



Mediterranean scrubland and elevation drive gene flow of a Mediterranean carnivore, the Egyptian mongoose *Herpestes ichneumon* (Herpestidae)

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Identifying the environmental features affecting gene flow across a species range is of extreme importance for conservation planning. We investigated the genetic structure of the Egyptian mongoose (*Herpestes ichneumon*) in Western Iberian Peninsula by analyzing the correlations between genetic distances and landscape resistance models. We evaluated several functional relationships between elevation, vegetation cover, temperature, and genetic differentiation under the original and reciprocal causal modelling approaches. Additionally, we assessed evidence of isolation-by-distance (IBD) in the mongoose population. Original causal modelling identified IBD as the best model explaining genetic patterns in the mongoose population. By contrast, reciprocal causal modelling supported high shrub cover at middle elevations as the best model explaining species gene flow. The results from reciprocal causal modelling demonstrate that the Egyptian mongoose is dependent of ecosystems dominated by Mediterranean shrub cover. Recent land-use changes related to rural abandonment promoted the growth of shrub areas, especially at middle elevations, facilitating genetic connectivity in the mongoose population in those areas, where anthropogenic activities are less intense. The present study should be considered as a model for landscape genetics studies of Mediterranean carnivores in the Iberian range with the aim of better understanding how recent land-use changes affect a broad guild of species. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 00, 000–000.

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INTRODUCTION

A landscape is an intricate and dynamic combination of distinct habitats, each one having possible impacts on the distribution of species (Turner, 1989; Taylor *et al.*, 1993; Stevenson-Holt *et al.*, 2014). Understanding those pattern–process relationships is of extreme importance for unravelling the ecological characteristics of a population, as well as for assessing 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 (Cushman, 2015) or as a

consequence of more or less deterministic mechanisms, such as those resulting from a response towards a changing environment (Colwell & Rangel, 2010). Land-use alterations have had large impacts on ecological processes across the globe (Vitousek *et al.*, 1997; Myers & Knoll, 2001; Balmford, Green & Jenkins, 2003). By transforming native ecosystems into altered landscapes, human-driven land-use practices dramatically affect biodiversity (Saunders, Hobbs & Margules, 1991; McGarigal & Cushman, 2002; Cushman & McGarigal, 2003; Cushman, 2006). In recent years, several studies have highlighted the effect of anthropogenic land-use changes on species distributions in Mediterranean Europe (Falcucci, Maiorano & Boitani, 2007; Moreira & Russo, 2007;

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Ruiz-González *et al.*, 2014; Mateo-Sánchez *et al.*, 2015). Some studies not only reported effects of past landscape change in Mediterranean countries, but also projected severe consequences of potential future land-use changes (Acevedo *et al.*, 2011).

Continued dispersal of individuals under a changing environment is critical to maintain genetic diversity within their population because dispersal constitutes one of the cornerstones for maintaining population viability (Frankham, Ballou & Briscoe, 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 the support for a large number of alternative hypotheses within a rigorous analytical framework (Cushman *et al.*, 2006, 2013a; Shirk *et al.*, 2010; Castillo *et al.*, 2014). The isolation-by-distance (IBD) method ignores the complexity of landscapes, which potentially plays a significant role in dispersal patterns and genetic structure on natural populations (Michels *et al.*, 2001; Spear *et al.*, 2005; Giordano, Ridenhour & Storfer, 2007; Wang, Savage & Bradley, 2009). Landscape genetics focuses on the effect of landscapes in the genetic structure of organisms by using spatial analysis with Geographic Information Systems. This method consists on the creation of landscape resistance hypotheses, which are then compared with genetic distances among individual organisms or populations, dramatically improving knowledge on the functional connectivity across a landscape matrix (Coulon *et al.*, 2006; Cushman *et al.*, 2006; Wasserman *et al.*, 2010; Watts *et al.*, 2010; Stevenson *et al.*, 2013) and contributing to the development of effective management and conservation strategies (Balkenhol *et al.*, 2009; Segelbacher *et al.*, 2010; Wasserman, Cushman & Wallin, 2012; Mateo-Sánchez *et al.*, 2015).

The Iberian Peninsula is one of the European regions where the Mediterranean maquis is commonly found. Broadly, the Mediterranean maquis is composed of deciduous broad-leaved trees or stiff-leaved evergreen shrubs, which recombine the Mediterranean forest, and it is mainly characterized by evergreens such as holm oaks and cork oaks (Minelli, 2003). In the Iberian Peninsula, the Mediterranean maquis supports a 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.

