



Tânia Sofia
Queirós Barros

**Ecologia molecular do sacarrabos
(*Herpestes ichneumon*) em Portugal**

**Molecular ecology of the Egyptian mongoose
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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Prof. Doutor Carlos Manuel Martins Santos Fonseca, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro.

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Aos meus pais.

Ao meu avô Rafael.

o júri

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palavras-chave

Alterações do uso do solo, carnívoro, DNA mitocondrial, expansão, diversidade genética, genética populacional, genética de paisagem, Herpestidae, microssatélites, Península Ibérica.

resumo

O desafio central no estudo de expansões populacionais é fornecer uma caracterização exaustiva dos indivíduos em expansão, assim como a sua dinâmica ao longo do território colonizado. A combinação de dados ecológicos com técnicas moleculares permite a compreensão dessa dinâmica, estrutura populacional e a biologia dos indivíduos em expansão. O objetivo geral desta tese foi analisar quais os fatores que contribuíram para a expansão do sacarrabos, analisar a estrutura genética e genética populacional desta espécie em expansão e relacionar fatores de paisagem com o *gene flow* da população de sacarrabos na Península Ibérica. A expansão foi analisada nos períodos 1980-1990, 1990-2000 e 2000-2010, projetando dados de presença/ausência da espécie e propondo quatro hipóteses explicativas agrupando fatores ambientais, geográficos e antropogénicos. Os resultados sugeriram que o efeito das alterações do uso do solo, temperatura e variações de precipitação foram os fomentadores principais da expansão da espécie ao longo das décadas. Por outro lado, barreiras geográficas e antropogénicas condicionaram a expansão da espécie. Os padrões genéticos, história demográfica e a estrutura genética da espécie foram avaliados através de DNA mitocondrial e microssatélites. A diversidade mitocondrial converge com um padrão de uma população histórica existente no sul da Península Ibérica, de onde dispersões ocorreram recentemente em direção a territórios a norte. A deteção de migrantes de longa distância de sul para norte foram suportados por 1) uma maior diversidade haplotípica e nucleotídica em territórios a norte, 2) uma distribuição heterogénea da diferenciação genética e 3) a quase ausência de isolamento por distância. Evidências de expansão rápida e espacial foram detetadas e reforçadas pelos resultados de *mismatch* e pelos testes de neutralidade marginalmente significativos. Por outro lado, as análises dos microssatélites evidenciaram uma população a centro-norte (*CNorth*) e uma população a sul (*S*), com uma diferenciação significativa. A expansão recente para territórios a norte foi suportada pelas frequências alélicas, os níveis de heterozigotia, por um *bottleneck* ocorrido após a expansão em *CNorth* e por um maior contributo dos indivíduos de *S* para a diversidade alélica. Inversamente ao DNA mitocondrial, os microssatélites evidenciaram um padrão de isolamento por distância juntamente com um padrão de isolamento por barreira devido à presença do Rio Tejo. O Rio foi identificado como uma semi-barreira ao *gene flow* e possivelmente levou à presença dos dois *clusters* genéticos. A presença de *S* e *CNorth* em locais opostos à sua distribuição geral pode estar relacionado com dispersantes de longa distância. Com o objetivo de investigar a relação entre a estrutura genética do sacarrabos e aspectos paisagísticos, uma análise de genética de paisagem foi realizada através das abordagens modelação causal original e modelação causal recíproca. A modelação causal original identificou o isolamento por distância como o modelo que melhor reflete os padrões genéticos da população de sacarrabos. A nova abordagem de modelação causal recíproca identificou áreas de elevado coberto arbustivo a altitudes médias como o melhor modelo. Estes resultados demonstram a elevada dependência do sacarrabos por ecossistemas dominados por coberto arbustivo Mediterrâneo. As recentes alterações no uso do solo em Portugal relacionadas com o abandono rural promoveram a conectividade entre as populações de sacarrabos em áreas com coberto arbustivo elevado em altitudes médias, onde a influência antropogénica é menor. Esta tese representa um estudo abrangente dos padrões dinâmicos desta espécie em expansão, com conhecimentos novos e essenciais sobre o sacarrabos na Península Ibérica. Estes resultados devem ser percecionados como um modelo para espécies em expansão num ambiente em mudança, especialmente carnívoros Mediterrânicos, com particular ênfase na avaliação dos fatores chave que contribuem para a expansão de uma espécie, no efeito das alterações da distribuição das espécies nos seus padrões genéticos e, por fim, na influência de fatores de paisagem no *gene flow* dessas espécies.

keywords

Carnivore, expansion, genetic diversity, Herpestidae, Iberian Peninsula, land-use changes, landscape genetics, microsatellites, mitochondrial DNA, population genetics.

abstract

The central challenge on studying population expansions is to provide an in-depth characterization of the expanding individuals and their dynamics across invaded ranges. By combining ecological data with molecular techniques, one can understand those dynamics, population structure and the biology of expanding species. The general aim of this thesis was assessing which factors contributed for the expansion of the Egyptian mongoose, analyse the genetic structure and population genetics of this expanding species and relate landscape factors with the gene flow within the mongoose population of the Iberian Peninsula. The expansion was evaluated across 1980-1990, 1990-2000 and 2000-2010, by projecting presence/absence data of the species and proposing four explanatory hypotheses gathering environmental, geographic and anthropogenic factors. The results suggested that land-use alterations coupled with temperature and rainfall variations over the decades were the main drivers promoting the mongoose expansion. Moreover, geographic barriers and human infrastructures acted as barriers towards the Egyptian mongoose expansion. Genetic patterns, demographic history and genetic structure of the species were evaluated by mitochondrial DNA and microsatellite markers. The distribution of mitochondrial diversity fitted the pattern of a historically diversified population in southern Iberian Peninsula, from where recent dispersals into northern areas occurred. Long-term dispersal migrants from south towards northern areas were evidenced by 1) higher levels of haplotype and nucleotide diversities in the northern areas, 2) heterogeneous distribution of population genetic differentiation, and 3) weak signal for isolation by distance. Sudden and spatial expansion scenarios were reinforced by mismatch analysis and marginally supported by neutrality tests. On the other hand, microsatellite analyses evidenced a central-northern subpopulation (*CNorth*) and a southern subpopulation (*S*) with significant differentiation. Allelic frequencies, levels of heterozygosity and evidences of bottleneck occurred after the expansion in *CNorth* supported the recent northwards range expansion, with *S* individuals contributing with a higher allelic diversity. Inversely to mitochondrial DNA, microsatellites evidenced a pattern of isolation by distance together with a pattern of isolation by barrier due to the Tagus River, which was identified as a semi-barrier towards gene flow which possibly caused the presence of the two genetic clusters. The presence of *S* and *CNorth* in opposite locations of their general distribution might be related with long-range dispersals. A landscape genetics approach was implemented under the original and reciprocal causal modelling frameworks with the objective of investigate the relation between genetic structure and landscape aspects. Isolation by distance was the best model identified by original causal modelling explaining genetic patterns within the mongoose population. The newer reciprocal causal modelling approach identified high shrub cover at middle elevations as the best model. These results demonstrate the high dependency of the Egyptian mongoose on ecosystems dominated by Mediterranean shrub cover. The recent land-use changes in Portugal related with rural abandonment promoted the connectivity among the mongoose populations in areas with high shrub cover at middle elevations, where anthropogenic disturbance is lower. This thesis represents a holistic study of the dynamic patterns of this expanding species, with new and essential findings of the Egyptian mongoose in the Iberian Peninsula. The results should be perceived as a model for expanding species under a changing environment, especially Mediterranean carnivores, with a special emphasis on assessing which key factors act as drivers for the expansion of species, the effect of the alterations of species' ranges on their genetic patterns, and ultimately how landscape influences gene flow of those species.

MOLECULAR ECOLOGY
OF THE
EGYPTIAN MONGOOSE
(*Herpestes ichneumon*)
IN PORTUGAL



Illustration by Ashvini Menon

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LIST OF ACRONYMS

ΔR	Rainfall variation for each decade
ΔT	Temperature variation for each decade
Π	Nucleotide diversity
AIC	Akaike Information Criterion
AMOVA	Analysis of Molecular Variance
Central IP	Central Iberian Peninsula
Central-northern IP	Central-northern Iberian Peninsula
Cluster CNorth	Cluster majorly distributed in central-northern areas of Western Iberian Peninsula
Cluster S	Cluster majorly distributed in the south of Western Iberian Peninsula
cr	Control region
cyt b	Cytochrome b
EBSP	Extended Bayesian Skyline Plot
F_{IS}	Inbreeding coefficient
F_{ST}	Fixation index
GLM	Generalized Linear Models
h	Number of haplotypes
Hd	Haplotype diversity
H_E	Expected heterozygosity
HE	High elevation
H_O	Observed heterozygosity
HPA	Hierarchical Partitioning Analysis
HS	High selectivity for dense shrub cover
HSF	High selectivity for dense forest cover
HT	High temperature
HWE	Hardy-Weinberg Equilibrium
IBB	Isolation by Barrier
IBD	Isolation by Distance
LE	Low elevation
LD	Linkage Disequilibrium

LS	Low selectivity for dense shrub cover
LSF	Low selectivity for dense forest cover
LT	Low temperature
MaxAltit	Maximum Altitude
MaxSlope	Maximum Slope
MCMC	Markov Chain Monte Carlo
ME	Medium elevation
MeanAltit	Mean Altitude
MeanSlope	Mean Slope
MinAltit	Minimum Altitude
MS	Medium selectivity for dense shrub cover
MT	Medium temperature
mtDNA	Mitochondrial DNA
N _A	Number of alleles
N _{PA}	Number of private alleles
P/A	Presence-absence data
PCR	Polymerase Chain Reaction
PopDens	Weighted Human Population Density per unit
r	Raggedness index
R _{ST}	Fixation index designed for microsatellites
S	Number of polymorphic sites
SAMOVA	Spatial Analysis of Molecular Variance
Southern IP	Southern Iberian Peninsula
SSD	Sum of Squared Deviations
TRness	Land Ruggedness
WClosedArea	Weighted Closed Areas per unit
WCrop	Weighted Crop Areas per unit
WForest	Weighted Forest Areas per unit
WIP	Western Iberian Peninsula
WOpenArea	Weighted Open Areas per unit
WScrub	Weighted Scrub Areas per unit
WRoad	Weighted Roads Extension per unit

WRiver	Weighted River Extension per unit
WUrban	Weighted Urban Area per unit

CHAPTER I

INTRODUCTION

OVERVIEW, STATE OF THE ART, SPECIES DESCRIPTION, THESIS AIMS AND STRUCTURE,

LIST OF PUBLICATIONS

1. INTRODUCTION

Overview, state of the art, species description, thesis aims and structure, list of publications

1.1. Species' ranges

Understanding what underlies behind a species' range is one of the cornerstones in Ecology (Gaston & Blackburn, 2008; Gaston, 2009a). Species' distributions greatly vary: while certain species present ubiquitous distributions [e.g., the common barn owl (*Tyto alba*) (BirdLife International, 2012)], others are confined to more limited ranges [e.g., the Andean Bear (*Tremarctos ornatus*) Goldstein et al., 2008]. Therefore, it is pivotal to disentangle what shapes a species' distribution. This subject is even more important nowadays, as the knowledge about this matter bears on some of the most significant and contemporaneous environmental challenges, including biological invasions, habitat loss and climate change (Gaston, 2009b).

Inherent to a species' range, there is a tight link with time and space (Pearman et al., 2007). Such time-space dynamic explains why a species' range may be influenced by the same factors but at different levels (e.g., Torres et al., 2012). Four classes have been proposed by Soberón & Peterson (2005) for grouping the potential factors that limit the distribution of a species: 1) *Abiotic factors*, including climatic, topographic and land-use features (e.g., Parmesan, 2006; Kinnaird & O'Brien, 2012; Di Virgillio et al., 2013); 2) *Biotic factors*, such as competition, predation and parasitism (e.g., Jaeger, 1970; Briers, 2003; Holt & Barfield, 2009); 3) *Dispersal potential*, which is the mechanism that dictates range expansions, and comprises both movement of individuals to new locations and their successful establishment in those areas. The identification of what limits the dispersal of species is the keystone of the study of biogeographical patterns (Gaston, 2009a; Wiens, 2011); lastly, 4) *Evolutionary factors* linked with the genetic traits that provide the ability of a population to adapt to new conditions (Etterson & Shaw, 2001).

Although the limits of a species' range may be influenced by one single factor, the combination of those factors frequently occur and act synergistically, antagonistically, or even independently of one another (Mott, 2010; Figure 1). This interface between factors was already described in certain species, proving that an assemblage of factors can affect the distributional patterns of a species (e.g., Barbosa et al., 2001; Virgós et al., 2001; Eggermann et al., 2011; Melles et al., 2011).

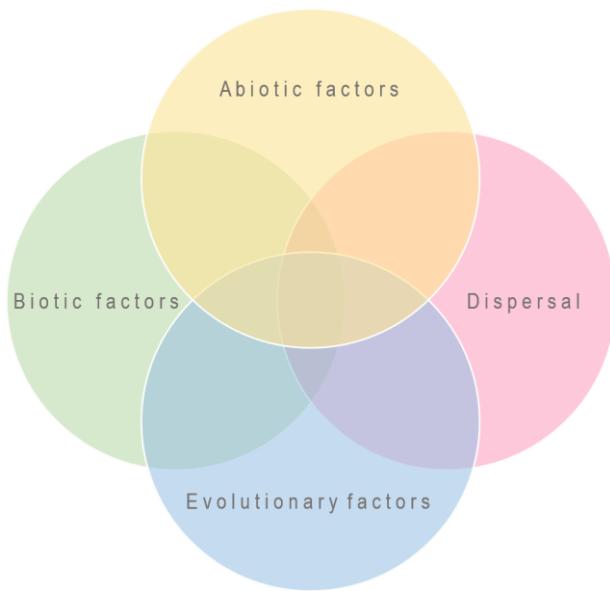


Figure 1. Illustration of the four classes grouping factors affecting a species' range. All factors may exert their influence alone, or in combination and limiting the distribution of a species.

1.1.1. Environmental causes underlying the mobility of a species' range

Species' ranges are extremely mobile (Sexton et al., 2009). The alteration of a species' range can reflect a consequence of deterministic processes, such as species' responses to changes in the environment (Colwell & Rangel, 2010). Those responses can result in range expansions and range contractions over time (Davis & Shaw, 2001; Gaston, 2003).

Today, one of the most pervasive factors altering species' distribution is anthropogenic disturbance. Its impact can be translated in habitat loss, alteration, degradation, pollution, disease, introduction of non-native species, over-harvesting and global climate change (Mott, 2010). In the last decade, many studies have interpreted variations in species' ranges under the climate change scenario (e.g., Levinsky et al., 2007; Seneviratne et al., 2006; Brook, 2008; Jeschke & Strayer, 2008; Guo et al., 2013). Rapid rates of global warming are drastically affecting biodiversity, leading to range shifts and even extinction (Lambers, 2015; Figure 2). In fact, in Biogeography, the effect of climate constitutes one of the most essential elements that naturally affects the distribution of biodiversity worldwide (Gaston, 2003; Pearson & Dawson, 2003). In this context, many species can adapt to new environments with different climatic conditions (Hone & Benton, 2005), however, in

general, species have greater chances of survival in areas with similar climatic conditions to those in their native ranges (Walther et al., 2009).

Due to its great influence on the distribution and abundance of animal and plant species (Gottfried et al., 1999; Jump et al., 2006; Morin et al., 2008; Rahel & Olden, 2008), climate change has been a permanent discussion matter in the scientific community. Both temperature and precipitation are proven to dictate species' success (Ontiveros et al., 2003; Peguero-Pina et al., 2007). Whilst the extinction risk of species subject to range contractions may occur in certain species (La Sorte & Jetz, 2010), other expanding species are benefiting from these environmental changes due to the increasing climatic resemblance of the newly colonized areas to their native areas (Acevedo et al., 2007).

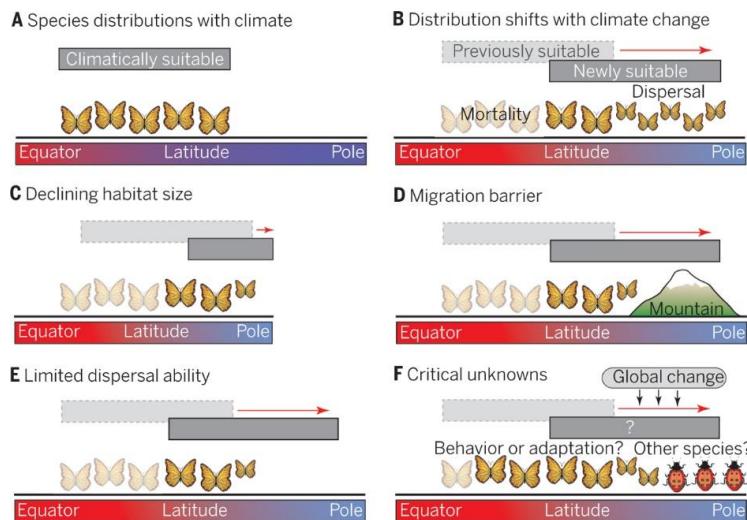


Figure 2. *Move, adapt, or perish.* Species distributions are generally determined by climate (A). They track climate change (red arrows) if populations can disperse and establish in newly suitable habitats, and disappear where climate has become unsuitable (B). Species may face extinction if habitat sizes shrink (for example, at the poles or at mountaintops) (C), or if migration barriers (D) or limited dispersal ability (E) prevent them from reaching newly suitable habitat. The ability of species to adapt (or modify their behaviour), species interactions, and other global change stressors represent key uncertainties (F) that affect our ability to predict biodiversity loss with climate change (from Lambers, 2015).

Furthermore, vegetation cover can dictate the distribution of a species due to the reduced levels of human interference as well as food availability, which makes this feature an important resource for wildlife. However, land use alterations and their consequences across the

globe have been greatly documented (Figure 3). Anthropogenic-driven changes are drastically transforming landscapes with native vegetation into altered environments, and consequently altering the distribution and survival of species (e.g., Pardini, 2004; Zao et al., 2006; Flynn et al., 2009). For instance, the Mediterranean landscape has recently suffered major alterations and several studies enhanced their impact on species and their habitats (e.g., Díaz et al., 1997; Suarez-Seoane et al., 2002; De Juana, 2004; Moreira & Russo, 2007; Acevedo et al., 2011). Due to the important role of the Mediterranean ecosystems on the activity of many species (see Mangas et al., 2008), it is imperative to analyse the impact of land use alterations in the viability of such ecosystems. The major causes of Mediterranean land-use alterations are the gradual substitution of traditional agriculture by land-use intensive practices, the use of broader areas for plantations and the crescent implementation of permanent pasture areas (Recenseamento Agrícola, 2011). Despite some species seem to benefit from agricultural farmland (e.g., Goldyn et al., 2003; Rosalino et al., 2005; Rondinini et al., 2006; Balestrieri et al., 2009), drastic consequences at landscape-level are occurring, including the decline of mosaic patches, landscape homogenization and loss of forest cover in marginal farming areas, which are important for several species (see Falcucci et al., 2007; Van Doorn & Bakker, 2007; De Aranzabal et al., 2008; Pita et al., 2009).

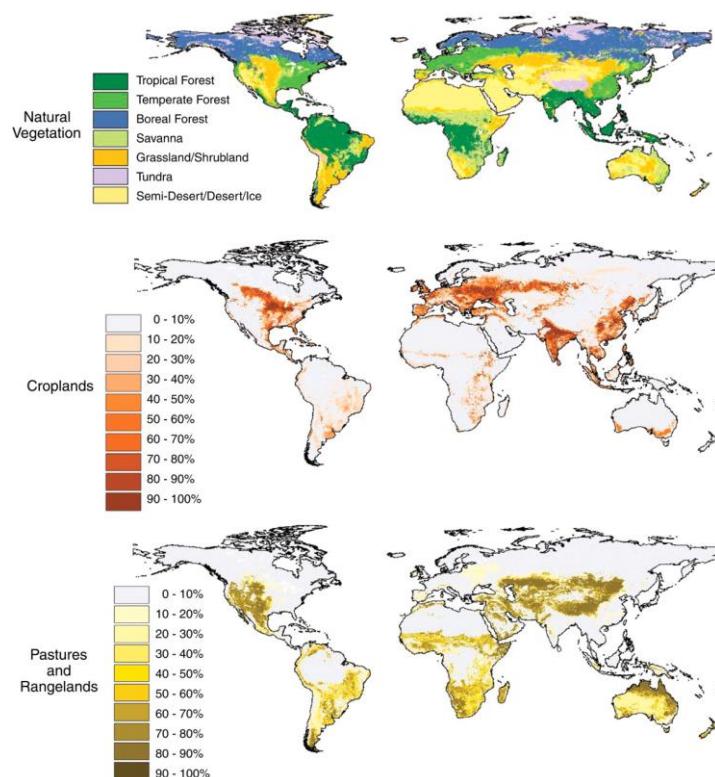


Figure 3. Worldwide extent of human land-use and land-cover change. These maps illustrate the geographic distribution of “potential vegetation” (top), vegetation that would most likely exist in the absence of human land use, and the extent of agricultural land cover (including croplands and pastures) (middle and bottom) across the world during the 1990s (from Foley et al., 2005).

On the other hand, anthropogenic activities related with urban infrastructures are regarded as one of the major causes of declines and contractions in wildlife populations (Riley et al., 2003; Venter et al., 2006; Cumo et al., 2013). Nevertheless, urbanization can have a dual effect on wildlife. Some species seem to benefit from being close to urban areas, for instance the red fox (*Vulpes vulpes*), the wild boar (*Sus scrofa*), and the racoon (*Procyon* sp). Indeed, some authors demonstrated that conservation of wild species and their management is possible where human presence is evident (Linnel et al., 2001), and those species can even benefit from using areas with high human interference (François et al., 2008). Contrastively, others species are negatively affected by man. In fact, generally, wild species tend to disappear from human-dominated landscapes with great human activity and urban areas, where habitat loss is most likely to occur (Gehrt et al., 2009). For instance, wild carnivores have a preference for areas with a reduced human presence (Woodroffe et al., 2005; Rosalino et al., 2009), as human population densities can distress the presence of those species (Treves & Karanth, 2003; Delinger et al., 2013). Currently, the crescent urbanization is frequently associated with the construction of roads. Road network has adverse impacts on wildlife and ecosystems, and it is a driving factor for biodiversity decline (Benítez-López et al., 2010) due to deforestation, habitat loss and habitat fragmentation (McKinney, 2002). In the particular case of terrestrial mammals, several studies confirm that these infrastructures can limit animal movement and also conditioning their survival (Gubbi et al., 2012; Barthelmess, 2014). Other human infrastructures can also cause negative impacts on species distribution. For instance, dam construction can difficult or even hinder species' passage by changing river flow, water volume, soil composition and vegetation destruction (Santos et al., 2008; McCartney, 2009).

The impacts of these drivers on biodiversity's distribution is an emerging question. Thus, the improvement through science on understanding their effects in biodiversity and ecosystems is crucial. Scientists must urge to explore interdisciplinary approaches designed to analyse the effects caused by multiple drivers, as well as evaluating the mechanisms behind those factors and their interaction over time and space. Under the global change scenario, this perspective is necessary to improve the understanding of species responses to environmental changes.

1.1.2. The importance of studying expanding species under a global change scenario

Species under range expansions across the globe are gaining amplified attention by the scientists, as they offer the chance to analyse which factors underlie the increasing of a species' area and also their population dynamics (Scheidt & Hurlbert, 2014). Many attention is given to the expansion of non-native species due to their great impact on the ecosystems (Bonesi & Palazon, 2007; Hays & Conant, 2007; Letnic et al., 2008), however, the assessment of the expansion of native species may be equally important (Putman & Moore, 1998). Population expansion is greatly influenced by dispersal of individuals, population growth, and by temporal and spatial variation in the environmental factors that affect both dispersal and population growth (Lubina & Levin, 1988). By understanding how a species, exotic or native, increases its range and colonize new habitats, it is possible to assess the driving mechanisms of their expansion and predict the consequences of their presence in areas where those species were absent. Additionally, new highlights concerning ecological aspects of expanding species can be assessed, as well as unravelling potential changes in both historical and newly colonized ranges that drove those native species to expand.

1.2. Causes of genetic variation across a species' range

There is a growing interest in quantifying the genetic variation across a species' geographical range, as certain genetic traits permit flexibility and survival of a population in a changing environment (Nature Education, 2014). At population level, genetic variation may be caused by multiple factors, including 1) *matting patterns*, which may shape the genetic combinations that appear in successive generations (Coates & Sokolowski, 1992); 2) *genetic drift* associated with random fluctuations in relative allele frequencies in a population (Hartl & Clark, 1997); 3) *migration events* that contribute with a flux of alleles that may alter the existing proportion of alleles in a population (Konnert & Bergmann, 1997); and 4) *distributional range* (Leniaud et al., 2010), which will be further explored in the next section.

1.2.1. Causes of genetic patterns in populations along their distribution

The genetic variation of a population with a continuous distribution may show great genetic variation and seldom has the same genetic pattern over its entire range (Eckert et al., 2008). When a population is colonizing a geographical gradient of environmental conditions, it generally shows high abundance at the centre of its range (Vucetich & Waite, 2003). In this case, the

distribution of the genetic diversity across the range of the population should vary, where geographically peripheral regions should exhibit lower genetic diversity and higher genetic differentiation than the central region. Consequently, this may limit the evolutionary potential of those marginal individuals, and potentially inhibiting adaptation to conditions beyond the range limit (Hoffman & Blows, 1994). Furthermore, in a wide range population, a decrease on the interbreeding between opposite ends of the range may occur, and the different forces in both ends may turn individuals at opposite locations genetically more distinct from one another. This matter is related with a phenomenon called Isolation by Distance (Wright, 1943), characterized by the decreasing chances of mating or gene flow as the geographic distance between individuals or populations increases (Hamilton, 2011; Figure 4). Isolation by Distance is recorded in many species across several taxa (e.g., Sharbel et al., 2000; Pogson et al., 2001; Pope et al., 2006). It is frequently established over great periods of time through an equilibrium between gene flow and drift, with a restricted dispersal and with limited barriers to gene flow across the range of a population (Pope et al., 2006, after Wright, 1943; Slatkin, 1993). However, species with great dispersal capability can suppress the geographic distance between individuals (see Wright, 1943). If individuals at either end of the range can contact and continue mating, the resulting genetic intermixing can contribute to more genetic variation overall. This may greatly affect the evolutionary and adaptive potential of populations (Slatkin, 1987) by increasing average fitness when environmental quality fluctuates in time and space, by reducing kin competition and inbreeding (Garant et al., 2007).

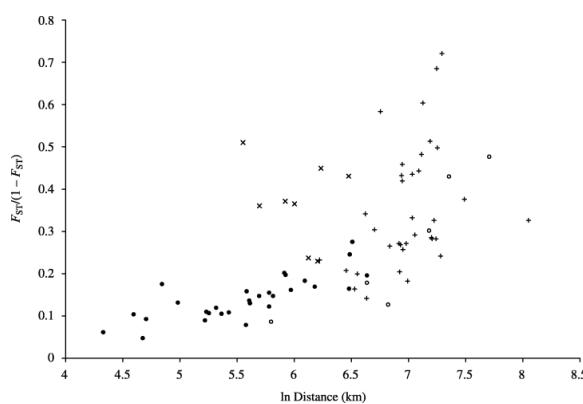


Figure 4. Plot of $F_{ST}/(1 - F_{ST})$ against the natural log of geographic distance among large European badger (*Meles meles*) populations and showing a pattern of isolation by distance. Black circles represent comparisons among British populations; crosses comparisons between Britain and Ireland; white circles comparisons within Western Europe; with the remainder of comparisons represented by plus symbols (from Pope et al., 2006).

Nonetheless, other factors besides distance and dispersal can have their impact on the genetic variation across a species' range. Factors intrinsically linked with anthropogenically altered landscapes are widely proven to alter the distribution of populations and consequently their genetic diversity (Johansson et al., 2007). The presence of habitat fragmentation can cause discontinuities along the landscape, including changes in vegetation patches which potentially influence gene flow in a continuous population (Dixo et al., 2009). For instance, the presence of grasslands and deserts have been proven to affect gene flow in the wide-ranging American puma (*Puma concolor*) in the southwestern area of United States of America, even knowing that the species can disperse through large distances (McRae et al., 2005). Further, anthropogenic barriers including roads lead to loss of connectivity between populations and consequently limit gene flow (Keller & Lagiardè, 2003). Epps et al. (2005) recorded a dramatic decrease of the genetic diversity and gene flow in the desert bighorn sheep (*Ovis canadensis nelsoni*) in the Mojave and Sonoran deserts due to the construction of interstate highways. Additionally, limits on gene flow can be caused by natural barriers. Topographical barriers such as mountains and water bodies may shape the genetic patterns of a population. One of the species that was found to be greatly influenced by water bodies is the red deer (*Cervus elaphus*) in Scottish Highland, as gene flow is mainly limited by sea lochs and favoured by rivers and inland lochs (Pérez-España et al., 2004).

Finally, one of the major factors altering genetic variation of populations is climate change. Species may respond to climate change by local adaptation (Davis et al., 2005), range shift and/or population reduction (Thuiller et al., 2005). These changes will reduce genetic diversity in populations and even species, possibly in such extreme situations to the point where genetic impoverishment will contribute to reduced population viability and extinction (Pauls et al., 2013). Pauls et al. (2013) stressed the importance of assessing cryptic diversity under the climate change scenario and the importance of including the assessment of genetic diversity in studies predicting species' responses to climate change.

However, global changes may have a distinct effect. Genetic diversity may alter due to changes in the distribution of genetic variants in space and time. Hence, as the ranges of populations change, changes in levels of phenotypic plasticity and evolutionary adaptation of individuals (and populations) occur, due to their response to new environmental conditions (Hoffman & Sgrò, 2011). In fact, under the global change scenario, micro-evolution mechanisms (meaning, the possibility of a species to genetically adapt to new conditions through mutations or selection of existing genotypes) and plasticity (which provides a very short-term response) operate

simultaneously (Bellard et al., 2012). As it has been explained by certain authors, the plasticity of a species is often incredibly vital under environmental changes (Hoffman & Sgrò, 2011). On the other hand, recent evidences show that evolution can be very rapid (Lavergne et al., 2010), especially introduced species, for which selection-driven phenotypic changes have enhanced the invasive potential (e.g., Phillips, 2009). Furthermore, global change, including land use and climate warming may turn unsuitable areas into more suitable areas for some species. For instance, a future increase of amphibian and reptile species is expected in the northern European range due to the warming of those cooler regions (Araújo et al., 2006). Such increasing may promote the gaining of genetic diversity of those populations and increase their survival chances under a changing environment.

1.2.2. The importance of assessing the genetic diversity of expanding populations

Due to the interaction between a population and the environment, population dynamics can be affected by environmental features (Gaston, 2009a). As it was stated above, range expansions can occur due to environmental alterations and, consequently, changes in the genetic patterns of a population may arise. For instance, it is known that spatial expansions can generate allele frequency gradients (Excoffier et al., 2009) and they can occur in three different contexts: range expansions from historical (postglacial) range expansions (Eckert et al., 2008); contemporary range expansions due to anthropogenic influence (Dlugosch & Parker, 2008); and natural range expansions (Figure 5), which can possibly possess a different genetic signature (McInerny et al., 2009; Hill et al., 2011).

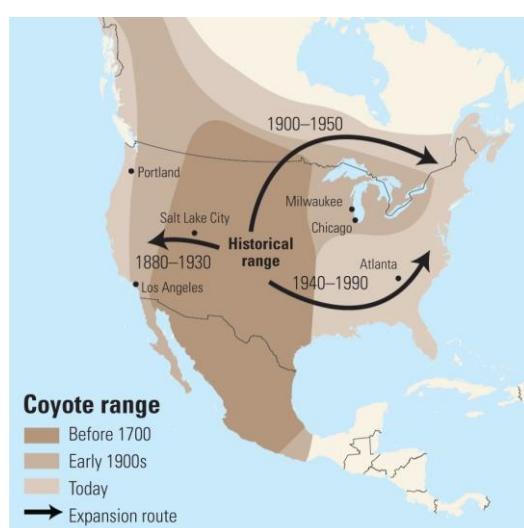


Figure 5. Range expansion of the coyote (*Canis latrans*) over time, an example of a contemporaneous and natural range expansion (from Morrell, 2013).

Whether a range expansion is historical, human-mediated or even natural, unravelling the genetic processes occurring during range expansion is essential, as those processes have different evolutionary consequences. For example, Swaegers et al., 2013 stated that a reduction in the genetic diversity of marginal populations can affect the adaptation to new ecological settings (Agashe et al., 2011), whereas genetic drift can promote or even block adaptation due to alleles surfing on the expansion front (Klopfstein et al., 2006; Burton & Travis, 2008). Hence, understanding the genetic processes associated with a range expansion due to current global changes is also motivated by conservation concerns. Many species are shifting their ranges as a consequence of those contemporary human-mediated changes around the globe. Therefore, the ability of a species to follow those changes is a major determinant of its fate, and assessing the genetic diversity of range expansions can aid on the prediction of species responses to future environmental changes. By studying real-time expansions, one can assess additional insights into the role of evolutionary processes of species. These studies should be conducted in collaboration between both fields of Biology and Global Change.

1.2.3. Assessing the genetic diversity of a population

Genetic methods based on molecular markers provide useful tools for inferring about the genetic patterns in an astonishing number of situations (DeYoung & Honeycutt, 2005). Those inferences may greatly depend on the questions to be raised, the availability of molecular markers, the sampling size and the financial cost.

The study of genetic variation is frequently analysed at different levels and there are distinct methods for differentiating those levels. Generally, the analysis of the genetic variation can be assessed 1) at individual-level, where genetic variation is described as either the percentage of heterozygous loci or allelic combinations at each locus (Hedrick, 1999), or 2) at population-level, in which the most common parameters are genetic structure, demographic assessments, polymorphism, average number of alleles per locus, or expected heterozygosity under Hardy-Weinberg Equilibrium (Nei, 1973; Lacy, 1997).

Broadly, molecular markers are classified by their inheritance, genome location, and by the implemented methods for their use. Currently, two of the most commonly used molecular markers are the mitochondrial DNA and microsatellites, in which we will focus in this section.

Mitochondrial DNA is a circular molecule comprising two ribosomal RNA genes, 22 transfer RNA genes, 13 protein-encoding genes and a noncoding control region (Brown, 1985;

Figure 6). Mitochondrial DNA is maternally inherited in vertebrates and reveals low levels or even no recombination (Harrison, 1989). Mitochondrial DNA was the first molecular marker of choice for assessing patterns of variation between populations in intraspecific phylogeography studies (Avise, 2000). Broadly, patterns of mitochondrial DNA variation provide clues regarding the formation of communities (Riddle & Honeycutt, 1990), population structure and genetic variation (Harrison, 1989), as well as insights regarding the influence of dispersal patterns on the genetic partitioning of haplotypes (Suzuki et al., 2013).

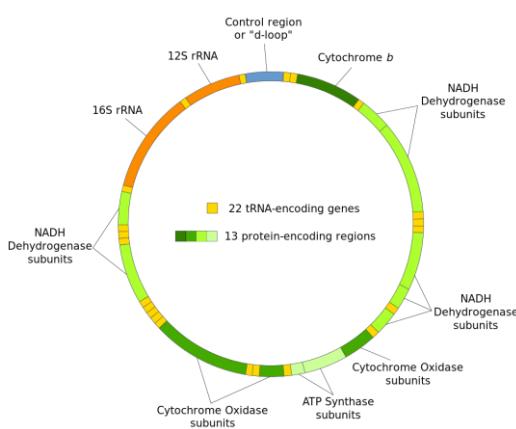


Figure 6. Illustration of the mitochondrial DNA structure. Mitochondrial DNA is mainly represented by the control region (d-loop), 22 transfer RNA encoding regions, 13 protein-coding regions and the cytochrome b (©Wikimedia Commons).

Microsatellites are short tandem repeats of DNA sequences (1-6 base-pairs) located in the genomes of most eukaryotes (Freeland et al., 2012; Figure 7). Variation at microsatellite loci is thought to result from slip-strand mispairing, a process that adds or removes repeat units during DNA replication, resulting in a great rate of mutation (DeYoung & Honeycutt, 2005). The high mutation rate at microsatellite loci usually results in high levels of polymorphism, which makes them useful for assessing the genetic structure of a population, genetic relatedness, gene flow, and genetic drift (Beaumont & Bruford, 1999). Currently, they have become the genetic marker of choice for studies of intraspecific variation (e.g., Bashyal et al., 2014; Wofford et al., 2014).

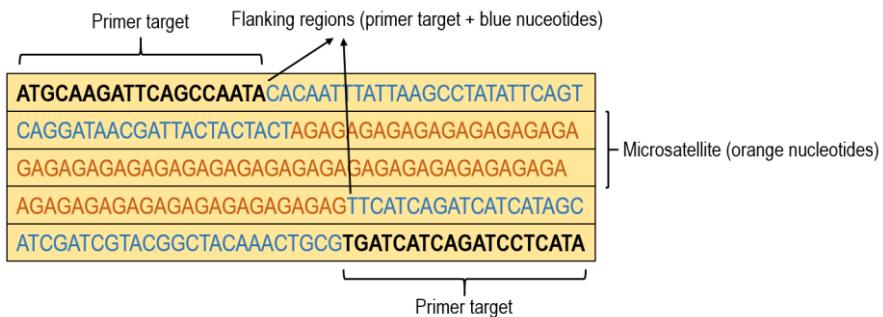


Figure 7. Scheme of the location of a microsatellite. Primer target represents the region of the nucleotide sequence where a single-stranded primer binds to start replication. Flanking regions represent the region of DNA that is adjacent to the each end of the gene (microsatellite). Microsatellite is represented by the repetition of adenine and guanine nucleotides.

Assessing patterns related with the demographic history of population expansions are among the most difficult events to analyse. This is due to the striking variation of effects of demographic bottlenecks on animal populations (DeYoung & Honeycutt, 2005). However, several recently developed analytical methods provide useful tools for assessing the demographic history of populations based on both nuclear microsatellites (e.g., tests for genetic bottlenecks; Cornuet & Luikart, 1996) and DNA sequence data (e.g., changes in population size and geographic distribution over time; Emerson et al., 2001; Ho & Shapiro, 2011).

Recent developed approaches may also increase our understanding of the genetic diversity across the distributional area of a population. For instance, the field of Landscape Genetics aim to inform on the interactions between landscape features and evolutionary processes, mainly gene flow and selection (Manel & Holderegger, 2013). The exploration of this subject is crucial, not only for improving ecological knowledge, but also for accurately managing the genetic diversity of populations (Manel et al., 2003). By using molecular markers such as microsatellites combined with statistical tools (e.g., geo-statistics, maximum likelihood and Bayesian approaches), Landscape Genetics enlightens our understanding of how geographical and environmental features influence genetic variation at both population and individual levels, and provides essential matter for the fields of Ecology, Evolution and Conservation Biology. Nowadays, the main objective of Landscape Genetics is to expand our knowledge of the effect of global change on genetic patterns with the aim of analysing (1) how recent global changes, including land use, land cover and climate change affected patterns of genetic variation; and 2) how species are likely to adapt to the evolving global change on an ecological time scale (Manel & Holderegger, 2013).

