection level on European wildcat's genetic integrity from NW continental metapopulation, our results suggest that GI tend to be lower in strictly protected areas: $GI_{strictProt} = 0.47\ [0.00 - 0.99]$, $GI_{modProt} = 0.77\ [0.08 - 0.99]$, $GI_{poorProt} = 0.83\ [0.10 - 0.99]$, $GI_{unProt} = 0.68\ [0.06 - 0.98]$ (Figure 1.5).

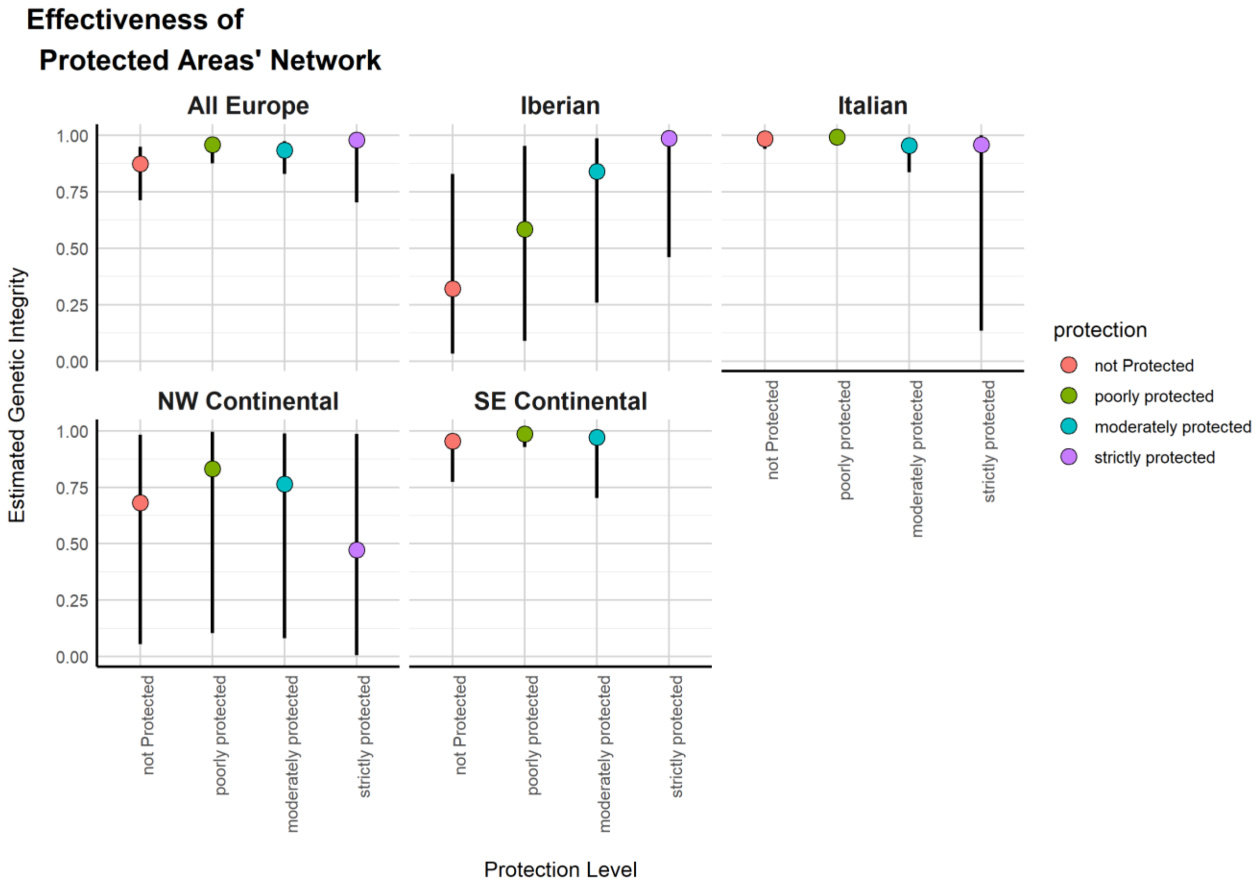

Figure 1.5. Europe-wide and metapopulation-specific estimated genetic integrity of putative European wildcats according to landscape protection level. Colors represent each protection level, and black line represents the 95% confidence intervals. Protection level categories: not protected – areas deprived of any type of protection; poorly protected – includes areas with IUCN categories V and VI; moderately protected – includes areas with IUCN categories II, III and IV; strictly protected – includes areas with IUCN categories Ia and Ib. Scottish metapopulation were not included due to small sample size (N = 17).

4. Discussion

Our findings suggest that at the range-wide scale, i.e. Europe, putative European wildcats tend to have high levels of genetic integrity (Tiesmeyer et al. 2020). However, this global pattern masks distinct realities at a metapopulation scale. Results highlight that there are high levels of baseline GI in NW continental, SE continental and Italian metapopulations. Previous studies have revealed inconsistent estimates of European wildcat-domestic cat hybridization for these metapopulations, suggesting a spatial structuring of the hybridization process at the regional scale and localized variation in threats to the integrity of some wildcat populations (Spassov et al. 1997; Lecis et al. 2006; Nussberger et al. 2014; Steyer et al. 2016). Despite previous reports of geographically localized high admixture (Hertwig et al. 2009, O'Brien et al. 2009, Nussberger et al. 2018), our results for these metapopulations are supportive of the scenario proposed by Nussberger et al. (2018), whereby introgression should be mostly caused by wildcat expansion, rather than by incursions of domestic cats into wildcat habitats, due to the high GI values of these metapopulations and the recent local range expansion (Streif et al. 2012; Say et al. 2012). Conversely, the Iberian and Scottish metapopulations revealed lower levels of genetic integrity and expose a more serious threat posed by hybridization. Beaumont et al. (2001) and Pierpaoli et al. (2003) had already revealed that the admixture between European wildcats and domestic cats was the higher in Scotland than what was known to occur in other parts of the species' range. However, our results suggest that admixture in the Iberian metapopulation should also be higher than previously thought. We estimate an average of 18% baseline GI among putative European wildcats for the sampled landscapes in Iberia. These figures are indicative of a baseline admixture rate higher than the 14% previously proposed by Oliveira et al. (2008a). However, the reduced precision of the baseline genetic integrity, which is accommodated in the models' intercept, exposes the variability not explained by landscape characteristics. There is very limited data, if at all existing, on most of potentially influential factors, such as population connectivity or demographic history (Beugin et al. 2018; Steyer et al. 2018). However, while the variability in European wildcat's genetic integrity could be strongly influenced by the effect of these unaccounted factors, our results demonstrate that GI is also shaped by habitat-related features. The worse scenario for the Iberian metapopulation depicted by our results (predicted GI of *ca*. 18%), when compared to the published patterns, could be related to the broader geographic range, larger sample size and finer molecular approaches accounted for in our study, likely providing a more thorough description of the Iberian scenario. However, caution is warranted in the interpretation of these results, as our model supports a habitat- and protection-dependent spatial structuring of wildcats' GI across Iberia, whereby genetically-integer individuals have a higher probability of occurring in favorable habitats and with higher levels of landscape protection (see discussion below; Figure 1.1, Appendix). Likewise, the Scottish metapopulation reveals high levels of background admixture (Beaumont et al. 2001; Senn et al. 2019), with an increase of hybridization in the last decades (Senn et al. 2019). Breitenmoser et al. (2019) concluded that the Scottish population is no longer viable, due to population's fragmentation, small occurrence extent, island isolation, and hybridization. These factors together with the low sample size limits the predictive capacity of the models, and a more robust inference on the spatial structure of Scottish GI.

4.1 Land Cover drivers influencing European wildcat genetic integrity

Ours results showed that the proportion of forest cover (in the simple and quadratic forms), human buildup areas are overarching drivers of the European wildcat genetic integrity, at the European scale. Specifically, GI is higher in areas with a high cover of forest and lower in regions dominated by anthropogenic infrastructures, a pattern that corroborates our initial hypothesis (H1). However, there are metapopulation-specific deviations from this general response. While anthropogenic interference arises as the most influential driver of the wildcat's GI for the Italian and NW Continental metapopulations, forest cover is more influential for the SE Continental metapopulation, and both drivers act synergistically to determine the genetic uniqueness of wildcat for the Iberian metapopulation. In the latter, GI is maximized by the proximity and by the amount of forest cover and restrained by the amount of anthropogenic buildup. However, and counterintuitively, in Italian metapopulation the wildcats' GI seems to increase in urbanized areas. Nevertheless, given the high baseline GI predicted for the Italian metapopulation (*ca*. 99%), any covariate positively correlated with our response variable effect will have only a marginal effect. Therefore, when placed into context, we interpret this as a near-null effect, supportive of the high introgression-resilient scenario proposed by Nussberger et al. (2018). The reduced available sample size limited the robustness of our inferences regarding the effect of landscape composition on wildcat's GI for the Scottish metapopulation. Regardless, shrublands emerge as a potentially relevant land cover class linked to wildcat's GI variability, which corroborated the finding of Kilshaw et al. (2016). But, although we acknowledge the importance of shrubland cover in shaping wildcats GI in Scotland, we cannot state, undoubtedly, that it promotes GI (since the 95%CI includes the zero).

The importance of forests for European wildcat's genetic integrity, at European and metapopulation scale, suggests that this habitat type can provide key conditions for this felid, e.g. prey availability, shelter and refuge, and, consequently, support a more robust wildcat populations, which enhance intraspecific reproduction opportunities (Sarmento et al. 2006; Germain et al. 2008; Klar et al. 2008; Monterroso et al. 2009; Lozano 2010; Beugin et al. 2016; Oliveira et al. 2018). Native forests have been thoroughly described as one the preferred European wildcat's habitats (Sarmento et al. 2006; Germain et al. 2008; Gil-Sánchez et al. 2015; Beugin et al. 2016; Oliveira et al. 2018), providing suitable feeding resources and breeding conditions, thus limiting animals' need to forage into other suboptimal habitats (e.g. agriculture fields), where there is a higher risk of interacting with domestic cats. Usually, habitat quality (e.g. high prey availability, low human disturbance, high shelter opportunities, and favorable and higher mate choice chances) is related to species occurrence (Verbeylen et al. 2003). European wildcat's GI metapopulation-specific patterns suggest that Central European and Italian forests could present better habitat conditions reaching high levels of GI, thus reducing the hybridization likelihood, even though habitat fragmentation, human disturbance, and local decreasing populations trends occur. Peripheric metapopulations, i.e. Iberian and Scottish, may have suboptimal environmental conditions that are reflected in lower wildcat fitness, and challenge animals to search for resources in more altered landscapes, where the risk of encountering domestic cats is higher. Metapopulations-specific patterns may also be structured by local landscape, whereby in regions with suitable habitat (e.g. low human disturbance and high forest cover), wildcats do not need to explore anthropic landscapes in search for resources (thus promoting encounter with domestic cats) and individuals may have higher fitness, and therefore populations are more resilient to threats. These patterns are coherent with results from other studies from Iberian Peninsula (Gil-Sanchez et al. 2015) and France (Beugin et al. 2020), where admixture levels between the two species are low, due to suitable habitat conditions.

We assumed that the human buildup area cover can be used as a surrogate of domestic cat abundance and anthropogenic disturbance, which produced a generalized negative effect on European wildcats' genetic integrity. This observation is coherent with the findings of Le Roux et al. (2015) for African wildcats, which showed a significant negative relationship between GI and human disturbance along Kruger National Park boundaries. The geographical contact between two species does not necessarily imply that hybridization occurs. For this process to arise, wildcat populations need to be permeable to domestic cats, i.e. with low densities, possibly due to fragmentation, human disturbance and other factors somehow linked to anthropogenic actions (Gil-Sanchez et al. 2015; Oliveira et al. 2018). Populations showing high permeability may indicate depletion of wildcat individuals. Thus, under Hubb's Desperation hypothesis (Hubbs, 1955), depleted populations may suffer from restricted mate choice, promoting mating with heterospecific, consequently leading to hybridization (Bohling and Waits 2015). Domestic cats, often associated with human presence, tend to set their home ranges near human settlements (Ferreira et al. 2011). However, they engage in foraging and exploratory bouts into wild habitats (e.g. Say and Pontier 2004), reaching distances than can vary from 2.5km to 6.8km from the settlements (Germain et al. 2008; Ferreira et al. 2011). These bouts promote encounters between wild and domestic cats, and thus increase the hybridization probability. On the other hand, male wildcats generally have higher home-ranges than females (Monterroso et al. 2009, Anile et al. 2018), and can tolerate lower habitat quality when compared to females (Oliveira et al. 2018). In fragmented landscapes, where native patches cannot provide enough resources, males may tend to explore anthropic patches/matrixes, increasing contact probability with domestic cats. This spatial gender-specific patterns may affect directionality of hybridization (Tiesmyer et al. 2020). In eastern Switzerland, Nussberger et al. (2018) determined that the rate of introgression may be explained by sex-biased dispersal of male wildcats into domestic cat ranges. Moreover, the directionality of hybridization (especially between male domestic cats and female domestic cats) may lead to the incorporation of hybrids into the wild population (e.g. when mating between female wildcat and male domestic cat occurs, the offspring will be raised as 'wildcat'; Oliveira et al. 2018). These sex-biased dispersal and hybridization directionality patterns are extremely important mechanisms to consider when dealing with hybridization processes and when defining conservation strategies for wildcats, especially for the Iberian and Scottish metapopulations.

Overall, our results support a negligible effect of shrublands on European wildcats' genetic integrity, although with a tendency to have a negative effect. Shrublands are a key element for the European wildcat in its Mediterranean range (Lozano et al. 2003; Lozano 2010; Oliveira et al. 2018). When structured in a mosaic landscape, shrublands favor European rabbit (*Oryctolagus cuniculus*) abundance (Lozano et al. 2003; Fernández 2005), wildcats' main prey in this region (Lozano et al. 2006), and therefore should provide rewarding hunting grounds for the European wildcat. However, extensive shrub-only landscapes tend to have the opposite effect, usually hosting limited prey abundance for European wildcats (Silva et al. 2013a). Furthermore, these areas are often associated to agricultural fields and farms, where domestic cats are kept as pets and to help control rodents around farm houses (Germain et al. 2008). In such a scenario, the contact between both cat species is likely to increase in this landscape context, potentially creating interbreeding opportunities.

4.2 Effectiveness of European protected areas' network

Despite having a common overarching goal of safeguarding wildlife, natural processes and ecosystems, protected areas' (PA) conservation effectiveness is highly variable across the globe, according to their management, geography, biodiversity patterns and landscape context (Joppa et al. 2008; Boitani et al. 2008; Locke and Dearden 2005). The IUCN defines distinct PA categories (Ia, Ib, II, III, IV, V, and VI), which have specific restrictions to humans' presence and activities, and management approaches to achieve conservation goals (Dudley 2008). It is therefore expected that the level of wilderness across PA's should be correlated to the protection level gradient inherent to its IUCN categories: Ia = Ib > II = III > IV = V > VI (Chape et al. 2005; Dudley 2008), with Ia having the highest protection and wilderness levels, and VI having the lowest.

At the larger scale, our results revealed that the effectiveness of PAs' network in safeguarding wildcats' GI was relatively independent of its protection level (Figure 1.5). However, given that the baseline European wildcat GI levels are high at the range-wide scale (*ca.* 87%), 'pure' wildcats may still be found if landscape conditions are favorable. Nevertheless, our results preclude us from inferring the role of protected areas in maintaining healthy wildcat populations and, therefore, assess if the objectives for PAs in Europe, in the wildcat conservation perspective, are being properly fulfilled. We did, however, identify a well-defined positive relationship between PA protection level and wildcats' GI for the Iberian metapopulation. Although based on low precision estimates, our data showed that unprotected Iberian landscapes seem to account for putative wildcats, with a baseline GI of *ca.* 32%, while Iberian PAs with sustainable use of natural resources (IUCN category VI), revealed low levels of wildcat's GI (*ca.* 58%), higher than non-protected landscapes. The primary objective of category VI PAs is to safeguard natural ecosystems and promote the sustainable use of natural resources, hence contributing to a balanced relationship between humans and nature (Dudley 2008). However, our results clearly suggest that these goals are not being met by category VI Iberian PAs, when considering the conservation of wildcat's genetic integrity. Human presence, and related practices, in this PA typology could be favoring the frequent contact, and consequent admixture, between the wildcat and its domestic counterpart. Furthermore, areas in this region with a strict landscape protection account for a predicted GI of *ca.* 99%. These areas comprise the Ia and Ib IUCN categories, with the main objective of conserve and protected outstanding ecosystems and/or species and long-term ecological integrity of natural areas that do not have anthropogenic activity (Dudley et al. 2008). This significant barrier to human activities, and therefore domestic cat presence, together with pristine habitat, may explain the high values of wildcat's GI in Iberian Peninsula Ia and Ib PA, highlighting the effectiveness of these PAs to European wildcat's conservation at long-term.

Between 1975 and 2014, there was a significant increase in human-influenced areas (human buildup) surrounding protected areas, together with a smaller, but threatening, increase within protected areas (Fuente et al. 2020). These increase of human activity around PAs, together with the presence of domestic cats inside PAs, at a significant distance from human settlements (Sarmento et al. 2009; Zwijacz-Kozica et al. 2017), may increase the probability of encounters, and therefore, admixture between the two species. Moreover, Leroux et al. (2010) revealed that some protected areas may not be managed accordingly to the objectives of their IUCN category guidelines and, therefore, some PAs with IUCN category I (i.e. Strict nature reserve) revealed low levels of naturalness. The wide range of wildcat's GI of Iberian and NW continental PAs may be somehow connected with these threats, and with a inter and intra-PA's variation in the effectiveness of implementing conservation guidelines, mentioned above (Figure 1.5).