One species that is intrinsically linked with Mediterranean maquis is the Egyptian mongoose (*Herpestes ichneumon*) (Palomares & Delibes, 1991). The Egyptian mongoose is a carnivore from the Herpestidae family present in the African continent, Middle East, and in the Iberian Peninsula (Cavallini & Palomares, 2008). In its Iberian distribution, the species has predominantly diurnal habits and is associated with Mediterranean habitats (Palomares & Delibes, 1993a, b), where the species' activities are frequently displayed in those areas (Palomares & Delibes, 1993a, b). Until the 1980s, the species was restricted to the southern territories of the Iberian Peninsula (Borrallho *et al.*, 1996). However, recent studies showed a rapid north-west expansion of the species (Barros, 2009; Talegón & Parody, 2009; Barros & Fonseca, 2011), greatly extending the limits of its traditionally known range in the Iberian Peninsula (Delibes, 1982). In Portugal, located in western Iberian Peninsula, the expansion was mainly driven by land-use change in shrub dominated ecosystems, forest clearing, and agricultural practices (Barros, 2009; Barros *et al.*, 2015). In addition, increasing temperatures recorded in this area have expanded the extent of potentially suitable range for the species in the Iberian Peninsula (Barros *et al.*, 2015).

The recent expansion of the Egyptian mongoose population in the Iberian range led to changes in its genetic patterns and demographic signatures (Barros *et al.*, 2016a, b). However, the influence of the current landscape on the genetic structure of the species has not yet been described. By adopting a landscape genetics framework, the primary goal of the present study was to investigate the effects of landscape connectivity on the genetic patterns of the Egyptian mongoose in Portugal; more specifically, in the western Iberian range of the species. Because the species is associated with Mediterranean habitats (Palomares & Delibes, 1993a), we hypothesize that the extent of shrub-dominated landscapes and forest cover would affect the gene flow of the species. Moreover, we expect that the recent land-use changes occurring in western Iberian Peninsula will influence the genetic connectivity of the species because it was previously reported that the expansion of the species is mainly related to land-use alterations related to an increase of shrub areas (Barros *et al.*, 2015). Additionally, physical barriers, elevation, and temperature may represent important environmental constraints for the species movement, hence impacting on gene flow in the mongoose population. We investigated the genetic structure of the mongoose population at an individual-level by analyzing the correlations between genetic distances based on microsatellite loci and cost distances via a number of landscape resistance hypotheses within a causal modelling framework

(Cushman *et al.*, 2006; Cushman & Landguth, 2010). To identify the most likely drivers of the observed patterns of genetic differentiation of the Egyptian mongoose in the study area, we assessed a number of alternative hypotheses, including IBD (Wright, 1943) and 191 landscape resistance hypotheses, comprising a combination of land cover, elevation, and temperature. We then compared all alternative hypotheses by applying two widely used analytical frameworks for landscape genetic analysis: the original causal modelling method (Cushman *et al.*, 2006) and the reciprocal causal modelling method (Cushman *et al.*, 2013a), with the aim of determining the best correlation between genetic distance and a given landscape resistance.

MATERIAL AND METHODS

STUDY AREA

The study area comprised the entire Portuguese continental territory (Fig. 1). Portugal has an area of

92 270 km² and is 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). The southern areas are mainly characterized by flatlands, although the Serra S. Mamede and Monchique mountain ranges constitute the two main exceptions. The north-western 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 approximately 1060–3094 mm year⁻¹; 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 Mediterranean sub-region, where the Egyptian mongoose is currently present (Barros *et al.*, 2015) and where the present study was carried out. This region is characterized by dry summers and wet winters (mean