1.3. The Egyptian mongoose

1.3.1. Species' identification

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a medium-sized carnivore included in the Herpestidae family (Figure 8). Herpestidae contains 34 species distributed among 14 genera (Veron et al., 2004), in which the Egyptian mongoose is included in the *Herpestinae* subfamily and in the genera *Herpestes* (Gilchrist et al., 2009). In 1914, Cabrera considered the mongoose distributed in the Iberian Peninsula as a well-defined subspecies, *Herpestes ichneumon widdringtonii*, whilst the subspecies *Herpestes ichneumon sangronizi* occupied the African continent and Near East. This division in two subspecies was due to the bigger size of *H. ichneumon widdringtonii* compared with *H. ichneumon sangronizi* (Blanco, 1998; Palomo & Gisbert, 2002).



Figure 8. Photography of an Egyptian mongoose (©José Luís Conceição, 2014).

The Egyptian mongoose is characterized by an elongated body with a dark and mixed coloured fur (Palomares, 2004). The tail is long and pointy at the end, finishing in a brush of black hairs (Palomares & Delibes, 1998). Males weight around 3.142 kg, while the females' average weight is 2.823 kg. The body length is around 53 cm, the tail measures approximately 45 cm and 43 cm in males and females, respectively, and some individuals can achieve a total length of 1 m (Palomares & Delibes, 1992). Paws have five fingers with non-retractable nails, the head is pointy and the snout terminates in a dark hairless nose (Palomo & Gisbert, 2002). The eyes are small and rounded, with an amber-like colour and the pupils are oval and horizontal, which constitutes a very rare characteristic within carnivore species (Blanco, 1998). Ears are rounded, large and short, with

approximately 34 mm (Palomares & Delibes, 1992). One of the most typical features of this species is the glandular pouch that surrounds the anus. It contains anal glands for territorial marking and for intra and interspecific recognition (Hefetz et al., 1984).

The Egyptian mongoose can live for 20 years in captivity and 12 years in the wild. Chromosome number is $2n=43$ for males and $2n=44$ for females (Palomo & Gisbert, 2002).

According to the IUCN Red List, the conservation status of the Egyptian mongoose is “Least Concern” (Cavallini & Palomares, 2008), and in Portugal is considered a game species (Decreto-Lei 136/96, Diário da República).

1.3.2. Global distribution

The Egyptian mongoose is mostly distributed in sub-Saharan Africa, from Senegal and Gambia to East Africa, Angola, Zambia, Malawi and Mozambique. It is absent from almost of the territory of southern Africa, but it is present in north-east Namibia, northern Botswana, northern and eastern Zimbabwe and in the South African coastline. In the North African range, the species is present in the coast line from Western Sahara to Tunisia, and also from northern and eastern Egypt, southwards to Ethiopia (Cavallini & Palomares, 2008). The species is also found from the Sinai Peninsula to the south of Turkey (Delibes, 1999). The species was present in southern and central Portugal (Borralho et al., 1996) and south-western Spain (Delibes, 1999), but recently the species suffered a rapid expansion towards northern areas in both countries (Barros & Fonseca, 2011; Balmori & Carbonelli, 2012), which will be further explored in the section 1.3.4. Figure 9 represents the global distribution of the species.

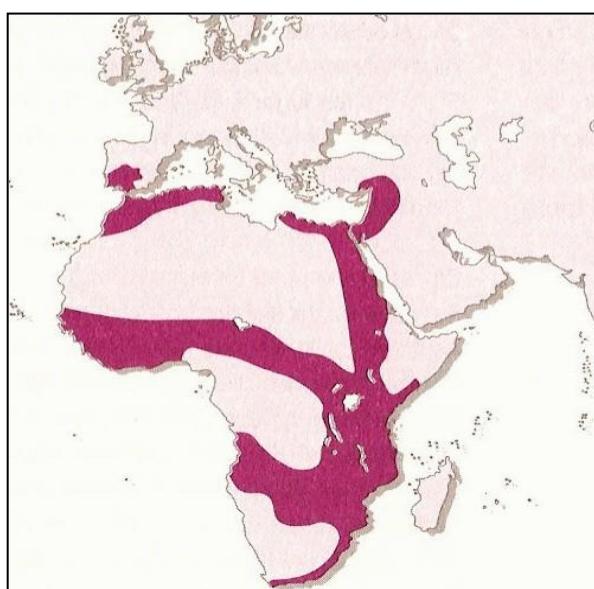


Figure 9. Global distribution of the Egyptian mongoose (adapted from © Blanco, 1998).

1.3.3. Origin of the species in the Iberian Peninsula

The debate between natural dispersal and human-mediated origin of the Egyptian mongoose in the Iberian Peninsula is a current issue. Two hypotheses on the origin of the Iberian population were postulated by different authors. Historical records and archaeological data support a human-mediated origin (Dobson, 1998). The oldest known European remain of the Egyptian mongoose was found in Sardinia (Italy) and dated from the Phoenician occupation in Italy between Vth and IVth centuries BC (Campanella & Wilkens, 2004; Carenti & Wilkens, 2006). In the Iberian Peninsula, the oldest fossil is dated from ca. VIIth century AC (Detry et al., 2011). Thus, different authors argue that the species was introduced during the Muslim occupation that occurred from VIII to XV centuries AC (Riquelme-Cantal et al., 2008; Detry et al., 2011). On the other hand, a study using mitochondrial DNA proposed that the Egyptian mongoose naturally dispersed to the Iberian Peninsula during the Late Pleistocene (Gaubert et al., 2011). The authors suggested that the time of the coalescence of an identified clade consisting in the Iberian and North African populations (median = 735 kyr) can be considered as a fair approximation of the divergence time between the North African and European clades. These results refute the idea that the Egyptian mongoose was historically introduced in Europe by the Muslims, supporting a scenario of sweepstake dispersal during Late Pleistocene sea-level fluctuations through the Strait of Gibraltar, followed by a long-term *in situ* evolution throughout the last glaciation cycles (Gaubert et al., 2011).

1.3.4. Distribution and expansion of the species in the Iberian Peninsula

In the Iberian Peninsula, the Egyptian mongoose is mainly associated with the Mediterranean maquis. The habitat of this species is commonly constituted by mastic (*Pistacia lentiscus*), rockroses (*Cistus* sp. and *Halimium* sp.), strawberry trees (*Arbutus unedo*), with dispersed oak trees (*Quercus ilex*), cork oaks (*Quercus suber*), lusitanian oaks (*Quercus lusitanica*), and sporadically pines (*Pinus* sp.) and eucalyptus (*Eucalyptus* sp.) (Palomares, 1993). The species avoids open areas and it is not a mountain animal, exceptionally in Ronda Highlands and in other mountains in Malaga where the presence is detected above 1000 meters of altitude (Palomares, 1993).

Delibes (1982) affirmed that the Egyptian mongoose occupied the south-eastern of the Iberian Peninsula. Concerning the Spanish range, references from the XIXth century reported that the species was already present in Extremadura, Cotos de Doñana y Del Rey, Guadalquivir, Strait of Gibraltar, Montes de Cádiz and Sevilla (Delibes, 1982). However, other references about the

presence of this carnivore pointed to its presence where currently the species is not documented, including Madrid, Asturias and Coruña. In 1982, an individual was captured in Páramo de Sil in León where, according to those references, the presence of the species was unknown since 1869. Regarding this record, some authors suggest that a mongoose population could have survived in that area (Palacios et al, 1992). Although with no confirmation, these records constitute intriguing facts regarding the distribution of the species in the Iberian Peninsula.

In Portugal, it was known that the Egyptian mongoose was distributed in the southern range of the country and the Tagus River functioned as a natural barrier, limiting the northern edge of the species' distribution (Borralho et al., 1996). Similarly to Spain, there are historical references about the presence of this species in locations where the species was absent in the 90s, such as Porto (Delibes, 1982). In fact, Borralho et al. (1996) suggested that the species suffered successive expansions and contractions through the years. Due to the increase of cereal production in the 30s, a decrease of favourable habitats for the mongoose yield to its regression and to its confinement to southern areas. In contrast, the recent abandon of crop lands and the migration of rural population to more urbanized areas possibly contributed for the growth of shrub habitats and consequently led to its expansion (Borralho et al, 1996).

In the last three decades, mongoose population in Portugal has dramatically expanded its range and gradually occupied central and northern territories (Barros & Fonseca, 2011). In the early 1990s, this carnivore started to expand northwards across the Tagus River and currently occupies almost the entire range of the country, excluding the north-western tip (Figure 10). Authors hypothesized that this expansion was associated with several factors, such as recent habitat modifications and land-use changes, absence of natural predators and also due to the ecological plasticity of the species (Barros, 2009; Barros & Fonseca, 2011).

The expansion of the species has also been documented in Spain. Recent sightings of the species in Zamora (Talegón & Parody, 2012), as well as the record of a specimen captured in León in 1982 and another in 2007 are of particular interest and may support the expansion of the species in the Spanish range. Authors point to two different scenarios: one solely indicating an expansion of the species from south to north, and another supporting the expansion scenario from south to north together with the presence of populations in the north of the country that remained unnoticed until nowadays (Balmori & Carbonelli, 2012).

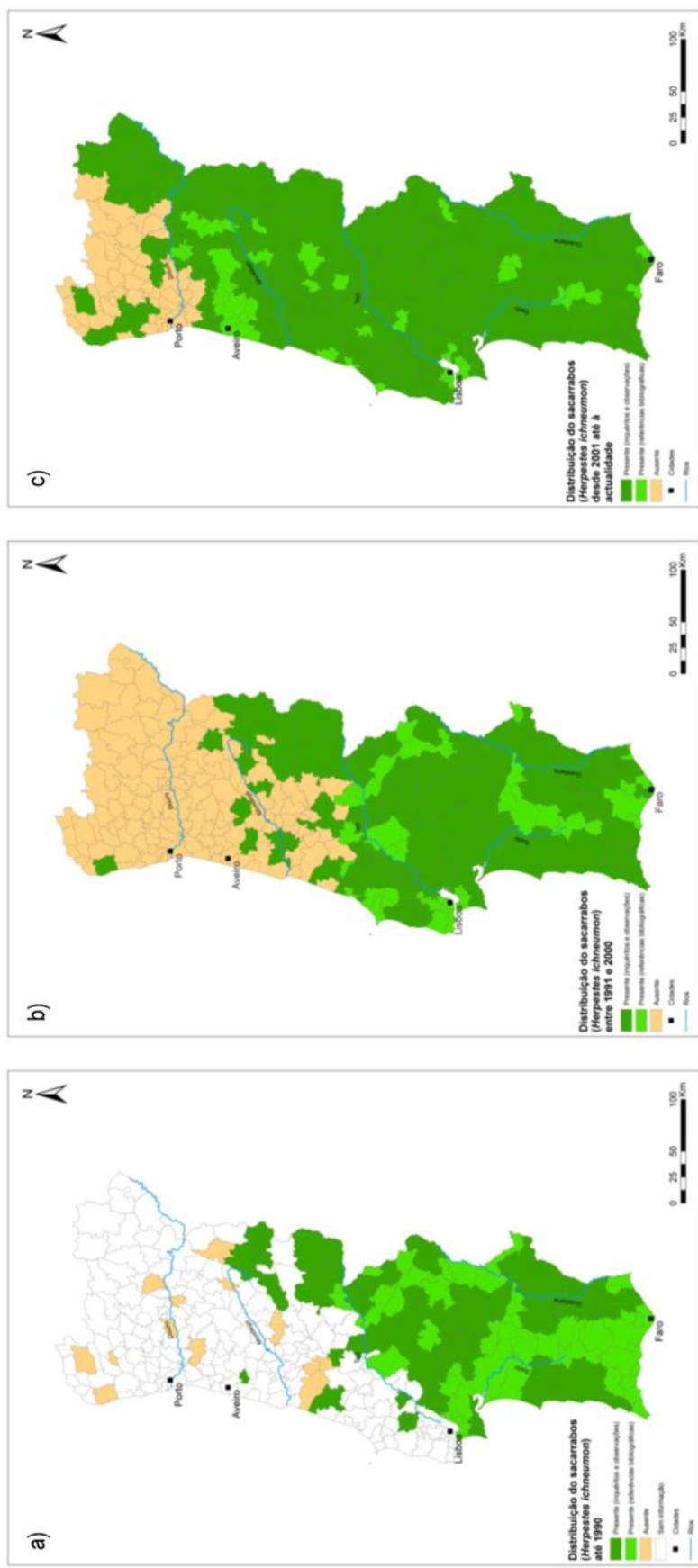


Figure 10. Maps of the distribution of the Egyptian mongoose in the last three decades and evidencing the rapid expansion of the species; (a) Distribution of the species in the 80s decade; b) Distribution of the species in the 90s decade; c) Distribution of the species between 2001 and 2010 (from Barros, 2009).

1.4. General and specific aims of the thesis

The main challenge on understanding population expansions is to provide a sufficiently exhaustive picture of the characterization of those expanding individuals, as well as understanding their dynamics and spread across invaded ranges. To achieve this goal, the combination of ecological data with molecular techniques is extremely useful for understanding those dynamics, population structure and the biology of expanding species. Since the Egyptian mongoose suffered a rapid expansion in the Iberian Peninsula, and knowing that population dynamics and chances of expansion success are partially shaped by the genetic characteristics and population demography of the species, the general aim of this thesis was assessing which factors contributed for the expansion of the Egyptian mongoose, analyse genetic structure and population genetics of this expanding species and relate landscape factors with the gene flow within the mongoose population.

One of the main topics in Ecology is unravelling the factors behind the distribution patterns of a species, and such topic is even more important to assess when a species that was confined to a limited range, suddenly expands, which was the case of the Egyptian mongoose in Portugal. Despite previous studies pointed to potential factors that may be behind the expansion of the species in Portugal, little is known about which specific factors drove its expansion towards central and northern territories. Environmental, geographic and anthropogenic variables were collected based on their potential effect in the increasing of the species' range. This study is the first comprising a meticulous analysis on the expansion of this species in Portugal. The results largely contribute for a broader knowledge about the ecology of the Egyptian mongoose, and will serve as guidelines for the management of this species. Additionally, this study will also aid on future studies of expanding species worldwide, native or exotic, with the emphasis on the dynamics between a species range and a changing landscape.

Furthermore, no investigation exclusively focusing on the genetic aspects of this species in the Iberian Peninsula has ever been envisaged. The study of such a historical, successful species in Europe allows leading investigations on real-time micro-evolutionary processes that shape populations at non-equilibrium with their environment. This study is the first to exclusively focus on the genetic characterization of the Egyptian mongoose in the Iberian Peninsula, a very important issue for assessing the genetic patterns related with the success of this species in a real-time environment. By assessing the genetic variability of the species in Portugal through an analysis combining mitochondrial DNA and microsatellites, this study unravels the demographic processes underlying the recent range expansion of this carnivore. Plus, this study constitutes a great and

unique opportunity to study ongoing processes of a successful carnivore in Portugal, and to provide applications in terms of management of expanding species at a larger scale.

The bulk of this thesis is the assessment of the genetic characterization of this expanding species in the Iberian Peninsula. This genetic assessment meets the state-of-the-art of the biology of expanding species because it is used (1) a top-down approach, from a global phylogeographic approach of the Egyptian mongoose in the Mediterranean Basin that should replace the Iberian population within a larger framework of introduction events (Gaubert et al., 2011) to a population genetics approach at a finer scale (Iberian Peninsula), where micro-evolutionary processes and demographic dynamics are characterized; and (2) a combination of markers with different inheritances to get a comprehensive picture of population structure and characterize in detail population-level parameters, such as gene flow and demographic dynamics. By assuming that population dynamics and chances of expanding populations are shaped by genetic characteristics and population demography, influencing notably the versatility of the ecological niche of the species, the achievements from these genetic analyses are expected to have applications in terms of management of expanding species in general, thus providing a critical model for science, society, hunting activity and wildlife management.

Moreover, under a changing environment, populations in expansion are largely influenced by the maintenance of their genetic diversity and the dispersal of individuals. Due to the rapid expansion of the Egyptian mongoose in Portugal and knowing that this phenomenon is potentially linked with environmental features, the use of Landscape Genetics approach greatly aims the assessment of the consequences of different landscape patterns on genetic differentiation and gene flow of the species in Portugal. By combining techniques from Geographic Information based on rasters of least-cost distances of the identified features underlying the expansion of the species in Portugal, and the genetic structure based on microsatellite loci at individual-level, it is possible to identify the most likely drivers of the observed patterns of genetic differentiation of this species in the Portuguese range.

Specifically, this thesis addressed the following specific objectives:

1. Analyse the patterns of the species' expansion over the last three decades and evaluate the relationship between environmental, geographic and anthropogenic variables and the expansion of the Egyptian mongoose in Portugal (*Manuscript I*);
2. i) Assess the population expansion of the Egyptian mongoose in the Iberian Peninsula by mitochondrial DNA analyses; and ii) to assess the genetic structure and demographic signatures and iii) revisit evidences of long-term presence of the species in the Iberian Peninsula (*Manuscript II*);
3. i) Analyse the genetic diversity associated with the range expansion of the species in the Iberian Peninsula occurred in the last three decades through the analyses of microsatellites; ii) search for evidences of genetic bottlenecks related with the dispersal events; and iii) assess evidences of isolation by distance and/or by barrier within the studied population (*Manuscript III*);
4. Explore the influence of the current landscape including vegetation cover, temperature and altitude on the genetic structure and gene flow of the Egyptian mongoose in its Portuguese range through a Landscape Genetics framework (*Manuscript IV*).

1.5. Global structure of the thesis

The structure of this thesis comprises seven chapters and their outline is described as follows:

CHAPTER ONE provides a review of literature, supplying a brief research background necessary for understanding the thesis. It is addressed a conceptual overview of the processes underlying a species' range and a description of the factors that limit the distribution of species. Next, it is explored the causes that lead to a species to shift its distribution, contract or expand. Those causes are focused on recent environmental alterations. Afterward it is described the need of studying expanding species, and why this question should comprise invasive and native species. This issue relates with the ongoing debate of the status of the Egyptian mongoose in Portugal as

introduced or native. Recent studies pointed that the species was not introduced, hence '*naturally dispersed*' is the assumed status for the species in this thesis.

Further, it is explored the causes underlying the genetic variation of species and the causes of genetic diversity along the distribution of a given population, again with an emphasis on the current global changes. Next, the importance of studying the genetic diversity of expanding populations is addressed, as well as what insights those assessments can provide for the study of the genetic consequences in species under the global change scenario. Lastly, it is presented a brief description of the molecular markers and the Landscape Genetics approach that are used in this thesis.

The next section describes the study species, including the general characteristics of the species, its global distribution, the origin of the species in the Iberian range, its distribution and expansion in the study area.

Finally, the aims of this thesis are described at the end of this chapter.

CHAPTER TWO briefly describes the study area (the Iberian Peninsula) and a general description of the methods is envisaged, including data collection and laboratory methods. Detailed methods used in each manuscript are described in the next four chapters.

CHAPTER THREE (*Manuscript I*) analyses the patterns of the species' expansion over the last three decades - between 1980 and 1990; between 1990 and 2000; and between 2000 and 2010 - and evaluates the relationship between the selected environmental, geographic and anthropogenic variables and the expansion of the Egyptian mongoose towards newly colonised areas in Portugal.

CHAPTER FOUR (*Manuscript II*) assesses the population expansion of the Egyptian mongoose in the Iberian Peninsula through an analysis of the mitochondrial DNA variation of the species; it also explores the genetic structure and demographic signatures within the expanding populations of the species and re-addresses evidences of long-term presence of the species in the Iberian Peninsula.

CHAPTER FIVE (*Manuscript III*) explores the analysis of the genetic diversity of the Egyptian mongoose associated with the range expansion of the species occurred in the last three decades through an analysis of microsatellites. It also addresses the search for evidences of genetic

bottlenecks related with the dispersal events and the genetic structure within the mongoose population, possibly associated with a pattern of isolation by distance and/or by an hydrographic barrier that was described as a possible barrier towards mongoose expansion – the Tagus River.

CHAPTER SIX (*Manuscript IV*) describes the use of the Landscape Genetics approach to explore the influence of the current landscape on the genetic structure of the Egyptian mongoose in its Portuguese range. It is investigated the effects of landscape connectivity related with vegetation cover, temperature and altitude on gene flow at individual-level, based on microsatellites. Under two widely used approaches in the Landscape Genetics framework - original causal modelling and reciprocal causal modelling - it is also addressed the evaluation of the best correlation between genetic distance and a given landscape resistance, and also between genetic distance and isolation by distance.

CHAPTER SEVEN summarizes all the research results and conclusions presented in the previous four chapters and highlights the achievements of this thesis, limitations, as well as the future application of its results. The proposition of future investigations of the expanding Egyptian mongoose are also envisioned.

The four chapters of this thesis related with the four manuscripts are in ‘paper format’ due to their previous submission to international peer-reviewed journals, and they will be referred to by their Roman numerals.

1.6. List of publications

The four following publications derived from this thesis:

MANUSCRIPT I

Barros, T., Carvalho, J., Pereira, M.J.R., Ferreira, J.P. & Fonseca, C. (2015). Following The Trail: Factors Underlying the Sudden Expansion of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal. PLoS ONE 10(8): e0133768. doi:10.1371/journal.pone.0133768.

MANUSCRIPT II

Barros, T., Gaubert, P., Rocha, R.G., Bandeira, V., Souto, L., Mira, A. & Fonseca, C. (2015). Mitochondrial demographic history of the expanding Egyptian mongoose in the Iberian Peninsula. Mammalian Biology. doi:10.1016/j.mambio.2015.09.003

MANUSCRIPT III

Barros, T., Ferreira, E., Rocha, R.G., Gaubert, P., Bandeira, V., Souto, L., Mira, A. & Fonseca, C. Genetic signature of the northward expansion of the Egyptian mongoose *Herpestes ichneumon* (Herpestidae) in the Iberian Peninsula. Accepted in Biological Journal Of The Linnean Society.

MANUSCRIPT IV

Barros, T., Cushman, S.A., Carvalho, J. & Fonseca, C. Mediterranean scrubland and elevation drive gene flow of the Egyptian mongoose in Portugal – a landscape genetics assessment.

Submitted to Landscape Ecology.

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CHAPTER II

MATERIAL & GENERAL METHODS

BRIEF DESCRIPTION OF THE STUDY AREA AND DATA COLLECTION

2. MATERIAL AND METHODS

Brief description of the study area and data collection

2.1. Study area

2.1.1. Iberian Peninsula

Here, the description of the study area comprises the entire Iberian Peninsula. In what manuscripts I and IV concern, the study area exclusively focuses in Portugal, whilst manuscripts II and III comprised the Iberian Peninsula territory (Figure 11).

The Iberian Peninsula is located in the southwest part of Europe (40°N 4°W) and has an area of approximately $582,000 \text{ km}^2$. The annual average values for mean air temperature vary between values below 2.5°C (in areas of high altitude) and values higher than 17°C (Charraza, 2011).

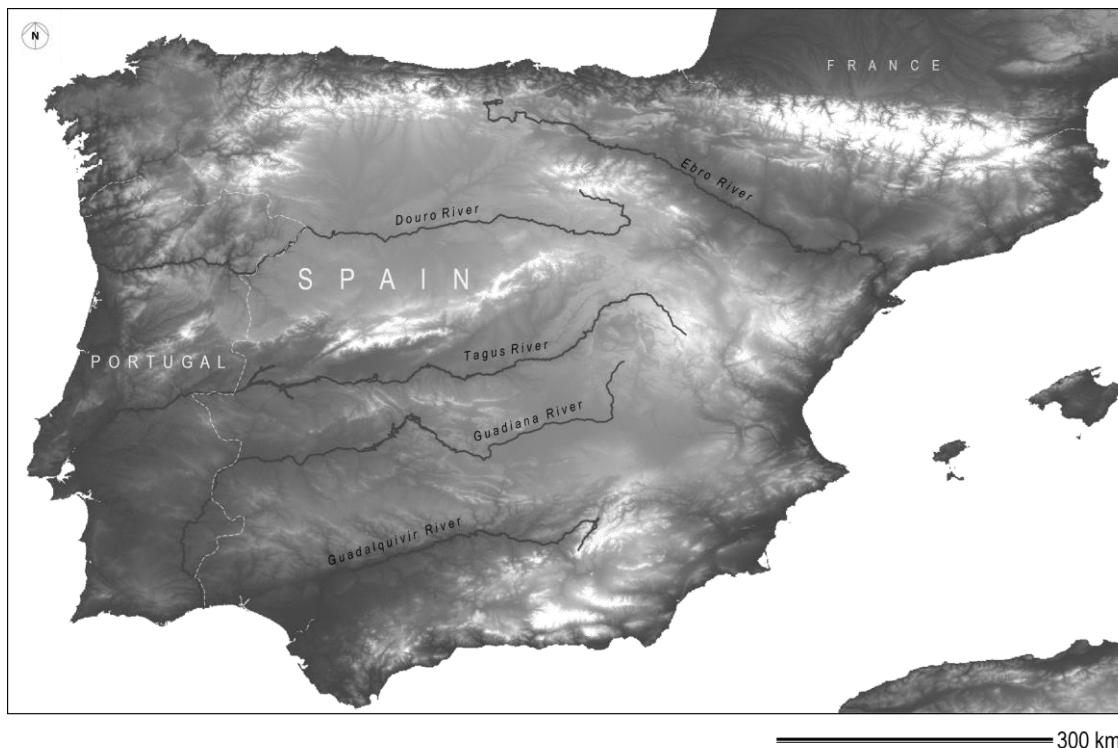


Figure 11. Map of the Iberian Peninsula with the main rivers and the administrative boundaries of Portugal and Spain.

The Eurosiberian Region is characterized by a temperate climate, with medium to high levels of precipitation and absence of a delimited dry season (Costa et al., 1998; Aguiar et al., 2008). The dominant vegetation is characterized by sessile oaks (*Quercus petraea*), English oaks (*Quercus robur*), European ash (*Fraxinus excelsior*), silver firs (*Abies alba*), beeches (*Fagus spp.*), birches (*Betula spp.*), maples (*Acer spp.*), and occasionally holm oaks (*Quercus ilex*) and bay trees (*Laurus nobilis*) (Costa et al., 1998; Aguiar et al., 2008). The remaining and the majority of the territory is within the Western Mediterranean Region, with hot and dry summers and with possible high precipitation levels in other seasons. The vegetation of the Mediterranean Sub-region is characterised by holm oaks (*Quercus rotundifolia*), cork oaks (*Quercus suber*), kermes oaks (*Quercus coccifera*), mastic (*Pistacia lentiscus*), laurustinus (*Virbunum tinus*), olive trees (*Olea europaea*), carob trees (*Ceratonia siliqua*), bay trees (*Laurus nobilis*) and shrub areas of narrow-leaved mock privet (*Phillyrea angustifolia*). A great part of the Mediterranean Region is constituted by the Mediterranean maquis, which is mainly characterized by oak trees and short evergreen shrubs (Costa et al., 1998; Aguiar et al., 2008).

2.2. Data collection and laboratory procedures

2.2.1. Presence/Absence data and data variables

For Manuscript I, new data on the presence-absence of the Egyptian mongoose across the Portuguese range was added on the previous data collected by Barros & Fonseca (2011). Also, new presence-absence data was added related with collected mongooses during 2008 and 2013 under the research project PTDC/BIA-BEC/104401/2008.

Data variables were retrieved from different sources: human population density from Instituto Nacional de Estatística; road extension from the Instituto Geográfico Português; geographic data from the U.S. Geological Survey; river extension via Sistema de Informação Nacional de Recursos Hídricos; climatic data from the European Climate Assessment & Dataset (ECAD); and land cover and vegetation variables were retrieved from the Corine Land Cover database (CLC06). All variables were used in Manuscript I, whilst geographic data, climatic data and land cover were used in Manuscripts I and also in Manuscript IV.

2.2.2. Sampling collection and genetic protocols

For both Manuscripts II and III, samples were obtained from individuals from road-kills, hunting activities and other death causes. All samples were obtained from collected mongooses under the research project “Genetic assessment of a successful invasion: Population genetics of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal” (PTDC/BIA-BEC/104401/2008). Samples were stored according to their characteristics: muscle samples were stored in ethanol at 70%, blood samples were stored in FTA® cards and hair samples were stored dry in envelops at room temperature.

DNA extraction was done depending on the characteristics of the sample and DNA amplification was yielded using the Qiagen Taq PCR Core Kit®, flowing the manufacturer's recommendations. For mitochondrial DNA amplification, two mtDNA fragments from cytochrome *b* and control region were amplified and for microsatellite amplification, 11 pairs of primers were used. PCR products were visualized by electrophoresis on 2% agarose gels.

Mitochondrial fragments were purified using ExoSap-IT® (USB Corporation) and sequenced in both directions on automatic sequencers ABI PRISM® 3730-XL DNA Analyser from Applied Biosystems™. Fragment analysis of the microsatellite products was performed using ABI Standard Dye-Set DS-33® (Figure 12).

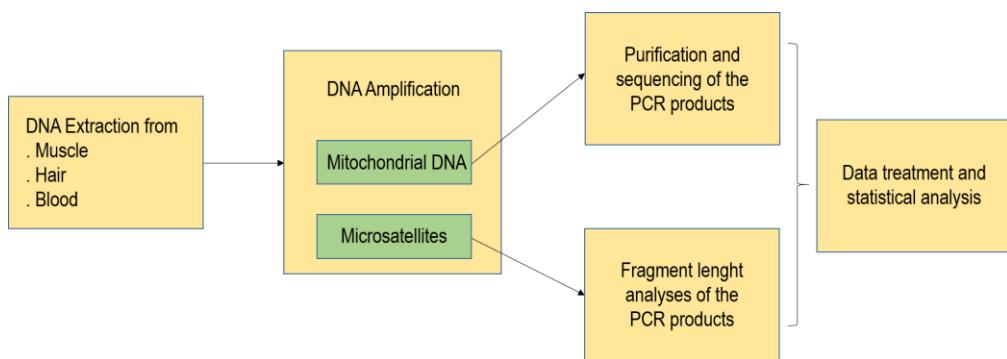


Figure 12. Scheme representing the general steps of the laboratory procedures previous to the statistical analyses of the genetic data.

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CHAPTER III

FOLLOWING THE TRAIL:

FACTORS UNDERLYING THE SUDDEN EXPANSION
OF THE EGYPTIAN MONGOOSE (*HERPESTES ICHNEUMON*) IN PORTUGAL

MANUSCRIPT I

CHAPTER THREE

Barros, T., Carvalho, J., Pereira, M.J.R., Ferreira, J.P. & Fonseca, C. (2015). Following The Trail: Factors Underlying the Sudden Expansion of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal. PLoS ONE, 10: e0133768. doi:10.1371/journal.pone.0133768

3. MANUSCRIPT I

Following The Trail: Factors Underlying the Sudden Expansion of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal

ABSTRACT

Species range-limits are influenced by a combination of several factors. In our study we aimed to unveil the drivers underlying the expansion of the Egyptian mongoose in Portugal, a carnivore that was confined to southern Portugal and largely increased its range during the last three decades. We evaluated the expansion of the species in three periods (1980-1990, 1990-2000 and 2000-2010), by projecting the presence/absence data of the species in each temporal range and proposed four hypotheses to explain this sudden expansion associated to changes in the barrier effects of human infrastructure and topographic features, and in the availability of suitable areas due to climate change or land use. An exploratory analysis was made using Spearman rank correlation, followed by a hierarchical partitioning analysis to select uncorrelated potential explanatory variables associated with the different hypotheses. We then ran Generalized Linear Models (GLM) for every period for each hypothesis and for every combination of hypotheses. Our main findings suggest that dynamic transitions of land-use coupled with temperature and rainfall variations over the decades are the main drivers promoting the mongoose expansion. The geographic barriers and the human infrastructures functioned as barriers for mongoose expansion and have shaped its distribution. The expansion of the Egyptian mongoose across the Portuguese territory was due to a variety of factors. Our results suggest a rapid shift in species range in response to land-use and climate changes, underlining the close link between species ranges and a changing environment.

KEYWORDS

Climate change; Distribution; Expansion; Herpestidae; Land-use alterations; Mediterranean carnivore; Species' range.

INTRODUCTION

Knowledge on the mechanisms controlling species' distribution patterns is central in Ecology (Gaston & Blackburn, 2008; Gaston, 2009a; Gaston, 2009b). For assessing those patterns, one can use different methods, including ecological surveys, museum specimens, bibliographical records, and interviews (e.g., Gil-Sánchez et al., 2001; Pérez et al., 2002; Hoving et al., 2003; Barja et al., 2005). By unravelling such patterns and identifying range shifts, both contractions and expansions, it is possible to create accurate measures for conservation and management with the emphasis on the species-environment relationship (Guisan & Thuiller, 2005). The diversity of factors affecting such distributional patterns have been categorized as either biotic – e.g., competition, predation, parasitism (e.g., Thomas et al., 2006; MacNeil et al., 2013; Zhang et al., 2014) -, and abiotic e.g., - climatic, topographic, land-use (e.g., Palomares et al., 2000; Acevedo et al., 2005; Sidorovich et al., 2005; Borkowski et al., 2008; Fulgione et al., 2009; Marcelli et al., 2012) -, and they are linked to time-space dynamics (Pearman et al., 2008). Animal and plant species have specific ecological requirements for their survival, and literature shows that the same factors impact different species at different levels, depending on temporal and spatial scales (e.g., Torres et al., 2012).

More specifically, several studies assert human presence, anthropogenic activities and urban infrastructures as some of the major causes of declines and contractions in wildlife populations due to their consequences for habitat fragmentation (Barbosa et al., 2001; McKinney, 2002; Basille et al., 2009). However, other authors present a more optimistic scenario, indicating that the conservation of wild species might be possible even where human presence is strong (Linnell et al., 2001; François et al., 2008).

Human-mediated changes to land-cover are now ubiquitous across the globe and are drastically transforming landscapes and, consequently, altering species distributions (e.g., Pardini, 2004; Zhao et al., 2006; Flynn et al., 2009). Many studies have dealt with the impact of land use changes on species' distribution in Mediterranean Europe (e.g., Díaz et al., 1997; Suárez-Seoane et al., 2002; De Juana, 2004; Moreira & Russo, 2007; Acevedo et al., 2007).

In recent decades, climate change has also been proven as a major cause of shifts in species' ranges (e.g., Levinsky et al., 2007; Brook, 2008; Jeschke et al., 2008; Guo et al., 2013). There is now ample evidence that climate change may lead to range contractions in many organisms (La Sorte & Jetz, 2010), either by reducing their optimal climatic envelope or due to the encroachment of the optimal climatic envelope of better-adapted invaders (Acevedo et al., 2007).

Under a scenario of anthropogenic-driven environmental changes, is essential to understand the processes beneath range shifts. Also, for expanding species, there may be a need to control for their potential impacts on the newly occupied territory (Roemer et al., 2001; Nogales et al., 2006; Hays & Conant, 2007; García-Díaz et al., 2013). Shifts in species' ranges may be modelled according to stochastic processes but also according to deterministic mechanisms, such as those resulting from a response to changes in the environment (Colwell & Rangel, 2010). Most studies in the literature focused on understanding rapid range expansions of exotic species during processes of invasion (With, 2002; Urban et al., 2007; Kadoya & Washitani, 2010). However, it is equally important to understand how and why a native species that was confined for a long time within a specific range, suddenly expands into new areas (e.g., Putman & Moore, 1998; Swenson et al., 1998). This is the case of the Egyptian mongoose, *Herpestes ichneumon* (Linnaeus, 1758). Though traditionally considered an exotic herpestid species in the Iberian Peninsula that was intentionally introduced by the invading Moors in the Middle Ages (García-Perea et al., 1996; Dobson, 1998; Detry et al., 2011), recent genetic studies showed that this carnivore probably naturally settled in Iberia during the Late Pleistocene sea-level fluctuations (Gaubert et al., 2011).

In Africa, the species is widely distributed, albeit absent from the Sahara Desert, the wet forests of central and West Africa and the deserts of South Africa (Blanco, 1998; Kingdon, 2003). It is also present in the Middle East, including Syria, Jordan, the Palestinian Territories, and Israel (Moreno et al., 2002).

Whether of Pleistocene origin or a Moorish introduction, until recently the Egyptian mongoose was restricted to the south of the Tagus River, which probably acted as a natural barrier to the expansion into northern territories (Borralho et al., 1996). However, in the late 1980s the Egyptian mongoose rapidly started expanding northwards beyond the Tagus River in Portugal (Barros, 2009; Barros & Fonseca, 2011), considerably extending the limits of its traditionally known range in this country (Delibes, 1982).

Knowing that species' ranges are limited by several factors, including vegetation cover and climatic aspects, and that those may change over time and space, we hypothesize that the sudden expansion of the Egyptian mongoose in Portugal is due to changes in either the i) barrier effects of human infrastructure and topographic features; ii) availability of suitable areas due to climate change; iii) availability of suitable areas and expansion corridors due to changes in land use; or iv) a combination of all of the above.

Using field data, questionnaires and literature, we analysed the patterns of the species' expansion over the last three decades (1980-1990; 1990-2000; 2000-2010) and evaluated the relationship between the explanatory variables chosen for each hypothesis and the expansion of the Egyptian mongoose to newly colonised areas.

The Egyptian mongoose is known to favour the Mediterranean *maquis* and it is well-adapted to the climate of southern Portugal, which presents warmer temperatures in comparison to central and northern regions. Hence, we trust that any occurred alterations across the Portuguese range especially comprising vegetation cover and climatic aspects consequently affected the distribution of this species. The thorough analysis of the species expansion across the last three decades will largely contribute with new information related with the ecology of the Egyptian mongoose, by emphasizing the link between the changing environment and the occurrence of this species.

METHODS

Study area

The study area encompasses the entire Portuguese continental territory ($35^{\circ}57'$ - $42^{\circ}10'$ N, $6^{\circ}12'$ - $9^{\circ}29'$ E). Central and northern areas are characterised by a mountainous landscape with the highest altitude in the Iberian Central Mountain Chain at Serra da Estrela (1993 m). Southern areas are characterised by flatlands and two major mountain chains: Serra de S. Mamede (1027 m) and Serra de Monchique (902 m). Climate and vegetation vary with the biogeographic sub-region: the Atlantic Mid-European Sub-region in the northwest has a temperate and humid climate, wet summers and high levels of precipitation. In the Atlantic Mid-European Sub-region the dominant species are oaks (*Quercus* spp.), beeches (*Fagus* spp.), birches (*Betula* spp.), ashes (*Fraxinus* spp.) and maples (*Acer* spp.). The remaining and the majority of the territory is within the Western Mediterranean sub-region, with hot and dry summers and with high precipitation levels in other seasons, ranging from 350 mm to 1500 mm. The Mediterranean Sub-region is characterised by oaks, mastic (*Pistacia lentiscus*), laurustinus (*Virbunum tinus*), olive trees (*Olea europaea*), carob trees (*Ceratonia siliqua*) and narrow-leaved mock privet (*Phillyrea angustifolia*) (Costa et al., 1998). However, in recent decades, intensive monocultures of eucalyptus (*Eucalyptus globulus*) and maritime pine (*Pinus pinaster*) have been planted throughout the country, significantly modifying forest composition (Águas et al., 2014).

Data collection, analysis and modelling procedures

Presence-absence data

We added new data on the presence-absence (P/A) of the Egyptian mongoose to that collected and published in Barros (2009) and Barros & Fonseca (2011). The new P/A data was assessed by collected specimens from hunting activities. For more details concerning the P/A data see Figs. A, B and C in Supporting Information 1. We also used additional information on the distribution of the mongoose available in the literature for confirmation purposes (Delibes, 1982; Borralho et al., 1996; Palomo & Gisbert, 2007).

We used those P/A data to calculate the occupied area from one decade to another and we generated three maps to reflect the distribution of the Egyptian mongoose in the following periods: between 1980 and 1990, between 1990 and 2000 and between 2000 and 2010. Since the presence of the Egyptian mongoose in Portugal was collected according to municipality, all P/A data was projected in a map with the Portuguese territory divided by this administrative unit. We then evaluated the expansion between the units where the mongoose was present in the previous period to the new units occupied by the mongoose in the following one. Maps were built in ArcGIS Version 10.2.

