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Combining movement and genetic data to assess a forest carnivore's response to forest fragmentation

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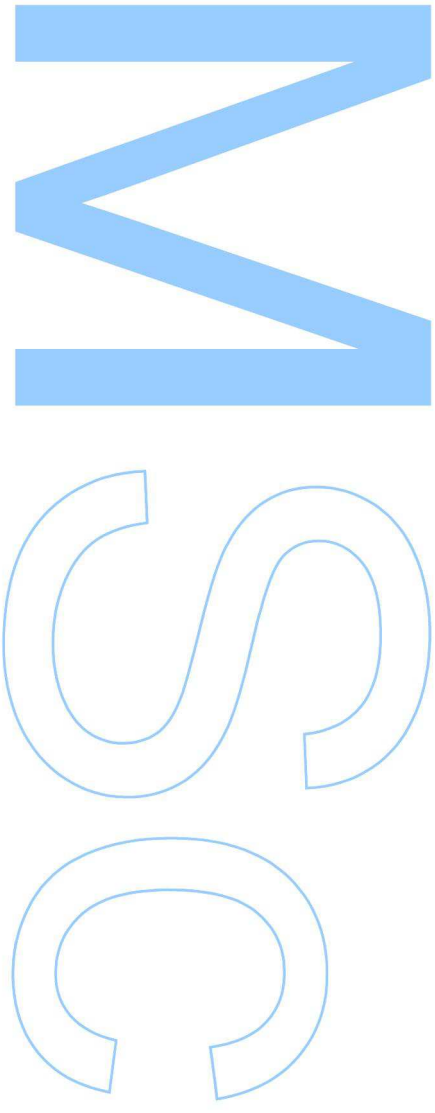

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RESUMO

A modificação de habitats naturais é atualmente reconhecido como uma grande ameaça à biodiversidade. A perda e a fragmentação dos habitats são reconhecidas como as principais causas da alteração da paisagem natural. Especificamente, a fragmentação do habitat modifica a configuração da paisagem, criando várias áreas de habitats desfavoráveis (matriz) para as espécies. A divisão de grandes e contínuas "manchas" em fragmentos mais pequenos rodeados por habitat não favorável, provoca um decréscimo na conetividade ao longo de uma determinada área. Esta interrupção do movimento, entre outras consequências negativas, bloqueia o fluxo génico entre diferentes áreas. Posteriormente, este fenómeno vai conduzir a um aumento da consaguineidade e diminuição da diversidade genética populacional entre individuos na mesma população, aumento o risco de extinção. De modo a combater estes efeitos negativos, medidas de mitigação devem de ser implementadas. Para poderem ser eficientes, estas medidas devem de ser suportadas por uma investigação científica robusta, e o aparecimento de novas áreas de investigação tais como a genética da paisagem, podem ter um papel fundamental na biologia da conservação. Atualmente, paisagens Mediterrânicas são dominadas por sistemas agro-forestais, onde uma grande proporção da floresta Mediterrânica original foi transformada em campos agrícolas. Por isso, animais como carnívoros florestais podem ser particularmente afetados por estas mudanças. Para entender como é que o fluxo génico é moldado por determinados tipos de habitats, é importante determinar com precisão a resistência que estes oferecem. Neste estudo usaram-se dados de telemetria de geneta (espécie de hábitos florestais) previamente recolhidos no âmbito de outro estudo num sistema agro-florestal no sul de Portugal, para estimar uma função de seleção de recursos de modo a avaliar objetivamente os efeitos da paisagem na variação genética nesta população de genetas. Foram colocadas três hipóteses fundamentais: (1) os dados de telemetria revelariam que as genetas usam mais zonas florestais comparativamente com outros tipos de habitats; (2) dados de parentesco e de movimento revelarão resultados discordantes relativamente aos efeitos da auto-estrada como barreira impermeável ao fluxo génico; e (3) modelos que assumem a heterogeneidade do habitat como um factor crucial que influencia a variação genética entre individuos serão mais suportados que modelos mais simplistas (por exemplo, modelos de isolamento por distância ou por barreira). Dezassete microsatélites foram genotipados com sucesso e com uma baixa taxa de erro para 74 amostras, de modo a estimar as distâncias genéticas entre os indivíduos amostrados. A equação calculada por uma regressão logística condicional revelou resultados do uso de habitats que são semelhantes àqueles obtidos por estudos anteriores, demonstrando que as genetas selecionam positivamente áreas com grande disponibilidade de recursos ecológicos (florestas de montado e galerias ripícolas) e evitaram significativamente zonas agrícolas e zonas próximas de perturbação humana. Apesar de a auto-estrada limitar os movimentos (de acordo com dados de telemetria),

esta população particular não sofreu subestruturação genética, provavelmente devido à recente construção desta barreira, não dando tempo suficiente para a população responder geneticamente. Contrariamente às expetativas iniciais, o modelo de isolamento por distância foi mais suportado do que modelos alternativos, apesar de apresentar baixos valores de correlação (Mantel r=-0.07; p<0.001). A agregação de grande parte das amostras recolhidas numa zona de habitat favorável relativamente contínuo provavelmente afectou a robustez deste modelo. O facto de as amostras estarem próximas espacialmente (aumentando a probabilidade de os indivíduos serem aparentados) numa zona favorável, não há grandes variações a nível genético entre os indivíduos nessa zona particular de amostragem. Isto implica que as diferentes variáveis de habitat consideradas estarão mal representadas nos cálculos das distâncias ecológicas entre diferentes indivíduos. Dessa forma, nessa zona altamente favorável aonde a maioria das amostras foram recolhidas, é provável que o fator principal que influencia a variação genética seja a distância geográfica. Para além disso, o papel que as galerias ripícolas desempenham como corredores de dispersão numa matriz desfavorável habitats desfavoráveis e limitações relacionadas com as técnicas de modelação usadas neste estudo, poderão ter tido alguma influência nos resultados obtidos. Melhorar a metodologia usada aqui no futuro, através da consideração de escalas espaciais e temporais apropriadas e que descrevem realisticamente processos de conetividade de fluxo génico, serão fundamentais para obter resultados mais robustos. É bastante importante ter isto em conta, para as autoridades de conservação no futuro atuarem de modo a reduzir os efeitos de fragmentação em espécies Mediterrânicas.

Palavras-chave: conetividade da paisagem; função de seleção de recursos; genética da paisagem; *Genetta genetta*; isolamento por resistência.

ABSTRACT

Landscape modification is actually recognized as major threat to biodiversity. Habitat loss and habitat fragmentation *per se* are acknowledged as the main negative consequences caused by landscape changes. Especially, habitat fragmentation modify landscape configuration, creating several areas of lower quality (matrix). The division of large continuous patches into smaller habitat patches surrounded by a low permeable matrix, greatly disturbs connectivity across the landscape. This movement disruption, among other negative consequences, impedes gene flow across the landscape. Eventually, this phenomenon will lead populations to inbreeding depression and loss of genetic diversity, reducing overall population fitness. In order to counteract these negative effects, conservation measures should be implemented. Nevertheless, accurate measures should be supported by solid scientific knowledge, and new research fields, like the landscape genetics prove to play an important role in conservation. Currently, Mediterranean landscapes are dominated by agro-forestry systems, where a great proportion of the original Mediterranean forest was transformed into agricultural fields. Therefore, genetic connectivity of several forest specialists, such as forest carnivores, may be particularly affected by these landscape changes. To understand the role of specific landscape features in shaping gene flow, it is fundamental to accurately quantify the resistance that these features impose to gene flow. Here, taking advantage of previously radio-tracking data collected for common genets (which are forest species) in an agro-forestry system in southern Portugal, a resource selection function (RSF) was estimated to objectively assess how landscape variables influenced genetic relatedness. Here, three hypotheses were tested: (1) radio-tracking data will reveal that common genets use more forested areas when compared with other types of habitats; (2) parental analysis and movement data will not present concordant results; and (3) models which assume habitat heterogeneity as a crucial factor that influences genetic variation between individuals will be more statistically supported than simpler models (for example, isolation-by-distance and isolation-by-barrier models). Seventeen microsatellites were successfully genotyped with low genotyping error rates for 74 samples, in order to estimate genetic relatedness between individuals. The conditional logistic regression equation calculated in the RSF was in accordance with previous studies, demonstrating that common genets select positively areas with high availability of ecological resources (*montado* forests and riparian corridors) and avoided agricultural fields and areas near human disturbance. Despite the highway disrupt movements (accordingly with radio-tracking data), this particular population is not genetically substructured probably due to the recent construction of this feature. Thus, the population did not have enough time to respond to the construction of the highway. Contrary to the initial hypotheses, the IBD (isolation-by-distance) model was more supported than the competing models, despite presenting low correlation values (Mantel $r=-0.07$; $p<0.001$). Most samples are clustered (non-random sampling) in a small region holding suitable contiguous habitat

which probably hampered the analyses. Since these samples are spatially close (increasing the probability of familiar relationships) in a favourable area, there are not great variations at the genetic level between individuals in that particular sampled zone. This implies that the different landscape features that were analysed are poorly represented in ecological distances calculations between individuals. Thus, in that particular region the geographical distance is probably the main factor influencing gene flow. Additionally, the role that riparian corridors may play as dispersal enhancers in unsuitable habitats and limitations concerning the modelling techniques used here, may have also contributed for the observed results. Refining the methodology employed here in the future, by accounting spatial and temporal scales that likely describe more realistically how processes responsible for genetic connectivity operate, is fundamental to obtain more robust results. This is very important, if conservation authorities want to reduce the effects of fragmentation in Mediterranean species in a near future..

Keywords: landscape connectivity; resource selection function; landscape genetics; *Genetta genetta*; isolation-by-resistance.

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LIST OF ABBREVIATIONS

AIC – Akaike Information Criterion
LD – Linkage Disequilibrium
FDR – False Discovery Rate
GIS – Geographical Information System
HW – Hardy-Weinberg

IBB – Isolation-by-barrier

IBD – Isolation-by-distance

IBR – Isolation-by-resistance

ITA – Information Theoretic Approach

LCP – Least Cost Path

MRDM- Multiple Regression on Distance Matrices

PCR – Polymerase chain reaction

PIM – Population Inbreeding Model

rho – Spearman-rank correlation coefficient

RSF – Resource Selection Function

RSPF – Resource Selection Probability Function

VHF – Very High Frequency telemetry

w(i) – Akaike weight

1-INTRODUCTION

1.1- Landscape modification and its effects on biodiversity

Biodiversity decline is currently recognized as a major environmental concern issue. Landscape modification largely contributed for this decline, reducing habitat suitability quantitatively and qualitatively at both local and global scales (Foley *et al.* 2005; Fischer & Lindenmayer 2007). Agriculture, urbanization, forest clearing or construction of infrastructures greatly contributed for the transformation of pristine habitat into artificial or semi-natural landscapes over the last decades, causing major changes in habitat spatial structure (Foley *et al.* 2005; Hanski 2010). Hence, heterogeneous landscapes are created with smaller and isolated habitat patches embedded within a landscape matrix (unsuitable area surrounding favourable habitat patches for the species of interest) (Fahrig 2003; Fischer & Lindenmayer 2007). This landscape modification is mainly caused by two important phenomena – habitat loss and habitat fragmentation *per se*. Despite some initial issues to delimit the conceptual boundaries of both processes (Holt *et al.* 1995; Schumaker 1996), probably the best definitions are provided by Bender *et al.* (1998) and Fahrig (2003) (see also "dissection" phenomenon in Bogaert *et al.* 2004; Fischer & Lindenmayer 2007). Habitat loss consists on the removal of native vegetation, while habitat fragmentation *per se* (the expression "*per se*" means that the effects of pure habitat loss are controlled) is defined as the division of a contiguous patch into multiple smaller patches separated by a non-natural matrix. In other words, habitat loss changes patch size attributes and fragmentation interferes with spatial configuration of patches. Despite presenting different properties, both phenomena cannot be seen as fully independent (Bender *et al.* 1998; Wiegand *et al.* 2005). Usually fragmentation follows habitat loss, augmenting their effects. Thus, both threats must be taken into account jointly by conservation authorities.

Three major effects resultant from the combination of both above described processes can be observed. First, decrease of patch size (perceived as loss of native vegetation area) is the main and most serious negative impact, caused primarily by habitat loss (Fahrig 2001; Fahrig 2003; but see also Wiegand *et al.* 2005). Patch size reduction implies a loss of functional space, decreasing the available area to be used by species/individuals. Under these new conditions, intra- and interspecific interactions are altered and there is a decrease of food resources and shelter availability, ultimately leading to a higher mortality rate and lower breeding success (Fahrig 2003; Swift & Hannon 2010). Second, patch isolation mediated by habitat fragmentation is the main driver of connectivity disruption. Day-to-day movements, long migration routes or juvenile dispersal are negatively affected since the adjacent matrix (generally unsuitable) coerces individuals to stay in a particular patch or to travel through an inhospitable matrix, eventually increasing mortality risk (Ricketts 2001; Bender & Fahrig 2005; Bonte *et al.* 2012). Thus, if movement between patches is blocked then gene flow is also interrupted. The greater isolation experienced by individuals within

fragmented patches leads to inbreeding depression and loss of genetic diversity, reducing individual fitness and the ability for a particular population to adapt to environmental changes (Frankham 2005; Delaney *et al.* 2010; Struebig *et al.* 2011). Third, edge effects are expressed through changes of biotic (change in vegetation communities or alteration of species interactions such as competition or predation) and abiotic (such as microclimatic changes in insulation, moisture, wind patterns) conditions in the periphery of a patch (Murcia 1995; Ewers *et al.* 2007). The "new" boundary environment is generally hazardous for native species, reducing habitat quality and availability within a given area. These major threats can lead a population to a sharp decline, eventually reaching an irreversible critical threshold (also called extinction vortex) where environmental (eg: natural variation and catastrophic unpredictable events), genetic (eg: genetic drift) and demographic stochasticity (eg: natural oscilitation on yearly breeding success) acquire a significant importance as local drivers of extinction (Lande 1993; Dennis 2002; Blomqvist *et al.* 2010).

Taxa intrinsic features also play an important role on determining extinction proneness. Despite being processes transversal to many taxonomical groups (Andrén 1994; Didham *et al.* 1996; Andrews & Gibbons 2005; Aguilar *et al.* 2006), the particular combination of biological characteristics exhibited by each taxon will determine a differential susceptibility to landscape modification (Crooks 2002; Cushman 2006; see also table 2 in Fischer & Lindenmayer 2007). Mammalian carnivores constitute a clear example of vulnerability to habitat loss and fragmentation, and the biological traits that make them susceptible are relatively well known (Sunquist & Sunquist 2001; Crooks 2002; Boitani & Powell 2012). Carnivore populations usually present low densities and slow growth rates derived from low reproductive outputs. Moreover, large area requirements and other anthropogenic pressures (eg: hunting) constitute additional factors that inflate the deleterious effects of landscape changes on this group. The great dispersal ability shown by carnivores (usually more pronounced on juveniles or sub-adults) may counter-balance or exacerbate these consequences. Patch isolation effects may be minimized by being able to move through different patches, but on the other hand, high mobility may imply a greater willingness to travel through unsuitable habitat, increasing energy costs and mortality risk (Bonte *et al.* 2012).

1.2-Maintaining landscape connectivity

1.2.1-Overview

Counteracting landscape changes effects has been a major challenge for conservation authorities. To implement effective conservation measures, one must have knowledge about several landscape features such as patch size and isolation, characteristics of the surrounding matrix and ecological requirements of the target species (Fahrig 2003; Fischer & Lindenmayer 2007). Preventing fragmentation and habitat loss is probably the best solution to maximize

conservation efforts (Crooks & Sanjayan 2006). This is rarely accomplished and, in most situations, conservation authorities are faced already with post-disturbance scenarios. Restoring habitat quality and the original amount of area would be the ideal solution to this problem (Mortelliti *et al.* 2010; Brückmann *et al.* 2010; Hodgson *et al.* 2011). However, logistical and budget constraints hamper the viability of many conservation projects and alternative solutions must be considered in order to mitigate the loss of pristine habitat.

One viable option to reduce the fragmentation effects on species population (especially isolation effects) is to increase landscape connectivity between habitat patches. Landscape connectivity is defined as a context/species-specific concept that quantifies how movement of a particular entity (eg: pollen, seeds, genes, individuals or species) is facilitated on a particular environment (Crooks & Sanjayan 2006). The concept can be decomposed in two components: structural and functional (see review in Baguette et al. 2013). Structural connectivity describes the physic properties of the landscape such as the arrangement of patches, isolation degree and topography. Functional connectivity assesses the ecological and biological responses of a particular species or entity (individual, genes, seeds, etc.) to the structural characteristics of the landscape.

Studies focusing on landscape connectivity started to increase on early 1990's, denoting the importance that this field acquired in conservation biology (Crooks & Sanjayan 2006). Complementary research fields such as metapopulation theory and landscape ecology, largely contributed for increasing the knowledge of important landscape processes (eg: matrix permeability, immigration patterns and dynamics of colonization and extinction patterns in patches) (Moilanen & Hanski 2006; Taylor *et al.* 2006). Additionally, development of GIS (Geographic Information System) and better modelling tools, as well the development of higher performance computers have allowed the generation of connectivity maps with finer resolution, contributing with more detailed information to researchers about spatio-temporal patterns of species response to fragmentation (Adriaensen *et al.* 2003; McRae 2006; Saura & Torné 2009).

During these last two decades, theoretical and empirical studies addressed the potential benefits of enhancing linkage between areas (Noss 1987; Beier & Noss 1998; Prevedello & Vieira 2010). Improving connectivity reduces movement costs (foraging movements, juvenile dispersal, migration), helps to prevent inbreeding depression, diminishes the risk of extinction in small isolated recipient populations and promotes ecological processes stability, such as natural disturbances, nutrient cycles or vegetation succession (Vilà *et al.* 2003; Crooks & Sanjayan 2006; Moilanen & Hanski 2006). Conversely, increasing connectivity in some situations can have biodiversity negative effects on biodiversity by promoting disease and exotic species spread and also can act as ecological traps, since they may increase exposure to several threats (eg: predator attraction) (Hess 1994; Crooks & Suarez 2006; McCallum & Dobson 2006). Outweighing the benefits and deleterious effects of such effort is a procedure that should be situation-specific; however, generally the advantages are obvious and cannot be disregarded. Despite its positive

effects on biodiversity, setting defragmentation measures into practice had lead to much controversy. Especially, corridor's use and efficiency for maintaining long-term populations' viability was questioned in the past (Simberloff *et al.* 1992; Beier & Noss 1998; Gilbert-Norton *et al.* 2010). The main criticisms concerned the small set of organisms that have been tested and the poorly implemented experimental designs (Beier & Noss 1998). However, Gilbert-Norton and colleagues (2010) on a review based on recent studies concluded, that in fact corridors are used by several species form different taxonomic groups. However, it is still not certain if corridors, as other conservation measures, such as stepping stone patches and management of sub-optimal matrix habitat (Fischer & Lindenmayer 2002; Baum *et al.* 2004; Prevedello & Vieira 2010) are efficient for maintaining long-term viability in populations (Hodgson *et al.* 2011). Improving not only modelling techniques, but also adopting robust management and assessment frameworks will enable scientists to effectively address connectivity issues. This is crucial to restore connectivity in fragmented landscapes.