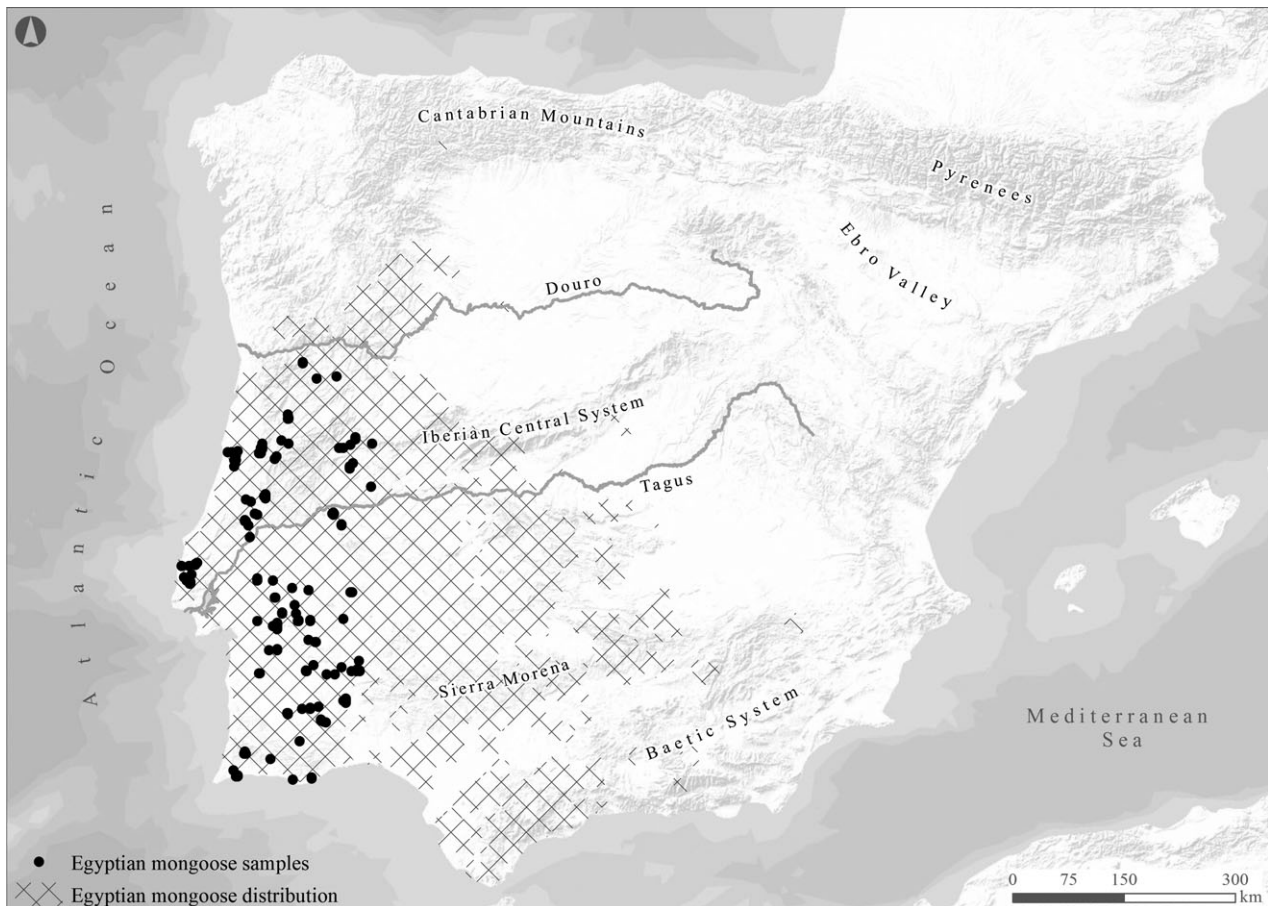


Fig. 1. Map representing the Iberian distribution of the Egyptian mongoose (*sensu* Talegón & Parody, 2009; Balmori & Carbonell, 2012; Barros *et al.*, 2015). The locations of the Egyptian mongoose samples are also indicated.

annual precipitation approximately 246–717 mm year⁻¹; Panagos *et al.*, 2015). The main flora in this sub-region includes species commonly found in Mediterranean regions, including 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

Sampling collection and genetic analyses were conducted prior to the present study and are specifically described in Barros *et al.* (2016b). A total of 167 samples of Egyptian mongoose from individuals captured during hunting activities, or as roadkills or dead from other causes, were collected across the species' distributional range (Fig. 1). Although passive capture methods may influence model outcomes, the selection of individuals to be analyzed by Barros *et al.* (2016b) was performed to reduce potential errors of sampling bias because the number of individuals captured in southern locations (historical distribution of the species) is higher than the number of individuals captured at central and northern regions (recently colonized areas). The location and details of the samples are presented in the Supporting Information, Appendix S1. DNA extraction from muscle samples was carried out using the salt-extraction method (Bruford *et al.*, 1992). DNA from blood and hair samples was isolated under a modified phenol–chloroform protocol (Sambrook, Fritsch & Maniatis, 1989) and under an adapted protocol of the CTAB method described by Rogers & Bendich (1988), respectively. Extracted DNA from each individual was quantified using a spectrophotometry method with a NanoDrop CD-1000 spectrophotometer.

A set of ten microsatellites developed for the Egyptian mongoose (Rodrigues *et al.*, 2009) was used for DNA amplification. Amplification was conducted using the Taq PCR Core Kit (Qiagen) in accordance with the manufacturer's conditions. Visualization of the polymerase chain reaction (PCR) products was conducted in 2% agarose gel and fragment analysis was performed using an ABI Standard Dye-Set DS-33. Allele calling was performed manually using GENEMAPPER, version 3.7 (Applied Biosystems).

We tested for stuttering and allele dropout in MICRO-CHECKER, version 2.2.3 (Van Oosterhout *et al.*, 2004). Stuttering is related to the presence of additional peaks in a profile as a result of stochastic effects of the PCR process. On the other hand, allele drop-out occurs when one or more alleles are not present in an analyzed sample, which may lead to scoring errors and incorrect profiles. Deviations from Hardy–Weinberg equilibrium (HWE) were tested

using the exact test in GENEPOP, version 4.2.1 (Rousset, 2008), with 100 batches and 1000 randomizations. We used ARLEQUIN, version 3.5.1.2 (Excoffier & Lischer, 2010) for estimating pairwise linkage disequilibrium for each pair of loci, with 10 000 permutations. Number of alleles (N_A), as well as observed (H_O) and expected (H_E) heterozygosity, were calculated using 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.* (2016b), the mongoose population across south-western Iberian Peninsula is sub-structured in two clusters. However, both clusters showed high admixture between them and are distributed across a broad geographical area (Barros *et al.*, 2016b), which may indicate a spurious result related to the use of clustering methods on a clinal population (Schwartz & McKelvey, 2009; Blair *et al.*, 2012). Thus, in the present study, we disregard the population-based clustering approach and, instead, use an individual-based gradient approach (Cushman *et al.*, 2006), which can robustly evaluate a wide range of alternative models, including IBD, isolation-by-barriers and isolation-by-resistance (Cushman & Landguth, 2010). Individual-based analyses in which individual genetic differences are associated with cost distances across alternative resistance hypotheses were shown to be a useful approach for understanding population connectivity (Coulon *et al.*, 2004; Cushman *et al.*, 2006, 2013a). Genetic distance among all individuals was calculated using GENALEX, using the Smouse and 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 a total of 191 resistance surfaces, comprising the factorial combination of four landscape features: temperature, altitude, shrub areas, and forest areas (Table 1; Supporting Information, Appendix S2). 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.*, 2015). Altitude is reported to greatly affect the distribution of organisms (Lomolino, 2001; Li, Song & Zeng, 2003; Wasserman *et al.*, 2010) because of its influence on temperature and habitat conditions (Whittaker, 1967; Barry, 1992; Körner, 2007; Evans & Cushman, 2009). The Egyptian mongoose avoids high altitudes (Borralho *et al.*, 1996) and the distribution of the species is linked with the