Overall, European wildcat GI tends to be lower when landscapes are deprived of environmental protection, but the relative effect of protection level is highly dependent on the baseline GI at the metapopulation level. Areas without legal protection are, in Europe, typically characterized by higher human presence and human-related activities. Therefore, the higher abundance of domestic cats (Reed and Merelender 2008), coupled with increased anthropic disturbance (Jones et al. 2018), and habitat destruction (Joppa and Pfaff 2011; Geldmann et al. 2014), deems these areas with higher propensity for interbreeding events and for genetic admixture between the wild and domestic forms of the European wildcat. This pattern has also been observed elsewhere. African wildcat populations exhibit higher hybridization rates outside protected areas, arguably due to the more pristine character and lower human disturbance of the landscapes located within park boundaries (Le Roux et al. 2015). All these factors may contribute synergistically to the overall negative effect of non-protected areas on wildcats' genetic integrity.

4.3 Implications to European wildcat conservation

Overall, PAs can act as important administrative and political conservation tools to the broader conservation of European wildcat populations in Europe. Our results suggest that for metapopulations with a greater baseline GI (e.g. Italian, Southeast and Northwest continental), the legal landscape protection level is not directly correlated with the European wildcat's GI, since these metapopulations revealed an overall high baseline GI. Alongside, regions deprived of legal protection that overlap with Italian and SE metapopulation ranges, presented high values of GI, which leads us to believe that some of those areas may have the necessary conditions to host genetically 'pure' populations of European wildcat, which should make these areas good candidates and also a priority for wildcat conservation. Nevertheless, the Italian strictly PAs and each NW PAs presented high GI variability, implying that hybridization may occur inside those areas. Thus, these results indicate that some PA's located within Italian and NW metapopulation range may not be fully accomplishing their conservation goals, when considering wildcats. Some conservation measures can be implemented to improve wildcat's integrity (and prevent the increase of hybridization rates), namely: 1) the regular genetic monitoring of wildcats; 2) the enforcement of stricter conservation measures to prevent PA's use by domestic cats (e.g. chipped, vaccinated and neutering; the later reduces considerably their activity pattern and home-range; Ferreira et al. 2020); 3) removal of feral cats that may be establishing their territory inside PA, and 4) reduce or mitigate human-induced land change/disturbance, especially if involving forest conversion.

The Iberian metapopulation presented high variability on baseline GI, despite the confounding effect of unaccounted factors. Our results support that in Iberia, PAs of levels I-IV i.e. moderately and strictly protected, tend to have wildcats with an average GI > 80%. These areas typically present a higher habitat quality and reduced human-disturbance (Fuente et al. 2020). Thus, we believe that the conservation of wildcats' GI in Iberian PAs is mediated not only by direct protection (i.e. species conservation action plan), but also indirectly, by the maintenance of good quality habitats inside PAs. This is further supported by our landscape-level analyses, that indicate a positive effect of forest habitats and negative effect of anthropogenic buildup areas. Regions without or with low-level legal protection exhibited a wide range of GI values, suggesting that the wider baseline GI values could be improved if habitat restoration and some degree of protection are implemented or upgraded.

The Scottish metapopulation revealed to be the most threatened population, with low levels of baseline GI (*ca*. 48%). With an estimated population size as low as 115 – 200 (Kilshaw et al. 2015; Mathews et al. 2018), and high levels of hybridization (Senn et al. 2019), it should be expected that the Scottish metapopulation may be no longer viable (Breitenmoser et al. 2019). This population needs significant and urgent attention by conservationist and managers and direct conservation measures should be implemented, mainly by re-introducing genetically ´pure´ wildcats and neutering and vaccination pet and feral domestic cats. Even with local conservations programs and the creation of five main priority conservation protected areas for wildcats, a survey conducted in 2017/18, revealed a ratio of wildcats to un-neutered hybrids of almost 1:6 (Breitenmoser et al. 2019). This scenario highlights the urgent need of reintroduction/reinforcement projects and rigorous protection to avoid hybridization. Nevertheless, despite these actions, an effort should be made to implement an efficient and significant control of feral cats and hybrids in areas target for reintroductions.

For both Iberian and Scottish metapopulations PAs fail to fulfill the Aichi Target 13 of the Strategic Plan for Biodiversity 2011-2020 (i.e., by 2020 minimize genetic diversity loss and maintaining genetic diversity of wildlife; CBD 2012; Hollingsworth et al. 2020), since the safeguarding of wildcat's GI is still inadequate. As we determined, the wildcat's GI for those metapopulations presents a significant low value, suggesting that the management and conservation guidelines of PAs located within their range are inadequate to assure the fulfilment of the Aichi Target 13. This underperforming scenario should motivate a serious concern among conservationists and lead to the implementation of strict measures to guaranty the preservation of these threatened felid.

4.4 Limitations

The biological samples used in this study were collected in locations that are considered suitable for the wildcat, and thus the results need to be interpreted as the probability of genetic integrity, given the species presence. Since this species presents fragmented populations, often confined to small regions, an unbiased approach is difficult to implement in such a wide scale. Nevertheless, we recognize that this sampling biased can influence our ability to detect some of the ecological drivers' effect.

We also recognize that the limited predictive power of some of our models can constraint our inferences. The GI can also be influenced by unaccounted historical factors (e.g. historical bottlenecks, diseases, etc.), population-specific fecundity, geographical isolation, population structure (e.g. sex-ratio, age structure). However, these factors are largely unattainable/unavailable for a wide geographical area and, therefore, their effect impossible to accommodate in a large-scale approach, such as ours. Regardless, of the potentially confounding effect of the unaccounted factors, our inference remains valid for the covariates included in our models and supports the landscape composition importance in shaping wildcat's GI across metapopulations.

5. Conclusions

Ours results highlighted that, at the broader scale, European wildcat GI is strongly influenced by habitat quality, particularly linked to the availability of higher forest cover and the amount humanized patches. However, our approach also showed that the genetic status of European wildcats is metapopulation-specific, hence conservation measures should be specifically tailored to address each metapopulations' explicit threats.

The main goals of Protected Areas include securing the survival of endangered species, as well as biodiversity as a whole, especially in man-dominated landscapes. Therefore, providing insights into protected area effectiveness to guarantee a species GI arises as a valuable tool to guide decision-making processes and contribute to management solutions. Our results demonstrate that European protected areas' network, tend to host 'pure' European wildcats, and are therefore contributing to its conservation. However, protection level and habitat quality emerge as particularly relevant when the landscape matrix hosts high wildcat-domestic admixture levels, such as in the Iberian metapopulation. Moreover, data from the Scottish metapopulation revealed a serious scenario regarding wildcat GI and it long-term survival, highlighting that urgent direct actions (e.g. reintroductions and control/sterilization of domestic/feral cats) are needed to conserve this threatened species. Whether high hybridization is the cause or the consequence of depressed European wildcat populations remains to be clarified, but it is clear that domestic cats' neutering, especially in landscapes with or in the vicinity of protected areas where wildcat populations exist is key to ensure wildcats' GI in the long-term.

This study provides another piece for the European wildcats' conservation puzzle, and contributes to assess the role of Protect Areas and landscape patterns to the silent deterioration of this small felid. Future studies should try to incorporate demographic and historical parameters in the analysis to provide deeper insights on the drivers of European wildcat's genetic integrity, allowing to optimize conservation actions and mitigate the main threats to this species.

Chapter II

Effectiveness of protected areas for wildcat conservation: case study of Mediterranean Natural Park (Montesinho - Northeast Portugal)

Matias, G.[1], **Rosalino, LM.**[1], **Rosa JL,**[2] **Monterroso, P.**[3]

[1] cE3c - Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal
[2] Instituto da Conservação da Natureza e Florestas, Parque Florestal, 5300-000, Bragança
[3] CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quinta 7, Vairão 3385-661 Portugal

Abstract

Population density data on depleted and endangered wildlife species is an essential tool to assure their effective management and, ultimately, conservation. The European wildcat is an elusive and threatened species inhabiting the Iberian Peninsula, with fragmented populations and living in low densities. We developed spatial capture-recapture (SCR) models, based on camera-trap data, to provide the first estimate of wildcat density in Portugal, and optimize this felid's detection protocol. The study was implemented in Montesinho Natural Park (NE Portugal), where we identified 9 individuals, with a total effort of 3477 trap-nights. The density estimate was 0.119 ± 0.065 wildcat/Km^2 for the entire study area. The wildcat population density tended to increase in sites far from humanized areas, often linked to lower human disturbance and domestic cat presence. This density estimate is within the range of values estimated in others published for protected areas elsewhere in the Iberian Peninsula. However, our estimates can be considered low in the European context, which highlights that European wildcats may be living in low population densities across the Iberian biogeographic region. During our survey, no domestic cat was detected, which can be considered an indicator of a rare/low admixture rate between the two species. We provided evidence that Montesinho Natural Park may be a suitable protected area to host a healthy wildcat population, and thus be an important PA in this species' conservation context.

Keywords: European wildcat, Population density, Spatial capture-recapture model, Conservation

1. Introduction

One of the fundamental requirements to efficiently manage and conserve any wildlife species is to have available robust local or regional abundance and/or density estimates (Stephens et al. 2015). Moreover, population density is one of the essential metrics to establish and comprehend the populations' variation or trend (Wright and Hubbell 1983), and to assess extinction risk (Purvis et al. 2000). Likewise, the International Union of Conservation of Nature criteria for defining specie's threaten status depend highly on population size (IUCN 2012), highlighting the importance of these metrics to species conservation. While these parameters are often difficult to obtain (Marques et al. 2013), the development of reliable and precise population's density is essential to conservation policies. Incorrect population estimates, or speculations and distrust linked to inaccurate values may affect conservation strategies (Hayward et al. 2015; Popescu et al. 2016). Those threats may lead to inefficiencies in conservations actions and inaccurate populations status (López-Bao et al. 2018). For threatened/rare species accurate density data is particularly important, especially because even with intensive surveys, low detectability makes it difficult to have good estimates (Foster and Harmsen 2012; Sollmann et al 2014).

The European wildcat (*Felis silvestris silvestris*, Schreber, 1777) is a good example of such as species for which density estimates are often unavailable. It is a small felid with a large distribution range, natively occurring from the Iberian Peninsula to Eastern Europe, and British Isles (Nowell and Jackson 1996, Yamaguchi et al. 2015). The current distribution of the European wildcat is fragmented across much of its range as a result of significant declines (Yamaguchi et al 2015), mainly due to habitat loss, roadkill, disease transmission, and hybridization with his domestic counterpart (Beaumont et al. 2001, Macdonald et al. 2010; Yamaguchi et al. 2015). The European wildcat is listed as 'Least Concern' by the IUCN red list, but is included in Habitat and Species directive (European Council Directive 92/43/EEC, Appendix IV of 21 May 1992), Bern convention (Appendix II, 1979) and CITES (Appendix II, UNEP-WCMC, 2013), since this species it is considered a threatened species at several regions of Europe e.g. Portugal, Germany, Switzerland, (Nussberger et al. 2018). This legal protection has reduced and locally eliminated much of the threats mentioned above, reducing the causes of its decline (Streif et al. 2012), leading to a recovery of a few wildcat populations across Europe (Steyer et al. 2016; Nussberger et al. 2018).

The apparent recent turnover in European wildcats' population trends (Steyer et al. 2016), with populations reaching high densities in some European regions (e.g. 0.29 ind/Km2 in Switzerland; Kéry et al. 2010, and 0.28 – 1.36 ind/Km2 in Sicily; Anile et al. 2012, 2014), appears not to be occurring in Iberian Peninsula. Here the wildcat populations are suspected to keep decreasing (Cabral et al. 2005; Lozano et al. 2007; Sobrino et al. 2009, Gil-Sánchez et al. 2020), due to loss of habitat, habitat fragmentation, human persecution and loss of its main prey in Mediterranean region, the European rabbit (Gil-Sánchez et al. 1999; Lozano et al. 2003, Gil-Sánchez et al. 2020). Moreover, the low densities of these species in Iberia (0.038 – 0.069 wildcat/Km^2; Gil-Sanchéz et al. 2020; Ferreras et al. in prep) highlight that European wildcat might be undergoing a significant population decline. In Portugal, the European wildcat is listed as 'Vulnerable' in the Portuguese red list (Cabral et al. 2005), with a suspected population decline $\geq$ 30% during the last 24 years as a result of the deterioration of habitat quality, populations fragmentation and hybridization with domestic cats. These threats are still acting in Iberia, and the profound gap that persists regarding the status, abundance and trends of the remnant Iberian wildcat populations preclude the implementation of efficient conservation actions and limits the capacity to engage the public administration and civil society in reverting this potentially ongoing silent extinction. The essential role of the Iberian protected areas in this peripheral metapopulation (Chapter I) highlight the need to determine the species' trend specially at protected areas.

Estimating the density or abundance of mammalian carnivore populations is a challenging task due to their typically large ranges, low densities, and elusive and nocturnal behavior, which deems detection by direct observation extremely difficult (Karanth et al. 2006). Remotely triggered cameras (henceforth camera-traps) have emerged as successful tool to overcome these limitations in the study of carnivore density, and are currently of widespread use (Sollmann et al. 2011; Rich et al. 2017; Steenweg et al. 2017). Camera-traps are considered a noninvasive method i.e. not involving animal's capture, and allow deployment designs that encompass large areas, permitting to feasibly study elusive and cryptic species, with large home-ranges and occurring at low densities (Noss et al. 2012).

Recently, new analytical tools have emerged to cope with the large amounts of data produced by new field methods such as camera-traps, particularly under the framework of hierarchical models (Royle et al. 2014; Kéry and Royle 2015). Spatial capture-recapture models (SCR) in particular, use a spatial point process to determine the abundance and distribution of individual's activity centers, i.e. individual's home range center during the survey (Efford 2004; Borchers and Efford 2008; Royle and Young 2008; Efford et al. 2009; Royle et al. 2014). SCR models also make inference about individual's capture probability as a function of the distance between the detectors (i.e. camera-traps in our study) and the activity centers (Royle et al. 2013). This capture probability includes two main parameters: the baseline detection probability (p_0) at its activity center, and the scale parameter (σ), which is the average of individual movement rate (Royle et al. 2014).

Although several studies have recently shed light into some important aspects of European wildcat's ecology in the Iberian metapopulation (Sarmento 1996; Sarmento et al. 2006; Oliveira et al. 2008a, b; Monterroso et al. 2009; Oliveira et al. 2018), robust assessments of its abundance, density range and trends are still missing. This study aims to fulfill this information gap by estimating European wildcat population density and abundance, as well assessed the spatial drivers of density variation, using SCR modeling approach based on camera trapping data from a Portuguese protected area (Montesinho Natural Park). Secondly, the Iberian protected areas revealed to have a significant role for wildcat's conservation (Chapter I), and thus we aim to assess if this protected area still hosts a wildcat population healthy enough to play a role in this species conservation in Iberia. To fill the study aims, we formulated two hypotheses: i) European wildcat density at Montesinho Natural Park will be within the range estimated for other Iberian protected areas, and ii) European wildcat density will be negatively associated with humans and anthropic activities presence and positively associated with native forest (see table 2.1).

2.Material and methods

2.1 Study area

The study was carried out in Montesinho Natural Park (*ca*.748 Km^2), Bragança, Portugal (Figure 2.1). The Park include a European Union Natura 2000 Site (Montesinho-Nogueira; PTCON0002). The Park is located in a typically Mediterranean region, dominated by a natural wooded landscape within a mountain region, ranging from 438 to 1481 m.a.s.l. The annual average monthly temperature ranges between 3°C and 21°C, and the precipitation 600 and 1500mm (Castro et al. 2010). The Park is characterized by a diverse vegetation, that included several forest species such as holm oak (*Quercus rotundifolia*), Pyrenean oak (*Quercus pyrenaica*), sweet chestnut (*Castanea sativa*) and different Pines species (*Pinus silvestris, Pinus nigra* and *Pinus pinaster*). The understory vegetation is dominated by gorse (*Ulex europaeus* and *Ulex minor*), gum rockrose (*Cistus ladanifer*) and heather (*Erica* spp.). The riparian vegetation is mainly composed by ash (*Fraxinus angustifolia*), white willow (*Salix salviifolia*), common alder (*Alnus glutinosa*) and black poplar (*Populus nigra*; Castro et al. 2010). Furthermore, the area includes some small villages (i.e, < 8000 people; Valente et al. 2014), and is crossed by two main rivers, Sabor and Onor. This region contain a highly diverse carnivore community, including threatened species, such as the Iberian wolf (*Canis lupus signatus*) and European wildcat (*Felis silvestris silvestris*), and species classified as 'Data deficient' by the Portuguese Red list (Cabral et al. 2005), such as the European polecat (*Mustela putorius*), pine marten (*Martes martes*), and ermine (*Mustela erminea*).