1.2.2-Landscape genetics as a tool to assess landscape functional connectivity

Landscape functional connectivity can be measured through studies on individual movement behaviour (e.g. radio-telemetry) to assess landscape resistance to dispersal, migration and daily movements (Kindlmann & Burel 2008). Although these metrics give information about permeability to movement, they fail essentially in resolving one key aspect – they do not provide information concerning successful reproduction of migrants (Mills & Allendorf 1996; Vilà *et al.* 2003; Jaquiéry *et al.* 2011). To answer questions such as "Does a corridor enables enough gene flow to prevent inbreeding depression in the recipient isolated population?" or "Does a road creates population substructuring?", the obvious approach is to gather information regarding gene flow. Hence, indirect gene flow assessment can act as surrogate measure of landscape permeability, providing at the same time information about genetic variability among subpopulations (Cushman *et al.* 2006; Pérez *et al.* 2009; Frantz *et al.* 2010). In order to help addressing these types of questions, the new research field of landscape genetics arose.

Following technological advances on molecular techniques, landscape genetics emerged as a promising research field tool that integrates landscape ecology, population genetics and spatial analyses. It is mainly concerned on investigating the impact of landscape features on species microevolutionary processes such as gene flow, genetic drift and adaptive genetic variation (Manel *et al.* 2003). The spatial and temporal scales involved on landscape genetics studies are smaller than other population genetic studies, such as phylogeography (Wang 2010). Thus, although holding a great potential to be applied to other research areas (Balkenhol *et al.* 2009), its applicability to address contemporary connectivity conservation issues has been recognized, leading to an increase of published papers on the last decade concerning this subject (Storfer *et al.*

2010). Landscape genetics statistics and its limitations have been addressed, leading to the improvement of spatial and genetic models employed at population and individual levels (Dyer *et al.* 2010; Cushman *et al.* 2013). All this research contributed for the establishment of a standard statistical framework. Basically, it comprises the correlation (eg: Mantel test, partial Mantel test) between pairwise (individual or populations) matrices of genetic and ecological distances (the term "effective distances" is also used in the literature) for the detection of genetic discontinuities and/or detection of the influence of particular landscape features on gene flow (eg: Coulon *et al.* 2004; Stevens *et al.* 2006; Graves *et al.* 2012). Following the causal modelling framework developed by Cushman *et al.* (2006), three types of models are usually tested: isolation-by-distance (IBD), isolation-by-barrier (IBB) and isolation-by-resistance (IBR). The only difference between the models relies on ecological distances calculations, since different distance metrics are employed (see below). The first two are considered as null hypotheses where correlation values are confronted with the alternative hypothesis represented by the IBR model. The latter is usually translated into several models that contain different combinations of landscape variables (Shirk *et al.* 2010; Garroway *et al.* 2011). In these tests, an adequate sampling that realistically describes the spatio-temporal processes operating in the landscape of interest, and additionally, the ability to accurately estimate genetic distances and ecological distances are crucial steps to robustly assess model performance.

The use of adequate molecular markers is the first step to obtain reliable differentiation measures between individuals or populations. In their review, Storfer and colleagues (2010) identified microsatellites (short tandem sequence repeats found in the nuclear genome) as the preferred molecular markers, at least in studies where the target species were animals (encompassing 70% of the reviewed papers). The fine temporal window (few to dozens of generations) that researchers face on landscape genetics studies requires markers with high mutation rates and hence, microsatellites constitute suitable candidates (Selkoe & Toonen 2006; Wang 2010). Genetic markers with higher mutation rates exhibit high allelic diversity within an evolutionary short period of time, retaining enough resolution to detect population microevolutionary responses to changes in landscape (Cushman *et al.* 2006; Garroway *et al.* 2011; Amos *et al.* 2012). Markers with lower mutation rates, such as sequences of mitochondrial and nuclear DNA, are more suitable to analyse events from a distant past such as the assessment of postglacial colonization genetic patterns or the evaluation of genetic isolation effects of a particular historical natural barrier (Shafer *et al.* 2010; Bryson *et al.* 2011). Despite their high resolution power, microsatellite genotyping is prone to a variety of errors such as: (1) the systematic non-amplification of an allele generally due to a point mutation on marker's primer binding regions – null alleles; (2) allele stochastic amplification failure – allele dropout; and (3) allele misgenotyping due to human factors or to the generation of PCR artefacts Taq polymerase slippage on early cycles of PCR - false alleles (reviewed for example in Pompanon *et al.* 2005; Hoffman & Amos 2005; Selkoe

& Toonen 2006). Several factors can contribute to the increase of a particular type of errors (see table 2 in Pompanon *et al.* 2005). For example, low DNA quality and quantity is a common cause of allele dropout, constituting a relevant issue on non-invasive genetics (Waits & Paetkau 2005; Beja-Pereira *et al.* 2009; but see allele dropout for high quality samples in Soulsbury *et al.* 2007). Errors are inevitable and the best solution is to establish a solid quality control system, either on the laboratory procedures and on data analysis (Goossens *et al.* 1998; Piggott *et al.* 2004; Dakin & Avise 2004; Johnson & Haydon 2007; Chybicki & Burczyk 2009). Failure to account these errors may lead, among others, to a false increase on observed number of genotyped homozygotes (caused by null alleles and allele dropout), miscalculations of allelic frequencies and interference with Hardy-Weinberg and parentage analysis (Viard *et al.* 1998; Dakin & Avise 2004; Van Oosterhout *et al.* 2006; Johnson & Haydon 2007). Consequently, spurious scientific conclusions may be extrapolated. Landscape genetics studies rely heavily on a free bias genotyping to accurately estimate genetic distances between genetic units (eg: Pérez-Espona *et al.* 2008; Braunisch *et al.* 2010; Shirk *et al.* 2010). Phenomena such as null alleles or allele dropout, as stated above, lead to miscalculations of genetic distances and may obscure true marker polymorphism. Polymorphism is acknowledged as an important feature on this research field, providing more resolution power to microsatellites to detect landscape effects on genetic structuring (Holderegger & Wagner 2008; Wang 2010; Landguth *et al.* 2012). Thus, when theses errors are not taken into account, false landscape gene flow relationships can be obtained, having serious repercussions at conservation planning and management.

Calculation of ecological distance matrices for IBD and IBB models is straightforward. The IBD model simply assumes that genetic differentiation between individuals is only dependent of geographical distance (Wright 1943). Hence, the model predicts that one particular individual is more related with geographically closer individuals than the ones far apart. A pairwise distance (distance expressed in geographic or map cell units) matrix is then constructed and correlated with a pairwise matrix of genetic distances (eg: Murphy *et al.* 2010; Phillipsen & Lytle 2012; Quaglietta *et al.* 2013). The IBB model is used to test the effects of a particular barrier (eg: highway or a river) on gene flow (Cushman *et al.* 2006; Shirk *et al.* 2010). Panmixia on either side of the barrier with no gene flow between different sides of the barrier is assumed by the IBB model. No distance costs are considered between individuals on the same side of the barrier, while it is assigned a disproportionate maximum cost to cross the barrier. Hence, it is expected a higher relatedness among individuals on the same side of the barrier than among samples from different sides. These models are fairly unrealistic for most of the times. The IBD model assumes that an animal perceives the surrounding environment homogeneously. This is false for most species (see Frantz *et al.* 2010 for opposite results), since there is a hierarchical habitat selection where suitable habitats are selected over hostile environments (McRae 2006; Broquet *et al.* 2006). IBB model is also rather too simplistic since it disregards completely landscape features that may influence gene

flow, besides the barrier itself. Additionally, not all barriers constitute completely impermeable features (Coulon *et al.* 2006; Frantz *et al.* 2010). Therefore assigning maximum resistance values to the barrier may be untruthful. The IBR model accounts for the heterogeneity of the landscape, being much more realistic in describing the underlying spatial processes that regulate gene flow (eg: Cushman *et al.* 2006; Wang *et al.* 2008; Garroway *et al.* 2011; Apodaca *et al.* 2012). To accurately estimate a pairwise ecological distance matrix from a resistance surface (raster or vectorial layer representing the different landscape features with varying permeability), two common algorithms are usually employed: least cost path (LCP) and circuit theory (Adriaensen *et al.* 2003; McRae 2006). LCP algorithm allows the calculation of a single optimal path between a pair of individuals (i.e., the path that holds a minimum value of cumulated resistance), being especially used on the last decade (eg: Cushman *et al.* 2006; Braunisch *et al.* 2010). The minimal values of ecological distances that were calculated are then translated into a pairwise matrix. Criticism concerning LCP increased on the last years, especially concerning the limitation of only accounting for one single path in distance calculations. This scenario is many times considered unrealistic, since it is unlikely that a particular animal has the knowledge to choose a single path that is necessarily the best one. To circumvent this problem, McRae (2006) developed Circuitscape software which uses an algorithm that borrows much of the mathematical foundations from circuit theory. Given that electricity has properties of a random walk in an electric circuit, then resistance parameters can be expressed as the probability of a random individual travelling through the cells that connect nodes (individuals or populations). Unlike LCP, circuit theory has the advantage of accounting for multiple possible pathways. Pairwise resistances are then calculated by averaging the cumulated resistance of each processed path among nodes. However, whether one algorithm is chosen over other, one of the biggest challenges that researchers face in landscape genetics still remains present: after selecting the variables of interest, one must assign specific resistance to movement values to each environmental variable (also called in the literature as parameterization of resistance values) (Spear *et al.* 2010; Zeller *et al.* 2012). So, one question must be posed "What criteria should be used to assign resistance scores?".

For resistance value assignment to a particular landscape, there are methods that are more suitable than others. Expert opinion is the easiest way to parameterize resistances surfaces. One or more researchers with experience or taking advantage of previous published papers regarding the biology of a particular organism, assign differential resistance to the landscape variables (Coulon *et al.* 2004; Murray *et al.* 2009; Spear & Storfer 2010). When empirical data about presence or dispersal is either absent or hard to obtain, this approach may be useful. However, this method is subjective and possibly inaccurate, specially due to possible species and habitat differences across regions (Spear *et al.* 2010). To improve on expert opinion resistance parameterization, some authors relied on model optimization (Cushman *et al.* 2006; Pérez-Espona *et al.* 2008; Shirk *et al.* 2010). Briefly, a resistance model including the same variables is tested

within a range of resistance values. The resistances models that best match with genetic data are objectively selected through model selection procedures such as AIC (Akaike Information Criterion) based multimodel inference. Optimization offers more power than the previous approach where a set of resistance hypotheses is tested instead of only one. Nevertheless, it suffers from the same type of bias, once the range of resistance values tested is limited and it is dependent of researchers' choice (Spear *et al.* 2010).

The use of non-genetic field empirical data (point counts, mark-recapture studies or radio/GPS telemetry for example) to estimate ecological distances, can be one way to avoid subjectivity. Indeed, using this data in habitat suitability modelling via resource selection functions (RSF) constitutes a valuable tool to address landscape connectivity (Coulon *et al.* 2008; Chetkiewicz & Boyce 2009; Sawyer *et al.* 2011). The underlying principle is based on the fact that habitat preference and movement are intimately linked. In this approach, landscape permeability scores for each variable are estimated through the habitat suitability models developed for species presence or intensity of use of each landscape unit. Thus, each map unit or pixel has a suitability or RSF score associated. Higher RSF values represent more permeable areas and lower otherwise. These scores can be easily converted to resistance values by simply inverting the RSF scale (eg: $[\text{RSF score}]^{-1}$), where now higher values represent more resistant to movement areas (Chetkiewicz & Boyce 2009; Shafer *et al.* 2012). At conservation management level, this can aid conservation managers to implement important decisions, such as the location of corridors in areas that maximize connectivity (Chetkiewicz & Boyce 2009; Pullinger & Johnson 2010; Squires *et al.* 2013). However, obtaining field data of species presence can be difficult for many elusive organisms (eg: nocturnal carnivores), require intensive sampling effort and are financially expensive, driving many researchers to choose other parameterization approaches. Additionally, there is not only uncertainty regarding the choice of the landscape variables that truly affect genetic variation in a particular study area, but there may be also a disconnection between spatial and temporal scales where/when field data was collected and the genetic processes that contributed for the observed genetic structure of a population(s) (Spear *et al.* 2010). Those reasons are likely explanations for the fact that few landscape genetic studies took advantage of empirical data to parameterize resistance surfaces (Wang *et al.* 2008; Cushman *et al.* 2011; Shafer *et al.* 2012; Reding *et al.* 2013).

Assigning resistance values to landscape variables for effective distances calculation can be performed using genetic data itself. Until now, probably only one study accomplished successfully this approach. By using standardized landscape attributes as predictive variables and pairwise genetic distances as response variable, Garroway *et al.* (2011) used multiple regression on distance matrices (MRDM) to construct a resistance surface. The authors found that the final multivariate surface provided high statistical power to explain population genetic differentiation across the landscape.

Choosing the most appropriate approach is not straightforward. Factors such as availability of empirical information, species nature or budget constraints may influence this choice (Spear & Storfer 2010; Zeller *et al.* 2012). Nevertheless, while it seems that ideally one must avoid expert opinion approaches, other methods such as genetic parameterization employed by Garroway *et al.* (2011) or the use of indirect gene flow predictors, such as habitat selection studies still require more studies to evaluate its performance.

1.3-Study species

The present study addresses the landscape effects on gene flow for common genets (*Genetta genetta,* Linnaeus 1758; Fig. 1). This species is a small arboreal carnivore that is mainly characterized by a long cat-like body with a yellow pale coat pattern, exhibiting longitudinal rows of dark spots and also a long tail with dark rings (Livet & Roeder 1987; Calzada 2007). The only exceptions are the rare melanic (grayish fur color) and albine (individuals with white fur coloration) phenotypes which were only detected in Europe (Gaubert & Mézan-Muxart 2010; Delibes *et al.* 2013). Intersex differences are little evident, although males are in general slightly bigger and heavier than females (Calzada 1998; Larivière & Calzada 2001).

Common genets belong to the Viverrinae subfamily (Mammalia, Carnivora, Viverridae) which includes, besides *Genetta* spp. genus, other groups such as civets and African linsangs (Gaubert *et al.* 2004b; Gaubert *et al.* 2005b). In Africa, *Genetta genetta* may be misidentified with other sympatric species (namely *Genetta felina*), but skull biometric measurements and recent molecular studies have helped to resolve systematic issues among these cryptic *Genetta* species (Gaubert *et al.* 2004a; Gaubert *et al.* 2005a). This taxonomic confusion between *Genetta genetta* and *Genetta felina* led scientists to create ambiguous distribution maps, especially for the former (Gaubert *et al.* 2004a). It is well accepted now that the small-spotted genet occurs in the Arabian Peninsula, and

Fig.1-*Genetta genetta* adult individual.

sub-Saharan Africa, excepting for areas with dense rain forests (central-west Africa; see Fig. 2) (Larivière & Calzada 2001; Gaubert *et al.* 2005a). Contrarily to other species of the genus, *Genetta genetta* is the only viverrid where its distribution range extends to Europe, occupying the most extensive geographical area observed in *Genetta* species (Larivière & Calzada 2001). However, they are not native in Europe since individuals from the Maghreb region were introduced by humans at multiple places in Southern Europe (at least in Andalusia and Catalonia), probably during Muslim invasions (Gaubert *et al.* 2009; Gaubert *et al.* 2011). Following initial human-mediated colonization, the relatively similar bioclimatic conditions exhibited by Maghreb and Iberian Peninsula (Dobson & Wright 2000) likely contributed for the successful European expansion. Low temperatures seem to be the main limiting environmental variable that limits its distribution in Europe (Virgos & Casanovas 1997; Virgós *et al.* 2001). Accordingly, current distribution encompasses the southern region of Europe, namely Iberian Peninsula (including Mallorca, Ibiza and Cabrera islands) and the southern region of France (Calzada 2007). Recent evidence points that common genets are spreading beyond France. Observation records in northern Italy are increasing, suggesting a natural spread in that territory (Gaubert *et al.* 2008b). There are also sporadic observations recorded in Germany, Belgium and Switzerland, but were likely animals used as pets that were abandoned or escaped (Livet & Roeder 1987).

Literature about biology and ecology of this species is poorly available for Africa (Rosevear 1974). Since the present work deals with genets in Portugal (section "2.1 Study area"), much of the information about these topics will be provided for the European range. *Genetta genetta,* like many carnivores species, is a solitary and nocturnal species with two major peaks of activity during the night – one after sunset and other before sunrise (Livet & Roeder 1987; Palomares & Delibes 1994). The exhibited solitary behaviour demands that olfactory signals (ano-urogenital secretions, latrines) play a major role on inter-individual communication, territory delimitation and reproduction (Roeder 1980; Barrientos 2006). Reproduction on common genets is well documented (Livet & Roeder 1987; Larivière & Calzada 2001). Breeding season spans from January to September with intensifying mating activity during February-March. Gestation period lasts for 10-11 weeks and cubs stay with the mother for two-four months. After that, juveniles start dispersing to establish their own territory, reaching sexual maturity at two years of age.

Common genets have an euryphagous diet (feed on a wide variety of food) which includes small mammals, arthropods (despite their low contribution on biomass) and birds as predominant prey groups, although other elements such as amphibians, reptiles, plants and fruits are often found in their scats (Delibes *et al.* 1989; Virgós *et al.* 1999; Rosalino & Santos 2002). Despite the variety of food items that they can intake, genets are labelled as small mammal specialist with the facultative ability to change its feeding habits towards different types of preys (Virgós *et al.* 1999).