Table 1. Resistance values for each land cover class of forest and shrub areas used in this study

Cover classes	Resistance levels for LSF	Resistance levels for HSF	Resistance levels for LS	Resistance levels for MS	Resistance levels for HS
Forest classes (description)					
Agro-forestry areas (annual crops or grazing land under the wooded cover of forestry species)	2	7			
Broad-leaved forest (vegetation mainly composed of trees, including shrub and bush understoreys, where broad-leaved species predominate)	6	1			
Coniferous forest (vegetation mainly composed of trees, including shrub and bush understoreys, where coniferous species predominate)	5	2			
Mixed forest (vegetation mainly composed of trees, including shrub and bush understoreys, where neither broad-leaved nor coniferous species predominate)	6	2			
Shrub classes					
Land principally occupied by agriculture, with significant areas of natural vegetation (areas principally occupied by agriculture, interspersed with significant natural areas)			3	3	5
Natural grasslands (low productivity grassland; often situated in areas of rough, uneven ground. Frequently includes rocky areas, briars and heatland)			2	3	4
Moors and heathland (vegetation with low and closed cover, dominated by bushes, shrubs and herbaceous plants; e.g. heather, briars, broom, gorse, laburnum, etc.)			8	6	2
Sclerophyllous vegetation (bushy sclerophyllous vegetation, including maquis and garrigue)			7	5	1
Transitional woodland-shrub (bushy or herbaceous vegetation with scattered trees; can represent either woodland degradation or forest regeneration/recolonization)			7	6	4

A description of each cover class is given in brackets (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).

extent of Mediterranean sub-region in the Iberian Peninsula (Palomares & Delibes, 1998).

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

projection in ARCMAP, version 10.1 (Environmental Systems Resource Institute).

We modelled the resistance of each variable to gene flow for each unit across three levels for altitude, temperature, and shrub areas, and two levels of forest areas. Landscape resistance as a result of elevation and temperature was modelled as an inverted Gaussian function (Cushman *et al.*, 2006), assuming a minimum of 1 and a maximum approaching an asymptote of 10, with an SD of the Gaussian function of 5°. We modelled elevation in low elevation (LE), medium elevation (ME), and high elevation (HE). For this purpose, we considered a minimum resistance of 1 at 100, 1000, and 2000 m

for LE, ME, and HE, respectively. Low temperature (LT), medium temperature (MT), and high temperature (HT) were modelled with a minimum resistance of 1 at 15, 20, and 25 °C, 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. We assigned resistance values to different vegetation types, including five categories for shrub cover and four categories for forest cover (Table 1). To assess the combination of factors that was most strongly related to the observed patterns of genetic differentiation, we factorially combined all variables used for the creation of resistance surfaces into all possible combinations (Cushman *et al.*, 2006). All variables were allowed to speak with equal weight using the fuzzy overlay tool in ARCMAP. In addition to the resistance surfaces, we tested for IBD by calculating the Euclidean distance using UTM coordinates between all individuals. For the isolation-by-landscape resistance premise, we created cost matrices reflecting the least-cost distance (m) between each mongoose location across each one of the 191 resistance surfaces, and, additionally, a matrix representing the Euclidean distance between all individuals. All matrices were built on R, version 3.1.2 (R Core Team, 2013) using the sGD package (Shirk & Cushman, 2011).

RELATIONSHIP BETWEEN GENETIC AND EUCLIDEAN DISTANCE: IBD

To determine whether genetic differentiation patterns followed a pattern of IBD, we calculated Mantel correlations between genetic distance and Euclidean distances. The correlation between both distance matrixes 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.*, 2013a), aiming to infer which landscape features best explain the genetic structure of the mongoose population in the Portuguese territory. We generated three explanatory models of causality containing 191 hypotheses and we tested each model against the IBD null model: isolation-by-resistance (Model 1), isolation-by-resistance by partialling out the effects of geographical distance (Model 2), and IBD by