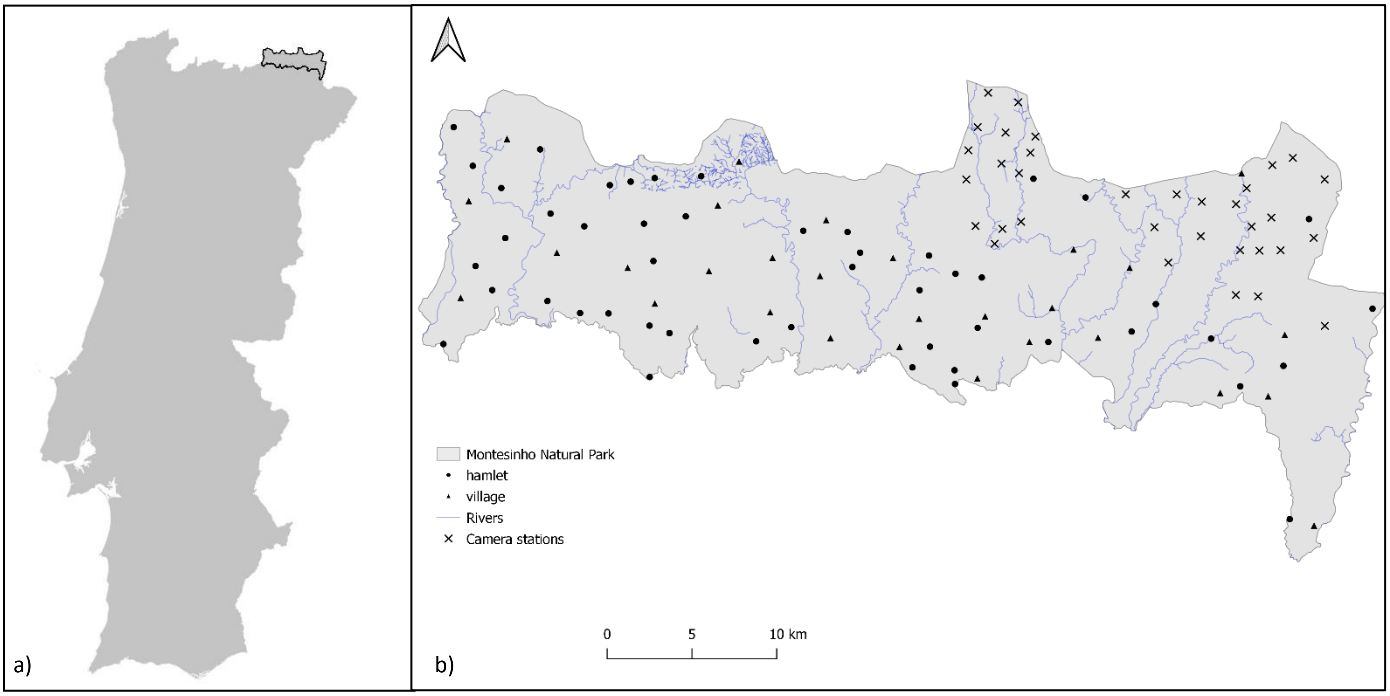

Figure 2.1. Location of the study area in Portugal - a) Map of Portugal with Montesinho Natural Park limits; b) Montesinho Natural Park limits, with the location of hamlets, villages, rivers and camera stations, represented by black circle and triangle, blue line and black cross, respectively.

2.2 Data collection

Fieldwork was conducted between October 2019 and March 2020. We deployed 34 camera-traps equipped with heat and motion PIR sensor. Three camera models were used: Cuddeback Model H-1453 (n = 14, Cuddeback Digital, De Pere, WI, USA), Moultrie M-990i (n = 14, Moultrie Products, Alabaster, AL, USA) and Browning Strike Force HD Pro (n = 20, model BTC-5HDP, Prometheus Group, Birmingham, AL, USA), which were placed at an inter-camera distance of 1590 ± 650 m (range: 1001 – 4344 m). Camera models were changed (n= 14) during the fieldwork due to logistic constrains. Cameras were attached to wooden sticks or tree trunks, at 40–80 cm above ground level, to achieve the best angle for capturing wildcat's pelage characteristics. We set cameras to take 3 consecutive photos per trigger event, with a delay of 10 seconds between events, recording the date and time of each photograph. All stations were lured with valerian extract and domestic cat urine, deployed on a wood stick 2m from the camera. This lure is known to be an effective attractant for cats (Monterroso et al. 2011; Steyer et al. 2013; Ferreras et al. 2018). We checked the cameras every 15-20 days, to replace SD cards and lure, exchange batteries and troubleshooting. Cat records were classified as potential European wildcat based on pelage characteristics

(Figure 2.2), as defined by Kitchener et al. (2005) and Ragni and Possenti (1996). Cats were identified to the individual level by coat morphology (e.g. number, dimension and shape of spots and bands on the limbs and trunk; Figure 2.2). Due to logistics constrains, we only used one camera per station; thus, two datasets were generated (left and right flank) to create the individual's recapture history. A detection record was considered as independent event if a record of the same species in the same camera had a minimum time interval greater than 30 minutes (unless animals were undoubtedly individually distinguishable; Rich et al. 2017).

Figure 2.2 – Example of individual identification using the coat pattern of the right leg, and body (e.g. shape, position and number of spots), at Montesinho Natural Park.

2.3 Drivers of density variation

The candidate covariates for explaining the density variations in the study area were separated into two ecological types: land cover and disturbance (Table 2.1). Despite our knowledge that prey availability is a key factor in determining European wildcat's presence (Sarmento 1996, Lozano et al. 2003; Monterroso et al. 2009; Silva et al. 2013a,b), such data was not available for our study area and, therefore, could not be tested in our models. Land cover and disturbance data were obtained from the Global Land Cover (raster; 100 m resolution; Buchhorn et al. 2020). To avoid overparameterizing in our models (see below), we tested a single land cover covariate – distance to the nearest forest edge –, hypothesized as the most relevant for European Wildcat presence at Mediterranean region (Lozano et al. 2003, Sarmento et al. 2006; Monterroso et al. 2009, Oliveira et al. 2018; See Chapter I). Despite our knowledge that shrubs are an important habitat feature to wildcat presence in Mediterranean region (Oliveira et al. 2018), native forest seems to be a significant key factor for wildcat presence (Sarmento et al. 2009; Oliveira et al. 2018) and genetically 'pure' populations in Iberian metapopulation (Chapter I). Forest was calculated as the sum of all forest types from Global Land Cover raster. To incorporate in our analysis the human-induced disturbance as a surrogate of unsuitable habitat and domestic cat presence (Germain et al. 2008; Ferreira et al. 2011), we selected one covariate: distance to human-buildup areas. We included the detector position i.e. on or off animal/human trail as a binary covariate to account for this effect on the baseline detection probability. The distance-based covariates were estimated as the Euclidean linear distance from each detector (n = 34) to the nearest landscape feature edges.

All spatial analysis were estimated using the software R Studio© version 1.1.463, R version 3.5.3 (R Development Core Team 2017)

2.4 Statistical analysis

To avoid model overparameterization, we use the criterium of a maximum 1:10 ratio between the number of estimated parameters (covariates coefficients) and sample size (n= 34), and thus, we used two covariates as drivers for density estimate (e.g. distance to the nearest forest and human-buildup patch edges) and one to detection probability (detector on/off trail) (Burnham and Anderson 2002; Table 2.1). Continuous predictors were scaled to ’z-scores’ to avoid dispersion bias and to facilitate numeric convergence of the models, and coefficient comparisons (Shiffler 1988; MacKenzie et al. 2005).

To test for multicollinearity among continuous covariates (e.g. distance to forest and human-buildup, and detector position), we performed a nonparametric Spearman’ correlation, using the psych R package (Revelle 2015).

We applied a SCR approach, combining both individual’s recapture history (left and right side) in the same model, to estimate wildcat’s density and detection probability using the oSCR package for R software (Sutherland et al. 2019). Conceptually, the SCR undertakes that each individual from a population has an activity center i i.e. individual’s home range center during the survey, (Royle et al. 2013), and the encounter probability is express by the Euclidean distance between i and a detector (e.g. camera trap) location j. The SCR methods considered that i follow a spatial point process (Royle et al. 2013). The spatial distribution of the activity centers i is assumed to be constant following a homogenous distribution $i \sim \text{Uniform}(S)$, wheres S is the ‘state-space’ (Royle and Young 2008). S is the area encompassing all detectors (e.g. camera-traps) and neighboring area (i.e. including unsampled areas of the survey), large enough that theoretically comprise all individuals that possibly have been detected in the survey (Royle et al. 2013). The oSRC framework uses two primary data, the Trap Deployment File (TDF) and Encounter Data File (EDF). The TDF contain, at least, the detector’s location and name. Besides, TDF can contain the detector operation data (i.e. binary data, 1- operational, 0 – not operational), and covariates from detector’s location. EDF contains the individuals’ encounter history data. EDF data is composed by the unique individual identifier (e.g. I1, I2, etc.), the detector name and the occasion when the individual was record (Sutherland et al. 2019). Such model assumes that within a state-space S, a total of N individuals has their specific activity center (i.e. individual’s home range center) during the survey. Moreover, the baseline probability of encountering an individual (p_0) by a detector is a function of the distance between the detector and the individual’s activity center. This function permits estimating the average of individual movement rate (σ) within S in relation to its activity center, such that the probability of detecting an individual decreases with increasing distance between its activity center and detector position (Efford et al. 2009; Royle et al. 2014). Therefore, density estimate D can be measure as $D = N/S$. These models provide a spatial perspective accounting for trap location and animal movement, representing an upgrade from traditional capture-recapture models (Royle et al. 2015).

The state-space resolution must be thin enough to approximate the continuous space relatively to the species’ movement (Royle et al. 2013). To estimate the state-space, we created a buffer with 3σ, where σ

the individual movement during the survey, is related to the extent of space used by each individual, which is typically determined by half of the mean maximum distance moved by captured individuals (Royle et al. 2013). The state-space resolution was a 1000m grid cells, encompassing the minimum know wildcat home-range in Mediterranean region (1.22 km^2; Oliveira et al. 2018). Both parameters (Density and detection probability) were estimated over 157 occasion, representing the total number of days that detectors were deployed and operational at Montesinho Natural Park.

Finally, we used maximum likelihood methods to jointly estimate each model parameters (Density and detection probability). The maximum likelihood allows to compare models formally using Akaike information criteria (AIC; Sutherland et al. 2019). Thus, we created a set of candidate models including all covariate combinations and a null model. Model parameters were estimated using a maximum likelihood estimation (MLE) approach using the oSCR package (Sutherland et al. 2019) in R. Model selection was based on Akaike's Information Criterion for small samples size (AICc), and on Akaike model weights (Burnham and Anderson 2002). Models with a ΔAICc < 7 (measure of each model performance relative to the best model; Burnham and Anderson 2002) were considered top models and having substantial support (Burnham and Anderson 2002). Whenever more than one model comprised a ΔAICc < 7, the model-averaged coefficients were calculated using the 'ma.coef' function of the package *oSCR* (Sutherland et al. 2019) to obtain the best estimates of covariates' effects from the top model(s) set. As an additional measure of each covariate's effect on the European wildcat's density and detection probability, the relative variable importance (RVI) was calculated as the sum of Akaike weights (ωi) of all models that included that covariate over the total ωi of the considered model set (Arnold 2010). RVI is scaled between 0 and 1, with values near 1 indicating a high support for a covariate to be highly influential to response variable variability, while RVI near 0 indicates little support (Burnham and Anderson 2002).

All statistical analysis were estimated using R Studio© version 1.1.463 and R version 3.5.3 (R Development Core Team 2017).

Table 2.1. Candidate covariates used in the modelling procedure to assess wildcat's density (D) and detection probability (p0), with the corresponding acronym, units and observed range, hypothesis reasoning, description, source, and references supporting the presented reasoning.

Model	**Covariate**	**Range and Units**	**Hypothesis**	**Description**	**Source**	**References**
Density (D)	Distance to Forest Patches **(D_For)**	[0.00 – 0.63] km	European wildcat density increases in areas near forest patches. This habitat is expected to be suitable for wildcat's population, since it as a higher prey availability and refuge, contributing to a higher wildcat density.	Euclidean distance from near forest edge	Global Land Cover https://lcviewer.vito.be/	Klar et al. 2008 Monterroso et al. 2009 Oliveira et al. 2018
	Distance to Human Buildup Patches **(D_urb)**	[0.17 - 8.53] Km	European wildcat density decreases near humanized regions. These regions are avoided by wildcats due to human disturbance and possible competition with domestic cat.	Euclidean distance from near urban area	Global Land Cover https://lcviewer.vito.be/	Klar et al. 2008
Detection (p0)	Trail **(trail)**	0 / 1	Detection probability increases when detectors are located in or at edge of trails. Trails can be used as energy efficient travel and increase scent mark encounter rate	On/off trail	Detector position	Kolowski and Forrester 2017 Rafiq et al. 2020 Bruggeman et al. 2007

3. Results

3.1 General results

We registered 24 independent European wildcat records, acquired from 9 stations over a total sampling effort of 3477 trap-nights. From the 24 independent wildcat records, 20 were valid for individual identification. These detection records resulted in 5 records from the left side and 9 from the right, allowing us to identify a minimum of 9 individuals. A total of 2457 independent detections from other wildlife were obtained. The red fox (*Vulpes vulpe*s, n = 767), European roe deer (*Capreolus capreolus*, n = 614), and red deer (*Cervus elaphus*, n = 593) were the species with a highest number of independent records, respectively (Table 2.2). The analysis of collinearity did not reveal any significant correlation among potential wildcat covariates.

Table 2.2. Number of independent events for all species detected and proportion of occupied stations, at Montesinho Natural Park (ranked by the number of events).

Species	Independent events	Occupied stations (%)
Red Fox (*Vulpes vulpes*)	767	100.0
Roe deer (*Capreolus capreolus*)	614	91.2
Red deer (*Cervus elaphus*)	593	91.2
Wild boar (*Sus scrofa*)	205	79.4
European rabbit (*Oryctolagus cuniculus*)	103	11.8
Iberian hare (*Lepus granatensis*)	75	20.6
Iberian Wolf (*Canis lupus signatus*)	48	38.2
Marten (*Martes* sp*)	47	35.3
European badger (*Meles meles*)	40	35.3
European wildcat (*Felis silvestris silvestris*)	24	26.5
Common genet (*Genetta genetta*)	22	17.6
Stone marten (*Martes foina*)	14	17.6
Red squirrel (*Sciurus vulgaris*)	14	11.8
Pine marten (*Martes martes*)	8	23.5
Total	2574	34 stations

*Unable to distinguish between stone (*Martes foina*) and pine marten (*Martes martes*)

3.2 Density and detection probability

The mean maximum distance moved by the European wildcat in our study area was $\sigma = 1430.4$ m. Thus, the buffer used to create the state-space was 4.3 km (3σ; Sutherland et al. 2019), resulting in a state-space of *ca.* 423 Km^2 (Figure 2.1, Appendix).

We generated eight models (Table 2.3), containing the two covariates (distance to the nearest human-buildup and forest edge) influencing the European wildcat's density (*D*) estimator, and the detector position for detection probability (p^0) estimator. Despite all models comprised the $\Delta\text{AICc} < 7$ criterion, thus were considered top-supported models, the null model was considered the best model, with $\omega i = 0.243$ (Table 2.3). The contribution of covariates tested for the *D* – distance to forest edge and human-buildup – and for p_0 – detector position - estimates was not sufficient enough to improve the models, when compared to the null model.

Table 2.3. Models explaining wildcats' density (*D*), detection probability (p_0) variation and spatial scale parameter (sig), which included the covariates distance to forest, distance to human buildup, and detector position. The degrees of freedom (k), log-likelihood (LogL), Akaike's Information Criterion for small samples (AICc), variation between the AICc from each model and the lower AICc value (ΔAICc), the Akaike weight (ωi), and the cumulative Akaike weight (Cumωi) are presented.

Model	**K**	**LogL**	**AICc**	**ΔAICc**	**ωi**	**Cumωi**
D (~ 1) p_0 (~ 1) sig (~ 1)	3	199	404	0.00	0.243	0.24
D (~ 1) p_0 (~ trail) sig (~ 1)	4	198	404	0.22	0.218	0.46
D (~ d_urb) p_0 (~ trail) sig (~ 1)	5	198	405	0.85	0.159	0.62
D (~ d_urb) p_0 (~1) sig (~1)	4	199	406	1.77	0.100	0.72
D (~ d_for) p_0 (~1) sig (~1)	4	199	406	1.83	0.097	0.82
D (~ d_for) p_0 (~ trail) sig (~1)	5	198	406	2.12	0.084	0.90
D (~ d_for + d_urb) p_0 (~ trail) sig (~1)	6	197	407	2.78	0.061	0.96
D (~ d_for + d_urb) p_0 (~1) sig (~1)	5	199	408	3.70	0.038	1.00

Although with relatively low precision, both state covariates (distance to forest and distance to urban) appear to have a positive effect, with a relative variable importance (RVI) of 0.28 and 0.36, respectively (Table 2.4). Camera placement presents a RVI of 0.52 (Table 2.4) and a positive effect on wildcat detection probability (i.e. detector on trail increase the wildcat detection probability; Table 2.4; Figure 2.3).