Data variables

We collected a total of 17 variables based on their possible effect on mongoose expansion in each studied period: Weighted Urban Area per unit (WUrban), Weighted Roads Extension per unit (WRoad), Weighted Human Population Density per unit (PopDens), Mean Altitude (MeanAltit), Maximum Altitude (MaxAltit), Minimum Altitude (MinAltit), Mean Slope (MeanSlope), Maximum Slope (MaxSlope), Land Ruggedness (TRness), Weighted River Extension per unit (WRiver), Temperature variation for each decade (ΔT), Rainfall variation for each decade (ΔR), Weighted Open Areas per unit (WOpenArea), Weighted Closed Areas per unit (WClosedArea), Weighted Forest Areas per unit (WForest), Weighted Scrub Areas per unit (WScrub) and Weighted Crop Areas per unit (WCrop). All variables were selected based on previous studies describing habitat requirements that influence mongoose presence and also on species having similarly-described habitat requirements (Palomares & Delibes, 1991a; Palomares, 1993; Palomares & Delibes, 1993a; Palomares & Delibes, 1993b). We did not include prey availability as a variable in our study due to the generalist nature of the diet of this species, including small mammals, reptiles, amphibians, invertebrates and occasionally berries and other fruits, and its significant variation

across its distribution range (Palomares & Delibes, 1991b; Delibes et al., 1984). The Egyptian mongoose is considered an opportunistic species, as it usually preys on the most abundant items available, which also causes significant variation in its diet along the year (Palomares & Delibes, 1991c).

Data from all the variables was obtained for each studied period. We retrieved human population density for each period from the Instituto Nacional de Estatística website (www.ine.pt/); data on road extension for each period was assessed from the Instituto Geográfico Português website (www.igeo.pt); geographic data was downloaded from the U.S. Geological Survey website (<http://srtm.usgs.gov/index.php>); river extension was assessed via Sistema de Informação Nacional de Recursos Hídricos (<http://snirh.apambiente.pt/>); climatic data for each period was compiled from the European Climate Assessment and Dataset (ECAD) website (<http://eca.knmi.nl/>); and land cover and vegetation variables for each period were retrieved from the Corine Land Cover, with a spatial resolution (pixel width) of 250 m (<http://www.eea.europa.eu/publications/COR0-landcover>).

Analysis and modelling procedures

To reduce the risk of overfitting (Harrell, 2001), an exploratory analysis was made by calculating the Spearman rank correlation to rank the variables in each studied temporal range. Variables showing a correlation above 0.7 were eliminated. We then used hierarchical partitioning analysis (HPA) (Chevan & Sutherland, 1991; MacNally, 1996), also for each temporal range, to select uncorrelated potential explanatory variables from those described above. HPA separates up to twelve variables with high independent correlations with the dependent variable from variables that show a high pairwise correlation with the dependent variable but that is due to the joint action of other independent variables (MacNally, 1996). This analysis was done using the R package ‘hier.part’ (Walsh & MacNally, 2013). Models with more than nine explanatory variables, as in our study, may present a “minor rounding error” (Walsh & MacNally, 2013). In identical cases like ours, some incongruities were found in terms of the ranking of the independent and co-dependent contributions of the variables depending on the order they enter the hierarchical analysis (Olea et al., 2010). To correct these errors, Olea et al. (2010) suggest that models should be run at least 100 times whilst reordering the variables. We adopted this approach in our study and then ranked the variables according to the number of times they showed the highest independent contribution towards the variation of the response variable, i.e. Egyptian mongoose expansion across the three periods. Once we were able to select a subset of significant potential predictors, we then grouped

the variables in different groups based on each one of the explanatory hypotheses. We considered different hypotheses aiming to evaluate the partitioning of the variance in relation to the response variable. Four hypothesis were considered: one hypothesis gathering variables related with anthropogenic activities and natural barriers (WUrban, WRoad and MeanAltit), one gathering climatic variables (ΔT and ΔR), one grouping environmental variables related with land use and their alteration across the three decades (WForest, WScrub, WCrop, WOpenArea and WClosedArea), and a Global Hypothesis, this final hypothesis being a combination of all the variables from the other three hypotheses (Table 1). We then used generalized linear models (GLM) with a binomial error distribution and logit link function to evaluate which hypothesis and set of variables best modelled the expansion of the Egyptian mongoose in each period. GLM is a rather flexible and robust technique, least susceptible to over-fitting than other methods (e.g., classification trees, regression splines) (Guisan & Zimmermann, 2000). Also, the GLM approach is able to deal with response variables that are not normally distributed (Millington et al., 2011).

With the aim of truly reflecting the expansion of the species from one decade to another, P/A data for every period was arranged in separated matrices. We selected P/A data for each period solely reflecting the newly colonised areas in each period. Thus, for the 1980-1990 matrix, P/A data reflects the presence of the species in the areas occupied in the first period, plus the absence of the species in areas occupied in the following two periods; for the 1990-2000 matrix, presence data corresponds to the colonisation of new areas in 1990-2000 and the absence of the species from those same areas in 1980-1990; and finally, for the 2000-2010 matrix, we selected presence data for the species in the newly-occupied areas in that period, plus the absence of the species from those same areas in the previous periods. Therefore, P/A data in each period does not reflect the cumulative distribution of the species across each decade, but the incremental expansion of the species that reflects the newly-occupied areas from one decade to another. Every model was run for these incremental areas against the variables associated with those increments. We used the Akaike Information Criterion (AIC) to rank the best-fitted models. All analyses were done in R Version 3.1.2 (R Core Team, 2013).

RESULTS

Egyptian mongoose in Portugal

The species expanded more intensively towards the central (both inland and along the coast) and north-eastern territories (Figure 13). From the 1980s to the 1990s, the range of the Egyptian mongoose in Portugal increased from approximately 210 km² to 245 km² (Figure 14). The steepest increase in the range of the species was documented in the last decade – between 2000 and 2010 - when its expansion continued further northeast, but also towards coastal areas with an increase of 55 km² relative to 1990-2000. Currently, the area occupied by the Egyptian mongoose in Portugal is ca. 300 km², and includes almost the entire Portuguese territory, with the exception of the northwest tip of the country.

Factors behind the sudden expansion

Highly correlated variables were eliminated. We eliminated MaxAltit, MinAltit, MeanSlope, MaxSlope, TRness and PopDens in the three temporal ranges. WCrop was eliminated from the 1980-1990 and 1990-2000 ranges, as well as WRiver1 from 1980-1990 ranges. The results of the HPA showed that for the 1980-1990 range the variables contributing most to the variation of the response variable were WClosedArea, ΔR90_80, WForest, WUrban, WScrub, MeanAltit, ΔT90_80 and WOpenArea. For the 1990-2000 range, the highest-contributing variables were MeanAltit, ΔT00_90, WClosedArea, WScrub, WForest, WRoad2, ΔR00_90 and WUrban. For 2000-2010, ΔT10_00, ΔR10_00, MeanAltit, WForest, WClosedArea, WScrub, WCrop, WUrban and WRoad3 contributed most to variation (Figure 15). The variable WRiver was removed from every analysed temporal range and WOpenArea was removed from the 2000-2010 range because their independent contributions to variation of the response variable were lower to the models' goodness-of-fit.

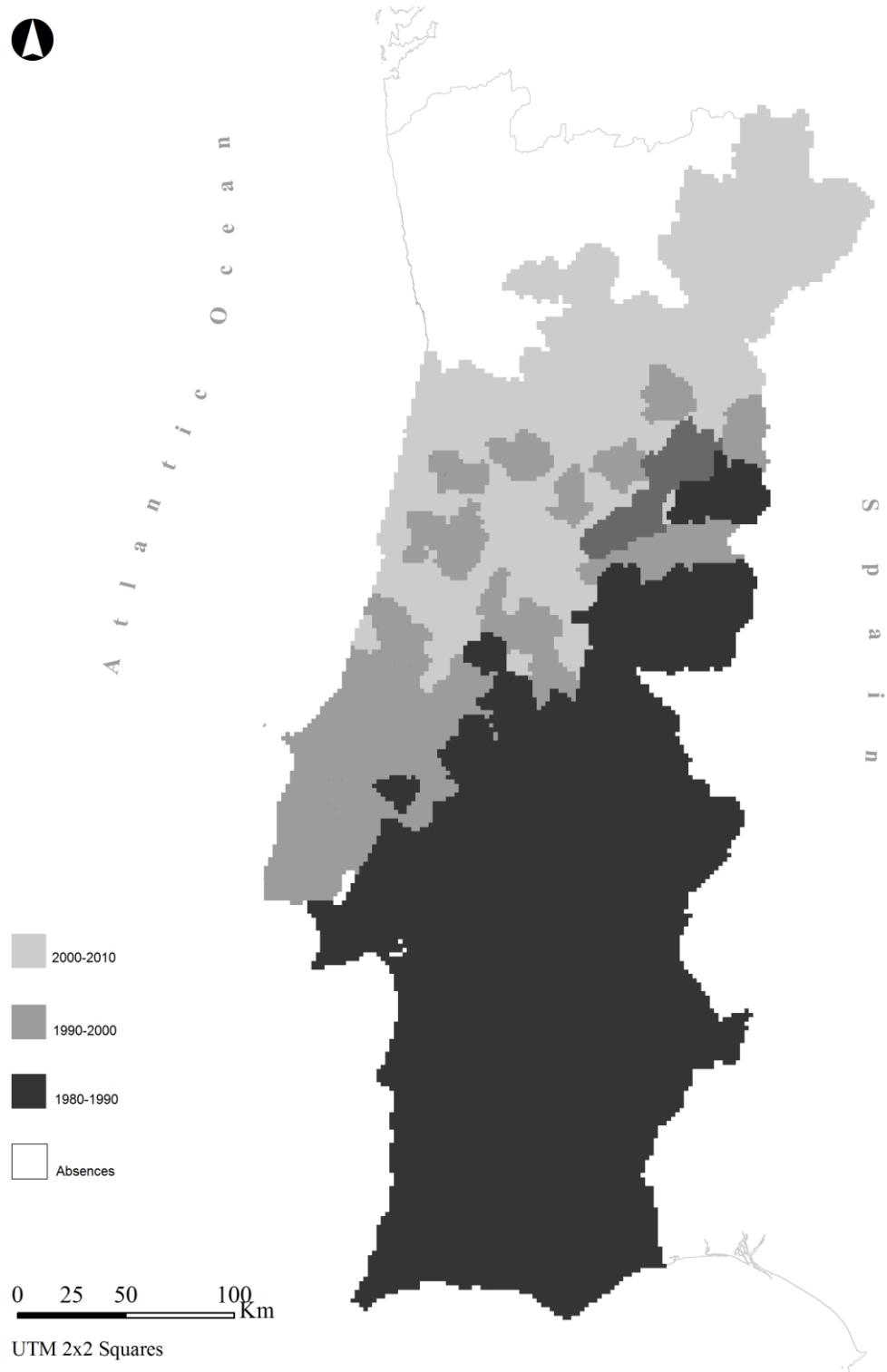


Figure 13. Map representing the expansion of the Egyptian mongoose across 1980–1990, 1990–2000 and 2000–2010 periods.

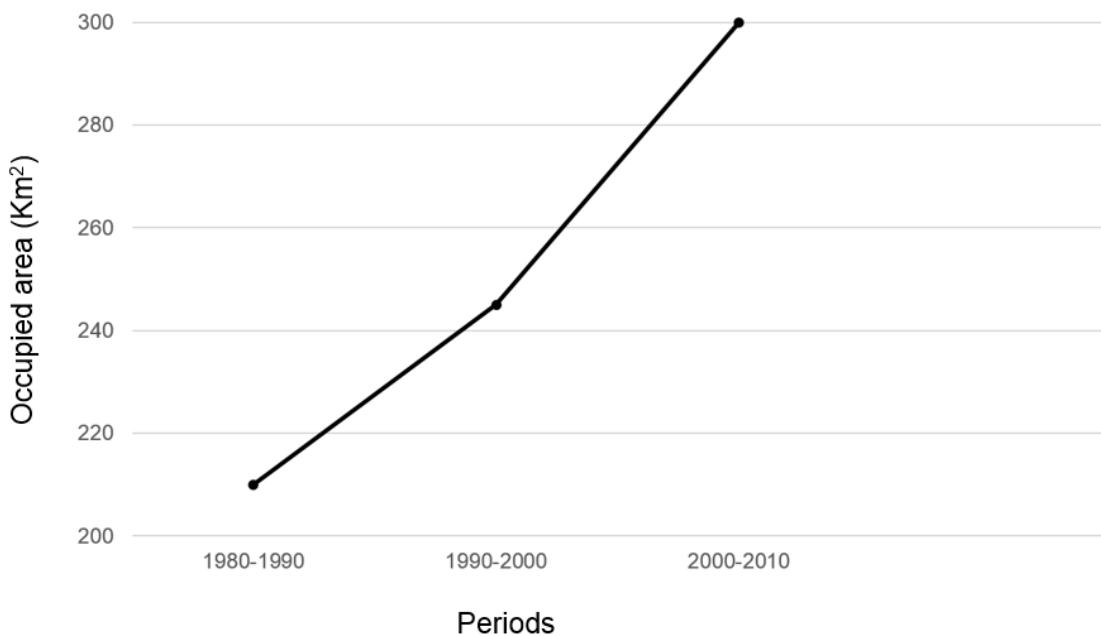


Figure 14. Graphical representation of the occupied area by the Egyptian mongoose in 1980-1990, 1990-2000 and 2000-2010 periods.

By testing the three hypotheses separately (AnthrGeo; Clim; LUChanges), in tandem (AnthrGeo_Clim; AnthrGeo_LUChanges; Clim_LUChanges) and combined (Global) (Table 1), GLM analyses generated a total of 57 models for the analysed temporal ranges (see Supporting Information 2). Table 2 shows the best models for each temporal range. The best models for the three temporal ranges include variables from the three hypotheses (Global Model), with the majority of the variables highly correlated with the expansion of the Egyptian mongoose. We found WUrban, WForest, WScrub, WOpenArea, WClosedArea and $\Delta R90_80$ as highly correlated variables with the expansion of the species between 1980 and 1990 ($p > 0.000$). MeanAltit was the least correlated variable ($p > 0.05$) in this temporal range. MeanAltit, $\Delta T00_90$, WClosedArea and WScrub were found to be highly correlated for 1990-2000 ($p > 0.000$), while WForest and WOpenArea ($p > 0.001$), WUrban ($p > 0.01$), $\Delta R00_90$ ($p > 0.05$) and WRoad2 ($p > 0.1$) were less correlated. In the temporal range between 2000 and 2010, MeanAltit, WClosedArea, WForest, WScrub and $\Delta T10_00$ were highly correlated with the expansion of the species ($p > 0.000$), whilst WCrop was less correlated ($p > 0.001$).

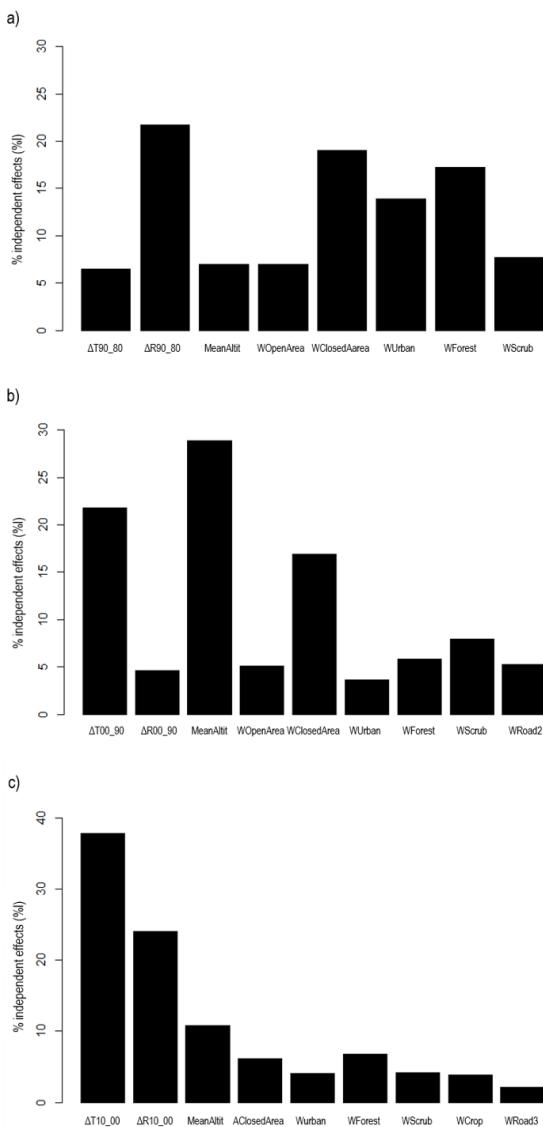


Figure 15. Graphics representing the percentage of the independent effect of the studied variables analysed in (a) 1980-1990, (b) 1990-2000 and (c) 2000-2010 periods, assessed by hierarchical partition analysis (HPA).

DISCUSSION

There is a clear link between the physical environment and the distribution of a species, in which the influencing factors may assume a major or minor role depending on a geographic-time gradient (Gaston, 1996; Elith & Leathwick, 2009). This explains why the best models found for each temporal range included variables expressing different effects on mongoose expansion in the Portuguese territory. Variables explaining mongoose expansion were mutable over time, except

MeanAltit. We found variables with the same effect across the Portuguese range in the 1980-1990 and 1990-2000 temporal ranges (MeanAltit (-), WForest (+), WScrub (+), and WClosedArea (-). WUrban (-), WOpenArea (-) and ΔR (-)). Temperature (ΔT) showed a variable effect in the 1990-2000 and 2000-2010 periods. WRoad2 (-) and WCrop (+) were only significant in the 1990-2000 and 2000-2010 periods, respectively. Our results suggest that the expansion of the Egyptian mongoose in the Portuguese territory is mostly associated with anthropogenically-driven changes in the landscape.

Table 1. Potential explanatory variables and their corresponding values for every hypothesis with their influence on Egyptian mongoose expansion in each temporal range.

Hypothesis (abbreviation)	Explanatory variables (abbreviation)	Unit	Temporal range			
			1980-1990	1990-2000	2000-2010	
			Mean			
Global (Global)	Anthropogenic and Geographic Barriers (AnthrGeo)	Weighted Urban Area per unit (WUrban)	m ²	0.0	0.1	0.0
		Weighted Extension of Roads per unit (WRoad)	m	WRoad1	WRoad2	WRoad3
		Mean Altitude (MeanAltit)	m	0.1	0.5	0.6
	Climate Change (Clim)	Temperature variation (ΔT)	°C	$\Delta T90_80$	$\Delta T00_90$	$\Delta T10_00$
		Rainfall variation (ΔR)	mm	0.4	3.6	-3.1
				$\Delta R90_80$	$\Delta R00_90$	$\Delta R10_00$
			mm	11	-13.3	10.4
	Land Use Changes (LUChanges)	Weighted Forest Area per unit (WForest)	m ²	0.3	0.2	0.3
		Weighted Scrub Area per unit (WScrub)	m ²	0.2	0.3	0.4
		Weighted Crop Area per unit (WCrop)	m ²	0.4	0.4	0.3
		Weighted Open Area per unit (WOpenArea)	m ²	0.5	0.5	0.5
		Weighted Closed Area per unit (WClosedArea)	m ²	0.3	0.3	0.5

Table 2. Best model generated by GLM for each studied period (significance codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 '').

Period	Estimate	Std. Error	z value	Pr(> z)
1980-1990				
(Intercept)	10.590	3.014	3.514	0.000 ***
MeanAlt	-0.003	0.001	-1.882	0.060 .
WUrban	-15.187	3.525	-4.308	1.64e-05 ***
WForest	15.028	3.663	4.103	4.07e-05 ***
WScrub	9.341	2.384	3.918	8.91e-05 ***
WOpenArea	-12.136	3.388	-3.582	0.000 ***
WClosedArea	-22.723	4.005	-5.673	1.40e-08 ***
ΔR90_80	-0.126	0.034	-3.690	0.000 ***
1990-2000				
(Intercept)	6.670	2.652	2.518	0.012 *
MeanAltit	-0.007	0.001	-5.913	3.35e-09 ***
WUrban	-6.401	3.055	-2.095	0.036 *
WRoad2	-1.550	0.989	-1.568	0.116967
ΔT00_90	0.452	0.124	3.638	0.000 ***
ΔR00_90	-0.040	0.020	-1.950	0.051146 .
WOpenArea	-8.407	2.881	-2.918	0.004 **
WClosedArea	-14.307	3.021	-4.735	2.19e-06 ***
WForest	6.473	2.497	2.592	0.009 **
WScrub	6.487	1.877	3.456	0.000 ***
2000-2010				
(Intercept)	-23.295	0.699	-3.332	0.000 ***
MeanAltit	-0.004	0.001	-5.195	2.04e-07 ***
WClosedArea	-73.690	15.658	-4.706	2.52e-06 ***
WCrop	28.106	0.998	2.817	0.005 **
WForest	108.074	19.718	5.481	4.23e-08 ***
WScrub	89.356	16.289	5.486	4.12e-08 ***
ΔT10_00	-0.443	0.048	-9.180	< 2e-16 ***

Human infrastructure vs mongoose expansion

In Portugal, rural areas exhibit lower human densities compared to the coastal region, where urbanisation is more intense (Diogo & Koomen, 2010). This dichotomy started to be even more evident in the 1990s with migration from inland to the coast, resulting in significant rural depopulation (Cavaco, 1996; Mitchell, 2001). We found a highly significant negative effect of urban areas on mongoose expansion across the two first studied temporal ranges (Table 2). Similarly to the majority of wild carnivores (Woodroffe, 2000; Cardillo et al., 2004), in the Iberian Peninsula the Egyptian mongoose avoids anthropic-disturbed areas with high human population densities (Borralho et al., 1996). In the first decade, the species was mainly present in the south-east where it was absent from intensely urbanized areas. In the second decade, the expansion was most notable towards the coast, and particularly the Lisbon district, but it was still absent from highly populated areas.

We also found a negative correlation between road density and the expansion of the species during the 1990-2000 period. Road networks can negatively affect wildlife and ecosystems (Reed, 1996; Rondinini & Doncaster, 2002; Iuell et al., 2003; Santos et al., 2011), limiting animal movements and causing a significant number of deaths by road-kills (Caro et al., 2000; Underhill, 2002; Cain et al., 2003; Kramer-Schadt et al., 2004; Rico et al., 2007). In the specific case of the Egyptian mongoose, data shows that this species is frequently reported as road-kill across the species' distributional range (Grilo et al., 2009; unpublished data). This underlines the negative impact the increasing construction of roads in the second period must have had on the Egyptian mongoose populations. In fact, as a result of European Union policies, there was a considerable investment in public works associated with the construction of highways and other main roads in order to facilitate access and transportation across the Portuguese territory during the 1990s (European Commission 2014 - http://ec.europa.eu/legislation/index_en.htm). In the 1980-1990 period, roads covered a significantly lower area of the species distributional range than in other decades (see Table 1), re-enforcing the idea that road construction had a significant negative effect on the expansion of the mongoose in the following periods.

The hurdle effect of altitude

During the 1980s the mongoose was confined to southern areas, with a tendency to occupy rural territories (see Figure 13). Low altitude plateaus characterise the majority of these territories, where mountainous landscapes are scarce. However, greater altitudes are found in

central and northern areas, which were occupied by the mongoose during 1990-2000 and 2000-2010. Besides the altitude variation across the Portuguese range, we found a negative correlation between altitude and the expansion of the Egyptian mongoose across the three studied periods. Climatic factors show large spatial discrepancies in mountainous areas and affect habitat conditions (Barry, 1992; Körner, 2007), leading to changes in floral composition (Shreve, 1924; Tang et al., 2006; Jump et al., 2009) and distressing animal species richness and abundance (Lomolino, 2001; Rahbek & Graves, 2001; Li et al., 2003; Yu et al., 2013). Indeed, the Egyptian mongoose seems to avoid high altitudes (Borralho et al., 1996), and seems to prefer the Mediterranean *maquis* (Palomares & Delibes, 1993a), where conditions of shelter, food availability and climatic elements are ideal for the species.

The lands they are a-changing

Land-use has been changing in the Mediterranean in the last four decades (Ales et al., 1992; Debussche et al., 1999; Falcucci et al., 2007), and in the case of the Iberian Peninsula these changes have been highly significant. Mediterranean *maquis* is commonly present in the southern territories of Portugal and it is a well-known and common habitat with an essential role for several medium-sized carnivores (e.g., Palomares & Delibes, 1991a; Virgós & Casanovas, 1997; Revilla et al., 2001).

Although Mediterranean woodland can also be found in central and north-eastern areas of Portugal, the establishment of monocultures of *Eucalyptus* sp. and *Pinus* sp. began to be commonplace throughout Portugal in the last two decades and is rapidly replacing the Mediterranean *maquis* (Águas et al., 2014; ICNF, 2013). Mongoose presence is commonly linked to the Mediterranean landscape across the Iberian Peninsula, and an important part of its activities (e.g., foraging, resting and sheltering) are displayed in areas with *maquis* vegetation (Blanco, 1998; Palomares & Delibes, 1993a; Palomares & Delibes, 1993b; Palomares & Delibes, 1992; Palomares & Delibes, 1998), which explains the positive correlation between forest areas and mongoose expansion in the three decades. Besides the crescent implementation of monocultures of pine and eucalyptus, we believe these land-use changes have also benefitted the Egyptian mongoose, as studies confirm that the Egyptian mongoose is found in areas with both tree species (Barros, 2009; Palomares & Delibes, 1993a). This suggestion could explain the positive correlation between mongoose expansion in the last two studied periods and forest areas.

Moreover, shrub areas became more frequent in the last three decades due to rural depopulation of the Portuguese countryside (OPSS, 2003). This trend was accentuated when national policies were influenced by the Common Agricultural Policy, which focused on productivity and greatly transformed traditional farming schemes (European Commission 2014 - http://ec.europa.eu/legislation/index_en.htm). In fact, during the 1980s this led to significant land abandonment, especially evident in the interior of the country, resulting in a decrease in human population density, while simultaneously promoting recovery of non-cultivated vegetation. Higher densities of Mediterranean scrublands began to be more frequent across the Portuguese countryside and this led to an increase in the availability of sheltering and foraging resources for the Egyptian mongoose in areas where they were traditionally less available, promoting the expansion of the species. There was an additional decrease in the area used for agriculture due to increasing land prices in the coast and the north-east (INE, 2011). Decreased crop production also promoted the expansion of shrub areas and the appearance of more heterogeneous and patchy mosaics, which are important for medium-sized carnivores like the Egyptian mongoose (e.g., Lozano et al., 2003; Mestre et al., 2006; Mangas et al., 2008; Rosalino et al., 2009).

We found a negative correlation between open areas and the species' expansion in the three periods. This result was predictable due to the diurnal habits of the species in the Iberian Peninsula and its avoidance of open areas, which can expose it to human interference and natural predators (Palomares & Delibes, 1993a; Palomares & Delibes, 1992; Palomares & Delibes, 1998). However, we found the same effect between closed areas and mongoose expansion. We hypothesize that this simultaneous negative correlation with open and closed areas is due to the categorization of Corine Land Cover data. If we defined 'Closed Areas' by a single category grouping forest areas and 'Open Areas' as shrub areas, crops and agricultural areas, we would discard other variables that we did not find them to be preponderant for answering the main questions of our study.

The hotter and drier, the better

The Egyptian mongoose is present in the Mediterranean and in the Afrotropic Region (Blanco, 1998). An extensive range of temperatures and precipitation occur in these regions. Yet, in Africa - from where the Egyptian mongoose radiated (Gaubert et al., 2011) -, the species is absent from the Sahara desert (Kingdon, 2003), where the climate is characterised by extreme temperatures and long dry seasons (Burt, 2004; Nicholson, 2011). The Egyptian mongoose is also absent from central-African rainforests (Kingdon, 2003), characterised by the highest rainfall levels in

that continent (Mitchell, 2001). It seems then that this species avoids extreme environments in both its ancient and present distributional range.

We found a positive correlation between temperature and mongoose expansion in Portugal during the 1990-2000 period, leading to the assumption that the expansion was driven also by temperature variations. Climate conditions in southern areas traditionally occupied by the mongoose are characterised by the highest annual temperatures (Miranda et al., 2006). Furthermore, several studies have already recorded climate change as an impact factor on species ranges in Portugal (Teixeira et al., 2002; Lima et al., 2007). Because the Egyptian mongoose has a preference for these warmer climates, this seems to explain why the species was confined to southern Portugal for several decades; however, during 1990-2000, a striking temperature increase of 3.6 °C was registered in central territories (see Table 1). Species undergoing expansion have greater chances of survival in areas where climatic conditions are analogous to those in their native ranges (Walther et al., 2009), so this temperature shift seems to have had a role in the sudden expansion of the mongoose by transforming the northern territories into more climatically suitable areas for this species.

Still, a negative correlation between temperature and mongoose presence was found during the 2000-2010 temporal range (Table 2). Temperature is often linked to altitude, and the territories occupied in that temporal range are frequently colder as they are greatly characterised by a mountainous landscape. A temperature decrease of 3.1 °C was recorded in the colonised territories during 2000-2010. We believe that the expansion of the Egyptian mongoose was negatively affected by temperature in the last period, as the species is not adapted to colder climates.

Similarly to temperature, variation in rainfall limits the presence of many species in a wide variety of habitats (e.g., Tews et al., 2004). We found rainfall to be a limiting factor for species expansion in the 1980-1990 and 1990-2000 temporal ranges (Table 2), as the Egyptian mongoose is better adapted to dry conditions. Southern areas have lower precipitation levels compared with northern areas (Miranda et al., 2006), but in the first studied decade, we found that the Egyptian mongoose was limited by rainfall and maintained its range in areas where rainfall levels were more favourable. Between 1990 and 2000, rainfall levels decreased significantly (see Table 1), providing a drier environment for the species, but still had a limiting effect on mongoose dispersal.

The current distribution of the Egyptian mongoose overlaps what is presently recognized as Mediterranean sub-region, while the species is still absent from the northwest, included in the

Atlantic sub-region. These regions have distinct climates, independently of the climatic variations found in the three temporal ranges studied here. Our data clearly indicates that the Mediterranean climate is much more suitable for the Egyptian mongoose.

CONCLUSION

The expansion of the Egyptian mongoose over the three last decades was influenced by a multitude of factors. We found that climatic factors, human-mediated factors, geographic and habitat features played a critical role in the sudden expansion and current distribution of this carnivore in Portugal. A similar pattern of interactions between several factors impacting on a species' distribution has been observed for other carnivores in Europe. Barbosa et al. (Barbosa et al., 2011) found spatial, environmental and human-mediated factors were considerable influences on the distribution of the otter (*Lutra lutra*); Virgós et al. (2001) revealed that altitude, climatic conditions and land cover play an important role for the presence of the genet (*Genetta genetta*); and Zabala et al. (2004) found that the expansion of the European mink (*Mustela lutreola*) was driven by environmental, land-use and interspecific competition.

Our study had some limitations. We compared three different spatial contexts but this cannot be overcome in studies concerning species range expansions across a temporal scale. Also, we restricted our study to Portugal and did not proceed with the analysis of the entire Iberian Peninsula due to the absence of updated data concerning the current distribution of the Egyptian mongoose in Spain. The expansion of the species in Spain might also be occurring, nevertheless, the inclusion of incomplete data of the current situation of the species in the Spanish territory would weaken our study.

Nevertheless, we believe our study contributes with valuable information to understanding the drivers underlying the distributional pattern of the Egyptian mongoose in Portugal. The applications of this study should reflect a top-down approach, from contributing to management guidelines for worldwide expanding species in an altered environment, to a finer scale, where the creation of potential management guidelines for the expanding mongoose populations in Portugal should be considered.

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Supporting Information 1. Presence/Absence data analysed in each studied period for each municipality. Each map represents the combinations of analysed data for each municipality. a) Presence/Absence data for 1980-1990 period; b) Presence/Absence data for 1990-2000 period; c) Presence/Absence data for 2000-2010 period. Data concerning inquiries, direct observations, and museum records/stuffed animals was collected by Barros (2009) and Barros & Fonseca (2011); Hunting records were obtained from Instituto da Conservação da Natureza e das Florestas (ICNF) and were collected by Barros (2009) and Barros & Fonseca (2011); Data concerning animal collection were obtained from collected mongooses from hunting activities in the last decade, under the project 'Genetic assessment of a successful invasion: Population genetics of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal (PTDC/BIA-BEC/104401/2008) (see details in <http://www.cesam.ua.pt/index.php?menu=200&language=eng&tabela=projectosdetail&projectid=380>); References were used for additional information on the distribution of the species across the three decades (Borralho et al., 1996; Delibes, 1982; Palomo and Gisbert, 2007). Absence data across the three decades were also confirmed by inquiries (Barros, 2009; Barros & Fonseca, 2911), hunting records (Barros, 2009; Barros & Fonseca, 2911) and bibliographic references (Borralho et al., 1996; Delibes, 1982; Palomo and Gisbert, 2007).



Supporting Information 2. Models generated for each studied temporal range and each AIC and Δ AIC value.

Table continues in the next page.

Model code	Variables	AIC	Δ AIC
<u>80_90</u>			
MAnthrGeo_80_90_a	MeanAlt+WUrban	297.95	108.79
MClim_80_90_a	$\Delta T_{90_80} + \Delta R_{90_80}$	289.50	100.34
MClim_80_90_b	ΔR_{90_80}	288.56	99.40
MLUChanges_80_90_a	WOpenArea+ClosedPond+WForest+WScrub	250.38	61.22
MLUChanges_80_90_b	ClosedPond+WForest+WScrub	251.06	234.06
MAnthrGeoClim_80_90_a	MeanAlt+WUrban+ $\Delta T_{90_80} + \Delta R_{90_80}$	249.96	60.80
MAnthrGeoClim_80_90_b	MeanAlt+WUrban+ ΔR_{90_80}	248.07	58.91
MAnthrGeoClim_80_90_c	WUrban+ ΔR_{90_80}	251.75	62.59
MAnthrGeoLUChanges_80_90_a	MeanAlt+WUrban+WForest+WScrub+WOpenArea+WClosedAread	203.39	14.23
MAnthrGeoLUChanges_80_90_b	WUrban+WForest+WScrub+WOpenArea+WClosedAread	221.71	32.55
MClimLUChanges_80_90_a	$\Delta T_{90_80} + \Delta R_{90_80}$ +WForest+WScrub+WOpenArea+WClosedAread	214.52	25.36
MClimLUChanges_80_90_b	$\Delta T_{90_80} + \Delta R_{90_80}$ +WForest+WScrub+WClosedAread	212.55	23.39
MClimLUChanges_80_90_c	ΔR_{90_80} +WForest+WScrub+WClosedAread	214.08	24.92
MGlobal_80_90a	MeanAlt+WUrban+WForest+WScrub+WOpenArea+WClosedArea+ $\Delta T_{90_80} + \Delta R_{90_80}$	189.70	0.54
MGlobal_80_90b	MeanAlt+WUrban+WForest+WScrub+WOpenArea+WClosedArea+ΔR_{90_80}	189.16	0
MGlobal_80_90c	WUrban+WForest+WScrub+WOpenArea+WClosedArea+ ΔR_{90_80}	190.97	1.81
<u>90_00</u>			
MAnthrGeo_90_00_a	MeanAlt+WUrban+WRoad2	403.40	88.54
MAnthrGeo_90_00_b	MeanAlt+WRoad2	401.90	87.04
MClim_90_00_a	$\Delta T_{00_90} + \Delta R_{00_90}$	417.66	102.80
MClim_90_00_b	ΔT_{00_90}	419.51	104.65
MLUChanges_90_00_a	WOpenArea+WClosedArea+WForest+WScrub	395.71	80.85
MLUChanges_90_00_b	WClosedArea+WForest+WScrub	395.39	80.53
MLUChanges_90_00_c	WClosedArea+WForest	399.22	84.36
MAnthrGeoClim_90_00_a	MeanAlt+WUrban+WRoad2+ $\Delta T_{00_90} + \Delta R_{00_90}$	341.67	26.81
MAnthrGeoClim_90_00_b	MeanAlt+WUrban+WRoad2+ ΔT_{00_90}	344.37	29.51
MAnthrGeoClim_90_00_c	MeanAlt+WRoad2+ ΔT_{00_90}	344.89	30.03
MAnthrGeoClim_90_00_d	MeanAlt+ ΔT_{00_90}	352	37.14
MAnthrGeoLUChanges_90_00_a	MeanAlt+WUrban+WRoad2+WOpenArea+WClosedArea+WForest+WScrub	351.78	36.92
MAnthrGeoLUChanges_90_00_b	MeanAlt+WUrban+WOpenArea+WClosedArea+WForest+WScrub	349.88	35.02
MClimLUChanges_90_00_a	$\Delta T_{00_90} + \Delta R_{00_90}$ +WForest+WScrub+WOpenArea+WClosedArea	371.61	56.75
MClimLUChanges_90_00_b	ΔT_{00_90} +WForest+WScrub+WOpenArea+WClosedArea	369.98	55.12
MClimLUChanges_90_00_c	ΔT_{00_90} +WForest+WOpenArea+WClosedArea	370.23	55.37
MClimLUChanges_90_00_d	ΔT_{00_90} +WForest+WClosedArea	375.03	60.17
MGlobal_90_00a	MeanAlt+WUrban+WRoad2+$\Delta T_{00_90} + \Delta R_{00_90}$+WOpenArea+WClosedArea+WForest+WScrub	314.86	0
MGlobal_90_00b	MeanAlt+WUrban+ $\Delta T_{00_90} + \Delta R_{00_90}$ +WOpenArea+WClosedArea+WForest+WScrub	315.32	0.46
MGlobal_90_00c	MeanAlt+WUrban+ ΔT_{00_90} +WOpenArea+WClosedArea+WForest+WScrub	315.47	0.61
MGlobal_90_00d	MeanAlt+WUrban+ ΔT_{00_90} +WOpenArea+WClosedArea+WScrub	321.37	6.51
<u>2000_2010</u>			
MAnthrGeo_00_10_a	MeanAlt+WUrban+WRoad3	535.20	125.15
MClim_00_10_a	$\Delta T_{10_00} + \Delta R_{10_00}$	474.07	64.02
MClim_00_10_b	ΔT_{10_00}	472.47	62.42
MLUChanges_00_10_a	WScrub+WForest+WCrop+WClosedArea	544.77	134.72
MLUChanges_00_10_b	WScrub+WForest+WClosedArea	551.28	141.23
MAnthrGeoClim_00_10_a	MeanAlt+WUrban+WRoad3+ $\Delta T_{10_00} + \Delta R_{10_00}$	439.28	29.23
MAnthrGeoClim_00_10_b	MeanAlt+WUrban+ $\Delta T_{10_00} + \Delta R_{10_00}$	437.94	27.89

MAnthrGeoClim_00_10_c	MeanAltit+WUrban+ΔT10_00	437.32	27.27
MAnthrGeoClim_00_10_d	MeanAltit+ΔT10_00	452.29	42.24
MAnthrGeoLUChanges_00_10_a	MeanAltit+WUrban+WRoad3+WClosedArea+WCrop+WForest+WScrub	504.83	94.78
MAnthrGeoLUChanges_00_10_b	MeanAltit+WUrban+WRoad3+WClosedArea+WForest+WScrub	504.60	94.55
MAnthrGeoLUChanges_00_10_c	MeanAltit+WUrban+WRoad3+WClosedArea+WForest	519.90	109.85
MClimLUChanges_00_10_a	ΔT10_00+ΔR10_00+WForest+WScrub+WCrop+WClosedArea	441.22	31.17
MClimLUChanges_00_10_b	ΔT10_00+WForest+WScrub+WCrop+WClosedArea	439.97	29.92
MClimLUChanges_00_10_c	ΔT10_00+WForest+WScrub+WClosedArea	444.53	34.48
MGlobal_00_10a	MeanAltit+WUrban+WRoad3+WClosedArea+WCrop+WForest+WScrub+ΔT10_00+ΔR10_00	414.99	4.94
MGlobal_00_10b	MeanAltit+WUrban+WClosedArea+WCrop+WForest+WScrub+ΔT10_00+ΔR10_00	413.04	2.99
MGlobal_00_10c	MeanAltit+WClosedArea+WCrop+WForest+WScrub+ΔT10_00+ΔR10_00	411.10	1.05
MGlobal_00_10d	MeanAltit+WClosedArea+WCrop+WForest+WScrub+ΔT10_00	410.05	0
MGlobal_00_10e	MeanAltit+WClosedArea+WForest+WScrub+ΔT10_00	416.61	6.56

CHAPTER IV

MITOCHONDRIAL DEMOGRAPHIC HISTORY OF THE EXPANDING EGYPTIAN MONGOOSE (*HERPESTES ICHNEUMON*) IN THE IBERIAN PENINSULA

MANUSCRIPT II

CHAPTER FOUR

Barros, T., Gaubert, P., Rocha, R.G., Bandeira, V., Souto, L., Mira, A. & Fonseca, C. (2015). Mitochondrial demographic history of the expanding Egyptian mongoose in the Iberian Peninsula. *Mammalian Biology*. doi: 10.1016/j.mambio.2015.09.003

4. MANUSCRIPT II

Mitochondrial demographic history of the expanding Egyptian mongoose (*Herpestes ichneumon*) in the Iberian Peninsula

ABSTRACT

Describing the genetic patterns and the demographic history of expanding species is essential for providing insights into the processes linked with range dynamics. We analysed the mitochondrial diversity of the Egyptian mongoose (*Herpestes ichneumon*) across the Iberian Peninsula, where the species is currently expanding north-west. A total of 242 individuals were analysed, together with nine representatives from the North African dispersal source. Haplotype segregation and strong differentiation between Iberian and North African populations confirmed the long-term presence of the species in the Iberian Peninsula. The distribution of mitochondrial diversity fitted the pattern of a historically diversified population in southern Iberia, from which the recent dispersals into northern areas may have occurred. Higher levels of haplotype and nucleotide diversities in the northern areas, together with the heterogeneous distribution of pairwise population differentiations and the weak signal for isolation-by-distance suggest the existence of long-dispersal migrants across the Iberian Peninsula. Sudden and spatial expansion scenarios of *H. ichneumon* in the Iberian Peninsula were supported by mismatch analysis and marginally supported by neutrality tests. However, the precise time of occurrence of the detected expansion remains unclear. Future studies should incorporate additional markers in order to further clarify the population dynamics of the Egyptian mongoose in its Iberian range.