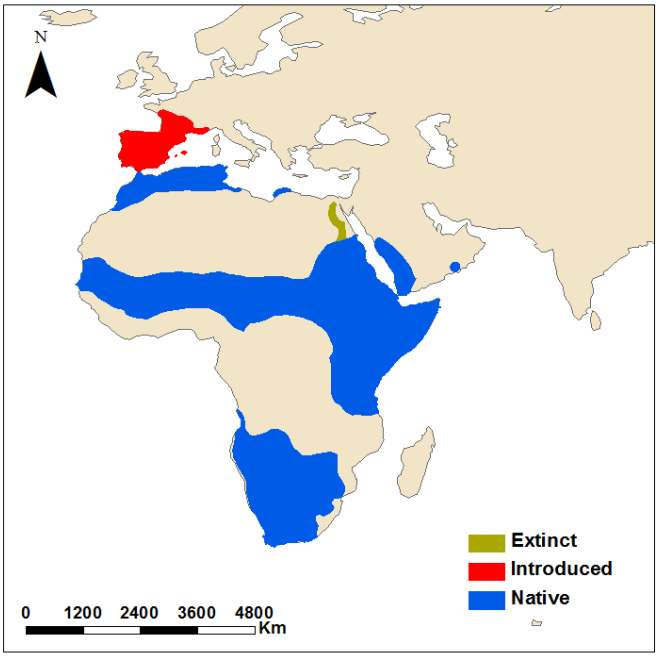

Fig.2-Worldwide distribution of the common genet. Adapted from Herrero & Cavallini (2008).

This species demonstrates a great flexibility regarding space use. They were observed in a varied habitat types including holm and cork oak *montado*, pine forests, riparian woodlands, olive groves, shrublands and rocky areas (Palomares & Delibes 1994; Larivière & Calzada 2001; Camps & Alldredge 2013). This flexibility in habitat use range it is not a synonym of habitat preference. There is a clear hierarchical habitat selection process, where forested areas with dense shrub cover (eg: holm oak forests, pine forests with dense underbrush) are primarily selected over other habitats. Two key features likely explain this preference: (1) trees and dense understory vegetation offer several potential places for resting sites (thickets, hollow trees, branches, dead trunks on the ground) and protection against predators; and (2) shrubby areas constitute a suitable habitat for small mammals (like *Apodemus sylvaticus*), guaranteeing high availability of food (Livet & Roeder 1987; Galantinho & Mira 2009; Camps 2011; Rosalino *et al.* 2011). Riparian woodlands assume also a great importance for genets (as for several other species), especially in Mediterranean environments for at least 3 reasons (Virgós 2001; Matos *et al.* 2009; Santos *et al.* 2011): (1) water is a limiting resource during the Mediterranean dry season, being confined to larger water bodies and major riparian streams. (2) associated trees and shrub cover provide shelter; and (3) riparian ecosystems can act as important dispersal corridors. Among the unsuitable habitats, it is known that genets actively avoid farmland areas and urban environments since they lack proper vegetation conditions or present high disturbance levels (Galantinho & Mira 2009; Pereira & Rodríguez 2010; Camps & Alldredge 2013). The differential habitat use exhibited by genets is crucial for the establishment of the home range, once habitat quality greatly influence the availability of food and shelter resources (Camps & Alldredge 2013). In general, there are little

inter-sexual differences between home ranges size (mean and standard deviation were 4.63 km^2 ± 1.1 km^2 and 4.70 km^2 ± 1.62 km^2, for five adult males and eight adult females, respectively in the study area), but territory overlap is minimal at intra-sexual levels (Livet & Roeder 1987; Palomares & Delibes 1994; Carvalho *et al.* in prep.).

On the last evaluation of IUCN (Herrero & Cavallini 2008), and contrarily to the general trend observed in most carnivores (Crooks 2002; Crooks *et al.* 2011), genets were classified as Least Concern (LC) due to their extensive distribution and their ecological tolerance. The only exception is Ibiza, where common genet is rated as Vulnerable (VU; Calzada 2007) due to fragmentation and habitat loss resulting from growing urbanization. Despite its worldwide status, several human related activities such as fur harvest, predator control, road-kills and habitat fragmentation may pose a considerable threat in a near future (Livet & Roeder 1987; Herrero & Cavallini 2008). In Portugal, genet is rated also as LC (Cabral *et al.* 2005). Studies concerning genets in Portugal addressed mainly space use, namely in the southern region of Portugal that encompasses Mediterranean habitat (Galantinho & Mira 2009; Matos *et al.* 2009; Sarmento *et al.* 2010; Santos *et al.* 2011). Results of these studies are in accordance with foreign literature, detecting a clear genet's preference towards forested areas with a dense shrub layer and riparian ecosystems.

1.4-Objectives and hypotheses

In southern Iberian Peninsula, original Mediterranean forests and shrublands have been transformed into agro-forestry systems (Fig. 3). Long-term human disturbance greatly fragmented the original holm and cork oaks forests, transforming the landscape into a mosaic of natural (original Mediterranean oak forests), semi-natural (holm and cork oak *montado*) and pasturelands and crops (Pinto-Correia & Mascarenhas 1999; Acácio *et al.* 2010). Cultures, livestock grazing and logging rapidly substituted areas where natural tree and shrub layers were dominant, into open farmland areas. Natural forest remnant patches and linear features such as riparian corridors are likely determinant in terms of population viability and connectivity for medium-size carnivores in fragmented Mediterranean ecosystems. Understanding how patterns of fragmentation influence carnivore persistence is a fundamental issue given the mesocarnivores susceptibility to landscape changes and the role that they play on ecosystems (Roemer *et al.* 2009).

Landscape genetic studies in Mediterranean fragmented landscapes are practically inexistent. Knowing to what extent agricultural landscapes may influence patterns of genetic variation is crucial to manage connectivity in these areas, allowing an improved conciliation between agricultural activities and biodiversity conservation. The present study aimed to provide primary insights regarding the spatial processes that affect genetic structure of a highly vagile carnivore, in a human altered Mediterranean landscape. Common genet was chosen as target species for this study due to two main reasons: (1) robust radio-tracking data was available within the study area

(Carvalho *et al.* in prep.; see Fig. S1); and (2) common genets are forest specialists for which is easier to develop landscape models, being a valuable surrogate for other native Mediterranean

Fig.3- *Montado* area, a common feature of agro-forestry systems in Mediterranean landscapes. Photo credit – Unit of Conservation Biology of University of Évora.

forest carnivores (Virgós *et al.* 2002; Santos-Reis *et al.* 2004; Pita *et al.* 2009; Matos *et al.* 2009). Specifically, it is intended to answer the following question: "Which landscape predictors possibly enhance or obstruct gene flow in an agro-forestry system". To address this question, three hypotheses were tested which are directly or indirectly related with the thesis' question. First, in accordance with previous studies of habitat selection and by using only the available radio-tracking data in the study area, it is hypothesized that genets will use more habitats where prey resources and shelter are presumably higher such as riparian corridors and *montado* forests, while anthropogenic disturbance (settlements and roads) and agricultural areas will be avoided. Second, it is hypothesized that a highway (see Fig. 4 and Fig. 5) present in the study area will not constitute a significant barrier to gene flow, despite the fact that movement data points to contrary conclusions (Fig. 4). The reason for this discrepancy lies in the fact that the highway is a very recent feature which does not possess structural characteristics to cause an evident population signal at short-term. Third, the IBR model which accounts for differential landscape permeability, will consistently outperform the IBD and IBB null models, exhibiting stronger correlations between ecological distances and genetic relatedness. To objectively calculate ecological distances and assign different values of permeability to the landscape features analyzed to test the IBR model, a resistance surface was derived from a RSF model. To construct this RSF model, the habitat selection analysis derived from movement data to test the first hypothesis was used.

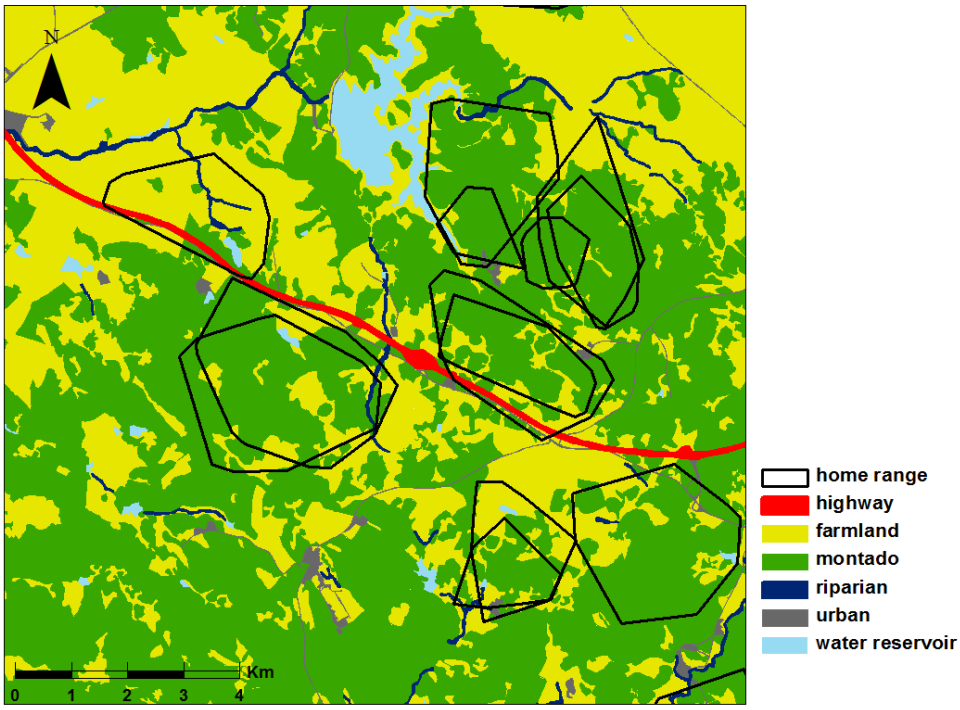

Fig.4- Home ranges near the highway are represented. It is possible to detect that some home ranges are bounded by the highway, indicating that this feature constitutes a behavioural barrier to movement.

2-METHODS

2.1-Study area

The study area is located in Alto Alentejo district (southern Portugal), encompassing about 2300 km^2 (longitude from X-551723 to X-616078 and latitude from Y-4245622 to Y-4306590; UTM WGS84 29 N; see Fig. 5). Climate is typically Mediterranean. The dry season lasts from May to September, with monthly average temperatures ranging from 20ºC-23ºC (although maximum daily temperature may reach 40ºC). The wet season extends from October to April with monthly average temperatures ranging between 10ºC-15ºC. Mean rainfall for dry and wet season are 80 mm and 500 mm respectively (Évora 2009-2012). Climate data was accessed from a local meteorological station (CGE, 2013). Topography is smooth and altitude varies between 100-400m. Two important categories of landcover are predominant in the area: Mediterranean evergreen oak forest (*montado*) and agricultural lands. *Montado* is a semi-natural habitat, comprising about 57% of the total study area. It constitutes a traditional agro-silvo-pastoral multiuse system which resulted from human alteration of the original Mediterranean forest, holding a great regional socio-economical importance (cork extraction and livestock production; Pinto-Correia & Mascarenhas 1999). It is mainly characterized by alone or mixed evergreen stands of cork oaks (*Quercus suber*) and holm oaks (*Quercus rotundifolia*). On the absence of human interference, sub-arboreal cover is mainly dominated by xerophytic shrubs such as *Cistus* spp. and *Erica* spp.. The remaining area is composed by agricultural lands such as cereal crops, vineyards, olive groves, orchards, meadows

and eucalyptus plantations. Density of human settlements is low and people are mostly located in three major cities (Évora, Montemor-o-Novo and Arraiolos) along with small villages and scattered farmhouses. Additionally, the area is located in the main terrestrial transportation corridor between Lisbon and Madrid being bisected by a highway. Other important national roads with medium/high traffic volumes and low travelled municipal roads are also located in the area. At southeast, the study area is delimited by a portion of the Natura 2000 site "Serra de Monfurado" (PTCON0031). The site has extensive well preserved *montado* areas of *Quercus suber* and *Quercus rotundifolia* (especially the former). Watercourses such as riparian ecosystems of *Fraxinus* spp. and *Salix* spp. transverse the area, exhibiting a good conservation status (ICN 2006). Previous faunistical research detected high species richness in "Serra de Monfurado", hosting several threaten species of vertebrate and invertebrate animals (ICN 2006). Among vertebrates, the carnivore community is in general diverse and abundant (eight species of carnivores), including the genet.

2.2-Sample collection for genetic analysis

In total, 76 samples were collected for genetic analysis from two sampling methods: roadkills and cage live-trapping. From those, 44 samples were obtained from roadkilled genets and 32 from trapped animals. High quality samples (muscle, n=38; blood, n=29) comprised 88% while hair samples (n=9) constituted 12% of the total dataset. Systematic road surveys were conducted within the scope of the project "MOVE – assessment of road effects on terrestrial vertebrates". An extent of approximately 50 km, comprising national and municipal roads with distinct traffic intensities, was travelled by vehicle in the morning on a weekly (2007) and daily basis (from 1st January 2008 to 31st March 2013). Other roadkilled samples were opportunistically collected in other roads not included on MOVE project. For each roadkilled genet, UTM coordinates were recorded by a hand-held global position system (GPS) with an accuracy of 5 meters. The carcasses that were found were taken to the laboratory in order to collect tissue

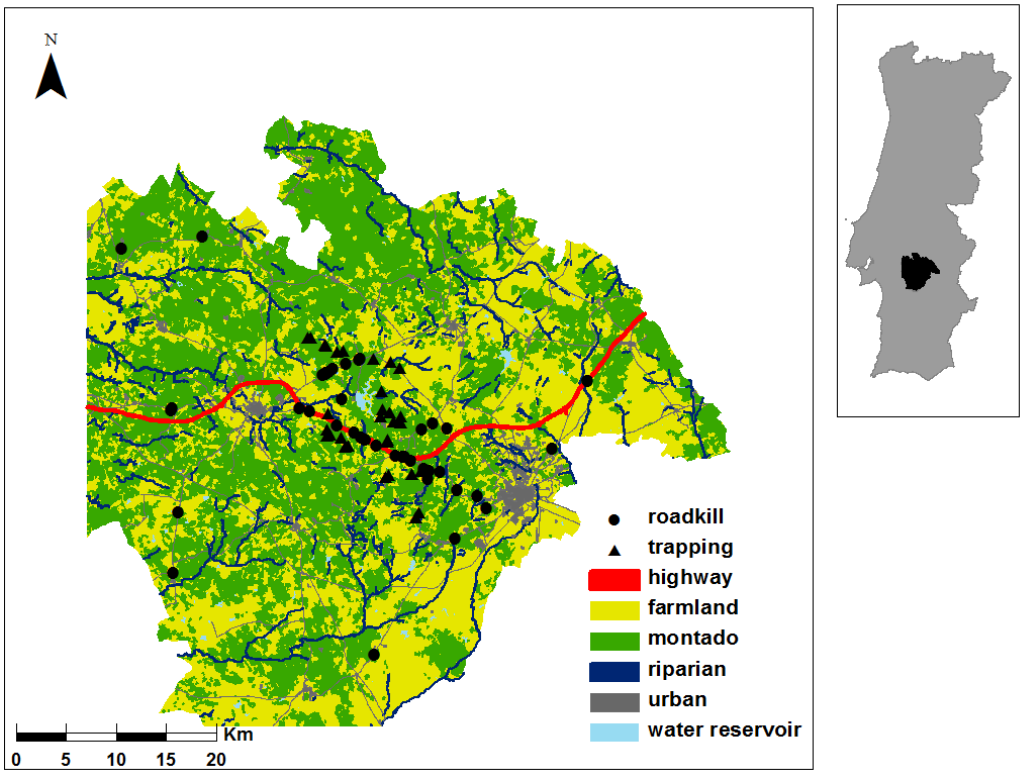

Fig.5- Study area limits and main land cover categories. Black dots and triangles show locations of the genet samples used in the genetic analysis. The highway tested in this study as a hypothetical barrier is also illustrated.

samples under good asepsis conditions. Tissue samples were stored in tubes containing 100% ethanol. On a few carcasses accidently found, the highly decomposition state or the lack of transportation conditions and chirurgical material to handle the carcass prevented proper obtainment of tissue samples. On those cases, hairs were plucked from the animal and stored dry inside paper envelopes. Genet trapping was performed in a smaller portion (about 500 km^2) of the study area (Carvalho *et al.* in prep.). Trapping was undertaken intensively from May 2010 to December 2011, except on particular periods (February-April 2011 and August-September 2011) due to logistical limitations and lower capture success rate (Zabala *et al.* 2001). Box-traps (30W x 30H x 90L cm) were baited (sardines, chicken eggs and road-killed small mammals and passerines) and placed (at least 500m apart) in suitable habitat for the species to increase capture success (Galantinho & Mira 2009; Sarmento *et al.* 2010). UTM coordinates were recorded for each box-trap location. Traps were daily visited for capture confirmation and/or to replace the bait. All captured genets were transported to the veterinarian hospital of Évora University. Standard handling procedures were carried out to immobilise and anesthetize the animal. Information regarding sex, age and biometric measurements were obtained, along with blood and hair samples. Hair samples were stored dry in paper envelopes while blood samples were kept frozen at -20ºC. Each genet was also radio-collared (models: lpm2700A, Wildlife Materials, US and TW-3, BioTrack, Wareham, UK) in order to follow its activity and movements in the aim of another study

(Carvalho *et al.* in prep; see also section "2.5.2-Resource selection function"). When all procedures were complete, genets were released on the original capture site after regaining conscience and full movement capacity. Captures and handling were carried out with the permission of the Portuguese Institute for Nature and Biodiversity Conservation and conformed to the guidelines approved by the American Society of Mammalogists for the use of wild mammals in research (Sikes *et al.* 2011).

2.3-Laboratory procedures

2.3.1-Marker selection

A set of 20 published microsatellites (see table 1) developed specifically for *Genetta genetta* were used (Gaubert *et al.* 2008a; Fernandes *et al.* 2009). Different loci were combined in multiplex sets. In order to combine them in the same multiplex, at least two criteria were taken into account: (1) avoiding the overlap size range between markers; and (2) prevent primer-dimer formation. Microsatellite alleles differ in size and consequently, allele scoring is based on that property. Due the high level of polymorphism, it is fairly hard to join a great number of microsatellites in the same multiplex since the probability of size range overlap increases. Length overlap between markers hinders scoring of alleles with similar sizes. To avoid this issue, a fluorescent dye (6-FAM, VIC, NED or PET) was added on the 5' end of each marker's forward primer (table 1). This allowed that alleles at loci tagged with different fluorescent dyes could be distinguished, even if they had similar extents. Markers possessing the same fluorescent tag and length overlap were mandatorily separated in different multiplex reactions. Besides the size criterion, it is also fundamental to guarantee that primer-dimer interactions between loci are absent or very low. High probability of primer-dimer formation leads to amplification of non-target regions. These side reactions will compete for PCR reagents, decreasing the amplification success of the target region (Markoulatos *et al.* 2002; Vallone & Butler 2004). Software AutoDimer (Vallone & Butler 2004) was employed to deal with this problem. The software attributes a score for each primer pair combination. This value represents the degree of interaction between primer oligonucleotides (higher scores represent higher complementarity). Pairs of markers that exhibited equal or higher values than 7 (default threshold score recommended by Vallone & Butler 2004) were separated in different multiplexes. Considering both criteria, multiplexes' performance was tested and PCR conditions were optimized using good quality tissue samples (see PCR details in section "2.3.2-Laboratory procedures").