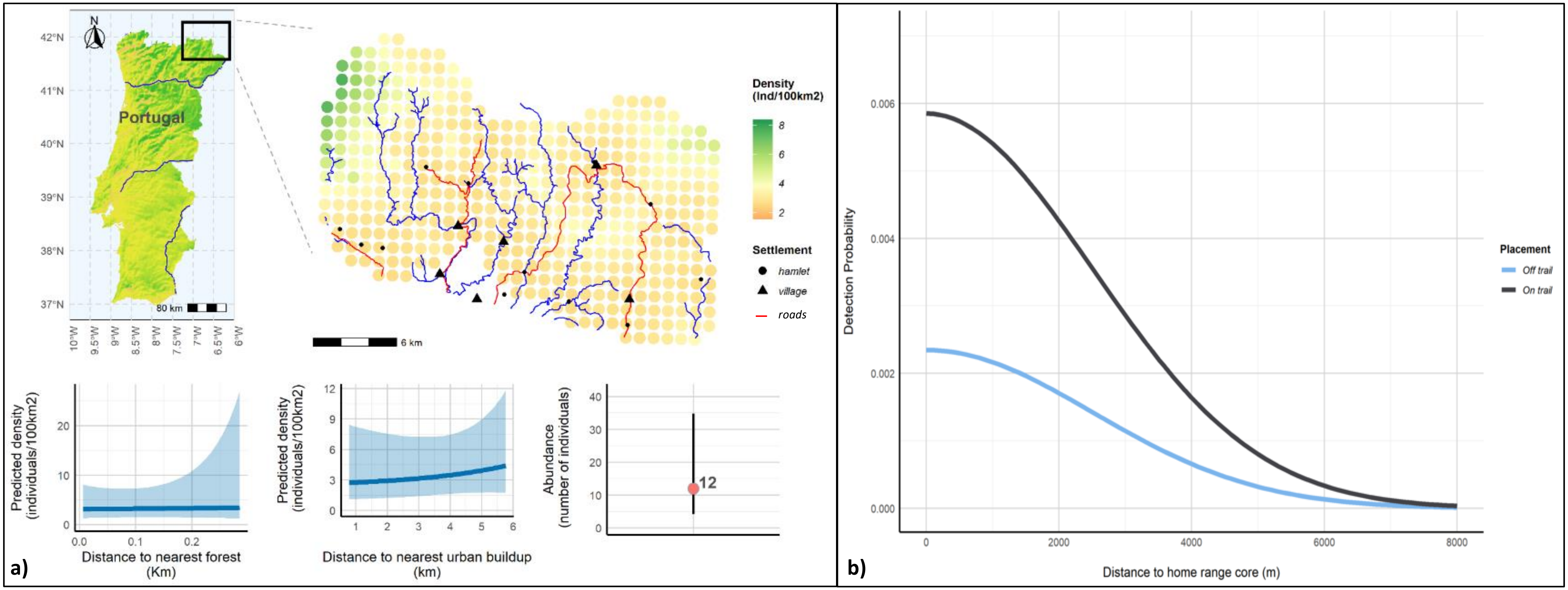

Figure 2.3. (a) -Predicted wildcat density (wildcat/100km^2), together with the location of the main rivers (blue lines), roads (red lines), hamlets (black dots) and villages (triangles) (upper right). Predicted wildcat density variation with the distance to the nearest forest patch and urban buildup patches and the number of individuals in the state space (bottom from left to right). (b) – Representation of the wildcat detection probability with the distance to home range core, when the detectors are on trail (black line) or off trail (blue line).

The mean European wildcat's density estimate (*D*) obtained from the model average was 0.119 ± 0.065 wildcats/Km^2 ranging from [0.04-0.34], resulting in an estimate of 12 wildcats for our study area, ranging from 4 to 34 (Figure 2.3). The baseline detection probability (p_0) was 0.002 ± 0.001 [0.001-0.006] and 0.006 ± 0.002 [0.002-0.014], for detector located off and on trail, respectively (Figure 2.3). The p_0 indicates the probability of detecting a European wildcat, when deploying one camera throughout one day at the center of individual's activity center (Sutherland et al. 2019).

Table 2.4. Covariates included in the top models set produced to explain the wildcats' density and detection probability, including the spatial scale parameter (Sig), their untransformed estimate, standard error (SE), and relative importance (RVI) (variables acronyms are described in Table 2.1).

Parameter	**Estimate**	**SE**	**RVI**
D0 (Intercept)	-3.44	0.42	1.00
P0 (Intercept)	-5.73	0.45	1.00
Sig (Intercept)	7.70	0.23	1.00
P0 ~ trail	0.73	0.50	0.52
D ~ d_for	0.05	0.37	0.28
D ~ d_urb	0.36	0.39	0.36

4. Discussion

Density and abundance are two of the most important population parameters needed for an effective species conservation. The lack of such information for elusive and threatened species, such as European wildcat, can be critical since the assessment of the efficacy of conservation measures is based on the analysis of population trend. Here, we provided a density estimate for the wildcat population in Montesinho Natural Park (NE Portugal), a region within one of the most critical wildcat metapopulations (Iberian) (Chapter I). This study also illustrates that data from camera-trap has the potential to estimate densities of an elusive species, and therefore, be a pivotal tool to generate baseline information to delineate management and conservation strategies.

The population density estimate derived from SCR model reached 0.119 ± 0.065 wildcat/Km^2. This wildcat density, which is the first estimate for Portugal, is within the range of values already estimated for other areas in Iberian Peninsula (Sayol et al. 2018; Gil-Sanchéz et al. 2020, Ferreras et al. in prep; Figure 2.4). However, it shows a low-density scenario when compared to other metapopulations of Europe (e.g. Italian and Northwest continental; Figure 2.4). Nevertheless, such inter-study comparisons need to be done cautiously, since the analytical methods and data used to estimate densities differ. Furthermore, these discrepancies may also be related to a misidentification of ´pure´ wildcat and hybrids. In Scotland, Kilshaw et al. (2015), used SCR models and determined a wildcat's density of 0.68 wild-living cats/Km^2, but this estimate also included hybrids. In our survey no phenotypic domestic cats were detected, and all captured individuals presented phenotypic characteristics of 'pure' wildcats (Kitchener et al. 2005). However, Ferreras et al. (in prep), in Spain, captured five individuals with ´pure´ characteristics, one of which was genetically identified as an F1 hybrid. If this pattern occurs in other populations, it could be a serious threat to wildcat conservation, since the true density of the species might be lower.

This peripherical metapopulation (Iberian) has high values of admixture between the European wildcat and its domestic counterpart, with an average observed genetic integrity of *ca.* 62% (Chapter I), thus the hybridization could be a severe threat in this region of wildcat's range. This scenario in conjunction with low population's density (Gil-Sanchéz et al. 2020, Ferreras et al. in prep), highlights that this peripheric population may be undergoing a silent extinction. Moreover, low wildcat density may reduce the likelihood of encounters between male and female wildcats during the mating season. This challenging situation regarding mate selection may increase the search of domestic cats to mate (desperation hypothesis; Hubbs, 1955), promoting hybridization (Bohling and Waits, 2015). Despite the legal landscape protection level at Montesinho, the predicted value of the average wildcat's genetic integrity for this protected area was *ca.* 47% (Figure 1.1, Appendix), ranging between 19% and 86%. Thus, it is possible that hybridization can occur in and around the Park, in regions with higher anthropic influence, and consequently domestic cat presence, and with lower proportion of native forests (Chapter I). Furthermore, the spillover of diseases from domestic cats to wildcats are an instrumental demographic factor for small and isolated populations (Ferreras et al. in prep). The seroprevalence of feline leukemia virus can act as a negative factor for small population of threatened felines (Millán and Rodriguez 2009; López et al. 2009) and found in several Iberian wildcat populations (Millán and Rodriguez 2009; Duarte et al. 2012). Despite the presumed absence (or low abundance) of domestic cats inside wildcat's occupancy range in Montesinho Natural Park, due to the absence of domestic cat detections during the survey, we detected several domestic cats in and around the human settlements within MNP, highlighting that diseases from domestic cats could pose a threat to wild taxa in this protected area if contact is frequent or intensified. This domestic cat presence nearby hamlets can also act as a hybridization promoter that can induce extra pressure on this wildcat population survival.

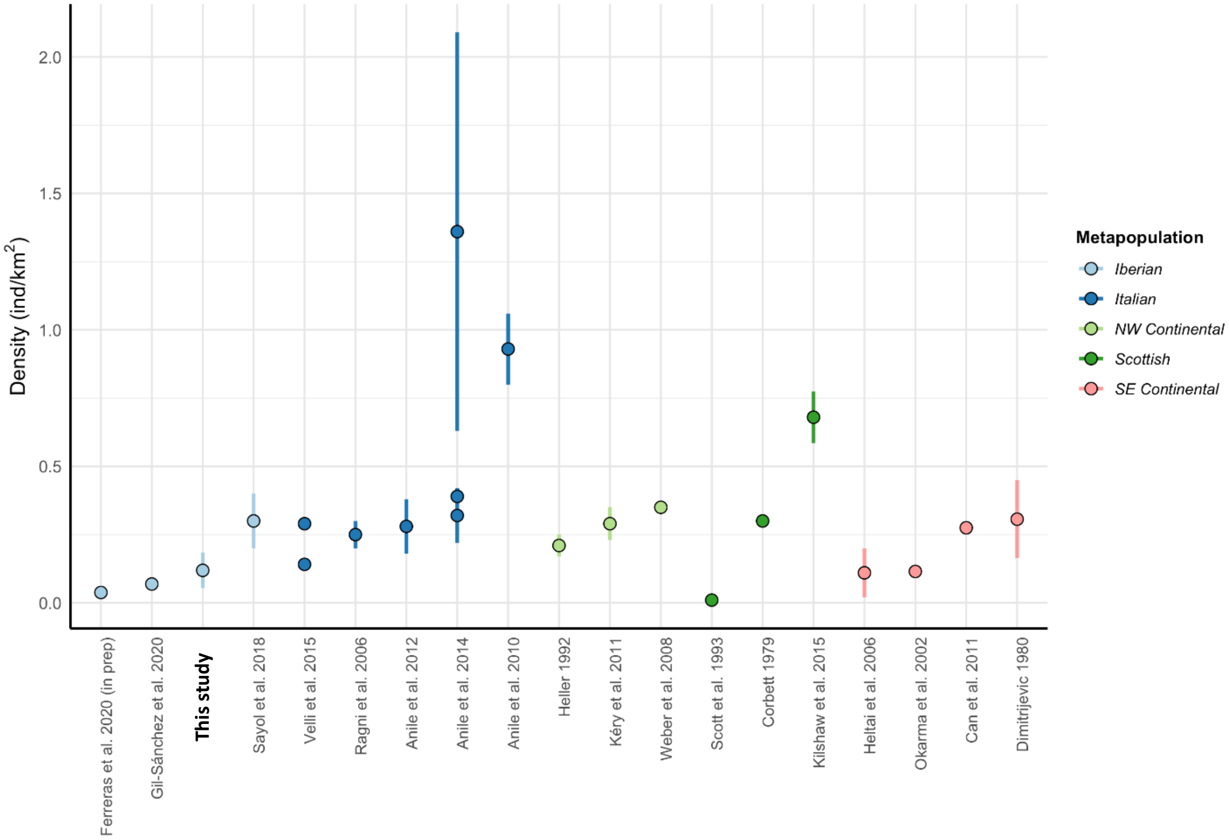

Figure 2.4. European wildcat's density (Ind/Km^2) gathered from different wildcat's metapopulation of Europe with the respective standard deviation. Some studies have not included the standard deviation. This study is presented in bold.

Interspecific competition with the Iberian lynx may constrain wildcat's abundance and occupancy in Iberia (Soto and Palomares 2014; Monterroso et al. 2020). However, although the Iberian lynx is absent from Montesinho Natural Park, it is possible that the presence of Iberian wolf (*Canis lupus signatus*) could also influence wildcat's spatial patterns. In this region, Figueiredo et al. (2020) showed that 6% of the wolf's diet was composed by domestic/wild cats. Furthermore, direct human persecution and road mortality should be low since the region shows strict wildlife protection (i.e. Natural Park) and low human presence (Valente et al. 2014). These patterns may act synergistically, resulting in a slightly higher wildcat density than observed elsewhere in Iberia (Figure 2.4). Furthermore, prey availability may also be concurring to the higher densities registered in our study area when compared to estimates from other studies in Iberian Peninsula (Gil-Sánchez et al. 2020 and Ferreras et al. in prep). Wildcat's main prey in the Mediterranean region is the European rabbit (Fernández 2005) and high rabbit density has been suggested as a key driver of wildcat's population density in Sicily (Anile et al. 2012) and in other Portuguese populations (e.g. south eastern Portugal; Monterroso et al. 2009). The European rabbit was the fourth species with highest number of independent events (n = 103; Table 2.2) in our study area, suggesting that this mammal could be abundant (although patchily distributed, as it was only detected in 12% of our cameras).

Inversely, the reduced estimated density when compared to other European locations, may be related to a high ungulate's abundance registered inside the park (ungulates were three of the four species with more events and present in more than 79% of sampling sites; see table 2.2). Ungulates indirectly may cause low wildcat abundance (Lozano et al. 2007), by reducing wildcat's prey (European rabbit and small mammal) abundance (Navarro-Castilla et al. 2017). For example, the wildcat's density in Sicily (0.28 – 1.36 wildcats/km^2; Anile et al. 2010,2012,2014) is above our estimate, and this island has a low abundance of ungulate, since only two species exist - Wild boar (*Sus scrofa*) and Fallow deer (*Dama dama*) - and they were reintroduced recently (in 1980 ; Massa and La Mantia 2007). Alternatively, low wildcat density may also be related to small mammal's density and availability at Iberian region. The Mediterranean region has relatively low rodent density and diversity when compared to central and eastern Europe (Krystufek and Griffiths 2002). Thus, the higher prey availability and density may promote wildcat's density at these European regions. Moreover, in the Mediterranean region, the wildcat's main prey is the European rabbit (Lozano et al. 2006). In such context, rodents are significantly less consumed when rabbit is present and available (Malo et al. 2004). A new emergence of rabbit hemorrhagic disease virus (Lagovirus europaeus/GI.2) reduced the rabbit abundance in Iberia (Monterroso et al. 2016). This decline (*ca*. 60-70%) in Iberia, had a significant negative impact in the Iberian lynx (*Lynx pardinus*) and Spanish Imperial eagle (*Aquila adalberti*) fecundities (Monterroso et al. 2016). The same scenario may be possible for Iberian wildcat's population, leading to this low-density value.

The result of our density model revealed that the distance to human-buildup patches has a positive effect on wildcat's density (Figure 2.3), i.e. higher wildcat's population density are registered in regions far away from humanized areas, corroborating our initial hypothesis. However, despite the small RVI, the distance to nearest forest patch edge revealed an unanticipated positive effect, as wildcat's in Mediterranean region set their home-ranges near native forests (Oliveira et al. 2018; Table 2.4). Forest habitats have been documented as an important landscape component for the European wildcat (Klar et al. 2008; Lozano 2010; Beugin et al. 2018; Jerosch et al. 2018). This habitat may provide key resources to wildcats e.g. high prey, refuge and shelter availability, and low human disturbance, (Sarmento et al. 2006;Germain et al. 2008; Klar et al. 2008; Oliveira et al. 2018). Inversely, the European wildcat is negatively associated with human presence and consequently human-related activities (Klar et al. 2008; Germain et al. 2008; Klar et al. 2009; Oliveira et al. 2018). During the survey, we detected a high abundance and presence of domestic dogs and cats surrounding the villages, which can add a significant disturbance effect constraining wildcat presence (Klar et al. 2008). However, these results need to be interpretated with caution, since the null model was considered the best model, thus more supportive (Table 2.3).

Finally, our results evidenced that there is an increase in detection probability when cameras are deployed on animal/human tracks. Such improvement in the detection probability of this threatened species by simply changing the sampling protocol is particularly important when dealing with low-density populations. A higher detectability on trails may be related to higher animals' visibility, since vegetation cover may challenge the detection of small and intermediate-sized mammals (Kolowski et al. 2017). Trails are also known to be used by predators and prey (Mann et al. 2015), although the width of the road/trail may contribute to the specific-species detection (Harmsen et al. 2010). Scent marking is widespread among felines and it is crucial to maintain the social organization and communication within populations (Sunquist and Sunquist 2017). Thus, trails or roads may act as a key location for olfactory information, being more efficient, increasing scent mark encounter rates (Rafiq et al. 2020). Likewise, trails may be used as an energy-efficient travel i.e. saving energy when traveling by trails/roads comparing to more rough terrain (Bruggeman et al. 2007), thus increasing the detection probability. The use of trails for camera-trap placement maximizes the detection probability of threatened and elusive species, such as European wildcat, therefore could be a positive contribution for sampling designs targeting low density species.

4.1 Limitations

The deployment of two camera traps per sampling station is recommended (McClintock et al. 2013) in photographic capture-recapture designs to obtain an accurate identification of individuals (i.e. recording both flanks), since individual's marks are usually bilaterally asymmetric (McClintock et al. 2013). Due to logistics constrains this was not possible in our study. Nevertheless, the use of one camera per station still allows to estimate reliable European wildcat's density given that cumulative detection probabilities are high enough (Gil-Sánchez et al. 2020).

The low number of independent events and of spatial recaptures per individual i.e. low captures of the same individual in different detectors (camera-trap), may be a significant limitation to accurately determine wildcat's density (Brassine and Parker 2015). However, the time period often recommended for capture-recapture studies targeting large felines (*ca.* 90 days) (Karanth and Nichols 2002), and species that occurs in low population densities (*ca.* 130 days) (Brassine and Parker 2015), was fulfilled in our study (five months, i.e *ca.* 150 days). Moreover, recent studies recommend a minimum surveyed area for SCR models larger than a single average home range (Sun et al. 2015). Our camera-trap survey fitted this scenario, as the area surveyed reached ca. 423 km^2, and the average wildcat's home range estimated for Iberia being 13.7 km^2 (Oliveira et al. 2018).