KEYWORDS

Demographic history; Genetic diversity; Iberian Peninsula; *Herpestes ichneumon*; Mitochondrial DNA.

INTRODUCTION

The genetic structure of a species can be substantially influenced by historical and environmental factors (e.g., Schwartz et al., 2003; Mora et al., 2007). Such dynamics cause a diversity of specific responses that can result in population stability or range shifts, which in turn produce signatures in the species' demographic history (Slatkin, 1993; Ramírez-Soriano et al., 2008; Excoffier et al., 2009).

Genetic patterns of colonizing populations are dependent on the size of the founding group and the timeframe of the colonization event (Arenas et al., 2012; Szűcs et al., 2014). Species under geographic expansion usually show a gradient of genetic diversity across their range, with a tendency of presenting a lower genetic diversity in newly colonized areas in comparison with areas where the species is long-established (Frankham & Ralls, 1998). Notwithstanding some exceptions exist, in which high genetic diversity is found in newly colonized areas (e.g., Zalewski et al., 2011; Gaubert et al., 2011).

The majority of studies concerning the genetic structure of expanding populations have focused on invasive and exotic species (e.g., Zalewski et al., 2009; Estoup & Guillemaud, 2010). This is notably the case introduced carnivores in the Iberian Peninsula (e.g., Lecis et al., 2008; Gaubert et al., 2009; Alda et al., 2013; Gaubert et al., 2015). These studies are of extreme importance for improving knowledge on the dynamics of introduced populations and for providing appropriate management guidelines. Nevertheless, it is also crucial to investigate the genetic patterns of naturally expanding species (e.g., Lubina & Levin, 1988; Swenson et al., 1998) to improve our knowledge on dispersal/colonization processes.

The Egyptian mongoose (*Herpestes ichneumon*) is a medium-sized carnivore (Herpestidae) that has recently spread in north-western Iberia. The origin of the species in the Iberian Peninsula is debated. Apart from the Iberian Peninsula, it is distributed across the African continent, the Middle East and southern Turkey (Palomares, 2013). It was traditionally considered as an introduced species in the Iberian Peninsula during the Muslim Invasions around the VIIIth century AC (Detry et al., 2011; Riquelme-Cantal et al., 2008). However, a recent study supported by mitochondrial DNA (mtDNA) data suggested that the Egyptian mongoose naturally dispersed into the Iberian Peninsula during the Late Pleistocene sea-level fluctuations, thus refuting the hypothesis of introduction into Europe (Gaubert et al., 2011). Moreover, demographic history and niche dynamic evidence suggest that European populations of Egyptian mongooses were likely stable and at equilibrium with their environment until recently (Gaubert et al., 2011; Papeş et al., 2015). The

situation has recently changed, as the species is expanding towards central and northern regions of Portugal (Barros & Fonseca, 2011) whereas thirty years ago, its range was restricted to the southern regions of the country (Borralho et al., 1996). This has been a fast ongoing phenomenon and the current range of the Egyptian mongoose seems to now encompass the majority of the Portuguese territory, except the north-western region (Barros & Fonseca, 2011). This expansion has also been observed in Spain, where the species was exclusively established in the south-western part of the country before ca. 1990 (Delibes, 1982) but has now gained the south-eastern and northern provinces (Gil-Sánchez et al., 2001; Talegón & Parody 2009; Balmori & Carbonell, 2012).

The species' activity patterns are dependent on the Mediterranean habitat in the Iberian range, including shrub areas and dense Mediterranean forest cover (Palomares & Delibes, 1993). Land-use alterations in the last three decades mainly related to the extension of shrub areas and warmer temperatures transformed new territories into suitable areas for the Egyptian mongoose, acting as driving factors for the species' dispersal in the Portuguese range (Barros et al., 2015). The distribution of other native carnivores across the Iberian range has shown an interaction with similar factors, including climate, land cover, anthropic factors and altitude (e.g., Barbosa et al., 2001, Virgos et al., 2001; Zabala et al., 2004).

This study aims to assess the signatures of population expansion of the Egyptian mongoose in the Iberian Peninsula through the study of mtDNA sequences. Through a significantly increased geographic sampling compared to a previous study on the species (Gaubert et al., 2011), we aim to readdress evidence for the long-term presence of the species in the Iberian Peninsula, as well as to reassess the genetic structure and demographic signatures within these expanding populations.

METHODS

Sample collection

A total of 203 new samples of the Egyptian mongoose were collected in the Iberian Peninsula (Figure 16). Portuguese samples were obtained from road kills or dead of natural causes. As the species is legally hunted in the Portuguese territory, samples were also obtained during hunting activities. Spanish samples were kindly shared by several colleagues that had access to dead animals collected from road kills. Samples were deposited at Departamento de Biología (DBIO) & Centro de Estudios do Ambiente e do Mar (CESAM), from Universidade de Aveiro, Portugal.

Tissue samples were stored in ethanol at 70%, blood samples from the collected animals were obtained and stored in FTA® cards and hair samples were stored dry in envelopes at room temperature. Additionally, we included in our analyses 39 sequences from the Iberian Peninsula and nine sequences from North Africa previously published (Gaubert et al., 2011; see Supporting Information 3 for more detailed information).

DNA extraction, amplification and sequencing

DNA extraction from fresh samples, including muscle, hair and blood, was carried out in DBIO & CESAM, and in the Service de Systématique Moléculaire, at the Muséum National d'Histoire Naturelle (MNHN) of Paris, France. DNA extraction from muscle samples was conducted using the salt-extraction method with sodium chloride (Bruford et al., 1992). For blood samples stored in FTA® cards, we used a modified protocol of the phenol-chloroform method described by Sambrook & Russell (2006). For hair samples, an adapted protocol of the CTAB method described by Rogers & Bendich (1988) was used.

Two mtDNA fragments namely cytochrome b (cyt b) and control region (cr) were amplified. A 402 bp fragment of the cyt b was amplified with the primer pair GVL14724 (modified from Irwin et al., 1991; Veron et al., 2004) and H15149 (modified from Kocher et al., 1989; Veron & Heard, 2000). A 400 bp of the first hypervariable region of cr was amplified using primer pairs HERP-DL93L (Gaubert & Zenatello, 2009) and HERP-DL656H (Gaubert et al., 2011). DNA amplification was done using the Qiagen Taq PCR Core Kit®, following manufacturer indications. Reaction mixtures were initially denatured at 94 °C for 3 min, followed by 34 amplification cycles (94 °C for 30 s; annealing for 30 s at 50 °C and 51 °C for cyt b and cr, respectively; extension for 45 s at 72 °C) and a final extension step at 72 °C for 10 min. PCR products were visualized by electrophoresis on 2% agarose gels. Mitochondrial fragments were purified using ExoSap-IT® (USB Corporation) and sequenced in both directions at Genoscope – Centre National de Séquençage (Ivry, France) on automatic sequencers ABI PRISM® 3730-XL DNA Analyser from Applied Biosystems™.

Sequences were manually aligned using BioEdit Version 7.1.3.0 (Hall, 1999) and MEGA version 5.10 (Tamura et al., 2011). The newly generated haplotypes were deposited in GenBank with the accession numbers KP878521-KP878523 (cyt b) and KP878524-KP878529 (cr).

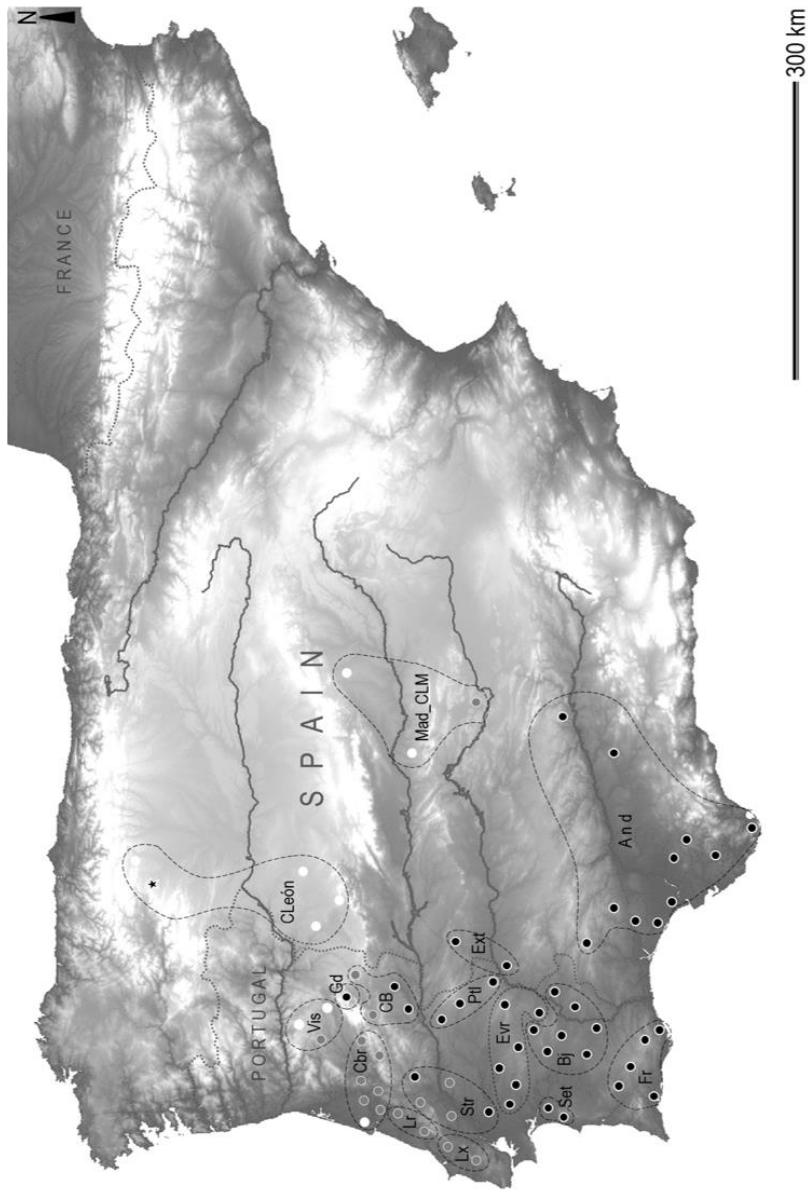


Figure 16. Location of the collected samples of the Egyptian mongoose. The colour of the dots express the presence and expansion of the Egyptian mongoose in the Iberian Peninsula. Black dots represent the samples from locations where the species is present before 1990; Grey dots represent the samples from locations to where the species expanded between 1990 and 2000; White dots represent the samples from the recently colonized areas between 2000 and 2010. The star symbol represents an individual found during the 80s decade in Páramo de Sil, Léon. Black dashed lines represent the 16 sampled regions (Portuguese districts and Spanish provinces) (Vis – Viseu; Gd – Guarda; Cbr – Coimbra; CB – Castelo Branco; Lr – Leiria; Lx – Lisboa; Str – Santarém; Ptl – Portalegre; Ev – Évora; Set – Setúbal; Bj – Beja; Fr – Faro; Cléon – Castilla-León; Mad_CLM – Madrid and Castilla-La Mancha; Ext – Extremadura; And – Andaluzia). Dark grey dotted lines represent the borders of the countries.

Genetic diversity analysis

To assess a complete and cohesive picture from the genetic profile of the Egyptian mongoose, analyses were divided in two distinct spatial scales: (1) one at a finer scale and comprising the range of the species in the Iberian Peninsula and (2) one at a larger scale and grouping both Iberian Peninsula and North Africa, the latter corresponding to the source of past dispersal of the species into Europe. Number of polymorphic sites (S), nucleotide diversity (π), number of haplotypes (h) and haplotype diversity (Hd) were calculated using DnaSP Version 5.10 (Librado & Rozas, 2009) for each dataset and for both spatial scales. This set of analyses was conducted independently for cyt b and cr, and also for concatenated sequences of both markers. For the Iberian Peninsula, 242 sequences were used and 251 sequences from the Iberian Peninsula + North Africa were used.

Genetic structure of the Egyptian mongoose

Since separate and concatenated analyses gave similar results, all the following analyses were conducted with the ‘coherent segment’ concatenating cyt b and cr sequences. A haplotype network was constructed following a median-joining (MJ) algorithm (Bandelt et al., 1999) using Network Version 4.6.1.1 (Polzin & Daneshmand, 2008). Mean genetic distance between the Iberian and North African mongoose populations was calculated on MEGA Version 5.10 (Tamura et al., 2011) by Kimura 2-parameter model method, with 500 bootstraps for assessing the confidence intervals.

A spatial analyses of molecular variance (SAMOVA) was conducted to assess the patterns of geographic structure of the Egyptian mongoose between the Iberian and North African populations. The analysis was run in SAMOVA Version 1.0 (Dupanloup et al., 2002) by allowing the number of K groups to vary between 2 and 20, using 500 simulations for testing the significance of the differences among groups, and assessing the significance of the highest value for F_{CT} statistics. Fixation index (F_{ST}) was determined by calculating pairwise differences between the putative populations from SAMOVA analysis. F_{ST} significance was assessed on Arlequin Version 3.5.1.2 (Excoffier & Lischer, 2010) with 10 000 pairwise permutations.

The analysis of the genetic structure within the Iberian population was conducted following two scales. First, we analysed the Iberian population by arbitrarily dividing the sampling area in Portuguese and Spanish provinces. Madrid and Castilla-La Mancha provinces were grouped in the same ‘region’ due to low number of samples (Madrid = 1). Second, and with the aim of specifically

addressing the expansion of the Egyptian mongoose in the Iberian Peninsula, the seventeen regions in the Iberian range were regrouped in three areas delineating the temporal expansion of the Egyptian mongoose in the last three decades, supported by previous studies concerning the sequential expansion of the species (Barros, 2009; Barros & Fonseca, 2011; Delibes, 1982; Gil-Sánchez et al., 2001; Talegón & Parody, 2009). We denominated “Southern IP” the occupied regions before 1990, “Central IP” the colonized regions between 1990 and 2000, and “Central-Northern IP” the recently colonized areas between 2000 and 2010 (Figure 16).

Diversity parameters, number of exclusive haplotypes, fixation index (F_{ST}) and mean genetic distances were calculated following the same conditions as detailed above. With the aim of testing the presence of Isolation by Distance (IBD), a Mantel test (Mantel, 1967) was performed using the ISOLDE option on Genepop version 4.2 (<http://genepop.curtin.edu.au/>). IBD calculation was conducted using two matrices, one with logarithmized geographic distances and another with linearized genetic distances after Slatkin (1995). Significance value for IBD was assessed through 1000 permutations. All the analyses were conducted for the 16 regions delineated across the Iberian territory as well as for the three areas depicting the species expansion except the IBD analysis, which was exclusively implemented for the 16 regions.

Demographic history within the Iberian range

Neutrality and demographic history in the Iberian population was evaluated using DnaSP (Librado & Rozas, 2009) through a battery of neutrality tests including *Tajima's D*, *Fu* and *Li's D** and *F**, *Ramos-Onsins e Rozas' R2*, *Fu's Fs*, *Nei's Hd*, *Wall's B*, *Wall's Q*, *Kelly's ZnS*, *Rozas' Za* and *Zz*. To determine the significance of each test, a total of 1000 replicas were simulated, assuming a coalescent process with a constant population size (Hudson, 1990). In order to detect a possible demographic event, the results were interpreted using the tables from Ramírez-Soriano et al. (2008). Mismatch distributions (distribution of pairwise genetic differences) of the European gene pools were carried out following the expected distribution under Roger's sudden expansion model (Rogers & Harpending, 1992) and following the spatial expansion model, as implemented in Arlequin (Excoffier, 2004). Traditionally, a unimodal distribution under the sudden expansion model is interpreted as a population expansion, while a multimodal distribution indicates population stability through time (Rogers & Harpending, 1992). However, populations with low migrations rates can have multiple peaks (Ray et al., 2003), thus the spatial expansion model developed by Excoffier (2004) was implemented for encompassing possible low migration rates within the mongoose population.

Sudden expansion and spatial expansion models were assessed by calculating the goodness of fit of the sum of squared deviations (SSD) between the observed and expected distributions, with 1000 bootstrap replicates. Harpending raggedness index (r) was calculated in DnaSP, with 1000 bootstrap replicates.

To detect demographic changes through time in the Iberian population, an Extended Bayesian Skyline Plot (EBSP) (Heled & Drummond, 2008) was constructed with BEAST Version 1.7 (Drummond et al., 2012). As EBSPs are sensitive to sampling (Heller et al., 2013), three sets of 100 samples each from the total dataset were randomly selected on BioStat[©]. Best models of nucleotide substitution for cyt b and cr were selected in jModelTest 0.1.1 (Posada, 2008), using the Akaike Information Criterion (AIC). AIC selected HKY (Hasegawa-Kishino-Yano) for both markers as the best evolution model. A lognormal relaxed clock (uncorrelated) was assumed, with a standard substitution rate for mammals of 27.4e-9 substitutions site-1 year-1 for cyt b (Pesole et al., 1999). A substitution rate of 1.5e-7 substitutions site-1 year-1 was estimated for the cr for other carnivores and used in this paper (e.g., Taberlet & Bouvet, 1994, Savolainen et al., 2002; Leonard et al., 2005). Priors were set according to the evolution model and all the parameters were used on BEAST analysis using the coalescent prior. Analysis was run for 1e9 and screened every 1e5 echo states. Convergence of the MCMC chains was verified in Tracer Version 1.6 (Rambaut & Drummond, 2013) by checking the effective sample size values (ESS).

RESULTS

Genetic diversity

Egyptian mongoose samples from the Iberian Peninsula yielded four haplotypes for cyt b and eight for the cr (Table 3). Two additional haplotypes for the control region in the Iberian populations were found compared to Gaubert et al. (2011). By gathering the samples from North Africa, we obtained a total of 5 haplotypes for the cyt b and 10 for cr. Haplotype diversity, number of polymorphic sites and nucleotide diversity were higher for cr than for cyt b, when considering both Iberian and Iberian plus North African datasets (Table 3).

Concatenated sequences of both cyt b and cr showed 14 mtDNA haplotypes across the European range of the Egyptian mongoose and two haplotypes exclusively from North Africa, with no haplotypes shared between both regions (Table 3). The number of polymorphic sites was found to be higher in Andalusia and Santarém. Number of haplotypes was the highest for both locations and

additionally Beja. The highest haplotype diversities were found in Andalusia, Portalegre, Coimbra and Castilla-León. Nucleotide diversity was higher in Beja, Coimbra and Guarda. Andalusia and Beja included four and two exclusive haplotypes, respectively (Table 4).

Concerning the expansion ranges, number of haplotypes in Southern IP was higher compared to the other two regions, and exclusive haplotypes were present only in this area (Table 5). A pattern of increasing haplotype and nucleotide diversities was found, from the historical locations (Southern IP) to the recently colonized range of the species (Table 5).

Table 3. Genetic diversity parameters of the Egyptian mongoose (cyt b – cytochrome b; cr – control region; N – number of individuals; bp – base pairs; S – number of polymorphic sites; h – number of haplotypes; *Hd* – haplotype diversity; π - nucleotide diversity).

		N	bp	S	h	<i>Hd</i>	π
Iberian Peninsula	cyt b	242	402	3	4	0.402	0.00108
	cr	242	400	5	8	0.644	0.00207
	cytb + cr	242	802	8	14	0.810	0.00157
Iberian Peninsula + North Africa	cyt b	251	402	4	5	0.443	0.00122
	cr	251	400	8	10	0.669	0.00268
	cyt b + cr	251	802	12	16	0.822	0.00195

Genetic structure of the mongoose populations

Median-joining network showed a clear separation between Iberian and North African haplotypes by four mutation steps (Figure 17). North African haplotypes H15 and H16 presented a genetic distance of 0.7% from the Iberian haplotypes. SAMOVA results suggested a partition in two groups ($k = 2$, $F_{CT} = 0.8$, $p = 0.000$), one group comprising the Iberian samples and one group comprising the North African samples (Figure 17). Genetic differentiation between the two groups was significant and suggested a great level of differentiation ($F_{ST} = 0.45486$, $p = 0.000$). Haplotype network for the Iberian populations showed a “star-like” configuration, with haplotypes separated exclusively by one mutation step (Figure 17). Haplotypes H2 and H5 were the most ubiquitous. Haplotype H8 was more common in Southern IP, while haplotypes H9 and H11 were more frequent

in Central and Central-northern IP. Haplotype H12 was more frequent in Central IP. Haplotype H1 was exclusively found in Southern IP (Andalusia, Beja and Évora). Haplotypes H3, H4, H6, H7, H10, H13 and H14 were rare and solely found in Southern IP (Andalusia and Beja), except for H10, which was also found in Central IP and Central-northern IP (Figure 17a).

Table 4. Mitochondrial diversity of the Egyptian mongoose for the considered regions in the Iberian Peninsula estimated from the concatenated sequences (N – number of individuals; bp – base pairs; S – number of polymorphic sites; h – number of haplotypes; Hd – haplotype diversity; Π - nucleotide diversity; Pt – Portugal; Sp - Spain).

Region	N	S	h	Number of exclusive haplotypes (number of individuals with exclusive haplotypes)	Hd	Π
Andaluzia (Sp)	36	5	8	4(6)	0.784	0.00141
Faro (Pt)	14	1	2	0	0.363	0.00045
Beja (Pt)	36	3	6	2(3)	0.770	0.00151
Extremadura (Sp)	4	2	2	0	0.500	0.00125
Setúbal (Pt)	5	2	2	0	0.600	0.00150
Évora (Pt)	25	3	4	0	0.657	0.00099
Portalegre (Pt)	8	3	4	0	0.786	0.00129
Castilla-La Mancha / Madrid (Sp)	3	0	1	0	0.000	0.00000
Lisboa (Pt)	11	1	2	0	0.545	0.00068
Santarém (Pt)	33	5	7	0	0.773	0.00140
Leiria (Pt)	2	0	1	0	0.000	0.00000
Castelo Branco (Pt)	20	2	3	0	0.695	0.00118
Coimbra (Pt)	27	4	5	0	0.803	0.00181
Guarda (Pt)	3	3	3	0	1.000	0.00249
Viseu (Pt)	10	3	3	0	0.378	0.00094
Castilla-León (Sp)	5	2	3	0	0.800	0.00125

Table 5. Mitochondrial diversity (cytochrome b + control region) of the Egyptian mongoose in the Iberian Peninsula, according to the species expansion ranges in the last three decades. Dots have correspondence with Figure 16.

	Southern IP ●	Central IP ●	Central-northern IP ○
Number of haplotypes in each location	14	6	6
Number of exclusive haplotypes	7	0	0
Number of individuals with exclusive haplotypes	22	0	0
Haplotype diversity (Hd)	0.792	0.804	0.835
Nucleotide diversity (Π)	0.00148	0.00163	0.00188

Significant F_{ST} values across the 16 considered regions suggested moderate to great differentiation ($0.05019 > F_{ST} > 0.72546$) across the Iberian range. Levels of differentiation values varied between populations pairs, independently of their geographic remoteness (Table 6). Mean genetic distances between each pair of regions ranged between 0.1 and 0.3% ($\pm 0.1\%$) (Supporting Information 4). A marginally significant value of IBD was found across the regions of the Iberian range. F_{ST} values were significant for the ‘temporal’ pairs “Southern IP/Central IP” and “Southern IP/Central-northern IP”, suggesting moderate differentiations ($0.04668 < F_{ST} < 0.07070$) (Table 7). Mean genetic distance between “Southern IP/Central IP”, “Southern IP/Central-northern IP” and “Central/Central-northern IP” was 0.2% ($\pm 0.1\%$).

Table 6. F_{ST} values for the 16 Portuguese districts and Spanish provinces. Bold values indicate significant genetic differentiation ($p < 0.05$) (Pt – Portugal; Sp – Spain).

	Andaluzia (Sp)	Faro (Pt)	Beja (Pt)	Extremadura (Sp)	Setúbal (Pt)	Évora (Pt)	Portalegre (Pt)	Castilla-La Mancha Madrid (Sp)	Lisboa (Pt)	Santarém (Pt)	Leiria (Pt)	Castelo Branco (Pt)	Coimbra (Pt)	Guarda (Pt)	Viseu (Pt)	Castilla-León (Sp)	
Andaluzia (Sp)	0.00000																
Faro (Pt)	0.20332	0.00000															
Beja (Pt)	0.12849	0.27531	0.00000														
Extremadura (Sp)	0.35365	0.70726	0.39720	0.00000													
Setúbal (Pt)	0.18403	0.43610	-0.08023	0.44523	0.00000												
Évora (Pt)	0.00976	0.27275	0.06827	0.42601	0.14896	0.00000											
Portalegre (Pt)	0.12166	0.47166	0.21630	0.18306	0.27421	0.13390	0.00000										
Castilla-La Mancha Madrid (Sp)	0.24097	0.05963	0.28400	0.70732	0.38776	0.35469	0.47787	0.00000									
Lisboa (Pt)	0.27632	0.66534	0.34595	-0.01012	0.49256	0.32670	0.25244	0.72546	0.00000								
Santarém (Pt)	0.05019	0.18222	0.14506	0.23159	0.17531	0.02402	0.02403	0.21837	0.18672	0.00000							
Leiria (Pt)	0.43696	0.81875	0.46349	0.52941	0.55224	0.53987	0.13271	1.00000	0.69891	0.36415	0.00000						
Castelo Branco (Pt)	0.09220	0.21610	0.19319	0.22794	0.24752	0.09119	0.14289	0.25000	0.16796	0.00317	0.52694	0.00000					
Coimbra (Pt)	0.13490	0.12452	0.21087	0.30342	0.19714	0.13449	0.10380	0.08456	0.31468	0.04812	0.29709	0.10687	0.00000				
Guarda (Pt)	0.04929	0.34743	0.14503	-0.09091	0.11765	0.08323	-0.18812	0.25000	0.11272	-0.12531	0.07692	0.08731	0.05268	0.00000			
Viseu (Pt)	0.38853	0.67263	0.40500	-0.07633	0.48673	0.44364	0.36653	0.68204	0.03476	0.29251	0.68165	0.24252	0.37434	0.15537	0.00000		
Castilla-León (Sp)	0.20858	0.55274	0.27850	0.33333	0.32927	0.25956	-0.10159	0.55224	0.47017	0.11716	0.11111	0.27916	0.11016	-	0.13208	0.51872	0.00000

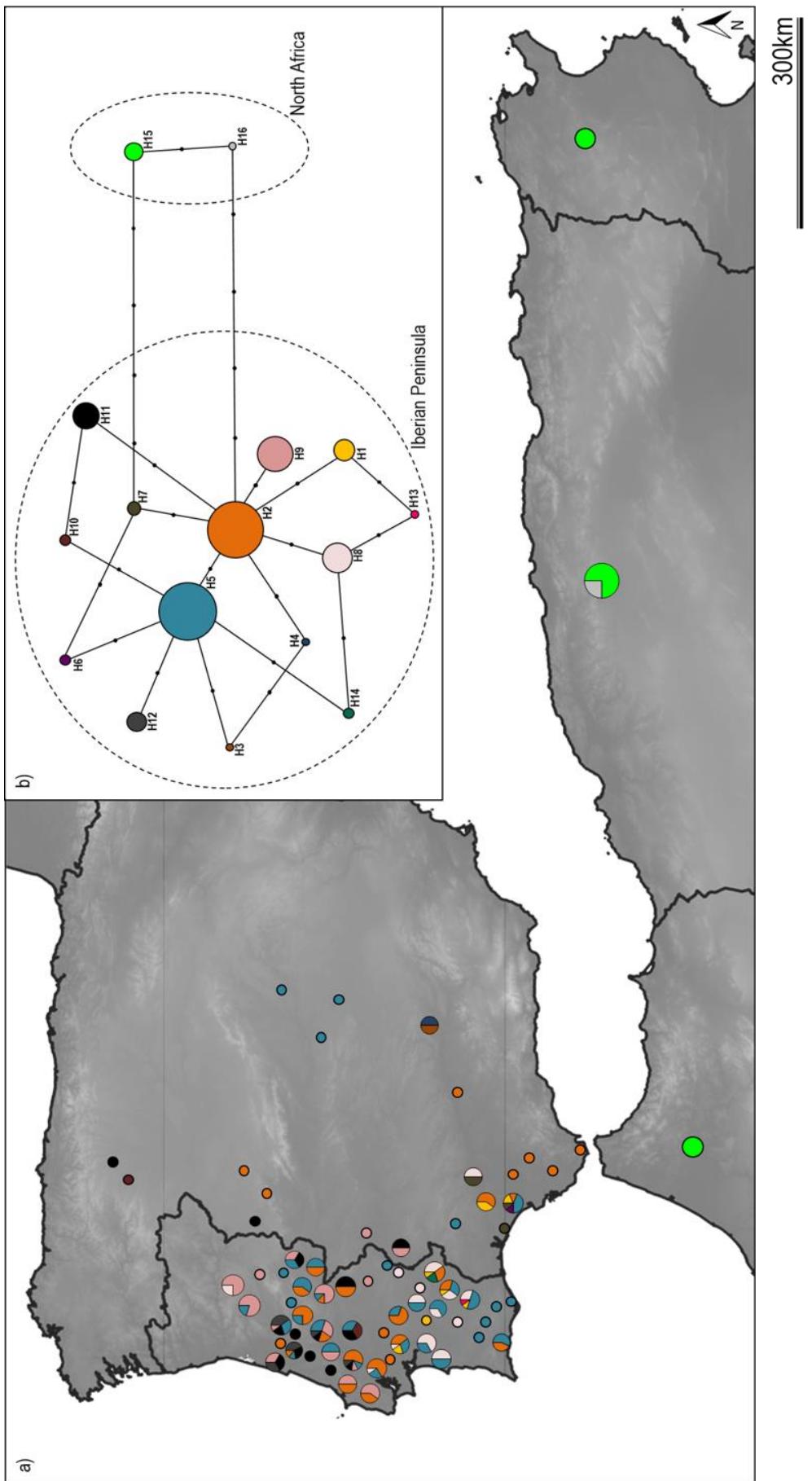


Figure 17. a) Haplotype distribution of the Egyptian mongoose populations from Iberian Peninsula and North Africa. b) Haplotype network of the Egyptian mongoose in the Iberian Peninsula and North Africa. Mutations steps between each haplotype pairs are represented by black dots. Dotted lines represent the best number of k groups from SAMOVA analysis concerning the Iberian and North African populations.

Demographic history of the Iberian population

Neutrality tests were generally not significant within the Iberian populations, although F_s showed marginal significance (0.053). Mismatch distributions showed unimodal distribution (Figure 18). For the sudden expansion model, the observed raggedness value r and SSD were significant, hence the hypothesis of a sudden expansion was not rejected (Table 8). The goodness of fit concerning both SSD and r under the spatial expansion model analysis was significant ($p = 0.000$ for both values). The three sets for EBSP gave identical results and did not show any observable departure from demographic stability (Supporting Information 5).

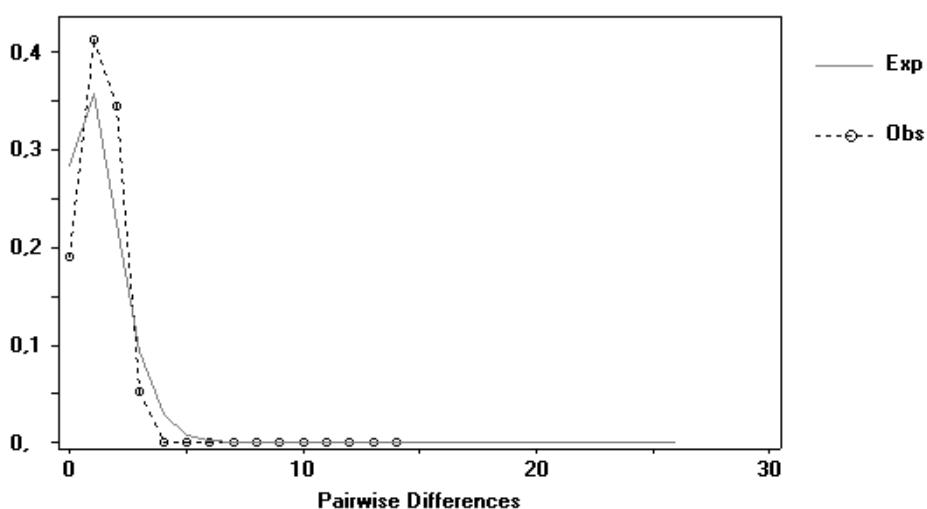


Figure 18. Mismatch distribution for the Egyptian mongoose population in the Iberian Peninsula. The solid curve represents the expected distribution under a sudden expansion model. Dashed curve indicates the observed frequency distribution of the pairwise differences.

Table 7. F_{ST} values (cytochrome b + control region) among the three populations of the Egyptian mongoose in the Iberian Peninsula representing the expansion ranges of the species within the last three decades. Bold values indicate significant genetic differentiation ($p < 0.05$).

	Southern IP	Central IP	Central-northern IP
Southern IP	0.00000	-	-
Central IP	0.04668	0.00000	-
Central-northern IP	0.07070	0.00236	0.00000

Table 8. Summary statistics applied to the mitochondrial diversity of the Egyptian mongoose in the Iberian population. Bold numbers indicate significant values ($p < 0.05$) (D - Tajima's D ; D^* - Fu and Li's D ; F^* - Fu and Li's F ; $R2$ - Ramos-Onsins e Rozas' $R2$; Fs - Fu's Fs ; Hd - Nei's Hd ; B - Wall's B ; Q - Wall's Q ; ZnS - Kelly's ZnS ; Za - Rozas' Za ; Zz - Rozas' Zz ; r - Harpending raggedness index; SSD – sum of squared deviations).

D	D^*	F^*	$R2$	Fs	Hd	B	Q	ZnS	Za	Zz	r	SSD
-0.0959	1.1944	0.8783	0.0788	-5.0163	0.8097	0.0000	0.0000	0.0120	0.0180	0.0060	0.1419	0.0230

DISCUSSION

Genetic diversity and structure of the Egyptian mongoose in the Iberian Peninsula

Our results reinforced the hypothesis of a long-term, Pleistocene separation between Iberian and North African populations (Gaubert et al., 2011) through (i) the strong differentiation among the two gene pools (SAMOVA and F_{ST} results; see Figure 17) and (ii) the absence of detection of haplotypes shared with North Africa despite the significant increase in the number of Iberian samples achieved through this study ($n = 203$).

Broadly, the overall population of the Egyptian mongoose in the Iberian Peninsula showed low levels of nucleotide and haplotype diversities. Low genetic diversity across Iberian carnivores was already reported in other species due to population bottlenecks and contraction into refugia occurred in the Pleistocene (e.g., Iberian lynx *Lynx pardinus*, Eurasian otter *Lutra lutra*; Ferrando et al., 2004; Johnson et al., 2004). The observed low genetic diversity in the Egyptian mongoose is a possible outcome of the bottleneck following its natural dispersal into Europe across the Strait of Gibraltar during the Late Pleistocene (Gaubert et al., 2011). This is in contrast with the common genet *Genetta genetta*, which shows a relatively high level of genetic diversity in its Iberian range due to multiple introduction events (Gaubert et al., 2011; 2015).

Three decades ago, the range of the Egyptian mongoose was confined to the southernmost regions of Portugal and Spain (Borralho et al., 1996; Delibes, 1982). Our results fit with the historical data, the southern territories having the highest number of haplotypes ($n = 14$) when compared to recently colonized areas ($n = 6$). Additionally, seven haplotypes were found to be exclusive to locations corresponding to the distribution of the species in Southern IP, whilst no exclusive haplotypes were found in regions colonized in the last two decades (Central IP and

Central-northern IP). Such pattern possibly indicates a northward expansion phenomenon, since the number of haplotypes tends to be higher in long-term, source populations than in recently dispersed ones (Allendorf & Lundquist, 2003). Thus, the high number of haplotypes and the exclusive haplotypes found in southern Iberian Peninsula can be directly linked to the historical presence of the species in this area.

On the other hand, the absence of exclusive haplotypes in central and northern areas as well as the fact that some haplotypes are more represented over others may be linked to the contemporaneous expansion of the species, which between 1990 and 2010 increased its territory towards central areas and the north-eastern tip of Portugal, and central and north-western regions in Spain (Barros & Fonseca, 2011; Balmori & Carbonell, 2012; Barros et al., 2015). Additionally, mismatch analyses did not refute the sudden and spatial expansion events, which supports the hypothesis of a spread of the species in the Iberian Peninsula.