Table 1- Characterization of 20 microsatellite loci selected to genotype *Genetta genetta* samples. Information regarding optimized multiplex sets and fluorescent labels used in this study is also provided.

Locus	Repeat motif	Primer sequence (5'-3')	Label	Multiplex	V (µl)	Reference
A104	$(CA)_{23}$	F:TGAAAGAATTGCTTGGTATGG R:GCATGGTTGGTGAACATTC	VIC	Panel 1	0.8	Gaubert *et al.* (2008a)
A108	$(CA)_{16}$ (TGCACACG CACGCG) $(CA)_{12}$	F:TGCATTACAATCACTCACTCTC R:TAGGTGGAAATCAATCTGTTG	6-FAM	Panel 1	0.8	Gaubert *et al.* (2008a)
C101	$(ATGG)_{12}$	F:TCCCACAGAAGGAACAGTC R:GCTTGTCCCATCAGAGTGT	VIC	Panel 1	1.6	Gaubert *et al.* (2008a)
D111	$(TAGA)_{14}$	F:TGCTTTTTCTTTAATCCCTCTC R:TATCCTCAGCAGTCCTCAGAG	6-FAM	Panel 1	0.8	Gaubert *et al.* (2008a)
D4	$(TATC)_{15}$	F:TTGGAGAGGATTTCACTGAC R:TAGGCTTAGGAGATTTAGCAAG	NED	Panel 1	0.8	Gaubert *et al.* (2008a)
Ggen 2.1	$(CTTT)_{27}$	F: CCACATAATAGCTGCTGT R: CAAAGGAGCTGAACACGT	PET	Panel 1	1.2	Fernandes *et al.* (2009)
Ggen 2.A16	$(TAGA)_{17}$	F: TCCCAGATTCATTCAGTC R: TTATGGGCCTCTCTCCACGA	NED	Panel 1	0.8	Fernandes *et al.* (2009)
Ggen 4.10	$(CTTT)_4$ CATT $(CTTT)_3$ $(CT)_2$ $(CTTT)_{13}$	F: CTCTGTTGGCCTTTCGTA R: GGTTCCTAAAACAGCTAC	VIC	Panel 1	1.6	Fernandes *et al.* (2009)
Ggen 4.12	$(TAGA)_{13}$	F: GTGAGCTTCCATAATAGC R: GCTTTTCCAGAGAAACAG	PET	Panel 1	1.2	Fernandes *et al.* (2009)
A110	$(AC)_{22}$	F:TCGTGCTGACGTGTTTAGC R:TTTGCCTTCCACAAAGAGG	6-FAM	Panel 2	1.1	Gaubert *et al.* (2008a)
A5	$(GT)_{16}$	F: GAACTCGGGGCTTAGATGTC R:CTGGAAAGATGAGGGGACTT	PET	Panel 2	1.2	Gaubert *et al.* (2008a)
B105	$(GA)_{18}$	F:CGTGTATGTGTGTGGTGTGTG R:CCCCTACCTTCTTCATCCAAC	VIC	Panel 2	0.8	Gaubert *et al.* (2008a)
Ggen 2.A13	$(TCTA)_{14}$	F: TAGGCCCCCAATCACATG R: ACTAGTCAGGTTCTCCAG	6-FAM	Panel 2	0.8	Fernandes *et al.* (2009)
Ggen 3.3	$(TCTA)_3$ TCA $(TCTA)_{18}$	F: CCTGTATATATTTATGGC R: TGAAAAATAGCTTTAGAC	NED	Panel 3	5.0	Fernandes *et al.* (2009)
A112	$(GT)_{21}$	F:CCAACTGCCTCTGTGACTC R:CCAAAACCTATCCGAGAATG	VIC	Panel 3	1.6	Gaubert *et al.* (2008a)
B104	$(AG)_{20}$	F:ATCTGCTACTGGCAAGTCAAC R:GCCTGTTTCAGTTTCTGTGTC	NED	Panel 3	3.2	Gaubert *et al.* (2008a)
Ggen 2.A15	$(TCTA)_{14}$ TCA $(TCTA)_3$	F: TATACCCCTCATAGCTCA R: CGAATCATATCAGGCTAG	VIC	Panel 3	1.6	Fernandes *et al.* (2009)
Ggen 1.30*	$(TTTC)_{17}$	F: ACATTATTAACTAAGCTA R: GTGGTGATTACATCAGTC	PET			Fernandes *et al.* (2009)
Ggen 2A2*	$(TCTA)_{14}$	F: GGACTCATAATCCACGGA R: CGAGTCACTAAACTCTAC	6-FAM			Fernandes *et al.* (2009)
Ggen 2.A25*	$(CTTT)_{17}$ $(CT)_8$ $(CTTT)_2$ $(CT)_2$ $(CTTT)_4$	F: TCTGGAGACTCCAATTTG R: GGCTTCTCAAGAAAACCT	6-FAM			Fernandes *et al.* (2009)

F - Forward; R – Reverse;

6-FAM (5' TGT AAA ACG ACG GCC AGT 3'); VIC (5' TAA TAC GAC TCA CTA TAG GG 3'); NED (5' TTT CCC AGT CAC GAC GTT G 3'); PET (5' GAT AAC AAT TTC ACA CAG G 3');

V (µl) – Volume added by each forward primer, reverse primer and fluorescent dye to the multiplex panel mix (pure H_2O was added to make up a total volume of 100 µl).

* Marker not included on further genetic analysis due to amplification inconsistency.

2.3.2-Sample genotyping

Extraction of genomic DNA was carried out from tissue, hair and blood samples using DNeasy Blood & Tissue Kit (QIAGEN), following the manufacturer's protocol. Once hairs present low DNA quantity and quality, about 10 hairs with visible root bulbs were used to increase probability of amplification success (Goossens *et al.* 1998; Beja-Pereira *et al.* 2009). The quality and quantity of extracted DNA were assessed by gel electrophoresis. Three µl of bromophenol blue were added to two µl of extracted DNA and then loaded into a 0.8% agarose gel containing GelRed (DNA fluorescent dye; BioTarget). Gels containing DNA were run at 300V and extracted DNA was visualised in a UV transilluminator device (Bio-Rad).

PCR was performed using the Multiplex PCR kit (QIAGEN) which is adequate for multiplex reactions. This kit contains a Multiplex PCR Master Mix (HotStarTaq DNA Polymerase), Multiplex PCR Buffer and Q-Solution. Each PCR reaction included five µl of Multiplex PCR Master Mix, three µl of pure H_2O, one µl of a multiplex panel mix (table 1) and one µl - three µl of genomic DNA, depending on the DNA concentration and quality. One – two µl of extracted DNA from tissue and blood samples were added into the PCR reaction while two – three µl of DNA were added when extracted from hair samples. A negative control was also used to identify possible DNA contaminations. The optimized PCR cycling conditions were equal across the three sets. First, an initial activation step at 95ºC for 15 min, followed by nine cycles of denaturation at 95ºC for 30 s, annealing at 58ºC with a temperature decrease of 0.5ºC each cycle and extension at 72ºC for 30 s. Following this, 28 cycles of denaturation at 95ºC for 30 s, annealing at 54ºC during one minute and extension at 72ºC for 30 s, finishing with eight additional cycles of denaturation at 95ºC for 30 s, annealing at 53ºC for 45 s and extension at 72ºC for 30 s. The protocol was completed with a final extension at 60ºC during 30 min. Amplification success was also evaluated via gel electrophoresis: three µl of bromophenol blue mixed with two µl of PCR product into a 2% agarose gel to ensure a good resolution power to discriminate small fragments of interest (150 bp - 300 bp). A 100–1000 bp DNA ladder (NZYTech) was added to the gel for product size comparison. Gels were run at 300V and amplified DNA was visualized in a UV transilluminator device (Bio-Rad). PCR products were run on an ABI 3130 capillary sequencer (Applied Biosystems), using a size standard LIZ 725 (Nimagen), and scored using GeneMapper version 4.0 (Applied Biosystems). A singleplex PCR was performed whenever a particular marker from a multiplex set failed to amplify in order to increase amplification probability. In this case, PCR was performed using five µl Multiplex PCR Master Mix, 2.8 µl of distilled H_2O, 0.4 µl of forward primer (diluted 1:100), 0.4 µl of reverse primer (dilueted 1:10) and 0.4 µl of marker specific fluorescent dye (diluted 1:10). Singleplex PCR cycling conditions were similar to those described for multiplex sets.

Laboratory procedures to minimize genotyping errors were carried out. Two different protocols were performed to assess allele dropout and false allele rates: one for high quality samples (tissue and blood) and other for non-invasive hair samples. The reason to employ different methodologies

lies essentially on the fact that the latter is much more affected by stochastic errors than the former (eg: Gagneux *et al.* 1997; Bonin *et al.* 2004; Hoffman & Amos 2005). Independent PCRs were employed a second time on a subset of high quality samples. This approach has been recognized as a valid one to estimate microsatellite errors (eg: Bonin *et al.* 2004; Dewoody *et al.* 2006). Twenty three samples (about 34% of the whole dataset) were re-genotyped. Mismatches between the two genotype datasets were dealt differently according with allele scoring results. If a homozygote (AA or BB) and a heterozygote (AB) were scored for a particular locus between different screenings, then the heterozygote would be considered as the true genotype. Favouring heterozygotes over homozygotes follows the assumption that that dropout is more likely to occur than the presence of false alleles (Broquet & Petit 2004). A heterozygote would also be considered as the definitive genotype if one obtained AA for the first replicate and BB for the second. For different heterozygotes (AB and AC), the PCR would be repeated using the marker individually. The third replicate (if in accordance with one of the previous repetitions) would be accepted for further analysis. All hair samples were re-genotyped twice to estimate dropout and false allele rates. Procedures to determine the true genotype were similar to the ones employed for blood and tissue samples, excepting on one situation. Since allele dropout rates are generally higher for this type of samples, a third replicate was performed for homozygote loci to increase confidence on results. For both protocols, when both alleles failed to amplify for a given locus, individual markers PCRs were carried out to increase PCR performance. Hair samples that consistently failed to give consensus genotypes were removed from further analysis.

Sex from each individual was genetically determined to improve parentage analysis (see section "2.4- Microsatellites data analysis"). Published primer sets that amplify conserved regions of X and Y chromossomes in mammals - DBY intron 7 (Hellborg & Ellegren 2003) and DBX intron 5 (Hellborg & Ellegren 2004) - were tested on a set of eight high-quality samples (five known males and three known females). PCR reactions were performed as follows: five µl Multiplex PCR Master Mix, 3.2 µl of distilled H_2O, 0.4 µl of forward primer (diluted 1:10), 0.4 µl of reverse primer (diluted 1:10) and 1 µl of DNA sample. Optimized PCR program consisted of an initial activation step at 95ºC for 15 min, followed by 12 cycles of denaturation at 95ºC for 30 s, annealing at 61ºC with a temperature decrease of 0.5ºC each cycle and extension at 72ºC for 45 s, followed by 23 cycles of denaturation at 95ºC for 30 s, annealing at 56ºC during 30 s, extension at 72ºC for 45 s and a final extension step at 60ºC during 5 min. Sex determination was performed by gel electrophoresis as described above. Although PCR products were obtained from DBX and DBY for some samples (product size superior to 300 bp and 400 bp respectively), they failed to amplify on samples of lower quality. To circumvent this problem, these PCR products were sequenced in order to re-design new primers – genX5 and genY7 (table 2). It is expected that primers that generate shorter amplified fragments will increase PCR efficiency. Successful amplifications were purified using ExoSAP (constituted by a mix of exonuclease I and Shrimp Alkaline Phosphatase enzymes;

Applied Biosystems). Sanger sequencing reactions were carried out on 10 µl reaction volumes with the following composition: 0.4 µl of termination reaction reagent BigDye (Applied Biosystems), one µl of BigDye buffer (Applied Biosystems), 0.5 µl of forward primer (diluted 1:10) and 7.1 µl of distilled H_2O. Sequence reaction profile was as follow: initial denaturation at 94ºC for three min, followed by 24 cycles of denaturation at 96ºC during 10 s, annealing at 50ºC for five seconds, finishing with a elongation step at 60ºC for four minutes. Reaction sequence products were sequenced on an ABI 3130 capillary sequencer (Applied Biosystems). Sequenced fragments were screened on BioEdit (Hall 1999). From these fragments, primers genX5 and genY7 were designed using Primer 3 (Untergasser *et al.* 2012). Primers were tested using the same samples in a single reaction. PCR reaction consisted in 5 µl of Multiplex PCR Master Mix, 1.5 µl of genY7 forward primer (diluted 1:100), 1.5 µl of genY7 reverse primer (diluted 1:10), 0.1 µl of genX5 forward primer (diluted 1:100), 0.1 µl of genX5 reverse primer (diluted 1:10), 1.6 µl of 6-FAM fluorescent dye (diluted 1:10) and 1-3 µl of extracted DNA. Optimized PCR cycling conditions consisted of an initial activation step at 95ºC for 15 min, followed by 12 cycles of denaturation at 95ºC for 30 s, annealing at 58ºC with a temperature decrease of 0.5ºC each cycle and extension at 72ºC for 30 s, followed by another set of 28 cycles of denaturation at 95ºC for 30 s, annealing at 53ºC during 30 s, extension at 72ºC for 30 s and a final extension step at 60ºC for 5 min. These primer sets proved to be more efficient than those initially tested for DBX intro 5 and DBY intron 7. Sex determination was performed via visual inspection using a UV transilluminator device (Bio-Rad) (Fig. 6).

Table 2- Characterization of the primers re-designed for sexing genets.

Primer	Primer sequence (5'-3')	Tm (ºC)	G/C%	Product size
genX5	F: TGT AAA ACG ACG GCC AGT AGCCTGGGGATTGGTTTTCT	59.21	50.00	189
	R: TCCCATCTCAACATCGCTGA	58.81	50.00	
genY7	F: TGT AAA ACG ACG GCC AGT AGTTGTTGGCATAAAATGTTTGA	55.83	30.43	250
	R: GGCGTCCGTATCTTCCATTT	58.05	50.00	

Tm - Melting temperature; G/C% - Percentage of guanines and cytosines;

2.4-Microsatellite data analyses

Allele dropout and false allele rates were estimated using software Pedant (Johnson & Haydon 2007). The software uses a maximum likelihood method to estimate jointly ε_1 (allele dropout rate) and ε_2 (false allele rate). Due to different error proneness, high quality samples and hair samples were assessed separately. Following author's recommendation, 10000 steps were set to perform maximum likelihood search. To estimate null allele frequency accurately, it is important to account for inbreeding since both measures are correlated. Both contribute to observed homozygosity excess and failure to account them simultaneously can introduce important bias on analysis (Dakin & Avise 2004; Chapuis & Estoup 2007). Undetected null alleles may inflate estimation of inbreeding coefficient (F), whereas disregarding inbreeding can lead to an overestimation of null

allele frequencies (Van Oosterhout *et al.* 2006; Campagne *et al.* 2012). Considering that most sampled individuals in the present study are concentrated in a small area, it is possible that related individuals are present in the dataset. Thus, it is important to account for the possibility of inbreeding. Here, the newly developed INEst software (Chybicki & Burczyk 2009) was used. INEst employs a maximum likelihood method that estimates jointly the inbreeding coefficient (F) and null allele frequencies (r). Population Inbreeding Model (PIM) with a jackknife procedure was used to estimate F and r for each locus. The output provides parameters estimates and the standard errors by locus. A standard one tailed z-test was then performed to test if the estimated r was significantly higher than zero (α=0.05).

After accounting for errors, markers must be tested for possible deviations of Hardy-Weinberg (HW) equilibrium and linkage disequilibrium (LD). The presence of related individuals in the dataset may bias the analysis, increasing the risk of committing type I errors (accepting erroneously the alternative hypotheses of Hardy-Weinberg disequilibrium and linkage disequilibrium). The Hardy-Weinberg model assumes that sampled individuals are not related on a random mating scenario (Robertson & Hill 1984; Allendorf and Luikart 2007). Genotypes between related individuals are much more similar than among unrelated ones, causing deviations from allelic frequencies predicted by the model (Robertson & Hill 1984; Bourgain *et al.* 2004). The nonrandom association of alleles between different loci (linkage disequilibrium) is inflated when analyzing samples with high levels of relatedness. Linkage equilibrium assumes genotypic independence among samples (Allendorf and Luikart 2007). This assumption is violated because genotypes from individuals that share familiar relationships are correlated, increasing covariance between alleles at different loci (Weir *et al.* 2006; Slatkin 2008). To avoid false positives, related individuals must not be included when checking for marker equilibrium departures. Taking advantage from genetic profiles, sexing, age information from trapped individuals and data about their home ranges (Carvalho *et al.* in prep.), familiar relationships could be inferred with a reasonable level of confidence, especially for cage-trapped individuals. COLONY software (Jones & Wang 2010) was used for this purpose. It applies a maximum likelihood method to infer jointly parent/offspring relationships and sibships. Markers deviating from Hardy-Weinberg equilibrium and linkage equilibrium expectations may lower the power of the analysis. However, the method is relatively robust if a reasonable number of polymorphic markers are used (Wang 2004). Three runs using the full likelihood method (with high likelihood precision) were performed. Only parent/offspring, full sibling and half sibling relationships with probabilistic values superior to 0.95 were considered. However, only parent/offspring and full sibling pairs were considered to filter the data in order to perform equilibrium tests. One must note that this data filtering meant to minimize familiar relationships with highest values of relatedness (parent/offspring and full sibling pairs). If two genets shared a parent/offspring or full-sibling relationship, one of them would be arbitrarily removed from equilibrium analyses.