Finally, the use of camera-trap data to identify individuals as wildcat, domestic and hybrid by phenotypic characteristics, may result in an unambiguous and difficult identification (Daniels et al. 2001). This misidentification can be a significant obstacle to wildcat conservation. However, during our survey, no phenotypic domestic cats was detected, and all wildcat photos exhibited wildcats' main phenotypic characteristics (Kitchener et al. 2005). Although we are confident on our results, we acknowledge that the use of genetic tools can be a better and accurate approach to determine wildcat's density (Anile et al. 2014), with an exact individual's identification, that allows accurate estimates.

4.2 Implications for European wildcat's conservation

Our results revealed that, despite Montesinho Natural Park being a strictly protected area, the recorded wildcat density value is low when compared to populations elsewhere in Europe, suggesting that this peripheral/edge population (Iberia) may be significant threatened, experiencing a considerable low densities and an increment of isolation.

Moreover, the implementation of strict protection measures targeting human disturbance and domestic cat presence inside the Natural Park, are needed to ensure a suitable habitat quality for wildcats, as this are two important drivers of this felid density. Finally, the creation of a long-term monitoring protocol targeting wildcat's density inside the PA could be a valuable tool to identify the species trend, early detect any density variation, and adjust conservation guidelines and Natural Park's management plans to actions outputs.

FINAL CONSIDERATIONS

This study identified relevant drivers influencing the wildcat's genetic integrity, across the species geographical range and metapopulation scales, while also determining the effectiveness of European and metapopulation's protected areas network as a conservation tool for wildcats. Results demonstrate the necessity to adopt different management approaches for each metapopulation, considering the different drivers influencing wildcat's GI and the effectiveness of PAs. Furthermore, this study has also an innovative character, since it provides the first wildcat density estimates for Portugal and highlighted some of the drivers shaping this population parameter.

Although acknowledging the study limitations, such as a sampling bias towards some landcover categories and protection areas, limited predictive power of some models, and the inability to test other factors that may also influenced wildcat's GI (e.g. historical bottlenecks, diseases, populations fertility, geographical isolation, etc.), we believe that it still provides a good contribution to the body of ecological knowledge regarding this threatened felid, and a useful tool to promote it conservation. However, we also recognize that it is necessary to replicate our approach i.e. identify the main drivers that influence wildcat's genetic integrity and estimate wildcat's density, ideally for each metapopulation and protected area where the wildcat is present, and to include the maximum number of factors that we were unable to include in our modeling procedure.

Despite the limited number of studies concerning wildcat's population density in Iberian Peninsula, our estimate is consistent with the values presented in those studies. However, our estimate is considered low when compared to other regions in Europe that present local positive trends, and consequently, high densities. Moreover, the Iberian metapopulation could be undergoing a possible silent extinction, mostly as a result of small and isolated populations (Chapter II) and a substantial admixture scenario between the wild feline and domestic cat (Chapter I). Our metapopulation-specific model predicts that Montesinho Natural Park revealed a low average of predicted wildcat's GI (*ca.* 47%), stressing the need to empirically quantify admixture patterns inside the Park.

Considering that the scenario of hybridization between the European wildcat and its domestic counterpart it is a significant threat to the wild species long-term survival, especially for Scottish and Iberian metapopulations, it is urgent to define priority conservation areas for this felid. This identification should be based on legal landscape protection, habitat quality and wildcat population density or abundance (e.g. Regions with high values of wildcat's genetic integrity and high values of wildcat's population density). Furthermore, it should be also a priority to create specific policies inside those priority areas concerning the minimization and mitigation of disturbances linked to human disturbance and domestic cat presence.

Finally, this study will contribute to the definition of more effective management policies and regulations throughout European and metapopulations' PAs, by combining wildcats genetic integrity, population density and the drivers shaping these parameters, in an integrative approach to wildcat's population long-term survival, across its entire range.

REFERENCES

According to Mammal Research

Abbott RJ (1992) Plant invasions, interspecific hybridization and the evolution of new plant taxa. Trends Ecol Evol 7:401–405. https://doi.org/10.1016/0169-5347(92)90020-C

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: Setting conservation guidelines. Trends Ecol Evol 16:613-622. https://doi.org/10.1016/ S0169-5347(01)02290-X

Anderson E (1953) Introgressive Hybridization. Biol Rev 28:280–307. https://doi.org/10.1111/j.1469-185X.1953.tb01379.x

Anderson E, Stebbins GL (1954) Hybridization as an evolutionary stimulus. Evolution (N Y) 8:378. https://doi.org/10.2307/2405784

Anile S, Amico C, Ragni B (2012) Population density estimation of the European wildcat (*Felis silvestris silvestris*) in Sicily using camera trapping. Wildl Biol Pract 8:1–12. https://doi.org/10.2461/wbp.2012.8.1

Anile S, Bizzarri L, Lacrimini M, et al (2018) Home-range size of the European wildcat (*Felis silvestris silvestris*): A report from two areas in Central Italy. Mammalia 82:1-11. https://doi.org/10.1515/mammalia-2016-0045

Anile S, Bizzarri L, Ragni B (2010) Estimation of European wildcat population size in Sicily (Italy) using camera trapping and capture-recapture analyses. Ital J Zool 77:241-246. https://doi.org/10.1080/11250000903419731

Anile S, Ragni B, Randi E, et al (2014) Wildcat population density on the Etna volcano, Italy: A comparison of density estimation methods. J Zool 293:252–261. https://doi.org/10.1111/jzo.12141

Arnold TW (2010) Uninformative parameters and model selection using Akaike's Information Criterion. J Wildl Manage 74:1175-1178. https://doi.org/10.2193/2009-367

Bartoń K (2019) MuMIn: Multi-Model Inference, Version 1.43.6. R Package. http://cran.r-project.org/package=MuMIn

Bates D, Sarkar D, Bates MD, Matrix L (2007) The lme4 Package: Linear Mixed–Effects Models Using S4 Classes. R package version 0.99875-9. https://cran.r-project.org/package=lme4.

Beaumont M, Barratt EM, Gottelli D, et al (2001) Genetic diversity and introgression in the Scottish wildcat. Mol Ecol 10:319–336. https://doi.org/10.1046/j.1365-294X.2001.01196.x

Beugin MP, Leblanc G, Queney G, et al (2016) Female in the inside, male in the outside: insights into the spatial organization of a European wildcat population. Conserv Genet 17:1405–1415. https://doi.org/10.1007/s10592-016-0871-0

Beugin MP, Salvador O, Leblanc G, et al (2018) Contrasted hybridization patterns between two local populations of European wildcats in France. BioRxiv 342576. https://doi.org/10.1101/342576

Beugin MP, Salvador O, Leblanc G, et al (2020) Hybridization between *Felis silvestris silvestris* and *Felis silvestris catus* in two contrasted environments in France. Ecol Evol 10:263-276. https://doi.org/10.1002/ece3.5892

Bohling JH, Waits LP (2015) Factors influencing red wolf-coyote hybridization in eastern North Carolina, USA. Biol Conserv 184:108–116. https://doi.org/10.1016/j.biocon.2015.01.013

Boitani L, Cowling RM, Dublin HT, et al (2008) Change the IUCN protected area categories to reflect biodiversity outcomes. PLoS Biol 6:436-438. https://doi.org/10.1371/journal.pbio.0060066

Bolker BM, Brooks ME, Clark CJ, et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135. https://doi.org/10.1016/j.tree.2008.10.008

Borchers DL, Efford MG (2008) Spatially explicit maximum likelihood methods for capture-recapture studies. Biometrics 64:377–385. https://doi.org/10.1111/j.1541-0420.2007.00927.x

Brassine E, Parker D (2015) Trapping elusive cats: Using intensive camera trapping to estimate the density of a rare african felid. PLoS One 10:20142508. https://doi.org/10.1371/journal.pone.0142508

Breitenmoser U, Lanz T, Breitenmoser-Würsten C (2019) Conservation of the wildcat (*Felis silvestris*) in Scotland: review of the conservation status and assessment of conservation activities. IUCN SSC Cat Specialist Group, Bern.

Bruggeman JE, Garrott RA, White PJ, et al (2007) Covariates affecting spatial variability in bison travel behavior in Yellowstone National Park. Ecol Appl 17:1411-1423. https://doi.org/10.1890/06-0196.1

Buchhorn M, Lesiv M, Tsendbazar NE, et al (2020) Copernicus global land cover layers-collection 2. Remote Sens. https://doi.org/10.3390/rs12061044

Burnham DA, Anderson KP (2002) Model selection and multimodel inference : a practical information-theoretic approach. Springer-Verlag, New York, NY

Cabral MJ, Almeida L, Almeida P, et al (2005) Livro vermelho dos vertebrado de portugal. Instituto da conservação da natureza. Lisboa (p. 659). Lisboa, Portugal: Instituto da Conservação da Natureza

Cabria MT, Michaux JR, Gómez-Moliner BJ, et al (2011) Bayesian analysis of hybridization and introgression between the endangered european mink (*Mustela lutreola*) and the polecat (*Mustela putorius*). Mol Ecol 20:1176–1190. https://doi.org/10.1111/j.1365-294X.2010.04988.x

Castro J, De Figueiredo T, Fonseca F, et al (2010) Montesinho Natural Park: General description and natural values. Springer 119-132. https://doi.org/10.1007/978-3-642-01577-9_15

CBD (2012). Strategic plan fro biodiversity 2011-2020 and the Aichi targets. Retrieved from www.cbd.int/doc/strategic-plan/2011-2020/ AIchi-Targets-EN.pdf

Chape S, Harrison J, Spalding M, Lysenko I (2005) Measuring the extent and effectiveness of protected areas as an indicator for meeting global biodiversity targets. Philos Trans R Soc Lond B Biol Sci 360:443-455. https://doi.org/10.1098/rstb.2004.1592

Chapin FS, Zavaleta ES, Eviner VT, et al (2000) Consequences of changing biodiversity. Nature 405:234–242. https://doi.org/10.1038/35012241

Chapman C, Chapman L (1996) Mid-elevation forests: a history of disturbance and regeneration. East African Ecosyst. Their Conserv. 385–400.

Crispo E, Moore JS, Lee-Yaw JA, et al (2011) Broken barriers: Human-induced changes to gene flow and introgression in animals: An examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. BioEssays 33:508–518. https://doi.org/10.1002/bies.201000154

Daniels MJ, Balharry D, Hirst D, et al (1998) Morphological and pelage characteristics of wild living cats in Scotland: Implications for defining the “wildcat.” J Zool 244:231–247.

https://doi.org/10.1017/S0952836998002088

Daniels MJ, Beaumont MA, Johnson PJ, et al (2001) Ecology and genetics of wild-living cats in the north-east of Scotland and the implications for the conservation of the wildcat. J Appl Ecol 38:146-161. https://doi.org/10.1046/j.1365-2664.2001.00580.x

Dinerstein E, Olson D, Joshi A, et al (2017) An ecoregion-based approach to protecting half the terrestrial realm. Bioscience 67:534-545. https://doi.org/10.1093/biosci/bix014

Dittmer CG, Allee WC (1931) Animal aggregations: A study in general sociology. J Educ Sociol. https://doi.org/10.2307/2961735

Dowling TE, Secor CL (1997) The role of hybridization and introgression in the diversification of animals. Annu Rev Ecol Syst 28:593–619. https://doi.org/10.1146/annurev.ecolsys.28.1.593

Driscoll CA, Menotti-Raymond M, Roca AL, et al (2007) The near eastern origin of cat domestication. Science 317:519–523. https://doi.org/10.1126/science.1139518

Duarte A, Fernandes M, Santos N, Tavares L (2012) Virological survey in free-ranging wildcats (Felis silvestris) and feral domestic cats in Portugal. Vet Microbiol 158:400–404. https://doi.org/10.1016/j.vetmic.2012.02.033

Dudley N (2008) Guidelines for applying protected area management categories. IUCN. Gland, Switzerland. Retrived from: https://portals.iucn.org/library/node/9243

Efford M (2004) Density estimation in live-trapping studies. Oikos 106:598-610. https://doi.org/10.1111/j.0030-1299.2004.13043.x

Efford MG, Borchers DL, Byrom AE (2009) Density estimation by spatially explicit capture–recapture: likelihood-based methods. Modeling demographic processes in marked populations 3:225-269. https://doi.org/10.1007/978-0-387-78151-8_11

Fernández N (2005) Spatial patterns in European rabbit abundance after a population collapse. Landsc Ecol 20:897-910. https://doi.org/10.1007/s10980-004-3976-7

Ferreira GA, Machado JC, Nakano-Oliveira E, et al (2020) The effect of castration on home range size and activity patterns of domestic cats living in a natural area in a protected area on a Brazilian island. Appl Anim Behav Sci 230:105049. https://doi.org/10.1016/j.applanim.2020.105049

Ferreira JP, Leitão I, Santos-Reis M, Revilla E (2011) Human-related factors regulate the spatial ecology of domestic cats in sensitive areas for conservation. PLoS One 6:1–10. https://doi.org/10.1371/journal.pone.0025970

Ferreras P, DÍaz-Ruiz F, Monterroso P (2018) Improving mesocarnivore detectability with lures in camera-trapping studies 45:505-517. Wildl Res. https://doi.org/10.1071/WR18037

Figueiredo AM, Valente AM, Barros T, et al (2020) What does the Wolf eat? Assessing the diet of the endangered Iberian Wolf (Canis lupus signatus) in northeast Portugal. PLoS One 15:1-15. https://doi.org/10.1371/journal.pone.0230433

Foster RJ, Harmsen BJ (2012) A critique of density estimation from camera-trap data. J Wildl Manage 76:224-236. https://doi.org/10.1002/jwmg.275

Fuente B de la, Bertzky B, Delli G, et al (2020) Built-up areas within and around protected areas: global patterns and 40-year trends. Glob Ecol Conserv 24:201291. https://doi.org/10.1016/j.gecco.2020.e01291

Geldmann J, Joppa LN, Burgess ND (2014) Mapping change in human pressure globally on land and within Protected Areas. Conserv Biol 28:1604-1616. https://doi.org/10.1111/cobi.12332

Germain E, Benhamou S, Poulle ML (2008) Spatio-temporal sharing between the European wildcat, the domestic cat and their hybrids. J Zool 276:195–203. https://doi.org/10.1111/j.1469-7998.2008.00479.x

Gil-Sánchez JM, Barea-Azcón JM, Jaramillo J, et al (2020) Fragmentation and low density as major conservation challenges for the southernmost populations of the European wildcat. PLoS One 15:1–21. https://doi.org/10.1371/journal.pone.0227708

Gil-Sánchez JM, Jaramillo J, Barea-Azcón JM (2015) Strong spatial segregation between wildcats and domestic cats may explain low hybridization rates on the Iberian Peninsula. Zoology 118:377-385. https://doi.org/10.1016/j.zool.2015.08.001

Gil-Sánchez JM, Valenzuela G, Sánchez JF (1999) Iberian wild cat predation on rabbit - functional response and age selection. Acta Theriol 44:421–428

Harmsen BJ, Foster RJ, Silver S, et al (2010) Differential use of trails by forest mammals and the implications for camera-trap studies: A case study from Belize. Biotropica 52:126-133. https://doi.org/10.1111/j.1744-7429.2009.00544.x

Hayward MW, Boitani L, Burrows ND, et al (2015) Ecologists need robust survey designs, sampling and analytical methods. J Appl Ecol 52:286-290. https://doi.org/10.1111/1365-2664.12408

Hertwig ST, Schweizer M, Stepanow S, et al (2009) Regionally high rates of hybridization and introgression in German wildcat populations (*Felis silvestris*, Carnivora, Felidae). J Zool Syst Evol Res 47:283–297. https://doi.org/10.1111/j.1439-0469.2009.00536.x

Holderegger R, Di Giulio M (2010) The genetic effects of roads: A review of empirical evidence. Basic Appl Ecol 11:522–531. https://doi.org/10.1016/j.baae.2010.06.006

Hollingsworth PM, O'Brien D, Ennos RA, et al (2020). Scotland’s biodiversity progress to 2020 Aichi targets: conserving genetic divsersity-development of national approach of addressing Aichi Biodiversity Target 13 that includes wild species. https://www.nature.scot/scotlands-biodiversity-progress-2020-aichi-targetsconserving-genetic-diveristy-development-national (accessed October 2020)

Horn JA, Mateus-Pinilla N, Warner RE, Heske EJ (2011) Home range, habitat use, and activity patterns of free-roaming domestic cats. J Wildl Manage 75:1177–1185. https://doi.org/10.1002/jwmg.145

Hothorn T, Bretz F, Westfall P, Heiberger R (2007) Package 'multcomp'. Simultaneous inference in general parametris models. Project for Statistical Computing, Vienna, Austria.

Hubbs CL (1955) Hybridization between fish species in nature. Syst Zool 4:1–20. https://doi.org/10.2307/sysbio/4.1.1

IUCN (2012) Guidelines for Application of IUCN Red List Criteria at Regional and National Levels, Version 4.0. Gland, Switzerland and Cambridge, UK.