Our results possibly indicate the existence of long-dispersal migrants from multiple source populations throughout the Iberian Peninsula, resulting in ‘admixed’ populations across the range of the species, especially in recently colonized areas. At a finer scale and considering the 16 regions of Iberian Peninsula, evidence for IBD was marginally significant, which might indicate a minor influence of the geographic distance across the range (Wright, 1943). However, the disparity of the F_{ST} values among the population pairs leaves little support for the existence of a consistent IBD. Further, recently colonized areas (Central IP and Central-northern IP) showed higher values of haplotype and nucleotide diversities than Southern IP, although a species naturally expanding is expected to show lower genetic diversity in its new range (e.g., Rollins et al., 2009). In line with our results, other studies had already reported a low number of haplotypes but high haplotype and nucleotide diversities in newly colonized ranges relative to the source population (e.g., Stepien et al., 2005; Gillis et al., 2009). These results can be due to an admixture of different source populations and long-distance dispersals of individuals from healthy source populations (Allendorf & Lundquist, 2003). The expansion of the Egyptian mongoose towards central and northern areas might have occurred from different source populations, thus contributing to a higher genetic diversity in those new areas. This phenomenon has been reported as the “paradox of the invasive species” (although it can apply to natural dispersers as in our case) and refers to the major impact of multiple introductions (in our case, dispersals) from distinct sources on the genetic patterns of a population in newly invaded ranges (Roman & Darling, 2007).

The dispersal ability of the Egyptian mongoose gives support to a scenario of long distance-dispersal migrants. Daily dispersals of 4–5 km have been reported, and individuals can exceptionally travel 10 km in one day (Palomares & Delibes, 1998). Nevertheless, our results concerning long-dispersal migrants from multiple source populations should be considered with caution, since haplotype and nucleotide diversities are frequency-dependent and distance-based statistics, respectively (Nei, 1987). In our case, population size and representativeness may have conditioned those two parameters, which in turn could have led to biased estimates (high diversity) in areas with lower numbers of samples.

F_{ST} values among the three areas describing the population expansion showed moderate differentiation. The absence of strong differentiation between source-sink populations is also a possible consequence of multiple source populations (see above), and might indicate the poor effect of barriers such as the Tagus River on the species' expansion (contra Borralho et al., 1996), which is in line with the reassessment of the species' range made by Barros et al. (2015). Overall, our results suggest that the Egyptian mongoose increased its distributional range despite low genetic diversity and indicate that high neutral genetic variability is not mandatory for the successful colonization of new areas (e.g., Zachos et al., 2007).

Signatures of demographic expansion throughout the Iberian range

A signature of demographic expansion of the Egyptian mongoose was detected in the neutrality tests with a marginally significant F_s value (compared to the significant F_s value in Gaubert et al., 2011), but was not evidenced through EBSP. Neutrality tests may be powerful for detecting ancient population growth (e.g., Fu's F_s , R_2), although they are sensitive to time and to number of generations (Ramos-Onsins & Rozas, 2002). A constant population size was rather suggested, reflecting the long-term stability of the Egyptian mongoose in the Iberian Peninsula. This fact is likely explained by the confined range of the species in the southern territories of Iberia Peninsula for a long period (Gaubert et al., 2011), which is in line with the stable climatic niche occupied by the Iberian populations in this area (Papeş et al., 2015). On the other hand, mismatch distributions, r index and SSD supported a scenario of sudden and spatial expansion of the species in the Iberian Peninsula. Whether it is a recent (Barros & Fonseca, 2011; Borralho et al., 1996; Delibes, 1982; Talegón & Parody, 2009) or a more ancient signature of expansion is still unclear, given the single locus (mtDNA) that we used in this study.

Although our results evidenced the genetic signature of an expansion of the Egyptian mongoose in the Iberian Peninsula, future research with other genetic markers are needed to complement our understanding of the genetic patterns accompanying the recent expansion of this species.

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Supporting Information 3. Details of the samples used in the study and number of haplotypes (cytochrome b + control region) per location (N - number of samples; DBIO & CESAM - Departamento de Biologia Universidade de Aveiro & Centro de Estudos do Ambiente e do Mar, Portugal). Table continues in the next two pages.

Location		N (this study/Gaubert et al.,, 2011)	Collection	Haplotypes (number of individuals)
Portugal	Faro	Loulé	1	DBIO & CESAM
		Monchique	3	H5(1) H5(3)
		Lagos	7	H2(3); H5(4)
		Olhão	2	H5(2)
		Silves	1	H5(1)
	Beja	Beja	3	H5(2); H8(1)
		Castro Verde	1	H8(1)
		Ferreira do Alentejo	1	H1(1)
		Mértola	10 (9/1)	H1(1); H5(5); H8(3); H13(1)
		Moura	10	H1(1); H2(3); H8(4); H14(2)
		Serpa	10	H1(1); H2(3); H5(3); H8(3)
		Vidigueira	1	H8(1)
Évora	Évora	Alandroal	1	H8(1)
		Évora	10	H2(7); H5(3)
		Montemor-o- Novo	10	H1(2); H2(4); H5(3); H8(1)
		Mora	1	H2(1)
		Portel	2	H5(1); H8(1)
		Vendas Novas	1	H2(1)
		Castelo de Vide	1	H9(1)
	Portalegre	Elvas	1	H5(1)
		Nisa	6 (5/1)	H2(3); H11(3)
Setúbal	Alcácer do Sal	3	H5(1); H8(2)	
	Grândola	2	H5(1); H8(1)	
Lisboa	Maia	6	H2(3); H9(3)	
	Torres Vedras	5	H2(2); H9(3)	
Santarém	Coruche	9	H2(6); H5(2); H8(1)	
	Ferreira do Zêzere	10	H2(2); H15(3); H9(3); H11(1); H12(1)	
	Tomar	3	H5(1); H10(1); H11(1)	

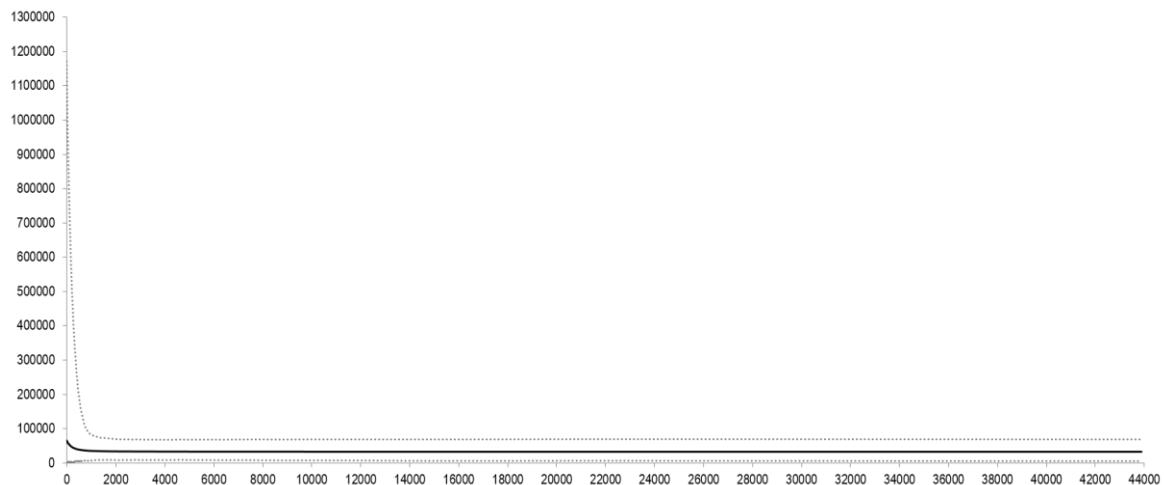
		Torres Novas Vila Nova de Ourém	9 2	DBIO & CESAM DBIO & CESAM	H2(5); H5(1); H9(1); H5(1); H9(1) ..
Leiria	Bombarral	1	DBIO & CESAM	H11(1)	
	Pombal	1	DBIO & CESAM	H11(1)	
Castelo Branco	Castelo Branco	8	DBIO & CESAM	H2(1); H5(1); H9(6)	
	Fundão	5	DBIO & CESAM	H2(2); H5(3)	
	Idanha-a-Nova	6(1)	DBIO & CESAM/ Gaubert et al., 2011	H2(3); H5(3)	
	Penamacor	1	DBIO & CESAM	H5(1)	
	Arganil	1	DBIO & CESAM	H5(1)	
Coimbra	Coimbra	10	DBIO & CESAM	H5(3); H9(1); H11(2); H12(4)	
	Condeixa-a-Nova	1	DBIO & CESAM	H11(1)	
	Figueira da Foz	3	DBIO & CESAM	H9(1); H11(1); H12(1)	
	Lousã	4	DBIO & CESAM	H2(3); H5(1)	
	Montemor-o-Velho	1	DBIO & CESAM	H2(1)	
	Soure	7	DBIO & CESAM	H2(1); H5(1); H11(2); H12(3)	
	Sabugal	3	DBIO & CESAM	H5(1); H9(1), H11(1)	
Viseu	Castro Daire	4	DBIO & CESAM	H8(1); H9(3)	
	Tondela	5	DBIO & CESAM	H5(1); H9(4)	
	Vila Nova de Paiva	1	DBIO & CESAM	H9(1)	
Spain	Andaluzia	Cádiz	7 (5/2)	DBIO & CESAM/Gaubert et al., 2011	H2(7)
		Córdoba	1	Gaubertetal., 2011	H2(1)
		PNDoñana	16	Gaubertetal., 2011	H1(3); H2(2); H5(7); H5(1); H7(1)
		Huelva	2	Gaubertetal., 2011	H2(1)
		Los Alcornocales	1	Gaubertetal., 2011	H7(1); H8(1)
		Sevilla	2	Gaubertetal., 2011	H1(2), H2(3)
		Torrecuadros	5	Gaubertetal., 2011	H3(1); H4(1)
		Valquemado	2	Gaubertetal., 2011	
	Extremadura	Badajoz	2 (1/1)	DBIO & CESAM/Gaubert et al., 2011	H9(1); H11(1)
		Cáceres	2	DBIO & CESAM	H9(2)
	Madrid	Madrid	1	Gaubertetal., 2011	H5(1)
	Castilla-La Mancha	Montes de Toledo	1	Gaubertetal., 2011	H5(1)
		Ciudad Real	1	Gaubertetal., 2011	H5(1)
		Salamanca	3	DBIO & CESAM	H2(2); H11(1)

	Castilla-León	Villablino Páramo de Sil	1 1	DBIO & CESAM Gaubert et al., 2011	H11(1) H10(1)
North Africa	Algeria		4	Gaubert et al., 2011	H15(3); H16(1)
	Tunisia		2	Gaubert et al., 2011	H15(2)
	Morocco		3	Gaubert et al., 2011	H15(3)

Supporting Information 4. Mean genetic distances among the 16 regions where the Egyptian mongoose is present in Iberian Peninsula, following Kimura-2 parameter model with 500 bootstraps for confidence interval estimates (And – Andalusia; Ext – Extremadura; CM/Mad – Castilla-La Mancha/Madrid; CL – Castilla-León; Pt – Portalegre; CB – Castelo Branco; Gd – Guarda; Ev – Evóra; Lx – Lisbon; Bj – Beja; Vs – Viseu; Lr – Leiria; Fr – Faro; Set – Setúbal).

	And	Ext	CM/Mad	CL	Pt	CB	Str	Cbr	Gd	Ev	Lx	Bj	Vs	Lr	Fr	Set
And		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Ext	0.002		0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CM/Mad	0.001	0.003		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.000	0.001
CL	0.002	0.002	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Pt	0.002	0.002	0.002	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
CB	0.001	0.002	0.001	0.002	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Str	0.001	0.002	0.001	0.002	0.001	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cbr	0.002	0.002	0.001	0.002	0.002	0.002	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Gd	0.002	0.002	0.002	0.002	0.002	0.002	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Ev	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Lx	0.002	0.001	0.002	0.002	0.001	0.001	0.001	0.002	0.001		0.001	0.001	0.001	0.001	0.001	0.001
Bj	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.001	0.002		0.001	0.001	0.001	0.001
Vs	0.002	0.001	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.001	0.002		0.002	0.001	0.001
Lr	0.002	0.002	0.003	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003		0.002	0.001
Fr	0.001	0.002	0.000	0.002	0.002	0.001	0.001	0.001	0.002	0.001	0.002	0.001	0.002	0.002		0.001
Set	0.002	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001	0.002	0.003	0.001	

Supporting Information 5. Extended Bayesian Skyline Plots (EBSP) for the Egyptian mongoose in the Iberian Peninsula showing the demographic history of the overall population. The y-axis indicates the population size and the x-axis the time from present in units of years. The black solid line is the median and the dashed lines show the 95% highest posterior density limits.



CHAPTER V

GENETIC SIGNATURE OF THE NORTHWARD EXPANSION
OF THE EGYPTIAN MONGOOSE
HERPESTES ICHNEUMON (HERPESTIDAE)
IN THE IBERIAN PENINSULA

MANUSCRIPT III

CHAPTER FIVE

Barros, T., Ferreira, E., Rocha, R.G., Gaubert, P., Bandeira, V., Souto, L., Mira, A. & Fonseca, C.
Genetic signature of the northward expansion of the Egyptian mongoose *Herpestes ichneumon*
(Herpestidae) in the Iberian Peninsula.

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5. MANUSCRIPT III

Genetic signature of the northward expansion of the Egyptian mongoose *Herpestes ichneumon* (Herpestidae) in the Iberian Peninsula

ABSTRACT

In the last three decades, the range of the Egyptian mongoose (*Herpestes ichneumon*) greatly increased in the Iberian Peninsula due to land-use alterations and climatic fluctuations. A panel of microsatellites was used to confront the patterns of genetic diversity of the species with the scenario of its recent northward expansion in its Iberian range. Evidences of substructure and significant genetic differentiation within the studied population were recorded, with a central-northern subpopulation (CNorth) and a southern subpopulation (S). Recent northward range expansion was supported by the observed allelic frequencies, diversity parameters and observed heterozygosity of the studied loci, with S showing a higher allelic diversity and a higher number of private alleles than CNorth. Patterns of Isolation by Distance and Isolation by Barrier due to the Tagus River on the northward spread were evidenced, suggesting that the river acted as a semi-permeable barrier and possibly leading to the genetic differentiation of the studied population. The observed individuals from CNorth in southern locations and individuals from S in central/northern areas might be an evidence of long-range dispersals across the studied range. A bottleneck arised after population expansion was supported by a significant heterozygosity deficiency in CNorth, which is in agreement with a scenario of possible founder events occurred in recently colonized areas related with the crossing of the Tagus River.

KEYWORDS

Carnivore; Genetic structure; Isolation by barrier; Isolation by distance; Microsatellites; Range expansion; Iberia.

INTRODUCTION

The identification of the factors behind the limits of a species' range has been a central question in Ecology and in Evolutionary Biology (Holt, 2003). Assessing the genetic structure of a population is critical for providing insights on the dynamics of the species' range and their ecological attributes (Hanski & Gilpin, 1997). By unravelling those genetic patterns, one can infer the influence of dispersal, genetic drift, and isolation on the genetic diversity of a population (Xu et al., 2013), as well as identifying the occurrence of bottlenecks and founder events (Dlugosch & Parker, 2008).

In wild populations, genetic patterns caused by range shifts may be influenced by intrinsic factors, such as the ability of a species to disperse (Clobert et al., 2001; Chambers & Garant, 2010). On the other hand, those patterns can also be greatly influenced by extrinsic features, for instance physical barriers (Lowe et al., 2004). In the light of the study of expanding species, understanding the mechanisms of range expansions is essential to predict how species shift their ranges in response to changing environments, as well as for designing management guidelines for those expanding populations (Schrey et al., 2014).

Sequential stepwise range expansions generally lead to a decrease of the genetic diversity in the population, while founder effects or bottlenecks increase genetic differentiation (Estoup et al., 2004; Short & Petren, 2011). Notwithstanding, the consequences of range expansions on the genetic patterns of a population are complex (Zenger et al., 2003), where local factors are capable of affecting the genetic patterns of expanding populations (Excoffier et al., 2009).

In expanding populations, loss of genetic diversity may occur if a low number of individuals expand into new areas. Moreover, if several dispersal events - possibly from different locations - occur into new areas, the genetic diversity could increase with the expansion event (Brown & Stepien, 2008; Parisod & Bonvin, 2008; Darling & Folino-Rorem, 2009; Bronnenhuber et al., 2011). Besides the dynamic character of range expansions, the genetic structure of widespread species with continuous populations can be broadly characterized by 1) a single genetic pool with observed genetic differences exclusively due to geographic distance and with a gradual genetic diversity turnover; or 2) by distinct genetic pools arising from the presence of barriers to gene flow, such as rivers or roads (Pérez-España et al., 2008; Tammeleht et al., 2010).

Due to the proximity between North Africa and the Iberian Peninsula, fauna exchanges were possible through natural dispersal (e.g., Carranza et al., 2006) or human-mediated (e.g., Morales, 1994). The Egyptian mongoose (*Herpestes ichneumon*) is a medium-sized carnivore from the Herpestidae family occurring in the African continent, Near East and in the Iberian Peninsula

(Kingdon, 2003). Archaeological remains found in Iberia have yielded investigators to propose a human-mediated origin of the species in the Iberian Peninsula during the Muslim occupation, ca. VIII – XV century AC (Dobson, 1998; Riquelme-Cantal et al., 2008; Detry et al., 2011). However, an exhaustive study conducted on the whole range of the species based on mitochondrial DNA (mtDNA) suggested the natural dispersal of the species from North Africa through the Strait of Gibraltar during the Late Pleistocene via sweepstake dispersal (Gaubert et al., 2011).

The case of the Egyptian mongoose is particularly relevant for the study of mammals in the course of natural range expansion. Mostly confined to the southern territories of its Iberian distribution until the 1980s, the species has continuously expanding its range (Barros, 2009). During the 1990s, it was assumed that the expansion of the species in Portugal was somewhat limited by the Tagus River, crossing the country from the Spanish border to the Portuguese coast (Borralho et al., 1996). Nevertheless, during the last three decades, the Egyptian mongoose gradually expanded into central and north-western areas of the Iberian Peninsula and is currently widespread across the region (Barros & Fonseca, 2011; Balmori & Carbonell, 2012). Such recent northward dispersal is likely due to temperature warming and land-use changes (Barros et al., 2015a). The genetic diversity associated with the expansion of the species has been analysed using mtDNA (Barros et al., 2015b). A higher number of haplotypes in the historical range of the species was encountered, but higher haplotype and nucleotide diversity in newly colonized areas was observed, possibly associated with long-distance dispersal (Barros et al., 2015b). MtDNA also supported a sudden, spatial and demographic expansion scenario, but it remains unclear to which period (i.e. Late Pleistocene or more recent) this signature refers to (Barros et al., 2015b).

We here further contribute to the genetic tracking of the northward dispersal signature of the Egyptian mongoose in the Iberian Peninsula through microsatellites analysis. The main aim of this study was to investigate the genetic structure in the currently widespread population of the Egyptian mongoose in the Iberian range, as the effects of the expansion and dispersal may reflect on the genetic diversity of the studied mongoose population. We also assessed the role of Isolation by Distance and isolation by the Tagus River in the genetic patterns of the mongoose population, as well as analysing the presence of structure within the Iberian range of the species. Specifically, we aimed to assess: (1) the distribution of the genetic diversity of the species in the Iberian Peninsula; (2) the signature of genetic bottlenecks related with northward dispersal; and (3) the patterns of isolation by Distance and/or by the Tagus River.

METHODS

Sampling and microsatellite genotyping

We analysed a total of 175 samples of Egyptian mongoose individuals from the Iberian Peninsula. The samples were obtained from individuals caught during surveys from legal hunting activities (only in Portugal), road kills, and dead individuals from natural causes. Samples were deposited at Departamento de Biologia (DBIO) & Centro de Estudos do Ambiente e do Mar (CESAM), Universidade de Aveiro. Tissue samples (muscle) were stored in ethanol at 70%, blood samples were collected and stored on FTA® cards, and hair samples were stored in dry envelops, at room temperature. Detailed information about the collected samples is available in Supporting Information 6. As most of the collected samples were from Portugal and from western Spain, the sampling area will be referred as Western Iberian Peninsula (WIP).

DNA was isolated from muscle samples using the salt-extraction method (Bruford et al., 1992). For blood samples, we used a modified protocol of the phenol-chloroform method (Sambrook et al., 1989). For hair samples, an adapted protocol of the CTAB method described by Rogers & Bendich (1988) was used. Samples were genotyped using a panel of eleven microsatellites developed by Rodrigues et al. (2009). Microsatellite loci were amplified by singleplex using the QIAGEN Taq PCR Core Kit®, following manufacturer's conditions. PCR products were visualized on 2% agarose gel and fragment analysis was performed using ABI Standard Dye-Set DS-33®. Allele calling was done on GeneMapper Version 3.7® and all alleles were checked manually. Locus 3.19 (Rodrigues et al., 2009) was excluded from analysis due to poor quality of the amplified products.

Genetic diversity and structure in the Western Iberian population

The program Micro-Checker Version 2.2.3 (Van Oosterhout et al., 2004) was used to test for stuttering and large allele dropout. Deviations from Hardy-Weinberg equilibrium (HWE) for all loci were tested using Genepop Version 4.2.1 (Rousset, 2008), with 100 batches and 1000 randomizations. Extent of linkage disequilibrium (LD) for all pairs of loci and inbreeding coefficients (F_{IS}) were estimated using Arlequin Version 3.5.1.2 (Excoffier & Lischer, 2010), with 10,000 permutations. Number of alleles (N_A), observed (H_0) and expected heterozygosity (H_E) were calculated using Genalex Version 6.501 (Peakall & Smouse, 2012). Bonferroni corrections were applied for all multiple tests.

Evidences of genetic structure of the Egyptian mongoose in WIP were tested in Structure Version 2.3.4 (Pritchard et al., 2000), using the admixture model with correlated allele frequencies.

No prior information about the original population of each individual was provided. The program was run for 1,000,000 iterations of the Markov Chain Monte Carlo, with a burn-in of 10,000 steps. The putative number of populations was simulated from $K = 1$ to $K = 10$, with 3 replicates for each K . Results obtained in Structure were analysed using Structure Harvester (Earl & VonHoldt, 2012), and the best K was estimated using the Evanno method (Evanno et al., 2005). The genetic variability for each inferred cluster based on the most likely K was assessed. Significant deviations from the HWE for each locus and LD for all pairs of loci was tested separately for each cluster. Allele number (N_A), number of private alleles (N_P), allele frequencies, observed (H_0) and expected heterozygosity (H_E) were calculated in Genalex, and allelic richness and gene diversity for each loci were also estimated for each inferred cluster in Fstat Version 1.2 (Goudet, 1995).

To assess the most conservative q value for pure assignment and the potential of the studied loci to detect admixed individuals, simulations were performed on HybridLab Version 1.0 (Nielsen et al., 2006). Multilocus genotypes were simulated by sampling alleles from the inferred populations evidenced in Structure, assuming random mating, linkage equilibrium and neutrality. Hence, the power of the Bayesian method implemented in Structure to identify admixture was tested. Given the most likely K for WIP is $K=2$ by the Evanno method, 20 individuals were selected from each one of the inferred subpopulations using a Tq threshold value of $Tq > 0.90$ (Pritchard et al., 2000). A total of 100 simulated genotypes were created for each admixed class: F1, F2 and backcrosses BxOa, BxOb. The assignment tests of simulated genotypes were performed under the same conditions than the real data set in Structure.

For assessing the partition of the genetic variation among the identified subpopulations, a standard analysis of molecular variance (AMOVA) for the inferred clusters was calculated. Significance of the inferred genetic structure was assessed through pairwise F_{ST} (Wright, 1951). Inbreeding coefficients F_{IS} and F_{IT} were also assessed. All analyses were performed using Arlequin Version 3.5.1.2, with 10,000 permutations. R_{ST} – analogous to F_{ST} but for microsatellites (Slatkin, 1995) – was calculated using Rst Calc Version 2.2 with 1000 randomized permutations (Goodman, 1997).

Isolation by Distance and Isolation by the Tagus River

Individual-based analyses for Isolation by Distance (IBD) and Isolation by Barrier (IBB) were conducted. Testing both models using individual genetic distances have proven to be a useful approach in other studies (e.g., Coulon et al., 2004; Cushman et al., 2006). For both IBD and IBB, a

square matrix of genetic distances among pairs of individuals was generated on Genalex using the Smouse & Peakall (1999) coefficient. In order to test for IBD, a square matrix of geographic distances was created on Genalex based on individual coordinates in latitude and longitude. For testing for IBB, a binary (0,1) square matrix was created. We attributed the value '1' for pairs of samples separated by the Tagus River and '0' for pairs of samples located in the same side of the river.

Correlations between genetic distance/geographic distance and genetic distance/barrier presence were calculated by means of a simple Mantel test (Mantel, 1967) using Pearson correlation, with 10 000 permutations for a 95% confidence interval. Simple Mantel tests were calculated using the Vegan package (Oksanen et al., 2007) for R Version 3.1.2 (R Core Team, 2013).

Detection of bottlenecks in the inferred subpopulations

Evidence of recent bottlenecks for each inferred cluster was tested using the software Bottleneck Version 1.2.02 (Cornuet & Luikart, 1996). Simulations were made using the two-phase model (T.P.M.), with 95% S.M.M. and 20% variance, and 1000 iterations. Wilcoxon sign-rank tests were applied to determine significance of each model. Evidence of bottlenecks was also tested using the ratio M , described by Garza & Williamson (2001). According to the authors, the value of the ratio is expected to be significantly lower in bottlenecked than in equilibrium populations: values of M lower than 0.66 constitute evidence of a bottleneck, whereas values greater than 0.8 would indicate no bottleneck events. Comparisons of the sampled M ratios against theoretical critical and equilibrium values were performed using the executable files provided by Garza & Williamson (2001). Significance tests were conducted using a set of conservative parameter values ($\Delta g = 3.5$; %SMM = 90%) and for a range of θ values (from $\theta = 0.01$ to $\theta = 5$). Both approaches were implemented due to their power for detecting bottlenecks at different time scales (Williamson-Natesan, 2005).

RESULTS

Microsatellite variation of the Egyptian mongoose in WIP

There was no evidence of significant allele dropout or stuttering for any of the loci. For the overall population, nine loci showed significant departure from HWE and 6/45 pairs of loci showed

significant LD after Bonferroni correction. All 10 loci were polymorphic in the overall population (Table 9). Hich5 was found to be the most polymorphic locus ($N_A=9$) and Hich7 the least one ($N_A=4$). Mean number of alleles across all loci was approximately 6.2. Hich9 was the locus with the lowest observed heterozygosity (0.287) and Hich2 showed the highest (0.704). Mean observed heterozygosity across all loci for WIP was 0.498, while expected heterozygosity was 0.621 (Table 9).

Table 9. Number of alleles (N_A), number of private alleles (N_{PA}), allelic richness and gene diversity for the overall studied population (WIP) and for the two inferred subpopulations (S, southern cluster 1; CNorth, central/northern cluster). Numbers between parentheses in the loci column are the respective loci names from Rodrigues et al. (2009).

Loci	WIP (2n =350)		S (2n=144)					CNorth (2n=146)				
	Loci range	N_A	Loci range	N_A	N_{PA}	Allelic richness	Gene diversity	Loci range	N_A	N_{PA}	Allelic richness	Gene diversity
Hich1 (1.95)	140-159	6	140-155	5	2	4.947	0.624	146-159	4	1	3.966	0.468
Hich2 (2.52)	178-201	7	178-201	7	2	7.000	0.860	182-197	5	0	4.941	0.721
Hich3 (3.22)	178-212	8	178-212	8	3	6.045	0.679	194-209	5	0	4.491	0.695
Hich4 (4.30)	248-268	6	252-268	5	0	4.987	0.754	248-268	6	1	5.135	0.632
Hich5 (4.59)	140-178	9	140-178	9	4	7.132	0.752	140-170	5	0	3.658	0.525
Hich6 (2.30)	215-229	5	215-229	5	1	4.687	0.577	215-225	4	0	3.514	0.152
Hich7 (4.27)	220-230	4	224-230	3	0	2.826	0.493	220-230	4	1	4.000	0.517
Hich8 (3.16)	189-218	5	189-218	5	0	4.460	0.703	189-218	5	0	4.911	0.730
Hich9 (4.47)	189-261	6	189-261	6	2	4.457	0.336	249-261	4	0	3.969	0.395
Hich10 (3.20)	161-181	6	161-181	5	0	4.690	0.709	161-181	6	1	4.811	0.521
Mean	-	6.2	-	5.8	1.4	5.123	0.648	-	4.8	0.4	4.339	0.535

Genetic diversity and structure of the inferred subpopulations

Results were consistent among replicate runs in Structure and the most likely model evidenced two genetic clusters ($K=2$) (Figure 19a; Supporting Information 7). A total of 30 individuals were found to be below the threshold value evidenced by Hybridlab ($Tq > 0.70$; Figure 19b). These

were considered as genetically admixed individuals and excluded from further analyses, except for the detection of IBD and IBB (individual-based analyses). Most individuals from the central/northern WIP were assigned to one cluster (Cluster CNorth; Figure 19a), while most of the individuals from the southwest were assigned to another (Cluster S; Figure 19a). Nine individuals sampled from the south were attributed to CNorth, while nine individuals from central and northern locations were attributed to S (Figure 20). The names for each cluster (CNorth and S) are exclusively related with the capture location of most individuals attributed to one or other genetic cluster.

Three of the 10 loci presented significant deviation from HWE genotype proportions in the S cluster and LD was found in 1/45 pairs of loci. Loci in CNorth cluster did not depart significantly from HWE and LD was found in 1/45 pairs of loci. Hich 2 and Hich 3 were the loci with the highest observed heterozygosity in S and CNorth, respectively. Hich9 and Hich6 showed the lowest observed heterozygosity in S and CNorth, respectively, and S presented the highest mean observed heterozygosity (Table 10). S showed the highest mean number of alleles, allelic richness and gene diversity. We found 14 private alleles at six loci in S and four private alleles at four loci in CNorth. Generally, the most frequent alleles in CNorth were also frequent in S (see Supporting Information 8). F_{IS} coefficient was significant for both clusters and higher in S (Table 10). Results from AMOVA showed that approximately 79% of the total genetic variation was attributed to differences within individuals, 12% to differences among individuals within the two subpopulations and 9% among groups. Global F_{IS} was significant ($F_{IS} = 0.132$; $p < 0.007$), as well as F_{IT} ($F_{IT} = 0.210$; $p < 0.001$). Pairwise F_{ST} and R_{ST} estimates among the two subpopulations were statistically significant ($F_{ST} = 0.091$, $P < 0.0001$; $R_{ST} = 0.095$, $P < 0.0001$), with F_{ST} evidencing a moderate differentiation level (Wright, 1978).

Wilcoxon rank tests for bottleneck analysis implemented in Bottleneck showed significant evidence of heterozygosity deficiency for CNorth (Table 11). M Ratio did not detect any evidence of the occurrence of bottlenecks. M values estimated with M Ratio for the two inferred clusters were greater than 0.8 (0.884 and 0.956 for CNorth and S, respectively), and none of the formal significance tests performed under different scenarios provided significant evidence of a recent bottleneck.

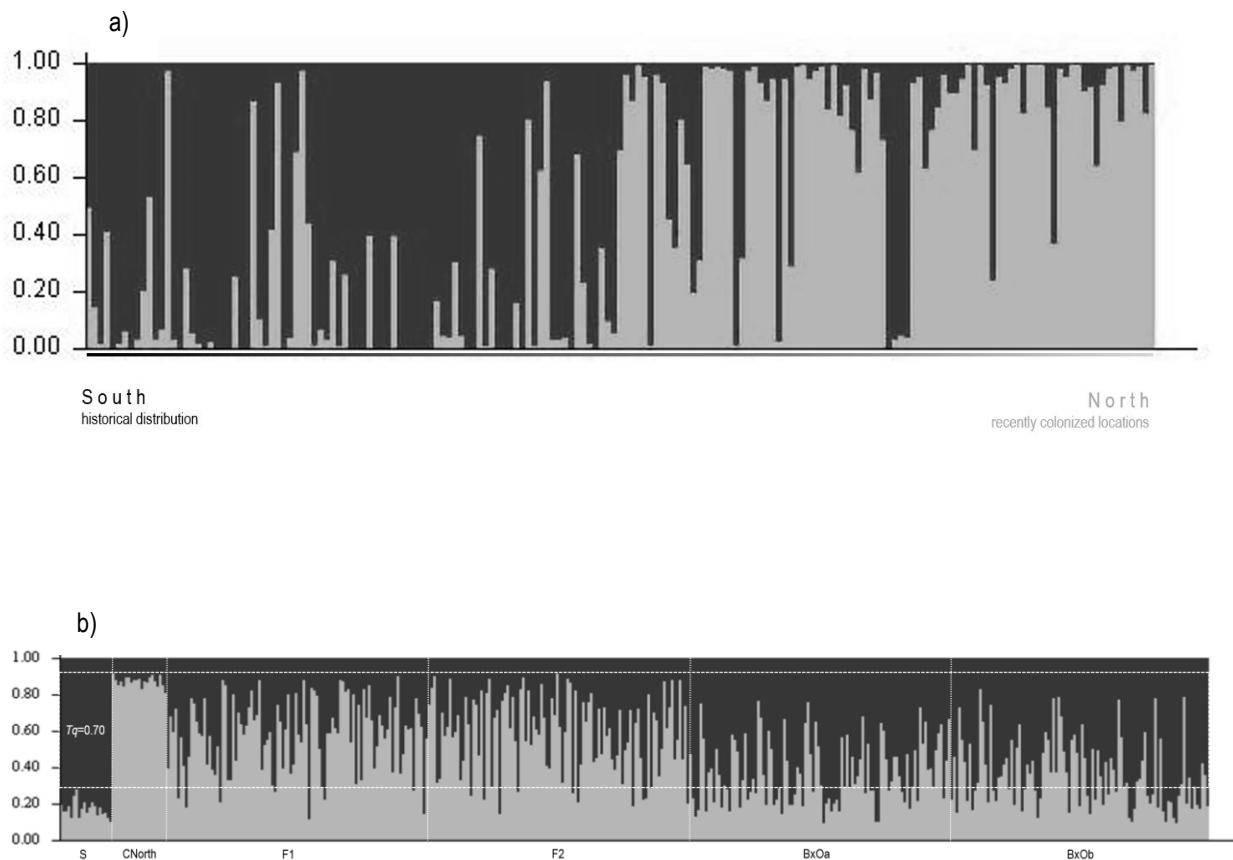


Figure 19. Proportion of each individual genotypes assigned to each genetic cluster inferred in Structure (for best $K = 2$). Each individual is represented by a single vertical bar; a) Probabilistic assignments of the observed genotypes in WIP. All individuals are presented by geographical order, from the most southern locations to the most northern location; b) Probabilistic assignments to each one of the S and CNorth parental gene pools and hybrid (F1, F2 and backcrosses) genotypes simulated using HybridLab. Horizontal dotted lines indicate the threshold value for the assignment of the simulated individuals created by HybridLab, which are based on the individual assignment for S and CNorth. Dotted vertical lines delimitate each class. Cluster S = dark grey; cluster CNorth = light grey.

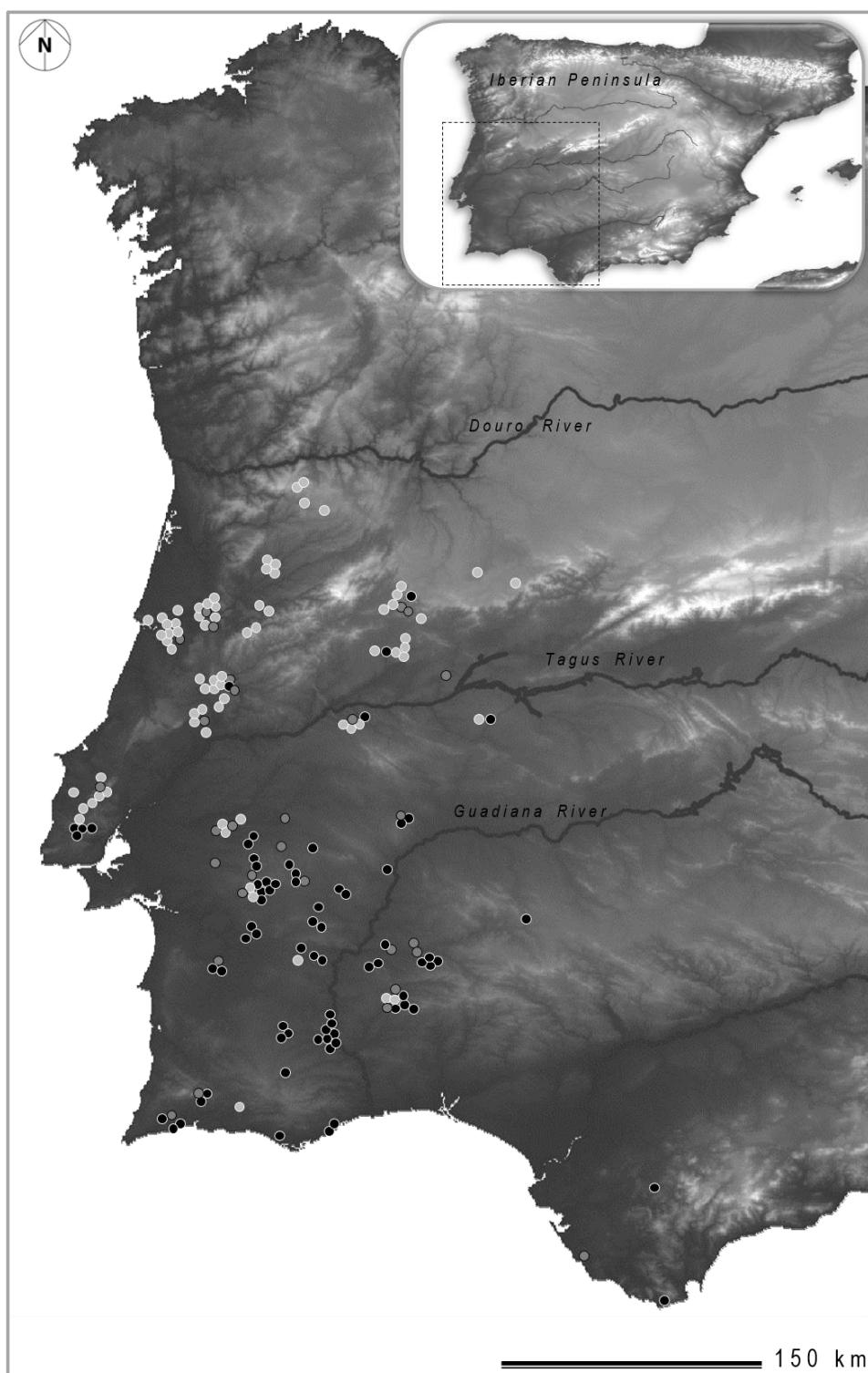


Figure 20. Capture locations of individuals (circles) assigned to each of the two clusters ($Tq > 0.70$) identified by Structure (Cluster S = black circles; CNorth = light grey circles; admixed individuals = dark grey circles).

Table 10. Deviations to HWE expectations, linkage disequilibrium (LD) and F_{IS} for the overall population (WIP) and for the two inferred subpopulations (S and CNorth clusters). Bold numbers indicate loci significantly departing from HWE after Bonferroni correction and significant F_{IS} ($P < 0.05$). Numbers between brackets stand for the number of individuals.