Exact tests of Hardy-Weinberg equilibrium and linkage disequilibrium were performed in GENEPOP 4.2 (parameters were set to dememorisation=10000, batch length=50000 and batch number=2000; Rousset 2008). Once multiple comparisons among loci pairs are carried out by these exact tests, the chance of making type I errors increase. To correct the level of significance (α) for multiple tests, the False Discovery Rate (FDR) method was used assuming α=0.05 (Benjamini & Hochberg 1995). This method is more accurate than the classical Bonferroni correction (Verhoeven *et al.* 2005). Number of alleles, observed and expected heterozygosities were calculated in GenAlEx 6.5 (Peakall & Smouse 2006; Peakall & Smouse 2012).

Fig.6- Sex identification of four samples in gel electrophoresis. Two females (1) and two males (2) are illustrated.

2.5-Spatial analyses

2.5.1-Environmental spatial variables

A vectorial layer containing ten classes of land cover for 2010 (CCDRA, 2012-2014) was reclassified into five cover types (Fig. 5). Especially, classes regarding hypothesized unsuitable areas such as farmlands (cultures, exotic plantations, pasturelands) and urban areas (roads and settlements) were merged in order to avoid model over-fitting (Burnham & Anderson 2002). The land cover map was converted into a vectorial polygon map with a grid cell size of 100 m. The grid resolution choice was a compromise between ecological accuracy and computing times. Kernel density estimates showed that step size (distance between two consecutive biangulations/triangulations; see section "2.5.2-Resource selection function") of 100 m constituted the maximum value of the probability density function for a period of 30 minutes. This means that in the available radio-telemetry dataset (Carvalho *et al* in prep.), a genet would move often a distance of about 100 m, every 30 minutes. Taking into consideration this distance and previous studies regarding movement (Palomares & Delibes 1988; Palomares & Delibes 1994), 100 m seemed a valid resolution unit that represents well the fine spatial scale at which common genets

perceive the surrounding environment while travelling through the landscape. Within each grid cell, four landscape variables (table 3) were calculated to develop the habitat suitability model and posteriorly estimate the RSF scores (see section "2.5.2-Resource selection function"). These four predictors were chosen based on its biological meaningfulness influence on genets' movement (eg: Galantinho & Mira 2009; Camps & Alldredge 2013; see also section "1.3-Study species"). For the habitat categorical variable, a pixel was assigned with 1 or 0 if the cell grid presented more than 50% of forest area or agricultural area, respectively. Distance to water bodies with standing water all year (here only water bodies with an area higher than 10000 m^2 were considered) such as reservoirs, ponds or dams was included in the analysis since they are additional water suppliers (besides riparian corridors) during the dry season (Rosalino *et al.* 2005). Similarly, only major urban areas (with an area superior to 30000 m^2) and roads were represented, given that they may constitute the major disturbance sources (Ditchkoff *et al.* 2006; Fahrig & Rytwinski 2009). All calculations were performed using QGIS version 2.1.0 (QGIS Development Team 2013) and accessory GIS tools, including GRASS version 6.4.3 (GRASS Development Team 2013) and PostGIS version 2.1.0 (PostGIS Project Steering Committee 2013). The centroids of each grid cell were used to calculate distance metrics variables.

Table 3- Description of the predictive landscape variables.

Variable	Code	Type	Description
Habitat	Hab	categorical	Presence (1) or absence (0) in forested areas. Forested areas include riparian vegetation and montado (with arboreal cover >30%). Non-forested areas are composed by agricultural lands (cultures, pastures, exotic plantations);
Distance to human disturbance	dist_human	Continuous	Distance to the nearest human disturbance (urban areas and all types of roads) with an area higher than 30000 m^2;
Distance to riparian vegetation	dist_rip	Continuous	Distance to the nearest riparian corridor;
Distance to water bodies	dist_water	Continuous	Distance to the nearest water body with an area higher than 10000 m^2 (excluding riparian ecosystems);

2.5.2-Resource selection function

A resource selection function (RSF) derived from the habitat suitability model where the four landscape variables constituted the predictor set, was used to parameterize resistance surfaces and calculate ecological distances between samples. Conditional logistic regression was employed here within a match case-control design framework in order to compare habitat use (presence points or observed paths) with available habitat (points or paths randomly generated) (Boyce *et al.* 2002; Johnson *et al.* 2006). This method differs from standard logistic regression in which each specific used location (scored as 1) is matched to a group of available locations (scored as 0), allowing the comparison between empirically observed locations to random locations that represent habitat availability. When animals use a particular habitat type with higher or lower

proportion than what is present in the available dataset, then the animal is selecting or avoiding that particular habitat type. The conditional logistic regression equation takes the exponential form (Johnson *et al.* 2006) showed on equation 1:

$$w(x)=\exp(\beta_1 x_1+ \beta_2 x_2+\ldots+ \beta_i x_i) \quad (1)$$

where β_i is the coefficient estimate for variable i, x_i is the measured variable i in a particular map unit (eg: raster pixel or a 100 m grid cell in our case) and w(x) is the RSF or suitability score associated to that map unit, weighting all analyzed variables. As demonstrated by Johnson *et al.* (2006), adapting the equation 1 to calculate probabilities of use (also called RSPF – Resource Selection Probability Function) is a valid approach and it can be accomplished by using equation 2:

$$w^*(x)=\frac{\exp(\beta_1 x_1+ \beta_2 x_2+\ldots+ \beta_i x_i)}{1+\exp(\beta_1 x_1+ \beta_2 x_2+\ldots+ \beta_i x_i)} \quad (2)$$

where w*(x) corresponds to the RSPF scores.

Here, the used dataset constituted by VHF (very high frequency) nocturnal radio-telemetry biangulations (two bearings taken in less than 5 minutes), triangulations (three bearings taken in less than 10 minutes) and diurnal resting sites (homing-in technique) locations (data gathered by Carvalho *et al.* in prep.), was compared with an available points dataset composed of randomly generated points (see below). This use-available design is performed at a scale within home ranges, being categorized as third/fourth-order habitat selection (Johnson 1980). Given the smooth topography of the study area, accuracy of locations determined by VHF radio-telemetry was relatively high (error inferior to 100 m) (Carvalho *et al.* in prep.). Few locations presented low spatial accuracy and were removed from the dataset. Movement data from 21 adult genets (9 males and 12 females) with stable home ranges (calculated through 100% Minimum Convex Polygon technique) was used to represent used locations sample set (Fig. S1). Radio-telemetry data is spatially and temporally autocorrelated, violating the independence assumption of most statistical methods. Violation of independence may introduce significant inference bias on habitat selection models, possibly leading to spurious conclusions (Nielsen *et al.* 2002; Fieberg *et al.* 2010). Independence among locations was assumed here, by considering only locations separated within a 4 hour interval (Palomares & Delibes 1994). Given the role that they play on genet's ecology and biology, resting sites provide important information about habitat selection. The choice of particular resting sites is largely conditioned by the surrounding habitat (Camps 2011). Ideally, the habitat surrounding resting sites must fulfil foraging (food and water intakes), reproductive and low disturbance requirements, and thus it is likely that the location of resting sites represent areas highly permeable (see also Camps & Alldredge 2013). Therefore, resting sites were also included

as used locations, which were also highly accurate contributing for the overall location accuracy. Buffers with 800 m radius were created around each used location using ArcGis 10 (ESRI 2011). The 800m represented the mean distance between independent biangulations/triangulations among all genets. To represent availability, software Geospatial Modelling Environment (Beyer 2012) was used to generate 20 random points within each buffer. To better represent availability inside a buffer, random points were set apart at least 100m following the same criteria used to set grid resolution. Usually, the criterion employed to choose the number of points is arbitrary (eg: Klar *et al.* 2008; Shafer *et al.* 2012). Here, the number of random locations was decided upon simulations performed by Northrup *et al.* (2013). Their simulations showed that a minimum of 20 points per buffer was enough to provide reliable coefficient estimates.

Predictive landscape variables were measured on each used and random point. Prior to statistical analyses, skewed variables were transformed to approach normality and to reduce the influence of extreme values, using logarithmic transformations. Given the possible range of distance values exhibited by continuous variables, it is expected that they have a greater influence on w(x) scores on equation 1 or 2 than the forest predictor. To minimize this unbalanced scale effect between a categorical and continuous predictors, all variables were standardized using a z-transformation (Quinn & Keough 2002). Multicollinearity between predictors was assessed with Spearman correlation and variance inflation factors (VIF). Predictive variables were considered correlated and eliminated if they presented values of $| r |>0.5$ and VIF>3 (Zuur *et al.* 2009). The best combination of variables (best model) was determined through an information-theoretic approach (ITA), following criteria established by Burnham & Anderson (2002). First, univariate conditional logistic regression was applied to remove unimportant predictors ($p>0.25$; Hosmer and Lemeshow 2000). Models including the remaining important landscape variables (i.e. $p<0.25$) were considered in the candidate model set, if they presented a $\Delta AIC<2$ (difference of AIC between a particular model and the best model). The best global model would be chosen if it presented an Akaike weight (w_i)>0.9 alone (probability of being the best model), otherwise model averaging would be applied to all models in the set (Akaike weight sum (w_i)>0.9), in order to obtain the best average parameter estimates across all variables (Burnham & Anderson 2002). Conditional logistic regression was fitted using the "clogit" function from survival package in R, while model selection was accomplished using MuMIn package (R Development Core Team 2012). The final logistical equation (in the form of equation 2) was applied to each grid cell to estimate RSF scores. Given that there are not records of genets crossing large water bodies, a RSF score of 0 was assigned to each cell containing water bodies.

Predictive capacity of the top model was evaluated using 5-fold cross validation (Boyce *et al.* 2002). The data from the 21 individuals (used and available locations) was divided into five bins (classes) where each bin contained approximately 20% of the total dataset. Data across bins was assumed independent because points belonging to a single individual were not scattered into

different bins (Hirzel *et al.* 2006). Conditional logistic coefficient estimates were recalculated using 80% (four bins) of the dataset as training data while withholding the remaining bin as test data. This procedure was repeated until all bins were used as testing data. Five logistical equations were estimated and re-applied to each cell. Hence, five new suitability maps expressing probability of use were created. To examine top model performance, the approach proposed by Boyce *et al.* (2002) was employed. For each one of the five maps, RSF probability scores were divided into four equal intervals (from a probability of 0 to 1.0) and ranked from 1 (interval with lowest RSF values) until 4 (interval with highest probability use values). Using R software (R Development Core Team 2012), Spearman-rank correlation test was performed between rank categories and the respective area adjusted frequencies of used points. The area adjusted frequencies are calculated by simply dividing the number of used points falling in grid cells exhibiting a particular rank interval by the total area (expressed in number of cells) that a particular rank/category occupies in the suitability map. If correlation between ranks and frequencies is high, it means that used locations fall within higher ranked areas (more suitable areas) indicating that the best model calculated using all data has a good predictive performance. Usually, the number of chosen rank intervals is arbitrary (eg: Pullinger & Johnson 2010; Kunkel *et al.* 2013). Here, only four intervals were chosen since testing datasets were relatively small, especially compared to other studies employing GPS telemetry. If several classes of RSF scores were created, there would be a higher risk of having categories with no observations (even if they were suitable) due to data sparseness (Wiens *et al.* 2008). Accordingly, keeping four suitability classes (classes can be interpreted as low, medium, high and very high probabilities of use) was considered as reasonable choice that conciliates model accuracy and simplicity.

2.5.3-Landscape genetics analyses

The suitability map calculated with the top model's conditional logistic equation was converted into a resistance vectorial map. Resistance scores in each grid cell were calculated by applying a simple formula to RSF probability scores:

$$R=[1-w^*(x)]100 \quad (3)$$

where R is the resistance score and w*(x) is the RSPF score. The resistance values range from 0 to 100. The vectorial IBR map was rasterized with ArcGis 10 (ESRI 2011) and exported to Circuitscape version 3.5.8 (McRae 2006) using ArcGis tool "Export to Circuitscape" (Jenness Enterprises 2010). Circuit theory based algorithms such as the one implemented in Circuitscape were chosen due to the ability to account for multiple possible paths to estimate resistance between nodes (here they represent individuals; see section "1.2.2- Landscape genetics as a tool

to assess landscape functional connectivity"). Node's spatial coordinates were assigned based on the origin of the sample (roadkill or trapping) or the centroid of the home range (table S1). The options of "pairwise mode across all pairs" and connection scheme of eight neighbors was set to perform pairwise calculations. Two additional raster maps simulating models of IBD and IBB were created. For both models, despite its particularities, water bodies had always a maximum resistance value. For the IBD map, a value of 1 was given to each cell, allowing that pairwise resistance calculated between nodes is mainly dependent of the mean number of pixels separating nodes. The IBB model will be tested by hypothesizing that the highway is a barrier to gene flow (see Fig. 4 and Fig. 5). Accordingly, maximum resistance values were assigned to pixels containing the highway and 0 was given to the remaining cells, simulating a panmitic scenario in both sides of the highway. Since not aligned pixels (horizontally and vertically) that represent the barrier will allow movement without additional costs (this concerns diagonal movements if the option of connection with eight neighbors is chosen), a connection scheme of four neighbors was set in Circuitscape. Pairwise matrices of relatedness coefficients of Queller & Goodnight (1989) were calculated using GenAlEx 6.5 (Peakall & Smouse 2006, Peakall & Smouse 2012).

The pairwise resistance matrices obtained from Circuitscape were correlated with the matrix of pairwise genetic relatedness, following causal modeling on resemblance matrices applied by Cushman *et al.* (2006) to landscape genetics. A first preliminary assessment for model support was conducted by performing a correlation between each matrix model and genetic distances matrix using simple Mantel tests (Mantel 1967). Then, a second complementary assessment using partial Mantel tests was performed. Partial Mantel analysis correlates two matrices while controlling the effects of a third matrix (Smouse *et al.* 1986). Here, each supported model by the Mantel test was correlated with genetic distances, while removing the effects of other model. Hence, if a particular model (eg: IBR) remains significant after controlling the effects of an alternative one (for example IBB and/or IBD), then the model tested as a great independent support, being considered as the model where ecological distances best conform with observed genetic data. Mantel and partial Mantel tests were performed with 10000 permutations using the ecodist R package (R Development Core Team 2012). In order to corroborate analysis regarding the hypothesis of the highway as a barrier to gene flow (i.e. support for the IBB model), complementary statistical methods were employed. Following results obtained by (Blair *et al.* 2012) concerning software performance evaluation to detect barriers to gene flow, the spatial bayesian clustering software Geneland (Guillot *et al.* 2005) was also used to infer the effects of the highway. Runs were performed with a number of possible populations set to k=2 and with 500000 iterations with a thinning procedure of 200. The correlated allele frequency model was chosen and three independent runs were performed.

3-RESULTS

3.1-Samples and microsatellites variability

During multiplex optimization, markers Ggen2.A25, Ggen 2.A2 and Ggen 1.30 were discarded since they continuously failed to amplify. In total, 17 microsatellites were multiplexed in three reaction sets (Table 1) and used in subsequent genetic analysis. Two hair samples (table S1) failed to generate consensus genotypes for some markers and consequently were removed from all statistical analysis. Overall, the remaining samples were successfully genotyped for the 17 loci selected (0.7% of missing data). Statistics concerning genotyping errors are presented on Table 4. Allele dropout and false alleles were not detected for high quality samples except for markers A108 (ε_1=0.051) and Ggen 2.1. In multiplex reactions, Ggen 2.1 presented high dropout and false alleles rates (ε_1=0.171 and ε_2=0.060). After identifying possible problematic alleles through assessment of deviations from expected allele frequencies in GenAlex 6.5, all blood and tissue samples were re-genotyped again using only this locus. The same subset of samples was scored twice to calculate ε_1 and ε_2. Allele dropout and false alleles were still detected but at lower rates (ε_1=0.022 and ε_2=0.026). As expected, hair samples exhibited higher frequency of stochastic errors (overall ε_1=0.097 and ε_2=0.047). In general, loci presented null allele frequencies statistically not different from zero (overall r=0.016), excepting Ggen 2.1 (r=0.060; p=0.014). Considering the dropout issues and the likely presence of null alleles, this marker was removed, and thus a total of 16 microsatellites were used in the following genetic analysis. COLONY maximum likelihood algorithm identified 12 pairs of genets sharing full sibling (n=1) or parent/offspring (n=11) relationships (Fig. 7). The maximum distance between two related individuals (full siblings or parent/offspring) was superior to 15 km (maximum distance of 15.8 km and mean distance between the 12 pairs is 5.4 km). Additionally, COLONY also identified 116 half-sibling relationships (Fig. 8). Maximum distance between two half-siblings was 41 km, while the mean distance was 10.3 km. To perform equilibrium tests, seven individuals exhibiting parent/offspring or full sibling relationships were discarded (see table S1). No locus showed Hardy-Weinberg equilibrium deviations (even when incorporating all samples). The loci pair constituted by Ggen 4.10 and Ggen 3.3 exhibited linkage disequilibrium (p=0.000057), but after pruning related genets from the sample set, the comparison was no longer significant (p=0.0034 for a multiple comparison adjusted threshold α=0.0003). Overall, genetic diversity was high (Ho=0.662 and He=0.670) and inbreeding coefficient was relatively low (F=0.035).

Table 4-Diversity measures and genotyping error statistics of the 17 loci genotyped.

Locus	Na	ASR	Ho	He	F	ε1 (hq)	ε2 (hq)	ε1 (hair)	ε2 (hair)	r
A104	4	133-147	0.676	0.605	-0.111	0	0	0.084	0	0
A108	3	149-153	0.338	0.395	0.154	0.051	0	0.369	0	0.048
C101	5	283-303	0.662	0.690	0.040	0	0	0.215	0	0
D111	7	201-225	0.608	0.704	0.133	0	0	0.099	0.081	0.044
D4	5	225-261	0.703	0.738	0.050	0	0	0.106	0	0.008
Ggen 2.1*	11	242-282	0.747	0.853	0.130	0.022	0.026	0.178	0	0.060
Ggen 2.A16	8	191-219	0.784	0.770	-0.010	0	0	0	0	0
Ggen 4.10	10	197-233	0.797	0.852	0.070	0	0	0	0	0.038
Ggen 4.12	5	175-191	0.595	0.678	0.148	0	0	0	0.073	0.051
A110	7	271-291	0.549	0.510	0.048	0	0	0.207	0	0
A5	3	155-159	0.662	0.651	0	0	0	0.023	0.128	0.004
B105	5	170-188	0.527	0.573	0.077	0	0	0.004	0.137	0.012
Ggen 2.A13	4	172-184	0.658	0.659	0.029	0	0	0.104	0	0.002
Ggen 3.3	7	133-147	0.732	0.713	0.044	0	0	0.139	0.195	0.029
A112	3	217-245	0.662	0.586	-0.120	0	0	0	0	0
B104	7	321-329	0.903	0.824	-0.035	0	0	0.123	0.181	0
Ggen 2.A15	8	263-277	0.743	0.778	0.059	0	0	0	0	0.026
Overall[1]	5.69		0.662	0.670	0.035	0.003	0.000	0.097	0.047	0.016

Na – number of alleles; ASR – allele size range; Ho – observed heterozygosity; He – expected heterozygosity; F – Wright's inbreeding coefficient; ε1 (hq) – allelic dropout rate per heterozygote for high quality samples; ε2 (hq) – false allele rate per genotype for high quality samples; ε1 (hair) – allelic dropout rate per heterozygote for hair samples; ε2 (hq) – false allele rate per genotype for hair samples; r – null allele frequency;

* Locus exhibiting Hardy-Weinberg deviations and null allele frequencies significantly higher than zero.