Jerosch S, Kramer-Schadt S, Götz M, Roth M (2018) The importance of small-scale structures in an agriculturally dominated landscape for the European wildcat (*Felis silvestris silvestris*) in central Europe and implications for its conservation. J Nat Conserv 41:88–96. https://doi.org/10.1016/j.jnc.2017.11.008

Jones KR, Venter O, Fuller RA, et al (2018) One-third of global protected land is under intense human pressure. Science 360:788-791. https://doi.org/10.1126/science.aap9565

Joppa LN, Loarie SR, Pimm SL (2008) On the protection of "protected areas." Proc Natl Acad Sci 105:6673-6678. https://doi.org/10.1073/pnas.0802471105

Joppa LN, Pfaff A (2011) Global protected area impacts. Proc R Soc B Biol Sci 278:1633-1638. https://doi.org/10.1098/rspb.2010.1713

Karanth KU, Nichols JD (2002) Statistical concepts: estimating absolute densities of tigers using capture-recapture sampling. In: Monitoring Tigers and their Prey: A Manual for Researchers, Managers and Conservationists in Tropical Asia. Center for Wildlife Studies: Bangalore, India

Karanth KU, Nichols JD, Kumar NS, Hines JE (2006) Assessing tiger population dynamics using photographic capture-recapture sampling. Ecology 87:2925–2937. https://doi.org/10.1890/0012-9658(2006)87[2925:ATPDUP]2.0.CO;2

Kéry M, Gardner B, Stoeckle T, et al (2010) Use of spatial capture-recapture modeling and DNA data to estimate densities of elusive animals. Conserv Biol 25:356-364. https://doi.org/10.1111/j.1523-1739.2010.01616.x

Kéry M, Royle JA (2015) Applied Hierarchical Modeling in Ecology: Analysis of distribution, abundance and species richness in R and BUGS. Volume 1: Prelude and Static Models. Academic Press.

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. Can J Zool 85:1049–1064. https://doi.org/10.1139/Z07-095

Kilshaw K, Johnson PJ, Kitchener AC, Macdonald DW (2015) Detecting the elusive Scottish wildcat Felis silvestris silvestris using camera trapping. ORYX 9:207-215. https://doi.org/10.1017/S0030605313001154

Kilshaw K, Montgomery RA, Campbell RD, et al (2016) Mapping the spatial configuration of hybridization risk for an endangered population of the European wildcat (Felis silvestris silvestris) in Scotland. Mammal Res 61:1-11. https://doi.org/10.1007/s13364-015-0253-x

Kitchener AC, Yamaguchi N, Ward JM, Macdonald DW (2005) A diagnosis for the Scottish wildcat (Felis silvestris): A tool for conservation action for a critically-endangered felid. Anim Conserv 8:223-237. https://doi.org/10.1017/S1367943005002301

Klar N, Fernández N, Kramer-Schadt S, et al (2008) Habitat selection models for European wildcat conservation. Biol Conserv 141:308–319. https://doi.org/10.1016/j.biocon.2007.10.004

Kolowski JM, Forrester TD (2017) Camera trap placement and the potential for bias due to trails and other features. PLoS One 12:20186679. https://doi.org/10.1371/journal.pone.0186679

Kryštufek B, Griffiths HI (2002) Species richness and rarity in European rodents. Ecography 25:120-128. https://doi.org/10.1034/j.1600-0587.2002.250114.x

Le Roux JJ, Foxcroft LC, Herbst M, Macfadyen S (2015) Genetic analysis shows low levels of hybridization between African wildcats (Felis silvestris lybica) and domestic cats (F. s. catus) in South Africa. Ecol Evol 5:288-299. https://doi.org/10.1002/ece3.1275

Lecis R, Pierpaoli M, Birò ZS, et al (2006) Bayesian analyses of admixture in wild and domestic cats (Felis silvestris) using linked microsatellite loci. Mol Ecol 15:119–131. https://doi.org/10.1111/j.1365-294X.2005.02812.x

Leroux SJ, Krawchuk MA, Schmiegelow F, et al (2010) Global protected areas and IUCN designations:

Do the categories match the conditions? Biol Conserv 143-609-616. https://doi.org/10.1016/j.biocon.2009.11.018

Lipshutz SE (2018) Interspecific competition, hybridization, and reproductive isolation in secondary contact: Missing perspectives on males and females. Curr Zool 64:75-88. https://doi.org/10.1093/cz/zox060

Locke H, Dearden P (2005) Rethinking protected area categories and the new paradigm. Environ. Conserv 32:1-10. https://www.jstor.org/stable/44520802

López-Bao J V., Godinho R, Pacheco C, et al (2018) Toward reliable population estimates of wolves by combining spatial capture-recapture models and non-invasive DNA monitoring. Sci Rep 8:1–8. https://doi.org/10.1038/s41598-018-20675-9

López G, López-Parra M, Fernández L, et al (2009) Management measures to control a feline leukemia virus outbreak in the endangered Iberian lynx. Anim Conserv 12:173-182. https://doi.org/10.1111/j.1469-1795.2009.00241.x

López-Martín JM, García FJ, Duch A, Virgós E, Lozano J, Duarte J, España AJ (2007) Felis silvestris Schreber, 1777. In: Atlas y Libro Rojo de los Mamíferos Terrestres de España 336-338. Palomo LJ, Gisbert J, Balnco JC (Eds). Madrid: Dirección 633 General para la Biodivsersidad-SECEM-SECEMU.

Lozano J (2010) Habitat use by European wildcats (Felis silvestris) in central Spain: What is the relative importance of forest variables? Anim Biodivers Conserv 33:143-150. https://www.raco.cat/index.php/ABC/article/view/214977

Lozano J, Malo AF (2012) Conservation of the European wildcat (Felis silvestris) in mediterranean environments: A reassessment of current threats. In: Williaam GS (ed) Mediterranean Ecosystems: dynamics, management and conservation. Nova Science Publisher's Inc., Hauppauge, 2-31

Lozano J, Moleón M, Virgós E (2006) Biogeographical patterns in the diet of the wildcat, Felis silvestris Schreber, in Eurasia: Factors affecting the trophic diversity. J Biogeogr 33:1076–1085. https://doi.org/10.1111/j.1365-2699.2006.01474.x

Lozano J, Virgós E, Cabezas-Díaz S, Mangas JG (2007) Increase of large game species in Mediterranean areas: Is the European wildcat (Felis silvestris) facing a new threat? Biol Conserv 138:321–329. https://doi.org/10.1016/j.biocon.2007.04.027

Lozano J, Virgós E, Malo AF, et al (2003) Importance of scrub-pastureland mosaics for wild-living cats occurrence in a Mediterranean area: Implications for the conservation of the wildcat (Felis silvestris). Biodivers Conserv 12:921–935. https://doi.org/10.1023/A:1022821708594

Lynch M, O'Hely M (2001) Captive breeding and the genetic fitness of natural populations. Conserv Genet 2:363–378. https://doi.org/10.1023/A:1012550620717

Macdonald DW, Yamaguchi N, Kitchener AC, et al (2010) Reversing cryptic extinction: the history, present, and future of the Scottish wildcat. Biol Conserv Wild Felids 22:471-491.

MacKenzie, Darryl I., Nichols, James D., and Pollock KH (2005) Occupancy estimation and modeling : Inferring patterns and dynamics of Species occurrence. Elsevier Academic Press. https://doi.org/10.1017/CBO9781107415324.004

Mallet J (2008) Hybridization, ecological races and the nature of species: Empirical evidence for the ease of speciation. Philos Trans R Soc B Biol Sci 363:2971–2986. https://doi.org/10.1098/rstb.2008.0081

Malo AF, Lozano J, Huertas DL, Virgós E (2004) A change of diet from rodents to rabbits (Oryctolagus cuniculus). Is the wildcat (Felis silvestris) a specialist predator? J Zool 263:401–407. https://doi.org/10.1017/S0952836904005448

Mann GKH, O'Riain MJ, Parker DM (2015) The road less travelled: assessing variation in mammal detection probabilities with camera traps in a semi-arid biodiversity hotspot. Biodivers Conserv 24:531-545. https://doi.org/10.1007/s10531-014-0834-z

Marques TA, Thomas L, Martin SW, et al (2013) Estimating animal population density using passive acoustics. Biol Rev 88:287-309. https://doi.org/10.1111/brv.12001

Massa B, La Mantia T (2007) Forestry, pasture, agriculture and fauna correlated to recent changes in Sicily. For - Riv di Selvic ed Ecol For 4:418-438. https://doi.org/10.3832/efor0495-0040418

Mathews F, Kubasiewicz LM, Gurnell J, Harrower CA, McDonald RA, Shore RF (2018) A review of the population and conservation status of british mammals. Natural England, Peterborough.

Mattucci F, Oliveira R, Lyons LA, et al (2016) European wildcat populations are subdivided into five main biogeographic groups: Consequences of Pleistocene climate changes or recent anthropogenic fragmentation? Ecol Evol 6:3–22. https://doi.org/10.1002/ece3.1815

Maucha G, Pataki R (2011) Development of methodology to eliminate contradictions between CLC-Change1990-2000 and CLC-Change2000-2006. Retrived from: http://atliekos.gamta.lt/files/Change_harmonization_report_final.pdf

Maxwell SE (1980) Pairwise Multiple Comparisons in Repeated Measures Designs. J Educ Stat 5:269-287. https://doi.org/10.3102/10769986005003269

Mazerolle MJ (2006) Improving data analysis in herpetology: Using Akaike's information criterion (AIC) to assess the strength of biological hypotheses. Amphib Reptil 27:169-180. https://doi.org/10.1163/156853806777239922

Mazerolle MJ (2017) Package ' AICcmodavg .' R Package. Version 2.3-1 https://cran.r-project.org/web/packages/AICcmodavg/AICcmodavg.pdf

McClintock BT, Conn PB, Alonso RS, Crooks KR (2013) Integrated modeling of bilateral photo-identification data in mark-recapture analyses. Ecology 94:1464-1471. https://doi.org/10.1890/12-1613.1

Millán J, Rodríguez A (2009) A serological survey of common feline pathogens in free-living European wildcats (Felis silvestris) in central Spain. Eur J Wildl Res 55:285–291. https://doi.org/10.1007/s10344-008-0246-z

Monterroso P, Alves PĆ, Ferreras P (2011) Evaluation of attractants for non-invasive studies of Iberian carnivore communities. Wildl Res 38:446-454. https://doi.org/10.1071/WR11060

Monterroso P, Brito JC, Ferreras P, Alves PC (2009) Spatial ecology of the European wildcat in a Mediterranean ecosystem: Dealing with small radio-tracking datasets in species conservation. J Zool 279:27–35. https://doi.org/10.1111/j.1469-7998.2009.00585.x

Monterroso P, Díaz-Ruiz F, Lukacs PM, et al (2020) Ecological traits and the spatial structure of competitive coexistence among carnivores. Ecology 101:1-16. https://doi.org/10.1002/ecy.3059

Monterroso P, Garrote G, Serronha A, et al (2016) Disease-mediated bottom-up regulation: An emergent virus affects a keystone prey, and alters the dynamics of trophic webs. Sci Rep 6:36072. https://doi.org/10.1038/srep36072

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proc Natl Acad Sci U S A 98:5446–5451. https://doi.org/10.1073/pnas.091093398

Navarro-Castilla Á, Diaz M, Barja I (2017) Does ungulate disturbance mediate behavioural and physiological stress responses in Algerian mice (Mus spretus)? a wild exclosure experiment. Hystrix 28:165-172. https://doi.org/10.4404/hystrix-28.2-12332

Noss AJ, Gardner B, Maffei L, et al (2012) Comparison of density estimation methods for mammal populations with camera traps in the Kaa-Iya del Gran Chaco landscape. Anim Conserv 15:527–535. https://doi.org/10.1111/j.1469-1795.2012.00545.x

Nowell K, Jackson P (1996) Status Survey and Conservation Action Plan Wild Cats. IUCN/SSC Cat Spec Group, Gland Switz

Nussberger B, Currat M, Quilodran CS, et al (2018) Range expansion as an explanation for introgression in European wildcats. Biol Conserv 218:49–56. https://doi.org/10.1016/j.biocon.2017.12.009

Nussberger B, Greminger MP, Grossen C, et al (2013) Development of SNP markers identifying European wildcats, domestic cats, and their admixed progeny. Mol Ecol Resour 13:447-460. https://doi.org/10.1111/1755-0998.12075

Nussberger B, Wandeler P, Weber D, Keller LF (2014) Monitoring introgression in European wildcats in the Swiss Jura. Conserv Genet 15:1219–1230. https://doi.org/10.1007/s10592-014-0613-0

O’Brien J, Devillard S, Say L, et al (2009) Preserving genetic integrity in a hybridising world: Are European Wildcats (Felis silvestris silvestris) in eastern France distinct from sympatric feral domestic cats? Biodivers Conserv 18:2351–2360. https://doi.org/10.1007/s10531-009-9592-8

Oliveira R, Godinho R, Randi E, et al (2008a) Molecular analysis of hybridisation between wild and domestic cats (Felis silvestris) in Portugal: Implications for conservation. Conserv Genet 9:1–11. https://doi.org/10.1007/s10592-007-9297-z

Oliveira R, Godinho R, Randi E, Alves PC (2008b) Hybridization versus conservation: Are domestic cats threatening the genetic integrity of wildcats (Felis silvestris silvestris) in Iberian Peninsula? Philos Trans R Soc B Biol Sci 363:2953–2961. https://doi.org/10.1098/rstb.2008.0052

Oliveira T, Urra F, López-Martín JM, et al (2018) Females know better: Sex-biased habitat selection by the European wildcat. Ecol Evol 8:9464–9477. https://doi.org/10.1002/ece3.4442

Ottoni C, Van Neer W, De Cupere B, et al (2017) The palaeogenetics of cat dispersal in the ancient world. Nat Ecol Evol 1:1-7. https://doi.org/10.1038/s41559-017-0139

Pierpaoli M, Birò ZS, Herrmann M, et al (2003) Genetic distinction of wildcat (Felis silvestris) populations in Europe, and hybridization with domestic cats in Hungary. Mol Ecol 12:2585–2598. https://doi.org/10.1046/j.1365-294X.2003.01939.x

Popescu VD, Artelle KA, Pop MI, et al (2016) Assessing biological realism of wildlife population estimates in data-poor systems. J Appl Ecol 53:1248-1259. https://doi.org/10.1111/1365-2664.12660

Purvis A, Gittleman JL, Cowlishaw G, Mace GM (2000) Predicting extinction risk in declining species. Proc R Soc B Biol Sci 267:1947–1952. https://doi.org/10.1098/rspb.2000.1234

QGIS Development Team (2016) QGIS Geographic Information System. Open Source Geospatial Found. Proj.

R Development Core Team (2017) R: A language and environment for statistical computing. Vienna, Austria

Rafiq K, Jordan NR, Meloro C, et al (2020) Scent-marking strategies of a solitary carnivore: boundary and road scent marking in the leopard. Anim Behav 161:115-126. https://doi.org/10.1016/j.anbehav.2019.12.016

Ragni B, Possenti M (1996) Variability of coat-colour and markings system in felis silvestris. Ital J Zool 63:285-292. https://doi.org/10.1080/11250009609356146

Reed SE, Merenlender AM (2008) Quiet, Nonconsumptive Recreation Reduces Protected Area Effectiveness. Conserv Lett 1:146–154. https://doi.org/10.1111/j.1755-263x.2008.00019.x

Revelle MW (2015) Package 'psych': The comprehensive R archive network. Version 1.9.12. https://cran.r-project.org/web/packages/psych/psych.pdf

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. Annu Rev Ecol Syst 27:83–109. https://doi.org/10.1146/annurev.ecolsys.27.1.83

Rich LN, Davis CL, Farris ZJ, et al (2017) Assessing global patterns in mammalian carnivore occupancy and richness by integrating local camera trap surveys. Glob Ecol Biogeogr 26:918–929. https://doi.org/10.1111/geb.12600

Royle JA, Chandler RB, Gazenski KD, Graves TA (2013) Spatial capture-recapture models for jointly estimating population density and landscape connectivity. Ecology 94:287-294. https://doi.org/10.1890/12-0413.1

Royle JA, Chandler RB, Sollmann R, Gardner B (2014) Fully Spatial Capture-Recapture Models. http://dx.doi.org/10.1016/B978-0-12-405939-9.00005-0

Royle JA, Sutherland C, Fuller AK, Sun CC (2015) Likelihood analysis of spatial capture-recapture models for stratified or class structured populations. Ecosphere 6:1-11. https://doi.org/10.1890/ES14-00148.1

Royle JA, Young K V. (2008) A hierarchical model for spatial capture recapture data. Ecology 89:2281–2289. https://doi.org/10.1890/07-0601.1

Sarmento P (1996) Feeding ecology of the European wildcat Felis silvestris in Portugal. Mammal Res 41:409–414. https://doi.org/10.4098/AT.arch.96-39

Sarmento P, Cruz J, Eira C, Fonseca C (2009) Spatial colonization by feral domestic cats Felis catus of former wildcat Felis silvestris silvestris home ranges. Mammal Res 54:31–38. https://doi.org/10.1007/bf03193135

Sarmento P, Cruz J, Tarroso P, Fonseca C (2006) Space and habitat selection by female European wild cats (Felis silvestris silvestris). Wildl Biol Pract 2:79-89. https://doi.org/10.2461/wbp.2006.2.10

Say L, Devillard S, Léger F, et al (2012) Distribution and spatial genetic structure of European wildcat in France. Anim Conserv 15:18–27. https://doi.org/10.1111/j.1469-1795.2011.00478.x

Say L, Pontier D (2004) Spacing pattern in a social group of stray cats: Effects on male reproductive success. Anim Behav 68:175-180. https://doi.org/10.1016/j.anbehav.2003.11.008

Sayol F, Vilella M, Bagaria G, Puig J (2018). El gat salvatge, Felis silvestris (Schreber, 1777), al Prepirineu oriental: densitat de les poblacions del Lluçanès i el Bisaura. Butlletí de la Institució Catalana d'Història Natural 82:185-191.

