Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Biologia

Tese de Doutoramento

Population dynamics and connectivity of an endangered small mammal in Mediterranean farmland

António Miguel Proença Ferreira

Orientador(es) | António Mira
Francisco Manuel Ribeiro Ferraria Moreira
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Abstract

Earth's ecosystems have been profoundly reshaped by human activities, with farmland landscapes standing as examples of long-term human-driven land use changes. Many species have adapted to these dynamic environments, for example by persisting as metapopulations. However, conservation efforts for rare and elusive species remain hindered by critical knowledge gaps, partly due to methodological limitations in studying small and cryptic local populations. This thesis investigates the drivers of population dynamics and landscape connectivity of the threatened Cabrera vole (*Microtus cabrerae*) in Mediterranean farmland, integrating ecological, genetic, and methodological developments to inform conservation strategies. Using genetic non-invasive sampling, a systematic survey was conducted across four seasons within a 462-ha area, allowing for an in-depth assessment of demographic parameters, dispersal patterns, and functional connectivity. Landscape composition and structure were found to be main drivers of demographic and contemporary gene flow dynamics. Notably, dirt road verge habitats were found to have a dual ecological function, increasing both survival and connectivity. Extensive pasture grasslands also had a positive effect on connectivity, while agricultural areas negatively affected survival. The seasonal dynamics of abundance and dispersal highlighted the high sensitivity of the species to habitat availability. These findings emphasize the ecological and conservation importance of maintaining extensive agricultural practices and naturalised linear habitats to support Cabrera vole populations. As agricultural intensification continues to threaten Mediterranean farmland biodiversity, this work reinforces the urgent need to sustainably reconcile agricultural activities with species conservation. Finally, it demonstrates the effectiveness of genetic non-invasive sampling for studying small mammals and develops techniques to model dispersal dynamics, contributing to methodological advances that further strengthen conservation efforts in rapidly changing landscapes.

Keywords: Cabrera vole; Demography; Dispersal; Metapopulation; Non-invasive genetics

Resumo

As atividades humanas transformaram profundamente os ecossistemas, alterações particularmente notórias em paisagens agrícolas. Muitas espécies conseguiram adaptar-se a estes ambientes dinâmicos, por exemplo persistindo como metapopulações. Contudo, a eficácia de conservação de espécies raras e difíceis de detectar continua limitada por lacunas de conhecimento, em parte devido a limitações metodológicas no estudo de populações pequenas e crípticas. Esta tese visa compreender os factores que influenciam a dinâmica populacional e a conectividade funcional do rato-de-Cabrera (*Microtus cabrerae*) em paisagens agrícolas mediterrânicas, integrando abordagens ecológicas, genéticas e inovações metodológicas para apoiar as estratégias de conservação. Com base em amostragem genética não-invasiva, monitorizou-se de forma sistemática uma área de 462 ha ao longo de quatro épocas, permitindo uma avaliação detalhada dos parâmetros demográficos, padrões de dispersão e conectividade funcional da paisagem. A composição e a estrutura da paisagem foram factores determinantes da dinâmica demográfica e do recente fluxo genético. Salienta-se que os habitats das bermas de estradas de terra-batida revelaram uma dupla função ecológica, aumentando tanto a sobrevivência como a conectividade. As áreas de pastagens extensivas também tiveram um efeito positivo na conectividade, enquanto as áreas agrícolas afectaram negativamente a sobrevivência. As dinâmicas sazonais de abundância e dispersão evidenciaram a elevada sensibilidade da espécie à disponibilidade de habitat. Estes resultados acentuam a importância de manter práticas agrícolas extensivas e faixas de habitats naturalizados para a persistência do rato-de-Cabrera. Face às ameaças à biodiversidade causadas pela crescente intensificação agrícola em paisagens agrícolas mediterrânicas, este trabalho reforça a urgência de conciliar de forma sustentável as actividades agrícolas com a conservação das espécies. Por fim, comprova a utilidade da amostragem genética não-invasiva no estudo de pequenos mamíferos e produz ferramentas para modelar a dinâmica de dispersão, reforçando assim os esforços de conservação em paisagens em rápida transformação.

Palavras-chave: Rato-de-Cabrera; Demografia; Dispersão; Meta-população; Genética não invasiva

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Chapter 1

General Introduction

In considering the study of physical phenomena, not merely in its bearings on the material wants of life, but in its general influence on the intellectual advancement of mankind, we find its noblest and most important result to be a knowledge of the chain of connection, by which all natural forces are linked together, and made mutually dependent upon each other; and it is the perception of these relations that exalts our views and ennobles our enjoyments.

Alexander von Humboldt, *Kosmos*

General introduction

Nature is patchy, heterogeneous, and dynamic (Wiens 1997). This spatial and temporal heterogeneity implies that organisms and populations must deal with environmental uncertainty. As a result, over time, species must have developed strategies for population organisation, allowing them to adapt to changes in their environment. Evolutionary adaptations to these environmental dynamics may, over time and generations of continuous selection, affect the life-history and traits of species, for example their habitat preferences, dispersal choices, breeding strategies, or spatial organisation (Olivieri and Gouyon 1997). Understanding how heterogeneity affects ecological systems requires knowing how organisms respond to structural and functional changes in their environment in both space and time (Fahrig et al. 2011). This has been the concern of naturalists, ecologists and biogeographers, since the inception of those subjects, decades and centuries ago, and continues to be the concern of conservationists today, when biodiversity and ecosystems are subject to a decline like no other since the Pleistocene (Barnosky et al. 2011), due to human activities (Chapin III et al. 2000; Díaz et al. 2019).

Few human impacts have been as widespread on the landscape as agriculture (Foley et al. 2005; Pereira et al. 2012). Agriculture is one of the main drivers of species' contractions and extinctions, but, over generations of human occupations, it simultaneously, and perhaps counterintuitively, allowed for other species to thrive (Fahrig et al. 2011). In fact, extensive farmlands, embedded in landscape mosaics that embraced naturalised habitats whilst allowing for farming, forestry, and pastoralism, have for generations allowed for the establishment and passive conservation of species which would have difficulties in thriving without human presence. Due to the constant land management that maintains specific habitats and prevents secondary succession (Blondel et al. 2010; Halada et al. 2011; Boivin et al. 2016), species either went extinct or became adapted to such farmland landscapes. This often created a positive feedback, where species persisted while providing important ecosystem functions to these extensively managed areas (Bürgi et al. 2014; Boivin et al. 2016). Persisting in those mosaic landscapes, subject to some degrees of perturbation and fragmentation, could also imply the subdivision of populations into small breeding units of local populations. These local populations may become more vulnerable to stochastic and extreme events, or to major land use changes, as those caused by the global intensification of agriculture of the past 150 years (Pereira et al. 2012). On the other hand, at a more regional level, land abandonment in some areas over the past 50 years may threaten habitats critical for farmland biodiversity (Macdonald 2000). Understanding how species are associated with agriculture and, more specifically, how species exhibiting metapopulation characteristics can be impacted by landscape changes and human activities is therefore necessary for the preservation not only of species and biodiversity as heritages, but also to maintain their function in the ecosystem.

1.1 From metapopulation and landscape ecology to species persistence

1.1.1 Metapopulation ecology

The term metapopulation was first coined by Levins in the late 1960s, to define a simple model of two interconnected populations (Levins 1969). However, the recognition of metapopulation ecology as a distinct subdiscipline within ecology only emerged in the late 1980s, after a conference on the conservation of animal populations held in Sweden (Gilpin and Hanski 1991). Not less surprisingly, at about the same time, the newly emerging discipline of conservation biology also started to take shape (Hanski and Simberloff 1997), which would prove decisive for advancing the understanding of metapopulation ecology in a world marked by the increasing global efforts to preserve species and ecosystems (Rands et al. 2010).

A metapopulation is roughly defined as a population of populations inhabiting discrete patches of habitat, mostly isolated from each other, but occasionally connected by dispersing individuals (Hanski and Simberloff 1997). Over the years, this concept has expanded to include a wide range of organisms across different environments, becoming crucial for addressing complex ecological dilemmas, such as the single large or several small (SLOSS) controversy (Fahrig 2019). According to classic metapopulation theory, the landscape is basically considered as having two main features: the patches and the matrix (Hanski and Simberloff 1997). Patches are considered the suitable and static habitats where organisms establish, having all the necessary conditions for survival and reproduction, like food and shelter. The matrix is the otherwise hostile environment for the focal species, where individuals cannot establish, but through which they may have to cross to move or disperse to other habitat-patches. The ability of organisms to move between patches is then defined as connectivity and landscape connectivity as *"the degree to which the landscape facilitates or impedes movement among resource patches"* (Taylor et al. 2006). Therefore, measuring connectivity between habitat patches requires assessing how organisms cross hostile areas in attempting to find suitable habitat, and which environments are better suited for crossing (Taylor et al. 2006).

1.1.2 Landscape ecology and connectivity

Classic metapopulation models are important for summarising and simplifying the complexity of ecological systems. However, they may overemphasise the relevance of the size and isolation of habitat-patches on the rates of local site occupancy, at the same time assuming that the landscape where suitable (and static) habitat-patches are embedded is a featureless and uniformly unsuitable matrix (Wiens 1997). The complexity of ecological systems and the need for a deeper understanding of their functioning led to a more comprehensive view of the landscape, where "*the effect of spatial patterns on ecological processes*" would be the main focus, hence laying the foundations of landscape ecology (Turner 1989). Within metapopulation research, landscape ecology mostly attempts to relate the structure and composition of the matrix to the cost of movement between habitat-patches, either by dispersers or when foraging for food, thereby evaluating its influence on the species regional dynamics and persistence (With 2004).

Two concepts of connectivity shape how studies are conducted and interpreted: structural and functional connectivity. Structural connectivity focuses on the physical and geological aspects of the environment. It characterises the permeability of the landscape, based on physical characteristics and configuration of features and elements that may be considered relevant for organisms to move (Hilty et al. 2019). In contrast, functional connectivity is viewed from the perspective of the species, being affected not only by the physical environment but, more importantly, by the species' traits, including, in the case of animals, their individual decisions and behaviours (Manel and Holderegger 2013). Therefore, functional connectivity may be defined as the degree to which landscapes and their features facilitate or hinder the movement of organisms from a particular species (Manel and Holderegger 2013). For example, a road crossing a landscape (a physical, structural feature) may be a huge barrier for a frog to leap across while being a more or less irrelevant obstacle for a raptor flying over it. The study of the functional connectivity is thus the primary focus of connectivity research in ecology, as landscapes may provide structural without having functional connectivity.

Dispersal plays a critical role in the context of functional connectivity. Defined as the process where individuals move from their birth location to establish and reproduce in another (Clobert et al. 2012), dispersal is key for population persistence, being especially important in fragmented landscapes (Manel and Holderegger 2013). Assessing the effective dispersal of individuals, or gene flow, and overall functional connectivity at relevant scales has been made possible by applying genetic methods and relating them with landscape characteristics (Manel et al. 2003; Balkenhol et al. 2015a). This is the basis for landscape genetics, generally defined as the quantification of the effects of the landscape (composition, configuration, and quality) on micro-evolutionary processes (like drift, selection, or gene flow), using genetic data, thus combining the techniques of landscape ecology and population genetics (Balkenhol et al. 2015b).

1.1.3 Species persistence in changing landscapes

The goal of conservation biology should be to predict (and eventually avoid) species extinction. The link between metapopulation and landscape ecology thus emerges from the need to understand not only how spatial patterns, like fragmentation or heterogeneity of land uses, relate to metapopulation dynamics, but also the casual factors associated with demography and dispersal that drive such interactions and, ultimately, metapopulation persistence (With 2004). Demographic and dispersal parameters impact metapopulation dynamics by affecting extinction and (re)colonisation events, local recruitment, and overall connectivity. In particular, dispersal may be affected not only by habitat amount or fragmentation (with further apart and smaller fragments having lower connectivity) but also by the amount and fragmentation of “movement-suitable” land uses (With 2004). Knowing which land-use quality and composition impact species demography, dispersal and connectivity is thus a first step in filling the board to understand species´ persistence.

Demographic parameters, for example survival or abundance, are highly dependent on habitat availability, with the loss of an occupied patch potentially leading to a decline in abundance up to a threshold where it affects the species’ persistence (With 2004). Spatial heterogeneity (shape, size, and quality) of habitats may also affect the demography of metapopulations, where higher quality and larger habitat-patches may promote population growth (*e.g.* with higher birth and survival rates) – source habitats –, while the opposite may occur in smaller and worse quality patches – sink habitats (With 2004). These source-sink dynamics can be influenced not only by the changes in local patch attributes (Pulliam 1988; Crispim-Mendes et al. 2024) but also by landscape dynamics (With 2004). The sensitivity of species to varying degrees of habitat and landscape change is thus crucial for the persistence of their metapopulations.

Real landscapes are dynamic in both space and time. Patches may appear, disappear, and reappear, whilst different land uses may also have different rates of change, all of which may impact metapopulations (With 2004). The importance of considering spatial and temporal environmental heterogeneity, especially when populations are spatially structured, is particularly relevant in highly dynamic landscapes such as farmlands. In these systems, human activities create constant changes in the amount and quality of habitat-patches and land uses in the matrix, which in turn affects the permeability to individuals movement among patches, and therefore overall metapopulation persistence (Somoano et al. 2022).

1.2 Mediterranean farmland and the Portuguese conundrum

The Mediterranean region is one of the 36 global biodiversity hotspots (Medail and Quezel 1999; Myers et al. 2000; Mittermeier et al. 2011; Noss et al. 2015). Simultaneously, it is also one of few biodiversity-rich areas where land cover is the outcome of millennia of human presence and intervention. Recent data reveal that more than a quarter (28%) of the region is currently occupied by agricultural land (FAO 2024). From burning to farming, cutting to ploughing, planting to fallowing, humans have extensively modified and shaped Mediterranean landscapes, building terraces and hedgerows, together shaping biological communities (Blondel et al. 2010). These long-term interactions between agriculture and nature, in particular those associated with traditional, low-intensity farming and pastoral systems, stemmed in the creation and preservation of biodiversity-rich habitats and ecosystems (Moreira et al. 2005; Plieninger 2006; Blondel et al. 2010), where up to half of local species (including endemic and threatened ones) depend on (Bignal and McCracken 1996; Halada et al. 2011). This is the case of the agroecosystems in the south of Portugal, including agro-sylvo-pastoral, savannah-like ecosystems known as “montados” in Portugal and as “dehesas” in Spain (Gonçalves et al. 2012), or the extensive cereal farming systems, characterised by a mosaic of planted cereal, ploughed fields, fallows, and grazed areas. These semi-natural areas show a recognised importance for local biodiversity conservation (Bugalho and Abreu 2008; Pina et al. 2017), and in some cases constitute historical nuclei of populations of threatened species (Delgado and Moreira 2000; Moreira et al. 2004; Pita et al. 2009; Rosalino et al. 2009; Morgado et al. 2010; Catry et al. 2012). The low-intensity farming that defines those systems is usually characterised by very little mechanisation and agro-chemical input, reduced nutrient use, low livestock density, the adoption of regional breeds of livestock, use of fallowing in crop rotations, and the absence of automated and widespread irrigation (Beaufoy et al. 1994). The importance of these systems for the conservation of nature impelled their classification as High Natural Value (HNV) farmlands (Bignal and McCracken 2000; Halada et al. 2011; Plieninger and Bieling 2013; Lomba et al. 2014; Lomba et al. 2023).

The farmland area of southwest Portugal offers a particularly interesting context due to its high ecological importance and agricultural interest. The region is partially located in the *Sudoeste Alentejano and Costa Vicentina* Natural Park and within the Special Area of Conservation *Costa Sudoeste*, classified under the EU Directive 92/43/CEE. Despite this apparent protection, agricultural practices have been intensifying since the early 1990s. Examples of this intensification include (i) increasing densities of cattle stock and the substitution of extensive pastures by intensive ones; (ii) increasing size of irrigated fields, which are mainly used for forage, such as corn and sorghum; (iii) increasing area occupied by vegetable and fruit crops, often grown in greenhouses; (iv) destruction of naturalised habitats such as temporary ponds and shrubland; (v) increasing development and improvement of paved road networks; and (vi) introduction of forest plantations for wood production, such as eucalyptus and pines (Beja and Alcazar 2003; Pita et al. 2009; Ribeiro et al. 2014; Pereira et al. 2011). At the same time this intensification takes place, some areas have been abandoned while others still maintain extensive agriculture (Pita et al. 2009). Land abandonment may create still another problem for farmland species adapted to traditional farming practices, due to subsequent shrub encroachment and consequent loss of habitats that result from extensive farmland management (Pita et al. 2014).

These drastic landscape changes may have a significant and detrimental impact not only directly on the reduction of available habitats for native species, but also on the quality of the surrounding landscape, by constraining or impeding individuals' movement outside of their preferred habitats, impacting species connectivity and ultimately affecting the overall biodiversity of such ecosystems (Tscharntke, Tylianakis, et al. 2012). The interplay between agriculture production, the social and economic stability of local communities, and biodiversity and ecosystem protection creates a challenging puzzle without an obvious solution (Tscharntke, Clough, et al., 2012).

1.3 Small mammals in ecological research

1.3.1 From ecosystem engineers to research models

Small mammals, defined as those mammal species weighing less than 5 kg, comprise half of mammal species (Stoddart 1979). Of all small mammals, 90% belong to order Rodentia, representing over 40% of mammal species (Lacher, Jr. et al. 2016). Although rodents play an essential role in the ecological function of terrestrial ecosystems (Ostfeld et al. 1996), they are usually relegated to the outskirts of media attention and public awareness. Small mammals, and rodents in particular, are keystone species in agroecosystems (Delibes-Mateos et al. 2008; Delibes-Mateos et al. 2011), acting as ecological engineers (Zhang et al. 2003), being important as herbivores, seed predators and dispersers (Jansen et al. 2012), pollinators, soil aerators and nutrient cyclers (Kinlaw 1999), not to mention constituting prey for several species (Lacher, Jr. et al. 2016). Apart from their relevance in providing crucial ecosystem functions, under certain conditions rodents may instead become a nuisance, for instance as crop pests or hosts of pathogenic diseases (Stoddart 1979; Lacher, Jr. et al. 2016; Fischer et al. 2018). Furthermore, due to their life cycles (high fecundity rates and short lifespans) or the relatively small areas they occupy, rodents have a faster response to environmental changes, making them excellent models for addressing complex ecological research questions (Barrett and Peles 1999). The importance of rodents makes their study paramount in both ecological and conservation theory, as well as in monitoring human impacts on ecosystems (Barrett and Peles 1999).

Research on small mammals has been made possible due to their very specific characteristics, such as relatively small home ranges, short dispersal distances, short generation times, and strong behavioural responses to temporal and spatial variations in their environment (Barrett and Peles 1999). Specifically, studies using voles as model organisms have provided important advances in the topics of population dynamics (Crespin et al. 2002), community ecology (de la Peña et al. 2003), dispersal (Stenseth et al. 1992), behaviour (Duarte et al. 2015), population cycles (Oli 2019), sociality (Lin et al. 2006), phylogeography (Barbosa et al. 2017), biogeography (Mestre et al. 2015), or metapopulation ecology (Lambin et al. 2004). A large number of studies focusing voles have also been developed in landscape ecology, either by analysing the effects of natural landscape features or by their experimental manipulation (Barrett and Peles 1999; Presley et al. 2019).

Particularly in landscape ecology, researchers only started to use voles as models after the 1977 paper by Hansson, leading to a myriad of studies that exploded during the 1990s providing evidence for the effects of patch configuration, corridors and edges on dispersal and population dynamics (Barrett and Peles 1999; Presley et al. 2019). It was also during the late 1990s studies that the landscape started to be viewed by scientists as more than a mere featureless and uncharacteristic matrix, helping to shape landscape ecology research for the following decades (Presley et al. 2019).

1.3.2 Sampling challenges

Data collection is a critical aspect of ecological analyses. While environmental data, such as land uses, topography, climate, or vegetation characteristics, are in general easy to collect (either in fieldwork or using digital repositories), data on species' traits, on the other hand, usually requires more demanding sampling methods, often entailing the capture of individuals. Determining for instance the sex, age, or body condition, all depend on the capture and then measurement and assessment of individuals, referred to as active sampling (McCleery et al. 2022). The use of live trapping has been the paramount method for the sampling and study of small mammals, either by using marks (like ear tags, PIT tags, or toe or hair clipping) for capture-mark-recapture (CMR) methods (Pierce et al. 2012) or of electronic tracking devices, for example for radiotracking (Banks et al. 1975). However, directly capturing individuals is in many situations a difficult or even impossible task, either due to technical or financial constraints, or owing to ethical reasons. For instance, in the case of rare or elusive species of conservation concern or those that are difficult or dangerous to handle (to the scientist or for the animal), live captures are mostly impractical and unlikely to provide sufficient data to robustly study their ecology and population dynamics. Finally, policies for legally capturing species of conservation concern have also become increasingly more demanding in providing licences for the capture and handling of individuals.

In an attempt to circumvent these difficulties, less direct methods of observation have been developed – deemed passive sampling –, at the cost of losing some data (*e.g.* biometric variation). Among these, camera trapping is, by and large, the most common method for collecting field data on rare, elusive, or otherwise uncatchable species. Briefly, this method consists in leaving a set of cameras in the study area and automatically taking and storing photos or videos, being particularly efficient for sampling medium- to large-sized mammals, with visual characteristics relatively easy to identify at the individual level. Despite the costs associated with camera traps, this method provides a more economical alternative to capturing individuals, mostly due to its effectiveness in collecting large amounts of data owing to the possibility of leaving the cameras in the field for a long time. Unfortunately, this method is considerably less effective for most small mammal species (but see Tye et al., 2015), given the difficulty in distinguishing and identifying individuals from photos or videos (McCleery et al. 2014).

Other passive sampling approaches like searching for tracks, scats, or even hair samples from small mammals often fail to provide unequivocal species or individual identification as well, contributing to the already limited knowledge on their ecology and conservation status (for a full summary of available methods for small mammal sampling, see Table 1.1). The collection of DNA from samples detected in the field (without direct contact with animals, *i.e.*, non-invasive), is particularly effective when active captures are challenging or limited. Genetic non-invasive sampling (gNIS) allows for the study of several individuals and populations without needing to contact, disturb or even directly see the organisms. It may be applied to samples collected through passive methods – scats, hair, urine, feathers –, and allows for the collection of a variety of information, not only the species, sex, and the identification of individuals, but also details on diet, hormones, or parasites (Beja-Pereira et al. 2009). Notwithstanding this potential, the quality and quantity of the DNA sample will be lower than when directly collected from a captured individual. For this reason, in the last 10 to 20 years, significant improvement in extraction techniques allowed for great development in the collection of data that would be otherwise lost, due to DNA degradation or genotyping errors. While these technical advances led to a profusion of research using gNIS (see Beja-Pereira et al., 2009 and references therein), their use in small mammal studies has been relatively less frequent when compared to larger-sized animals, with live-trapping still being the most used sampling technique. Only during the last decade has gNIS been applied to the study of small mammals – with some studies showing relevant and more precise data as compared with live-trapping (Sabino-Marques et al. 2018) and their cost-effectiveness for monitoring rare or elusive species (Ferreira et al. 2018). However, by the time of this research no known studies focusing on small mammals had evaluated individual- (*e.g.* survival, dispersal) and population-level (*e.g.* gene flow, metapopulation connectivity) variations using gNIS.

Table 1.1 – Methods used to monitor small mammals and ecological information potentially provided. Abundance may refer to relative or absolute, depending on the method used. Asterix refers to information that can be obtained from genetic material if sampled using that technique. Adapted from McCleery et al., 2022.

	Ecological information						
Method	**Behaviour**	**Genetic**	**Diet**	**Abundance**	**Demographics**	**Movement**	**Community structure**
Active							
Traps		x	x	x	x	x	x
Passive							
Tracks			x	x			
Tracking plates	x		x	x			x
Scats and signs	x	x	x	x	x	x*	
Nest boxes					x		
Owl pellets		x		x	x*		x*
Hair sampling tubes		x		x*	x*	x*	x*
Scent dog				x			
Acoustics	x			x	x	x	
Camera	x			x	x	x	x
eDNA		x					x*

1.3.3 Analysis of demography and connectivity

There is a wide array of methods for analysing data collected in the field (Krebs 1989; McCleery et al. 2022). Analyses aimed at estimating demographic and dispersal parameters, apart from depending on the type and quality of sampled data (as seen in the previous section), are also contingent on the quantitative method used. Furthermore, even the choice for collecting and sampling data in the field is, for the most part, a function of the analytical technique to be applied at later stages (McCleery et al. 2022). In particular for small mammals, estimates on demography have been relying on statistical techniques such as minimum number alive – which analyses samples collected several trapping sessions, without accounting for capture probabilities (Efford 1992) –, distance sampling – which estimates abundance from the probability of seeing an animal at certain distances from the observer (Burnham and Anderson 1984) –, removal methods – which relies on physically removing animals from a population (Erlinge et al. 1983), commonly used for pest species –, and capture-mark-recapture (CMR) analysis – the most used method in ecology, relying on the capture and recapture of previously marked individuals (Lebreton et al. 1992).

Of the mentioned methods, CMR is the only that allows for the estimation of population size and demographic traits, also admitting the use of active or passive sampling procedures. CMR methods may be applied either in closed or open models CMR models, where the former assume no change in population size during the sampling period (hence only estimating population abundance or density) while the latter admit population size changes, thus also allowing for the estimation of birth, death, immigration, and emigration parameters. Regardless of the type of CMR method, they all share the basic conceptual idea: capturing a number of individuals, marking them, releasing them back into the population, and then determining the ratio (*i.e.* the proportion of marked to unmarked individuals) of the population when marked and unmarked individuals are captured at a later date (for a detailed overview of analyses available, see (Williams et al. 2002; Cooch and White 2017)[1]. In addition to the scarcity of the use of gNIS in small mammal research (mentioned in 1.3.2), the use of more general genetic sampling in mark-recapture studies also remains underused in studies on small mammals (Jung et al. 2020), thus having a strong potential for becoming increasingly used in studies on population dynamics (Lukacs and Burnham 2005).