Locus	WIP (2n = 350)		S (2n = 144)		CNorth (2n = 146)	
	H_o	H_E	H_o	H_E	H_o	H_E
Hich1	0.440	0.533	0.530	0.626	0.400	0.463
Hich2	0.704	0.803	0.774	0.852	0.632	0.715
Hich3	0.645	0.684	0.657	0.674	0.695	0.690
Hich4	0.524	0.707	0.529	0.747	0.559	0.627
Hich5	0.583	0.666	0.681	0.746	0.457	0.530
Hich6	0.329	0.434	0.485	0.572	0.095	0.150
Hich7	0.297	0.580	0.280	0.486	0.310	0.505
Hich8	0.632	0.744	0.619	0.696	0.594	0.723
Hich9	0.287	0.365	0.232	0.322	0.355	0.414
Hich10	0.542	0.691	0.531	0.702	0.452	0.519
Mean	0.498	0.621	0.532	0.642	0.455	0.533
LD	6/45		1/45		1/45	
F_{IS}	0.165		0.146		0.115	

Table 11. Significance of heterozygosity excess and deficiency estimated under the Two Phase Model (T.P.M.), using Bottleneck, for the two inferred subpopulations (S, southern cluster; CNorth, central/northern cluster). Parameters for T.P.M.: Variance=20; Probability of single-step mutations =95%. Bold numbers stand for significant departures.

	Clusters	
	S1	CNorth
Wilcoxon Test		
Heterozygosity excess	0.539	0.903
Heterozygosity deficiency	0.500	0.016

Evidences of Isolation by Distance and Isolation by Barrier between the inferred clusters

A pattern of IBD was demonstrated by a significant and positive correlation between the genetic distances and the geographic distances between all pairs of individuals ($r = 0.217$; $p = 9.999e-5$). Isolation by the Tagus River (IBB) was also evidenced by a positive and significant correlation between the genetic distances and the inferred barrier ($r = 0.124$; $p = 9.999e-5$).

DISCUSSION

Genetic structure of the Egyptian mongoose in WIP

Results demonstrate the occurrence of two clusters, CNorth and S, majorly distributed above and below the Tagus River, respectively (Figure 20), with a significant genetic differentiation between them. These results suggest a semi-permeable barrier effect of the river on the genetic structuring of the expanding mongoose population. Indeed, Borralho et al. (1996) suggested that the Tagus River was a possible barrier towards the expansion of the Egyptian mongoose to central and northern locations in Portugal. On the other hand, a recent study concerning the effect of environmental and geographic factors in the expansion of the Egyptian mongoose in Portugal concluded that the species' expansion was mainly conditioned by human interference, climatic variations and land-use changes, but not significantly by rivers (Barros et al., 2015a). Nevertheless, the evidences of IBB in this study suggest a barrier effect of the Tagus River on the genetic differentiation of the studied mongoose population, potentially causing the observed genetic structure within WIP. The effect of rivers as a barrier on the genetic structure of populations has already been shown for other mammals (e.g., Chambers & Garant, 2010; Rogic et al., 2013) and even for medium-sized carnivores (e.g., Cullingham et al., 2009). In our case, the swimming ability of the Egyptian mongoose (Osborn & Helmy, 1980; Delibes, 1982; Gaubert et al., 2011) could explain the dispersal of the species across the river, possibly a reduced number of dispersers.

The Egyptian mongoose has good dispersal abilities for a medium-sized carnivore (Palomares & Delibes, 1998). Food availability as well as certain aspects of its social behaviour can greatly determine the dispersal of the species (Palomares, 1993). Nevertheless, daily movements can reach 4 km in females, or 5 km in males, and great oscillations can occur with daily dispersals of 10 km (Palomares & Delibes, 1998). Despite its dispersal ability, a significant level of IBD across WIP was evidenced, a pattern that has been evidenced in other carnivores with great dispersal ability (e.g., Aspi et al., 2006). According to a stepping-stone model (Kimura & Weiss, 1964), if a species expands gradually, it is expected to find a correlation between the geographical distance separating populations and the genetic distance, which translates in a pattern of Isolation by Distance (Wright, 1943).

Furthermore, a more complex genetic structure may arise. If long distance dispersal occurs through single colonizers that generate genetic structuring, populations may have low and altered diversity due to local founder effects (Nichols & Hewitt, 1994; Bialozyt et al., 2006). In our case, long-range dispersals across the Tagus River might have occurred, which was already evidenced through

mtDNA analyses (Barros et al., 2015b). This allied to the semi-permeable barrier effect of the Tagus River might explain the presence of individuals from S located in central/northern areas and individuals from CNorth located in southern areas. Additionally, the width of Tagus River varies along its course, from Spain to Portugal, or throughout the year, in which smaller widths in drier seasons might facilitate isolated colonization events of individuals from one margin to another.

Northward expansion and bottleneck in WIP

The observed evidences of LD in the overall WIP population are a potential consequence of population structure (see Nei & Li, 1973). Likewise, departure from HWE might be explained by a Wahlund effect that leads to an excess of homozygotes in the aggregate population compared to what would be expected if we assume a non-structured, random mating population (Freeland et al., 2012). Structure results revealed that the Egyptian mongoose population in WIP is structured, which explains departures from HWE in the global population. Both clusters showed LD and S showed departures from HWE, probably due to the ongoing northward expansion of the species.

As genetic diversity is supposed to decline from a source population to a newly occupied area (e.g., Hawley et al., 2008), it may be possible to assess the pattern of colonization by screening the amount of diversity present in each area (Kawamura et al., 2006). Allelic frequencies in S and CNorth revealed signs of expansion following a southern-northern orientation, as frequent alleles in S tend to increase their frequencies in CNorth, and rare alleles in southern clusters tend to become rarer or disappear in CNorth (see Supporting Information 7).

On the other hand, the high allelic richness and gene diversity in S compared with CNorth corroborates the hypothesis of the historical presence of the species in southern Iberian Peninsula (Delibes, 1982; Borralho et al., 1996). Our data fitted the results from previous mtDNA studies (Gaubert et al., 2011; Barros et al., 2015b), in which a high number of haplotypes was found in southern areas compared with recently/northern colonized areas. Lower allelic richness and gene diversity in CNorth could be related to the expansion phenomenon, as lower genetic variability is usually encountered in recently colonized areas (Dlugosch & Parker, 2008). Moreover, the high level of private alleles found in S is in agreement with the south-north expansion scenario.

Presence of private alleles in CNorth could be a result of un-sampled source populations, as some locations across the Iberian range of the species could not be sampled. This led us to another inference indicating possible multiple source populations from the southern range as contributors for the colonization of central and northern areas, a fact that was already described in

other studies (e.g., Stepien et al., 2005; Gillis et al., 2009), and for the Egyptian mongoose as well (Barros et al., 2015b).

Range expansion may result in a succession of bottlenecks due to the movement of founders to new areas, followed by an increase in number and then move on continuously (Rollins et al., 2009). In our case, the recent expansion towards central and northern locations was supported by the Bottleneck results that evidenced heterozygosity deficiency in CNorth (see Luikart & Cornuet, 1998), as this may be related with a drastic population expansion similar to those that occur after a bottleneck event.

FINAL REMARKS AND CONSERVATION IMPLICATIONS

The recent expansion of the Egyptian mongoose in the western Iberian range was patent in our results. We found a significant differentiation between two subpopulations, southern individuals contributing with a higher allelic diversity than central and northern individuals, thus evidencing southern Iberia as the source from which the species spread northward. The barrier effect of the Tagus River was demonstrated in our results, as well as the occurrence of Isolation by Distance, leaving room for further analysis concerning other landscape features as possible determinants of the genetic structure of the species. For this, the emerging field of landscape genetics provides essential tools for inferring the effect of landscape features on the genetic connectivity of populations, with valuable insights for management guidelines for expanding species under a changing environment. Moreover, population genetics of the mongoose population in Europe could be further explored through a modelling approach (e.g., Cushman, 2015; Papeş et al., 2015), by simulating scenarios of spatial genetics combined with the recent ongoing land-use alterations and climatic fluctuations that shaped the range of the species in Portugal in the last three decades.

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Supporting Information 6. Number and locations of the analysed samples in Western Iberian Peninsula (N, number of samples). Table continues in the next page.

Country	District	Municipality	Parish	N
Portugal	Faro	Loulé	Almancil	1
		Monchique	Monchique	3
		Lagos	Bensafrim	1
			Lagos	3
		Olhão	Moncarapacho	2
	Beja	Silves	S.Bartolomeu de Messines	1
		Almodôvar	Santa Cruz	1
		Beja	S. Matias	3
		Castro Verde	Castro Verde	3
		Mértola	Alcaria Ruiva	3
			Mértola	5
		Moura	Amareleja	4
	Évora		Moura	3
			Safara	1
		Serpa	Pias	2
			Vila Nova de S. Bento	8
		Vidigueira	Selmes	1
Portugal	Évora	Arraiolos	Arraiolos	1
			Vimieiro	1
		Alandroal	Terena	1
		Évora	Evora	3
			Nossa Senhora da Graça do	3
			Torre de Coelheiros	1
		Montemor-o-Novo	Ciborro	2
	Mora		Nossa Senhora da Vila	2
			Santiago do Escoural	10
		Mora	Pavia	1
	Portalegre	Portel	Monte do Trigo	2
		Vendas Novas	Herdade dos Mares	1
	Setúbal	Elvas	Vila Boim	3
		Nisa	Montalvão	5
	Setúbal	Alcácer do Sal	Torrão	3
		Grândola	Azinheira de Barros	3
Portugal	Lisboa	Mafra	Mafra	3
			Malveira	1
			Tapada Nacional de Mafra	1
			Sobral da Abelheira	1
	Torres Vedras		Maxial	1
			Monte Redondo	3
			Silveira	1

			Turcifal	1
Portugal	Santarém	Coruche	Coruche	4
			Couço	1
		Ferreira do Zêzere	Areias	7
		Tomar	Beselga	1
			Casal das Freiras	1
		Torres Novas	Lapa	1
			Pedrógão	3
			Torres Novas	1
	Vila Nova de Ourém		Caxarias	1
			Urqueira	1
Portugal	Castelo Branco	Castelo Branco	Lardosa	2
		Fundão	Pêro Viseu	3
			Salgueiro	1
		Idanha-a-Nova	Santa Margarida	1
			S. Miguel de Acha	3
			Zebreira	1
		Penamacor	Penamacor	1
	Coimbra	Arganil	S. Martinho da Cortiça	1
			Sarzedo	1
		Coimbra	Assafarge	1
			Castelo Viegas	1
			Coimbra	7
		Figueira da Foz	Figueira da Foz	1
			Maiorca	1
Portugal	Lousã	Lousã	Lousã	1
			Vilarinho	1
		Montemor-o-Velho	Montemor-o-Velho	1
			Verride	1
		Soure	Samuel	6
			Vinha da Rainha	1
	Guarda	Sabugal	Casteloiro	3
	Viseu	Castro Daire	Mezio	2
			Mões	1
		Tondela	Mouraz	3
			Tondela	1
		Vila Nova de Paiva	Vila Nova de Paiva	1
Spain	Andaluzia	Cádiz	Alcalá de los Gazules	1
			Algar	1
			Tarifa	1
	Extremadura	Badajoz	Zafra	1
		Cáceres	Cáceres	2
	Castilla-León	Salamanca	Castraz de Yeltes	1
			San Felices de los Gallegos	1

Supporting Information 7. Results from Structure simulations (I, II and III) (K = number of clusters being considered; replicates = number of replicated runs; burn-in and run lengths = length of each period in number of iterations; best K = K number of clusters with higher likelihood; $\ln P(D)$ = likelihood; ΔK , delta K).

Simulation	K tested	Replicates	Burnin lenght	Run lenght	Best K	$\ln P(D)$	ΔK
I	1-10	3	1e5	1e6	2	-3730.3	18.594
II	1-10	3	1e5	1e6	2	-3732.0	49.546
III	1-10	3	1e5	1e6	2	-3730.2	44.579

Supporting Information 8. Allele frequencies for all the studied loci for the three clusters and for the overall population (N – number of individuals sharing the analysed loci; WIP, western Iberian Peninsula). Table continues in the next page.

Locus	Alleles	Clusters		WIP
		S	CNorth	
Hich1	N	66	60	150
	140	0.038		0.017
	142	0.114		0.050
	146	0.561	0.708	0.657
	150	0.189	0.092	0.137
	155	0.098	0.158	0.117
	159		0.042	0.023
Hich2	N	62	68	152
	178	0.153		0.066
	182	0.137	0.169	0.148
	186	0.153	0.294	0.250
	190	0.194	0.397	0.296
	193	0.129	0.103	0.109
	197	0.105	0.037	0.063
	201	0.129		0.069
Hich3	N	70	59	155
	178	0.007		0.003
	190	0.007		0.003
	194	0.143	0.085	0.123
	197	0.507	0.441	0.481
	201	0.143	0.220	0.181
	205	0.021	0.008	0.013
	209	0.164	0.246	0.194
	212	0.007		0.003
Hich4	N	70	68	168
	248		0.007	0.003
	252	0.057	0.088	0.068
	256	0.293	0.022	0.14
	261	0.093	0.029	0.063
	264	0.264	0.419	0.339
	268	0.293	0.434	0.387
Hich5	N	69	70	168
	140	0.130	0.257	0.182
	144	0.348	0.629	0.512
	149	0.029	0.007	0.018

	154	0.007	0.014	0.012
	164	0.051		0.024
	166	0.007		0.006
	170	0.319	0.093	0.185
	175	0.101		0.06
	178	0.007		0.003
Hich6	N	66	63	152
	215	0.091	0.016	0.063
	219	0.098	0.024	0.059
	223	0.614	0.921	0.737
	225	0.182	0.040	0.122
	229	0.015		0.02
Hich7	N	50	29	91
	220		0.052	0.027
	224	0.360	0.672	0.451
	227	0.620	0.172	0.462
	230	0.020	0.103	0.06
Hich8	N	63	64	155
	189	0.095	0.336	0.223
	208	0.302	0.031	0.148
	211	0.413	0.281	0.352
	215	0.183	0.281	0.245
	218	0.008	0.070	0.032
Hich9	N	69	62	157
	189	0.007		0.003
	211	0.007		0.003
	249	0.014	0.073	0.041
	254	0.138	0.048	0.096
	258	0.812	0.750	0.787
	261	0.022	0.129	0.07
Hich10	N	64	73	166
	161		0.014	0.009
	164	0.352	0.247	0.295
	168	0.242	0.644	0.431
	172	0.336	0.014	0.172
	176	0.055	0.075	0.084
	181	0.016	0.007	0.009

CHAPTER VI

MEDITERRANEAN SCRUBLAND AND ELEVATION DRIVE GENE FLOW OF THE EGYPTIAN MONGOOSE IN PORTUGAL

A LANDSCAPE GENETICS ASSESSMENT

MANUSCRIPT IV

CHAPTER SIX

Barros, T., Cushman, S.A., Carvalho, J. & Fonseca, C. Mediterranean scrubland and elevation drive gene flow of the Egyptian mongoose in Portugal – a landscape genetics assessment.

Submitted to *Landscape Ecology*

6. MANUSCRIPT IV

Mediterranean scrubland and elevation drive gene flow of the Egyptian mongoose in Portugal – a landscape genetics assessment

ABSTRACT

In a changing environment, the identification of environmental features affecting patterns of gene flow across a species' range is of extreme importance. In this study, we investigated the genetic structure of the Egyptian mongoose (*Herpestes ichneumon*) in Portugal by analysing the correlations between genetic differences and landscape resistance models. We evaluated several functional relationships between elevation, shrub cover, forest cover and temperature, and genetic differentiation. Additionally, we assessed evidence of Isolation by Distance (IBD) within the mongoose population. All hypotheses were analysed under the original causal modelling and reciprocal causal modelling approaches. Results from original causal modelling identified IBD as the best model explaining genetic patterns within the mongoose population. In contrast, the newer reciprocal causal modelling approach supported high shrub cover at middle elevations as the best model explaining the gene flow within the mongoose population. These results further demonstrate that the Egyptian mongoose is very strongly dependent on ecosystems dominated by Mediterranean shrub cover. Recent land-use changes in Portugal, related with rural abandonment and increase of intensive agricultural practices results in highest connectivity among the mongoose populations in areas with high shrub cover at middle elevations, where anthropogenic activities are of lesser intensity. These results should be considered as model to replicate in studies of landscape genetics of other Mediterranean medium-sized carnivores in the Iberian range to better understand how recent land use and climate change affects a broad guild of species.

KEYWORDS

Altitude; Causal modelling; *Herpestes ichneumon*; Land-use changes; Mediterranean maquis; Microsatellites.

INTRODUCTION

A landscape is an intricate and dynamic combination of distinct habitats, each with possible impacts on ecological processes and species' distributions (Taylor et al., 1993; Turner 1989; Stevenson-Holt et al., 2014). Understanding such pattern-process relationships is of extreme importance for unravelling the ecological characteristics of a population, including rates and patterns of gene flow across its distributional range (Storfer et al., 2007). For example, sudden range shifts may be modelled according to stochastic processes (e.g., Cushman, 2015) or they can occur as a result from a response towards a changing environment (Colwell & Rangel, 2010). Land use alterations have had global large impacts on ecological processes (Vitousek et al., 1997; Myers & Knoll 2001; Green et al., 2003). By transforming native ecosystems into altered landscapes, human-driven land use practices are dramatically affecting biodiversity (e.g., Saunders et al., 1991; McGarigal & Cushman, 2002; Cushman & McGarigal, 2003; Cushman, 2006). Recently, several studies have stressed the effect of anthropogenic land use changes on species distributions in Mediterranean Europe (e.g., Falcucci et al., 2007; Moreira & Russo, 2007; Ruiz-González et al., 2014; Mateo-Sánchez et al., 2015). Some studies not only reported effects of past landscape change in Mediterranean countries, but projected severe consequences of future land use changes (e.g., Acevedo et al., 2011).

The Iberian Peninsula is one of the European regions where the Mediterranean maquis is commonly found. Broadly, the Mediterranean maquis is composed by deciduous broad-leaved trees or stiff-leaved evergreen shrubs which recompose the Mediterranean forest, mainly composed by evergreens like holm oak and cork oak (Minelli, 2003). In the Iberian range, the Mediterranean maquis supports an atypically high biodiversity value given the species richness associated with it, including several medium-sized carnivores (Cuttelod et al., 2008; Mangas et al., 2008). Hence, it is critical to evaluate the effect of environmental features and land use alterations within this ecosystem type and the consequences of these changes on species that depend upon it.

Continued dispersal of individuals under a changing environment is critical to maintain genetic diversity within their population, and constitutes a cornerstone for maintaining population viability (Frankham et al., 2002; Lada et al., 2008). To assess the consequences of different landscape patterns on genetic differentiation and gene flow, methods from the emerging field of Landscape Genetics (Manel et al., 2003; Balkenhol et al., 2015) provide the ability to evaluate support for large numbers of alternative hypotheses within a rigorous analytical framework (Cushman et al., 2006; Shirk et al., 2010; Castillo et al., 2014; Cushman et al., 2013). While the

Isolation by Distance (IBD) method ignores the complexity of the landscape – which potentially plays a significant role in dispersal patterns and genetic structure on natural populations (Michels et al., 2001; Spear et al., 2005; Giordano et al., 2007; Wang et al., 2009) -, Landscape Genetics typically uses spatial analysis with Geographic Information Systems to calculate least-cost distances across landscape resistance hypotheses that are then compared with genetic distances among individual organisms. This dramatically improves knowledge of the functional connectivity through a landscape matrix (Coulon et al., 2006; Cushman et al., 2006; Watts et al., 2010; Wasserman et al., 2010; Stevenson et al., 2013), as well as for the development of effective management and conservation strategies (Balkenhol et al., 2009; Segelbacher et al., 2010; Mateo-Sánchez et al., 2015; Wasserman et al., 2012).

One of the species that is intrinsically linked with Mediterranean maquis is the Egyptian mongoose (*Herpestes ichneumon*) (Palomares & Delibes, 1991). Until very recently the species was restricted to the southern territories of the Iberian Peninsula (Borralho et al., 1996), but recent studies showed a rapid northward expansion in Portugal (Barros, 2009; Barros & Fonseca, 2011) that greatly extended the limits of its traditionally known range in the Iberian Peninsula (Delibes, 1982). This rapid expansion was mainly driven by land-use changes related with an increase of shrub-dominated areas, coupled with climate changes (Barros et al., 2015a).

The northward expansion of the species in the Iberian Peninsula led to changes in the genetic patterns of its population (Barros et al., 2015b; Barros et al., Manuscript III). However, the influence of the current landscape on the genetic structure of the Egyptian mongoose in its Portuguese range has not been described. By adopting a landscape genetics framework, our primary goal was to investigate the effects of landscape connectivity on the genetic patterns of the Egyptian mongoose in Portugal. We hypothesized that the extent of shrub-dominated landscapes, forest cover, temperature and altitude would affect the species' gene flow, since it is associated with Mediterranean habitats, including shrub areas and lowland forest mosaics (Palomares & Delibes, 1993a). In this study, we investigated the genetic structure of the mongoose population at individual-level (Rousset, 2000) by analysing the correlations between genetic differences based on microsatellite loci and cost-distances, through a number of landscape resistance hypotheses within a causal modelling framework (Cushman et al., 2006; Cushman & Landguth, 2010). In order to identify the most likely drivers of the observed genetic patterns of the Egyptian mongoose in the Portuguese range, we assessed a number of alternative hypotheses including IBD (Wright, 1943) and 191 landscape resistance hypotheses, comprising a combination of elevation, temperature and land

cover effects. We compared all alternative hypotheses with two widely used analytical frameworks for landscape genetics analysis: the original causal modelling (Cushman et al., 2006) and the reciprocal causal modelling method (Cushman et al., 2013), with the aim of looking for the best correlation between genetic distance and a given landscape resistance.

METHODS

Study Area

The study area consists of the entire Portuguese continental territory, encompassing an area of 92,270 km² located between 35°57' and 42°10' N, and 6°12' and 9°29' E. The central and northern areas are characterized by a mountainous landscape with the highest altitude in the Iberian Central Mountain Chain at Serra da Estrela (1993 m). Southern areas are mainly characterized by flatlands, although the Serra S. Mamede and Monchique mountain ranges constitute the main exceptions. The northwestern portion of the country is in the Atlantic Mid-European sub-region with a temperate and moist climate, wet summers and high levels of precipitation (mean annual precipitation ca. 1060-3094 mm/year; Costa et al., 1998; Panagos et al., 2015). Vegetation of this sub-region is characterized by forests dominated by oaks (*Quercus* sp.), beeches (*Fagus* spp.), birches (*Betula* spp.), ashes (*Fraxinus* spp.) and maples (*Acer* spp.). The majority of Portugal is within the Western Mediterranean sub-region, with dry summers and wet winters (mean annual precipitation ca. 246-717 mm/year; Panagos et al., 2015). The main flora in this climatic zone includes oaks (*Quercus* sp.), mastic (*Pistacia lentiscus*), laurustinus (*Virbunum tinus*), olive trees (*Olea europaea*), carob trees (*Ceratonia siliqua*) and narrow-leaved mock privet (*Phillyrea angustifolia*) (Costa et al., 1998).

Sample Collection and Microsatellite Analysis

All sampling and genetic analyses were carried out prior to the analyses reported in this paper and are described in Barros et al. (Manuscript III). A total of 167 genetic samples of Egyptian mongoose were used in the present study. Distribution and details of the used samples are presented in Supporting Information 9. DNA from muscle was isolated using the salt-extraction method (Bruford et al., 1992), DNA from blood and hair samples was isolated under a modified phenol-chloroform protocol (Sambrook et al., 1989) and by an adapted protocol of the CTAB method

(Rogers & Bendich, 1988), respectively. Extracted DNA from each individual was quantified using a spectrophotometry method with NanoDrop CD-1000[©] (Thermo Fisher Scientific Inc, 2008).

Genotyping was yielded by using a set of ten microsatellites developed for the Egyptian mongoose (Rodrigues et al., 2009). Amplification was conducted using the QIAGEN Taq PCR Core Kit[®], following the manufacturer's conditions. Visualization of the PCR products was conducted in 2% agarose gel and fragment analysis was performed using ABI Standard Dye-Set DS-33[®]. Allele calling was done manually on GeneMapper Version 3.7[®].

Stuttering and allele dropout were tested in Micro-Checker Version 2.2.3 (Van Oosterhout et al., 2004). Deviations from Hardy-Weinberg equilibrium (HWE) were tested using Genepop Version 4.2.1 (Rousset, 2008), with 100 batches and 1000 randomizations. Estimates of pairwise linkage disequilibrium (LD) for each pair of loci was tested on Arlequin Version 3.5.1.2 (Excoffier & Lischer, 2010), with 10,000 permutations. Number of alleles (N_A), observed (H_O) and expected heterozygosity (H_E) were calculated on Genalex Version 6.501 (Peakall & Smouse, 2012). Significance levels were adjusted with the Bonferroni correction for multiple tests (Rice, 1989).

According to Barros et al. (Manuscript III), the mongoose population across southwest Iberian Peninsula is sub-structured in two clusters. However, both clusters showed high admixture between them and they occupy a broad geographical area (Barros et al., Manuscript III), which may indicate a spurious result of using clustering methods on a clinal population (e.g., Schwartz & McKelvey, 2009; Blair et al., 2012). Thus, in this paper, we disregard the population-based clustering approach and instead use an individual-based gradient approach (e.g., Cushman et al., 2006) which can robustly evaluate a wide range of alternative models, including IBD, isolation by barriers and isolation by resistance (e.g., Cushman & Landguth, 2010). Individual-based analyses in which individual genetic differences are associated with the cost distances between them across alternative resistance hypotheses have proven to be a useful approach to understand population connectivity (e.g., Coulon et al., 2004; Cushman et al., 2006; Cushman et al., 2013). Genetic distance among all individuals was calculated on Genalex Version 6.51, using the Smouse & Peakall coefficient (Smouse & Peakall, 1999), resulting in a square matrix of genetic distances among all pairs of sampled mongooses.

Variables selection and creation of resistance surfaces

We created 191 resistance surfaces comprising the factorial combination of four landscape features: temperature, altitude, shrub areas and forest areas (Table 12). The Egyptian mongoose

has a preference for dry and warm climates, which is reflected in its current distribution in the Mediterranean sub-region and in the Afrotropic region (Blanco, 1998; Kingdon, 2003; Barros et al., 2015a). Altitude is a major factor that can affect the distribution of organisms (e.g., Lomolino 2001; Li et al., 2003; Wasserman et al., 2010) due to its influence on temperature and habitat conditions (e.g., Whittaker, 1967; Evans & Cushman, 2009; Barry, 1992; Körner, 2007). The Egyptian mongoose avoids high altitudes (Borralho et al., 1996) and the distribution of the species is linked to the extent of Mediterranean climatic and vegetation zones in the Iberian Peninsula (Palomares & Delibes, 1998).

Each landscape variable was represented by Geographic Information System raster maps. The digital elevation data was gathered from the shuttle radar topography mission (<http://srtm.usgs.gov/index.php>). Mean annual temperature with a spatial resolution of 30 arc-second (ca. 1km²), was compiled from the WordClim website (<http://www.worldclim.org/bioclim>). Land cover comprising shrub and forest cover was retrieved from Corine Land Cover (CLC06) (<http://www.eea.europa.eu/publications/COR0-landcover>). All raster maps were resampled to 250 meters pixel size and set to the ETRS89PortugalTM06 projection in ArcMap Version 10.1.

The resistance of each variable to gene flow was modelled for each unit across three levels for altitude, temperature and shrub areas, and two levels for forest areas. Landscape resistance due to elevation and temperature was modelled as an inverted Gaussian function (e.g., Cushman et al., 2006), assuming a minimum of 1 and a maximum approaching an asymptote of 10 and with a 5 degree standard deviation of the Gaussian function. For elevation, low elevation (LE), medium elevation (ME) and high elevation (HE) were modelled, in which was considered a minimum resistance of 1 at 100, 1000 and 2000 meters of elevation, respectively. Low temperature (LT), medium temperature (MT) and high temperature (HT) were modelled with a minimum resistance of 1 at 15, 20, 25 degrees Celsius, respectively. Levels of resistance for land cover – shrub cover and forest cover – were modelled assuming a minimum resistance value of 1 and a maximum resistance value of 10. Resistance values were assigned to different vegetation types, including five categories for shrub cover and four categories for forest cover (Table 12). All variables used for the creation of resistance surfaces were factorially combined into all combinations (e.g., Cushman et al., 2006), with the aim of determining the combination of factors that were most strongly related to the observed patterns of genetic differentiation. All variables were allowed to speak with equal weight using the fuzzy overlay tool in ArcMap. In addition to the resistance surfaces, we also tested for IBD, in which the Euclidean distance using UTM coordinates between all individuals was calculated. We created

cost matrices reflecting the least-cost distance in meters between each mongoose location and across each one of the 191 resistance surfaces for the isolation by landscape resistance premise (Supporting Information 10). A matrix representing the Euclidean distance between all individuals was also created. All matrices were built on R Version 3.1.2 (R Core Team, 2013) using the sGD package (Shirk & Cushman, 2011).

Table 12. Resistance values for cover classes of forest and shrub in the study area (LS – low selectivity for dense shrub cover; MS – medium selectivity for dense shrub cover; HS – high selectivity for dense shrub cover; LSF - low selectivity for dense forest cover; HSF – high selectivity for dense forest cover).

Cover classes	Resistance levels for LSF	Resistance levels for HSF	Resistance levels for LS	Resistance levels for MS	Resistance levels for HS
Forest classes					
Agro-forestry areas	2	7			
Broad-leaved forest	6	1			
Coniferous forest	5	2			
Mixed forest	6	2			
Shrub classes					
Land principally occupied by agriculture, with significant areas of natural vegetation			3	3	5
Natural grasslands			2	3	4
Moors and heathland			8	6	2
Sclerophyllous vegetation			7	5	1
Transitional woodland-shrub			7	6	4

Relationship between genetic and Euclidean distance – Isolation by Distance (IBD)

Mantel correlations were calculated between genetic distance and Euclidean distances to determine if patterns of genetic differentiation follow a pattern of IBD. The correlation between both distance matrices was calculated by means of a simple Mantel test (Mantel, 1967) as implemented in the Vegan package (Oksanen et al., 2007) in R, using the Pearson correlation with 10 000 permutations for a 95% confidence interval.

Original causal modelling and reciprocal causal modelling framework

As a comparative framework, we conducted both original (Legendre, 1993; Cushman et al., 2006) and reciprocal causal modelling (Cushman et al., 2013) in order to infer which landscape features best explain the genetic structure of the mongoose population (Figure 21).

Three explanatory models of casualty containing 191 hypotheses were created and tested against the IBD null model: Isolation by resistance (Model 1), isolation by resistance by partialling out the effects of geographic distance (Model 2) and IBD by partialling out the effects of each one of the resistance surfaces (Model 3) (Figure 21). To measure the support of the three models, simple Mantel tests were conducted for Model 1 between the genetic distance and each one of the landscape resistance surfaces, and Models 2 and 3 were generated by calculating partial Mantel tests (Smouse et al., 1986) between genetic distance, Euclidean distance and the landscape resistance surfaces. To infer the effect of a landscape resistance scenario on dispersal, we followed Cushman et al. (2006) by selecting the landscape resistance hypotheses in which the Mantel tests in Model 1 and 2 were statistically significant and in Model 3 the Mantel tests had to be negative or with a non-significant correlation. All tests were conducted using the Vegan package for R. All tests were calculated using the Pearson correlation with 10,000 permutations for a 95% confidence interval. All models were then ranked – both significant and non-significant - according to their support based on the magnitude of the Mantel r correlation coefficient as in Cushman et al. (2006).

We also used partial Mantel tests in the reciprocal causal modelling approach for inferring the effect of landscape structure on the genetic patterns and on the genetic distance between all the individuals. Due to the increased risk of spurious correlations using simple Mantel tests (Cushman & Landguth, 2010), Cushman et al. (2013) proposed reciprocal causal modelling, as this method decreases Type I error rates in landscape genetics analysis (Castillo et al., 2014). In reciprocal causal modelling, every alternative resistance hypothesis is tested against all others though partial Mantel tests. The outcome is a matrix of relative support calculated by the difference between a partial Mantel test of each candidate model partialling out each alternative model, and a partial Mantel test of the alternative model partialling out the candidate model (Cushman et al., 2013). The supported hypothesis(es) would present positive values of this difference with all the alternative models, and all alternative models would have negative values compared to the supported model. All analyses for reciprocal causal modelling were conducted using the Ecodist (Goslee & Urban, 2007) and Adegenet (Jombart, 2008) packages in R Version 3.1.2.

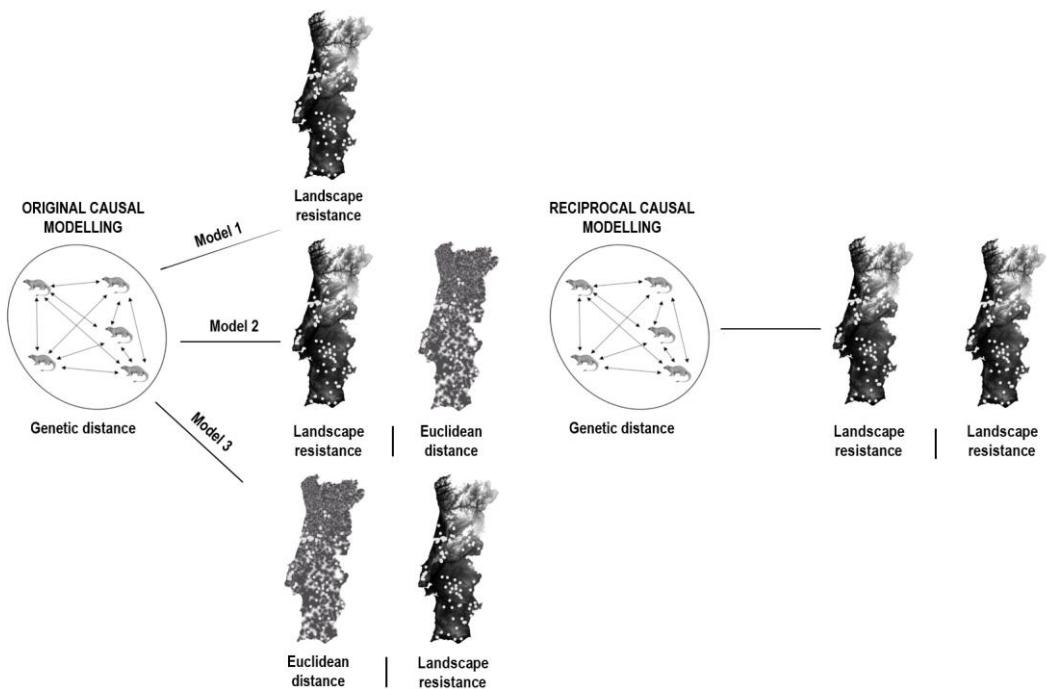


Figure 21. Scheme of the two causal modelling approaches tested in our study. Original causal modelling: Model 1, isolation by landscape resistance; Model 2, isolation by landscape resistance partialling out the Euclidean distance between the sampled individuals; Model 3, isolation by distance partialling out landscape-resistance; Reciprocal causal modelling, isolation by landscape resistance partialling each one of the landscape resistance matrices.

RESULTS

Genetic diversity

There was no evidence of significant allele dropout or stuttering. The overall mongoose population showed a significant deficit of heterozygotes as compared with HWE expectations, consistent with a Wahlund effect caused by localized breeding and dispersal as a result of IBD or isolation by resistance. Six pairs of loci out of 45 pairs showed LD after Bonferroni correction. All 10 loci showed polymorphism for the overall studied population. Hich5 was found to be the most polymorphic locus and Hich7 the least one. Mean number of alleles across all loci was approximately six. Results can be viewed in Supporting Information 11.

Mantel correlations - Isolation by Distance (IBD) and Isolation by Resistance

A pattern of IBD between the studied individuals was evidenced by a significant and positive correlation between the genetic distances and Euclidean distances between the individuals

(Supporting Information 12). All of the 191 simple Mantel tests from Model 1 were significant for all the landscape resistance hypotheses, showing a correlation between genetic distance and the resistance landscapes (Supporting Information 12). Partial Mantel analyses for Model 2 showed 23 significant landscape resistance hypotheses after partialling out the effects of Euclidean distance (Supporting Information 12). The correlation between genetic distance and Euclidean distance was significant when 137 resistance landscapes were partialled out (Model 3; Supporting Information 12).

Hypotheses ranking according to their partial r value showed IBD (*EucDist*) as the hypothesis with the strongest relationship with genetic differentiation, followed by the twenty three significant hypotheses with Euclidean distance partialled out (Supporting Information 13). The landscape resistance hypothesis with the highest r value was *hyp16* which exclusively includes ME. The hypothesis with the lowest r value is *hyp77*, combining HS, HSF and LE (Supporting Information 13), indicating very weak support for any relationship between these landscape features and gene flow.

Original causal modelling and reciprocal causal modelling

Results from the original causal modelling showed twenty three landscape resistance hypotheses supported under this framework (Supporting Information 12). These hypotheses included all levels of elevation and shrub areas, LT and LSF (see Supporting Information 10). On the other hand, the new method of reciprocal causal modelling showed *hyp16*, *hyp43*, *hyp46*, *hyp109*, *hyp136* and *hyp172* as supported landscape resistance hypotheses out of the 36,964 combinations of the 192 candidate models, including IBD (Figure 22). The six models supported by reciprocal causal modelling all indicated gene flow is maximum at ME, LT, and is limited by LSF and facilitated by dense shrub cover (MS, HS) (see Supporting Information 10). IBD was not supported under the reciprocal causal modelling framework. The relative support of each of the six models was calculated by subtracting the mean of r values of the respective row (partial Mantel results for the alternative models by partialling out the candidate model) from the mean of r values from the respective column (partial Mantel results for each candidate model by partialling out each alternative model). Results showed the best supported model is *hyp43*, with the highest value of relative support (Table 13). *Hyp43* includes HS and ME, suggesting that mongoose gene flow is highest in middle elevation shrub cover and declines at lower and higher elevations and in non-shrub cover types.

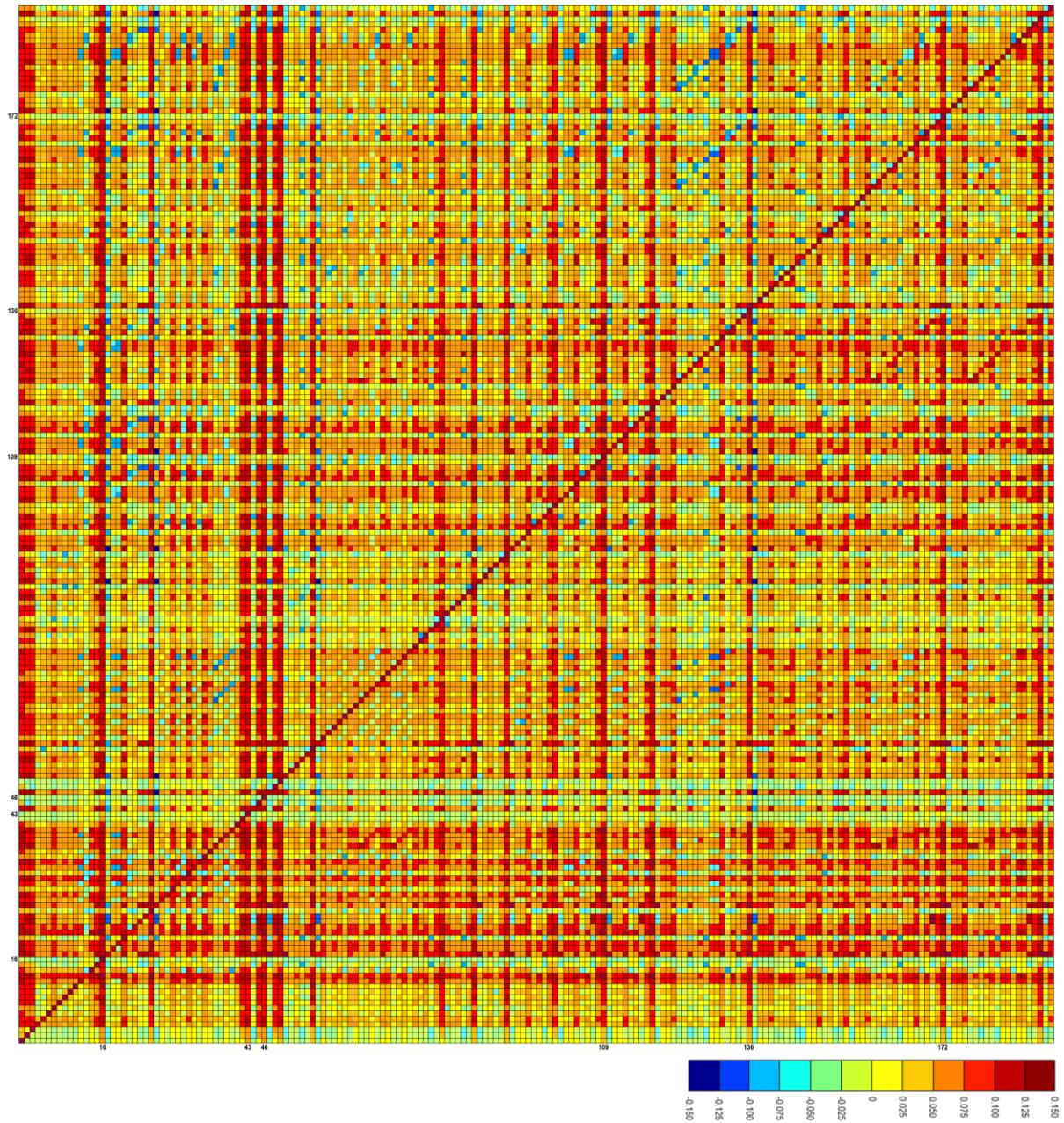


Figure 22. Mantel correlogram showing reciprocal causal modelling results. Models in the figure are in numerical order, the last being Isolation by Distance. *Hyp16*, *hyp43*, *hyp46*, *hyp109*, *hyp136* and *hyp172* are the best supported models. Columns indicate focal models, and rows indicate alternative models. The colour gradient indicates support for the focal model independent of the alternative model. Model codes are associated with the codes on Supporting Information 10.