Seehausen O, Takimoto G, Roy D, Jokela J (2008) Speciation reversal and biodiversity dynamics with hybridization in changing environments. Mol Ecol 17:30–44. https://doi.org/10.1111/j.1365-

294X.2007.03529.x

Senn H V., Ghazali M, Kaden J, et al (2019) Distinguishing the victim from the threat: SNP-based methods reveal the extent of introgressive hybridization between wildcats and domestic cats in Scotland and inform future in situ and ex situ management options for species restoration. Evol Appl 12:399-414. https://doi.org/10.1111/eva.12720

Senn H V., Pemberton JM (2009) Variable extent of hybridization between invasive sika (Cervus nippon) and native red deer (C. elaphus) in a small geographical area. Mol Ecol 18:862–876. https://doi.org/10.1111/j.1365-294X.2008.04051.x

Shiffler RE (1988) Maximum z scores and outliers. Am Stat 42:79-80. https://doi.org/10.1080/00031305.1988.10475530

Silva AP, Kilshaw K, Johnson PJ, et al (2013a) Wildcat occurrence in Scotland: Food really matters. Divers Distrib 19:232–243. https://doi.org/10.1111/ddi.12018

Silva AP, Rosalino LM, Johnson PJ, et al (2013b) Local-level determinants of wildcat occupancy in Northeast Scotland. Eur J Wildl Res 59:449–453. https://doi.org/10.1007/s10344-013-0715-x

Sobrino R, Acevedo P, Escudero MA, et al (2009) Carnivore population trends in Spanish agrosystems after the reduction in food availability due to rabbit decline by rabbit haemorrhagic disease and improved waste management. Eur J Wildl Res 55:161–165. https://doi.org/10.1007/s10344-008-0230-7

Sollmann R, Furtado MM, Gardner B, et al (2011) Improving density estimates for elusive carnivores: Accounting for sex-specific detection and movements using spatial capture-recapture models for jaguars in central Brazil. Biol Conserv 144:1017–1024. https://doi.org/10.1016/j.biocon.2010.12.011

Sollmann R, Linkie M, Haidir IA, Macdonald DW (2014) Bringing clarity to the clouded leopard Neofelis diardi: First density estimates from Sumatra. Oryx 48:536-539. https://doi.org/10.1017/S003060531400043X

Soto CA, Palomares F (2014) Surprising low abundance of european wildcats in a mediterranean protected area of Southwestern Spain. Mammalia 78:57-65. https://doi.org/10.1515/mammalia-2013-0054

Spassov N, Simeonovski V, Spiridonov G (1997) The wild cat (Felis silvestris Schr.) and the feral domestic cat: problems of the morphology, taxonomy, identification od the hybrids and purity of the wild population. Hist Nat Bulg 8:101–120.

Stahl P, Artois M (1994) Status and conservation of the wild cat (Felis silvestris) in Europe and around the Mediterranean rim. In: Convention on Wildlife and Natural Habitat Conservancy in Europe: 61. Strasbourg: Council of Europe.

Steenweg R, Hebblewhite M, Kays R, et al (2017) Scaling-up camera traps: monitoring the planet's biodiversity with networks of remote sensors. Front Ecol Environ 15:26–34. https://doi.org/10.1002/fee.1448

Stephens PA, Pettorelli N, Barlow J, et al (2015) Management by proxy? The use of indices in applied ecology. J Appl Ecol 52:1-6. http://dx.doi.org/10.1111/1365-2664.12383

Steyer K, Kraus RHS, Mölich T, et al (2016) Large-scale genetic census of an elusive carnivore, the European wildcat (Felis s. silvestris). Conserv Genet. https://doi.org/10.1007/s10592-016-0853-2

Steyer K, Simon O, Kraus RHS, et al (2013) Hair trapping with valerian-treated lure sticks as a tool for genetic wildcat monitoring in low-density habitats. Eur J Wildl Res 17:1183-1199.

https://doi.org/10.1007/s10344-012-0644-0

Steyer K, Tiesmeyer A, Muñoz-Fuentes V, Nowak C (2018) Low rates of hybridization between European wildcats and domestic cats in a human-dominated landscape. Ecol Evol 8:2290–2304. https://doi.org/10.1002/ece3.3650

Storfer A, Murphy MA, Spear SF, et al (2010) Landscape genetics: Where are we now? Mol Ecol 19:3496–3514. https://doi.org/10.1111/j.1365-294X.2010.04691.x

Streif S, Kraft S, Veith S, et al (2012) Monitoring and research of the European wildcat (Felis silvestris) in Baden-Württemberg. Säugetierkundliche Informationen 8:411-416.

Sun CC, Fuller AK, Royle JA (2015) Trap configuration and spacing influences parameter estimates in spatial capture-recapture models. PLoS One 9:e88025. https://doi.org/10.1371/journal.pone.0088025

Sunquist M, Sunquist F (2017) Wild Cats of the World. University of Chicago press. Chicago, Illinois

Sutherland C, Royle JA, Linden DW (2019) oSCR: a spatial capture–recapture R package for inference about spatial ecological processes. Ecography 42:1459-1469. https://doi.org/10.1111/ecog.04551

Sutherland WJ, Armstrong-Brown S, Armsworth PR, et al (2006) The identification of 100 ecological questions of high policy relevance in the UK. J Appl Ecol 43:617–627. https://doi.org/10.1111/j.1365-2664.2006.01188.x

Tiesmeyer A, Ramos L, Manuel Lucas J, et al (2020) Range-wide patterns of human-mediated hybridisation in European wildcats. Conserv Genet 21:247-260. https://doi.org/10.1007/s10592-019-01247-4

Todesco M, Pascual MA, Owens GL, et al (2016) Hybridization and extinction. Evol Appl 9:892–908. https://doi.org/10.1111/eva.12367

Trigo TC, Freitas TRO, Kunzler G, et al (2008) Inter-species hybridization among Neotropical cats of the genus Leopardus, and evidence for an introgressive hybrid zone between L. geoffroyi and L. tigrinus in southern Brazil. Mol Ecol 17:4317–4333. https://doi.org/10.1111/j.1365-294X.2008.03919.x

UNEP-WCMC and IUCN (2019) Protected Planet: The World Database on Protected Areas (WDPA) [October/2019], Cambridge, UK: UNEP-WCMC and IUCN. Available at: www.protectedplanet.net

UNEP-WCMC (2013) A guide to using the CITES Trade Database. Version 8. United Nations Environment Programme-World Conservation Monitoring Centre. Cambridge, UK. Available at:www.trade.cites.org

Valente AM, Fonseca C, Marques TA, et al (2014) Living on the edge: Roe deer (Capreolus capreolus) density in the margins of its geographical range. PLoS One 9:1–7. https://doi.org/10.1371/journal.pone.0088459

Veall MR, Zimmermann KF (1996) Pseudo-R2 measures for some common limited dependent variable models. J Econ Surv 10:241-259. https://doi.org/10.1111/j.1467-6419.1996.tb00013.x

Vellend M, Harmon LJ, Lockwood JL, et al (2007) Effects of exotic species on evolutionary diversification. Trends Ecol Evol 22:481–488. https://doi.org/10.1016/j.tree.2007.02.017

Verbeylen G, De Bruyn L, Adriaensen F, Matthysen E (2003) Does matrix resistance influence Red squirrel (Sciurus vulgaris L. 1758) distribution in an urban landscape? Landsc Ecol 18:791-805. https://doi.org/10.1023/B:LAND.0000014492.50765.05

Wierzbowska IA, Olko J, Hedrzak M, Crooks KR (2012) Free-ranging domestic cats reduce the effective protected area of a Polish national park. Mamm Biol 77:204–210.

https://doi.org/10.1016/j.mambio.2012.01.004

Williams ES, Miller MW, Kreeger TJ, et al (2002) Chronic wasting disease of deer and elk: A review with recommendations for management. J Wildl Manage 66:551. https://doi.org/10.2307/3803123

Wright SJ, Hubbell SP (1983) Stochastic extinction and reserve size: A focal species approach. Oikos 41:466. https://doi.org/10.2307/3544106

Yamaguchi N, Kitchener A, Driscoll C, Nussberger B (2015) Felis silvestris, Wild cat. IUCN Red List Threat Species 2015:e.T60354712A50652361. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T60354712A50652361.en

Zuur AF, Ieno EN, Walker NJ, et al (2009). Mixed effects models and extensions in ecology with R. Springer-Verlag, New York

Zwijacz-Kozica T, Wazna A, Muñoz-Fuentes V, et al (2017) Not European Wildcats, But Domestic Cats Inhabit Tatra National Park. Polish J Ecol 65:415-521. https://doi.org/10.3161/15052249PJE2017.65.4.010

APPENDICES

Table 1.2. Number and proportion of wildcat biological samples collected per country (n=964) and number of samples per type (N).

Country	**Total number of samples (%)**	**Sample Type (N)**
Austria	15 (1.55)	Hair (8), phenotypic (7)
Belgium	76 (7.88)	Hair (4), phenotypic (72)
Bulgaria	17 (1.76)	Hair (13), phenotypic (4)
France	1 (0.10)	Hair (1)
Germany	324 (33.61)	Hair (54), phenotypic (270)
Greece	20 (2.07)	Hair (1), phenotypic (19)
Italy	172 (17.84)	Hair (4), phenotypic (168)
Luxembourg	41 (4.25)	Hair (25), phenotypic (16)
Netherlands	9 (0.94)	Hair (9)
Portugal	64 (6.65)	Phenotypic (63), scat (1)
Romania	53 (5.50)	Hair (18), phenotypic (35)
Spain	155 (16.09)	Hair (42), phenotypic (100), scat (13)
United Kingdom	17 (1.76)	Tissue (17)

Table 1.2. Relation between the protection level of the sampled areas and continuous covariates *forest, urban* and *shrubs*. For each meta population, the effect of each protection level is showed (Estimate), together with the standard error (SE), and *p* value. Significant *p* value (< 0.01) are at bold.

Forest

Metapopulation	**Variable**	**Estimate**	**SE**	***p* value**
Europe	(Intercept)	0.343	0.119	**0.004**
	Moderately protected	-0.011	0.156	0.942
	Not protected	-0.437	0.146	**0.003**
	Strictly protected	3.198	0.442	**< 0.001**
Iberian Peninsula	(Intercept)	-0.050	0.213	0.814
	Moderately protected	0.129	0.263	0.624
	Not protected	-0.628	0.284	0.028
	Strictly protected	2.138	0.522	**<0.001**
Italian	(Intercept)	0.433	0.325	0.185
	Moderately protected	0.425	0.470	0.366
	Not protected	-1.076	0.522	0.041
	Strictly protected	12.297	1.561	**<0.001**
SE Continental	(Intercept)	0.091	0.272	0.738
	Moderately protected	-1.134	0.697	0.107
	Not protected	-0.938	0.373	**0.010**
NW Continental	(Intercept)	0.742	0.133	**<0.001**
	Moderately protected	-0.283	0.160	0.079
	Not protected	-0.444	0.147	**0.003**
	Strictly protected	-0.464	0.532	0.383

Urban

Metapopulation	**Variable**	**Estimate**	**SE**	***p* value**
Europe	(Intercept)	-4.196	0.113	**<0.001**
	Moderately protected	-0.473	0.148	**0.001**
	Not protected	0.638	0.139	**<0.001**
	Strictly protected	-2.033	0.419	**<0.001**
Iberian Peninsula	(Intercept)	-5.593	0.284	**<0.001**
	Moderately protected	-0.522	0.350	0.137
	Not protected	1.579	0.378	**<0.001**
	Strictly protected	-1.314	0.695	0.060
Italian	(Intercept)	-3.440	0.220	**<0.001**
	Moderately protected	-0.954	0.317	**0.003**
	Not protected	0.218	0.353	0.538
	Strictly protected	-3.524	1.055	**0.001**
SE Continental	(Intercept)	-4.459	0.185	**<0.001**
	Moderately protected	-0.288	0.473	0.545
	Not protected	0.982	0.254	**<0.001**
NW Continental	(Intercept)	-3.683	0.140	**<0.001**
	Moderately protected	-0.020	0.169	0.908
	Not protected	0.234	0.155	0.130
	Strictly protected	-0.300	0.560	0.592

Shrubs

Metapopulation	**Variable**	**Estimate**	**SE**	***p* value**
Europe	(Intercept)	-4.573	0.144	**<0.001**
	Moderately protected	-0.144	0.189	0.447
	Not protected	-1.277	0.177	**<0.001**
	Strictly protected	0.172	0.534	0.748
Iberian Peninsula	(Intercept)	-1.794	0.135	**<0.001**
	Moderately protected	-0.162	0.167	0.331

	Not protected	-0.997	0.180	**<0.001**
	Strictly protected	-1.260	0.330	**<0.001**
Italian	(Intercept)	-3.967	0.140	**<0.001**
	Moderately protected	-0.079	0.203	0.698
	Not protected	-0.510	0.225	0.025
	Strictly protected	-1.586	0.673	0.020
SE Continental	(Intercept)	-5.480	0.348	**<0.001**
	Moderately protected	1.002	0.891	0.264
	Not protected	0.873	0.478	0.071
NW Continental	(Intercept)	-6.928	0.060	**<0.001**
	Moderately protected	-0.079	0.072	0.275
	Not protected	-0.068	0.066	0.302
	Strictly protected	0.021	0.238	0.929

Table 1.3. Number of biological samples per genetic integrity class (genetically ‘pure’ wildcat, hybrid and domestic cat), and legal landscape protection level, for Europe and metapopulations.

Number of biological samples	**Europe**	**Iberian**	**Italian**	**Scottish**	**SE Continental**	**NW Continental**
Wildcat	758	130	149	2	79	398
Hybrid	59	75	16	14	7	57
Domestic	147	14	7	1	4	11
Unprotected area	429	64	42	8	44	271
Poorly protected area	216	59	66	1	39	60
Moderately protected area	302	95	61	8	7	131
Strictly protected area	17	10	3	-	-	4

Table 1.4. Landscape composition according to the potentially relevant covariates for European wildcat's genetic integrity for Europe and metapopulations. The mean sampled landscape proportion, standard error and range are presented.

Covariates	**Europe**	**Iberian**	**Italian**	**Scottish**	**SE Continental**	**NW Continental**
Forest cover	54.4 ± 24.9% [0.2 / 100.0]	49.3 ± 29.2% [2.54 / 95.9]	58.9 ± 25.9% [0.3 / 100.0]	23.3 ± 16.4% [0.4 / 48.8]	44.8 ± 27.1% [0.2 / 96.6]	58.4 ± 20.0% [2.9 / 98.4]
Shrubland cover	3.8 ± 8.1% [0.0 /64.0]	13.5 ± 12.3% [0.0 /64.0]	2.3 ± 1.9% [0.0 /17.2]	1.1 ± 2.7% [0.0 /11.5]	2.8 ± 3.5% [0.0 /17.4]	0.0 ± 0.03% [0.0 /0.3]
Cropland cover	29.1 ± 23.2% [0.0 / 95.0]	22.8 ± 25.7% [0.0 / 84.5]	27.7 ± 24.2% [0.0 / 88.1]	19.6 ± 21.4% [0.0 / 64.3]	40.7 ± 27.1% [0.1 / 95.0]	30.6 ± 19.4% [0.0 / 92.2]
Human buildup area	4.5 ± 23.2% [0.0 / 75.4]	3.8 ± 10.9% [0.0 / 73.9]	6.0 ± 7.2% [0.0 / 17.2]	0.6 ± 0.5% [0.0 / 15.5]	3.8 ± 8.7% [0.0 / 75.4]	4.5 ± 5.6% [0.0 / 44.1]

Table 1.5. Spearman's correlation matrix between the response variable (wildcat genetic integrity- wc) and continuous covariates (see table 1.1 for the variables acronym definition).

	wc	Crops	Forest	Shrubs	d_forest	d_urban	d_crops	d_shrub
wc	1.00	0.04	0.13	-0.20	-0.02	0.05	0.06	-0.00
Crops		1.00	-0.76	-0.19	0.02	0.07	0.09	0.17
Forest			1.00	-0.15	-0.00	0.03	0.02	-0.12
Shrubs				1.00	-0.21	-0.38	-0.35	-0.15
d_tree					1.00	0.02	-0.14	0.16
d_urb						1.00	0.56	0.50
d_crops							1.00	0.44
d_shrub								1.00

Table 1.6. Differences between the model explanation (R^2) including, or not, distance covariate for the European range and for each metapopulation.