When it comes to dispersal, data may also be obtained using the previously mentioned techniques, some to directly measure movements (*e.g.* telemetry) while others only for inferring it (*e.g.* gNIS). Due to its definition (see 1.1.2), dispersal is a difficult process to observe, particularly when individuals are hard to detect, follow, or recapture (Bode et al. 2018), mostly because it implies the establishment of individuals in a particular location, which may take time to occur (Cayuela et al. 2018). Several single-value parameters may be used to characterise dispersal movements (*e.g.* the mean distance or maximum dispersal rate, Driscoll et al., 2014). However, dispersal kernels are the most explanatory characterisation of dispersal distances, by incorporating all individual information on a probability density function, describing both shorter, mean, and long-distance dispersal events (Nathan et al. 2012). Apart from their utility in the description of dispersal patterns,

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[1] **Apparent survival probability**

Due to their assumptions and limitations in methodology, most CMR models do not distinguish mortality from immigration. Throughout this thesis, both terms are used interchangeably but, except when directly noted, they refer always to the product of the probabilities of true survival and study area fidelity – apparent survival probability (Lebreton et al. 1992).

dispersal kernels are also commonly incorporated in further analyses in ecology and conservation studies (Driscoll et al. 2014; Cayuela et al. 2018). However, unlike CMR methods for estimating demography and abundance (*e.g.* software MARK, or the *R* packages *RMark*, *capwire*, and *secr*; White and Burnham 1999; Laake 2013; Pennell et al. 2013; Efford 2024) there are no robust and accessible software applications for estimating dispersal kernels in ecological studies. Such applications would be of interest not only for small mammal research but also for several other taxa.

1.4 The model system – Microtus cabrerae

1.4.1 General description and distribution

The Cabrera vole (*Microtus cabrerae*, Thomas 1906) is a medium-sized Arvicolinae, with a mean body mass of ca. 52 g, varying from 30 g to 78 g, and a mean body length of 118.3 mm, ranging between 100.0 and 135.0 mm (Ventura et al. 1998; Pita et al. 2014). It has long and thick fur, with a brown to olive colour on the dorsal side and a yellow shade on the ventral side (Pita et al. 2014). Endemic to the Iberian Peninsula, the distribution of Cabrera voles is fragmented in four distinct population cores (Garrido-Garcia et al. 2013) (Fig. 1.1).Within its distributional range, the Cabrera vole occurs in regions with medium rainfall values (600 to 1200 mm) and humidity values lower than 85% (Mira et al. 2008).

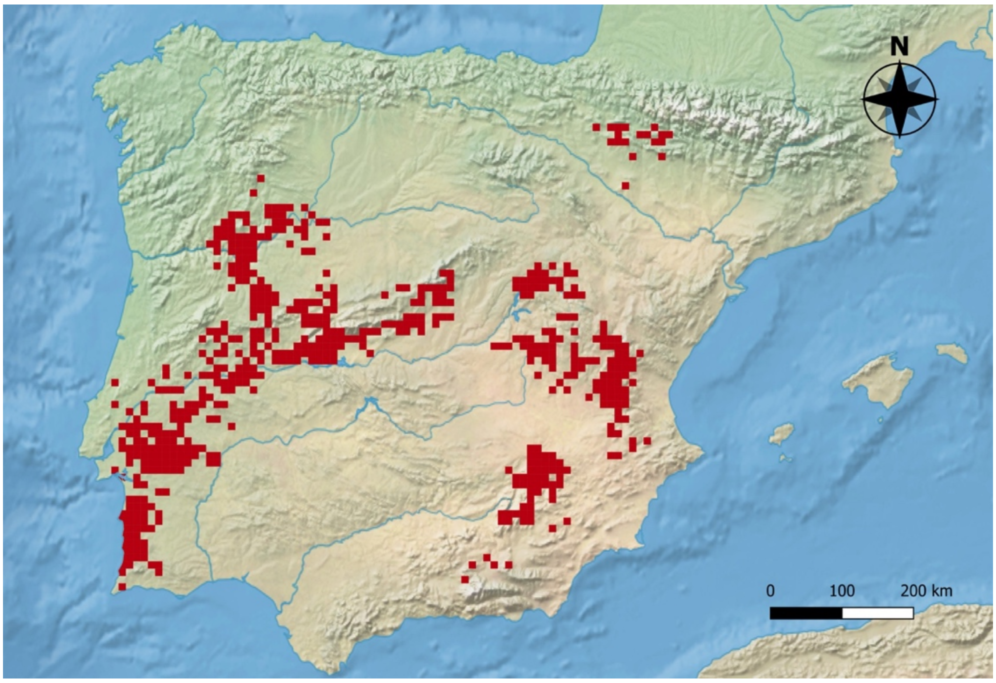

Fig. 1.1 – Current distribution area of the Cabrera vole (*Microtus cabrerae*, Thomas 1906). Data obtained from Bencatel et al. (2019) and GBIF (2023).

1.4.2 Demography and ecology

Research on population dynamics in Mediterranean farmland suggests an increase in abundance in the wet season (from early winter to the end of spring), decreasing in the most dry and hot seasons. Estimates of population densities, however, have shown a high variability in different studies using capture-mark-recapture techniques (Landete-Castillejos et al. 2000; Fernández-Salvador et al. 2005; Rosário 2012), which may be due to regional differences in habitat quality and stability. Cabrera voles' populations usually occupy patches of wet habitat between 500 and 2000 m^2, but are frequently found in patches smaller than 500 m^2 (Fernández-Salvador 1998; Pita et al. 2006; Santos et al. 2006; Pita et al. 2007). Within habitat-patches, residence times in general do not exceed 4 months, although individuals may persist for up to 12 months in large patches (Fernández-Salvador et al. 2005).

In the Mediterranean farmlands of southwest Portugal, Cabrera voles are generally found in cork oak (*Quercus suber*, L.) and holm oak (*Q. ilex*, L.) dominated agro-sylvo-pastoral systems and in open farmlands. Voles usually occur in habitat-patches characterised by wet and dense grasslands located along field margins, road verges, ditches, temporary ponds, or small streams (Pita et al. 2006) (Fig. 1.2). Within those patches, it selects microhabitats with tall (> 30 cm) and dense (near 100%) herbaceous cover, which offers protection from weather and predation (Pita et al. 2006; Santos et al. 2006). Populations are spatially structured, with individuals grouped in distinct population units (also referred to as colonies or subpopulations) and exhibiting strong fidelity to small home-ranges with mean areas of 400 m^2 (and often not larger than 1000 m^2) (Pita et al. 2007; Pita et al. 2014).

Evidence has been found for local extinction-colonisation events, largely driven by the appearance or disappearance of habitat-patches, either due to natural and stochastic events (such as seasonal floods or droughts) or to land management activities (as ploughing, planting, or grazing by cattle) (Pita et al. 2006; Pita et al. 2007). These fluctuations together with a degree of responsiveness to the size and isolation of habitat-patches (Pita et al. 2007), as predicted by classic metapopulation theory, suggest that metapopulation dynamics may play a role in the persistence of the species (Pita et al. 2007). In addition, the spatial structuring of its population seems to be influenced by the landscape characteristics of the matrix (Pita et al. 2007), eventually due to how it influences the individuals' motivation to move and, ultimately, their capacity to disperse.

Fig. 1.2 – Examples of typical habitat-patches suitable for Cabrera vole occurrence. Top left, patch embedded in cork-oak system; bottom left, patch within open extensive farmland; right, patch found along a hedgerow bordering a dirt-road (Photos by PERSIST and NETPERSIST projects).

1.4.3 Behaviour and reproduction

Cabrera voles show a predominantly diurnal activity, with peaks varying around noon during the wet season (October to April), and at dawn and dusk during the dry season (May to September) (Pita et al. 2011), highlighting a behavioural adaptation to the hottest hours of the day during summer. Besides adaptations to weather, the activity of Cabrera voles has also been shown to change in the presence of superior competitor voles, such as the water vole (*Arvicola sapidus*, Miller 1908) (Pita et al. 2011). In terms of reproduction, the species may breed throughout the year, although activity is higher during the wet season, with reports of ceasing completely during the hot season (mainly in summer), particularly in very dry years (Pita et al. 2014). This decline in reproduction activity is mostly associated with a decline in food quality and availability due to low rainfall and high temperatures during summer months. Studies on the physiology of this species revealed a high adaptation to high temperatures and summer droughts, which characterise the Mediterranean climate region. In particular, the resting metabolic rate of Cabrera voles is much lower than other *Microtus* species of temperate regions, with the species being able to approximate its body temperature to the outside temperature during hot periods (Mathias et al. 2003). With a gestation length of about 23 to 24 days and a mean litter size of 4 (+- 0.89) individuals, it shows low natality rates (k-strategy of reproduction), compared to other *Microtus* species (Fernández-Salvador et al. 2001).

As opposed to most rodents, including other arvicoline species, the Cabrera vole shows a predominantly monogamous mating system (Pita et al. 2014). Physically, this is supported by equal body sizes of males and females, and small size of testis in adult males (Ventura et al. 1998). In terms of behaviour and socio-spatial organization, monogamy is supported by the equal size and highly overlapping home ranges and core areas between breeding pairs (Pita et al. 2010), together with the reduced behaviour of scent-marking in paired animals in comparison with non-paired ones, or the even sex-ratios detected in many populations (Fernández-Salvador et al. 2005; Pita et al. 2014). However, it is still uncertain whether monogamy is genetic or social, and whether other mating systems may also be at play under certain socio-ecological contexts (Pita et al. 2010; Pita et al. 2011)

1.4.4 Conservation

The conservation status of the Cabrera vole has shown a decline over the last two decades, even as more detailed information was gathered (Barbosa et al. 2023). While the International Union for the Conservation of Nature (IUCN) classifies it as “Near-Threatened”, it is considered “Vulnerable” in both the Portuguese and Spanish mammal red books, its only occurrence countries, showing a reduction in population of over 30% in the last 10 years in Portugal. The population contraction is most likely a result of the destruction, fragmentation, and degradation of their optimal habitat, fundamentally because of land use changes caused by agricultural intensification and over-grazing, but also due to urbanisation and road construction or expansion (Pita et al. 2014). The Cabrera vole is protected in the European Union (included in the Habitat Directive, Annexes II and IV) and is listed in the Convention on the Conservation of European Wildlife and Natural Habitats, both implying strict protection of the species, with the former also entailing the designation of Special Conservation Areas. Due to its very specific ecological niche and life-history characteristics (*e.g.* low natality rates) (Pita et al. 2014), Cabrera voles are predicted to be affected by ongoing climate changes (Mestre et al. 2015; Mestre et al. 2017).

Best practices in the conservation of Cabrera voles have been directed at addressing the protection of its habitats, with particular focus on larger-sized patches – likely to contain several breeding pairs (Pita et al. 2010) –, mainly by preventing or limiting farming operations such as mowing, ploughing, or the spraying of herbicides within potential habitat-patches, as well as preventing the intensification of grazing areas. At a broader scale, conservation efforts should focus on preventing the isolation of local populations by managing the spatial arrangement of habitat-patches (Pita et al. 2007). Finally, the landscape mosaic outside habitat-patches has also been referred to as significant for the conservation of the species, mainly due to its importance in keeping landscape connectivity for the long-term persistence of populations (Pita et al. 2007). For this reason, understanding how each land use influences population connectivity of Cabrera voles is of utmost importance for better deciding on which land use types more conservation efforts should be allocated.

1.4.5 Sampling

CMR studies with the Cabrera vole, in particular in Mediterranean farmland, have revealed the challenges of live-trapping, due to very low capture probabilities (Ferreira et al. 2018; Sabino-Marques et al. 2018). As such, detailed data on the metapopulation ecology and dynamics of the species have been relying mostly on inferences from owl pellet analysis (Mira et al. 2008; Oliveira et al. 2023) or from presence sign surveys for patch occupancy analyses (Pita et al. 2006; Pita et al. 2007; Pita et al. 2007; Pita et al. 2010; Pita et al. 2011), sometimes also used to estimate relative abundance (Santos et al. 2006; Peralta et al. 2022) (Fig. 1.3).

Fig. 1.3 – Cabrera vole droppings.

Molecular markers (microsatellites) for species and individual identification of Cabrera voles through gNIS were already developed for the study of this species and proved to be highly cost-effective, allowing for the collection and analysis of a large number of samples (Ferreira et al. 2018) and improving demography estimates when compared to live-trapping (Sabino-Marques and Mira 2011). These promising results, together with the trap-shy behaviour of Cabrera voles, and its conservation and ecological importance, make this species ideal for showing the utility of genetic non-invasive sampling as a tool to collect more detailed data, specifically on metapopulation dynamics and connectivity.

1.5 Aims and objectives

This thesis aims at improving the knowledge on demography, dispersal, and metapopulation connectivity of the Cabrera vole in highly fragmented and dynamic Mediterranean farmland ecosystems, to better inform conservation planning and management of the species in these ecosystems. Simultaneously, it also aims at proving the utility of gNIS used in small mammal population ecology research (particularly for rare and elusive species, such as the Cabrera vole), and at developing a software application for analysis of dispersal data, thus combining both empirical and methodological approaches. To achieve these goals, I pursued the following research objectives:

i. Identify and understand the environmental and individual-level drivers influencing voles' survival in a fragmented farmland metapopulation;

ii. Assess how, which, and at what scales different land uses affect gene flow and functional connectivity of voles;

iii. Describe vole yearly dispersal patterns by developing a software application specifically for estimating dispersal kernels;

iv. Devise general management recommendations that contribute to the persistence of Cabrera voles in Mediterranean agricultural landscapes.

In tandem with these objectives, I intend to show the relevance of genetic non-invasive sampling for the study of small mammals, more specifically how it may provide important information on demography and connectivity for rare and elusive species, such as the Cabrera vole.

Overall, I expect this thesis will help to improve our understanding of how habitat and landscape variables impact vole population dynamics and connectivity in Mediterranean farmland. Specifically, voles' survival and gene flow are predicted to increase close to land uses that are more similar to the wet grass habitats that the species typically prefers, specifically natural ponds, extensive grassland pastures, but also verge habitats (*i.e.* dirt-roads) and areas with water. Areas associated with increased human activity (*i.e.* urban and agricultural lands) and with a more intensive land use (*i.e.* forest plantations and intensive pastures) should negatively affect both the survival and connectivity of the vole metapopulation. I also expect that basic determinants of metapopulation (*i.e.* patch amount and fragmentation) theory should affect vole survival. Abundance and survival are anticipated to follow the seasonal dynamics of the area, namely increasing in wetter seasons and decreasing in dryer. Reflecting a high degree of association with water vole presence, Cabrera voles' survival should also be lower in patches where both species occur.

1.6 Thesis structure

This thesis is organised into six chapters. The current chapter presents the scope, background, and motivation of the research. Of the following four chapters, Chapters 2, 3, and 5 detail the research developed to accomplish the objectives referred to above, while Chapter 4 introduces the software application for fitting dispersal kernels (Fig. 1.4). All four chapters comprise scientific papers published (Chapters 2 and 4) and in preparation for publication (Chapters 3 and 5) in peer-reviewed journals.

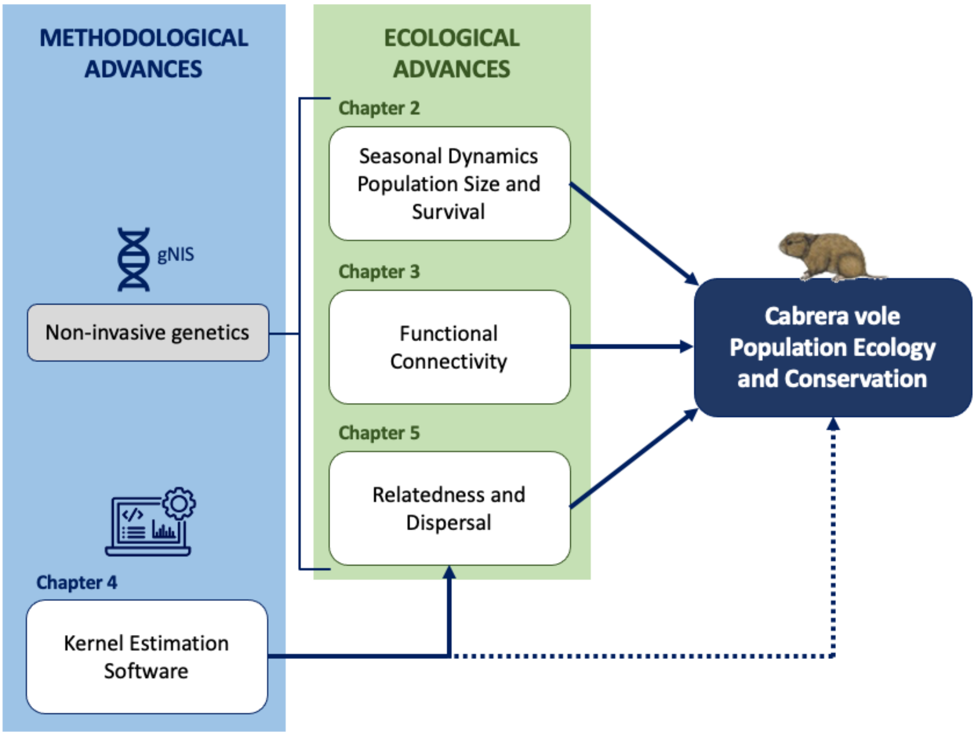

Fig. 1.4 – Diagram of the thesis structure.

In Chapter 2, seasonal population size and survival of the Cabrera vole were analysed along a year cycle concerning individual, patch, and landscape characteristics in a farmland area in southwest Portugal. To achieve this, genetic non-invasive sampling was applied over four seasons, with genotyping of samples providing individual information which was then used in a capture-mark-recapture model approach. Abundance was expected to follow seasonal variations in food and shelter availability, known to influence vole recruitment, while survival was expected to be positively influenced by bigger, less isolated, and stable habitat-patches, and negatively influenced by patches associated with higher human-disturbance. The chapter demonstrates the use of gNIS within a CMR framework, validating its application in obtaining demographic data from elusive small mammals. It further allows for more informed conservation planning in areas highly impacted by human activities.

Chapter 3 analyses the functional connectivity of a metapopulation of Cabrera vole in the breeding season, by relating land uses with the genetic and geographic pairwise distances of each detected individual. Using genetic non-invasive sampling and an information-based algorithm for estimating how different the land uses influence gene flow. Functional connectivity was expected to increase closer to land uses more similar to vole's typical habitat patches, whereas land uses associated with unsuitable conditions or higher degree of changes should have a neutral influence or even reduce vole gene flow. This chapter provides relevant insights on the conservation of voles and other species with similar habitat needs, in areas subject to agricultural intensification.

Chapter 4 introduces a software application designed for fitting dispersal kernels, specifically developed to support dispersal analyses of ecological studies. Following a general overview of dispersal ecology and dispersal kernels, software usage is briefly described with an example based on simulated data. Since no software application previously existed to fit and compare commonly used dispersal kernels, this application can be of great use for researchers studying dispersal ecology across many different taxa and ecosystems.

Chapter 5 explores the yearly dynamics of dispersal kernels of Cabrera voles in Mediterranean farmland, by using the software application mentioned in the previous chapter. Dispersal kernels were expected to follow the variations in food and habitat availability, known to influence vole population dynamics and breeding ecology. The chapter sustains the use of gNIS and relatedness analysis to examine dispersal patterns and dynamics in elusive species. It also underscores the importance of accounting for dispersal variation when applying conservation measures.

Finally, Chapter 6 provides an integrative synthesis of the findings from the previous chapters, presenting the main conclusions, ecological implications, and conservation recommendations of the thesis, concluding with suggestions on further research to better understand how metapopulations of threatened species may persist in intensively used Mediterranean farmlands.

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Chapter 2

Drivers of survival in a small mammal of conservation concern: an assessment using extensive genetic non-invasive sampling in fragmented farmland

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Abstract

Although important to guide conservation management, detailed demographic studies on rare or elusive species inhabiting fragmented, human-dominated landscapes are often hampered by the species' low densities, and the logistic and ethical constraints in obtaining reliable information covering large areas. Genetic non-invasive sampling (gNIS) provides cost-effective access to demographic information, though its application to small mammals is still scarce. We used gNIS to infer on the demography of an endemic small mammal, the Cabrera vole (*Microtus cabrerae*), occurring as a spatially-structured population in a 462-ha Mediterranean farmland landscape. We intensively sampled fresh vole feces in four seasons, extracted the DNA, and performed individual identification based on genotypes built using nine microsatellites. We then estimated population size and individual survival relative to environmental variables, controlling for heterogeneity in capture probabilities using capture-mark-recapture modelling. Population size increased during the wet season and decreased during the dry season, while survival remained constant across the study period. Individuals captured along road-verges and around water-bodies survived longer than those captured near agricultural fields. The use of gNIS on a heterogeneous landscape such as our study area allowed us to demonstrate that human land-use activities affect Cabrera vole demographic parameters in Mediterranean farmland, with implications for conservation planning towards its long-term persistence. Our approach can be widely applied to other elusive small mammals of conservation concern, but for which informative demographic data are still scarce.

2.1 Introduction

Estimating large-scale demographic patterns (*e.g.* abundance, population growth, survival) of animal species in relation to both individual traits (*e.g.* sex, age, weight) and environmental factors (*e.g.* climate or land-use change) is a difficult but necessary goal to understand species ecology and sustain conservation policies (Smallwood and Schonewald 1998; Williams et al. 2002). This is particularly true for species occurring in agricultural landscapes where major declines in biodiversity due to agricultural intensification have been reported worldwide (Tscharntke et al. 2012). However, achieving these goals is often difficult due to a number of technical, ethical, and logistic constraints in data collection, particularly for species that are rare, elusive, or otherwise hard to capture or observe.

Capture-Mark-Recapture (CMR) is one of the most popular methods to assess demographic parameters in animal populations (Lebreton et al. 1992), and hence to understand species' biology and ecology in different environments (Smallwood and Schonewald 1998). Traditional CMR studies have been mostly based on live-trapping techniques, which are usually logistically difficult to implement over large spatial and temporal scales and often expensive (Cheng et al. 2017). In addition, because live-trapping implies both physical confinement and handling of animals, it often involves behavioural and physiological responses due to trapping-induced stress. Stress responses can be reduced with the use of minimally invasive techniques such as camera-trapping, which is expected to be more time-efficient than live-trapping and does not require physical capturing and handling of animals (Mondol et al. 2009; De Bondi et al. 2010). However, camera trapping is unsuitable for CMR studies in species that are difficult to morphologically identify at the individual level, which is the case of most small mammal species (Glen et al. 2013). Furthermore, in the case for rare and elusive species, both live- and camera-trapping often yield insufficient data to be used in CMR models, thus hampering proper evaluation of their population status and trends (Mondol et al. 2009; Burgar et al. 2018).

Genetic non-invasive sampling (gNIS) has been increasingly used to estimate demographic parameters of species that are difficult to trap, mainly due to decreased field sampling effort, ever decreasing lab costs, and increasing DNA amplification success (Beja-Pereira et al. 2009; Marucco et al. 2011). Despite its limitations in retrieving information on relevant individual traits like age, body mass, or reproductive condition, gNIS can provide a more cost-effective solution than traditional live-trapping (Cheng et al. 2017; Ferreira et al. 2018). DNA extracted from non-invasive samples (*e.g.* feces, hairs, feathers) allows the identification of individuals, providing data that can be easily combined with CMR methods to obtain population parameters that otherwise would be difficult to obtain over large spatial scales (Petit and Valiere 2006; Cheng et al. 2017). However, to date, applications of gNIS in CMR studies have mostly focused on large and medium-sized mammal species, and often provide snapshots of population size estimates rather than variations over time (but see Brøseth et al., 2010 for an example). Furthermore, very few studies have used gNIS to estimate other important population parameters such as survival (Marucco et al. 2012; Zielinski et al. 2013; Lampa et al. 2015). In the case of small mammals, while some recent studies have used gNIS to estimate population density (Gillet 2016; DeMay et al. 2017; Sabino-Marques et al. 2018) or to infer dispersal (Gillet 2016; Ferreira et al. 2018), to our knowledge no study has yet explored the application of this method to understand how demographic parameters relate to large-scale environmental variation.

In this study, we combined gNIS and CMR methods to assess the seasonal variations in abundance, and to evaluate factors affecting survival probability of an elusive small mammal species in a Mediterranean farmland landscape. We focused on the 'near-threatened', Iberian endemic Cabrera vole (*Microtus cabrerae*, Thomas 1906), for which genotyping protocols based on faecal samples have been recently optimized (Barbosa et al. 2013; Ferreira et al. 2018). Additionally, previous studies have also shown the ability of gNIS to provide reliable density estimates for this species (Sabino-Marques et al. 2018). Based on repeated surveys of Cabrera vole feces, we explored the potential of gNIS to (i) assess the seasonal variation in population abundance; and (ii) estimate capture and survival probabilities in relation to variables reflecting survey conditions (genotyping success and season), individual traits (sex), and local and landscape environmental features. We considered variables that might affect survival both positively (*e.g.* patch area and presence of water) and negatively (*e.g.* isolation, patch persistence, interactions with the competitor *Arvicola sapidus*, and human disturbances) (Pita et al. 2014) (see Table 2.1 for a full description and rationale of covariates considered). Overall, our study illustrates the use of gNIS within a CMR framework, demonstrating its application to retrieve demographic data from elusive small mammals, thus enhancing conservation planning in areas that have been highly modified by human activities.

Table 2.1 – Definition and summary statistics of covariates used in Cormack-Jolly-Seber (CJS) models to estimate recapture probabilities (p) and survival (ϕ) of the Cabrera vole in a Mediterranean farmland landscape of SW Portugal. Underlying hypotheses regarding covariate effects tested on p and ϕ are also presented. All environmental covariates were measured for each individual at the time of first capture (see text). * indicates variables selected for the CJS modelling procedure (see *Data analyses*).