partialling out the effects of each one of the resistance surfaces (see Supporting Information, Appendix S3). To measure the support of the three models, we calculated simple Mantel tests for Model 1 between the genetic distance and each one of the landscape resistance surfaces, and Models 2 and 3 were generated by means of partial Mantel tests (Smouse, Long & Sokal, 1986) between genetic distance and Euclidean distance, and between each landscape resistance surface. In original causal modelling, each organizational model has a diagnostic set of statistical tests to evaluate its support through significant Mantel tests. To infer the scenario of landscape resistance on dispersal, we followed the original causal modelling from Cushman *et al.* (2006) by selecting the landscape resistance hypotheses in which, in Model 1 and 2, the Mantel tests should be statistically significant and, in Model 3, the mantel tests should be negative or presenting a nonsignificant correlation. All tests were conducted using the Vegan package for R by calculating the Pearson correlation with 10 000 permutations for a 95% confidence interval. All models (both significant and nonsignificant) were ranked according to their support based on the magnitude of the Mantel *r* correlation coefficient as in Cushman *et al.* (2006).

For inferring the effect of landscape structure on the genetic patterns and on the genetic distance between the individuals, we implemented partial Mantel tests under the reciprocal causal modelling approach. Because of the increased risk of spurious correlations using simple Mantel tests (Cushman & Landguth, 2010), Cushman *et al.* (2013a) proposed the reciprocal causal modelling method because it 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 via 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.*, 2013a). The supported hypotheses 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.

RESULTS

GENETIC DIVERSITY

There was no evidence of significant allele dropout or stuttering. The overall mongoose population showed

a significant deficit of heterozygotes compared to HWE expectations. Six pairs of loci out of 45 pairs showed linkage disequilibrium after Bonferroni correction. All 10 loci showed polymorphism for the overall studied population. Hich5 was found to be the most polymorphic locus ($N_A = 9$) and Hich7 was the least polymorphic locus ($N_A = 4$). The mean number of alleles across all loci was 6. Results related to the genetic diversity of the studied population are provided in the Supporting Information, Appendix S4.

ISOLATION-BY-DISTANCE AND ISOLATION-BY-RESISTANCE THROUGH MANTEL CORRELATIONS

A pattern of IBD between the studied individuals was indicated by a significant and positive correlation between the genetic distances and Euclidean distances between all the individuals (see Supporting Information, Appendix S5). Original causal modelling showed a significant correlation between genetic distance and resistance landscape (Model 1) for all of the 191 simple Mantel tests (see Supporting Information, Appendix S5). Partial Mantel analyses for Model 2 showed 23 significant landscape resistance hypotheses after partialling out the effects of Euclidean distance (see Supporting Information, Appendix S5). The correlation between genetic distance and Euclidean distance was significant when 137 resistance landscapes were partialled out (Model 3; see Supporting Information, Appendix S5).

Hypothesis ranking according to r values showed that IBD (*EucDist*) was the hypothesis with the strongest relationship with genetic differentiation, followed by the 23 significant hypotheses with Euclidean distance partialled out (Model 2) (see Supporting Information, Appendix S6). The landscape resistance hypothesis with the highest r value was *hyp16*, which exclusively includes middle elevations (ME). The hypothesis with the lowest r value is *hyp77*, which combines high selectivity for both dense shrubs cover (HS) and forest cover (HSF) at low elevations (LE) (see Supporting Information, Appendix S6), indicating very weak support for any relationship between this combination of landscape features and gene flow.

ORIGINAL CAUSAL MODELLING AND RECIPROCAL CAUSAL MODELLING

The results from the original causal modelling showed 23 landscape resistance hypotheses supported under this framework (see Supporting Information, Appendix S5). These hypotheses included all levels of elevation (LE, ME, and HE) and shrub areas (LS, MS, and HS), low temperatures (LT), and

low selectivity for dense forest cover (LSF) (see codes in Supporting Information, Appendix S2). On the other hand, the new method of reciprocal causal modelling showed *hyp16*, *hyp43*, *hyp46*, *hyp109*, *hyp136*, and *hyp172* as the supported landscape resistance hypotheses out of 36 964 combinations of the 192 candidate models, including IBD (Fig. 2). All six models supported by reciprocal causal modelling included middle elevations (ME), low temperature (LT), low selectivity for forest cover (LSF), and dense shrub cover (MS, HS), indicating that gene flow is maximum, and hence facilitated, by a combination of two or more of these resistance categories (see codes in Supporting Information, Appendix S2). IBD was not supported under the reciprocal causal modelling framework. To address the best supported model, the relative support of each one 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) (Cushman *et al.*, 2013a, b; Cushman *et al.*, 2014; Castillo *et al.*, 2014). The results showed that the best supported model is *hyp43*, with the highest value of relative support (Table 2). *Hyp43* includes high selectivity for dense shrub cover (HS) and middle elevations (ME) and suggests that mongoose gene flow is highest in middle elevation shrub cover and declines at lower and higher elevations and in non-shrub cover types.

DISCUSSION

GENETIC DIVERSITY

The main result of the present study suggests that gene flow in the Egyptian mongoose population is determined by a combination of elevation and land cover effects. Despite the good dispersal ability of the species (Palomares & Delibes, 1998), the identified landscape features may lead to a Wahlund effect by creating limited neighbourhoods for dispersal and mating. The recent expansion of the species (Barros *et al.*, 2015) might also contribute to the observed disequilibrium in Hardy–Weinberg proportions (Waits *et al.*, 2000; Cushman, 2015).