[1] The locus Ggen 2.1, which deviated from HWE, was excluded from overall estimates.

3.2-Model selection and validation

In total, 19866 points comprising used (n=946) and random (n=18920) locations were used to perform a conditional logistic regression. The used dataset was constituted by 356 nocturnal locations (biangulations and triangulations), with 4 hours interval, and 590 diurnal resting sites locations (mean used locations per animal, $\bar{x}$=45.05). The number of observed records was much greater in forested habitat (n=808; mean used locations per animal in forest, $\bar{x}$=38.48) than agricultural areas (n=144; mean used locations per animal in agricultural fields, $\bar{x}$=6.86). Mean distance to landscape features was similar among the three variables tested (distance to riparian vegetation - $\bar{x}$=1.151 m; distance to water reservoirs - $\bar{x}$=1084 m; distance to human disturbance - $\bar{x}$=969 m). There wasn´t evidence of multicollinearity, therefore all predictive variables were retained for model selection (table S2 and table S3). Univariate analysis revealed that "distance to water bodies" variable was a poor predictor of genet presence locations (p=0.34) and

consequently, it was removed. Model selection revealed that the model with all remaining variables incorporated, significantly outperformed the others with a w_i=1 (see table 5 and 6). All variables in the model were highly significant (p<0.0001) and presented non-overlapping zero 95% confidence intervals, reinforcing their biological importance. See Fig. 9 and Fig. 10 for maps expressing probability of use and resistance to movement, respectively. Forests were significantly more selected than agricultural areas. Common genets also selected zones near riparian ecosystems but the opposite pattern was observed for man-made structures, as expected. Hence, for model validation, ranks 1 and 2 corresponded majorly to agricultural fields and areas distant from riparian vegetation, while higher ranks (3 and 4) comprised areas mainly constituted by forests and proximity with riparian ecosystems. Top model's predictive performance was reasonably good based on these 4 ranks (table 7). Used locations from bin 1 and 5 had a maximum correlation value (rho=1) with RSF rank, while testing data from bins 2 and 3 presented a relatively high correlation (rho=0.8) but it was not significant. Model 4 was the only model that presented a poor correlation value (rho=0.6). Nevertheless, considering that 4 out of 5 models presented high correlation values, it was considered that the top model has relatively high predictive performance (nevertheless see section "4.2.1- Best model and RSF validity").

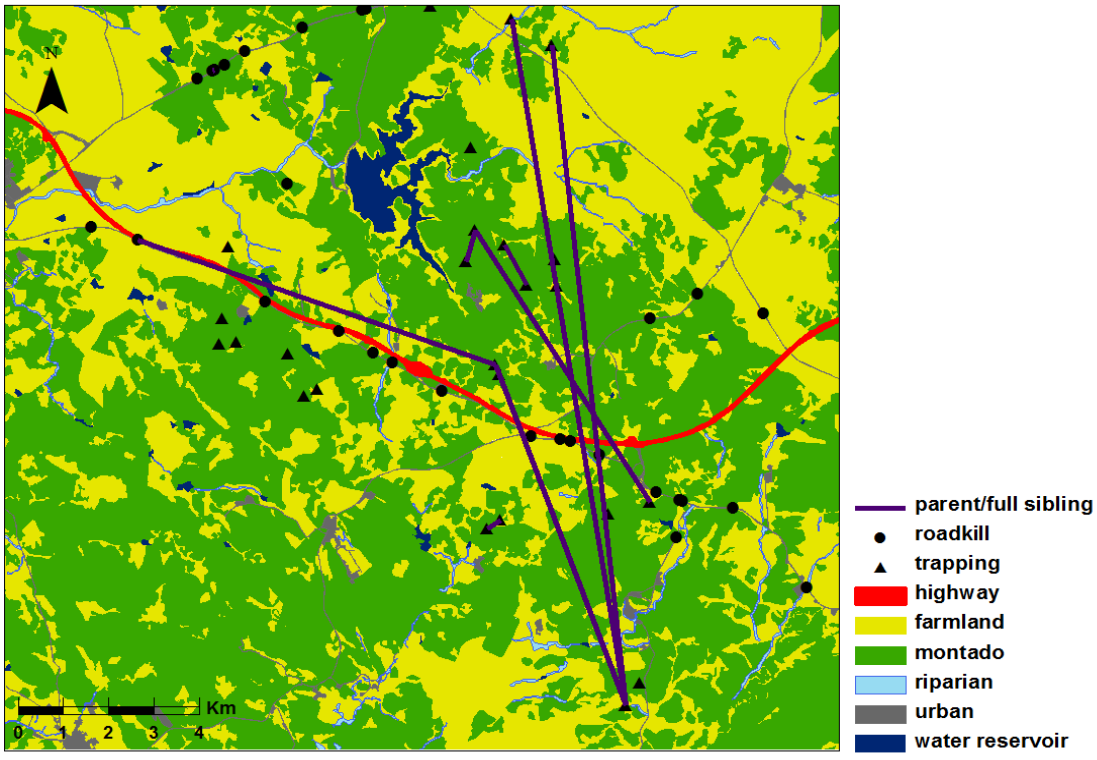

Fig.7- Representation of all parent/offspring and full sibling pairs estimated in COLONY. Note that related individuals are in different sides of the highway, revealing that the highway was successfully crossed probably during dispersal events.

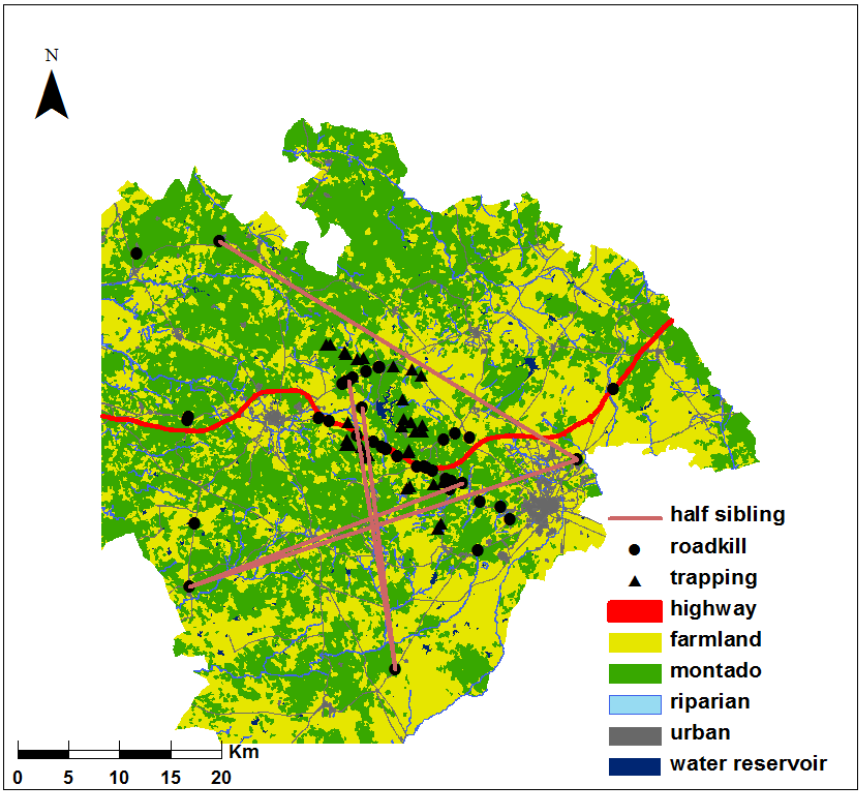

Fig.8- Representation of five half sibling relationships exhibiting the highest pairwise geographic distance.

Table 5- Model ranking based on ΔAIC and Akaike weights (w_i).

Rank	Model topology	AIC	ΔAIC	w_i
1	hab(forest)+dist_rip+dist_human	5589.542	0	1
2	hab(forest)+dist_rip	5606.351	16.809	0
3	hab(forest)+dist_human	5662.620	73.078	0
4	dist_rip+dist_human	5669.842	80.300	0
5	hab(forest)	5677.540	87.998	0
6	dist_rip	5690.184	100.642	0
7	dist_human	5742.996	153.454	0

Table 6-Top model conditional logistic regression parameters.

Variable	β	Exp(β)	SE	P	95% CI
hab(forest)	0.374	1.454	0.044	0	(0.288, 0.461)
dist_rip	-0.327	0.721	0.035	0	(-0.395, -0.258)
dist_human	0.227	1.254	0.056	<0.001	(0.117, 0.336)

Exp(β)- exponential coefficients; SE- Standard error; 95% CI- coefficient 95% confidence interval;

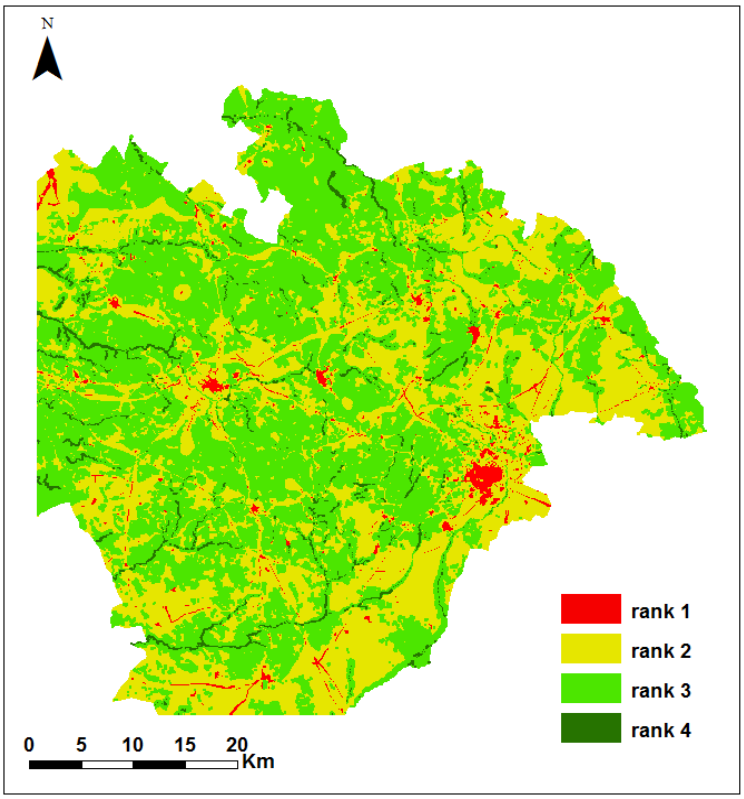

Fig.9- Suitability map constructed using conditional logistic equation from the top model. To facilitate map representation, each rank represent one quartile of probability of use (rank 1 – [0-0.25]; rank 2 – [0.26-0.50]; rank 3 – [0.51-0.75]; rank 4 – [0.76-1]). Greener areas represent *montado* forests and riparian corridors.

3.3-Mantel and partial Mantel results

The IBD (Mantel r=-0.129; p<0.001) and IBR (Mantel r=-0.110; p<0.001) models presented significantly negative correlations with genetic relatedness matrix, despite the relatively low Mantel r coefficients (table 8; see also Fig. 11 and Fig. 12 which represent output maps from Circuitscape). Negative correlation coefficients indicate that individuals with lower pairwise relatedness coefficients, are separated in general by higher pairwise effective resistance distances. The IBB model (Mantel r=-0.002; p=0.915) was not significant and consequently it was discarded from partial correlation analysis. This result is corroborated by Geneland which showed no genetic evidence for population structuring caused by the highway (Fig. 13). Partial Mantel tests (table 8) revealed that the IBD model (Mantel r=-0.07; p<0.001) was the most statistically supported model, because it was still significant after controlling the effects of the IBR model. The opposite situation was not verified, since the IBR model (Mantel r=0.01; p=0.642) was no longer significant after controlling for the effects of the IBD model.

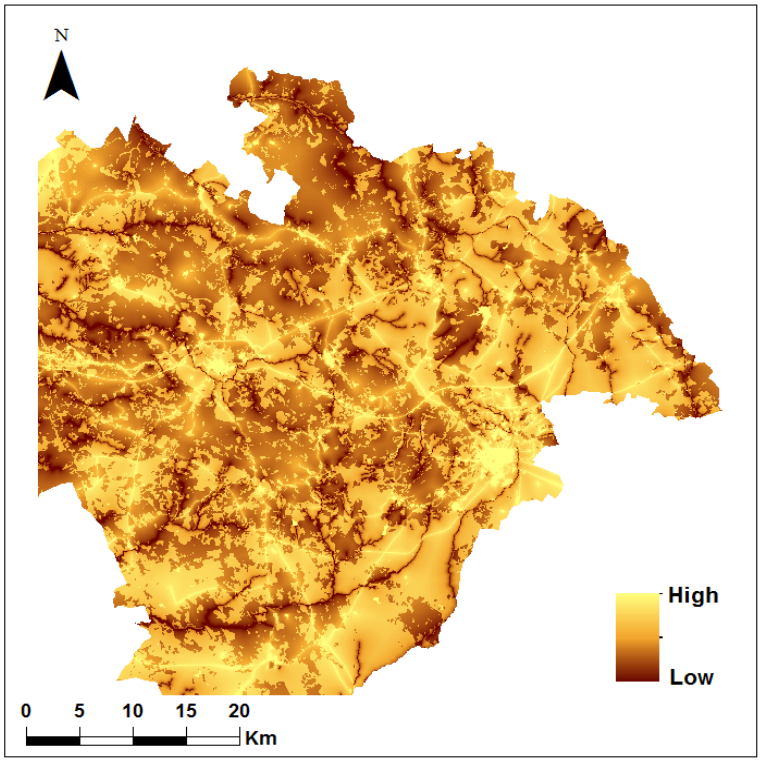

Fig.10- Resistance map constructed using conditional logistic equation from the top model. Whiter areas present higher resistance values and are mainly correspondent to urban areas and roads. Darker areas represent suitable areas that have low resistance values such as *montado* forests and riparian corridors.

4-DISCUSSION

4.1-Microsatellite performance

Several studies have reported a broad interval of rates in microsatellite genotyping (Pompanon *et al.* 2005; Beja-Pereira *et al.* 2009). Given the lower quality and quantity of DNA, it is expected that non-invasive genetics samples (such as hairs) will present higher error rates. Despite being recorded occasionally low error rates such as 2% (Bonin *et al.* 2004), genotyping errors superior to 10% (Broquet *et al.* 2007) and 20% (Gagneux *et al.* 1997; Johnson & Haydon 2007) can be frequently registered. This issue is less noticeable when using high quality samples (Bonin *et al.* 2004; Pompanon *et al.* 2005), despite that great dropout rates have been registered on special cases (Soulsbury *et al.* 2007). Consensus regarding the establishment of a critical error threshold has not been reached, where some argue that even low genotyping errors levels such as 1% may introduce significant biases (Hoffman & Amos 2005), while others advocate that an error <2% is acceptable (Bonin *et al.* 2004). Trying to establish a critical acceptable threshold constitutes a dubious approach once the severity of the effects that errors cause is conditioned by the scientific goals of

Table 7- Model 5-fold cross validation using Spearman-rank correlation test (rho).

Model	Rank	frequencies	Rho	p
Partition 1	1	0.00000	1.0	0.04
	2	0.00050		
	3	0.00100		
	4	0.00383		
Partition 2	1	0.00071	0.8	0.17
	2	0.00064		
	3	0.00081		
	4	0.00385		
Partition 3	1	0.00000	0.8	0.17
	2	0.00045		
	3	0.00120		
	4	0.00081		
Partition 4	1	0.00081	0.6	0.21
	2	0.00057		
	3	0.00101		
	4	0.00092		
Partition 5	1	0.00000	1.0	0.04
	2	0.00039		
	3	0.00114		
	4	0.00230		

Table 8-Mantel and partial Mantel correlation results.

Model topology	Mantel r	P
G ~ IBD	-0.129	<0.001
G ~ IBB	-0.002	0.915
G ~ IBR	-0.110	<0.001
G ~ IBD | IBR	-0.070	<0.001
G ~ IBR | IBD	0.010	0.642

G- genetic distances;
The | symbol separates the genetic and model matrices from the covariate matrix. For example, G~IBR | IBD means that a partial Mantel test was performed between G and IBR model, while removing the effects of the IBD matrix.

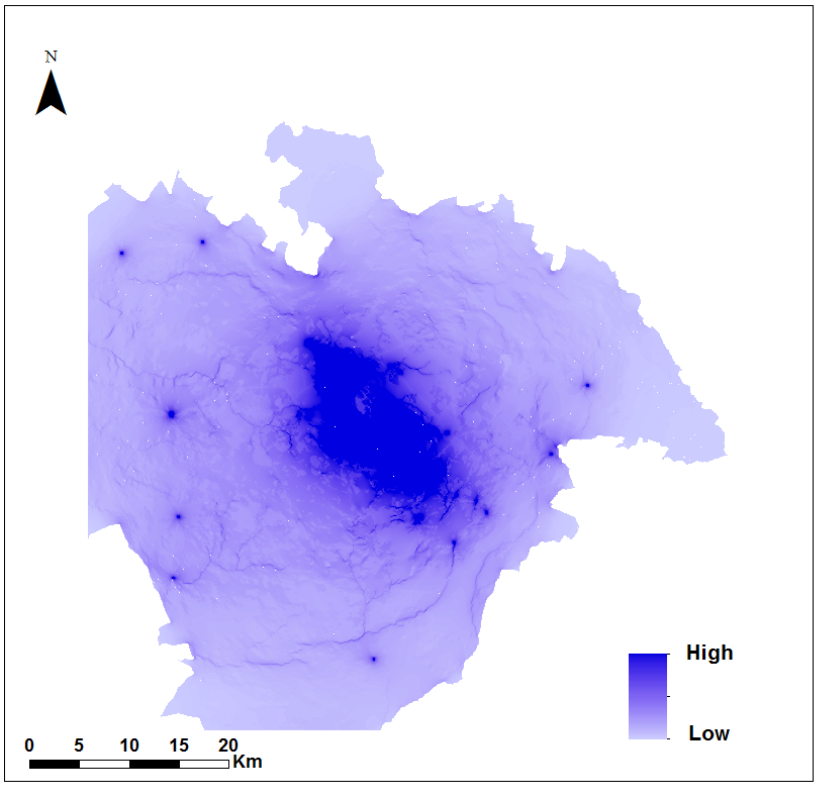

Fig.11- Current map created by Circuitscape for the IBR model. Dark blue areas represent higher values of current (i.e., areas highly permeable to movement) and lighter areas represent low values of current (i.e., with lower probability of being crossed by a random walker).