Covariate Code	**Description**	**Type**	**N**	**Mean ± SE [range]**	**Parameter**	**Underlying hypothesis (based on Pita et al., 2014, except where indicated)**
*GenSuccess**	Mean genotyping success of analysed samples in each patch	Proportion (0-1)	307	0.5 ± 0.01 [0.1 – 1]	p	p is higher in patches where genotyping success was higher.
*Time**	Transition between seasons	Categorical (EWS-LWS; LWS-EDS; EDS-LDS)	307	–	p ϕ	p and ϕ differ among seasons, being greater in spring when animals are more active, and habitat and food availability are greater; and lower in the summer when habitat and food resources are scarcer, and animals are less active.
*Sex**	Sex of individuals	Categorical (M/F)	M: 152 F: 155	–	p ϕ	p is similar between sexes, as home-ranges vary little between males and females, and both sexes are expected to equally mark their territories. ϕ is similar between sexes, as expected for monogamous mammals (Clutton-Brock and Isvaran, 2007).
PArea	Area of the patch where animals were captured (m^2)	Continuous (> 0)	307	9834.0 ± 658.93 [106.2−51764.0]	p ϕ	p decreases with patch area, as animals tend to concentrate their territories within particular areas of habitat-patches. ϕ increases with patch area, as larger patches should provide more habitat and food resources.
PIsolation	Mean distance from the patch to the 3 nearest patches (m)	Continuous (> 0)	307	34.7 ± 2.06 [3.4−187.9]	ϕ	ϕ decreases with increasing patch Isolation, due to increased mortality during movement attempts between patches further apart from each other.
PPermanence	Permanence of the patch in the following seasons	Categorical (0/1)	0: 73 1: 234	–	ϕ	ϕ is lower if patch disappears in the following seasons.
*RoadVerge**	Binary coding of whether the patch is a roadside habitat (<10m from a road)	Categorical (0/1)	0: 66 1: 241	–	ϕ	ϕ is higher in roadside habitats, as these are usually less disturbed by farming operations.
*Water**	Presence of a permanent water-body within or at the border of the patch	Categorical (0/1)	0: 278 1: 29	–	ϕ	ϕ is higher where water is present, as the species prefers wet vegetation providing both refuge and fresh food.
*Arvicola**	Occurrence of *Arvicola sapidus* in the patch	Categorical (0/1)	0: 102 1: 205	–	ϕ	ϕ is lower where the competitively superior *A. sapidus* is present.
AgroDist	Distance from patch to the nearest agricultural area (m)	Continuous (≥ 0)	307	27.9 ± 3.20 [0−248.9]	ϕ	ϕ is lower in patches closer to agricultural fields, as these are associated to increased disturbance resulting from farming operations.
UrbDist	Distance from the patch to nearest urban area (m)	Continuous (≥ 0)	307	36.9 ± 3.07 [0−247.6]	ϕ	ϕ is lower in patches closer to urban areas, as these are associated to increased human disturbance and/or predation by domestic cats and dogs.

2.2 Material and Methods

2.2.1 Study area and species

The study was carried out in a 461.8 ha area within the coastal plateau of south-western Europe, Portugal (37° 21’ - 38° 04’ N, 08° 51’ - 08° 30’ W) (Fig. 2.1). The region is included in the thermo-Mediterranean bioclimatic zone (Rivas-Martínez 1981), with a mean annual temperature of 16.5°C (monthly temperatures ranging from 6 to 29°C), and an annual rainfall of about 650 mm (of which >80% falls between October and March) (Pita et al. 2006; Pita et al. 2007). The landscape is mostly flat (56-76m above sea level) and land cover is dominated by pastures and annual irrigated crops (Pita et al. 2007). Forest cover is limited to a few woodlots and hedges with pines and eucalyptus, while natural woodlands, shrubs, and marshy vegetation are most frequent along road verges (mostly dirt-roads with low traffic), and in the surroundings of extensive agricultural fields (Pita et al. 2009). Over the past three decades, agricultural practices have been strongly intensified, particularly through the expansion of cultivated land, associated with the

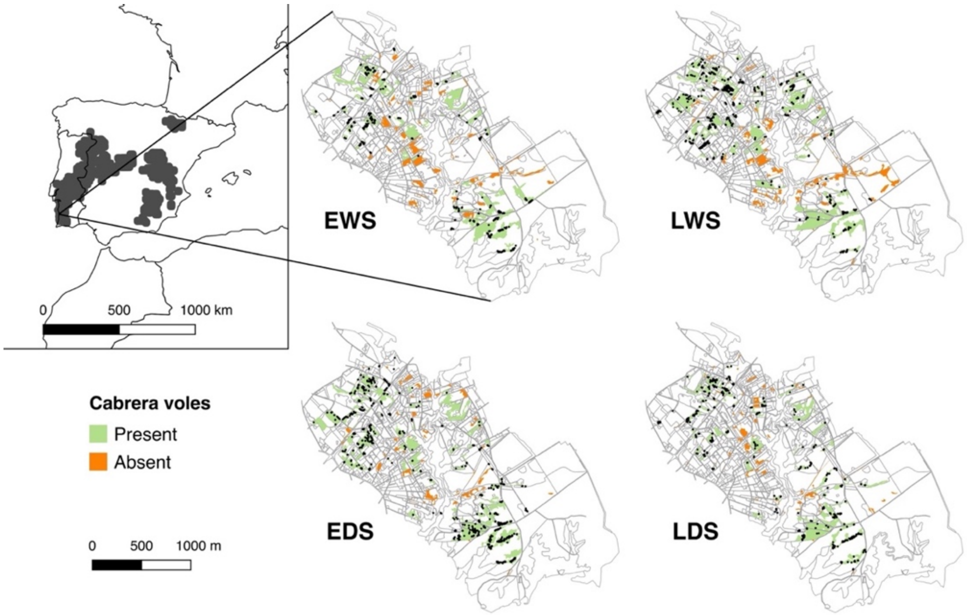

Fig. 2.1 – On the upper left, Cabrera vole distribution (in dark-grey, adapted from (Mestre et al. 2015) and study area location. On the right, four seasonal surveys made in a 461.8 ha area within the coastal plateau of SW Portugal. EWS - early wet season, November and December 2013; LWS - late wet season, February and March 2014; EDS - early dry season, May and June 2014; LDS - late dry season, September and October 2014. Coloured polygons represent identified suitable habitat-patches for voles. Black dots represent fresh-faeces samples collected for genetic analyses.

frequent use of pesticides and chemical fertilizers, with detrimental impacts on biodiversity (Pita et al. 2009; Ferreira and Beja 2013; Peralta et al. 2016).

Previous studies in the region have shown that Cabrera voles typically show a metapopulation-like spatial structure (Pita et al. 2007), occurring within damp habitat patches densely covered by tall wet herbs and shrubs along small streams, temporary ponds, field margins, and roadside verges (Pita et al. 2006; Pita et al. 2014). Population densities within patches are typically low (Pita et al. 2010; Pita et al. 2011), and individuals are rarely present in the same area for more than 4 months (Rosa Fernández-Salvador et al. 2005). Within habitat-patches, the Cabrera vole is often organized in monogamous breeding pairs and tend to exhibit home-ranges of only a few hundred square meters (Pita et al. 2010).

2.2.2 Vole surveys and environmental variables

Following the studies by Ferreira et al. (2018) and Sabino-Marques et al. (2018) conducted previously in a smaller area (78 ha) within the same study region, we surveyed Cabrera voles on four main occasions (seasons) along one year: early wet season (EWS, November-December 2013); late wet season (LWS, February-March 2014); early dry season (EDS, May-June 2014); and late dry season (LDS, September-October 2014). In each season, we used a two-step procedure, first identifying and mapping all habitat patches potentially used by Cabrera voles (damp areas with vegetation patches dominated by dense and tall herbs) using both *Bing Maps™* aerial photographs (retrieved in 2012) and ground validation (Fig. 2.1). Then, a survey was conducted over the whole area of each patch, to detect the presence of the species from its characteristic signs (faecal pellets and grass clippings), and to collect fresh faeces for genetic analysis. Within each patch, two samples were collected (if available) every 5 m (in order to maximize the chances of detecting different individuals) (Fig. 2.1), using sterilized tweezers, and stored in the field in individual 2 mL microtubes with 96% alcohol, and later kept at -20°C until DNA extraction. To minimize cross-contamination from conspecifics, faeces were collected from small latrines (<20 faecal pellets). The presence of the competitively superior southern water vole (*Arvicola sapidus*) was recorded based on the presence of similar but larger faecal pellets than those from the Cabrera voles, which are easily recognizable (Peralta et al. 2016). The surveying of the study area followed the same direction (from NW to SE) in each sampling season, such that habitat-patches were sampled following approximately the same order and time-intervals.

All information regarding the voles' habitat, matrix land-uses, and sample geographic location was stored in a vector-based Geographic Information System (GIS; QGIS, version 2.14.10 - Essen, QGIS Development Team, 2016).

2.2.3 DNA extraction and genotyping

Due to budget restrictions, only a limited number of faecal samples could be analysed. The selection of samples for genetic analyses followed a stepwise approach to reduce costs while aiming to achieve a comprehensive spatial coverage of each patch, hence maximising the number of captures and recaptures of individuals (Ferreira et al. 2018). In patches where less than six samples were collected, all samples were analysed. In each of the remaining patches, we selected at least 60% of the samples, evenly spread throughout the patch. When more than one sample was collected every 5 m, only one was initially analysed. If genotyping failed for the first sample, the second sample was analysed in order to obtain a minimum number of genotypes per patch of at least 40% of all sampling sites.

Vole DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (OMEGA bio-tek) following the manufacturers' instructions, with an initial digestion step using a lysis washing buffer (Maudet et al. 2004) for 15 minutes at 56°C. Samples were genotyped for a set of nine microsatellites characterized by high levels of polymorphism (Ho = 0.79; He = 0.81), *low probability of identity of unrelated* (PI= 3.2×10^{-12}) and related individuals (PIsibs= 9.2×10^{-5}), and high probability of exclusion (PE=0.99), and two small sized sex-linked introns (Supp. Table 2.1). These markers have been optimised for application to gNIS of Cabrera voles faeces (Ferreira et al. 2018), and provide accurate individual identifications and population estimates (Sabino-Marques et al. 2018). We followed the protocol described by Ferreira et al. (2018), which includes an initial screening of DNA quality using three species-specific microsatellite loci. The samples that amplified for the three loci were then amplified for the additional six microsatellite loci and two sex-linked introns. To account for genotyping errors (*e.g.* allele dropout and false alleles) and obtain a consensus genotype, each multiplex reaction was replicated a minimum of four times (three times for the sex-linked introns). To confirm species identification, a small fragment of cytochrome-*b* gene was amplified in all genotyped samples following Barbosa et al. (2013). To evaluate eventual biases in the estimation of genotyping success rate due to misidentification of faeces in the field, we also performed genetic species identification in at least 20% of the samples that failed during genotyping. The extractions and PCR reactions were conducted in a physically isolated room, where all the equipment was sterilized with bleach and ethanol, and exposed to UV light before and after usage. Negative controls were included in each manipulation, maintaining conditions to monitor and reduce the risk of DNA contamination (Beja-Pereira et al. 2009; Barbosa et al. 2013; Costa et al. 2017). All products were sequenced on a ABI3130 Capillary Sequencer (Applied Biosystems). Allele calling of the microsatellite loci and sex chromosome introns was performed using *GeneMapper* (v.4.0; Applied Biosystems). Cytochrome-*b* gene sequences were analysed in *Geneious 8* (Kearse et al. 2012).

Consensus genotypes for the successfully genotyped samples were obtained by analysing all replicate genotypes with *Gimlet* v.1.3.3 (Valiere 2002). For genotypes differing only by one or two loci or with up to two missing data, additional PCR replicates were performed to complete genotypes with missing data, and to check for genotyping errors. Consensus genotypes for each sample were then compared with each other to identify individuals. Following the criteria detailed in Ferreira et al. (2018) and Sabino-Marques et al. (2018), only samples that differed in more than two loci were assigned as new individuals. Genotyping error rates were estimated using Pedant (Johnson and Haydon 2007), with 10 000 search steps. Since the software only compares two replicates at a time, we carried out all possible pairwise comparisons and then averaged the results.

2.2.4 Data analysis

To estimate vole abundance (*N*), we considered each seasonal survey as a single sampling event and used ‘continuous-occasion’ closed-population CMR models allowing for multiple captures of the same individual within each survey. Specifically, we used the *capwire* estimator based on urn models (Miller et al. 2005; Pennell et al. 2013), considering two alternative formulations: the equal capture probability model (ECM) and the two innate rates model (TIRM). In ECM, all individuals are considered equally likely to be captured on each survey, while in TRIM there is a mixture of two types of individuals with different capture probabilities. A likelihood-ratio test was used to evaluate the fit of both models and determine the best fit (Miller et al.

2005). We used a parametric bootstrap test with 1000 samples to generate the 95% confidence interval for population estimates of the best model for each season. Both ECM and TRIM were fitted and compared in the package *capwire* (version 1.1.4) (Pennell et al. 2013) for *R* (version 3.3.2) (R Core Team 2018). For comparison purposes, we also used Chao's lower bound estimator assuming individual heterogeneity in capture probabilities (M_h-Chao) (Chao 1989). Although the M_h-Chao estimator assumes different capture occasions, it uses only the capture frequency, so it may be applied to our data (Miller et al., 2005). This estimator is thought to outperform *capwire* for large datasets (N > 200) (Miller et al. 2005), such as that used in this study (see Results). For this, we used the R package *Rcapture* (version 1.4.2) (Baillargeon and Rivest 2007).

To estimate monthly recapture (*p*) and survival (ϕ) probabilities we used the Cormark-Jolly-Seber (CJS) open-population model approach (Lebreton et al. 1992), implemented in *RMark* (v 2.2.4; Laake, 2013), an R interface for software package *MARK* (White and Burnham 1999). For this, we first collapsed within-season capture histories for each genotyped individual into a single value (0/1), denoting whether it was identified or not at each season (McCrea et al. 2015). We then tested the goodness-of-fit on a fully time-dependent CJS model (McCrea et al. 2015) using the *R2ucare* (v 1.0.0; Gimenez et al., 2017), which suggested a good fit of the data (χ^2=2.34, p-value=0.311). Potential predictors of CJS parameters included the effects of genotyping success on *p*; the effects of time, sex, and patch area on both *p* and ϕ; and the effects of patch isolation, patch permanence, presence of road-verges, presence of permanent water-bodies, detection of southern water voles, distance to the nearest agricultural area (vegetable gardens, orchards, or ploughed fields), and distance to the nearest urban area (*e.g.* houses, social areas, buildings) on ϕ (see full description and rationale of covariates in Table 2.1). Environmental covariates were specified at the individual level, considering the moment of first capture.

Before model building and selection, we assessed the correlations among predictors of *p* and among predictors of ϕ, retaining in the analyses only the predictors with correlation coefficients <0.30 (*i.e.* low correlations; Graham, 2003). Such a conservative criterion was chosen because in CJS models, as the number of predictors increases, so will the number of model parameters, and hence the number of possible models under evaluation, which decreases the ability to distinguish between informative and spurious variables (Doherty et al. 2012). Among the potential predictors of *p*, we found a negative correlation between genotyping success and patch area (Supp. Table 2.2). Because p should be most critically affected by variables more directly related to the sampling design, we retained genotyping success in the analyses to the detriment of patch area. As regards to potential predictors of ϕ, we found some degree of multicollinearity among the presence of road-verges, patch area, patch permanence, and patch isolation (Supp. Table 2.2), suggesting an association of road verge habitats to larger, more stable, and less isolated patches. Because the presence of road-verges was considered to provide a reliable descriptor of local environmental variations directly linked to human land-use activity, this variable was carried out to the CJS modelling procedure. We also retained the covariate distance to agricultural fields instead of the distance to urban areas (positively cross-correlated; Supp. Table 2.2), as agricultural land-use was predominant, and potentially most relevant land-use in the study area. Sex, presence of water, and detection of water voles were only weakly correlated to other predictors, and were therefore also retained in the analyses (Supp. Table 2.2).

We then evaluated a set of CJS models translating different combinations of hypotheses regarding uncorrelated factors possibly affecting *p* and ϕ (Lebreton et al. 1992). Given the relatively large number of possible models considering all possible combinations of main effects in both *p* and ϕ (*i.e.*, 512 models), we combined the most plausible submodels found separately for each parameter (Bromaghin et al. 2013). Plausible submodels for both *p* and ϕ were identified in each case, by first building and ranking all possible submodels defined by all possible additive combinations of main effects (three in the case of *p* and six in the case of ϕ). This resulted in eight submodels describing *p*, and 64 submodels describing ϕ. For the final set of candidate models to be evaluated, we retained in each case the set of submodels with a difference of Akaike's Information Criteria corrected for small sample size (ΔAICc) smaller than 2 relative to the respective top-ranked submodel, regarded as the most supported (Burnham and Anderson 2002). This plausible combination approach reduced the number of candidate models to about 3% (n=15) in relation to the all possible combinations strategy (see Results), thus reducing the potential incidence of spurious results (Doherty et al. 2012). Finally, from the most supported models (*i.e.* ΔAICc<2 relative to the top-ranking model) within this final set of 15 candidate models, we discarded those including uninformative covariates (*i.e.* with 85% confidence intervals of effect size estimates including zero; Arnold, 2010). This resulted in the selection of one single best model, from which we estimated the predicted monthly p and ϕ relative to each informative covariate included in that model.

2.3 Results

The amount of suitable habitat increased from the early wet season (EWS; 36 ha) to the late wet season (LWS; 46 ha), and declined both in the early dry season (EDS; 41 ha), and in the late dry season (LDS; 29 ha) (Fig. 2.1). The percentage of occupied patches was of 45% (n= 131) in EWS, 51% (n=138) in LWS, 61% (n= 137) in EDS, and 54% (n=149) in LDS (Fig. 2.1).

We collected a total of 2 711 faecal samples (mean±SE per season = 678±54), of which 48.4% (n=1312; 328±24 per season) were selected for DNA extraction and genotyping. Average genotyping success rate was 33.9% (444 samples), with the highest values obtained for samples collected in EDS (42.4%, n=153), followed by EWS (38.4%, n=140), LWS (26.8%, n=87), and LDS (24.4%, n=64). Overall genotyping errors were low (dropout rate: 0.68-2.8%; false allele rate: 0-0.18%; Supp. Table 2.1), with higher genotyping errors recorded in the seasons with lower genotyping success. From the randomly selected 23% of samples that failed amplification (i.e. 163 out of 704 samples), about 86% were identified as Cabrera voles, while the others belonged to other rodent species (7.3%) or were contaminated with human or ungulate DNA (6.7%). Another 164 samples were also contaminated despite being successfully amplified. The 444

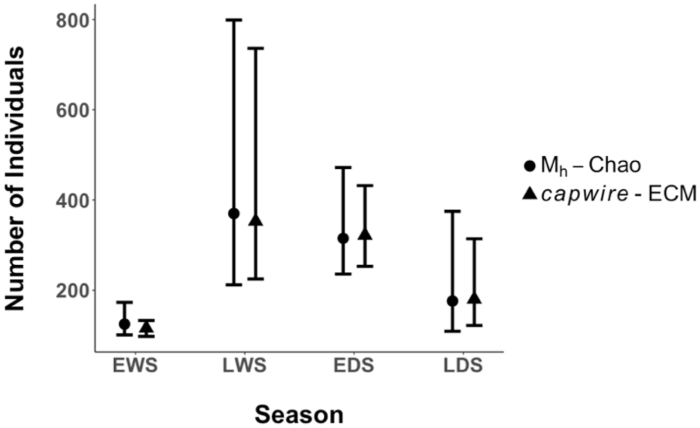

Fig. 2.2 – Seasonal population abundance of Cabrera voles in a Mediterranean farmland, based on genetic non-invasive sample, estimated using the M_h-Chao estimator (Chao) and the *capwire*'s equal capture probability model (ECM). EWS - early wet season, November and December 2013; LWS - late wet season, February and March 2014; EDS - early dry season, May and June 2014; LDS - late dry season, September and October 2014. Bars indicate 95% confidence intervals.

samples that were successfully amplified and were not contaminated were assigned to a total of 307 individuals, with 81 (EWS), 77 (LWS), 122 (EDS), and 64 (LDS) individuals per season. From the 137 recaptures identified across all surveys, 120 (87.6%) were in the same habitat patch of the previous (re)capture. The mean seasonal sex-ratio was even (M:F = 1.08), with no marked seasonal changes (between 1.03 and 1.13).

The likelihood-ratio tests indicated that ECM was more supported than TRIM for estimating abundances in all seasons ($p > 0.16$). Abundances estimated by ECM (range: 116-353) were very similar to those obtained using M_h-Chao (range: 125-370). There was an over 4-fold increase in vole abundance from EWS to LWS, with little change through to the EDS, followed by about a 2-fold decrease until the LDS (Fig. 2.2). Except for EWS, abundance estimates derived from both ECM and M_h-Chao had relatively wide confidence intervals.

The PC model selection approach resulted in the retention of five recapture probability submodels and three survival probability submodels (Table 2.2). After applying the model selection procedure on the final set of 15 plausible models and excluding models with uninformative covariates, the most supported model retained no covariates affecting recapture and included four covariates affecting survival (Table 2.3). Monthly recapture probability was estimated as 0.54 (0.20-0.85 CI 95%), while monthly survival was estimated as 0.52 (0.39-0.65 CI

Table 2.2 – Set of best plausible models (with a difference of Akaike's Information Criteria corrected for small sample size < 2) obtained from the set of 8 submodels describing p (keeping ϕ fixed) and 64 submodels describing ϕ (keeping p fixed).

Varying *p*, fixed ϕ	**AICc**	**k**
~1	178.1	9
~Time	178.4	11
~Sex + Time	179.8	12
~Sex	179.9	10
~GenSuccess	179.9	10
Varying ϕ, fixed *p*		
~RoadVerge + AgroDist + Sex + Water	178.1	10
~RoadVerge + AgroDist + Sex + Water + Time	179.9	12
~RoadVerge + AgroDist + Sex + Water + Arvicola	180.0	11

AICc –Akaike's Information Criteria corrected for small sample size
k – degrees of freedom
ϕ – apparent survival probability
p – recapture probability

95%), being 1.5-times higher in males than in females (Fig. 2.3-A). Survival was also affected by habitat conditions, being 2-times higher on road-verges than elsewhere (Fig. 2.3-B), 1.5-times higher in the presence of (or bordered by) water-bodies (Fig. 2.3-C), and 2-times higher 300m away from agricultural areas (Fig. 2.3-D).

Table 2.3 – Ordered set of best ranked plausible models (n = 15), obtained by fitting models separately for recapture (*p*) and apparent survival (ϕ) probabilities (more details in the Material and Methods section and in Bromaghin et al., 2013). Among the three most supported models (with ΔAICc < 2), the top-ranked model (in bold, AICc = 172.24) was the only one not including uninformative covariates, being thus retained as the best model. See definition of covariates in Table 2.1.

ϕ	*p*	ΔAIC$_c$	w$_i$	k
~AgroDist + RoadVerge + Sex + Water	**~1**	**0**	**0.29**	**6**
~AgroDist + RoadVerge + Sex + Water	~Sex	1.76	0.12	7
~AgroDist + RoadVerge + Sex + Water	~GenSuccess	1.80	0.12	7
~AgroDist + RoadVerge + Sex + Water + Arvicola	~1	2.05	0.10	7
~AgroDist + RoadVerge + Sex + Water	~Time	3.07	0.06	8
~AgroDist + RoadVerge + Sex + Water + Arvicola	~Sex	3.74	0.04	8
~AgroDist + RoadVerge + Sex + Water + Time	~1	3.83	0.04	8
~AgroDist + RoadVerge + Sex + Water + Arvicola	~GenSuccess	3.84	0.04	8
~AgroDist + RoadVerge + Sex + Water	~Sex + Time	3.89	0.04	9
~AgroDist + RoadVerge + Sex + Water + Time	~Time	4.00	0.04	10
~AgroDist + RoadVerge + Sex + Water + Arvicola	~Time	5.05	0.02	9
~AgroDist + RoadVerge + Sex + Water + Time	~Sex + Time	5.43	0.02	11
~AgroDist + RoadVerge + Sex + Water + Time	~GenSuccess	5.60	0.02	9
~AgroDist + RoadVerge + Sex + Water + Time	~Sex	5.65	0.02	9
~AgroDist + RoadVerge + Sex + Water + Arvicola	~Sex + Time	5.83	0.02	10

ΔAICc - Difference of Akaike's Information Criteria corrected for small sample size
w_i - model relative Akaike weight
k - degrees of freedom.

2.4 Discussion

We demonstrated for the first time the usefulness of large-scale genetic non-invasive sampling combined with capture-mark-recapture methods to estimate and identify the factors affecting small mammal demographic parameters and infer their population dynamics. Using the near-threatened Cabrera vole in Mediterranean farmland, we showed that our approach provides key information to improve conservation planning of elusive small mammals, especially those threatened by human activities and that are difficult to sample using traditional methods (Cheng et al. 2017).

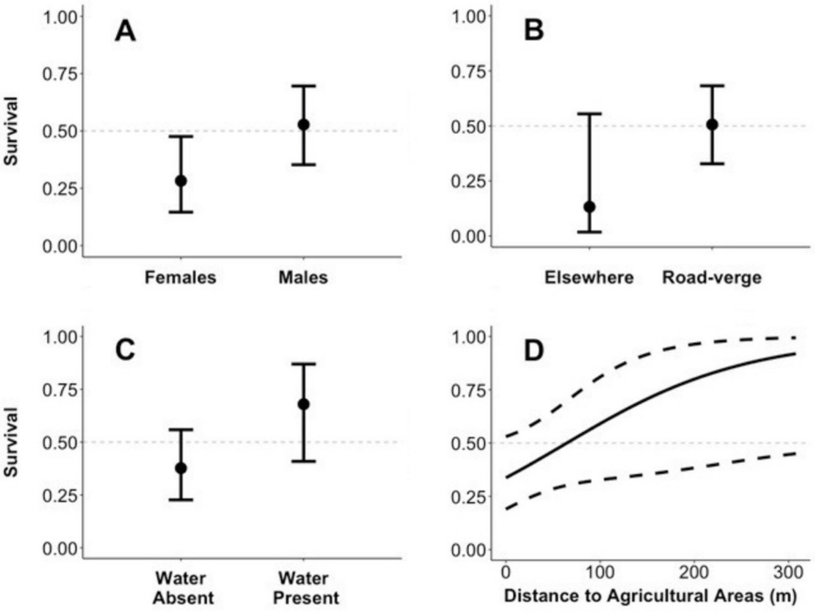

Fig. 2.3 – Apparent survival probability of Cabrera voles relative to sex (A); road verges (B); presence of water (C); and distance to agricultural areas (D), based in genetic non-invasive sampling in a Mediterranean farmland, during the period between November 2013 and October 2014. Black bars and dashed lines represent 95% confidence intervals. Grey dashed line represents average monthly apparent survival (= 0.52).