Landguth *et al.* (2010) showed that individual-based analyses in landscape genetics are much more powerful than population-based analyses with respect to detecting the effects of landscape features on gene flow and suffer much less from time lags before such effects become important, which constituted the primary motivation for using individual-based analysis rather than a population-based

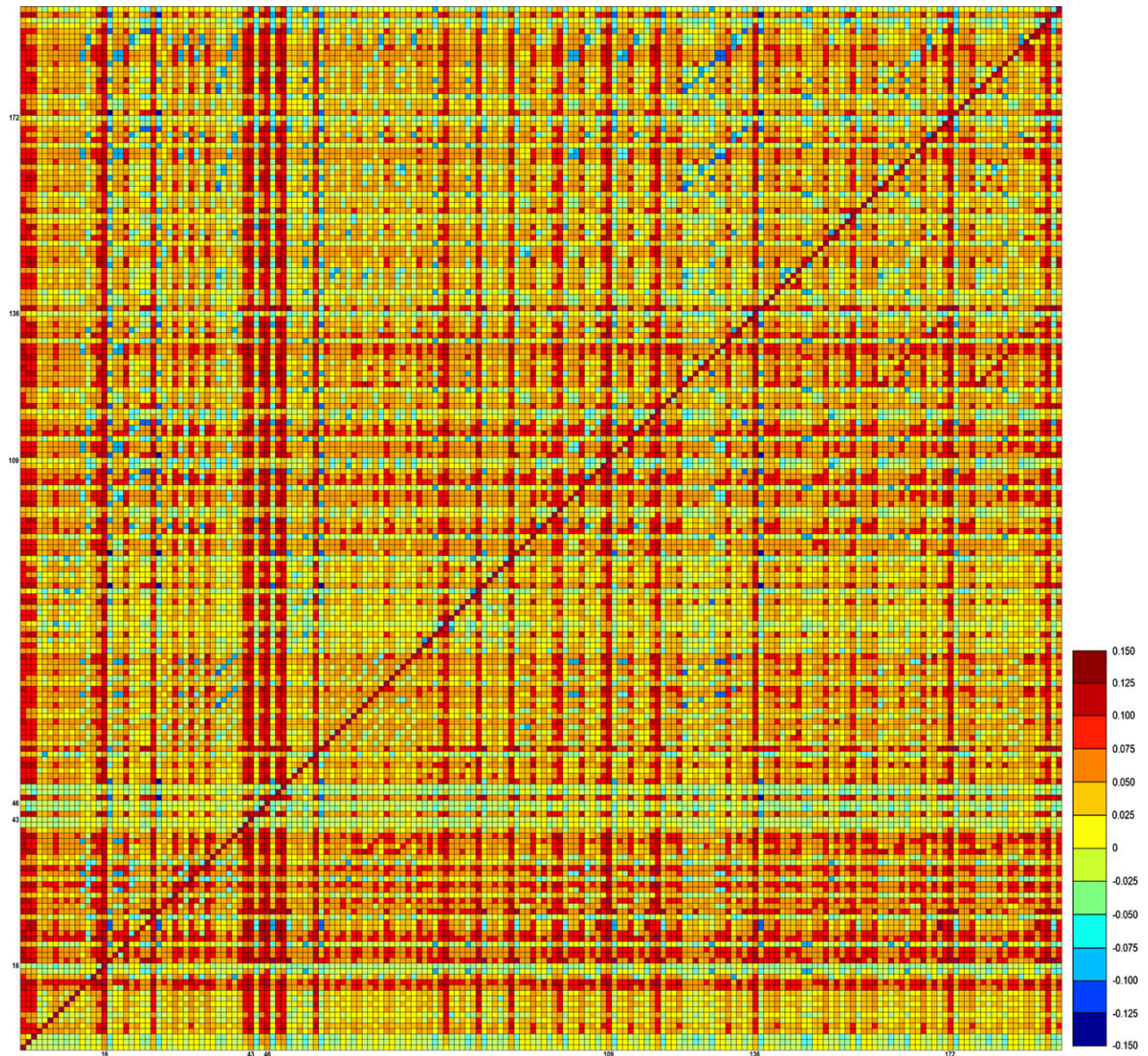


Fig. 2. Mantel correlogram with reciprocal causal modelling results. All models are in numerical order, with the last being isolation-by-distance. Hypotheses Hyp16, hyp43, hyp46, hyp109, hyp136 and hyp172 are the best supported models. Columns indicate focal models, and rows indicate alternative models. The colour gradient indicates support for the focal model independent of the alternative model. Model codes are associated with the codes in the Supporting Information, Appendix S2.

approach in the present study. Indeed, population-based inferences about genetic structure are highly vulnerable to inferential error when there are clinal based patterns of gene flow related with IBD or isolation-by-resistance (Schwartz & McKelvey, 2009; Blair *et al.*, 2012). Recent studies have suggested that individual-based landscape genetics analyses using partial Mantel tests in an optimization framework accurately identify the landscape features acting as drivers for gene flow (Shirk, Cushman & Landguth, 2012; Cushman *et al.*, 2013a; Castillo

et al., 2014). An individual-based approach enables a direct comparison of landscape resistance, barrier, and IBD hypotheses, which greatly improves clarity of inferences (Cushman & Landguth, 2010; Wasserman *et al.*, 2010).