DISCUSSION

Genetic diversity

We found evidence of LD was found in the overall population. However, LD can occur when the population is structured (Nei & Li, 1973) or presents admixture (Hartl & Clark, 2007). The observed departures from HWE might be explained by the Wahlund effect which leads to more homozygotes that would be expected in a non-structured, randomly mating population (Freeland et al., 2012). Our main result is that mongoose gene flow is limited by a combination of elevation and land cover effects, which leads to a Wahlund effect by creating limited neighborhoods for dispersal and mating. The ongoing expansion of the species (Barros et al., 2015a) also contributes for disequilibrium in the Hardy-Weinberg proportions (see Waits et al., 2000; Cushman, 2015).

This non-equilibrium state of range expansion and limitations to gene flow by isolation by resistance were primary motivations for using an individual-based rather than a population-based approach. Landguth et al. (2010) showed that individual-based analyses in landscape genetics are much more powerful than population-based analyses in detecting effects of landscape features on gene flow and suffer much less from time lags before such effects become important. In addition, population-based inferences about genetic structure are highly vulnerable to inferential error when there is actually clinal based patterns of gene flow related to IBD or isolation by resistance (e.g., Schwartz & McKelvey, 2009; Blair et al., 2012). Moreover, recent studies suggested that individual-based landscape genetics analyses using partial Mantel tests in an optimization framework accurately identify the landscape drivers of gene flow (Shirk et al., 2012; Cushman et al., 2013; Castillo et al., 2014). Such approaches enable direct comparison of landscape resistance, barrier and IBD hypotheses, which greatly improves clarity of inferences (e.g., Wasserman et al., 2010; Cushman & Landguth, 2010).

Table 13. Results for the relative support of the supported hypotheses in the reciprocal causal modelling. Model codes are associated with the codes on Supporting Information 10.

Hypotheses	Average R for the column	Average R for the row	Relative support
<i>hyp16</i>	0.100101	-0.018947	0.081154
<i>hyp43</i>	0.093502	-0.005886	0.087616
<i>hyp46</i>	0.096795	-0.016392	0.080403
<i>hyp109</i>	0.093461	-0.009327	0.084134
<i>hyp136</i>	0.090053	-0.018630	0.071423
<i>hyp173</i>	0.090486	-0.020840	0.069646

Original causal modelling versus reciprocal causal modelling

Our results re-enforced that the reciprocal causal modelling has a higher power for discrimination with landscape resistance hypotheses that better explains the observed genetic patterns. Plus, reciprocal causal modelling reduces Type I errors in comparison with simple Mantel tests and the original form of causal modelling introduced by Cushman et al. (2006), which has previously been demonstrated in other studies (e.g., Cushman et al., 2013; Castilho et al., 2014; Cushman et al., 2014). While original causal modelling supported 23 landscape resistance models (Supporting Information 12), reciprocal causal modelling identified six landscape resistance models, thus reducing Type I errors by 75%. Ranking these hypotheses by relative support identified one hypothesis that was most highly supported (*hyp43*; Figure 22; Table 13). Reciprocal causal modelling is the most recent and most thoroughly evaluated approach for inferring landscape resistance to gene flow in wild populations, and our results confirm it provides greater clarity than previous methods for discriminating between a large number of correlated alternative hypotheses.

Possibly as a result of the great dispersal ability of the species (Palomares & Delibes, 1998), simple Mantel tests identified IBD hypothesis as the model with the highest correlation with the genetic patterns of the Egyptian mongoose in the study area. Similar results were also obtained in a previous study, where a pattern of IBD in the mongoose population was recorded (Barros et al., Manuscript III). When causal modelling was employed, the results clearly identified the influence of landscape variables on gene flow of the Egyptian mongoose. It has frequently been seen that landscape resistance models outperform IBD when these hypotheses are rigorously competed, such as in a causal modelling framework (e.g., Cushman et al., 2006; Wasserman et al., 2010; Shirk et al.,

2010; Castillo et al., 2012; Ruiz-González et al., 2014). Indeed, the best model from reciprocal causal modelling indicates that genetic structure of the Egyptian mongoose is strongly influenced by high dense shrub cover at middle elevations, with no independent support for the IBD hypothesis. These results highlight the importance of including landscape features beyond the traditional IBD model for evaluating spatial genetic patterns (Holderegger & Wagner, 2008).

Selection of scrublands at middle elevations over altered habitats at low elevations

Results from reciprocal causal modelling advance knowledge of population connectivity of the Egyptian mongoose in the Iberian Peninsula. The Egyptian mongoose is a species highly dependent on Mediterranean landscapes characterized by shrub areas of mastic (*Pistacia lentiscus*), rockroses (*Cistus* sp. and *Halimium* sp.), strawberry bushes (*Arbutus unedo*) and dense vegetation of Rosaceae species (Palomares & Delibes, 1993a; Palomares & Delibes, 1993b; Blanco, 1998). Due to its diurnal habits in the Iberian range, the Egyptian mongoose avoids open areas which can expose the species to potential threats (Palomares & Delibes, 1992; Palomares & Delibes, 1993a; Palomares & Delibes, 1998). Studies have confirmed that foraging, hunting and resting are activities associated with the Mediterranean vegetation types (Palomares & Delibes, 1992; Palomares & Delibes, 1993a; Palomares et al., 1993b; Palomares & Delibes, 1998). It is not surprising that we found that the genetic patterns of the species are highly influenced by high shrub cover; gene flow in Egyptian mongoose is maximized in areas with high shrub cover and is lower in areas with low shrub cover and high extent of closed canopy forest.

Our results indicate that the genetic structure of the Egyptian mongoose is also influenced by elevation, with optimal gene flow at middle elevations. Initially, this result appears to be puzzling since the species is not a mountain animal across the Iberian range, except in Ronda Mountains (Spain) where the species is present at altitudes above 1000 meters (Palomares, 1993). Mountainous landscapes were found to exert a barrier effect towards the mongoose expansion across the Portuguese range (Barros et al., 2015a). However, the observation that gene flow of the species is promoted by high shrub cover at middle elevations might be linked with the decrease of human population density at higher altitudes (Cunha, 2007), which favours the Egyptian mongoose due its avoidance from anthropogenic activity (Palomares & Delibes, 1993b; Borralho et al., 1996), a behaviour commonly observed in carnivores (e.g., Woodroffe, 2000; Cardillo et al., 2004). Extensive rural depopulation together with the abandonment of traditional agricultural practices in middle elevation landscapes has promoted the regrowth of natural vegetation, hence higher densities of

scrublands at middle elevations (Barros et al., 2015a). Thus, the increasing availability of shelter and forage resources for the Egyptian mongoose might greatly influence gene flow of the species in middle elevation landscapes. On the other hand, Mediterranean Europe has experienced profound land use changes in the last two decades (Ales et al., 1992; Debussche et al., 1999; Falcucci et al., 2007). A large decline in farming have led to farmland abandonment and to a decline of traditional agricultural activities (Meeus, 1993). This trend became more evident when Portugal joined the European Union in the 1980s and adopted the Common Agricultural Policy that promoted productivity, which profoundly transformed traditional agricultural practices (European Commission 2014 - http://ec.europa.eu/legislation/index_en.htm). As a result, traditional agriculture was converted to intensive agriculture, where broader areas at lower altitudes are used for plantations as well as for permanent pasture areas (see Recenseamento Agrícola 2009 – Instituto Nacional de Estatística), environments that the Egyptian mongoose tends to avoid (Palomares & Delibes, 1993b). Thus, we believe that the joint effects of rural depopulation at higher altitudes and the implementation of intensive agriculture at lower altitudes may have led to influence the genetic patterns of the species, as it may prefer shrub areas at middle elevations, where anthropogenic disturbance is lower and shrub cover is higher.

Besides changes in agricultural practices and rural depopulation, the overall Portuguese Mediterranean scrubland is experiencing extensive alterations. One of the causes is related with the increased establishment of monocultures of *Eucaliptus* sp. and *Pinus* sp. in the last two decades which is gradually replacing the Mediterranean maquis (ICNF, 2013; Águas et al., 2014). Further, certain practices financed by the European Union were implemented to eliminate Mediterranean shrub areas due to their high probability of fire (Mangas et al., 2008), and also due to the fact that scrubland is not considered productive agricultural land (Delibes et al., 1999). The removal of shrub areas is commonly implemented over large areas and is implemented without regard to their role in the conservation of biodiversity (Terradas, 1996; Camprodón, 2001). The non-selective elimination of shrub areas can compromise many animal species dependent on the Mediterranean scrubland, which supports a diverse carnivore community, including the Egyptian mongoose. Due to the reduced level of human interference, as well as higher food availability compared to areas with low vegetation cover, other medium-size carnivores (e.g., the badger *Meles meles*; Revilla et al., 2001, the genet *Genetta genetta*; Virgós & Casanovas, 1997, and the polecat *Mustela putorius*; Mestre et al., 2007) are examples of species highly dependent on the Mediterranean scrubland. In fact, management guidelines have been proposed for the preservation of scrubland for the viability of

carnivores, such as the wildcat *Felis silvestris* (Lozano et al., 2003). Carnivores can be considered as indicators or even umbrella species (Gittleman et al., 2001; Caro, 2003), hence it is important to consider the preservation of Mediterranean scrublands for the conservation of Mediterranean carnivores. However, Mediterranean scrubland is widely considered to be of less importance for conservation than other types of vegetation in official programs for habitat conservation (Mangas et al., 2008). In the landscape genetics context, the importance of establishing guidelines in forest management plans to preserve the connectivity of populations was demonstrated by Koen et al. (2012) for medium-sized carnivores. In the specific case of the Egyptian mongoose, the preservation of shrub areas at lower elevations is not only important for dispersal, but also for the maintenance of gene flow and connectivity between the populations. Despite the fact that the species receives a 'Least Concern' conservation status (Cavallini & Palomares, 2008), our results stress the relevance of scrublands for mongoose. Management guidelines for this species should reflect a more holistic perspective by including the Mediterranean shrub areas and the observed contemporaneous alterations of this habitat type.

CONCLUSION

The contribution of landscape genetics to a broader knowledge about how landscape features influence gene flow is now well established. This study demonstrates the utility of applying rigorous multi-model approaches in landscape genetics analysis of the Egyptian mongoose gene flow. By combining genetic data with ecological variables, we demonstrated that the genetic patterns of the Egyptian mongoose are not simply explained by the geographic distance between those populations, but that a combination of vegetation cover and elevation drive gene flow across its Western Iberian range. Preserving landscape connectivity is crucial for biodiversity and the mongoose populations in Portugal are highly dependent of the connectivity of shrubland areas. Our study is one of the first to evaluate the landscape genetics of Mediterranean carnivores in the Iberian Peninsula, and provides valuable insights to develop habitat connectivity guidelines in the Mediterranean landscape, under the light of the recent documented land use alterations in this ecosystem.

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Supporting Information 9. Number and location of the analysed samples from Barros et al. (Manuscript III).

Tables continues in the next page.

District	County	Parish	N
Faro	Loulé	Almancil	1
	Monchique	Monchique	3
		Bensafrim	1
	Lagos	Lagos	3
	Olhão	Moncarapacho	2
	Silves	S.Bartolomeu de Messines	1
Beja	Almodôvar	Santa Cruz	1
	Beja	S. Matias	3
	Castro Verde	Castro Verde	3
		Alcaria Ruiva	3
	Mértola	Mértola	5
		Amareleja	4
	Moura	Moura	3
		Safara	1
		Pias	2
	Serpa	Vila Nova de S. Bento	8
Évora	Vidigueira	Selmes	1
		Arraiolos	1
	Arraiolos	Vímieiro	1
	Alandroal	Terena	1
		Évora	3
	Évora	Nossa Senhora da Graça do Pilar	3
		Torre de Coelheiros	1
		Ciborro	2
	Montemor-o-Novo	Nossa Senhora da Vila	2
		Santiago do Escoural	10
Portalegre	Mora	Pavia	1
	Portel	Monte do Trigo	2
	Vendas Novas	Herdade dos Mares	1
	Elvas	Vila Boim	3
	Nisa	Montalvão	5
Setúbal	Alcácer do Sal	Torrão	3
	Grândola	Azinheira de Barros	3
Lisboa		Mafra	3
		Malveira	1
	Mafra	Tapada Nacional de Mafra	1
		Sobral da Abelheira	1
		Maxial	1
	Torres Vedras	Monte Redondo	3
		Silveira	1

		Turcifal	1
Santarém	Coruche	Coruche	4
		Couço	1
	Ferreira do Zêzere	Areias	7
		Beselga	1
	Tomar	Casal das Freiras	1
		Lapa	1
	Torres Novas	Pedrógão	3
		Torres Novas	1
		Caxarias	1
	Vila Nova de Ourém	Urqueira	1
Castelo Branco	Castelo Branco	Lardosa	2
		Pêro Viseu	3
	Fundão	Salgueiro	1
		Santa Margarida	1
	Idanha-a-Nova	S. Miguel de Acha	3
		Zebreira	1
	Penamacor	Penamacor	1
Coimbra	Arganil	S. Martinho da Cortiça	1
		Sarzedo	1
		Assafarge	1
	Coimbra	Castelo Viegas	1
		Coimbra	7
	Figueira da Foz	Figueira da Foz	1
		Maiorca	1
	Lousã	Lousã	1
		Vilarinho	1
	Montemor-o-Velho	Montemor-o-Velho	1
		Verride	1
		Samuel	6
	Soure	Vinha da Rainha	1
Guarda	Sabugal	Casteleiro	3
Viseu	Castro Daire	Mezio	2
		Môes	1
	Tondela	Mouraz	3
		Tondela	1
	Vila Nova de Paiva	Vila Nova de Paiva	1

Supporting Information 10. Codes for the landscape resistance surfaces with the respective variables (HS – high selectivity for dense shrub cover; MS – medium selectivity for dense shrub cover; LS – low selectivity for dense shrub cover; HSF – high selectivity for dense forest cover; LSF - low selectivity for dense forest cover; HT – high temperature; MT – medium temperature; LT – low temperature; HE – high elevation; ME - medium elevation; LE – low elevation). Tables continues in the next page.

Hypotheses	Variables	Hypotheses	Variables	Hypotheses	Variables
<i>hyp1</i>	HS	<i>hyp65</i>	MS + HSF + LT	<i>hyp129</i>	LSF + HT + HE
<i>hyp2</i>	MS	<i>hyp66</i>	MS + LSF + HT	<i>hyp130</i>	LSF + HT + ME
<i>hyp3</i>	LS	<i>hyp67</i>	MS + LSF + MT	<i>hyp131</i>	LSF + HT + LE
<i>hyp4</i>	HSF	<i>hyp68</i>	MS + LSF + LT	<i>hyp132</i>	LSF + MT + HE
<i>hyp5</i>	LSF	<i>hyp69</i>	LS + HSF + HT	<i>hyp133</i>	LSF + MT + ME
<i>hyp6</i>	HS + HSF	<i>hyp70</i>	LS + HSF + MT	<i>hyp134</i>	LSF + MT + LE
<i>hyp7</i>	HS + LSF	<i>hyp71</i>	LS + HSF + LT	<i>hyp135</i>	LSF + LT + HE
<i>hyp8</i>	MS + HSF	<i>hyp72</i>	LS + LSF + HT	<i>hyp136</i>	LSF + LT + ME
<i>hyp9</i>	MS + LSF	<i>hyp73</i>	LS + LSF + MT	<i>hyp137</i>	LSF + LT + LE
<i>hyp10</i>	LS + HSF	<i>hyp74</i>	LS + LSF + LT	<i>hyp138</i>	HS + HSF + HT + HE
<i>hyp11</i>	LS + LSF	<i>hyp75</i>	HS + HSF + HE	<i>hyp139</i>	HS + HSF + HT + ME
<i>hyp12</i>	HT	<i>hyp76</i>	HS + HSF + ME	<i>hyp140</i>	HS + HSF + HT + LE
<i>hyp13</i>	MT	<i>hyp77</i>	HS + HSF + LE	<i>hyp141</i>	HS + HSF + MT + HE
<i>hyp14</i>	LT	<i>hyp78</i>	HS + LSF + HE	<i>hyp142</i>	HS + HSF + LT + HE
<i>hyp15</i>	HE	<i>hyp79</i>	HS + LSF + ME	<i>hyp143</i>	HS + HSF + MT + ME
<i>hyp16</i>	ME	<i>hyp80</i>	HS + LSF + LE	<i>hyp144</i>	HS + HSF + LT + ME
<i>hyp17</i>	LE	<i>hyp81</i>	MS + HSF + HE	<i>hyp145</i>	HS + HSF + MT + LE
<i>hyp18</i>	HT + HE	<i>hyp82</i>	MS + HSF + ME	<i>hyp146</i>	HS + HSF + LT + LE
<i>hyp19</i>	HT + ME	<i>hyp83</i>	MS + HSF + LE	<i>hyp147</i>	HS + LSF + HT + HE
<i>hyp20</i>	HT + LE	<i>hyp84</i>	MS + LSF + HE	<i>hyp148</i>	HS + LSF + HT + ME
<i>hyp21</i>	MT + HE	<i>hyp85</i>	MS + LSF + ME	<i>hyp149</i>	HS + LSF + HT + LE
<i>hyp22</i>	MT + ME	<i>hyp86</i>	MS + LSF + LE	<i>hyp150</i>	HS + LSF + MT + HE
<i>hyp23</i>	MT + LE	<i>hyp87</i>	LS + HSF + HE	<i>hyp151</i>	HS + LSF + MT + ME
<i>hyp24</i>	LT + HE	<i>hyp88</i>	LS + HSF + ME	<i>hyp152</i>	HS + LSF + MT + LE
<i>hyp25</i>	LT + ME	<i>hyp89</i>	LS + HSF + LE	<i>hyp153</i>	HS + LSF + LT + HE
<i>hyp26</i>	LT + LE	<i>hyp90</i>	LS + LSF + HE	<i>hyp154</i>	HS + LSF + LT + ME
<i>hyp27</i>	HS + HT	<i>hyp91</i>	LS + LSF + ME	<i>hyp155</i>	HS + LSF + LT + LE
<i>hyp28</i>	HS + MT	<i>hyp92</i>	LS + LSF + LE	<i>hyp156</i>	MS + HSF + HT + HE
<i>hyp29</i>	HS + LT	<i>hyp93</i>	HS + HT + HE	<i>hyp157</i>	MS + HSF + HT + ME
<i>hyp30</i>	MS + HT	<i>hyp94</i>	HS + HT + ME	<i>hyp158</i>	MS + HSF + HT + LE
<i>hyp31</i>	MS + MT	<i>hyp95</i>	HS + HT + LE	<i>hyp159</i>	MS + HSF + MT + HE
<i>hyp32</i>	MS + LT	<i>hyp96</i>	HS + MT + HE	<i>hyp160</i>	MS + HSF + MT + ME
<i>hyp33</i>	LS + HT	<i>hyp97</i>	HS + MT + ME	<i>hyp161</i>	MS + HSF + MT + LE
<i>hyp34</i>	LS + MT	<i>hyp98</i>	HS + MT + LE	<i>hyp162</i>	MS + HSF + LT + HE
<i>hyp35</i>	LS + LT	<i>hyp99</i>	HS + LT + HE	<i>hyp163</i>	MS + HSF + LT + ME
<i>hyp36</i>	HSF + HT	<i>hyp100</i>	HS + LT + ME	<i>hyp164</i>	MS + HSF + LT + LE
<i>hyp37</i>	HSF + MT	<i>hyp101</i>	HS + LT + LE	<i>hyp165</i>	MS + LSF + HT + HE
<i>hyp38</i>	HSF + LT	<i>hyp102</i>	MS + HT + HE	<i>hyp166</i>	MS + LSF + HT + ME
<i>hyp39</i>	LSF + HT	<i>hyp103</i>	MS + HT + ME	<i>hyp167</i>	MS + LSF + HT + LE
<i>hyp40</i>	LSF + MT	<i>hyp104</i>	MS + HT + LE	<i>hyp168</i>	MS + LSF + MT + HE

<i>hyp41</i>	LSF + LT	<i>hyp105</i>	MS + MT + HE	<i>hyp169</i>	MS + LSF + MT + ME
<i>hyp42</i>	HS + HE	<i>hyp106</i>	MS + MT + ME	<i>hyp170</i>	MS + LSF + MT + LE
<i>hyp43</i>	HS + ME	<i>hyp107</i>	MS + MT + LE	<i>hyp171</i>	MS + LSF + LT + HE
<i>hyp44</i>	HS + LE	<i>hyp108</i>	MS + LT + HE	<i>hyp172</i>	MS + LSF + LT + ME
<i>hyp45</i>	MS + HE	<i>hyp109</i>	MS + LT + ME	<i>hyp173</i>	MS + LSF + LT + LE
<i>hyp46</i>	MS + ME	<i>hyp110</i>	MS + LT + LE	<i>hyp174</i>	LS + HSF + HT + HE
<i>hyp47</i>	MS + LE	<i>hyp111</i>	LS + HT + HE	<i>hyp175</i>	LS + HSF + HT + ME
<i>hyp48</i>	LS + HE	<i>hyp112</i>	LS + HT + ME	<i>hyp176</i>	LS + HSF + HT + LE
<i>hyp49</i>	LS + ME	<i>hyp113</i>	LS + HT + LE	<i>hyp177</i>	LS + HSF + MT + HE
<i>hyp50</i>	LS + LE	<i>hyp114</i>	LS + MT + HE	<i>hyp178</i>	LS + HSF + MT + ME
<i>hyp51</i>	HSF + HE	<i>hyp115</i>	LS + MT + ME	<i>hyp179</i>	LS + HSF + MT + LE
<i>hyp52</i>	HSF + ME	<i>hyp116</i>	LS + MT + LE	<i>hyp180</i>	LS + HSF + LT + HE
<i>hyp53</i>	HSF + LE	<i>hyp117</i>	LS + LT + HE	<i>hyp181</i>	LS + HSF + LT + ME
<i>hyp54</i>	LSF + HE	<i>hyp118</i>	LS + LT + ME	<i>hyp182</i>	LS + HSF + LT + LE
<i>hyp55</i>	LSF + ME	<i>hyp119</i>	LS + LT + LE	<i>hyp183</i>	LS + LSF + HT + HE
<i>hyp56</i>	LSF + LE	<i>hyp120</i>	HSF + HT + HE	<i>hyp184</i>	LS + LSF + HT + ME
<i>hyp57</i>	HS + HSF + HT	<i>hyp121</i>	HSF + HT + ME	<i>hyp185</i>	LS + LSF + HT + LE
<i>hyp58</i>	HS + HSF + MT	<i>hyp122</i>	HSF + HT + LE	<i>hyp186</i>	LS + LSF + MT + HE
<i>hyp59</i>	HS + HSF + LT	<i>hyp123</i>	HSF + MT + HE	<i>hyp187</i>	LS + LSF + MT + ME
<i>hyp60</i>	HS + LSF + HT	<i>hyp124</i>	HSF + MT + ME	<i>hyp188</i>	LS + LSF + MT + LE
<i>hyp61</i>	HS + LSF + MT	<i>hyp125</i>	HSF + MT + LE	<i>hyp189</i>	LS + LSF + LT + HE
<i>hyp62</i>	HS + LSF + LT	<i>hyp126</i>	HSF + LT + HE	<i>hyp190</i>	LS + LSF + LT + ME
<i>hyp63</i>	MS + HSF + HT	<i>hyp127</i>	HSF + LT + ME	<i>hyp191</i>	LS + LSF + LT + LE
<i>hyp64</i>	MS + HSF + MT	<i>hyp128</i>	HSF + LT + LE	<i>EucDist</i>	Euclidean Distance

Supporting Information 11. Deviations from Hardy Weinberg conditions, linkage disequilibrium (LD), loci range and number of alleles for each locus for the overall population, published by Barros et al., 2015c. Bold numbers indicate loci significantly departing from HWE after Bonferroni correction. (H_o - observed heterozygosity; H_E - expected heterozygosity; N_A - number of alleles).

Locus	Range	H_o	H_E	N_A
Hich1	140-159	0.440	0.533	6
Hich2	178-201	0.704	0.803	7
Hich3	178-212	0.645	0.684	8
Hich4	248-268	0.524	0.707	6
Hich5	140-178	0.583	0.666	9
Hich6	215-229	0.329	0.434	5
Hich7	220-230	0.297	0.580	4
Hich8	189-218	0.632	0.744	5
Hich9	189-261	0.287	0.365	6
Hich10	161-181	0.542	0.691	6
Mean	-	0.498	0.621	6.2
LD		6/45		

Supporting Information 12. Results from the original causal modelling. Bold hypotheses indicate the hypotheses that are supported by the causal modelling approach. Model codes are associated with the codes on Supporting Information 10. 'Eucdist' refers to the Isolation by Distance hypothesis. Table continues in the next five pages.

Hypotheses	Simple Mantel		Partial Mantel			
	MODEL 1 - Genetic x Landscape		MODEL 2 - Genetic ~ Landscape Euclidian distance		MODEL 3 - Genetic ~ Euclidean distance Landscape	
	R	P < 0.05	R	P < 0.05	R	P < 0.05
hyp1	0.1506	9.999e-05	0.06072	0.00089991	-0.004199	0.56664
hyp2	0.1471	9.999e-05	0.05809	0.0026997	-0.02764	0.86771
hyp3	0.1464	9.999e-05	0.05816	0.0052995	-0.03151	0.90151
<i>hyp4</i>	0.1193	9.999e-05	-0.01002	0.63644	0.07095	0.0029997
<i>hyp5</i>	0.1213	9.999e-05	-0.035	0.88031	0.07531	0.0013999
<i>hyp6</i>	0.1285	9.999e-05	0.006203	0.40866	0.05168	0.018098
<i>hyp7</i>	0.1375	9.999e-05	0.01214	0.29527	0.01857	0.23368
<i>hyp8</i>	0.1257	9.999e-05	-0.0001279	0.49765	0.05781	0.010699
<i>hyp9</i>	0.1279	9.999e-05	-0.03581	0.90971	0.06366	0.0070993
<i>hyp10</i>	0.1264	9.999e-05	0.001798	0.47375	0.05638	0.014599
<i>hyp11</i>	0.1281	9.999e-05	-0.03182	0.87851	0.06107	0.0069993
<i>hyp12</i>	0.1105	0.0019998	0.007901	0.41216	0.08381	0.00049995
<i>hyp13</i>	0.0964	0.0041996	0.004941	0.44706	0.09956	9.999e-05
<i>hyp14</i>	0.1281	9.999e-05	0.02395	0.21028	0.05737	0.012799
hyp15	0.1417	9.999e-05	0.06255	0.0055994	-0.05396	0.9871
hyp16	0.159	9.999e-05	0.08978	0.00019998	-0.04186	0.9588
<i>hyp17</i>	0.1073	0.00039996	-0.0713	0.9883	0.1128	9.999e-05
<i>hyp18</i>	0.1105	0.0012999	0.007901	0.41526	0.08381	0.00079992
<i>hyp19</i>	0.1103	0.0012999	0.007705	0.40946	0.08404	0.00039996
<i>hyp20</i>	0.1402	9.999e-05	0.03652	0.074993	-0.02764	0.86511
<i>hyp21</i>	0.09687	0.0029997	0.004848	0.44636	0.0991	9.999e-05
<i>hyp22</i>	0.1092	0.0011999	0.02186	0.26747	0.08789	0.00039996
<i>hyp23</i>	0.117	0.00029997	-0.05284	0.9618	0.09081	0.00029997
<i>hyp24</i>	0.117	0.00019998	-0.05284	0.9664	0.09081	0.00029997
hyp25	0.1503	9.999e-05	0.06375	0.010199	0.02231	0.17838
<i>hyp26</i>	0.1084	0.00059994	-0.06974	0.9917	0.1107	9.999e-05
<i>hyp27</i>	0.1189	9.999e-05	0.008252	0.40076	0.0714	0.0028997
<i>hyp28</i>	0.1073	0.00089991	0.00849	0.39476	0.08797	0.00039996

<i>hyp29</i>	0.1391	9.999e-05	0.03581	0.062394	0.03194	0.10099
<i>hyp30</i>	0.1156	0.00029997	0.009	0.40116	0.07673	0.0010999
<i>hyp31</i>	0.1016	0.0009999	0.006917	0.40596	0.09443	0.00019998
<i>hyp32</i>	0.14	9.999e-05	0.03879	0.073093	0.0313	0.10309
<i>hyp33</i>	0.116	0.00049995	0.009578	0.37466	0.0761	0.0014999
<i>hyp34</i>	0.1021	0.0014999	0.007615	0.40876	0.09386	0.00019998
<i>hyp35</i>	0.1382	9.999e-05	0.03614	0.091191	0.03604	0.071693
<i>hyp36</i>	0.1286	9.999e-05	0.009284	0.38166	0.05188	0.018398
<i>hyp37</i>	0.1098	0.00029997	-0.01646	0.69343	0.08598	0.00029997
<i>hyp38</i>	0.1143	0.00019998	-0.01633	0.69593	0.07979	0.0009999
<i>hyp39</i>	0.1122	0.00069993	0.008525	0.40636	0.08165	0.00059994
<i>hyp40</i>	0.1056	0.00049995	-0.007773	0.58624	0.08997	0.00039996
<i>hyp41</i>	0.1274	9.999e-05	-0.003499	0.53865	0.05405	0.014599
<i>hyp42</i>	0.1512	9.999e-05	0.06293	0.00049995	-0.0111	0.66853
<i>hyp43</i>	0.1568	9.999e-05	0.07587	9.999e-05	-0.01285	0.69603
<i>hyp44</i>	0.114	0.00029997	-0.06653	0.989	0.1028	9.999e-05
<i>hyp45</i>	0.1483	9.999e-05	0.06284	0.0016998	-0.03154	0.89691
<i>hyp46</i>	0.1578	9.999e-05	0.084	0.00019998	-0.03389	0.92131
<i>hyp47</i>	0.112	0.00049995	-0.06245	0.982	0.1024	9.999e-05
<i>hyp48</i>	0.148	9.999e-05	0.0646	0.0022998	-0.03622	0.92631
<i>hyp49</i>	0.1575	9.999e-05	0.08309	0.00019998	-0.03302	0.91571
<i>hyp50</i>	0.113	0.00019998	-0.0603	0.979	0.1001	9.999e-05
<i>hyp51</i>	0.1177	9.999e-05	-0.01439	0.68053	0.07421	0.0019998
<i>hyp52</i>	0.1263	9.999e-05	0.004802	0.43886	0.05665	0.010399
<i>hyp53</i>	0.1132	0.00019998	-0.07905	0.9957	0.1121	9.999e-05
<i>hyp54</i>	0.1216	9.999e-05	-0.03454	0.87281	0.07462	0.0016998
<i>hyp55</i>	0.1497	9.999e-05	0.05918	0.014099	-0.01086	0.66443
<i>hyp56</i>	0.1078	0.00039996	-0.0708	0.9907	0.112	9.999e-05
<i>hyp57</i>	0.1334	9.999e-05	0.01043	0.35906	0.03788	0.064294
<i>hyp58</i>	0.1185	9.999e-05	-0.01057	0.63834	0.07237	0.0013999
<i>hyp59</i>	0.1265	9.999e-05	-0.001271	0.51575	0.05596	0.013899
<i>hyp60</i>	0.1203	0.00019998	0.009226	0.38856	0.06904	0.0029997
<i>hyp61</i>	0.1136	9.999e-05	-0.006066	0.58034	0.07944	0.00069993
<i>hyp62</i>	0.1323	9.999e-05	-0.006516	0.60964	0.04058	0.051595
<i>hyp63</i>	0.1305	9.999e-05	0.009936	0.36316	0.04693	0.028897
<i>hyp64</i>	0.1145	9.999e-05	-0.01096	0.63204	0.07865	0.00089991
<i>hyp65</i>	0.1239	9.999e-05	-0.003389	0.54605	0.06173	0.0087991

<i>hyp66</i>	0.1173	0.00069993	0.009909	0.38316	0.07424	0.0015998
<i>hyp67</i>	0.1108	0.00019998	-0.003528	0.53235	0.08309	0.0012999
<i>hyp68</i>	0.1343	9.999e-05	0.005876	0.41206	0.03327	0.088191
<i>hyp69</i>	0.1305	9.999e-05	0.009937	0.36296	0.0468	0.033297
<i>hyp70</i>	0.1147	0.00039996	-0.01047	0.62804	0.07818	0.0018998
<i>hyp71</i>	0.1226	9.999e-05	-0.004412	0.55504	0.06439	0.0066993
<i>hyp72</i>	0.1177	0.00039996	0.01047	0.37286	0.07354	0.0012999
<i>hyp73</i>	0.1114	0.00019998	-0.002729	0.52765	0.08232	0.00089991
<i>hyp74</i>	0.1344	9.999e-05	0.00672	0.39196	0.03323	0.091891
<i>hyp75</i>	0.1272	9.999e-05	0.0008522	0.48945	0.05448	0.016298
<i>hyp76</i>	0.1343	9.999e-05	0.0186	0.23698	0.0375	0.068393
<i>hyp77</i>	0.1172	9.999e-05	-0.07906	0.9972	0.1079	9.999e-05
<i>hyp78</i>	0.1376	9.999e-05	0.01336	0.28347	0.01857	0.22398
<i>hyp79</i>	0.15	9.999e-05	0.06255	0.0023998	-0.02114	0.80452
<i>hyp80</i>	0.1284	9.999e-05	-0.0287	0.86291	0.0588	0.009599
<i>hyp81</i>	0.1259	9.999e-05	-0.001192	0.51435	0.05747	0.012999
<i>hyp82</i>	0.1316	9.999e-05	0.013	0.31917	0.04431	0.039796
<i>hyp83</i>	0.1161	9.999e-05	-0.0741	0.9956	0.1056	9.999e-05
<i>hyp84</i>	0.1287	9.999e-05	-0.02981	0.86941	0.05878	0.009899
<i>hyp85</i>	0.1499	9.999e-05	0.06431	0.0062994	-0.02632	0.85451
<i>hyp86</i>	0.1124	9.999e-05	-0.0618	0.984	0.1016	9.999e-05
<i>hyp87</i>	0.1254	9.999e-05	-0.001862	0.52235	0.05841	0.0093991
<i>hyp88</i>	0.131	9.999e-05	0.012	0.32357	0.04596	0.031397
<i>hyp89</i>	0.1175	9.999e-05	-0.02038	0.74053	0.07597	0.0016998
<i>hyp90</i>	0.1292	9.999e-05	-0.02607	0.82922	0.0559	0.014199
<i>hyp91</i>	0.15	9.999e-05	0.06414	0.0070993	-0.02527	0.84232
<i>hyp92</i>	0.1134	0.00039996	-0.05969	0.9752	0.09927	9.999e-05
<i>hyp93</i>	0.1189	9.999e-05	0.00825	0.39466	0.0714	0.0039996
<i>hyp94</i>	0.1188	0.00019998	0.008205	0.39836	0.07157	0.0036996
<i>hyp95</i>	0.14	9.999e-05	0.03485	0.084092	-0.02643	0.85481
<i>hyp96</i>	0.1073	0.00049995	0.008483	0.38976	0.08796	0.00029997
<i>hyp97</i>	0.1188	9.999e-05	0.02614	0.20548	0.07571	0.0016998
<i>hyp98</i>	0.1174	9.999e-05	-0.05871	0.9806	0.0939	0.00019998
<i>hyp99</i>	0.1392	9.999e-05	0.03605	0.061994	0.03172	0.10279
<i>hyp100</i>	0.1469	9.999e-05	0.05414	0.0073993	0.01962	0.22368
<i>hyp101</i>	0.1142	9.999e-05	-0.06651	0.9889	0.1026	9.999e-05
<i>hyp102</i>	0.1156	0.00019998	0.009001	0.38586	0.07673	0.0019998