Our approach showed that Cabrera vole abundance varied greatly across an annual cycle, confirming a large increase in vole numbers along the wet season (67%), and a substantial decline through the dry season (44%), as reported elsewhere from live-trapping data collected at more confined scales (Rosa Fernández-Salvador et al. 2005; Rosário 2012). These changes seemed largely unrelated to seasonal genotyping success, which lowered by the end of the wet season and by the end of the dry season, likely due to increased DNA degradation under higher rainfall and sunlight exposure, respectively (Santini et al. 2007). Seasonal variation in vole numbers was consistent with the described breeding period for this species, which suggests a lower activity of individuals during the dry season, when reproduction may even cease completely (Rosa Fernández-Salvador et al. 2005; Pita et al. 2006). Comparable seasonal fluctuations have also been found in other rodent species inhabiting highly seasonal Mediterranean environments (Cohen-Shlagman et al. 1984; Gomez et al. 2016), as well as in other Iberian endemic herbivores, like the Iberian rabbit (*Oryctolagus cuniculus algirus*) (Gonçalves et al. 2002). This pattern is thought to be related to variations in habitat and food availability, which in our study area are generally reduced during the dry season (Pita et al. 2014). This was reflected in our data by the

37% decrease in habitat availability from the end of the wet season to the end of the dry season, in accordance with the natural seasonal variation in climate, and was also observed for other voles in intensively managed Mediterranean farmlands [*e.g.* common vole *Microtus arvalis*; (Rodríguez-Pastor et al. 2016)]. Pedigree and sibship analyses indicated that our gNIS was able to detect animals from different generations within each season (Ferreira et al., unpublished data), suggesting no serious bias towards any particular age-class of the population. While we acknowledge that the two-month duration of our seasonal surveys warrants some caution regarding abundance estimates obtained within a closed CMR framework, these estimates likely describe the broad patterns of seasonal population change at the surveyed landscape and its surroundings (Boulanger and McLellan 2001). Therefore, our study suggests that estimating vole population size based on gNIS and CMR modelling allows for the drawing of inferences on abundance variation across time.

Estimates of apparent survival of Cabrera voles based on gNIS in Mediterranean farmland were relatively low (0.39-0.65 CI 95%), though still within the range usually observed in other semi-fossorial *Microtus* species living in agricultural landscapes across different geographical regions. For instance, the survival of *M. agrestis* in field margins in fragmented farmland from northeast Scotland ranged between 0.42 and 0.69 (Renwick and Lambin 2011), while that of *M. arvalis* in agricultural landscapes from central western France varied between 0.22-0.69 (Bonnet et al. 2013). Similar survival rates (0.25-0.64) were also reported for *M. pennsylvanicus* in forage crops from Illinois (Getz et al. 2007). Surprisingly, despite the presumed lower habitat quality in our study area during the dry season, and the observed temporal variation in Cabrera vole abundance, survival was virtually constant across time. Variation in population abundance, despite constant survival rates, suggests that population recruitment (newborn and immigrants) increased during the wet season (when abundance increased), and decreased during the dry season, probably until it became insufficient to compensate the low survival rates, thereby resulting in pronounced decreases in vole numbers. Although we did not assess recruitment explicitly and our gNIS approach inherently prevents the identification of individuals' age, the observed seasonal variations in abundance agrees with the monthly fluctuations in recruitment rates observed in Cabrera vole populations from other Mediterranean areas (Rosa Fernández-

Salvador et al. 2005), as well as in other vole species from Mediterranean-like climates (Cockburn and Lidicker Jr 1983; Cohen-Shlagman et al. 1984; Paradis and Guédon 1993).

Survival was however not constant across space. Voles detected closest to farmed areas showed a 53% lower survival probability than voles captured furthest away, suggesting a negative impact of agriculture management on voles. Agriculture activities in this area are highly dynamic, involving for instance the conversion of fallow areas to farmed areas, resulting in the destruction of habitat-patches. While the putative negative effects of agricultural intensification on Cabrera vole populations have been widely suggested (R. Fernández-Salvador et al. 2005; Rosa Fernández-Salvador et al. 2005; Pita et al. 2006), our study provides the first quantitative evidence that the distance of grass-rich fragments occupied by voles to unsuitable farmed habitat affects individual survival probability. Further studies analysing fecundity variation across space and time are however needed to fully understand how such effect impacts overall population persistence in farmland areas. According to our initial predictions, voles captured in road-verge habitats showed higher survival probabilities than voles captured elsewhere. While road-verge habitats can be viewed as suboptimal for voles (Santos et al. 2007) it is likely that in intensively-used Mediterranean farmland, these habitats provide refuges for the species, as their vegetation is often left undisturbed for relatively long-time periods compared to surrounding fields (Ruiz-Capillas et al. 2013). This is supported by the association of road-verge habitats to larger and more stable habitats in our study area, as well as by the low traffic volume associated to the roads. The value of road-verges as refuges for small mammals in farmlands has also been noted in other Mediterranean environments with varying levels of land management (Sabino-Marques and Mira 2011; Ascensão et al. 2012; Ruiz-Capillas et al. 2013), being generally attributed to increased habitat quality, and/or to predation release effects, in the case of roads with more intensive traffic (Rytwinski and Fahrig 2007).

The positive effects of the presence of permanent water-bodies within or bordering habitat-patches on voles' survival was in accordance to the prediction that damper habitats provide better resources for Cabrera voles (Pita et al. 2011). This may be associated with the presence of fresh green vegetation providing high-quality food and shelter across the dry landscape (Santos et al. 2007). While the presumed superior competitor water vole also prefers habitats with dense and wet vegetation, and may affect Cabrera voles' occupancy patterns (Pita et al. 2016), we found no evidence for inter-specific effects on Cabrera voles' survival. It is also interesting to note that, despite their influence in shaping Cabrera voles' occupancy dynamics and abundance (Pita et al. 2007), patch size and isolation were poor predictors of individual survival. This has also been reported in other small mammal species, for which habitat quality was also a better surrogate for survival than patch size (Mortelliti et al. 2014).

Besides environmental factors, and contrary to our expectations based on the predominantly monogamous mating systems of Cabrera voles (Pita et al. 2014), apparent survival was also related to sex, with support for higher survival in males. In the case of monogamous species that are not sexually dimorphic and with both males and females sharing parental care, survival generally tends to be similar across sexes or in some cases male-biased, thus highlighting an interesting exception to the general rule of male-biased mortality, typical in polygynous mammals (Clutton-Brock and Isvaran 2007). Higher male survival in monogamous species may result from reduced competition among males compared to polygynous animals, and the fact that even in the presence of parental care, the costs of raising off-spring are likely to be endured primarily by lactating females (Clutton-Brock and Isvaran 2007; Lukas and Clutton-Brock 2013). Testing this hypothesis explicitly would require information on individuals' reproductive condition and age, which are not obtainable from gNIS approaches such as ours. However, because male-biased survival did not result in any male-biased sex-ratios, and apparent survival is the product of true survival and site fidelity (Sandercock 2006), we cannot rule out the possibility that the observed male-biased survival could also reflect a female-biased dispersal (or permanent emigration), which is also common in monogamous species (Mabry et al. 2013). Clearly, more studies are needed to fully elucidate on possible male-biased survival and/or female-biased dispersal in the Cabrera vole. This warrants the recommendation that future gNIS-based studies aiming to assess survival of elusive species, should combine other methods providing information on individual reproductive status and age, so as to improve inferences on their population dynamics.

2.5 Conclusions

Overall, our study provides empirical evidence that gNIS is a useful tool to monitor small mammal population parameters, and to identify management actions that may prove necessary to maintain their populations. Regarding the Cabrera vole, our results support the idea that conservation measures aimed to increase its survival in Mediterranean farmland, should promote low intensity agricultural management near occupied patches (encompassing longer fallow periods, low-disturbed margins, and high density of permanent water-bodies), in order to allow the continued existence of suitable habitats for the species, particularly during the stressful dry-season, when habitat availability is lower. We consider that our approach may be applied to other elusive small or medium mammals requiring conservation action, but for which informative demographic data across large spatial and temporal scales are still lacking.

Author contributions

Conception (PCA, PB, AM, RP); Design (XL, PCA, PB, AM, RP); Data Collection (APF, IL, CF, JP, HSM, SB); Data Analysis (APF, CF, RP); Writing (APF, RP); Revision (APF, CF, JP, PCA, HSM, SB, XL, PB, FM, AM, RP).

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Supplementary Material

Supp. Table 2.1 – Genetic diversity and genotyping error rates of the nine microsatellites used for genotyping Cabrera vole faecal samples collected in a Mediterranean farmland. N - number of samples where Locus occurred; EWS - early wet season, November and December 2013; LWS - late wet season, February and March 2014; EDS - early dry season, May and June 2014; LDS - late dry season, September and October 2014.

Locus	**GenBank**	**Season**	**N**	**Number of alleles**	**Expected Heterozygosity**	**Observed Heterozigosity**	**Dropout rate**	**False allele rate**
Mc18	MH264524	EWS	81	8	0.826	0.827	0.232%	0.000%
		LWS	77	8	0.824	0.805	1.615%	0.000%
		EDS	122	8	0.825	0.762	0.639%	0.000%
		LDS	54	7	0.832	0.722	2.433%	0.000%
		All	*307*	*8*	*0.828*	*0.779*	*0.865%*	*0.000%*
Mc24	MH264526	EWS	81	12	0.616	0.679	0.139%	0.000%
		LWS	78	10	0.582	0.667	2.085%	0.000%
		EDS	117	12	0.680	0.701	1.332%	0.000%
		LDS	52	12	0.650	0.654	0.121%	0.000%
		All	*301*	*14*	*0.638*	*0.668*	*0.895%*	*0.000%*
Mc30	MH264528	EWS	80	10	0.820	0.800	0.799%	0.207%
		LWS	78	10	0.819	0.731	1.121%	0.131%
		EDS	121	9	0.816	0.843	2.015%	0.147%
		LDS	54	9	0.795	0.815	2.197%	0.000%
		All	*306*	*12*	*0.818*	*0.804*	*1.499%*	*0.140%*
Ma25	EF177204	EWS	81	17	0.858	0.827	1.570%	0.000%
		LWS	74	16	0.851	0.797	6.799%	0.328%
		EDS	120	18	0.868	0.850	1.521%	0.061%
		LDS	53	15	0.871	0.868	3.309%	0.397%
		All	*301*	*19*	*0.864*	*0.837*	*2.750%*	*0.124%*
Mar76	EF666987	EWS	81	9	0.858	0.914	1.213%	0.000%
		LWS	78	10	0.859	0.795	1.254%	0.000%
		EDS	122	10	0.858	0.828	0.507%	0.000%
		LDS	54	8	0.840	0.852	0.550%	0.000%
		All	*308*	*11*	*0.858*	*0.847*	*0.874%*	*0.000%*
Mc02	MH264520	EWS	81	8	0.826	0.827	1.187%	0.000%
		LWS	77	8	0.834	0.844	3.122%	0.000%
		EDS	122	10	0.841	0.795	0.000%	0.000%
		LDS	54	9	0.834	0.833	1.727%	0.000%
		All	*307*	*10*	*0.837*	*0.811*	*1.200%*	*0.000%*
MSMM-3	AB016154	EWS	81	9	0.796	0.765	1.261%	0.215%
		LWS	76	9	0.799	0.803	2.625%	0.273%
		EDS	118	11	0.817	0.814	0.468%	0.000%
		LDS	53	9	0.821	0.755	3.885%	0.370%
		All	*301*	*11*	*0.808*	*0.781*	*1.629%*	*0.177%*

Mar03	EF666981	EWS	81	9	0.794	0.852	0.080%	0.000%
		LWS	78	9	0.786	0.756	0.935%	0.000%
		EDS	122	10	0.828	0.836	0.610%	0.000%
		LDS	54	9	0.805	0.759	1.928%	0.000%
		All	*308*	*10*	*0.812*	*0.805*	*0.677%*	*0.000%*
Mar16	EF666983	EWS	81	9	0.856	0.889	0.858%	0.158%
		LWS	78	9	0.861	0.821	1.190%	0.000%
		EDS	122	11	0.846	0.803	1.128%	0.000%
		LDS	54	9	0.838	0.759	1.650%	0.000%
		All	*308*	*11*	*0.854*	*0.818*	*1.122%*	*0.048%*
Mean		**EWS**		**10.111**	**0.805**	**0.820**		
		LWS		**9.889**	**0.802**	**0.780**		
		EDS		**11.000**	**0.820**	**0.804**		
		LDS		**9.667**	**0.809**	**0.780**		
		All		***11.778***	***0.813***	***0.794***		

Supp. Table 2.2 – Correlations among predictors of p and among predictors of ϕ. For variable definition, please see Table 2.1. Correlations between continuous variables, between continuous and categorical variables, and between categorical variables are Spearman correlations, point-biserial correlations and Cramer's V, respectively (Hinkle et al. 2002). Moderate to high correlations (≥0.30; Graham 2003) are indicated in bold. * indicates variables carried out to the CJS modelling procedure (see main text).

	Sex*	PArea	PIsolation	PPermanence	RoadVerge*	Water*	Arvicola*	AgroDist*	UrbDist
GenSuccess	-0.02	**-0.32**							
Sex*		-0.02	-0.04	0.05	0.01	0.01	-0.01	0.07	0.02
PArea			**-0.36**	0.17	**0.37**	0.17	0.00	0.17	0.02
PIsolation				-0.08	**-0.32**	-0.17	0.00	0.22	0.28
PPermanence					**0.38**	0.08	-0.04	0.13	-0.05
RoadVerge*						0.03	-0.04	0.10	-0.05
Water*							0.11	-0.22	-0.28
Arvicola*								0.08	-0.07
AgroDist*									**0.67**

Hinkle, D.E., Wiersma, W., Jurs, S.G., 2002. Applied Statistics for the Behavioral Sciences. Wadsworth Publishing Company.

Chapter 3

Extensive pastures and dirt-road verges promote landscape connectivity for a threatened rodent in Mediterranean farmland

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Keywords: Agricultural matrix; Animal movement; Gene flow;
Genetic non-invasive sampling; Resistance surface.

Abstract

Understanding functional connectivity in fragmented landscapes is crucial for conserving species and guiding management practices. This is particularly important in human-modified ecosystems such as farmland, where suitable habitat is often patchily distributed and surrounded by mosaics of inhospitable agricultural land. Studying gene flow in these landscapes offers valuable insights into how specific land uses influence individual dispersal and, consequently, the functional connectivity of populations. However, such approaches remain relatively scarce given the number of farmland species potentially threatened by agricultural intensification. Here, we assessed landscape functional connectivity for a small mammal of conservation concern, the Cabrera vole (*Microtus cabrerae*), in fragmented farmland in southwest Portugal. We used genetic non-invasive sampling to identify individuals and then related genetic and geographical distances between each pair. We then assessed how different land uses influenced gene flow based on resistance surface modelling. Our results revealed a weak population division into two clusters along an east-west cline separated by a riverine valley, as well as a weak but significant isolation by distance. This genetic configuration was best explained by proximity to extensive pastures, which exhibited the lowest resistance to gene flow (higher connectivity). Dirt roads with low traffic and their associated grassy verges were also associated with lower resistance to gene flow, facilitating vole gene flow across the farmland. Overall, these results suggest that maintaining functional connectivity for Cabrera voles in farmland requires the protection of extensive pastures in the matrix and the preservation of dirt-road grassy verges habitat, linking otherwise separated habitat patches. The ongoing trends of agricultural intensification, involving the destruction of many high-quality habitats, make these results especially relevant for conserving Cabrera voles and other similar species in Mediterranean farmland.

3.1 Introduction

Agricultural intensification leads to the loss and degradation of natural habitats, significantly altering resource availability for native biodiversity (Lambin and Meyfroidt 2011). The resulting habitat destruction typically leads to habitat fragmentation into smaller, isolated fragments surrounded by a matrix of human-transformed land cover (Haddad et al., 2015; but see Fahrig et al., 2019). As intensification grows, land use changes pose additional challenges for many organisms by restricting their ability to move across these altered landscapes (Foley et al. 2005). Together, these changes can reduce gene flow, genetic diversity and fitness, ultimately leading to population declines and increasing extinction risk (Balkenhol et al. 2015). Understanding how landscape composition affects gene flow is therefore crucial for maintaining functional connectivity, and thus mitigate the risks of inbreeding depression and genetic drift in threatened taxa (Allendorf and Luikart 2007). Landscape genetic research can provide spatially explicit insights into how gene flow is affected by landscape features at different spatial scales (Cushman and Landguth 2010; Segelbacher et al. 2010; Zeller et al. 2012). However, such approaches remain relatively underexplored in studies assessing how habitat changes in agricultural areas affect landscape functional connectivity for native farmland species (Somoano et al. 2022). This is particularly true for the Mediterranean region, given its rich biodiversity, large extent of altered and dynamic habitats, and unique agricultural practices (Blondel et al. 2010; Debolini et al. 2018).

Mediterranean agroecosystems such as those in southern Iberia, have historically been dominated by extensively managed pastures linked to beneficial environmental outcomes, including biodiversity and non-marketable ecosystem services (*e.g.* Bugalho and Abreu, 2008; Debolini et al., 2018). Extensive pastures, often associated with sparse, traditionally managed orchards or oak stands, play a key role in the conservation of High Nature Value (HNV) farmland in Mediterranean regions, being highly relevant in both environmental and social terms (Bernués et al. 2022; Lomba et al. 2023). However, in recent years, extensive pastures have been either abandoned or converted to croplands or intensive pastures. Such changes can significantly impact functional connectivity for many native farmland species, potentially leading to increased resistance to gene flow, and possibly ultimately affect the overall biodiversity value of such landscapes. In this context, marginal, low-managed linear strips delimiting agricultural fields (*e.g.*

along rural dirt roads, small water bodies, or ditches) may take greater importance as landscape elements promoting connectivity for farmland species (Fitzgibbon 1997; Marshall and Moonen 2002; Redon (de) et al. 2015; Jumeau et al. 2017). Solid empirical evidence examining how various land uses affect the functional connectivity of Mediterranean agricultural landscapes is scarce and negatively impacts the effectiveness of conservation strategies within these ecosystems.

The Cabrera vole (*Microtus cabrerae*, Thomas 1906) is a globally near-threatened rodent endemic to the Iberian Peninsula, found in isolated areas within the Mediterranean bioclimatic region, and closely associated with extensive farming systems (*e.g.* Pita et al., 2014). Locally, the species occurs in patchy habitats characterized by high soil moisture and dense cover by tall herbaceous vegetation. Agricultural intensification has been identified as the main driver of both historic and ongoing declines in its populations and habitats (Pita et al. 2014; Garrido García et al. 2017). Research based on the species' occurrence data in local habitat patches suggests the critical role of extensive pastures for the persistence of the species in Mediterranean farmland (Pita et al. 2007; Pita et al. 2016). In addition, there is evidence that individuals using habitats along the verges of dirt-roads have survival rates 2-times higher than in habitats located elsewhere (Chapter 2 - Proença-Ferreira et al. 2019). Thus, the size of extensive pastures and the extension and connectivity of road verges may contribute to increasing landscape functional connectivity for Cabrera voles. Testing these predictions, however, remains difficult due to the elusive behaviour of this species and the challenges in gathering reliable data through live-trapping or telemetry (Pita et al. 2014). In this context, genetic non-invasive sampling (gNIS) has emerged as a valuable alternative to identify individuals and infer their movements (*e.g.* Ferreira et al., 2018), making it an effective approach to assess how land use variation affects the contemporary gene flow.

Here, we used a Cabrera vole (meta)population in Mediterranean farmland as a model-system to evaluate how different land uses affect functional connectivity for native wildlife. Specifically, we analysed gNIS of vole faeces across an agricultural landscape from southwest Portugal (*e.g.* Ferreira et al., 2018; Chapter 2 - Proença-Ferreira et al., 2019) and to infer gene flow resistance at multiple spatial scales (*e.g.* Bauder et al., 2021). We tested the prediction that functional connectivity for Cabrera voles increases in or close to extensive pastures and to marginal habitats along the verges of rural dirt roads and water bodies (*e.g.* ponds), as these are the most similar land uses to their typical habitat-patches (*e.g.* Luque-Larena and López, 2007; Pita et al., 2011; Santos et al., 2006). Conversely, we predict that more altered land uses (*e.g.* cultivated land, improved pastures, planted forests, urban areas) or those associated with unsuitable conditions (*e.g.* river gallery, scrubland) should have little or no effect on enhancing connectivity for Cabrera voles, or may even contribute to reduce gene flow (see Table 3.1). If true, these predictions would provide important insights into how specific management strategies could be implemented to support the viability of this and other species with similar habitat requirements under agricultural intensification.

3.2 Methods

3.2.1 Study Area

The study was carried out in a 461.8 ha farmland area within the coastal plateau of south-western Portugal (37° 21' - 38° 04' N, 08° 51' - 08° 30' W) (Fig. 3.1). The study area is included in the thermo-Mediterranean bioclimatic zone (Rivas-Martínez 1981), with a mean annual temperature of 16.5°C (mean monthly temperatures ranging from 6 to 29°C), and an annual rainfall of about 650 mm (with over 80% falling between October and March) (*e.g.* Pita et al. 2006; Pita et al. 2007). The landscape is mostly flat (56-76m above sea level), with land cover dominated by extensive pastures and irrigated annual crops. This contrasts to some extent with the broader land use intensification trends observed in the region over the past three decades, including the expansion of cultivated land and/or of greenhouses, associated with the frequent use of pesticides and chemical fertilizers, with detrimental impacts on biodiversity (Pita et al. 2009; Ferreira and Beja 2013; Peralta et al. 2016). Forest cover is limited to a few woodlots and hedges with pines and eucalyptus, while natural woodlands, shrubs, and marshy vegetation are most frequent along road verges (mostly dirt-roads with very low traffic) surrounding agricultural fields (Pita et al. 2009).

Previous research in the study region has shown that Cabrera voles typically present a metapopulation-like spatial structure (Pita et al. 2007), occurring within small damp habitat patches (generally < 0.2 ha) densely covered by tall wet herbs and shrubs along small streams, temporary ponds, field margins, and roadside verges (Pita et al. 2006; Pita et al. 2011). Population densities within patches are typically low (< 20 ind/ha, Sabino-Marques et al., 2018), and individuals are rarely present in the same area for more than 4 months (Fernández-Salvador et al. 2005). Within habitat-patches, Cabrera voles are often organized in monogamous breeding pairs and tend to use home-ranges of only a few hundred square meters (Pita et al. 2010).

Table 3.1 – Summary statistics of the 18 main land uses in the 461.8 ha study area, with the predicted impact on enhancing the functional connectivity of Cabrera vole. A **+** indicates a positive impact, while **–** indicates no impact or negative impact.

Land use	Cover (ha)	Cover (% of total)	Predicted impact on connectivity
Urban areas	28.50	6.42	–
Paved roads	1.22	0.27	–
Eucalyptus plantations	19.62	4.42	–
Pine plantations	21.47	4.84	–
Cultivated land	4.69	1.06	–
Tilled land	0.40	0.09	–
Dirt roads	15.21	3.43	+
Bare soil	2.72	0.61	–
Intensive pastures	24.62	5.52	–
Non-farmed orchards	4.69	1.06	+
Farmed orchards	4.02	0.91	–
Extensive pastures	227.36	51.21	+
River-gallery	10.88	2.45	–
Shrubland	27.75	6.25	–
Natural ponds	1.22	0.27	+
Oaklands	47.99	10.81	+
Irrigation ponds	1.40	0.32	+
Irrigation ditches	0.27	0.06	+

3.2.2 Vole and land use surveys

We identified and mapped a total of 18 land uses across the study area based on aerial photos and ground validation (see Table 3.1 and Fig. 3.1). Within this area, Cabrera voles were surveyed following the procedure detailed in Chapter 2 (Proença-Ferreira et al. (2019). Data were collected during February-March 2014, coinciding with the expected peak of reproduction activity (Pita et al. 2014). In brief, all habitat-patches potentially used by Cabrera voles (n = 138; total area = 45.5 ha) were identified, mapped, and surveyed to detect and collect fresh faecal pellets for genetic analysis. Since land uses and suitable habitat-patches were mapped independently, the mapped suitable habitats often overlap different land-use types, according to the species requirements within the broader landscape context. Genetic non-invasive sampling was carried out within each patch, involving the collection of two samples (if available) every 5 m (to maximize the chances of detecting different individuals), using sterilized tweezers. All samples were stored in the field in individual 2 mL microtubes with 96% alcohol, and later kept at -20°C until DNA extraction (Chapter 2 - Proença-Ferreira et al., 2019). All information regarding the matrix land uses, voles' habitat, and sample location was stored in a vector-based Geographic Information System (GIS; QGIS, version 2.14.10 - Essen, QGIS Development Team, 2016).