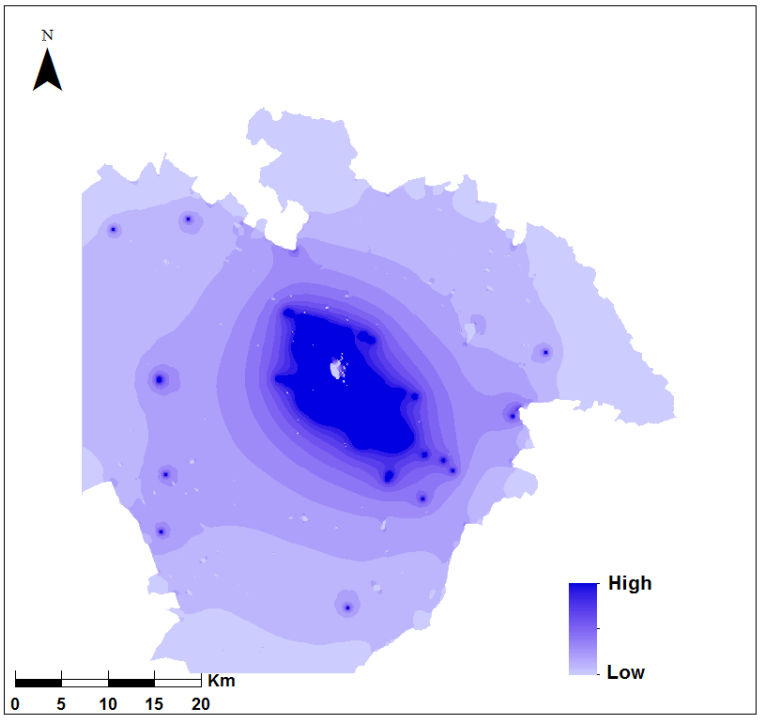

Fig. 12– Current map created by Circuitscape for IBD model. Similarly to Fig. 11, darker areas correspond to high current zones, while lighter pixels represent low current areas.

a particular study (Taberlet *et al.* 1999). Consequently, each particular case must be careful analyzed.

In this work, hair samples presented high allele dropout rates, where eight markers exhibited more than 10% (table 4). This highlights the importance of conducting quality procedures to minimize errors in low quality samples. The protocol used here to control hair genotyping errors was similar to the ones employed in another studies (Frantz *et al.* 2004; Mullins *et al.* 2010). Given that three replicates were performed for homozygotes, allied with the removal of problematic hair samples and the use of protocols that diminish dropout (Goossens *et al.* 1998), it is believed that the presence of dropout in hair multilocus genotypes was minimal. A third allele was scored on seven occasions among replicates, increasing false allele rates. Scored genotypes were completely distinct, i.e. did not involve adjacent alleles, which allows to discard miscoring due to stutter patterns (Dewoody *et al.* 2006). Since third replicates unambiguously corroborated all the second replicates, it is possible that a human factor (possibly contamination) during the first genotyping contributed for the observed "extra alleles" (Hoffman & Amos 2005). It is hard to ascertain the possible cause, but third replicates likely reduced the possibility of false alleles. High quality samples showed little genotyping error rates. This confirms the expectations that for these types of samples, less rigorous quality control protocols such as re-genotyping of a subset of samples can constitute a valid tool when logistical resources are limited (Dewoody *et al.* 2006). Nevertheless, two markers presented error rates higher than zero in high-quality samples: Ggen 2.1 and A108 (table 4). The latter marker A108 had a concerning dropout rate >5% for this kind of samples. From the 23 high quality samples re-genotyped, only one had a genotype not concordant with the first scoring. The reason for the relative high rate of allele dropout present by this marker may be related to the way that error rates were estimated. Allele dropout per heterozygote calculations are weighted by the proportion of available heterozygotes (Wang 2004; Johnson & Haydon 2007). Accordingly, if there is a low proportion of heterozygotes (A108 presented a Ho=0.333), ε_1 errors will be inflated by each heterozygote where an allele fails to amplify. Additionally, given the high quality sample and the small product size generated by A108 (Sefc *et al.* 2003; Hoffman & Amos 2005), here it was considered that this marker had an insignificant impact in the multilocus dataset. Initially, marker Ggen 2.1 exhibited very high levels of dropout in multiplex for tissue and blood samples. Performing singleplex reactions twice for all samples allowed the discovery of problematic alleles, decreasing the dropout rate to about 2%. However, and given the high expected heterozygosity presented by Ggen 2.1, there was a homozygote excess that caused deviations from HW equilibrium, even if related individuals were experimentally removed from equilibrium analysis. A significantly different from zero null allele frequency (accounting for inbreeding using software INEst) was detected, constituting another possible explanation for the observed homozygote excess. In this particular case, knowing if this marker was only affected by dropout (and consequently null allele presence is considered as a "false

positive" result caused by high rates of dropout) or if it was affected by both processes is hard to ascertain. Soulsbury *et al.* (2007) reported similar problems regarding allele dropout for specific loci amplified on high quality samples, supporting the idea that more research is needed concerning this problematic. Clearly, the most viable option was to remove this marker from posterior statistical procedures.

Marker polymorphism constitutes a key characteristic to retain genetic signatures caused by recently emerged landscape features that negatively affect dispersal (Wang 2010). Here, markers exhibited a moderately high heterozygosity levels and moderate allele numbers (mean allele number disregarding marker Ggen 2.1 was 5.69; number of alleles ranged from three to ten), considering the sample size and the small spatial scale that most individuals were sampled. Simulation analysis performed by Landguth *et al.* (2012) revealed that allele number is fundamental to increase statistical power for partial Mantel tests. However, it can be compensated by employing a high number of markers. Other landscape genetics studies conducted at similar spatial scales and using genetic markers with comparable diversity values (mean number of alleles ranged from 5.4 to 7.5 among the cited studies), had resolution enough to assess the effects of landscape features on gene flow (Hepenstrick *et al.* 2012; Apodaca *et al.* 2012; Koen *et al.* 2012). Common genets have great dispersal ability and short generation times (2 years), which are biological features that increase the power to reliably detect recent genetic signals (Landguth *et al.* 2010; Landguth *et al.* 2012). Accordingly, based on the procedures used here to minimize genotyping errors and on the comparable diversity values found on previous studies, it is unlikely that landscape genetics analysis was compromised by a high genotyping error rate or due to hypothetical insufficient polymorphism levels.

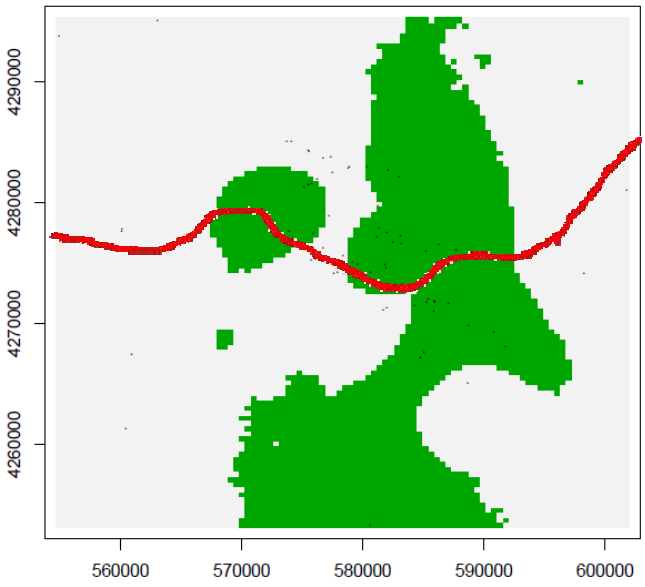

Fig. 13 – Estimated cluster membership in Geneland. X and Y axis represent UTM coordinates. Given that the highway cross the area in a west-east axis, it is visible by this figure that the population is not structured by the highway. Small black dots represent samples locations.

4.2- Landscape genetics analyses

4.2.1-Best model and RSF validity

The match case-control design employed here detected landscape variables that were positively associated with the presence of genets, constituting a result in accordance with previous studies conducted in Mediterranean environments (eg: Larivière & Calzada 2001; Galantinho & Mira 2009; Matos *et al.* 2009). Genets significantly selected for forest cover over agricultural areas, which confirms the previous importance given by other researchers to forest habitats (including riparian corridors) on meeting food, shelter, reproductive and hydric requirements (Galantinho & Mira 2009; Santos *et al.* 2011; Camps & Alldredge 2013). On the contrary, it has been advocated that agricultural habitat types such as vine yards, pastureland and farmland in general lack proper native shrub and tree cover, presenting low availability of resting sites and food resources (Pereira & Rodríguez 2010; Camps 2011). A low distance to the nearest urban features was a variable that was associated with less used locations. Again, this result meets previous expectations that common genets avoid urban areas (Galantinho & Mira 2009; Camps & Alldredge 2013). It is known that anthropogenic areas with high disturbance levels (eg: roads, villages) may repel carnivores within a "buffer-disturbance zone", reducing the functional available habitat in the vicinity of these areas (eg: Forman & Deblinger 2000; Crooks 2002; Ditchkoff *et al.* 2006). Finally, the variable "distance to water bodies" was the only predictor that was not significantly related to genet's presence. Water in riparian ecosystems are restricted to small pools during the summer (Matos *et al.* 2009; Santos *et al.* 2011). Considering the harsh conditions faced by species in Mediterranean areas during the dry season, additional sources of a limiting resource such as water can be valuable. Two reasons could have contributed for the non-significant results obtained for this variable. First, if water reservoirs are indeed selected by genets (or other carnivores), then a positive selection would be expected only during the dry season. Some studies managed to analyse their data by constructing different models for each season (eg: Shafer *et al.* 2012; Squires *et al.* 2013). Confronting different temporal models (eg: summer vs winter) is appropriate to assess habitat selection in regions where there is a relevant temporal contrast of resource availability (McLoughlin *et al.* 2010). However, in the current work, the number of observed locations on dry season only comprised about 30% of the total dataset used in the logistic equation. Accordingly, and given the small dataset used, partitioning the data into two seasonal small unequal subsets could have introduced important bias on equation's coefficients (Rice *et al.* 2013). The second reason is concerned with an intimate connection between human water use and agricultural practices. Water reservoirs in Mediterranean areas play an important role in agriculture, where water use is intrinsically associated with culture irrigation and water source for cattle. The presence of unsuitable habitat such as irrigated cultures nearby water reservoirs and cattle herding, will likely difficult the access of genets to these landscape elements (Brotons *et al.* 2004; Mestre *et al.* 2007; Pita *et al.* 2009).

Model evaluation was performed using a common approach: k-fold cross validation. This approach is especially useful when one does not possess independent data to validate the estimated landscape model (Boyce *et al.* 2002; Chetkiewicz & Boyce 2009; Kunkel *et al.* 2013). In this validation three out of five data subsets failed to give significant results despite presenting relatively high correlation coefficients (table 7). The third partition did not presented significant correlations because areas with high use probabilities (rank 3) presented a higher frequency of used points than more suitable zones (rank 4). Nevertheless, the higher frequencies were detected at the most suitable ranks and thus, it was considered that this bin was acceptable (it also presented a Spearman-rank correlation of 0.8). The other two partitions (partition 2 and partition 4) exhibited non-significant correlations due to high frequencies on rank 1 (unsuitable areas). In both cases, results were largely affected by one single animal which represented special cases. The testing dataset from partition 2 contained one subadult individual that comprised about 70% of the total observations in rank 1 habitat, contributing for the observed high frequency on this rank. Additionally, the animal held a home range where 70% of area has probability suitability scores inferior to 0.5 (rank 1 and rank 2). Due to intraspecific competition, it is common that sometimes subadults are forced by adults to establish their territories in suboptimal areas (Mergey *et al.* 2011). This particular individual also represented 73% of the observations in rank 4 habitat. Accordingly, despite being surrounded by unsuitable habitat, its territory also comprised highly suitable areas, probably being fundamental for the subadult's survival. In partition 4, the majority (about 70%) of observed records in unsuitable habitat (rank 1) belong to an adult female. Indeed, this female foraged often in agricultural areas during the radio-tracking period, probably taking advantage during the winter of a greater abundance of migratory birds such as lapwings (*Vanellus vanellus*) in farmland habitats (Moreira *et al.* 2005; Sánchez *et al.* 2008; Carvalho pers. observation). Doing a simple modelling exercise, by removing these two cited individuals, predictive performance in partition 2 and 4 rises to significant maximum values (rho=1). Accordingly, it was considered that the general model had a good predictive ability in the study area.

4.2.2-Isolation-by-barrier

Matching initial expectations, the barrier model presented the lowest fit with genetic data. The results presented by the IBB model are corroborated by parental analysis (Fig. 7) and Geneland results (Fig. 13), reinforcing the little genetic effects caused by this linear infrastructure. From radio-tracking data, it was observed that all home ranges from animals living nearby were bounded by the highway, with almost none locations obtained in the opposite side of the highway, reinforcing at least the behavioural barrier effect (see Fig. 4 and Fig. S1; Carvalho *et al.* in prep.). This observation gives some clues about the permeability of this particular feature, being apparent that it interferes with movement to some degree. Disrupting movement and gene flow is fundamentally dependent of species and barrier attributes (Holderegger & Giulio 2010). The

behaviour exhibited towards roads (road avoidance, car avoidance) plays an important part in determining species ecological and genetic sensitivity towards this phenomenon (Fahrig & Rytwinski 2009; Jackson & Fahrig 2011). On the other hand, the width of the physical structure, traffic volumes, barrier's age and presence of movement conductors (eg: culverts) may determine the overall effects that a given linear feature may exert on a species (Jaeger *et al.* 2005; Jaarsma *et al.* 2006). Empirical studies showing the genetic effects of particular barriers have been published (eg: Riley *et al.* 2006; Zalewski *et al.* 2009; Frantz *et al.* 2010). However, there are important differences regarding infrastructure characteristics. The highway analyzed in this study presents a mean monthly daily traffic of about 6400 vehicles (InIR 2011) and a width of 30 m and thus, these characteristics are not comparable with previous research: (1) it has lower width and considerably lower traffic volume (for example daily traffic volume is 15 times lower than the motorway assessed in Riley *et al.* 2006); (2) it is crossed by a large number of culverts and viaducts and it does not have tall fences, which increase permeability for wildlife movement (Ascensão & Mira 2007; Hepenstrick *et al.* 2012); (3) the lag time (time between barrier construction/formation and the genetic response) is also an important factor to be considered (Landguth *et al.* 2010). Since its construction, only about 7-8 generations in common genets have experienced highway effects, which may be not sufficient time for the population to genetically respond to the new disturbance feature. This highlights the temporal disconnection between movement data and population genetic responses (Anderson *et al.* 2010; Spear *et al.* 2010). Once movement data describes patterns during a specific period of time where they were measured, population genetic structure has a lag time response towards the changing landscape. Hence, the combination of the three factors previously mentioned has likely contributed for the absence of a genetic response, suggesting that immigration and genetic connectivity between northern and southern areas are not seriously affected by this highway, at least at short term (Mills & Allendorf 1996; but see also Vucetich & Waite 2000). Nevertheless, the eventual small effects on genets of this particular barrier may not be applicable to other carnivore species. Other medium-sized carnivores presenting smaller population sizes such as polecats (*Mustela putorius;* Cabral *et al.* 2005), may experience more rapidly the barrier effects caused by this particular structure. On these species, overall population genetic diversity may decrease much faster, not only due to the barrier effects mediated by road mortality, but also through home ranges pile-up which increases the difficulties for dispersing individuals to establish a territory, and consequently the mortality rate may grow (Riley *et al.* 2006; Holderegger & Giulio 2010). Reduction in genetic diversity may decrease population fitness and cause a loss of evolutionary potential (Frankham 2005). Future monitoring of highway effects may be advisable for these sensible species.

4.2.3-Isolation-by-distance and IBR model performance

Despite IBD and IBR models being significantly supported by Mantel tests, partial Mantel analysis depicted a higher independent support for a pattern of isolation-by-distance. The partial Mantel results go against to what was initially hypothesized. Direct spatial measurements of movement obtained in telemetry studies only document that an individual travelled a particular distance through particular habitat(s). Spatial and temporal scales at which both processes operate can be completely different, possibly leading to incongruence between gene flow and dispersal (Anderson *et al.* 2010; Spear *et al.* 2010). For example, movement reflects contemporaneous patterns of dispersal, where gene flow represents historical dispersal events in the landscape during multiple generations. Thus, assessing connectivity with different types of data (genetic or movement) may yield different results. Additionally, biological features such as sex, life stage and other specific traits from a given species may present different patterns of dispersal and gene flow, and failing to integrate that data could hamper the results obtained (Coulon *et al.* 2004; Fedy *et al.* 2008). Therefore caution must be taken when using empirical movement data. This issue is fairly discussed for example by Spear and colleagues (2010) in the scope of resistance surface parameterization. So far, studies that managed to combine landscape genetics and empirical dispersal data assumed that the latter is an indirect surrogate of gene flow (Shafer *et al.* 2012; Reding *et al.* 2013). This assumption is legit several times, once both metrics are generally correlated (Bohonak 1999) and the landscape variables selected for foraging and other ecological activities likely represent in most cases, suitable area that is used by a species for reproductive purposes (being informative of landscape permeability to gene flow) (Beier *et al.* 2008; Cushman & Lewis 2010; Baguette *et al.* 2013). Based on previous studies in Mediterranean environments, there is no reason to reject the hypothesis that the most suitable habitats, such as forest and riparian corridors, are in general habitats used by genets (males and females) for reproductive and survival purposes (Larivière & Calzada 2001; Barrientos 2006; Camps & Alldredge 2013; Filipe *et al. in prep;* but see below). However, if the RSF represents realistically habitats conductive or resistant to gene flow, why the IBR model performed worse than the IBD model? Several explanations or their combination could explain this result.