Model explanation (R^2)	**Europe**	**Iberian**	**Italian**	**Scottish**	**SE Continental**	**NW Continental**
With distances	0.175	0.343	0.078	0.157	0.05	0.02
Without distances	0.179	0.179	0.080	0.157	0.05	0.02

Table 1.7. Best model types for each metapopulation and the entire European dataset, for explaining European wildcat genetic integrity variation. Models for Europe, Italian, Scottish, SE Continental and NW Continental included covariates *forest*, *forest*^{2}, *shrubs*, *shrubs*^{2}, and *urban*. Models for Iberian Peninsula metapopulation included the covariates *forest*, *forest*^{2}, *shrubs*, *shrubs*^{2}, *urban*, and *distance to forest, shrubs, crops and urban. The degrees of freedom* (df)*, log-likelihood (LogLik), Akaike's Information Criterion for small samples (AICc), variation between the AICc from each model and the lower AICc value (ΔAICc), and the Akaike weight (Weight) are presented.*

Metapopulation	**Best model**	***df***	**LogLik**	**AICc**	**ΔAICc**	**Weight**
Europe	GLMM – ecoregion	8	-2673.747	5363.6	0.00	1.00
	Linear Model	7	-2710.676	5435.5	71.82	0.00
	GLMM – biome	8	-2718.596	5453.3	89.70	0.00
	GLMM – sample type	8	-2718.596	5453.3	89.70	0.00
Iberian Peninsula	GLMM – ecoregion	11	-345.718	715.9	0.00	0.73
	GLMM – sample type	11	-346.732	717.9	2.03	0.24
	GLMM – biome	11	-353.109	730.7	14.78	0.00
	Linear Model	10	-354.822	731.7	15.78	0.00
Italian	Linear Model	7	-393.717	802.1	0.00	0.76
	GLMM – ecoregion	8	-393.827	804.5	2.42	0.23
	GLMM – sample type	8	-396.560	810.0	7.88	0.01
	GLMM – biome	8	-398.022	812.9	10.81	0.00
Scottish	Linear Model	7	-21.361	69.2	0.00	0.98
	GLMM – ecoregion	8	-22.771	79.5	10.38	0.01
	GLMM – biome	8	-22.771	79.5	10.38	0.01
SE Continental	Linear Model	7	-231.549	478.5	0.00	0.76
	GLMM – ecoregion	8	-232.469	482.7	4.25	0.10
	GLMM – biome	8	-232.625	483.0	4.56	0.08
	GLMM – sample type	8	-232.664	483.1	4.64	0.06
NW Continental	Linear Model	7	-1255.183	2524.6	0.00	0.49
	GLMM – ecoregion	8	-1254.405	2525.1	0.51	0.38
	GLMM – biome	8	-1255.473	2527.3	2.65	0.13
	GLMM – sample type	8	-1258.872	2534.1	9.45	0.00

Table 1.8. Best models (ΔAICc<7) for each metapopulation and the entire European dataset for explaining European wildcat genetic integrity variability. Models for Europe, Italian, Scottish, SE Continental and NW Continental included covariates *forest*, *forest^2*, *shrubs*, *shrubs^2*, and *urban*. Models for Iberian Peninsula metapopulation included the covariates *forest, forest^2, shrubs, shrubs^2, urban,* and *distance to forest, shrubs, crops and urban. The degrees of freedom* (df), *log-likelihood (LogLik), Akaike's Information Criterion for small samples (AICc), variation between the AICc from each model and the lower AICc value (ΔAICc), and the Akaike weight (Weight) are presented. The proportion of model explanation are represented by R^2.*

Metapopulation	**Top models**	***df***	**LogLik**	**AICc**	**ΔAICc**	**Weight**	**R^2**
Europe	Frst + Urb	5	-2671.965	5454.99	0.00	0.639	0.175
	Frst + Frst^2 + Urb	6	-2672.284	5356.66	2.66	0.169	0.177
	Frst + Shrb + Urb	6	-2672.633	5357.36	3.36	0.119	0.176
	Frst + Frst^2 + Shrb + Urb	7	-2672.862	5359.84	5.85	0.03	0.177
Iberian Peninsula	Frst + Frst^2 + Urb + D_Frst + D_Crops + D_Urb	9	- 346.572	712.8	0.00	0.139	0.343
	Frst + Frst^2 + Urb + D_Frst + D_Urb	8	-347.791	721.9	0.10	0.133	0.332
	Frst + Frst^2 + Urb + Shrb + D_Frst + D_Urb	9	-346.664	713.0	0.18	0.127	0.343
	Frst + Frst^2 + Urb + Shrb + D_Frst	8	-348.029	713.4	0.57	0.105	0.328
	Frst + Frst^2 + Urb + Shrb + D_Frst + D_Crops + D_Urb	10	-345.752	713.5	0.74	0.096	0.350
	Frst + Frst^2 + Urb + D_Frst	7	-349.376	713.8	0.97	0.086	0.315
	Frst + Frst^2 + Urb + Shrb + D_frst + D_Crops	9	-347.812	715.3	2.48	0.040	0.329
	Frst + Frst^2 + Urb + Shrb + Shrb^2 + D_frst + D_Urb	10	-346.711	715.4	2.66	0.037	0.343
	Frst + Frst^2 + Urb + D_frst + D_Crops	8	-349.100	715.5	2.72	0.036	0.316
	Frst + Frst^2 + Urb + Shrb + Shrb^2 + D_frst	9	-348.072	715.8	3.00	0.021	0.328
	Frst + Frst^2 + Urb + Shrb + Shrb^2 + D_frst + D_Urb + D_Crops	11	-345.718	715.9	3.10	0.030	0.351
	Frst + Frst^2 + Urb + D_Urb	7	-351.056	717.1	4.33	0.016	0.294
	Frst + Frst^2 + Urb + Shrb + D_Urb	8	-350.056	717.4	4.63	0.014	0.303
	Frst + Frst^2 + Urb + Shrb + Shrb^2 + D_frst + D_crops	10	-347.838	717.7	4.91	0.012	0.329
	Frst + Frst^2 + Urb + D_Urb + D_Crops	8	-350.512	718.3	5.54	0.009	0.297
	Frst + Frst^2 + D_frst + D_Urb	7	-351.814	718.6	5.85	0.007	0.285
	Frst + Frst^2 + D_frst + D_Crops	8	-350.695	718.7	5.91	0.007	0.295
	Frst + Frst^2 + Urb + Shrb	7	-351.922	718.8	6.06	0.007	0.283
	Frst + Frst^2 + Urb + Shrb + D_Urb + D_Crops	9	-349.636	718.9	6.13	0.007	0.305
	Frst + Frst^2 + Urb	6	-353.152	719.0	6.27	0.006	0.270
Italian	Frst + Frst^2 + Urb	5	-394.359	799.080	0.00	0.307	0.082
	Frst + Frst^2 + Shrb + Urb	6	-393.808	800.126	1.05	0.182	0.088
	Frst + Frst^2	4	-396.198	800.635	1.55	0.141	0.062
	Frst + Frst^2 + Shrb	5	-395.500	801.362	2.28	0-098	0.069
	Frst + Frst^2 + Shrb + Shrb^2 + Urb	7	-393.717	802.118	3.03	0.067	0.089
	Frst + Frst^2 + Shrb + Shrb^2	6	- 395.332	803.174	4.09	0.040	0.071
	Frst + Shrb + Urb	5	-396.607	803.575	4.49	0.032	0.057
	Frst + Shrb + Shrb^2 + Urb	6	-395.699	803.907	4.83	0.027	0.067
	Frst + Shrb	4	-398.100	804.439	5.36	0.021	0.041
	Frst + Shrb + Shrb^2	5	-397.056	804.473	5.39	0.021	0.052
	Shrb	3	-399.459	805.062	5.98	0.015	0.026
	Shrb + Shrb^2	4	- 398.475	805.189	6.11	0.144	0.037
Scottish	Null model	2	-23.189	51.235	0.00	0.325	0.000
	Shrb	3	-21.728	51.303	0.07	0.314	0.158
	First	3	-23.166	54.178	2.94	0.075	0.003
	Urb	3	-23.189	54.224	2.99	0.073	0.000
	Shrb + Urb	4	-21.574	54.481	3.25	0.064	0.173
	Frst + Shrb	4	-21.593	54.520	3.28	0.063	0.171
	Shrb + Shrb^2	4	-21.669	54.671	3.44	0.058	0.164
	Frst + Frst^2	4	-22.997	57.327	6.09	0.015	0.024
	Frst + Urb	4	-23.158	57.650	6.42	0.013	0.004
SE Continental	First	3	-233.538	473.355	0.00	0.148	0.054
	Frst + Urb	4	-232.544	473.559	0.20	0.134	0.075
	Frst + Shrb	4	-232.681	473.832	0.48	0.117	0.072
	Frst + Shrb + Urb	5	-231.637	473.989	0.63	0.108	0.093
	Urb	3	-234.439	475.157	1.80	0.060	0.035
	Shrb + Urb	4	-233.396	475.262	1.91	0.057	0.057

	Frst + Frst^2	4	-233.535	475.540	2.19	0.050	0.054
	Frst + Frst^2 + Urb	5	-232.543	475.801	2.45	0.044	0.075
	Frst + Shrb + Shrb^2	5	- 232.643	476.000	2.65	0.040	0.073
	Frst + Frst^2 + Shrb	5	-232.662	476.037	2.68	0.039	0.073
	Frst + Shrb + Shrb^2 + Urb	6	-231.576	476.165	2.81	0.036	0.095
	Frst + Frst^2 + Shrb + Urb	6	-231.583	476.178	2.82	0.036	0.095
	Null model	2	236.052	476.242	2.89	0.035	0.000
	Shrb	3	-235.052	476.383	3.03	0.033	0.022
	Shrb + Shrb^2 + Urb	5	-233.094	476.902	3.55	0.025	0.064
	Shrb + Shrb^2	4	-234.760	477.990	4.64	0.015	0.028
	Frst + Frst^2 + Shrb + Shrb^2	6	-232.637	478.285	4.93	0.013	0.073
	Frst + Frst^2 + Shrb + Shrb^2 + Urb	7	-231.549	478.464	5.11	0.012	0.095
NW Continental	Urb	3	-1257.204	2520.460	0.00	0.273	0.020
	Frst + Frst^2 + Urb	5	-1255.359	2520.849	0.39	0.225	0 .028
	Shrb + Urb	4	-1257.076	2522.239	1.78	0.112	0.021
	Frst + Urb	4	-1257.099	2522.285	1.83	0.109	0.021
	Frst + Frst^2 + Shrb + Urb	6	-1255.183	2522.549	2.09	0.096	0.029
	Frst + Shrb + Urb	5	-1256.968	2524.066	3.61	0.045	0.021
	Shrb + Shrb^2 + Urb	5	-1257.067	2524.264	3.80	0.041	0.021
	Frst + Frst^2 + Shrb + Shrb^2 + Urb	7	-1255.183	2524.610	4.15	0.034	0.029
	Frst + Shrb + Shrb^2 + Urb	6	-1256.959	2526.102	5.64	0.016	0.021
	Frst + Frst^2	4	-1259.187	2526.460	6.00	0.014	0.012

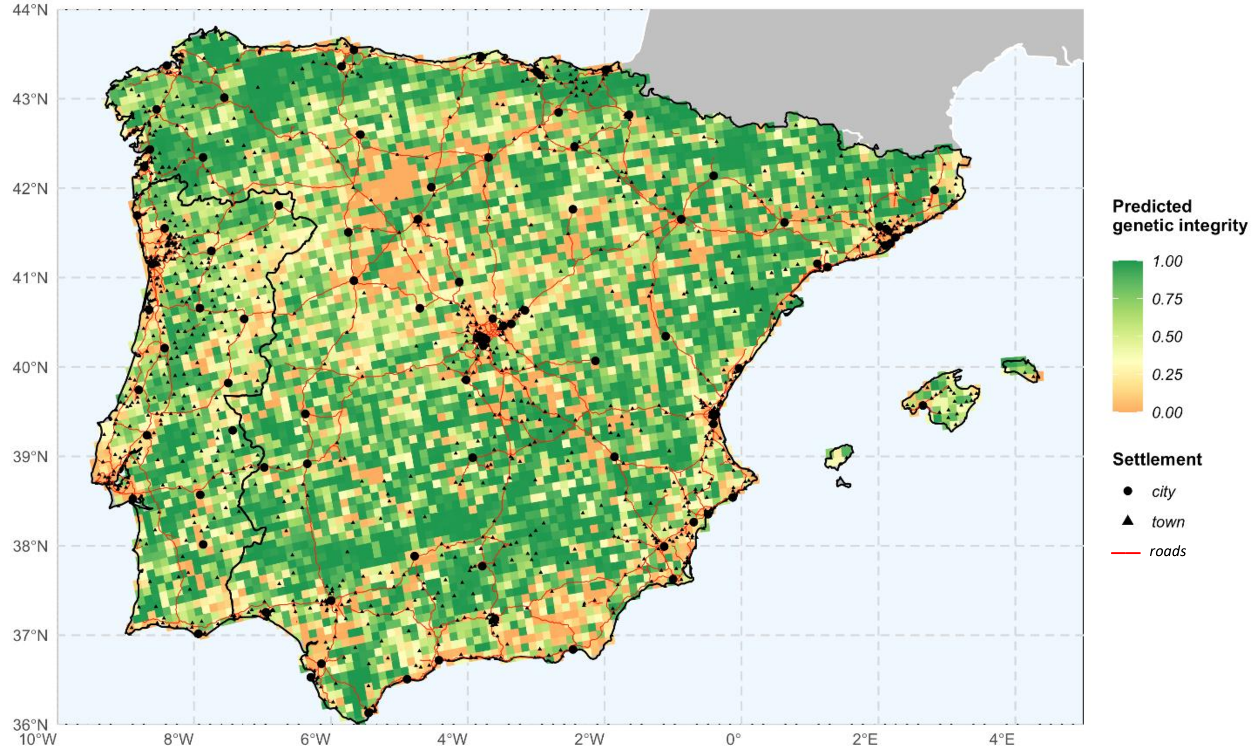

Figure 1.1. Iberian metapopulation predicted genetic integrity, based on the best models including covariates: forest, forest^2, distance to forest, shrubs, shrubs^2, urban, distance to urban, and distance to croplands. Cities and towns are represented as black dots and triangles, respectively. Main roads are represented by red lines.

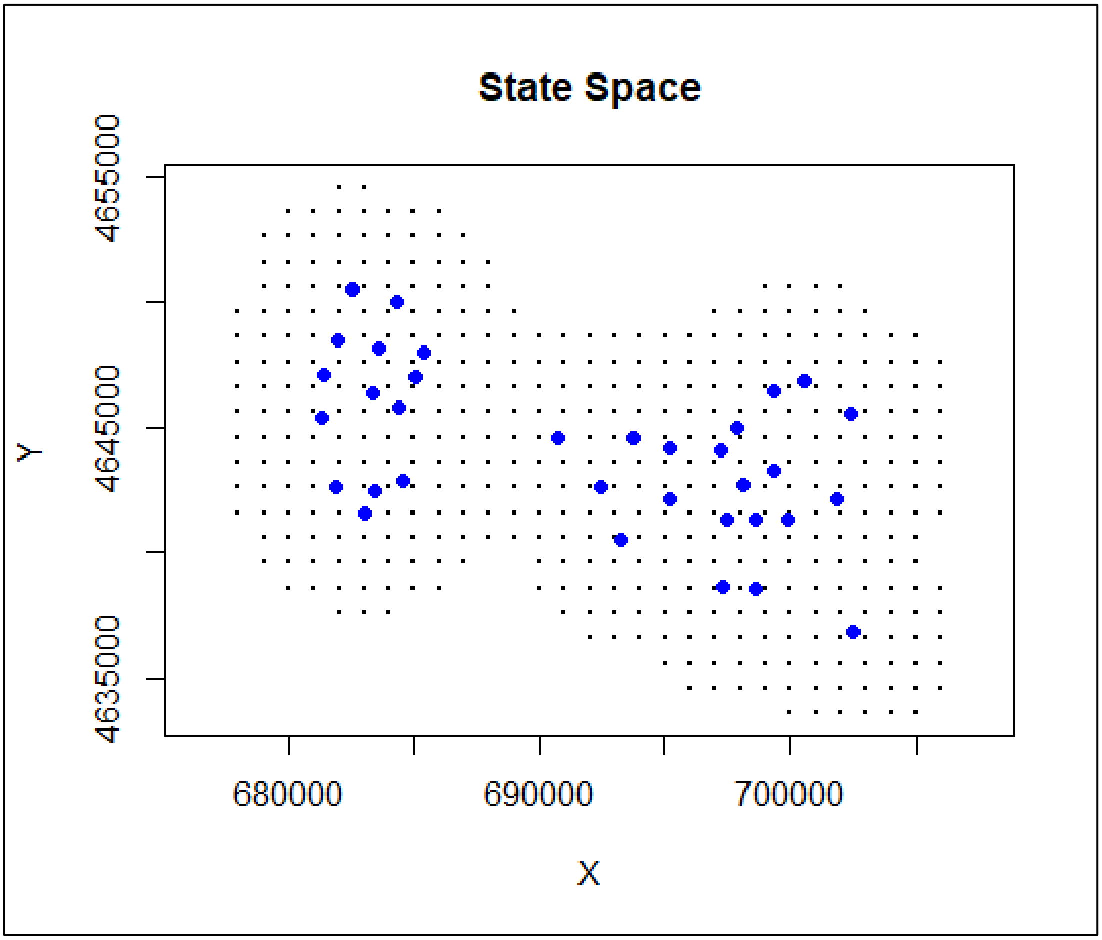

Figure 2.1. Visualization of the prescribed state space. Blue circle are the locations of the detectors (camera-trap) and grey points are the pixel centroids (hypothetically individual's activity center). The state space resolution is 1 km, and the buffer is 4.3 km.