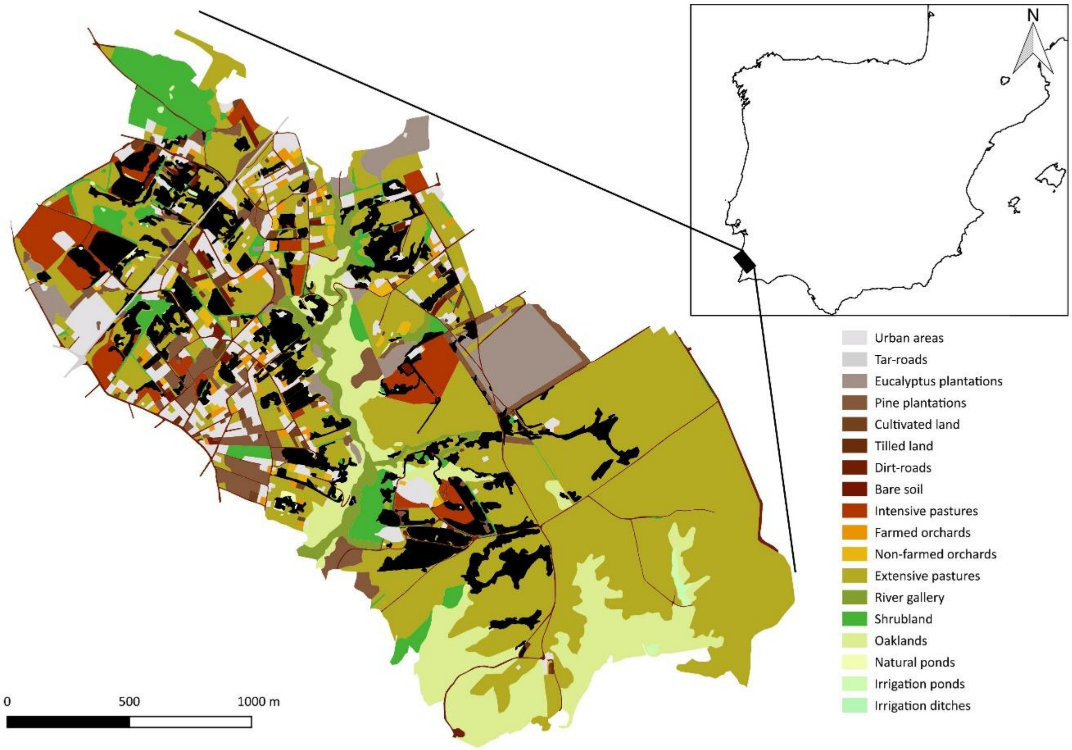

Fig. 3.1 – Study area in southwestern Portugal (461.8 ha). The 18 land uses were identified in the field and used in connectivity estimation for the Cabrera vole. Habitat patches suitable for Cabrera voles are shown in black polygons. More details about the land uses are provided in Table 3.1.

3.2.3 Genotyping

Vole DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (OMEGA bio-tek) following the manufacturers' instructions, with an initial digestion step using a lysis washing buffer (Maudet et al. 2004) for 15 minutes at 56°C. Samples were genotyped for a set of nine microsatellites characterized by high levels of polymorphism (Ho = 0.79; He = 0.81), low probability of identity of unrelated (PI= $3.2x10^{-12}$) and related individuals (PIsibs= $9.2x10^{-5}$), and high probability of exclusion (PE=0.99), and two small sized sex-linked introns. These markers have been optimised for application to gNIS of Cabrera vole faeces (Ferreira et al. 2018), and provide accurate individual identifications and population estimates (Sabino-Marques et al. 2018; Chapter 2 - Proença-Ferreira et al. 2019). We followed the protocol described by Ferreira et al. (2018), which includes an initial screening of DNA quality using three species-specific microsatellite loci. The samples that were amplified for the three loci were then amplified for the additional six microsatellite loci and two sex-linked introns. To account for genotyping errors (*e.g.* allele dropout and false alleles) and obtain a consensus genotype, each multiplex reaction was replicated a minimum of four times (three times for the sex-linked introns).

To confirm species identification, a small fragment of cytochrome-*b* gene was amplified in all genotyped samples following Barbosa et al. (2013). To evaluate eventual biases in the estimation of genotyping success rate due to misidentification of faeces in the field, we also performed genetic species identification in at least 20% of the samples that failed during genotyping. The extractions and PCR reactions were conducted in a physically isolated room, where all the equipment was sterilized with bleach and ethanol, and exposed to UV light before and after usage. Negative controls were included in each manipulation, maintaining conditions to monitor and reduce the risk of DNA contamination (Beja-Pereira et al. 2009; Barbosa et al. 2013; Costa et al. 2017). All products were sequenced on a ABI3130 Capillary Sequencer (Applied Biosystems). Allele calling of the microsatellite loci and sex chromosome introns was performed using GeneMapper (v.4.0; Applied Biosystems). Cytochrome-*b* gene sequences were analysed in Geneious 8 (Kearse et al. 2012).

Consensus genotypes for the successfully genotyped samples were obtained by analysing all replicate genotypes with Gimlet v.1.3.3 (Valiere 2002). For genotypes differing only by one or two loci or with up to two missing data, additional PCR replicates were performed to complete genotypes with missing data and to check for genotyping errors. Consensus genotypes for each sample were then compared with each other to identify individuals. Following the criteria detailed in Ferreira et al. (2018) and Sabino-Marques et al. (2018), only samples that differed in more than two loci were assigned as new individuals. Genotyping error rates were estimated using Pedant (Johnson and Haydon 2007), with 10 000 search steps. Since the software only compares two replicates at a time, we carried out all possible pairwise comparisons and then averaged the results.

3.2.4 Genetic structure analysis

We calculated patterns of contemporary population structure through two methods with different model assumptions: (i) the Bayesian clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000) and (ii) the Discriminant Analysis of Principal Components (DAPC; (Jombart et al. 2010) implemented in the R package *adegenet* v2.1.1 (Jombart 2008). Since evidence of population structure may be (falsely) detected when closely related individuals (namely first-order relatives: full siblings and parent-offspring) are included in a sample (Anderson and Dunham 2008), we used R package *related* (Pew et al. 2015), which is an R language implementation of the software COANCESTRY (Wang 2011), to estimate the Wang relatedness coefficient of all pairs of individuals. We removed from this analysis one individual per pair with relatedness > 0.45, maximizing the number of individuals kept.

We used STRUCTURE as a Markov chain Monte Carlo (MCMC) Bayesian clustering model, computing runs with K values between 1 and 10, with 20 independent runs performed for each K, each with 500 000 generations after a burn-in of 100 000 generations, using a no admixture model with correlated allele frequencies. The most likely number of clusters (K) were selected using the methods of Evanno et al. (2005) and Pritchard et al. (2000). As for the DAPC analysis, we first ran a PCA (Principal Component Analysis) on the genetic data, followed by a processing of the principal components with a linear discriminant analysis. Since DAPC needs an a priori identification of the groups from where each individual belongs, we estimated the genetic clusters by running the K-means clustering algorithm, from 1 to 10, then determining the optimal number of clusters by selecting the lowest value of the Bayesian Information Criterion (BIC). After the estimation of the population clusters of each method, we also calculated the pairwise F_{ST} between each group, and overall inbreeding in the population (F_{IS}) using the R package *hierfstat* (Goudet 2015).

3.2.5 Landscape genetic analysis

Isolation by distance was tested to assess the influence of geographic distance on gene flow. We first used a Mantel test with R package *ecodist* (Goslee and Urban 2007), comparing individual pairwise geographic distances with individual pairwise genetic distances obtained from genotyping. Secondly, using the same R package, we calculated a Mantel correlogram based on

12 distance classes. Spearman correlations with 9999 permutations were performed to estimate the significance and confidence intervals of each analysis.

We used the *ResistanceGA* R package (Peterman 2018) to evaluate the effect of each land-use (or resistance surface) on differences in genetic distances between sampled individuals. This algorithm adaptively explores parameter space to find the combination of resistance surface values and transformations that maximise the statistical relationship between matrices of pairwise cost-distances (predictor) and genetic distances (response). This method starts from an untransformed resistance surface (for example reflecting the presence of a certain land use or its percentage cover in each pixel) and applies a computer optimization based on genetic algorithms that reflect the actual estimated resistance surface for that land use. This avoids using the subjective, “expert opinion” assignment of values to resistance surfaces, which many times leads to inaccuracies (Zeller et al. 2012), favouring a more unconstrained approach. Individual-based genetic distances from the genotypes of each sampled individual were calculated using PCA-based metrics, in two stages. First, by computing the principal components (PC) for all alleles in the sampled population and then by calculating the distance matrix of the Euclidian distances between individuals among the first 64 PC axes. This method was deemed as an accurate genetic distancing method when having a low sample size and high dispersal, due to its higher sensitivity and inclusion of information otherwise lost in other methods (Shirk et al. 2017a).

Afterwards, a cost-based distance (*i.e.* effective distance) was iteratively calculated for each sampling pair using a least-cost path analysis based on the *gdistance* v1.2-2 R package (van Etten 2017). The *ResistanceGA* package algorithm then applied a linear mixed effects model with maximum likelihood population effects (MLPE), relating the genetic to the effective distance matrices, and ranks each resistance surface according to their AICc value (Akaike Information Criteria corrected for low sample size). This regression technique accounts for non-independence among the pairwise data and performs better than other regression methods commonly employed in landscape genetics (Shirk et al. 2017b). During optimisation, models were compared based on an objective function (we used the default option, log-likelihood) across different transformations and parameters over “generations” until there is no improvement, thus indicating the best optimized surface.

Raster surfaces were calculated from the 18 land uses identified in the field, stored in vector format, which were afterwards transformed into raster format for modelling. We derived three types of surfaces: (1) the presence-absence of each land use, (2) the percentage cover of each land use, and (3) the distance from each pixel to each land use. In addition, we considered three spatial scales when computing the percentage cover in each pixel, by calculating the land use cover in 10-, 20-, and 50-meter pixels. To reduce computational time, we then calculated the correlations between each surface, selecting the ones considered to have the most ecological meaning (for land uses with Pearson coefficient > 0.3; Graham, 2003).

We first performed a single-surface optimisation for each of the land uses selected using as response variable the pairwise genetic distance and, as predictors, pairwise cost-distance matrices calculated using the *commuteDistance* function implemented in the R package *gdistance* (van Etten, 2017), which is functionally equivalent to the CIRCUITSCAPE resistance distance. Two null models were also incorporated to examine model performance: a distance model, to test for isolation-by-distance (IBD, where each pixel is given a value of 1), and an intercept-only (or null) model (which implied gene flow was not affected by distance). Model performance was examined through the Akaike Information Criterion corrected for small sample sizes (AICc), and models were ranked according to ΔAICc (the difference in AICc between a given model and the top-ranked model) (Burnham and Anderson, 2002). We then performed a multiple surface optimisation to evaluate whether multivariate models (*i.e.* combinations of land-uses) better explain the observed patterns of genetic differentiation than univariate models. For the multiple surface optimisation, due to computation limitations, we selected only single surfaces with an AICc less than 10 relative to the best-performing surface. We conducted one run using the same optimisation parameters, with a total of 1000 bootstrap iterations in which individuals, sampling locations, and cost-distance matrices are sub-sampled in each iteration, to estimate each model's averaged Akaike weight (w_i) and the average marginal R^2 (or the percentage of explained variation).

3.3 Results

A total of 737 faecal samples were collected, of which 324 (44.0%) were selected for DNA extraction and genotyping. From these, 87 samples were successfully amplified (corresponding to a 26.8% success rate), corresponding to a total of 77 individuals. Overall genotyping errors were low (dropout rate: 0.68–2.8%; false allele rate: 0–0.18%) (Table 3.2). The mean observed heterozygosity and expected heterozygosity across loci were 0.780 and 0.802, respectively.

Table 3.2 – Genetic diversity and genotyping error rates of the nine microsatellites used for genotyping Cabrera vole faecal samples collected in a Mediterranean farmland.

Locus	**GenBank**	**N**	**Nr of alleles**	**H_e**	**H_o**	**Dropout rate**	**False allele rate**
Mc18	MH264524	76	8	0.803	0.828	1.615%	0.000%
Mc24	MH264526	77	10	0.675	0.590	2.085%	0.000%
Mc30	MH264528	77	10	0.740	0.825	1.121%	0.131%
Ma25	EF177204	73	16	0.794	0.859	6.799%	0.328%
Mar76	EF666987	77	10	0.805	0.866	1.254%	0.000%
Mc02	MH264520	76	8	0.855	0.841	3.122%	0.000%
MSMM-3	AB016154	75	9	0.800	0.805	2.625%	0.273%
Mar03	EF666981	77	9	0.753	0.789	0.935%	0.000%
Mar16	EF666983	77	9	0.818	0.867	1.190%	0.000%
		Mean	9.889	0.802	0.780		

N - number of samples where locus occurred

H_e - Expected heterozygosity

H_o - Observed heterozygosity

3.3.1 Genetic structure

Relatedness analysis detected 35 pairs of individuals closely related, resulting in a total of 60 sufficiently unrelated individuals for the genetic structure analyses. Both the STRUCTURE and DAPC population structure analyses were consistent, suggesting an optimal clustering of K = 2. Despite some admixed individuals, this underlined an east-west cline, with the river gallery largely separating the two groups (Fig. 3.2). F_{st} between the two clusters was similar in STRUCTURE (0.055) and DAPC analyses (0.060), indicating low to moderate genetic differentiation (Hartl and Clark 2007). F_{IS} was estimated as 0.023, indicating low inbreeding.

Fig. 3.2 – Collected samples of Cabrera voles with relatedness <0.4. Pie charts colours represent average proportion of assignment probability to K=2 genetic clusters, estimated by STRUCTURE.

3.3.2 Landscape genetic analysis

Isolation by distance analyses showed a weak but significant positive correlation between genetic distance and geographic distance (Mantel r = 0.219 (LCI 0.182 - LCI 0.264), p-value = 0.0002) (Fig. 3.3 - A). Considering the Mantel correlogram, we found a significant positive correlation for three distance classes up to about 500 m (Mantel r range = 0.077 - 0.100), as well as a significant negative correlation for distance classes between 1300 and 1700 m (Mantel r range = -0.069 - 0.081) (Fig. 3.3 - B).

Optimization and model selection results showed that the best-supported models were the multi-surface model including distance to extensive pastures and to dirt road resistance surfaces, and the single-surface model with only distance to extensive pastures (Table 3.3; for full set of models see Supp. Table 3.1 in Supplementary Material). Both models indicated lower resistance to gene flow when closest to this land use (Fig. 3.4). Although having a similar behaviour, revealed by the selection of the same monomolecular model function, the scale of estimated resistance is upwards to 20x higher when far from extensive pastures, when compared to dirt roads. Furthermore, it increases more steeply in extensive pastures (the maximum value is reached at a 50-meter distance to pastures, while for dirt roads the maximum is reached at circa 250 meters). Models with these surfaces had better performance than the null-model or the IBD model, indicating a good degree of robustness. Marginal R^2 values of the two most supported models (with ΔAICc < 2) were fairly low, varying between 0.035 and 0.058, while conditional R^2 values varied between 0.27 and 0.30. Mean contributions of each surface for the multi-surface model were 55.3% for dirt-roads and 44.7% for extensive pastures. Surfaces considering the

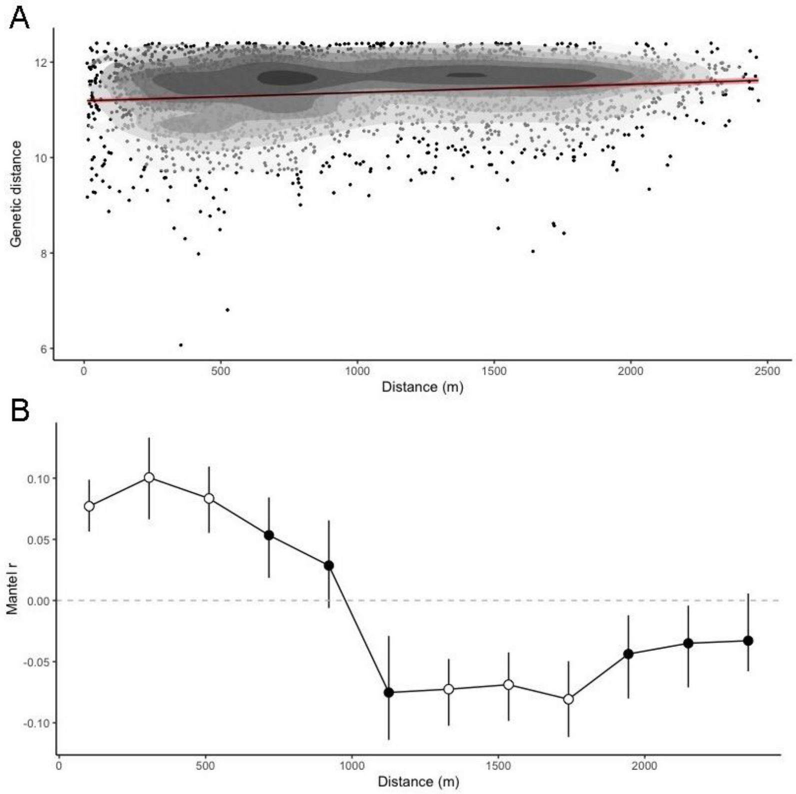

Fig. 3.3

(A) Isolation-by-distance of pairwise geographic distance vs genetic distance between each individual, red line and shading showing a linear regression and 95% confidence intervals, respectively. Grey cloud shows the density of points, with darker shade indicating the most common pairwise distances.

(B) Correlogram of spatial autocorrelation for 12 distance intervals between sampled locations of Cabrera vole faeces. Points represent the mean pairwise Mantel r, with 95% confidence intervals. Significant values of autocorrelation are indicated with empty circles.

Table 3.3 – Model selection results of the optimized land use resistance surfaces for the individual genetic difference of a Cabrera vole population in Southwest Portugal. Only models with ΔAICc < 7 are shown (for the full set of results see Supp. Table 3.1 in Supplementary Material). Bold font indicates highly supported models i.e. ΔAICc ≤ 2.

Surface	AICc	ΔAICc	R^2_m	R^2_c	w_i
dirt-roads distance + extensive pastures distance	**4020.28**	**0**	**0.035**	**0.266**	**0.454**
extensive pastures distance	**4020.29**	**0.011**	**0.058**	**0.296**	**0.430**
dirt-roads distance	4025.44	5.157	0.043	0.266	0.029

R^2_m - marginal R^2 (fixed effects only)

R^2_c - conditional R^2 (including both fixed and random effects)

AIC_c - Akaike Information Criterion (with finite sample size correction)

ΔAIC_c - AIC_c difference (difference between the 'best' model AIC_c and each model AIC_c)

w_i - Akaike weight (relative model likelihood/support across tested models for all resistance surfaces).

cover and presence/absence of different land uses had consistently lower explanatory power than distance-based surfaces, except in the case of dirt roads. This suggests a higher importance of the proximity to specific land uses than the composition of the surrounding landscape in explaining gene flow.

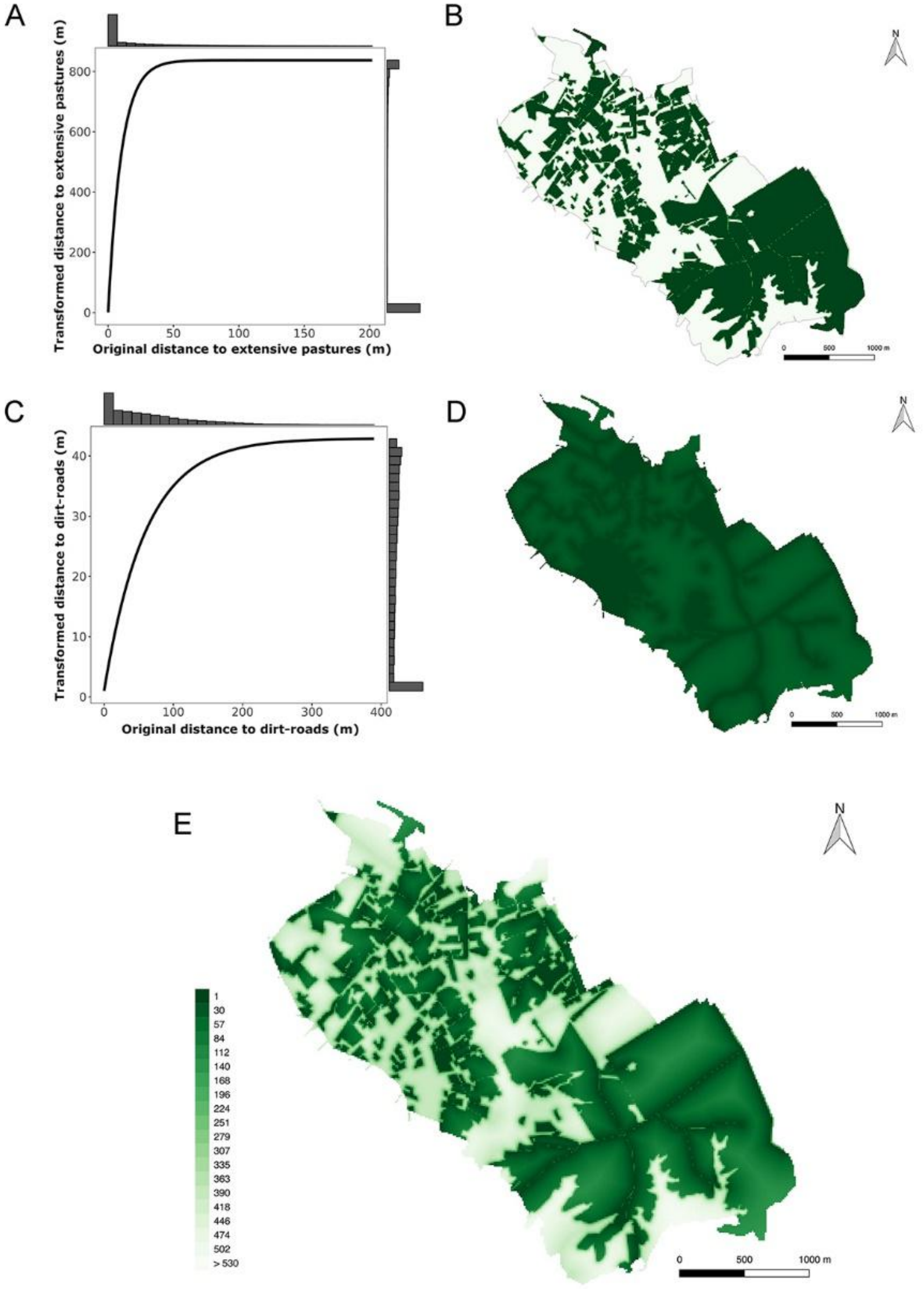

Fig. 3.4 – Resistance optimization curves and corresponding estimated raster surfaces for single-surface models for the gene flow of Cabrera voles in SW Portugal, based on the distance to extensive pastures (A and B) and on the distance to dirt-roads (C and D). At the bottom (E), the composite resistance surface including distance to extensive pastures and distance to dirt-roads. The colour scale is the same on all surfaces, varying continuously from dark green (low resistance) to white (high resistance).

3.4 Discussion

Our study showed a strong reliance on extensive pastures and linear dirt-road verges for Cabrera vole connectivity in Mediterranean farmland during the breeding season. This suggests that adequate management of these land uses is essential for maintaining connectivity and contributing to species' persistence in the area. To our knowledge, ours is the first study inferring small mammal movements and fine-scale genetic structure across the landscape based uniquely on genetic non-invasive sampling, showing that this approach may provide relevant data for modelling functional connectivity for elusive species.

3.4.1 Landscape genetic structure

We found a significant, albeit low genetic differentiation in our Cabrera vole population, with some spatial subdivision in two genetic clusters and a general pattern of isolation by distance. Although the weak genetic cline could be attributed to the relatively small study area, it is worth mentioning that this area extends over more than 4500 times the maximum home range estimated in the same region (Pita et al. 2010). Such area size potentially encompasses dispersal movements within the range inferred from empirical data (Bowman et al. 2002), suggesting that dispersal distances beyond 1km should be relatively infrequent (see Pita et al. 2014 and references therein). Despite the lack of a strong population structure, our results suggest that land uses less readily exploited or crossed by voles might contribute to variation in the population's genetic structure. This may be the case of the river gallery dissecting the study area, which is less suitable for the species and likely hosts a higher abundance of potential predators, eventually limiting voles' movement (*e.g.* Pita et al., 2009). However, this land use was not selected in the resistance models. Thus, it is also possible that the detected moderate population structure, together with the significant positive spatial correlation in individuals' relatedness at distances below 500m and the negative correlation at distances around 1500m, indicate the scale at which the studied metapopulation may begin to differentiate (Lambin et al. 2004). Apart from that, while current genetic patterns suggest relatively extensive gene flow in the study area, our results supported the prediction that certain land uses associated with low-management intensities significantly contributed to increased landscape functional connectivity of the species.

3.4.2 Landscape connectivity

As previously anticipated based on the species' ecological requirements (*e.g.* Pita et al., 2014), one of the clearest signals of our landscape resistance analyses was the importance of grasslands and verges in maintaining connectivity for Cabrera voles. Specifically, both the proximity to extensive pastures and dirt-road verges positively influenced the functional connectivity of the species. Notably, the results indicated that functional connectivity seems to be influenced not only by those two land uses considered individually, but also by their additive effects. At the same time, those effects seem to apply irrespectively of the scale used, eventually reflecting their throughout availability across the study area. This is particularly noticeable in the case of extensive pastures, which represent about 50% of all land-use cover in the study area, thus explaining the high degree of connectivity within the landscape. Despite this, our results also show a steep decrease in connectivity starting at distances as low as 50 meters away from extensive pastures.

As regards to dirt roads and their low-managed verge habitats, the observed positive effect on functional connectivity for Cabrera voles resonates with well-established literature, referring to the importance of these landscape elements on many farmland species (*e.g.* Ascensão et al., 2012; Benítez-López et al., 2010; Carthew et al., 2013). Nevertheless, this study is one of the few showing the positive effect of road verges on small mammal connectivity in the Iberian Peninsula (Galantinho et al. 2020; Ouédraogo et al. 2020). Extensively managed roadside verges are often considered to have great potential to increase the connectivity between specific grassland habitat-patches scattered in a landscape, such as those used by Cabrera voles. A plausible explanation for the generally positive effect of dirt roadside verges on landscape connectivity may be related to the small home-ranges of the species (Pita et al. 2010). This, coupled with the relatively stable high-quality habitat these linear elements often provide (see *e.g.* Pita et al., 2006), allows individuals that use and move through these habitats to have relatively high survival rates (Chapter 2 - Proença-Ferreira et al. 2019)

3.4.3 Management implications

Our results seem to reflect the reliance of Cabrera voles on extensive pastures for their dispersal, suggesting important conservation implications to farmland management, possibly including agri-environmental schemes where farmers are financially stimulated following the selection of land parcels dedicated to extensive grazing (Beaufoy and Poux 2014; Moreira et al. 2019). The importance of the presence of extensive pastures in matrix habitats for Cabrera vole populations has been referred in previous studies (*e.g.* Pita et al. 2007; Pita et al. 2016) and has been shown to be also important to other farmland species with preferences for open habitats such as insects (Pina et al. 2017), birds (Pereira et al. 2014; Morgado et al. 2021), or other mammals (Pita et al. 2009; Rosalino et al. 2009). In this context, the decline of extensively used pastures in our study region may be considered a major threat to farmland biodiversity, given its likely importance for landscape connectivity of many taxa. Importantly, because the expansion of intensive agriculture in southwest Portugal is ongoing and increasing (Silva et al. 2023), the high resistance values of other more intensively used areas may be somewhat conservative, given that recent isolation may not be fully reflected by current genetic distances (Jiménez-Franco et al. 2022).