<i>hyp103</i>	0.1155	0.00039996	0.008792	0.40136	0.07691	0.0014999
<i>hyp104</i>	0.1402	9.999e-05	0.03652	0.075592	-0.02764	0.86641
<i>hyp105</i>	0.1016	0.0020998	0.006931	0.41496	0.09441	0.00039996
<i>hyp106</i>	0.1136	0.00039996	0.02429	0.23038	0.0828	0.00029997
<i>hyp107</i>	0.1179	9.999e-05	-0.05111	0.9593	0.08873	0.00029997
<i>hyp108</i>	0.14	9.999e-05	0.03872	0.071193	0.03136	0.10279
<i>hyp109</i>	0.1537	9.999e-05	0.06862	0.0030997	0.009258	0.35336
<i>hyp110</i>	0.1121	0.00029997	-0.0627	0.9814	0.1026	9.999e-05
<i>hyp111</i>	0.1161	0.00039996	0.009579	0.38456	0.0761	0.0011999
<i>hyp112</i>	0.1159	0.00029997	0.009368	0.39786	0.07628	0.0014999
<i>hyp113</i>	0.1402	9.999e-05	0.03652	0.076392	-0.02764	0.87111
<i>hyp114</i>	0.1021	0.0017998	0.007629	0.40916	0.09384	9.999e-05
<i>hyp115</i>	0.1136	0.00049995	0.02424	0.22998	0.08278	0.00059994
<i>hyp116</i>	0.1182	0.00029997	-0.05043	0.9584	0.08785	9.999e-05
<i>hyp117</i>	0.1383	9.999e-05	0.03636	0.084592	0.03586	0.072193
<i>hyp118</i>	0.1521	9.999e-05	0.06572	0.0047995	0.01413	0.28487
<i>hyp119</i>	0.113	9.999e-05	-0.06075	0.9804	0.1003	0.00019998
<i>hyp120</i>	0.1286	9.999e-05	0.009279	0.37686	0.05189	0.019698
<i>hyp121</i>	0.1284	9.999e-05	0.008933	0.38076	0.05221	0.018698
<i>hyp122</i>	0.1392	9.999e-05	0.02331	0.18368	-0.01579	0.73853
<i>hyp123</i>	0.1096	0.00049995	-0.01679	0.69233	0.08631	0.00049995
<i>hyp124</i>	0.116	9.999e-05	-0.006545	0.57444	0.07584	0.0011999
<i>hyp125</i>	0.1197	9.999e-05	-0.06716	0.9924	0.09655	9.999e-05
<i>hyp126</i>	0.1141	9.999e-05	-0.01672	0.70003	0.08016	0.0009999
<i>hyp127</i>	0.1239	9.999e-05	0.002955	0.45945	0.06165	0.0082992
<i>hyp128</i>	0.1143	9.999e-05	-0.07806	0.9967	0.1103	9.999e-05
<i>hyp129</i>	0.1122	0.00069993	0.008526	0.40416	0.08165	0.00049995
<i>hyp130</i>	0.112	0.0010999	0.008312	0.40206	0.08189	0.00089991
<i>hyp131</i>	0.1402	9.999e-05	0.03652	0.076692	-0.02764	0.86691
<i>hyp132</i>	0.1056	0.00059994	-0.007756	0.59404	0.08994	9.999e-05
<i>hyp133</i>	0.1235	9.999e-05	0.01824	0.27647	0.06495	0.0057994
<i>hyp134</i>	0.1171	9.999e-05	-0.0528	0.9655	0.09072	0.00019998
<i>hyp135</i>	0.1277	9.999e-05	-0.002964	0.53305	0.05319	0.020198
<i>hyp136</i>	0.1519	9.999e-05	0.06693	0.0068993	-0.02085	0.79152
<i>hyp137</i>	0.1087	9.999e-05	-0.06926	0.9868	0.1101	0.00019998
<i>hyp138</i>	0.1334	9.999e-05	0.01039	0.35066	0.03791	0.067693
<i>hyp139</i>	0.1333	9.999e-05	0.01026	0.35556	0.03811	0.063594

<i>hyp140</i>	0.1392	9.999e-05	0.02356	0.17008	-0.01624	0.74673
<i>hyp141</i>	0.1185	0.00019998	-0.01058	0.63184	0.07238	0.0019998
<i>hyp142</i>	0.1266	9.999e-05	-0.001167	0.50765	0.05585	0.014899
<i>hyp143</i>	0.1248	9.999e-05	0.002377	0.45975	0.05988	0.009699
<i>hyp144</i>	0.1347	9.999e-05	0.01963	0.22578	0.03687	0.071993
<i>hyp145</i>	0.1196	9.999e-05	-0.07281	0.9948	0.1006	9.999e-05
<i>hyp146</i>	0.1173	9.999e-05	-0.07903	0.9982	0.1078	9.999e-05
<i>hyp147</i>	0.1203	0.00019998	0.009225	0.38476	0.06904	0.0023998
<i>hyp148</i>	0.1202	0.00039996	0.009177	0.38406	0.06922	0.0037996
<i>hyp149</i>	0.14	9.999e-05	0.03485	0.081192	-0.02643	0.85401
<i>hyp150</i>	0.1136	0.00029997	-0.006058	0.57504	0.07941	0.00059994
<i>hyp151</i>	0.1269	9.999e-05	0.01715	0.27097	0.05763	0.011199
<i>hyp152</i>	0.1174	9.999e-05	-0.05863	0.981	0.09378	0.00039996
<i>hyp153</i>	0.1327	9.999e-05	-0.005304	0.57874	0.03931	0.057994
<i>hyp154</i>	0.1446	9.999e-05	0.04356	0.029597	-0.005631	0.59094
<i>hyp155</i>	0.1142	9.999e-05	-0.06645	0.989	0.1025	9.999e-05
<i>hyp156</i>	0.1305	9.999e-05	0.009931	0.37016	0.04694	0.032697
<i>hyp157</i>	0.1303	9.999e-05	0.009655	0.37016	0.04725	0.025597
<i>hyp158</i>	0.1392	9.999e-05	0.02331	0.17958	-0.01579	0.73403
<i>hyp159</i>	0.1143	0.00029997	-0.01123	0.64484	0.07892	0.0011999
<i>hyp160</i>	0.1201	0.00019998	-0.00102	0.50015	0.06876	0.0032997
<i>hyp161</i>	0.1198	9.999e-05	-0.0673	0.9915	0.0965	0.00019998
<i>hyp162</i>	0.124	9.999e-05	-0.003273	0.54075	0.0616	0.0083992
<i>hyp163</i>	0.1316	9.999e-05	0.01491	0.29867	0.04501	0.039896
<i>hyp164</i>	0.1162	0.00019998	-0.07424	0.9967	0.1056	9.999e-05
<i>hyp165</i>	0.1173	0.00039996	0.00991	0.37496	0.07424	0.0019998
<i>hyp166</i>	0.1171	0.00019998	0.00967	0.38756	0.07445	0.0017998
<i>hyp167</i>	0.1402	9.999e-05	0.03652	0.076192	-0.02764	0.87151
<i>hyp168</i>	0.1109	0.00029997	-0.003506	0.53725	0.08305	0.00039996
<i>hyp169</i>	0.1273	9.999e-05	0.02235	0.22758	0.05846	0.010999
<i>hyp170</i>	0.1179	9.999e-05	-0.05108	0.9579	0.08864	0.00059994
<i>hyp171</i>	0.1345	9.999e-05	0.006369	0.39926	0.03243	0.09889
<i>hyp172</i>	0.1521	9.999e-05	0.0719	0.0011999	-0.03251	0.90681
<i>hyp173</i>	0.1124	0.00029997	-0.06206	0.9787	0.1018	9.999e-05
<i>hyp174</i>	0.1305	9.999e-05	0.009932	0.37336	0.0468	0.030997
<i>hyp175</i>	0.1304	9.999e-05	0.009655	0.37546	0.04711	0.027997
<i>hyp176</i>	0.1392	9.999e-05	0.02331	0.17918	-0.01579	0.73223

<i>hyp177</i>	0.1145	0.00029997	-0.01087	0.63584	0.07859	0.0016998
<i>hyp178</i>	0.1204	0.00019998	-0.000367	0.51075	0.06825	0.0039996
<i>hyp179</i>	0.1203	9.999e-05	-0.06607	0.9904	0.09506	9.999e-05
<i>hyp180</i>	0.1225	9.999e-05	-0.00443	0.55014	0.06446	0.0054995
<i>hyp181</i>	0.1302	9.999e-05	0.01323	0.32347	0.04845	0.028797
<i>hyp182</i>	0.1168	9.999e-05	-0.07296	0.9948	0.104	9.999e-05
<i>hyp183</i>	0.1177	0.00039996	0.01047	0.37416	0.07354	0.0027997
<i>hyp184</i>	0.1176	0.00029997	0.01025	0.37346	0.07373	0.0018998
<i>hyp185</i>	0.1114	0.00029997	-0.002707	0.53775	0.08229	0.00069993
<i>hyp186</i>	0.1273	0.00019998	0.02227	0.22988	0.05851	0.010999
<i>hyp187</i>	0.1273	9.999e-05	0.02227	0.22448	0.05851	0.0089991
<i>hyp188</i>	0.1183	9.999e-05	-0.05038	0.9596	0.08776	0.00029997
<i>hyp189</i>	0.1345	9.999e-05	0.007117	0.38986	0.0325	0.09739
<i>hyp190</i>	0.1514	9.999e-05	0.06879	0.0030997	-0.02874	0.87241
<i>hyp191</i>	0.1134	0.00039996	-0.06005	0.9789	0.09946	9.999e-05
<i>EucDist</i>	0.1382	9.999e-05				

Supporting Information 13. Ranking of all hypotheses according to their R value from the partial Mantel tests by partialling out the Euclidean Distance. Bold hypotheses were significant ($p < 0.05$). Model codes are associated with the codes in Supporting Information 10. 'Eucdist' refers to the Isolation by Distance hypothesis. Tables continues in the next page.

Hypotheses	R	Hypotheses	R	Hypotheses	R
Eucdist	0.1382	<i>hyp184</i>	0.01025	<i>hyp71</i>	-0.004412
hyp16	0.08978	<i>hyp69</i>	0.009937	<i>hyp180</i>	-0.00443
hyp46	0.084	<i>hyp63</i>	0.009936	<i>hyp153</i>	-0.005304
hyp49	0.08309	<i>hyp174</i>	0.009932	<i>hyp150</i>	-0.006058
hyp43	0.07587	<i>hyp156</i>	0.009931	<i>hyp61</i>	-0.006066
hyp172	0.0719	<i>hyp165</i>	0.00991	<i>hyp62</i>	-0.006516
hyp190	0.06879	<i>hyp66</i>	0.009909	<i>hyp124</i>	-0.006545
hyp109	0.06862	<i>hyp166</i>	0.00967	<i>hyp132</i>	-0.007756
hyp136	0.06693	<i>hyp157</i>	0.009655	<i>hyp40</i>	-0.007773
hyp118	0.06572	<i>hyp175</i>	0.009655	<i>hyp4</i>	-0.01002
hyp48	0.0646	<i>hyp111</i>	0.009579	<i>hyp70</i>	-0.01047
hyp85	0.06431	<i>hyp33</i>	0.009578	<i>hyp58</i>	-0.01057
hyp91	0.06414	<i>hyp112</i>	0.009368	<i>hyp141</i>	-0.01058
hyp25	0.06375	<i>hyp36</i>	0.009284	<i>hyp177</i>	-0.01087
hyp42	0.06293	<i>hyp120</i>	0.009279	<i>hyp64</i>	-0.01096
hyp45	0.06284	<i>hyp60</i>	0.009226	<i>hyp159</i>	-0.01123
hyp15	0.06255	<i>hyp147</i>	0.009225	<i>hyp51</i>	-0.01439
hyp79	0.06255	<i>hyp148</i>	0.009177	<i>hyp38</i>	-0.01633
hyp1	0.06072	<i>hyp102</i>	0.009001	<i>hyp37</i>	-0.01646
hyp55	0.05918	<i>hyp30</i>	0.009	<i>hyp126</i>	-0.01672
hyp3	0.05816	<i>hyp121</i>	0.008933	<i>hyp123</i>	-0.01679
hyp2	0.05809	<i>hyp103</i>	0.008792	<i>hyp89</i>	-0.02038
hyp100	0.05414	<i>hyp129</i>	0.008526	<i>hyp90</i>	-0.02607
hyp154	0.04356	<i>hyp39</i>	0.008525	<i>hyp80</i>	-0.0287
<i>hyp32</i>	0.03879	<i>hyp28</i>	0.00849	<i>hyp84</i>	-0.02981
<i>hyp108</i>	0.03872	<i>hyp96</i>	0.008483	<i>hyp11</i>	-0.03182
<i>hyp20</i>	0.03652	<i>hyp130</i>	0.008312	<i>hyp54</i>	-0.03454
<i>hyp104</i>	0.03652	<i>hyp27</i>	0.008252	<i>hyp5</i>	-0.035
<i>hyp113</i>	0.03652	<i>hyp93</i>	0.00825	<i>hyp9</i>	-0.03581
<i>hyp131</i>	0.03652	<i>hyp94</i>	0.008205	<i>hyp188</i>	-0.05038
<i>hyp167</i>	0.03652	<i>hyp12</i>	0.007901	<i>hyp116</i>	-0.05043
<i>hyp117</i>	0.03636	<i>hyp18</i>	0.007901	<i>hyp170</i>	-0.05108
<i>hyp35</i>	0.03614	<i>hyp19</i>	0.007705	<i>hyp107</i>	-0.05111
<i>hyp99</i>	0.03605	<i>hyp114</i>	0.007629	<i>hyp134</i>	-0.0528
<i>hyp29</i>	0.03581	<i>hyp34</i>	0.007615	<i>hyp23</i>	-0.05284
<i>hyp95</i>	0.03485	<i>hyp189</i>	0.007117	<i>hyp24</i>	-0.05284
<i>hyp149</i>	0.03485	<i>hyp105</i>	0.006931	<i>hyp152</i>	-0.05863
<i>hyp97</i>	0.02614	<i>hyp31</i>	0.006917	<i>hyp98</i>	-0.05871

<i>hyp106</i>	0.02429	<i>hyp74</i>	0.00672	<i>hyp92</i>	-0.05969
<i>hyp115</i>	0.02424	<i>hyp171</i>	0.006369	<i>hyp191</i>	-0.06005
<i>hyp14</i>	0.02395	<i>hyp6</i>	0.006203	<i>hyp50</i>	-0.0603
<i>hyp140</i>	0.02356	<i>hyp68</i>	0.005876	<i>hyp119</i>	-0.06075
<i>hyp122</i>	0.02331	<i>hyp13</i>	0.004941	<i>hyp86</i>	-0.0618
<i>hyp158</i>	0.02331	<i>hyp21</i>	0.004848	<i>hyp173</i>	-0.06206
<i>hyp176</i>	0.02331	<i>hyp52</i>	0.004802	<i>hyp47</i>	-0.06245
<i>hyp169</i>	0.02235	<i>hyp127</i>	0.002955	<i>hyp110</i>	-0.0627
<i>hyp186</i>	0.02227	<i>hyp143</i>	0.002377	<i>hyp179</i>	-0.06607
<i>hyp187</i>	0.02227	<i>hyp10</i>	0.001798	<i>hyp155</i>	-0.06645
<i>hyp22</i>	0.02186	<i>hyp75</i>	0.0008522	<i>hyp101</i>	-0.06651
<i>hyp144</i>	0.01963	<i>hyp8</i>	-0.000128	<i>hyp44</i>	-0.06653
<i>hyp76</i>	0.0186	<i>hyp178</i>	-0.000367	<i>hyp125</i>	-0.06716
<i>hyp133</i>	0.01824	<i>hyp160</i>	-0.00102	<i>hyp161</i>	-0.0673
<i>hyp151</i>	0.01715	<i>hyp142</i>	-0.001167	<i>hyp137</i>	-0.06926
<i>hyp163</i>	0.01491	<i>hyp81</i>	-0.001192	<i>hyp26</i>	-0.06974
<i>hyp78</i>	0.01336	<i>hyp59</i>	-0.001271	<i>hyp56</i>	-0.0708
<i>hyp181</i>	0.01323	<i>hyp87</i>	-0.001862	<i>hyp17</i>	-0.0713
<i>hyp82</i>	0.013	<i>hyp185</i>	-0.002707	<i>hyp145</i>	-0.07281
<i>hyp7</i>	0.01214	<i>hyp73</i>	-0.002729	<i>hyp182</i>	-0.07296
<i>hyp88</i>	0.012	<i>hyp135</i>	-0.002964	<i>hyp83</i>	-0.0741
<i>hyp72</i>	0.01047	<i>hyp162</i>	-0.003273	<i>hyp164</i>	-0.07424
<i>hyp183</i>	0.01047	<i>hyp65</i>	-0.003389	<i>hyp128</i>	-0.07806
<i>hyp57</i>	0.01043	<i>hyp41</i>	-0.003499	<i>hyp146</i>	-0.07903
<i>hyp138</i>	0.01039	<i>hyp168</i>	-0.003506	<i>hyp53</i>	-0.07905
<i>hyp139</i>	0.01026	<i>hyp67</i>	-0.003528	<i>hyp77</i>	-0.07906

CHAPTER VII

CONCLUSIONS AND FUTURE PERSPECTIVES

OVERVIEW, MANAGEMENT APPLICATIONS AND CONCLUSIONS

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Overview, management applications and conclusions

7.1. General outlines of the expansion of the Egyptian mongoose in Portugal

In studies focusing the assessment of the distribution of organisms, it is clear that observed changes in a species' range can be directly linked with its response to alterations in the environment where the species resides (Sexton et al., 2009). Prior to this study, a great gap concerning the factors behind the expansion of the Egyptian mongoose in Portugal was notorious. Through statistical modelling of the collected data concerning the distribution of the species in 1980-1990, 1990-2000 and 2000-2010, it was possible to shed light on the main factors affecting the species' range across the three mentioned decades. Overall, the results obtained from this work converge with what is known about the ecology of the Egyptian mongoose. Due to the intensive land-use changes occurred in Portugal, the abandonment of traditional agricultural landscapes promoted the growth of non-cultivated vegetation, leading to higher densities of scrublands. The crescent availability of sheltering and foraging resources for the Egyptian mongoose promoted the establishment of the species in almost the majority of the Portuguese country (Manuscript I). Similarly, recent climatic changes in Portugal also promoted the expansion of the species in Portugal (Manuscript I). The study concluded that the increasing of the mean temperature across central and northern areas in Portugal led to an increased availability of warmer habitats. On the other hand, the results also showed that the expansion of the Egyptian mongoose was negatively influenced by human activities, more specifically urbanized areas. Lastly, the preference of the species for areas with lower altitudes was also patent in the results, as altitude has been suggested to act as a barrier towards the expansion of the species (Borralho et al., 1996).

As it was stated in section 1.1. of the first chapter of this thesis, biotic factors are among the main factors that potentially limit the distribution of certain species. However, the effect of potential predators in the expansion of the Egyptian mongoose was not envisaged in this study. The Egyptian mongoose can be occasionally killed by the Iberian lynx (*Lynx pardinus*) as a result of intraguild predation (Palomares & Delibes, 1998). However, the species was virtually extinct from the Portuguese territory until very recently, when conservation plans for the re-introduction of the species began to be implemented (Serra et al., 2005). Hence, the current density of the Iberian lynx does not

conflict with the presence of the Egyptian mongoose. Besides the Iberian lynx, there are few predators of the Egyptian mongoose in Portugal. The species is an occasional prey for the golden eagle (*Aquila chrysaetos*) and the Bonelli's eagle (*Aquila fasciata*) (Palomares & Delibes, 1998), although both species exist in very low densities (Haller & Sackl, 1997; Real, 2004). Besides predator-prey interaction, interspecific competition was also disregarded in this study. There is no record of interspecific competition between the Egyptian mongoose and other species. In fact, the species easily co-habits with other carnivores in the Iberian Peninsula, e.g., the red fox (*Vulpes vulpes*) and the badger (*Meles meles*) (Fedriani, 1993; Borralho et al., 1996).

For assessing which factors influenced the expansion of the species in each decade, we selected generalized linear models (GLM) as the statistic method. Other statistical methods could be used for answering the proposed question, for instance Spatial Autologistic. Spatial Autologistic is one of the most used methods for modelling species distribution under a spatial and temporal framework. However, autologistic regression models are often associated with an underestimation of the effect of environmental variables and frequently gives biased estimates compared to a non-spatial logistic regression (Dormann, 2007). After a thorough bibliographic search, GLM was selected as the fittest method for the nature of the analysed data. All models were run for each decade (temporal component) and for each new colonized range (spatial component). Thus, each model for each decade not only deals with different values concerning the presence/absence of the species in that specific time and space, but also each model deals with distinct effects of the considered variables in that specific time and space. The distinct contribution of the same set of variables in the studied temporal ranges helped on understanding their influence on the distribution of the species in the three periods. According to the literature, GLM is the most flexible technique of regression models due to its robustness, it is able to deal with response variables that are not normally distributed and whose variance is unaffected by the size of the independent variable. The output of GLM is easily interpreted and there are several statistical procedures to assess the model performance (Guisan & Zimmerman, 2000; Millington et al., 2011). Nevertheless, the use of any statistical model has limitations because they are simplifications of reality, on which they depend on the quality of the data and their own mathematical construction (Torres, 2011). According to Torres (2011), the knowledge of the species' ecology, the selection of the field survey method, the ability of measure ecological variables and the selection of the statistical method is preponderant for the quality of the model output.

Several authors hypothesize that the contemporaneous global changes will gradually continue to affect biodiversity worldwide (Bellard et al., 2012), which I believe they would potentially continue to affect the range of the Egyptian mongoose. Currently, the species is absent in the northwest tip of Portugal, possible due to the current lack of suitable conditions for the species (Barros, 2009). The north-western area of Portugal is characterized by a temperate and humid climate with high levels of precipitation, which is typical of the Atlantic Mid-European Sub Region (Costa et al., 1998). Nevertheless, if the ongoing environmental alterations convert this area into a suitable area for the Egyptian mongoose, there is a great chance of the species to reach those north-western areas of Portugal.

7.2. The role of molecular tools and Landscape Genetics on studying the genetic patterns of the Egyptian mongoose, and their application in wildlife studies

Despite the difficulty of assessing the genetic patterns associated with population expansions, the evolution of genetic tools and techniques provides the access to essential data for understanding the processes behind a range expansion, including 1) assessing the mechanisms of expansion (e.g., direction of dispersal, patterns of dispersal between genders, gene flow and identification of barriers towards expansion) and 2) identifying the consequences of range expansions in the genetic diversity of populations (e.g., distribution of the genetic variation across the expanding range, presence of structure, bottleneck events and fluctuations in the demographic history of populations).

Due to the distinct characteristics of mitochondrial markers and microsatellites, phylogeographical features and historical patterns of populations are commonly traced through mitochondrial DNA, while the codominant nature of microsatellites can unravel more recent genetic patterns of populations as well as their genetic structure. However, complex biogeographic histories can influence the power of genetic markers. For instance, while mitochondrial DNA was found to have weak power addressing the demographic history of species with complex biogeographic histories (e.g. Paulo et al., 2002; Recuero et al., 2007), other studies showed the accuracy of nuclear markers to unscramble the evolutionary history of a species when mitochondrial DNA was not effective (e.g. Graciá et al., 2013). Hence, the combination of different molecular markers helps on disentangle complex biogeographical traits and mutually aid on filling gaps on the life history of a species. In this thesis, recently developed analytical methods for both mtDNA sequence data (see

Manuscript II) and microsatellite DNA (see Manuscript III) proved to be fundamental for assessing the demographic history of the expanding Egyptian mongoose population in the Iberian Peninsula, and provided a broad picture of the genetic patterns of this species. By combining two molecular markers with different modes of inheritance and evolution rates (Sunnucks, 2000), the results generated through this study greatly complemented the assessment of the recorded expansion of the species, proving that describing the genetic patterns and the demographic history of expanding species is essential for providing insights about the processes linked with range dynamics.

The observed genetic diversity of the species fits with the pattern of a historically diversified population in southern Iberia, reflecting the long-term presence of the species in the mentioned area (Manuscript II). A spatial and demographic expansion was evidenced through mitochondrial DNA analyses, though the period in which this scenario occurred remained unclear (Manuscript II). However, microsatellites analyses identified a population expansion after a bottleneck in the recently colonized areas, which can be linked with the recent expansion of the species towards those areas (Manuscript III). Isolation by distance was identified by microsatellites, which might be related with long-range dispersals (Manuscript III), a scenario that was also supported by mitochondrial DNA, together with the hypothesis of multiple source populations (Manuscript II). An interesting outcome of this genetic assessment was the presence of genetic substructure possibly related with the presence of the Tagus River, which might be functioning as a semi-permeable barrier towards gene flow of the species (Manuscript III). As described above, different markers can shed different light on the genetic patterns of a species, and these results underline the importance of using distinct molecular markers in molecular ecology studies.

Furthermore, the field of Landscape Genetics constituted an essential tool for directly assessing the influence of landscape features on the genetic structure of the Egyptian mongoose in Portugal. As individual movements within the mongoose population are not random across landscapes but influenced by several factors (Manuscript I), the combination of principles related to landscape ecology and population genetics of the species aid to understand fine-scale habitat and landscape features influencing the species' distribution across the Portuguese range. The recent land-use changes in Portugal promote the connectivity among the mongoose populations in areas with high shrub cover at middle elevations, where there is lesser anthropogenic disturbance (Manuscript IV). Landscape Genetics uses genetic data (genetic distances) that mainly reflect historical patterns of gene flow (Manel & Holderegger, 2013), hence the observed results would reflect past patterns related with historical landscapes. Notwithstanding, recent studies have shown

that genetic distances can reflect contemporary landscapes better than historical landscapes (e.g., Zellmer & Knowles, 2009; Landguth et al., 2010), hence, we trust that our results do not suffer from time lag and reflect the contemporaneous landscape alterations observed in Portugal.

Overall, genetic assessments are gaining an increasing importance in the study of ecology and management of wildlife species, which is resulting in a crescent indissoluble relation between ecology and evolutionary studies (Hedrick, 2009). The usage of genetic tools in studies of natural populations will only increase as a greater number of comparative genome projects are completed and further technological innovations increase the automation and speed of analyses (DeYoung & Honeycutt, 2005). The integration of genetic methods into wildlife ecology and management studies should become more and more implemented by wildlife professionals. I do believe that the combined fields of Genetics and Ecology greatly improves the knowledge of the species' biology and allows 1) a more comprehensive perspective of wild organisms, their population dynamics and history; 2) the implementation of cohesive and more complete management and conservation guidelines, when needed; and 3) can aid on understanding the effect of contemporaneous alterations in global biodiversity (e.g., climate change). On the other hand and as it has been proven, the coalescence of different fields of Biology can result in the ascendance of new and helpful methods that are approachable to both wildlife ecologists and geneticists (e.g., Landscape Genetics).

The progressive technical and analytical feasibility of genetic analyses in wildlife studies also raises another challenge, which is the acquisition of samples. By recognizing the undeniable value of molecular studies of wild species, wildlife researchers should implement more effort on collecting and archiving samples, with the aim of projecting long-term, comprehensive studies of those species.

7.3. Limitations

Certain constraints were encountered during the development of this study. Similarly to Portugal, the expansion of the Egyptian mongoose in the Spanish territory towards central and northern areas is occurring (Talegón & Parody, 2009; Balmori & Carbonell, 2012). Therefore, it would be crucial to analyse the factors behind the expansion of the species in the whole Iberian Peninsula. However and contrastively with Portugal, the limits of the current distribution of the species in Spain are not completely updated. The lack of consistent presence-absence data that could accurately correspond to the evolution of the distribution of the species in Spain did not allow doing a study comprising both countries.

The sampling method constituted a restraint for a more complete and coherent study of the genetic diversity of the species in the Iberian range. This situation was especially critical for the analyses with microsatellites, as the success of obtaining amplified samples was considerably lower (175 amplified samples) than for mitochondrial DNA (203 amplified samples). When designing a study in Molecular Ecology, one of the most important aspects is to determine the appropriate number of samples for ensuring that the results are shaped by real ecological patterns rather than by stochastic factors. However, several aspects can influence the sampling effort including money, time, field logistics, the actual ecology of the species, as well as marker types and their variability. For intra-species and population-level analyses, two approaches can be executed: 1) if the sampling locations are widely distributed, a sample of 20-50 individuals per location is desirable; or 2) if the study area reflects a broad geographical range, a reduced number of individuals (5-10 per site) can be sampled, but the number of populations must be large (>30) (Freeland et al. 2012). The last approach has the disadvantage of not all alleles or haplotypes are sampled from any particular population (Freeland et al., 2012). Regarding Portugal, the sampling strategy was to cover the entire range of the species with the same effort in each location, which would present a continuous sampling across the species' range. However, the sampling effort was not equal in every location due to the fact that the main source of sampling was from hunting activities. The number of hunted individuals was larger in locations comprising the historical distribution of the Egyptian mongoose (southern locations), where the species is commonly hunted and also where higher densities of the species occur. Hence, several locations where the species is currently present lacked of sampling, which resulted in an unequal number of samples across the species' range. However, before any laboratory procedure and for avoiding a biased interpretation of the results, an attempt of analysing an equal number per location was made. Nevertheless, I believe that an increased number of samples from certain locations – mainly from central and northern locations - would strength the genetic assessment of the Egyptian mongoose and would re-enforce the effect of the recent expansion of the species in its genetic patterns.

Although *a priori* the objective was to solely analyse the molecular ecology of the species in Portugal, I realized it would me more coherent to assess the molecular ecology of the species across its Iberian distribution. During the development of this study, I collected Spanish samples that were kindly shared by biologists and forest managers. However, the number of Spanish samples for mitochondrial DNA was larger than for microsatellites. For mitochondrial DNA, I had access to DNA

sequences previously published by Gaubert et al. (2011). Unfortunately, it was not possible to obtain a sample from the same individuals for microsatellite analyses.

Microsatellite amplification did not generate quality data for the entire analysed samples. The exact cause is unclear. I did not conduct multiplex polymerase chain reaction (PCR) due to several problems during its temperature optimization that consequently led to non-amplified products. Unfortunately, financial constraints did not allow a longer optimization phase for multiplex PCR and I opted for an economic and simpler choice (singleplex PCR). I hypothesized that the low success on obtaining quality data for microsatellites might be related with some unidentified cause during the process of amplifying each microsatellite separately and then gathering all the amplified products in a single tube for fragment length analysis. I believe this should be further explored.

7.4. What is new? – Achievements and management guidelines

The outcomes of this research developed during my PhD are vital for answering several questions regarding the molecular ecology the species, not only in Portugal but comprising the Iberian Peninsula. The pillar of this study was the combination of ecological surveys with genetic assessments. By combining those fields, it was obtained a clear picture of the link between the expansion of the Egyptian mongoose and the observed genetic patterns of the studied populations. This study was the first to exhaustively document the expanding patterns of the species in the studied area. Specifically, this study contributed for:

- 1) Unravelling the evolution of the species' range in the last three decades;
- 2) Specifically identifying the factors that influenced the expansion of the species in each studied decade;
- 3) Contributing with valuable information of the species by linking contemporary global alterations with the range expansion and genetic connectivity of the species;
- 4) Demonstrating the effect of the range expansion of the species in the genetic structure of the studied populations;
- 5) Assessing the genetic variation of the expanding populations which can aid on understanding spatial and temporal patterns of colonisation and dispersal;
- 6) Contributing with essential data for predicting future range expansions of the species by forecasting and managing the studied populations, and analysing management implications

concerning the rapid expansion of the species and its possible consequences in the newly colonized areas;

7) Creating key arguments that can be applicable to wildlife management of other expanding species;

8) Providing outcomes that should be perceived as a model for Mediterranean carnivores, as those species are located in a very specific biome which lately has been greatly influenced by man.

Based on this study and on previous published literature regarding the ecology of the Egyptian mongoose, the following management guidelines and recommendations are presented:

i) *Maintenance of Mediterranean shrub areas*

This thesis stressed the importance of shrub areas not only as a drive for the species' expansion, but also for the maintenance of the genetic connectivity within the mongoose population. The importance of establishing guidelines in forest management plans to preserve the connectivity of populations of medium-sized carnivores was already demonstrated in other studies (e.g., Koen et al., 2012), therefore it is important to consider the preservation of Mediterranean scrublands. I believe that potential management guidelines should include the preservation of Mediterranean shrub areas and the observed contemporaneous alterations of this habitat type, which would benefit other Mediterranean carnivores besides the Egyptian mongoose that are dependent on the Mediterranean scrubland (Mangas et al., 2008).

ii) *Evaluating the impact of the species in new colonized areas*

Although the majority of the studies concerning the impact of range expansions are related with invasive species, assessing spatial and temporal patterns of colonisation and dispersal in a naturally expanding species is essential. Besides the Egyptian mongoose appears to not compete with other carnivores, the spread of predatory species can have particularly deleterious costs on other species in the invaded range, and there is an increasing awareness and necessity for management action to minimise these impacts (e.g., Bryce et al., 2011). Though the Egyptian mongoose is characterized as an opportunistic and generalist predator (Delibes, 1984; Palomares & Delibes, 1991a; Palomares & Delibes, 1991b), the encounter of new preys or even different densities of them can compromise those species. Stomach contents of mongoose individuals were found to

contain species with *Near Threatened* status by the IUCN Red List (2015), including the ocellated lizard (*Timon Lepidus*), the garden dormouse (*Eliomys quercinus*), the European rabbit (*Oryctolagus cuniculus*), and also prey species with *Least Concern* status (IUCN Red List, 2015), but with a decreasing population trend, such as the Western three-toed skink (*Chalcides striatus*), the Southern smooth snake (*Coronella girondica*), the viperine snake (*Natrix maura*), the Iberian painted frog (*Discoglossus galganoi*), the natterjack toad (*Epidalea calamita*) and the European tree frog (*Hyla arborea*) (Bandeira et al., unpublished data; Santos et al., 2015). Additionally, the presence of endemisms in the Portuguese range – Golden-striped salamander (*Chioglossa lusitanica*) – and in the Iberian range – Iberian frog (*Rana iberica*) – with a *Near Threatened* conservation status and with a decreasing population trend (IUCN Red List, 2015) are also species that should gain attention regarding the expansion of the Egyptian mongoose. The potential consequence of the predator behaviour of the Egyptian mongoose should be monitored and the potential consequences in the species described above should be prevented.

iii) Hunting and predator control pressure in recently colonized hunting areas

The Egyptian mongoose is a hunted species within the Portuguese range and it is frequently hunted during ‘predator control’ activities. Predator control is a common practice in hunting areas and has the aim of controlling game species that are injurious to human interests, meaning, species that can affect other game species with economic interest (Reynolds & Tapper, 1996). However, predator control within the mongoose range should be carefully analysed, especially in the newly colonized areas that overlap hunting areas, where the species might exist in reduced population densities. It is known that hunting pressure may trigger responses in game species and change their dynamics (e.g., Gamelon et al., 2011; Massei et al., 2015). Predator control should be implemented after a careful evaluation of the population dynamics of the Egyptian mongoose in those new areas, with the aim of keeping a balance between the species densities and economic purposes of the hunting areas.

iv) Clarifying the status of the species

The status of the Egyptian mongoose in the Iberian range as a historically introduced species should be reviewed. Gaubert et al. (2011) already stressed the presence of the Egyptian mongoose in the Iberian Peninsula considerably earlier than the Muslim occupation, which possibly implies a distinct classification of the species in the Iberian Peninsula. The labelling of a species as

'introduced' should be done consciously, due to the social perception of this concept that can radically define the predisposition for its conservation (Pérez et al., 2011; Graciá et al., 2013). The current status of the species in the Iberian range should be re-considered.

Furthermore, during the development of my research, I realized that the hunting community perceives the Egyptian mongoose as a pest and as an undesired species. This perception is due to the fact that one of the mongoose preys is the European rabbit (*Oryctolagus cuniculus*), which is an extremely valuable game species in Portugal. This perception should be changed through awareness campaigns, with the goal of demystifying the negative idea that hunters have towards mongooses. Those campaigns should focus the important ecological role of the Egyptian mongoose as an integrant piece of the Mediterranean ecosystem, which embraces an unique carnivore biodiversity in the Iberian range.

7.5. Future perspectives

During this study, several questions were raised, not only regarding the questions related with the range expansion of the species, but also the genetic diversity of the mongoose population in the Iberian Peninsula. Here I present the major questions that I believe they should be addressed in a near future.

Analysis of the expansion of the Egyptian mongoose in Spain

As it was described in section 7.3 of this chapter, the Egyptian mongoose also experienced an expansion phenomenon in Spain, although its current limits are poorly known. As the species seems to encompass a continuous range comprising both Portugal and Spain, the assessment of 1) the current distribution of the species in Spain, 2) the evolution of its expansion across the years in those territories and 3) unravelling the factors underlying the range expansion are vital for a wider knowledge concerning the ecology of the species in the Iberian range. I believe that the factors behind the species' expansion in the Spanish territory would resemble the ones encountered in Portugal. The Iberian range is integrated in the Mediterranean region that had suffered profound land-use alterations across the years, which are possibly affecting Mediterranean Spain. Plus, I believe that this assessment would also aid on a possible cross-border plan management for the Egyptian mongoose between both countries.

Going further with the analysis of the genetic patterns of the expanding Egyptian mongoose

One of the major questions in Landscape Genetics is assessing how a species spreads into new ranges. However, the genetic study of a species under a fast range expansion is complex due to several issues regarding the mechanisms behind founder events, the disequilibria process of contagious population spread from the source site, and others. Cushman (2015) developed a new simulation method with the aim of better illustrate the spatial dynamism of the genetic patterns of expanding species. This approach models contagious range expansion from the initial source as a function of landscape resistance. This predicts the frequency of dispersal events as a function of the source population size, location and cumulative cost of movement through the landscape. I believe the implementation of this method would contribute for enlighten the genetic patterns of the species during its range expansion in Portugal, which would top off the genetic assessment of the species.

Origin of the species in the Iberian Peninsula: how many times did the Egyptian mongoose entered the Iberian range?

One of the main issues that I believe it should be explored is the clarification of how the Egyptian mongoose arrived to the Iberian Peninsula. Was it solely during the Late Pleistocene sea-level fluctuations? Or was it introduced during the Late Pleistocene and during the Muslim Invasions? The origin of the Egyptian mongoose in the Iberian range is quite complex, as the debate between its natural dispersal (Gaubert et al., 2011) or human-mediated introduction during the Muslim Invasions (Dobson, 1998; Riquelme-Cantal et al., 2008; Detry et al., 2011) remains open. Nevertheless, these two theories might not be mutually exclusive. Evidences of distinct introductions of a species in the same range through time were already evidenced by other authors (e.g., Calmet et al., 2001; Meixner et al., 2002; Zalewski et al., 2010; Gaudeul et al., 2011), and also comprising the Iberian range (e.g., Cardoso et al., 2013). The task of associating the scenarios of natural dispersal and human-mediated to each expansion event is challenging, and the development of accurate methodology to unravel the expansion processes of a species is crucial. However, through a molecular analysis combining both mitochondrial and nuclear markers and by greatly expanding the sampling area to North Africa, the evaluation of the number of introduction events (natural and human-mediated) of the species in the Iberian range constitutes, in my opinion, one of the most interesting issues regarding the history of this species in the Iberian Peninsula.

Analysis of the effect of historical and future climate change on the genetic variation of the Egyptian mongoose

It is undeniable the effect of future climate change in biodiversity worldwide. However, despite the importance of genetic diversity for species' persistence and adaptive capacity, the genetic effects are frequently disregarded in climate change studies (Pauls et al., 2013). Following Razgour et al. (2013), an integrated approach combining Approximate Bayesian Computation (ABC) and Ecological Niche Models (ENMs) could be applied to the Egyptian mongoose in the Iberian range. As the species 1) is present in the Iberian range since the Late Pleistocene, 2) possibly suffered a pattern of contractions and expansions through the years and 3) its current expansion is related with temperature increasing (Manuscript I), the implementation of this approach would be based on the prediction of the effects of future climate on the genetic diversity across the species' range, as well as on the analysis of how historical processes shaped the present distribution of the species' genetic variation.

7.6. Conclusion

Under the striking increase of the anthropic influence in biodiversity and ecosystems, it is urgent the identification of the factors and the consequences of shifts in species' ranges. Plus, the inclusion of molecular data is crucial for identifying the consequences of range alterations in the genetic diversity of species. Integrated studies using an interdisciplinary approach clearly benefits wildlife research and, in this case, the study of species going through range shifts. This study has shown the clear evidence of human-mediated changes on the range expansion of the Egyptian mongoose in Portugal, a species that was undoubtedly influenced by these contemporaneous global alterations. Its genetic patterns related with the dispersal of the species towards new ranges and the unquestionable link between the genetic differentiation of the species and the landscape alterations occurred in the Portuguese territory re-enforces the consequences of anthropogenic disturbance. The information generated in this study can be used for management strategies from a smaller scale concerning the range expansion of the Egyptian mongoose to a larger scale, where management plans concerning the whole ecosystem affected by the recent land-use changes should be projected. In the future, I believe that the gradual increase of the species deserves a closer look. The consequences of the presence of the species in new ecosystems, its response to the contact with

different ecological dynamics and how those ecosystems will respond to the presence of the Egyptian mongoose are issues that should gain attention.

The consequences of man on wildlife is well-patent in our times. It is therefore our responsibility, as wildlife researchers, to develop and advance knowledge on the consequences of global alterations for management, conservation and, most important, for prevention purposes.

7.7. References

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