The most likely contributing factor that explains lack of support of the landscape resistance model is derived from sampling scheme. The fact that samples locations were obtained from locations not specifically adjusted for this particular study possibly hampered a proper investigation of the role of habitat on gene flow. The majority of samples are clustered in a northwest-southeast strip of relatively contiguous *montado* forest with about 20 km of length (Fig. 5). Accordingly, pairwise node calculations between those individuals involve mainly suitable forested areas (*montado*), don't giving the chance to test the effects of other low permeable landscape features. Recently, this was shown by Oyler-McCance *et al.* (2013) where they performed simulations and detected that clustered sampling designs are not adequate for landscape genetics studies.

Additionally, by joining the current map from IBR model (highly permeable areas are concordant with the corridor; Fig. 9) and genet's great dispersal ability, as corroborated by radio-tracking data (in the study area mean daily dispersal was about 2.5 km, while maximum dispersal distance was about 10 km; Carvalho *et al.* in prep) and the distance between related individuals (Fig. 7 and Fig. 8), this issue in the sampling design becomes more evident. Thus, the combination of these factors likely contributed for highest support for the IBD model. Other studies also found more support for the IBD model (Broquet *et al.* 2006; Koen *et al.* 2012). In those studies, despite the distribution of samples was not clustered, sampling was performed across areas strongly covered by suitable habitat, promoting high gene flow rates. In those cases, the population genetic differentiation becomes mainly dependent of the geographical distance separating individuals, partially being similar to what happened in this study.

Another feature that may have contributed for the results obtained is the importance of riparian ecosystems as enhancers of dispersal. The food and water supplies provided and the availability of resting sites, make riparian areas important areas for carnivore survival, even if surrounded by an inhospitable matrix (Virgós 2001; Matos *et al.* 2009; Santos *et al.* 2011). Probably the best study that empirically demonstrates the value of linear corridors for dispersal in agricultural mosaics was conducted in Spain by Pereira & Rodríguez (2010). Despite the higher density and use of other linear habitat features such as hedgerows, their study illustrates well the importance that riparian corridors may present in assuring functional connectivity. The large use of riparian corridors by the genet may allow them to perceive fragmented habitat as functionally continuous if forest areas are connected by these elements. If this is the case, then genes "movement" is mainly determined by geographical distance in patches separated by farmlands but also connected by riparian ecosystems. Riparian habitat is extensive and well preserved in the study area. These areas are also accounted as highly permeable habitat features by the RSF model, emphasizing their biological and ecological importance. Furthermore, in the study dataset, two dispersers walked significant distances (approx. 3km and 7km, respectively) in these corridors surrounded by farmlands, constituting additional evidence of the importance of linear elements in Mediterranean agro-forestry systems (Carvalho *et al.* in press.). However, this connectivity pattern may be poorly represented by the multi-path algorithm implemented in software Circuitscape. The level of pairwise resistance assigned by the software is proportional to the number of pathways connecting two nodes (McRae 2006). Assuming that both nodes (individuals) are best connected by a single optimal path (riparian corridor) integrated in an inhospitable matrix, the overall effective distance between them may be overestimated since the number of possible paths providing inter-node connectivity are limited to the cells containing low resistance (i.e. represented by riparian vegetation) (see also Spear *et al.* 2010). Hence, circuit theory can perform worse than LCP on these particular cases, contributing for a lower performance of the IBR model (McRae 2006).

A third limitation of the approach employed here concerns with the number of variables used. It is possible that the final IBR model could be improved by calculating a RSF relying on a different set of landscape variables (Zeller *et al.* 2012). Obtaining a balanced trade-off between model simplicity and fitness is crucial for model selection and validation procedures, once complex models may cause model overfitting (Burnham & Anderson 2002; Wiens *et al.* 2008). The variables used here are undoubtedly important in genet's biology and ecology. However, the topology used here to represent them may not have been the ideal one. In general, Mediterranean carnivores and especially common genets, largely favour forested areas with a dense understory cover (Pita *et al.* 2009; Galantinho & Mira 2009). The presence of shrubs promotes the presence of rodent species such as the wood mouse (*Apodemus sylvaticus*), one of the preferred prey item of genets (Virgós *et al.* 1999; Rosalino *et al.* 2011). Some *montado* areas are managed for economical activities where ground cover is grazed and ploughed (Pinto-Correia & Mascarenhas 1999). The removal of a shrub layer may reduce shelter and food resources, being determinant for the use of these areas by carnivores. Unfortunately, vectorial data discriminating both types of montado (with and without understory shrubs) was not available because part of these areas is highly dynamic and may have strong seasonal and interannual changes (Pinto-Correia & Mascarenhas 1999; Acácio *et al.* 2009); therefore the effects of different types of shrub ground cover for dispersal and genetic connectivity may be not completely clear. Additionally, radio-tracking records showed that on specific situations, a habitat-matrix (forest-agricultural fields) paradigm does not fully represent how genets perceive the surrounding environment (Carvalho *et al.* in press.). The use of marginal agricultural fields for foraging activities by carnivores was documented on another conservation studies (Gehring & Swihart 2003; Šálek *et al.* 2010) and consequently, resistance in peripheral forested areas could be overestimated. This ecological tolerance buffer from forest edges can make a crucial difference between two in close range forest patches being functionally connected or not, implying that in practice, areas with low levels of fragmentation may be perceived as homogeneously suitable (Thornton *et al.* 2011; Driscoll *et al.* 2013). Similarly, not decomposing human disturbance into different components (eg: roads or urban centres) could also have decreased the statistical power of the IBR model. Here, the variable "distance to human disturbance" included all types of anthropogenic features. Preliminary exploratory analysis (not included in this previous work), showed that modelling this variable in function of human disturbance instead of the distance to urban centres or roads, consistently presented higher model support (lower AIC values) in modelling habitat selection. However, not accounting for example that different types of roads (eg: national roads, municipal roads and highways) present different structural properties (eg: traffic volume, road width; Jaarsma *et al.* 2006; Fahrig & Rytwinski 2009) could have created artificially high resistance areas in low travelled roads. Nevertheless, high resistance values erroneously attributed to municipal roads can be a lesser problem, once few nodes were separated by municipal roads. Similarly, and considering the present results where it was demonstrated that this

highway did not constitute a significant barrier to movement, genets exhibited similar behaviours (at home ranges level since they were bounded by these features) between the highway and the roads. Hence, actual resistance that the highway imposes to movement may not be much greater than national roads. Accordingly, this last issue regarding human disturbance may had lesser effect than the other two hypothetical variables.

The RSF model did not perform as expected likely due to the reasons enumerated in the previous paragraphs. Still, some considerations concerning RSFs statistical framework must be also taken into account. Procedures such as representation of habitat availability and model evaluation have greatly relied on subjective decisions, and only more recently, scientific debate concerning these issues arose in order to increase RSF performance (eg: Wiens *et al.* 2008; Northrup *et al.* 2013). Additionally, inferring movement (and indirectly gene flow) from RSFs relying on point data (as used here) instead of pathway data (and thus inferences can be made from direct dispersal) may have decreased IBR model's accuracy (Zeller *et al.* 2012). Robust pathway data can be much more easily obtained with GPS telemetry (Coulon *et al.* 2008; Reding *et al.* 2013), equipment which was too expensive to be used here. Additionally, accounting for inter-individual habitat selection can also be important to model resistance and genetic connectivity. Life stage (subadults, adults or juveniles) or ecological niche variation of particular individuals comparatively with the global population, as shown by this study, can alter model performance (Spear *et al.* 2010; Mergey *et al.* 2011; Araújo *et al.* 2011). Mixed modelling has been considered as a valuable tool in Ecology to deal with inter-individual variation of a particular measured variable, through the addition of random effects which confer several advantages over standard fixed effects methods (Zuur *et al.* 2009). Recently, mixed effects conditional logistic regression has been accounted as a valid extension from standard conditional logistical regression (Duchesne *et al.* 2010). This statistical method can be especially useful to deal with inter-individual variation in habitat selection, as well with unbalanced datasets between different individuals. Hence, the RSF equation estimated can reflect more realistically the permeability of the landscape to animal movement, and indirectly, assess more reliably genetic connectivity. However, this statistical method is very recent and literature regarding this method is scarce (see also Craiu *et al.* 2011). Consequently, technical information, model selection procedures and software to perform this kind of analysis is still poorly available and highly complex for ordinary modelling.

4.3-Final remarks and future prospects

The present study constituted one of the first landscape genetics studies conducted in Mediterranean landscapes. Trying to understand how medium-sized forest carnivores are affected by the transformation of Mediterranean forests into agricultural landscapes is crucial to implement conservation programmes aiming their persistence. Here, it was intended to assess how gene flow

was affected in agro-forestry systems by joining two strongly conceptually related fields: habitat selection and landscape genetics. So far, few authors managed to take advantages of combining both research fields. In fact, as Spear *et al.* (2010) pointed out, one of the attractions of landscape genetic studies is exactly the possibility of obtaining reliable connectivity information, without experiencing the logistical and hard working troubles of getting non-genetic empirical data. Nevertheless, that does not imply that genetic data and field data should be used separately. In this study, through the use of genetic and movement data, important information regarding the highway as a barrier was retrieved. Despite pointing to opposite results, it does not mean that they are incompatible. In fact, by complementing both types of data, it was clear that the highway does not constitute an impermeable barrier to gene flow although some animals were affected by it. Concerning the lack of support for the IBR model, possible issues related with sampling design may have hampered the utility of RSF as a tool to parameterize resistance surfaces in this study. However, this methodological bias does not necessarily invalidate the utility of RSFs in landscape genetics, but clearly limits the information that can be retrieved from the genetic data. However, important hints can be extrapolated from the results obtained. The weak pattern of isolation-by-distance detected, probably caused by the overrepresentation of individuals in an area limited forested contiguous patch, reinforces the importance of this type of habitat for connectivity in genets and probably for other sympatric forest mesocarnivores with similar ecological requirements (Santos-Reis *et al.* 2004; Mestre *et al.* 2007; Santos *et al.* 2011). The results are partially limited by some sampling bias derived by the use of opportunistic samples gathered in the framework of a larger study. Thus, the present conclusions should be interpreted with careful. Moreover, this study is illustrative of the importance of fully taking into account species biological and ecological characteristics, and include them in a spatiotemporal context that is adequate to design an unbiased sampling scheme (Graves *et al.* 2013). By accomplishing this, and integrating new improved statistical methods from habitat selection studies such as modelling of pathway data (through increasingly available GPS telemetry) and mixed effects conditional logistic regression (Duchesne *et al.* 2010; Zeller *et al.* 2012), RSFs can prove to be excellent tools which complemented with landscape genetics, may help us understand the patterns of genetic connectivity in Mediterranean agro-forestry systems. Getting more information about genetic connectivity patterns of carnivores in Mediterranean environments, may greatly increase the effectiveness of a future defragmentation measures that eventually will be needed in these landscapes.

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6-SUPPLEMENTARY MATERIAL

Table S1-Information about samples used on genetic analysis.

ID	Sex	Type	Source	Circuit_coord	UTM X	UTM Y
MG2	male	muscle	Roadkill	roadkill site	575787	4281626
MG3	female	muscle	roadkill	roadkill site	585927	4271838
MG4	female	muscle	roadkill	roadkill site	583250	4273209
MG5	male	muscle	roadkill	roadkill site	588657	4265063
MG6	male	muscle	roadkill	roadkill site	585842	4271007
MG8	female	muscle	roadkill	roadkill site	585210	4275953
MG9	male	muscle	roadkill	roadkill site	575172	4281302
MG10	male	muscle	roadkill	roadkill site	578896	4282932
MG11	male	muscle	roadkill	roadkill site	582620	4273270
MG12	female	muscle	roadkill	roadkill site	576720	4276266
MG13	female	muscle	roadkill	roadkill site	585902	4271851
MG14	male	muscle	roadkill	roadkill site	578797	4282902
MG15	male	muscle	roadkill	roadkill site	578359	4275613
MG16	female	muscle	roadkill	roadkill site	579546	4274900
MG17	female	muscle	roadkill	roadkill site	585399	4272022
MG18	male	muscle	roadkill	roadkill site	588741	4269885
MG19	male	muscle	roadkill	roadkill site	585962	4271821
MG20	female	muscle	roadkill	roadkill site	583489	4273166
MG21	female	muscle	roadkill	roadkill site	575555	4281501
MG22	female	muscle	roadkill	roadkill site	560103	4277637
MG23	male	muscle	roadkill	roadkill site	560132	4277851
MG24	female	muscle	roadkill	roadkill site	577489	4282490
MG25	male	muscle	roadkill	roadkill site	591783	4268153
MG26	male	muscle	roadkill	roadkill site	579127	4275118
MG27*	male	muscle	roadkill	roadkill site	573899	4277628
MG28	female	muscle	roadkill	roadkill site	590799	4269336
MG29	female	muscle	roadkill	roadkill site	586268	4276537
MG30	female	muscle	roadkill	roadkill site	572875	4277923
MG32	male	muscle	roadkill	roadkill site	575509	4281480
MG33	female	muscle	roadkill	roadkill site	601777	4281015
MG34	male	muscle	roadkill	roadkill site	598265	4274124
MG35	male	muscle	roadkill	roadkill site	577195	4278937
MG38	male	muscle	roadkill	roadkill site	563039	4295087
MG40	female	muscle	roadkill	roadkill site	580605	4253356
MG57	male	muscle	roadkill	roadkill site	584135	4272853
MG58	female	muscle	roadkill	roadkill site	576221	4281931
MG60	male	muscle	roadkill	roadkill site	587086	4271686
MG61	female	muscle	roadkill	roadkill site	580642	4274274
SG1	male	blood	trapping	trapping site	584766	4267180
SG2	female	blood	trapping	home range centroid	585076	4267688
SG3	female	blood	trapping	trapping site	584766	4267180
SG4*	female	blood	trapping	trapping site	585265	4271789
SG5	male	blood	trapping	home range centroid	575898	4277504
SG6	female	blood	trapping	home range centroid	581904	4274659
SG7*	male	blood	trapping	home range centroid	581822	4274885
SG8*	male	blood	trapping	home range centroid	581145	4277207
SG9	male	blood	trapping	home range centroid	581682	4271162
SG10	female	blood	trapping	home range centroid	582484	4276702
SG11	female	blood	trapping	home range centroid	581971	4271354

SG12	male	Blood	trapping	home range centroid	581336	4277925
SG13*	male	Blood	trapping	trapping site	581987	4277597
SG14	female	Blood	trapping	home range centroid	583152	4276679
SG15	male	blood	trapping	home range centroid	583101	4277281
SG16	male	blood	trapping	trapping site	575728	4275303
SG17	male	blood	trapping	trapping site	575782	4275863
SG18	female	blood	trapping	trapping site	576102	4275343
SG19	male	blood	trapping	home range centroid	577903	4274289
SG20	female	blood	trapping	home range centroid	577603	4274137
SG21	female	blood	trapping	trapping site	577238	4275090
SG22	female	blood	trapping	home range centroid	577367	4283813
SG23	male	blood	trapping	home range centroid	574078	4285081
SG24	female	blood	trapping	home range centroid	575567	4284219
SG25	female	blood	trapping	home range centroid	573715	4285095
SG26*	female	blood	trapping	home range centroid	582991	4282132
SG27*	male	blood	trapping	trapping site	582094	4282721
SG28	female	blood	trapping	home range centroid	580312	4283003
SG29	female	blood	trapping	trapping site	581240	4279801
PG1[1]	male	hair	trapping	roadkill site	575441	4265795
PG2	male	hair	trapping	home range centroid	584335	4271526
PG3	female	hair	roadkill	roadkill site	560425	4261346
PG4	female	hair	roadkill	roadkill site	554934	4293825
PG5	female	hair	trapping	home range centroid	576723	4283693
PG6	female	hair	trapping	trapping site	575480	4284369
PG7	female	hair	roadkill	roadkill site	587724	4276095
PG11[1]	undefined	hair	roadkill	roadkill site	604531	4284471
PG13	undefined	hair	roadkill	roadkill site	560906	4267437

Circuit_coord – Criterion employed to define UTM X and UTM Y coordinates. These coordinates were set as nodes in Circuitscape.
[1]Samples not included in genetic analysis.
*Sample not included in HW and LD tests.

Table S2-Spearman correlation matrix.

	hab	**dist_rip**	**dist_water**
dist_rip	0.299		
dist_water	0.121	0.208	
dist_human	0.131	-0.012	0.086

Table S3- VIF scores of landscape variables.

Variable	**VIF**
hab	1.103
dist_rip	1.083
dist_water	1.037
dist_human	1.022

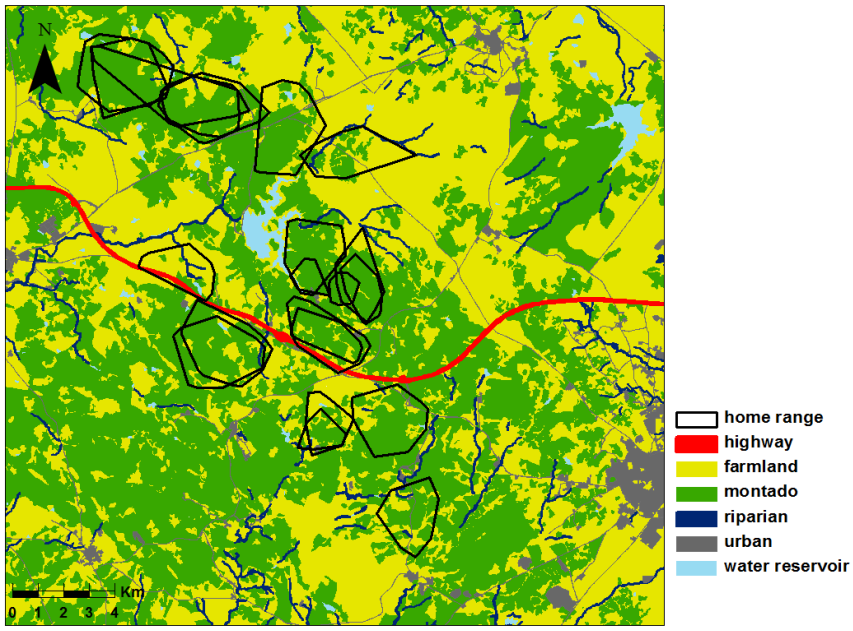

Fig.S1- Small portion of the study area illustrating the home ranges calculated for 21 genets. The home ranges that overlap belong to individuals form different sex.