The preservation and restoration of linear field margins such as those found along dirt roads may also play a pivotal role in conserving species such as the Cabrera vole, particularly in Mediterranean landscapes facing agricultural intensification. However, despite their potential benefits to farmland biodiversity, uncultivated field margins along dirt roads or field fences are just one component of the farmed landscape. Hence, their positive effects may be masked by the management of surrounding fields and the quality and spatial extent of the habitats that they connect (*e.g.* Feber et al., 2019; Galantinho et al., 2022). Nevertheless, while further research is needed to confirm the effectiveness of dirt road verges in promoting landscape connectivity in more intensive farming systems, our results provide evidence that due to their particular microhabitat and microclimate conditions, hydrology regimes, soil composition, low management, and minimal traffic volume, dirt-road verges may contribute to conservation efforts directed to small native fauna in Mediterranean farmland (Carthew et al. 2013; Gomez et al. 2016).

3.4.4 Conclusion

Future research should focus on evaluating the consistency of these results across different seasons, as well as on assessing critical thresholds in the amount and spatial arrangement of those land uses identified as beneficial, while considering different intensification levels in matrix habitats. Also, the inclusion of other land use classes or other environmental descriptors not assessed here could help improve the explanatory power of our models. This may include multispectral satellite data describing the vegetation composition and structure (Valerio et al. 2020), as well as its physiological condition at higher spatial and temporal resolutions (Fleming et al. 2021). In summary, our gNIS-based approach to infer gene flow and optimize a resistance surface model for the threatened Cabrera vole in Mediterranean farmland highlights the importance of considering specific land uses and their spatial configuration when addressing management priorities for the sustainable development of these systems. In particular, when conservation efforts target small fauna associated with specific grassland habitats, as is the case of the Cabrera vole, the presence of large extensions of extensive pastures across the agricultural matrix, together with low-managed field margins along dirt-roads delimiting agricultural fields, seems to be crucial for promoting landscape functional connectivity, and hence for retaining biodiversity in intensively used Mediterranean farmland.

Author contribution

Conceptualization: APF, PCA, PB, AM, RP; Design: APF, XL, PCA, PB, AM, RP; Data collection: APF, IL, CF, JP, HSM, SB; Data analyses: APF, CF, RP; Writing: APF, RP; Reviewing: APF, IL, CF, JP, PCA, HSM, SB, XL, PB, FM, AM, RP.

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Supplementary Material

Supp. Table 3.1 – Full list of univariate and multivariate resistance surfaces optimisation models after removing correlated surfaces.

Surface	AICc	ΔAICc	R^2_m	R^2_c	LL
distance dirt roads + distance extensive pastures	4020.28342	0	0.03533487	0.26651193	-2002.3417
distance extensive pastures	4020.29506	0.011645	0.05812429	0.29583421	-2005.8736
distance dirt roads	4025.4401	5.156687	0.04303584	0.2666796	-2008.4461
20% cover river-gallery	4037.97927	17.695856	0.02099816	0.24069005	-2014.7157
10% cover river-gallery	4044.1929	23.909487	0.01980772	0.23937795	-2017.8225
distance river-gallery	4045.55236	25.268947	0.02072499	0.23786434	-2018.5022
distance irrigation ponds	4046.00507	25.721656	0.0239978	0.25260481	-2018.7286
distance oaklands	4046.78488	26.50146	0.02799836	0.24780877	-2019.1185
presence intensive pastures	4047.07468	26.791262	0.01917051	0.23853528	-2020.3752
20% cover intensive pastures	4047.77581	27.492391	0.0200731	0.23891739	-2019.6139
10% cover intensive pastures	4048.94879	28.665372	0.01934679	0.23857896	-2020.2004
presence dirt roads	4049.26945	28.986029	0.01948019	0.23998183	-2021.4726
50% cover cultivated	4049.42983	29.146414	0.01813799	0.24118781	-2020.4409
20% cover urban	4051.17288	30.889461	0.01831411	0.23901982	-2021.3125
presence extensive pastures	4052.6664	32.382982	0.02515129	0.25401562	-2023.171
20% cover farmed orchards	4052.77862	32.495204	0.04329216	0.24630021	-2022.1153
presence eucalyptus	4052.9238	32.640387	0.01725663	0.23866802	-2023.2997
Distance model	4053.55442	33.271006	0.01664176	0.23772192	-2024.6972
presence irrigation ditches	4054.04754	33.764126	0.03775644	0.2501072	-2023.8616
10% cover urban	4054.07784	33.794426	0.01798845	0.23883573	-2022.7649
50% cover irrigation ponds	4054.28239	33.998968	0.01747158	0.23804269	-2022.8672
20% cover pines	4054.3664	34.082987	0.01750216	0.23888238	-2022.9092
50% cover soil	4054.50034	34.216922	0.02013008	0.24079382	-2022.9762
distance tilled	4054.54712	34.263698	0.01621541	0.2384234	-2022.9996
presence irrigation ponds	4055.4367	35.153283	0.0166946	0.23757523	-2024.5562
presence shrubland	4055.44257	35.159152	0.01670053	0.23778025	-2024.5591
presence paved roads	4055.6689	35.385481	0.01665296	0.2377441	-2024.6723
50% cover orchards	4055.73987	35.456451	0.01670659	0.23824433	-2023.596
presence tilled	4055.7516	35.468179	0.01663087	0.23771279	-2024.7136
presence cultivated	4055.80113	35.517708	0.01660366	0.23770204	-2024.7384
presence ditches	4055.84226	35.558838	0.01665535	0.2375168	-2024.759
10% cover pines	4055.90676	35.62334	0.01681034	0.23811786	-2023.6794
10% cover natural ponds	4055.98755	35.704136	0.0272517	0.24109626	-2023.7198
50% cover shrubland	4056.09756	35.81414	0.01665667	0.23790372	-2023.7748

20% cover soil	4056.20837	35.92495	0.02262977	0.23962217	-2023.8302
20% cover irrigation ponds	4056.55277	36.269352	0.0168573	0.23764696	-2024.0024
50% cover pines	4056.623	36.339583	0.01693514	0.23815738	-2024.0375
50% cover farmed orchards	4056.80608	36.522658	0.03848164	0.24479529	-2024.1291
20% cover natural ponds	4056.81062	36.527205	0.02273883	0.23856354	-2024.1313
10% cover shrubland	4056.82431	36.540893	0.01649983	0.23765488	-2024.1382
50% cover natural ponds	4057.18492	36.901504	0.01695574	0.23782332	-2024.3185
20% cover shrubland	4057.31606	37.032638	0.01672636	0.23780081	-2024.3841
10% cover irrigation ponds	4057.3246	37.041178	0.01671853	0.23758235	-2024.3883
20% cover orchards	4057.359	37.075586	0.01652533	0.23797479	-2024.4055
20% cover cultivated	4057.38876	37.105344	0.0166098	0.2381756	-2024.4204
10% cover soil	4057.43095	37.147529	0.01954937	0.23847431	-2024.4415
presence river-gallery	4057.56357	37.280152	0.01523268	0.23455209	-2025.6196
10% cover orchards	4057.80122	37.517802	0.01665501	0.23773875	-2024.6266
50% cover ditches	4057.83148	37.548058	0.01683377	0.23740291	-2024.6418
10% cover ditches	4057.86707	37.583652	0.01684807	0.23762573	-2024.6596
presence farmed orchards	4057.8835	37.600084	0.01652777	0.23787371	-2025.7796
10% cover paved roads	4057.88443	37.601016	0.01665538	0.23774899	-2024.6682
20% cover ditches	4057.90507	37.621655	0.01665303	0.23774291	-2024.6786
20% cover paved roads	4057.91542	37.631998	0.01663091	0.2378265	-2024.6837
50% cover tilled	4057.91886	37.635446	0.01664648	0.23770279	-2024.6855
50% cover paved roads	4057.93957	37.656151	0.01663177	0.23776081	-2024.6958
10% cover cultivated	4057.94237	37.658951	0.01664176	0.23772192	-2024.6972
10% cover tilled	4057.94237	37.658951	0.01664176	0.23772192	-2024.6972
10% cover farmed orchards	4057.94237	37.658951	0.01664176	0.23772192	-2024.6972
presence pine	4058.26034	37.976925	0.01584486	0.23693588	-2025.968
presence tilled	4059.31842	39.035003	0.05208105	0.2712079	-2026.497
presence urban	4060.72281	40.43939	0.04973267	0.27610554	-2027.1992
presence orchards	4071.167	50.883586	0.05755404	0.29767629	-2032.4213
Null model	4103.02017	82.736752	0	0.22164757	-2050.4838

Chapter 4

dispfit: an R package to estimate species dispersal kernels

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Abstract

Dispersal of organisms is a ubiquitous aspect of the natural world, with wide implications across scales and organization levels. Interest in dispersal has risen sharply over the past 30 years, mostly due to the multiple and rapid global changes ecosystems face. Among the various aspects that may characterize a dispersion event, dispersal distance is considered a key descriptor in a wide variety of studies across taxonomic groups. Typically, dispersal distances are defined in the form of dispersal kernels describing the dispersal distance distribution according to probability density functions. Although numerous methods providing dispersal data exist, there is still a lack of intuitive and comprehensive approaches and tools to estimate dispersal kernels from such data. Here we present the *dispfit* package, an `R` software application developed to fill this gap. *dispfit* fits and compares different families of parameterized functions to describe and predict dispersal distances. It includes 9 well-known and commonly used distributions, computes goodness-of-fit and model selection statistics, and estimate each distribution's parameters, along with their first four moments (mean, standard deviation, skewness, and kurtosis). We describe the main functions included in *dispfit* and provide an example to illustrate the workflow of the typical analyses performed within the package. We believe that *dispfit* will critically contribute to improving the modelling of species’ dispersal distances, thus enhancing the understanding of the ecological and evolutionary processes involving dispersal movement.

4.1 Introduction

Dispersal is defined as the movement of organisms from a source location (either after birth or breeding) to a different location where they might establish and potentially reproduce (Nathan, Schurr, et al. 2008; Clobert et al. 2012; Matthysen 2012). It has long been recognized as an important life-history trait affecting the dynamics and evolution of populations. Dispersal evolves from a combination of species and individual characteristics, and their responses to changes in biotic and abiotic environments (Bowler and Benton 2005; Benard and McCauley 2008). Theoretical and empirical advances on its causes, consequences, mechanisms, and patterns suggest that dispersal is tightly linked to other life-history traits (*e.g.* survival, niche breadth, trophic level, the timing of reproduction, mating strategies, fecundity), and can therefore be understood as a complex syndrome of traits, consisting of the integrated expression of many morphological, physiological, and behavioural aspects (Ronce and Clobert 2012). The ecological and evolutionary consequences of dispersal and related life-history traits span over multiple levels (from individuals to whole ecosystems) and have major implications for conservation and environmental management (Driscoll et al. 2014). The rapid changes that most ecosystems are currently undergoing have increased the interest in understanding how dispersal shapes species' responses to those changes.

Among the multiple aspects that may characterize a dispersion event (*e.g.* mechanism, post-dispersal survival, gene flow, triggering time), the distance between the start and end points (*i.e.* net displacement) remains a key spatial descriptor of dispersal on which many studies rely on (Nathan et al. 2012). In particular, dispersal distance is considered a crucial parameter to understand, for instance, the potential for local adaptation and genetic differentiation, the structuring of metapopulation and metacommunity networks, the dynamics of invasive species, the shifting in species distribution ranges, and the species' ability to track environmental conditions such as landscape or climate change (Ronce 2007; Nathan, Getz, et al. 2008). Including dispersal distance in studies focussing on these processes is thus of critical importance to improve their significance and predictive ability. In this context, the dispersal kernel concept has been central to analysing dispersal processes and spatial patterns. The dispersal kernel describes the distribution of dispersal distances based on probability density functions and is usually characterized by parameters describing the shape and form of those functions (Bullock et al. 2016). Currently, dispersal kernels are commonly incorporated in ecological and evolutionary studies dealing with dispersal across various taxonomic groups (Clark et al. 1999; Chapman et al. 2007; Coombs and Rodríguez 2007; Krkošek et al. 2007), but are also needed to inform policy to proper habitat management and conservation (Jongejans et al. 2008; Driscoll et al. 2014; Saura et al. 2014). Also, there has been an increasing number of probability distribution functions proposed to describe dispersal kernels with different properties (*e.g.* Hovestadt et al., 2012). However, the choice of the most appropriate function to fit empirical data is still largely based on personal preference or experience, suggesting a lack of coherence in approaches to model dispersal distances (Bullock et al. 2016).

Despite their great popularity, there is a paradoxical absence of tools to fit and compare different dispersal kernel functions from empirical dispersal data in a straightforward fashion. This is somehow surprising, given the considerable technical developments in movement ecology for improving the accuracy and precision of dispersal distance measures (Driscoll et al. 2014), including capture-recapture (Serrano et al. 2019), isotope analysis (Hall and Beissinger 2017), GPS and satellite tracking (Cadahía et al. 2010; Krieger et al. 2012) or molecular data (Bode et al. 2018). Here we present the `R` package *dispfit*, which was developed to fill this gap by providing an easy-to-use application to compare the fit of dispersal distance data to different families of parameterized functions commonly used to describe the relations between dispersal and distance. Package *dispfit* uses data obtained from dispersal distances measured directly (*e.g.* capture-recapture methods or other biologging techniques – such as radio, GPS, or satellite tracking) or indirectly (*e.g.* parentage analysis from genetic data). While many of the functions considered may be found scattered in multiple statistical software and packages [*e.g.* `R` packages *fitdistplus* (Delignette-Muller and Dutang 2015) and *gamlss* (Rigby and Stasinopoulos 2005), or program *CurveExpert* (Hyams 2010)], *dispfit* specifically uses functions adapted to species dispersal, providing the necessary measures to describe and compare dispersal kernels, which are not directly obtained from other software sources. We describe the functionalities, usage, and performance of *dispfit*, and recommend its use in ecological and evolutionary studies requiring information on dispersal distance.

4.2 Package overview

The *dispfit* package is written in the `R` language and is freely available on GitHub. It can be used under `R` version 3.5.1 or higher (R Core Team 2017), and may be installed using package *devtools*:

```
> devtools::install_github("https://github.com/apferreira/dispfit")
> library(dispfit)
```

The functions available in dispfit depend on packages *ggplot2* (Wickham 2016), *msm* (Jackson 2011), *numDeriv* (Gilbert and Varadhan 2019), and *reshape* (Wickham 2007).

4.2.1 Dispersal kernel formulation in dispfit

Assuming that a single point is the site of origin of all dispersers from a population, then the dispersal distance of each disperser is the Euclidean distance between the origin and its end point. The dispersal distances of all dispersers thus reflect a continuous parametric distribution, or probability density function (pdf), that characterizes the studied population. We can then define a dispersal kernel as the pdf of the distribution of the values of the Euclidean distances between the source and the final location of a dispersal event. There are several characterizations of a dispersal kernel, for instance Nathan et al. (2012) distinguish between "dispersal distance kernel, K_D", and "dispersal location kernel, K_L". We will follow the latter in this paper and briefly review these concepts in Box 1.

4.2.2 Distributions included in dispfit

The *dispfit* package fits 9 well-known distributions for estimating dispersal kernels (Clark et al. 1999; Nathan et al. 2012) (Table 4.1). The simplest functions considered are the single parameter Rayleigh and exponential, which are the most popular in mathematical developments for studying spatial dynamics theory (Gilbert et al. 2014). The remaining 7 functions are two-parameter distributions, often referred to as better depicting real dispersal kernels than the Rayleigh and exponential functions (Clark 1998; Clark et al. 1999; Bullock and Clarke 2000) (Table 4.1).

Box 4.1 – Dispersal kernel formulation in *dispfit*

We briefly review the concepts of "dispersal distance kernel, K_D", and "dispersal location kernel, K_L" (Nathan et al., 2012). Although it is very well known, we derive the radial expression of $K_D(r)$ in the special case of an isotropic binormal distribution because it illustrates an important difference between K_D and K_L: when plotted as a function of a radial distance, $K_D(r)$ peaks at r ≠ 0 and it goes through zero at the origin, a characteristic that is somehow counter-intuitive, and often underappreciated.

Following Nathan et al. (2012), we assume that a single point is the origin site of the dispersers. Therefore, the dispersal distance of each disperser is the Euclidean distance between the origin and its end point. Assuming that the distribution of the distance of the dispersers (K_D) follows an isotropic binormal distribution centred in the origin then, in cartesian coordinates (x, y), it can be expressed as:

$$K_D(x,y) = \frac{1}{2\pi\sigma^2} e^{\left(-\frac{1}{2\sigma^2}(x^2+y^2)\right)}, \tag{1}$$

this is shown in Fig. 4.1-A and could represent, for instance, the idealized distribution of the distances travelled by seeds of a tree located at the origin (x = 0, y = 0), where the surface maximum occurs. Often, it is more convenient to use polar coordinates, with distance from the origin r > 0 and angle 0 < θ ≤ 2π, such that x = r cos θ and y = r sin θ. In this case, to obtain the corresponding $K_D(r,\theta)$ one needs to calculate the Jacobian of the transformation, which in this case is r (e.g., Rice, 2007). Substituting x and y in eq. (1) by their expressions in terms of r and θ, and multiplying by the Jacobian, the $K_D(r,\theta)$ is:

$$K_D(r,\theta) = \frac{r}{2\pi\sigma^2} e^{\left(-\frac{r^2}{2\sigma^2}\right)}. \tag{2}$$

Because we assumed isotropy, the angle θ is irrelevant and we can integrate eq. (2) over θ to obtain $K_D(r)$:

$$K_D(r) = \int_0^{2\pi} \frac{r}{2\pi\sigma^2} e^{\left(-\frac{r^2}{2\sigma^2}\right)} d\theta$$

or

$$K_D(r) = \frac{r}{\sigma^2} e^{\left(-\frac{r^2}{2\sigma^2}\right)}. \tag{3}$$

Eq. (3) is the Rayleigh distribution. An important feature of this distribution is that it goes through zero at the origin and, consequently, the maximum occurs for r ≠ 0 (Fig. 4.1-B), even when the corresponding two-dimensional distribution has its maximum at the origin (as seen in Fig. 4.1-A). It may be more intuitive to understand this result if we consider that the data are being collected in rings of equal radius, Δr, as illustrated in Fig. 4.2. In general, a small number of points are located at rings far from the origin (because few propagules get that far), thus K_D attains small values and for very large distances it is, for practical purposes, equal to zero. When we move towards the origin (x = 0, y = 0) the number of points in each ring increases, thus contributing to an increase of K_D, but, simultaneously, the area of each ring becomes increasingly smaller, as illustrated in Fig. 4.2. From these two opposite trends (an increase in the number of points but a decrease in the area of the rings) results a maximum of K_D for an intermediate value of r. The continuous limits assumed in the above equations imply that there is a maximum somewhere when r ≠ 0, and that K_D(r = 0) = 0 (see Fig. 4.2).

Finally, Nathan et al. (2012) defined the dispersal location kernel, $K_L(r)$ in two dimensions and assuming isotropy, as:

$$K_L(r) = \frac{1}{2\pi r} K_D(r)$$

or, for the used Rayleigh distribution, if $a^2 = 2\sigma^2$,

$$K_L(r) = \frac{1}{\pi\, a^2}\ \mathrm{e}^{-\left(\frac{r}{a}\right)^2}, \tag{4}$$

shown in Fig. 4.1-C. By dividing K_D by $2\pi r$, dispersal readily becomes understood in terms of number of points per unit of area, *i.e.*, a density of points. The two kernels are then characterized as the probability that a movement from a source arrives at a distance r, $K_D(r)$, and of the density of movements at distance r, $K_L(r)$ (Peart 1985).

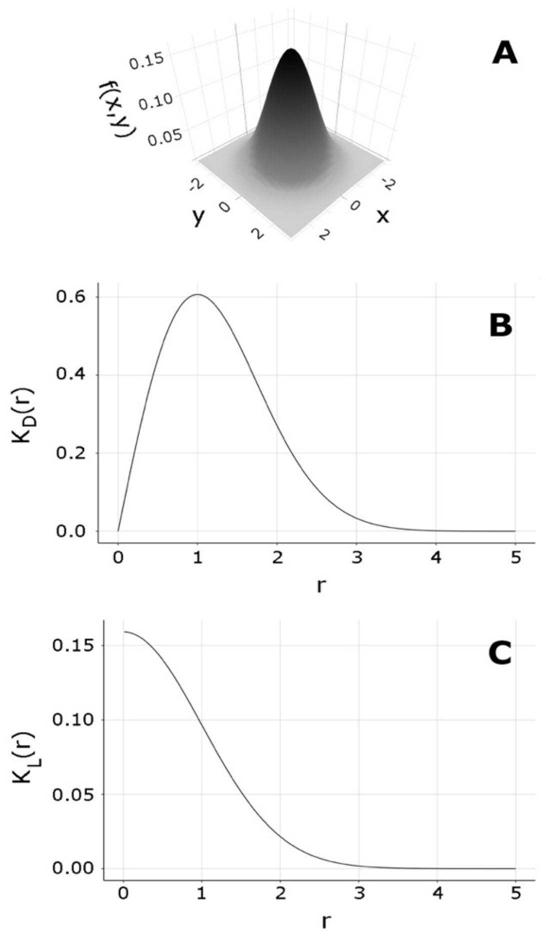

Fig. 4.1

(A) Example of the distribution of dispersers following an isotropic binormal distribution centred in the origin: $f(x,y) = \frac{1}{2\pi\sigma^2} e^{\left(-\frac{1}{2\sigma^2}(x^2+y^2)\right)}$.

(B) Under the assumption of isotropy and using polar coordinates, the above distribution can be written as a function of r as $f(r) = \frac{r}{\sigma^2} e^{\left(-\frac{r^2}{2\sigma^2}\right)}$, which is the Rayleigh distribution using the 'dispersal distance kernel', $k_D(r)$.

(C) The 'dispersal distance kernel' and the 'dispersal location kernel' are related such that $k_D(r) = 2\pi r k_L(r)$, so that the example Rayleigh distribution may be converted in such a way that, if $a^2 = 2\sigma^2$, $k_L(r) = \frac{1}{\pi\, a^2}\, \mathrm{e}^{-\left(\frac{r}{a}\right)^2}$.

See Box 4.1.

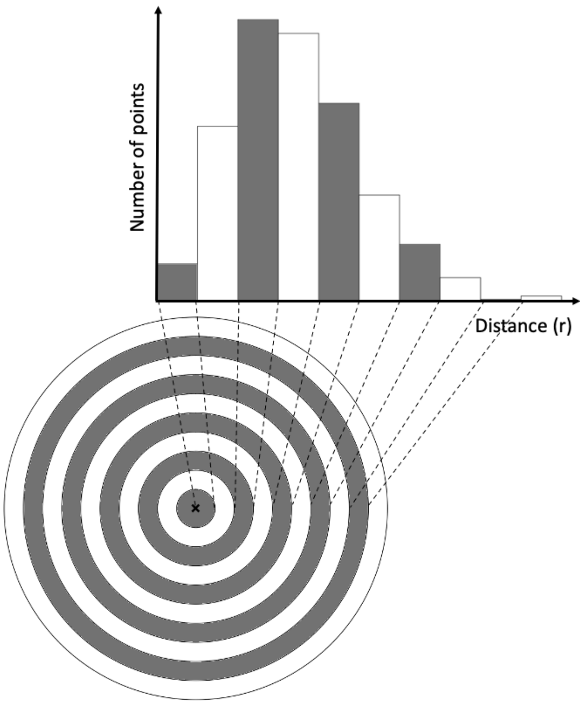

Fig. 4.2 – A histogram showing the distribution of dispersal distance by class. For example, seeds dispersing from a tree (represented by **X**, the origin), will fall within the area surrounding that tree. Data points will then be the seeds collected in each ring of equal Δr, and a histogram of these data will show the number of seeds collected in each ring. When we start collecting far from the origin, we have a small number of points (because few seeds get that far). When moving towards the origin, we will find more points, so the bars representing the number of points also increase. However, because Δr is constant, when moving towards the origin the area of the rings also becomes smaller. Therefore, close to the origin, the area of the rings is so small that the number of points collected within a ring is very low (even if the density of points is high). From the opposite trends in the increase in the number of points towards the origin, but a reduction in the area size of rings, results a maximum for a value of *r* different from zero.

4.2.3 ***dispfit* main functions**

dispfit includes one main function, `dispersal.kernel`, which covers most of the functionalities of the package. This function fits one or more dispersal kernels with 1-2 parameters, by estimating the distribution of kernel parameters, a vector θ (with all elements $\theta_j > 0$), maximizing the likelihood function, defined as

$$\mathcal{L}(\theta) = \prod_{i=1}^{n} f(x_i|\theta)$$

with x_i being the *n* observations of variable *X* and $f(\cdot\,|\theta)$ the probability density function of the distribution. The function `dispersal.kernel` provides parameter estimates of each fitted distribution, as well as model selection statistics, including the Akaike Information Criteria (AIC), the Akaike Information Criteria for small sample sizes (AICc), the Bayesian Information Criteria (BIC), and the Akaike weights of the relative strength of each model (w_i) (Burnham and Anderson 2002; Burnham et al. 2011). Finally, it also presents goodness-of-fit (GOF) measures of the distributions, computing the Chi-squared and Kolmogorov-Smirnov two-sample (K-S) tests, and their associated *p*-values (Press 1992; Sokal and Rohlf 1995). If *p*-values are above the threshold defined by the user, then the estimated distribution does not differ significantly from the data and is therefore accepted as a good approximation. The GOF measures may be used either isolated or in tandem, allowing for more robust interpretations. Because kernel parameters are specific for each family of distributions, they cannot be directly compared among different families, even when using the same dataset (Austerlitz et al. 2004). Therefore, to compare quantitatively the shape of different distributions, `dispersal.kernel` also computes the first four moments of each distribution: mean μ, standard deviation σ, skewness α_3, and kurtosis α_4 (Clark et al. 1999) (see Table 4.2 for their definition and equations).