ORIGINAL CAUSAL MODELLING VERSUS RECIPROCAL CAUSAL MODELLING

Our results re-enforced the suggestion that the reciprocal causal modelling has a higher power than the

Table 2. Results for the relative support of the supported hypotheses in the reciprocal causal modelling

Hypotheses	Mean <i>r</i> for the column	Mean <i>r</i> for the row	Relative support
hyp16 (ME)	0.100101	−0.018947	0.081154
hyp43 (HS + ME)	0.093502	−0.005886	0.087616
hyp46 (MS + ME)	0.096795	−0.016392	0.080403
hyp109 (MS + LT + ME)	0.093461	−0.009327	0.084134
hyp136 (LSF + LT + ME)	0.090053	−0.018630	0.071423
hyp173 (MS + LSF + LT + LE)	0.090486	−0.020840	0.069646

Model codes are associated with the codes provided in the Supporting Information, Appendix S2. (HS, high selectivity for dense shrub cover; MS, medium selectivity for dense shrub cover; LSF, low selectivity for dense forest cover; LT, low temperature; ME, medium elevation; LE, low elevation).

original causal modelling approach for discriminating which landscape resistance hypotheses better explain the observed genetic patterns. 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 (Cushman *et al.*, 2013a, b; Castillo *et al.*, 2014). Although original causal modelling supported 23 landscape resistance models (see Supporting Information, Appendix S5), reciprocal causal modelling identified six landscape resistance models, thus reducing Type I errors by 75%, and posterior ranking of these six models according to relative support identified one hypothesis as the most highly supported (*hyp43*) (Fig. 2, Table 2). Although recent studies question the use of Mantel tests and causal modelling in landscape genetics (Graves, Beier & Royle, 2013), reciprocal causal modelling is the most recent and most thoroughly evaluated approach for inferring landscape resistance to gene flow in wild populations (Cushman *et al.*, 2013a) and our results confirm that it provides greater clarity than previous methods for discriminating between a large number of correlated alternative hypotheses.

Perhaps as a result of the great dispersal ability of the species (Palomares & Delibes, 1998), simple Mantel tests identified the IBD hypothesis as the model with the highest correlation with the genetic patterns of the Egyptian mongoose. Similar results were also obtained in a previous study, where a pattern of IBD in the mongoose population distributed in the western Iberian Peninsula was recorded

(Barros *et al.*, 2016b). 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 shown that landscape resistance models outperform IBD when these hypotheses are rigorously competed, such as in a causal modelling framework (Cushman *et al.*, 2006; Wasserman *et al.*, 2010; Shirk *et al.*, 2010; Castillo *et al.*, 2014; Ruiz-González *et al.*, 2014). Indeed, the best model from reciprocal causal modelling indicates that the 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 obtained from reciprocal causal modelling advance knowledge on population connectivity of the Egyptian mongoose in the Iberian Peninsula. The Egyptian mongoose is a species highly dependent on the 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, b; Blanco, 1998). Because of its diurnal habits in the Iberian range, the Egyptian mongoose avoids open areas that can expose the species to potential threats (Palomares & Delibes, 1992, 1993a, 1998). Studies have confirmed that foraging, hunting, and resting are activities displayed by the species in Mediterranean vegetation types (Palomares & Delibes, 1992, 1993a, b, 1998). The known ecological habits of the species converge with the results from the present study indicating that the genetic patterns of the species are highly influenced by high shrub cover; gene flow in the Egyptian mongoose population is maximized in areas with high shrub cover and is lower in areas with low shrub cover and a 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 because 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 m (Palomares, 1993). Indeed, mountainous landscapes were shown to exert a barrier effect towards the mongoose expansion occurring in the western Iberian range in the last three decades

(Barros *et al.*, 2015). However, the observation that species gene flow is promoted by high shrub cover at middle elevations is potentially linked with the decrease of human population density at higher altitudes (Cunha, 2007), which favours the Egyptian mongoose as a result of its avoidance of anthropogenic activity (Palomares & Delibes, 1993b; Borralho *et al.*, 1996), a behaviour that is commonly observed in carnivores (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 resulting in higher densities of scrublands at middle elevations (ICNF, 2013; Barros *et al.*, 2015). Thus, the increase of the availability of sheltering and foraging resources for the Egyptian mongoose possibly influences the species gene flow in middle elevation landscapes. On the other hand, in the last two decades, Mediterranean Europe has experienced profound land-use changes (Ales *et al.*, 1992; Debussche, Lepart & Dervieux, 1999; Falcucci *et al.*, 2007). There has been a large decline in farming and traditional agricultural activities, which had led to the increased abandonment of farmland (Meeus, 1993). In relation to the Iberian Peninsula, this trend started to become more evident in the 1980s when the European Union implemented the Common Agricultural Policy, which profoundly transformed traditional agricultural practices (European Commission 2014; http://ec.europa.eu/legislation/index_en.htm). As a result, traditional agriculture was transformed to intensive agriculture, where broader areas at lower altitudes are used for plantations and for permanent pasture areas (Recenseamento Agrícola 2009 – Instituto Nacional de Estatística), which are environments that the Egyptian mongoose tends to avoid (Palomares & Delibes, 1993b). Hence, we consider that the joint effects of rural depopulation at higher altitudes and the implementation of intensive agriculture at lower altitudes influence the genetic patterns of the species because it may prefer shrub areas at middle elevations, where anthropogenic disturbance is lower and shrub cover is higher.