Pointwise confidence intervals based on profile-likelihood (Venzon and Moolgavkar 1988) are estimated for each kernel parameter. Confidence *envelopes* for predicted values are computed by generating a set of distributions based on a cross-sequence value of the sequence of one of the parameter values ranging from the lower to the upper confidence intervals of each parameter (*i.e.* for each value of the sequence of parameter *a*, distributions are calculated for all values of the sequence of parameter *b*). For each predicted value, that calculation's minimum and

Table 4.1 – Families of distributions implemented in the *dispfit* R software package. For each kernel, the $K_L(r)$ "dispersal location kernel" and the $K_D(r)$ "dispersal density kernel" used by *dispfit* to fit the supplied data are presented, along with its general properties. Notice that $K_D(r) = 2\pi r\, K_L(r)$. Notation was adapted from Nathan et al., 2012. In the package outputs, parameters *a* and *b* are always referred to as Parameter 1 and Parameter 2, respectively. Depending on the distribution, *a* or *b* might refer to location, shape, or scale parameters (see Forbes et al., 2011 for a detailed explanation). Except where noted, *a* and *b* are equal to the respective parameters of each distribution defined in Forbes et al., 2011). r is the dispersal distance.

Kernel	$K_L(r)$	$K_D(r)$	Properties and notes
Rayleigh	$\frac{1}{\pi\, a^2}\, \mathrm{e}^{-\left(\frac{r}{a}\right)^2}$	$\frac{2\, r}{a^2}\, \mathrm{e}^{-\left(\frac{r}{a}\right)^2}$	Characterized by a single parameter, *a*, where $a = \sigma\sqrt{2}$, with σ being the standard deviation of the isotropic bivariate normal distribution.
Exponential	$\frac{1}{2\, \pi\, a^2}\, e^{-\frac{r}{a}}$	$\frac{r}{a^2}\, e^{-\frac{r}{a}}$	Characterized by a single parameter, *a,* where $a = \gamma$, *i.e.*, the exponential scale parameter.
Generalised Normal	$\frac{b}{2\pi a^2\, \Gamma\left(\frac{2}{b}\right)}\, e^{-\left(\frac{r}{a}\right)^b}$	$\frac{b\, r}{a^2\, \Gamma\left(\frac{2}{b}\right)}\, e^{-\left(\frac{r}{a}\right)^b}$	Also called Exponential Power distribution. Γ is the gamma function (Abramowitz and Stegun 1965). The parameter *a* is the scale parameter, while *b* is the shape parameter, that affects the "fatness" of the tail of the distribution. When *b* < 1 the distribution is fat-tailed, while when *b* > 1 the distribution is thin-tailed, meaning fewer long-distance dispersal events. It's worth noting that when *b* = 2 this pdf is equal to the Rayleigh distribution, and when *b* = 1 it's equal to the Exponential distribution.
Bivariate Student's t (2*Dt*)	$\frac{(b-1)}{\pi a^2}\left[1+\left(\frac{r}{a}\right)^2\right]^{-b}$	$\frac{2r(b-1)}{a^2}\left[1+\left(\frac{r}{a}\right)^2\right]^{-b}$	Defined for values of *a* > 0 and *b* > 1. It has a leptokurtic (*i.e.* fat-tail) shape and is concave near the origin. It's described to be the best fit for seed dispersal in several tree species (Clark et al. 1999).
Geometric	$\frac{(b-2)(b-1)}{2\pi a^2}\left(1+\frac{r}{a}\right)^{-b}$	$\frac{r(b-2)(b-1)}{a^2}\left(1+\frac{r}{a}\right)^{-b}$	Defined for all values of *a* > 0 and *b* > 2. It shows a fat tail, being less leptokurtic than the Weibull distribution, but more than the 2*Dt*.
Lognormal	$\frac{1}{(2\pi)^{3/2} b\, r^2} e^{-\frac{log\left(\frac{r}{a}\right)^2}{2b^2}}$	$\frac{1}{\sqrt{2\pi}\, b\, r} e^{-\frac{log\left(\frac{r}{a}\right)^2}{2b^2}}$	Defined for all positive values of *a* and *b*.
Wald	$\frac{\sqrt{b}}{\sqrt{8\pi^3 r^5}} e^{-\frac{b(r-a)^2}{2a^2 r}}$	$\frac{\sqrt{b}}{\sqrt{2\pi\, r^3}} e^{-\frac{b(r-a)^2}{2a^2 r}}$	Also termed as Inverse Gaussian distribution, is defined for all positive values of *a* and *b*.
Weibull	$\frac{b}{2\pi a^b} r^{b-2} e^{-\left(\frac{r}{a}\right)^b}$	$\frac{b}{a^b} r^{b-1} e^{-\left(\frac{r}{a}\right)^b}$	Defined for all positive real numbers of *a* and *b*. The distribution is thin-tailed when *b* > 1 and fat-tailed otherwise. Like the Generalized Normal distribution, when *b* = 2 it degenerates to the Rayleigh distribution. However, when *b* = 1 it is not equal to the Exponential distribution. The Weibull distribution has a highly leptokurtic shape (Austerlitz et al. 2004).
Gamma	$\frac{1}{2\pi a^2\, \Gamma(b)}\left(\frac{r}{a}\right)^{b-2} e^{-\frac{r}{a}}$	$\frac{1}{a^b\, \Gamma(b)}\, r^{b-1}\, e^{-\frac{r}{a}}$	Defined for all positive values of *a* and *b*. It has an exponential-like tail and equals the Exponential distribution when *b* = 2.

Table 4.2 – Equations used by the *dispfit* R software package to calculate the first four moments (if they are defined) of the implemented $K_L(r)$ kernels (Press 1992; Clark et al. 1999; Austerlitz et al. 2004) (see Table 4.1 for details).

Name	Equation	Definition
Mean (1st moment)	$\mu = 2\pi \int_0^\infty r^2\, K_L\,(r)\, dr$	Arithmetic mean of a distribution.
Standard Deviation (2nd moment)	$\sigma = \sqrt{2\pi\, r \int_0^\infty (r-\mu)^2\, K_L\,(r)\, dr}$	The degree of spread about the mean.
Skewness (3rd moment)	$\alpha_3 = \frac{2\pi \int_0^\infty r^4\, K_L\,(r)\, dr}{\sigma^3}$	Degree of asymmetry. The higher the value, the longer the tail is to the right of the central maximum, and so the higher the long-distance dispersal.
Kurtosis (4th moment)	$\alpha_4 = \frac{2\pi \int_0^\infty r^5\, K_L\,(r)\, dr}{\sigma^4}$	Degree of peakedness. A distribution with a relatively high peak is called leptokurtic.

maximum outcomes are then defined as the lower and upper confidence *envelopes*. The R function `plot` draws the tested distributions, yielding a visual comparison of their shapes, while the function `predict` will return a table of predicted values according to the best fitting models.

4.3 Worked example

The following example demonstrates the usefulness and functionality of *dispfit*. We simulated a dataset with a known distribution and then tested the performance of *dispfit* in identifying the original distribution from which the dataset was generated.

Input data should be a vector representing the observed dispersal distances. For this example, we created a random vector of 200 values from a log-normal distribution, with parameters mean $\lambda = 5$ and SD = 1, using the function `rlnorm`, from the *stats* R package (we used the `set.seed` function so that the data is reproducible):

```
> set.seed(1111)
> simulated.data <- rlnorm(200, meanlog = 5, sdlog = 1)
```

Next, the data can be fitted to some or all of the available distributions by using the function `dispersal.kernel`:

```
> kernel.test <- dispersal.kernel(simulated.data, distribution =
"all", order.by = "AICc")
```

In this example, all the available distributions were fitted and the results ordered according to their minimum AICc value, but the user may choose the distributions to be fitted as well as the criterion to rank the fitted distributions.

```
> kernel.test
```

Two summary tables are printed by calling the resulting object. Firstly, a table listing the fitted distributions with the corresponding information criteria values and GOF tests is presented (Table 4.3). Model selection should take into consideration both the model selection criteria and the GOF tests. In this example, four of the distributions (Weibull, gamma, exponential, and Rayleigh) were rejected by the Chi-squared test and two by the KS test (exponential and Rayleigh), when 0.05 is defined as the threshold for a good approximation. From the accepted distributions (log-normal, Wald, geometric, generalized normal, and 2Dt), the log-normal distribution was chosen as providing the best fit describing the data according to all information criteria, with an AICc difference from the second best distribution above 2, indicating strong support (see Burnham et al., 2011).

A second table with the estimated parameters and moments for each fitted distribution is also printed by calling the object (*e.g.* `kernel.test$distribution.parameters`). Note that, in the specific case of function `rlnorm`, the `meanlog` parameter (μ) is equal to the logarithm of Parameter 1 and the `sdlog` parameter (σ) is equal to Parameter 2 of the log-normal distribution from the *dispfit* package. In this example, the estimated parameters for the log-normal distribution were very close to the parameters used to simulate the data, such that if Parameter 1 = 185.94 (95% Confidence Intervals: 159.80 - 216.40) then μ = 5.22 (CI95: 5.07 - 5.38), and Parameter 2 = σ = 1.09 (CI95: 0.99 - 1.20) (Table 4.4).

The fitted distributions can be plotted using the `plot` function, to aid in a visual interpretation of the results:

```
> plot(kernel.test, fit.criteria = "AIC", criteria.dif = 4, envelopes
= TRUE, plot.data = TRUE)
```

For this example, we selected the three distributions with an AICc difference from the top model lower than 4 (log-normal, Wald and geometric) and chose to plot the lower and upper 95% confidence *envelopes* for all selected distributions, although other criteria may be chosen by the user (Fig. 4.3). Together with the fitted distributions, the plot function also displays the kernel density of the distribution of the original dataset (see black line in Fig. 4.3). Since the plot is generated using the *ggplot2* package, some visual characteristics can be modified using that package's syntax (*e.g.* changing the colour of the lines, setting another background, or modifying

Table 4.3 – Comparison of model selection indexes provided by the *dispfit* `R` software package, calculated for the example random dataset obtained with `R` function `rlnorm`, as detailed in the text.

	AIC	Delta AIC	AICc	Delta AICc	BIC	Delta BIC	wi	Chi-squared value	Chi-squared significance	K-S value	K-S significance
Log-Normal	5521.16508	0	5521.22599	0	5527.76171	0	0.73324881	29.406801	0.34145474	0.07901554	0.57188932
Wald	5524.59847	3.43339055	5524.65938	3.43339055	5531.19511	3.43339055	0.13173467	35.9006321	0.11748657	0.08882979	0.42895325
Geometric	5524.97887	3.81378773	5525.03978	3.81378773	5531.5755	3.81378773	0.10891756	29.1231457	0.35495313	0.09126984	0.3931462
Generalized Normal	5528.18504	7.01996139	5528.24596	7.01996139	5534.78168	7.01996139	0.0219223	36.4421684	0.1059597	0.0711658	0.70240902
2Dt	5531.50143	10.3363506	5531.56234	10.3363506	5538.09807	10.3363506	0.00417582	33.8740036	0.16974068	0.12282609	0.11096344
Weibull	5549.34754	28.1824646	5549.40846	28.1824646	5555.94418	28.1824646	5.57E-07	86.8411055	3.33E-08	0.09471503	0.34159592
Gamma	5550.6644	29.499317	5550.72531	29.499317	5557.26103	29.499317	2.88E-07	150.826674	0	0.10512821	0.2252158
Exponential	5625.25037	104.085291	5625.27057	104.044579	5628.54869	100.786974	1.87E-23	25472.8757	0	0.24180203	1.82E-05
Rayleigh	5875.32328	354.1582	5875.34348	354.117488	5878.6216	350.859882	9.32E-78	8.4184E+10	0	0.42089744	1.33E-15

Table 4.4 – Estimates of parameter and moments for each distribution calculated by the *dispfit* `R` software package for the example random dataset obtained with `R` function `rlnorm`, as detailed in the text.

	Parameter 1	Parameter 1 lower CI	Parameter 1 upper CI	Parameter 2	Parameter 2 lower CI	Parameter 2 upper CI	Mean	Standard Deviation	Skewness	Kurtosis
Log-Normal	185.937023	159.798618	216.399506	1.08890836	0.99023214	1.20486987	336.389171	507.161449	7.94997725	211.03066
Wald	331.60259	275.211655	416.388932	154.163652	125.917084	186.418459	331.60259	486.335392	678.299046	32.2646667
Geometric	194.841192	127.06945	308.432555	4.08634381	3.52358111	4.95926705	358.709996	39.4755846	Inf	Inf
Generalized Normal	4.98045587	1.06514581	15.3739986	0.41010331	0.33399696	0.4974134	326.494973	391.228386	6.60270657	42.8791378
2Dt	117.619944	91.2459872	151.256623	1.60885183	1.48222177	1.77351144	617.783877	Inf	Inf	Inf
Weibull	321.560993	274.379398	375.236681	0.94351222	0.84967892	1.04099532	330.180957	350.127053	2.18286586	7.28336875
Gamma	331.6612	268.23901	418.048935	0.99915816	0.83735732	1.18255234	331.381993	331.521567	2.00084238	6.00505531
Exponential	165.690975	150.462823	183.047805	NA	NA	NA	165.690975	165.690975	6	24
Rayleigh	528.101853	493.5199	566.919448	NA	NA	NA	468.018082	244.64391	13.3716823	43.4273298

the axis labels and title).

Finally, the `predict` function may be used to estimate values predicted by the selected models. Similarly to the `plot` function, the `predict` function will return a table with the dispersal probability and corresponding upper and lower confidence *envelopes* of a set of values (from 1 to the maximum value in the original data):

```
> predict(kernel.test, fit.criteria = "AIC", criteria.dif = 4,
envelopes = TRUE)
```

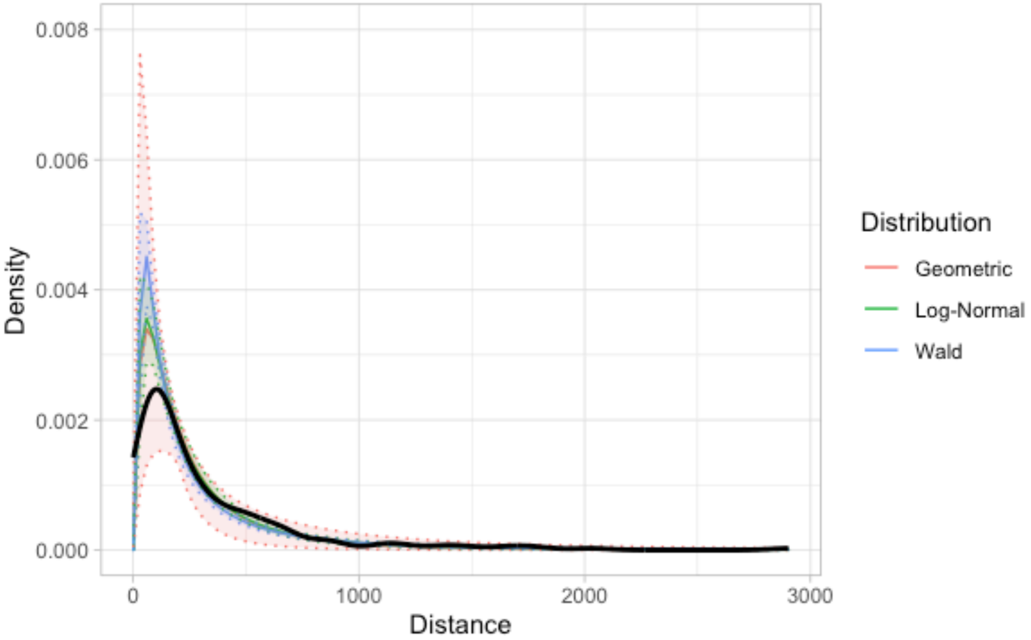

Fig. 4.3 – Distributions with an AICc < 4 fitted with *dispfit* R software package for the simulated dataset obtained with R function `rlnorm` (see text for details). Each colour matches a distribution, as detailed in the legend. Since the distributions yielded similar estimates, they appear almost overlaid. Solid lines show the distributions with parameters estimated in Table 4.4. Dotted lines show the 95% confidence envelopes of each distribution, as detailed in the text. The black line shows the real density or distribution of the analysed data.

4.4 Discussion

Accurate estimates of dispersal kernels are needed to understand and model processes such as (meta)population dynamics and persistence in fragmented landscapes (Morales et al. 2010), invasion spread and direction (Wilson et al. 2009), environmental and climate change impacts on species (Travis et al. 2013), community assemblages (Hill et al. 2017), gene flow (Browne et al. 2018), and evolutionary change (Ochocki and Miller 2017). While a variety of functions have been proposed and implemented to estimate dispersal kernels, researchers still often rely on a single or only a few distributions to fit observed data on dispersal distances, even though dispersal kernels are likely to vary greatly among and within study species and systems (Bullock et al. 2016). This inhibits proper identification of the best dispersal kernel given the data, which in turn may affect the predictive ability of models incorporating dispersal kernels to investigate ecological and evolutionary processes (Bowler and Benton 2005), impacting the necessary knowledge to address ecological management and conservation (Driscoll et al. 2014).

The R package *dispfit* presented here provides a useful and effective tool to implement and compare the fit of 9 well-known dispersal kernels from observed data, thereby offering a straightforward framework for kernel distribution selection that improves inferences on the fundamental processes affected by species dispersal distance. In addition, if available data exist, *dispfit* may be particularly convenient for assessing possible variations in the distribution of species dispersal, by fitting separate dispersal kernels according to individual covariates (*e.g.* age class, or sex) and environmental contexts (in space and time), as well as for comparing differences in how multiple species (or traits) may disperse and distribute under environmental change. Also, because the variety of methods to obtain data on dispersal distance (*e.g.* capture-recapture, habitat occupancy, telemetry, molecular information) may lead to some uncertainty (Driscoll et al. 2014), it may be of interest to assess how dispersal kernels may be affected by different sampling methods. These and many other ecological, evolutionary, and methodological research questions regarding species dispersal distance can be easily explored in *dispfit*, by taking advantage of the standardized outputs produced, allowing direct comparisons among fitted distributions.

Although *dispfit* considers a large set of distributions, we acknowledge that many more dispersal distributions are available and used in the literature (Nathan et al. 2012; Capdevila et al. 2018). While some of these consist of special cases of more general distributions already implemented in *dispfit* (*e.g.* exponential function), other functions (*e.g.* general mixture function) were not included in the current version of the package. Although mixture functions have been referred to provide useful descriptions of dispersal distance (*e.g.* Bullock & Clarke, 2000), they also comprise increased dimensionality, which can lead to overfitting and lack of generality (Bullock et al. 2017). However, future versions of the package may include these and other distributions that can be useful for dispersal kernel estimation. Likewise, future developments of *dispfit* will incorporate methods to deal with missed long-distance dispersal events that may occur to locations outside a predefined study area (*e.g.* Barrowclough, 1978; Terui, 2020) and that may lead to biased estimates of dispersal, or other vital rates such as survival (Barrowclough 1978; Zimmerman et al. 2007). Therefore, we advise researchers to acknowledge these assumptions and limitations in their studies, even when not explicitly accounting for them in the models. Also, in cases where the form and extent of the tail of the dispersal kernel are characterized by extreme value distributions (Bullock et al. 2016; García and Borda-de-Água 2017; Tung et al. 2017), we recommend the use of other existing tools that already allow for the fitting of extreme value distributions [see for *e.g.* `R` packages *evd* (Stephenson 2002) or *extRemes* (Gilleland and Katz 2016)].

Overall, package *dispfit* offers an intuitive approach based on simple functions for researchers interested in having representative and informative dispersal kernels for a given population. It can be applied in ecological and evolutionary research dealing with species dispersal, but its applications might be further expanded to a wider array of studies and disciplines, including species migration, geomorphology (*e.g.* transport of sediments), or even epidemiology. We consider that its effectiveness for quantitative and comparative proposes may contribute to better synthesising and set the research agenda on the empirical-based estimation of dispersal kernels.

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Supplementary Material

Dispfit package

All code available at: https://github.com/apferreira/dispfit

From the Readme page:

R package to estimate species dispersal kernels

The *dispfit* package is an R software application developed to intuitively and comprehensively estimate dispersal kernels from dispersal data. *dispfit* fits and compares different families of parameterized functions to describe and predict dispersal distances. It includes 9 well-known and commonly used distributions, computing goodness-of-fit and model selection statistics, and estimating each distribution's parameters, along with their first four moments (mean, standard deviation, skewness, and kurtosis).

To install *dispfit*, run the following commands in R:

```
# Install 'devtools' package, if needed
#install.packages("devtools")
#devtools::install_github("https://github.com/apferreira/dispfit")
#library(dispfit)
```

Chapter 5

Seasonal dispersal dynamics of an endangered small mammal in Mediterranean farmland

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Keywords: Cabrera voles; Dispersal kernels; Genetic relatedness; Movement ecology

Abstract

Dispersal plays a critical role in species population dynamics and persistence, particularly for metapopulations in fragmented landscapes. Here we investigated the seasonal dispersal patterns of the Cabrera vole (*Microtus cabrerae*) in south-western Portugal based on genetic non-invasive sampling (gNIS) and relatedness analysis. Dispersal kernel fitting revealed that the Weibull and Gamma distributions best described inferred movements across seasons. Mean dispersal distances were relatively limited during the early wet season (≈292m), likely due to lower population density and reduced competition. In contrast, mean dispersal distance increased in the late wet and early dry seasons (≈650m), when population abundance and competition for resources and mates increase, with some individuals undertaking movements longer than 2000 m. In the late dry season, mean dispersal distances stabilized but long-distance dispersal increased, probably as a result of lower habitat availability. Overall, our study highlights the influence of seasonality on dispersal behaviours of Cabrera voles, emphasizing the need to maintain habitat connectivity, especially during peak dispersal periods to support successful colonization. Importantly, our findings demonstrate the utility of gNIS and relatedness analysis in studying dispersal in elusive species, offering valuable insights for conservation planning.

5.1 Introduction

Dispersal, the movement of individuals from their birthplace to a new location where they establish and reproduce, is a key life-history trait with significant implications for population dynamics and persistence (Clobert et al. 2012). Dispersal directly influences and is influenced by multiple morphological, physiological, and behavioural traits (Baguette et al. 2013), which interact with environmental factors, such as habitat fragmentation and land use intensity (Ronce and Clobert 2012). In metapopulations, where colonization and extinction dynamics depend on movement between habitat patches, understanding dispersal is crucial for conservation, particularly for species inhabiting changing environments.

Dispersal distance is typically examined through dispersal kernels, defined by distribution functions describing the spread of distances travelled (Bullock et al. 2016). These kernels are widely used in conservation research but remain difficult to estimate, particularly for rare or elusive species that are difficult to detect and capture (Chapter 4 - Ferreira et al. 2023). Genetic non-invasive sampling (gNIS) provides a promising solution, allowing inferences on dispersal movements without direct observations or physical captures. Yet, empirical research using gNIS to estimate dispersal kernels remains limited, especially regarding how these may vary with environmental change (Ferreira et al. 2018).

Traditional genetic methods to infer dispersal typically focus on long-term migration rates, making them ineffective for detecting recent dispersal movements (Durand et al. 2011). Alternatively, kinship-based methods, such as relatedness analysis, can identify dispersal events over recent generations (Waser and Hadfield 2011; Escoda et al. 2017; Hill et al. 2023). High relatedness between individuals from different locations may thus provide insights for estimating dispersal kernels and inform conservation planning (Escoda et al. 2017).

Here, we explore the use of gNIS and relatedness analysis to estimate dispersal kernels for the threatened Cabrera vole (*Microtus cabrerae*) in Mediterranean farmland from SW Portugal. The species shows a metapopulation-like structure in the region, largely shaped by fragmented habitat patches surrounded by intensive agriculture, which constrains dispersal movements (Pita et al. 2007; Pita et al. 2016). These patches are frequently altered or destroyed by farming practices, prompting voles to disperse both following these disturbances and after rearing. Despite the critical role of dispersal in vole population persistence (Mestre et al. 2017), its range and frequency remain poorly understood (Pita et al. 2014). Based on estimates of vole relatedness obtained through gNIS across different seasons, we aimed to describe how the dispersal kernels of this metapopulation vary seasonally, evaluating whether emergent patterns align with the species' reproductive ecology and population dynamics.

5.2 Methods

We used 444 successfully genotyped faecal samples from 307 individual Cabrera voles, collected between November 2013 and September 2014 in a 461.8 ha farmland area from south-western Portugal (37°21' – 38°04' N, 08°51' – 08°30' W), as part of a study on the species demography based on gNIS (Chapter 2 - Proença-Ferreira et al. 2019). The data included 140 genotyped samples from 81 individuals surveyed in November-December 2013 (early wet season, EWS), 87 samples from 77 individuals surveyed in February-March 2014 (late wet season, LWS), 153 samples from 122 individuals surveyed in May-June 2014 (early dry season, EDS), and 64 samples from 64 individuals surveyed in September-October 2014 (late dry season, LDS). Samples were obtained through systematic surveys of Cabrera vole faeces in all habitat patches identified as suitable in each season, which included between 225 and 291 patches covering 29 to 46ha, as detailed in Chapter 2 (Proença-Ferreira et al. 2019). Genotyping was based on nine highly polymorphic microsatellites (Ho=0.79; He=0.81) and low probability of identity for unrelated (PI=3.2E-12) and related individuals (PIsibs=9.2E-5), combined with two small sized sex-linked introns, resulting in low overall genotyping errors (dropout rate: 0.68-2.6%; false allele rate: 0-0.18% (Chapter 2 - Proença-Ferreira et al. 2019). Consensus genotypes were compared to identify individuals, such that only samples differing in more than two loci were assigned as new individuals (Ferreira et al. 2018; Sabino-Marques et al. 2018; Chapter 2 - Proença-Ferreira et al. 2019).