Besides the recorded changes in agricultural practices and rural depopulation, the overall Western Mediterranean scrubland in the Iberian Peninsula is experiencing extensive alterations. For example, one reason for this in Portugal is the gradual replacement of Mediterranean maquis by monocultures of *Eucalyptus* spp. and *Pinus* spp. (ICNF – Instituto de Conservação da Natureza e das Florestas, 2013; Águas *et al.*, 2014). Furthermore, other practices were implemented to eliminate Mediterranean shrub areas as a result of their high probability of fire (Mangas *et al.*, 2008) and the exclusion of scrubland

as productive agricultural land (Delibes, Rodríguez & Ferreras, 1999). The removal of shrub areas is commonly implemented over large areas and is implemented without regarding the role of those areas in biodiversity conservation (Terradas, 1996; Camprodon, 2001). The nonselective elimination of shrub areas can compromise many animal species dependent on the Mediterranean scrubland, which supports a diverse carnivore community, including the Egyptian mongoose. Because of the reduced level of human interference, as well as higher food availability compared to areas with low vegetation cover, medium-size carnivores (e.g. the badger *Meles meles*: Revilla, Palomares & Fernández, 2001; the genet *Genetta genetta*: Virgós & Casanovas, 1997; and the polecat *Mustela putorius*: Mestre, Ferreira & Mira, 2007) are linked to the Iberian Mediterranean scrubland. Indeed, 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 occurring in the Iberian range. However, Mediterranean scrubland is widely considered to be of less importance for conservation than other types of vegetation in official programmes 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 important for the maintenance of gene flow and population connectivity, which rely on the dispersal of individuals. Despite the fact that the species receives a 'Least Concern' conservation status (Cavallini & Palomares, 2008), the results of the present study highlight the relevance of scrublands for the Egyptian 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. The current Mediterranean landscape structure can exert a great influence in other medium-sized carnivores and enable or hinder dispersal and gene flow. Thus, understanding the interactions between the Mediterranean landscape and animal movement can be pivotal for the conservation of their populations. Hence, the study should be regarded as a baseline for the preservation of Mediterranean habitats in the Iberian territory, as well as a starting point for future studies investigating the landscape

genetics of other medium-sized carnivores dwelling in the Mediterranean Iberian Peninsula.

CONCLUSIONS

The contribution of landscape genetics to a broader knowledge about how landscape features influence gene flow is now well established. The statistical performance of microsatellite markers is dependent on the number of loci, the level of polymorphism of each locus, and the sample size. The low number of polymorphic loci used in the present study may constitute a limitation. However, microsatellites are the standard molecular markers used in recent landscape genetic analyses and only eleven pairs of microsatellite markers are currently described for the Egyptian mongoose. The present study demonstrates the utility of applying rigorous multimodel approaches in landscape genetic analysis of the Egyptian mongoose gene flow. By combining genetic data with ecological variables, we have demonstrated that the genetic patterns of the species are not explained simply by the geographical distance between those populations. A combination of vegetation cover and elevation can also drive genetic differentiation across its Western Iberian range. Preserving landscape connectivity is crucial for biodiversity and the Egyptian mongoose is highly dependent of the connectivity of shrubland areas. Additionally, the present study will serve as a baseline for exploring more complex issues regarding simulations of population dynamics occurred during range expansion of the Egyptian mongoose in the Iberian Peninsula, as has been explored recently for other species (Cushman, 2015). The present study is one of the first to evaluate the landscape genetics of Mediterranean carnivores in the Iberian territory, 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

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Supplementary data.

Table S1. Number and location of the analyzed samples from Barros *et al.*, 2016b.

Table S2. Geographic coordinates of the analyzed individuals.

Appendix S2. 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.

Appendix S3. 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 surfaces.

Appendix S4. Deviations from Hardy–Weinberg conditions, linkage disequilibrium (LD), loci range, and number of alleles for each locus for the overall population (Barros *et al.*, 2016b). Bold numbers indicate loci significantly departing from Hardy–Weinberg equilibrium after Bonferroni correction. H_O , observed heterozygosity; H_E , expected heterozygosity; N_A , number of alleles.

Appendix S5. Results from the original causal modelling. Bold hypotheses indicate the hypotheses that are supported by the causal modelling approach. ‘*Eucdist*’ refers to the isolation-by-distance hypothesis. 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.

Appendix S6. 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, Appendix S2. ‘*Eucdist*’ refers to the Isolation-by-Distance hypothesis.