We estimated pairwise relatedness for all dyads (*i.e.* pairs of individuals) in each season based on allele frequencies of the population, using the R package *related* v.1.0 (Pew et al. 2015), which implements program COANCESTRY (Wang 2011). Among the seven available estimators, we selected the wang coefficient based on highest correlation between expected and simulated relatedness values (with 250 simulated dyads, details in Pew et al. 2015). We then assessed contemporary dispersal distances considering only dyads with relatedness coefficients ≥0.36, from the lower 95% confidence bound of parent-offspring simulations with the wang estimator (Wang 2002; Blouin 2003), corresponding to a recent separation of individuals belonging to the same original habitat patch. We calculated the Euclidian distance separating each dyad in each season, and used the R package *dispfit* v.0.1 (Chapter 4 - Proença-Ferreira et al. 2023) to fit the nine alternative dispersal kernels available. From these, we selected the best-performing distribution(s) for each season based on the Akaike Information Criteria corrected for small sample sizes (AICc) (Burnham et al. 2011) and on chi-squared and Kolmogorov-Smirnov two-sample Goodness-of-fit (GOF) tests (Chapter 4 - Proença-Ferreira et al. 2023). Kernels with AICc values within 2 units of the top-ranked distribution were considered equally supported (Burnham and Anderson 2002), while those with significant p-values in both GOF tests were discarded. Finally, we compared dispersal kernels among seasons by visually analysing their distributions, and through their first four moments (mean, standard deviation, skewness, and kurtosis) (Chapter 4 - Proença-Ferreira et al. 2023).

Table 5.1 – Best-performing dispersal kernels for each season with delta AICc < 2 and at least one non-significant GOF test.

Season	Kernel	AICc	Chi-squared significance	K-S significance
EWS	Weibull	2427.617	0.000	0.228
	Gamma	2427.814	0.000	0.163
LWS	Weibull	2849.235	0.016	0.078
	Gamma	2849.675	0.008	0.078
EDS	Weibull	4160.220	0.027	0.098
	Gamma	4160.769	0.012	0.070
LDS	Weibull	757.660	0.082	0.724
	Gamma	757.665	0.075	0.724
	Generalized Normal	759.033	0.049	0.444

5.3 Results and Discussion

Our findings indicate seasonal differences in Cabrera voles' dispersal distances, with patterns best explained by the Weibull and Gamma distributions and occasionally by the Generalised Normal (Table 5.1). During the EWS, the Weibull distribution provided the best fit (Table 5.1), with a relatively low mean dispersal distance of 292m, and low skewness and kurtosis, compared to other seasons (Table 5.2; Fig. 5.1). These short dispersal distances may reflect the lower population density typically reported for Cabrera voles at the onset of this season (Pita et al. 2014), a pattern also observed in the studied metapopulation (Chapter 2 - Proença-Ferreira et al. 2019). With fewer conspecifics, competition for mates and resources is likely lower, reducing the need for long-distance movements. Additionally, this season marks the beginning of improved habitat suitability and resource availability (Pita et al. 2014), allowing individuals to access higher-quality patches within relatively shorter ranges. As a result, movements during the EWS may be closely linked to mate searching and territory establishment within nearby familiar areas.

During the LWS, the Weibull distribution also provided the best-fitting kernel (Table 5.1). However, the mean dispersal distance increased to approximately 662m, along with higher skewness and kurtosis (Table 5.2), indicating a broader and more variable distribution of dispersal movements (Fig. 5.1). These patterns likely reflect the higher vole abundance observed during the LWS (Chapter 2 - Proença-Ferreira et al. 2019), due to increased reproductive activity (Fernández-Salvador et al. 2005; Pita et al. 2014). This may lead to more widespread dispersal movements by both juveniles and adults in search of less competitive or unoccupied areas. Dispersal distances reduced slightly in the EDS, with the Weibull distribution also providing the best fit (Table 5.1) and the mean distance reaching approximately 631m (Table 5.2; Fig. 5.1). The higher skewness and kurtosis suggest a distribution with more pronounced tails, likely reflecting the sustained high population abundance observed in this season, despite the early signs of declining habitat availability (Chapter 2 - Proença-Ferreira et al. 2019), which may have intensified competition for resources, further promoting dispersal (Crispim-Mendes et al. 2024).

Finally, during the LDS, the Weibull was again the best fit (Table 5.1), and the mean dispersal distance stabilized at 633m (Table 5.2; Fig. 5.1), despite declines in habitat availability and population abundance (Chapter 2 - Proença-Ferreira et al. 2019), a pattern commonly observed for the species during hot, dry summers (Pita et al. 2014), when reproduction and habitat availability are at their lowest levels (Ventura et al. 1998). Notwithstanding, the heavier-tailed distribution suggests that a few individuals may still undertake longer movements, possibly reflecting both individuals actively seeking new territories and the less predictable availability of resources.

Overall, the seasonal shifts in Cabrera voles' dispersal kernels reflect the species' reproductive patterns and population dynamics in Mediterranean farmland. It is important however to recognise that the spatial extent of the study area may not fully capture the range of possible movements, especially those involving rare long-distance dispersal. Additionally, the short duration of the study period may have also limited the ability to detect those events. For instance, simulation-based estimates from empirical occupancy data collected over ca. 3 years in Mediterranean farmland (Pita et al. 2007) predicted median dispersal distances reaching up to 2km (Mestre et al. 2017), suggesting that longer-term research over larger extents may be necessary to fully capture the dispersal patterns of voles. Despite these limitations, our findings provide valuable insights into the dispersal ecology of Cabrera voles, highlighting the importance of maintaining habitat connectivity, especially during peak dispersal seasons (LWS, EDS, and LDS) to ensure successful colonization of suitable patches. Notably, our study supports the use of gNIS and relatedness analysis to investigate dispersal patterns and dynamics in elusive species.

Table 5.2 – Estimated parameters and moments of best-performing dispersal kernels for each season. Depending on the distribution, parameters 1 and 2 might refer to location, shape, or scale parameters (see Chapter 4 - Proença-Ferreira et al. 2023). Values in brackets refer to the lower and upper confidence *envelopes*.

Season	Kernel	Parameter 1	Parameter 2	Mean	Standard Deviation	Skewness	Kurtosis
EWS	Weibull	292.99 (234.72-363.6)	1.01 (0.85-1.18)	291.85	289.15	1.97	8.82
	Gamma	313.20 (211.57-418.72)	0.96 (0.76-1.3)	300.60	306.83	5.04	6.25
LWS	Weibull	676.02 (548.81-827.57)	1.05 (0.89-1.23)	661.98	628.03	1.85	8.03
	Gamma	557.43 (447.9-880.47)	1.15 (0.82-1.39)	639.34	596.98	4.87	5.23
EDS	Weibull	633.89 (530.13-754.01)	1.01 (0.88-1.15)	630.95	623.97	1.97	8.78
	Gamma	563.10 (472.25-832.84)	1.08 (0.81-1.27)	609.33	585.76	4.92	5.54
LDS	Weibull	608.51 (376.14-959.28)	0.92 (0.66-1.23)	632.62	687.96	2.27	10.91
	Gamma	701.35 (407.64-1502.50)	0.89 (0.52-1.38)	625.04	662.09	5.12	6.73
	Generalised Normal	2.04 (0.00-77.62)	0.33 (0.17-0.61)	664.78	924.91	7.04	60.93

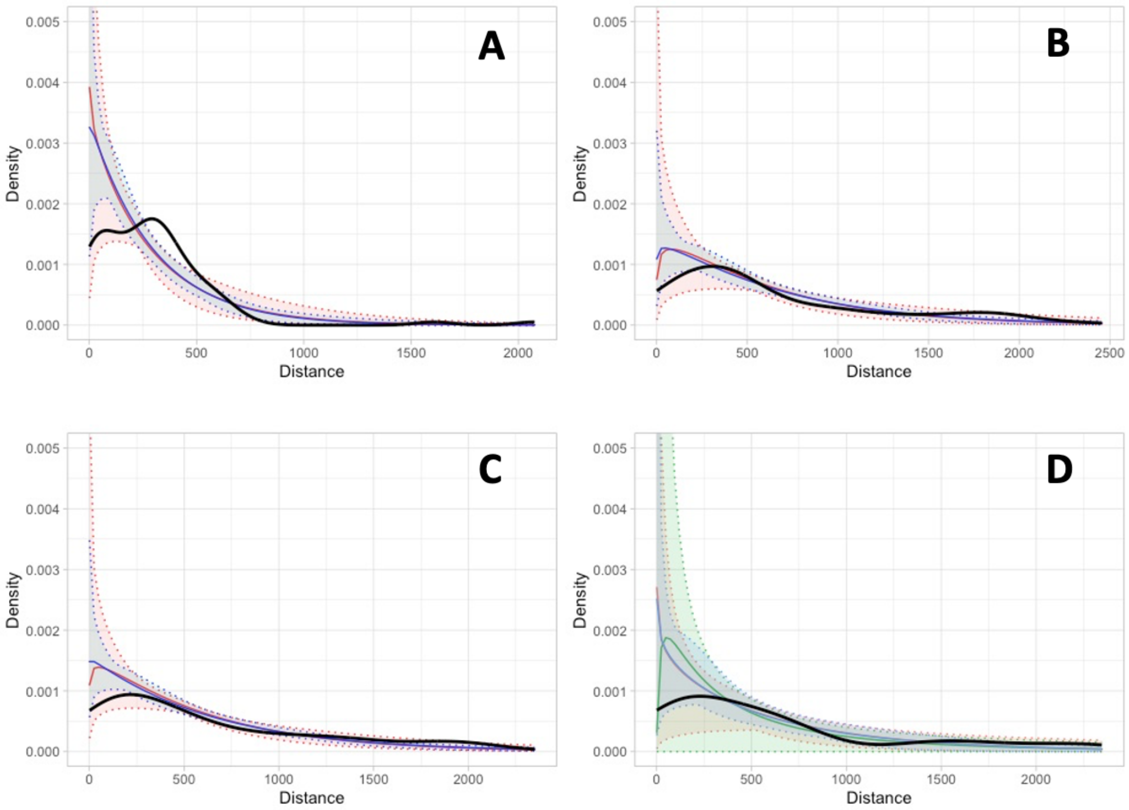

Fig. 5.1 – Estimated dispersal kernels selected as best-performing distributions for Cabrera voles sampled through gNIS in Mediterranean farmland in the EWS (A), LWS (B), EDS (C) and LDS (D). Estimates are based on individuals showing Wang's genetic relatedness coefficients ≥0.36. Estimated kernels are represented in colour: Weibull in blue, Gamma in red, and Generalised Normal in green. Black lines show the density of the data for each season.

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Chapter 6

General Discussion

[…] we attempt to treat the same problem with several alternative models each with different simplifications but with a common biological assumption. Then, if these models, despite their different assumptions, lead to similar results we have what we can call a robust theorem which is relatively free of the details of the model. Hence our truth is the intersection of independent lies.

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General Discussion

The Mediterranean region, and its agroecosystems in particular, are a paradigm for how human activities and high biodiversity levels may coexist in equilibrium, with habitat management sustaining biodiversity and, in turn, biodiversity supporting essential ecosystem functions (Blondel et al. 2010). Nevertheless, such an equilibrium has been threatened in recent decades, either by agriculture intensification or by land abandonment (Halada et al. 2011). As researchers, conservationists, farmers, and society in general became more aware of such threats, the demand for deeper insights into the interactions between ecosystems, species, and humans has also grown. No conservation programme, as detailed as it may be, will be successful if the bases for its execution are not well-established and grounded on empirical observations and analyses (Cardinale et al. 2012; Sutherland et al. 2019). Furthermore, biodiversity conservation in human-dominated landscapes such as agroecosystems, also requires recognizing the role and considering the needs of local communities (Niemelä et al. 2005; Henle et al. 2008). Understanding the impacts of agricultural activities and overall human structures on the ecology of species inhabiting mosaic, fragmented landscapes is thus critical for engaging with all parties with relevant information for balancing conservation with human activities. The farmland area from southwest Portugal examined in this thesis is no exception. Its highly dynamic and fragmented landscape, coupled with the recent agricultural changes towards intensification and over-grazing at a more regional scope (Beja and Alcazar 2003; Pita et al. 2009), highlights the importance of studies such as this in uncovering the relationships between farmed landscapes and metapopulation dynamics.

6.1 In a Nutshell

In line with the main goals outlined in the objectives, this thesis yields important insights into the ecology of the Cabrera vole and significant advances in methodological procedures for the study of small mammals and other taxa. Particularly, it provides a detailed description of the population dynamics of voles over the course of a year in a fragmented Mediterranean farmland, while exploring and explaining the drivers influencing those dynamics. At the same time, it evaluates the consequences of those findings for the conservation and management of the species, thereby providing relevant information that can be used by agricultural and environmental policymakers to better plan and manage current and future agricultural activities. Moreover, it involved the development of a user-friendly software application that allows for the analysis of dispersal data collected from any taxon and ecosystem, while also demonstrating the suitability of genetic non-invasive sampling (gNIS) for deriving relevant demographic information for rare and elusive species. As such, this thesis is expected to contribute with critical information to improve the balance between agricultural production and biodiversity conservation goals, in particular for the persistence of the Cabrera vole in these areas.

Specifically, based on gNIS data, this thesis shows that the landscape composition and structure are main drivers of the demography and connectivity of Cabrera voles, with marginal habitats – such as those occurring along dirt-roads – and agricultural areas – such as extensive pastures and agriculture fields – significantly influence vole metapopulations in farmlands. Particularly, the positive influence of extensive pasture areas adds to previous literature showing that these types of extensive practices are paramount for Cabrera voles' metapopulation persistence (and possibly to other species) in the Mediterranean agroecosystems. At the same time, linear strips of habitat, such as dirt-road verges, have shown an important dual ecological role, by promoting both individuals' survival and metapopulation connectivity in dynamic, human-dominated landscapes. Finally, it demonstrates that integrating gNIS data, genetic relatedness analysis, and kernel distribution modelling effectively allows for drawing inferences on species dispersal movements. In the case of the Cabrera vole, this approach highlighted the seasonal differences in the species dispersal patterns, which closely align with demographic and reproduction patterns along the year cycle.

6.2 Landscape ecology of the Cabrera vole

6.2.1 Voles in Mediterranean farmland

The influence of the agroecosystem landscape on the studied metapopulation of Cabrera voles was clear, as anticipated, given the habitat-patches occupied by the species are embedded in highly dynamic land uses, which can affect patch persistence. Nevertheless, only a few land uses included in the analyses were found to influence survival or connectivity. Specifically, agricultural activities and irrigation ponds impacted survival estimates, while extensive pasture areas and dirt-road verges affected both survival and connectivity estimates.

The connectivity of Cabrera voles was notably influenced by extensive pastures (Chapter 3). This is revealed by resistance to movement reaching the maximum functional cost at distances as close as 50 meters away from pastures. Conceptually, a functional cost refers to the Euclidian cost of movement within a given area. This means that, for voles, crossing a pixel (*i.e.* 10 meters) located just outside extensive pastures would be equivalent to moving 800 meters. Conversely, the functional cost for crossing extensive pastures was estimated to be negligible, that is to say, the same as the Euclidian distance. At the same time, extensive pastures were by far the most prevalent land use, covering nearly half of the study landscape, which may help explain the lack of an effect of patch characteristics on voles' survival. In fact, neither patch size nor isolation were detected as influencing the survival of Cabrera voles (Chapter 2 - Proença-Ferreira et al. 2019). This appears to be in slight contrast with previous studies in the same area, where those two variables were identified as predictors of occupancy (although to a lesser degree than landscape variables; see Pita et al., 2007; Pita et al., 2016). Patch size and isolation (or fragmentation) are considered main predictors of metapopulation dynamics in classic metapopulation theory, and the matrix is considered a plain, featureless, and hostile environment (Hanski and Simberloff 1997). By contrast, if certain matrix features have an effect on the connectivity of the landscape, then the effects of patch characteristics on demography can be diluted, since the colonisation events will be influenced by changes in movement ability of individuals across different land uses (With 2004). This may be the situation in this system, where a highly permeable and widespread land use (extensive pastures) may allow for easier movement of individuals between patches (in fact, more than 12% of recaptured individuals were found in different patches than their previous capture, (Chapter 2 - Proença-Ferreira et al. 2019). The ease of mobility across certain land uses in the matrix may therefore waive the effect of the size and isolation of suitable habitat-patches on survival. This can explain why survival was mostly influenced matrix characteristics, rather than patch-level descriptors (as suggested in Chapter 2 - Proença-Ferreira et al. 2019). These results emphasise the importance of considering landscape variables when analysing demographic parameters and population characteristics across space and time (Fahrig et al. 2011).

Proximity to agricultural fields was revealed as detrimental to survival, while irrigation ponds and dirt-roads had a positive effect. The influence of these land uses on survival may be understood in light of the dynamics of suitable habitat-patches in their close proximity.

Agricultural fields are associated to greater human intervention and management, which may create more instability in nearby habitat-patches, compared to dirt-road verge habitats or habitats bordering permanent artificial ponds, which are rarely cut or tilled. Habitat-patches with short life-spans are usually associated to limited population growth (Crispim-Mendes et al. 2024), likely reflecting reduced individual survival. The Cabrera vole thus seems to be adapted to human presence, and especially to human-made features, up to a degree where humans are not present and create very low levels of disturbance.

6.2.2 The dual ecological role of verges

As mentioned, dirt-road verges had a potential ecological role both as habitat-patches associated with increased individual survival and as corridors connecting habitat-patches. The recognition of these roles of dirt-road verges in fragmented farmland shaped by constant habitat turnover is key to inform future management and conservation efforts.

Corridors have long been considered important for landscape connectivity and species persistence (Hilty et al., 2019). Notably, many man-made landscape features (like hedgerows, road verges, or ditches) may be used as corridors despite not being explicitly designed as such, providing routes for species to move between otherwise isolated habitat areas (Hilty et al. 2019). Paradoxically, while these features often result from the destruction or conversion of important habitats for other species which avoid edge habitats or require large home ranges, they also provide opportunities for species like the Cabrera vole, not only by facilitating movement across the landscape, but also by providing breeding habitats. Indeed, although verge habitats are typically considered lower-quality habitats for voles (Santos et al. 2007), remnants of good- to high-quality habitats can persist in these unmanaged strips (Pita et al. 2006), highlighting their importance as seminatural habitats in the context of land use intensification (Barão et al. 2022).

The importance of verge habitats for the persistence of species is emphasised in the context of increasing agricultural intensification across ecosystems. If farmland areas are expected to become more widespread and connected, verges dividing different farmlands will tend to be eliminated, along with the verge habitats they harbour, reaching a threshold where the species might get locally extinct (Renwick and Lambin 2011). On the other hand, even in areas with a high degree of farming intensity, verges might become the only available habitat for voles (Renwick and Lambin 2011), by being the only remnant of semi-natural habitat undisturbed or with very low management.

Finally, it is worth noting that the dual function of road verge habitats has been seldom detected in temperate or arid climates, despite the large sum of studies on tropical and especially on northern climates (Ouédraogo et al. 2020). Furthermore, since previous studies did not find proof for the use of highway verges as corridors in two small mammal species inhabiting a Mediterranean area (Grilo et al. 2016), this study appears to be the first to demonstrate the function of road verges as both corridors and refuges for a small mammal species in the Iberian Peninsula (but see Galantinho et al., 2020).

6.2.3 Metapopulation ecology

Previous studies have consistently suggested for the existence of a metapopulation-like structure in Cabrera voles across SW Portugal farmland (Pita et al., 2007; Pita et al 2016). These evidences were mostly based on an overall population characterised by the existence of small colonies, or even pairs of individuals, inhabiting spatially separated habitat-patches, together with the extinction and colonisation events of habitat-patches (Pita et al. 2014). With this thesis, a further development of how this species is structured was achieved by examining the fine-scale demographic processes driving the population dynamics and gene flow within a selected landscape, rather than relying on occupancy data. This allowed for the identification of some population structuring encompassing two larger sets of patches, even considering the relatively high connectivity observed, as shown by the considerable gene flow across the landscape (Chapter 3). Despite this, it seems that the metapopulation-like dynamics in the area have not resulted in genetic separations between colonies or breeding pairs inhabiting habitat-patches, suggesting effective movement of individuals among patches, despite the observed seasonal variation in dispersal patterns (Chapter 5). This result hints that colonies behaving like metapopulations may themselves be part of larger sub-populations that allow for the overall persistence of voles in this area.

6.2.4 Population dynamics and social behaviour

Given Cabrera voles' high sensitivity to variations in habitat quality and food availability, a seasonal effect was expected to be detected in both abundance and survival estimates. However, abundance was the only demographic parameter showing temporal variability (Chapter 2 - Proença-Ferreira et al. 2019). The clear and substantial difference in abundance estimations from wet or post-wet seasons, as compared to dry or post-dry seasons, has been similarly detected in other studies relying on live-trapping (Fernández-Salvador et al. 2005; Rosário 2012), most likely related to food availability (Pita et al. 2014) and to habitat dynamics occurring in the area. Interestingly, there was a slight relation between the amount of available habitat in each season and the estimated population size, with abundance values following the same pattern as total habitat area (except in the end of the dry season) (Chapter 2 - Proença-Ferreira et al. 2019). Although more data from multiple years would allow for more robust inferences, this is consistent with the lower activity patterns generally found during the dryer seasons (Fernández-Salvador et al., 2005; Pita et al., 2006; Pita et al., 2011), (Fernández-Salvador et al. 2005; Pita et al. 2006) which reduce local recruitment, hence impacting abundance.

Past studies have suggested that Cabrera voles have a monogamous mating system, based on both captive (Fernández-Salvador et al. 2001; Fernández-Salvador et al. 2005) and free-living populations (Pita et al., 2010; Ferreira et al. 2018) (although some hints at deviations from a classic monogamous mating system have also been made; see Pita et al., 2011). Here, further evidence has been found for their monogamous behaviour, specifically in the higher apparent survival estimates of males (Chapter 2 - Proença-Ferreira et al. 2019), which could be due to female-based mortality, dispersal, or both (Clutton-Brock and Lukas 2012; Lukas and Clutton-Brock 2013).

6.3 Methodological advances

6.3.1 Genetic non-invasive sampling

This work shows how genetic non-invasive sampling (gNIS) applied to small mammal research allows for distinct ecological analysis. Before using gNIS to collect individual data on voles in the study area, our team employed live-trapping methods and found very low capture probabilities, even with a high sampling effort (Ferreira et al. 2018). In addition to collecting a large number of potential samples with relatively low sampling effort, gNIS allowed for a comprehensive survey over large areas, providing detailed data that can be used in a plethora of ecological analyses. As shown, this method provided adequate data for estimating Cabrera voles' population abundance dynamics and survival through CMR methods (both on Chapter 2 - Proença-Ferreira et al. 2019), providing also important insights on connectivity and population structuring (Chapter 3), and finally on dispersal (Chapter 5).

Two main problems may be identified with the sampling procedure used in this thesis. Financial constraints were, at the time of analyses, still an important limitation in DNA extraction from faecal samples (Ferreira et al. 2018). This led to the need to select samples based on their vicinity and apparent quality, and thus to the possibility of losing important information. As better techniques and procedures are developed, it is expected that the cost of extracting samples will gradually reduce. Also, as the utility and effectiveness of gNIS for small mammal monitoring are proven, more financing may be assigned for their use, thus allowing for the expansion of study areas accessed and the number of samples processed. Secondly, from the analysed samples, genotyping success was relatively low, when compared with other studies with larger-sized species (Tsaparis et al. 2014; Lampa et al. 2015; Mengüllüoğlu et al. 2019). As referred to in Chapter 2 (Proença-Ferreira et al. 2019), this was expected due to the small amount of available DNA, resulting not only from the small pellet size, but also from its rapid degradation caused by exposure to a wet environment at the time of collection. As experience and protocols evolve, however, this problem may be less relevant. Newer extraction methods that may improve success or improved field sampling protocols will also be a factor to consider (Lampa et al. 2013). Working as a team, the lab and field researchers may also help each other to produce better results. As a personal observation, my field colleagues and I were in constant contact with the lab researchers, which led to an adaptation of the field procedure between seasons. The visual inspection of the pellet (or set of pellets), together with ceasing to collect samples during rainy periods, led to an increase in genotyping success. Finally, different genotyping methods, such as Single Nucleotide Polymorphisms (SNPs), which use a different extraction technique and thus have different analysis costs (but also different potentialities, *e.g.* see Anderson & Garza, 2006; Norman & Spong, 2015), could be an alternative for samples with small amounts of non-degraded genetic material (Schultz et al. 2022).

The ability to sample large areas through gNIS is especially important when studying rare or elusive animals, for which very little data exists, thus fully opening the door for more widespread use of this technique in the study of other species, improving our knowledge of their ecology and better-applying conservation and management actions.

6.3.2 Dispersal kernels

Dispersal has long been a topic of interest in ecological research. In fact, since the release a little more than two years ago of the *dispfit R* package my colleagues and I developed (Chapter 4 - Proença-Ferreira et al. 2023), three different published papers analysing dispersal already applied it in their analyses, specifically on pollen dispersal and plant invasion (Chen and Pannell 2024; Fanal et al. 2024; Yamamoto and Jones 2024). This proves the utility of what was identified as a gap in dispersal kernel analytical applications. The collection of several commonly applied dispersal kernels, as well as measures to quantify the shape of those kernels, may allow for an improvement in comparing the dispersal of different years or seasons, or even in different populations and species. Adopting unified (and thus comparable) analytical methods, being this one or others, is decisive when relating differences in species occupancy or population dynamics in diverse areas, regions, and ecosystems. Finally, the usability of methodological procedures ultimately defines their utility. By allowing for an easy-to-use method, the *R* package *dispfit* also promotes research on dispersal, which is key for tracking pressing global conservation challenges related to climate and environmental change (Driscoll et al. 2014).

6.4 Conservation and management

Overall, results provided support for both habitat patch network and matrix land use effects on Cabrera voles' metapopulation dynamics and persistence ability in Mediterranean farmland landscapes. These insights contribute to a broader understanding of such agroecosystems, where biodiversity-friendly management actions favouring mosaics of habitat types are essential for their preservation. The benefits of habitat mosaics for farmland biodiversity should therefore be duly considered in conservation management planning, particularly given the current trends in land use intensification and abandonment across many agricultural systems (Altieri 1999). Specifically, farmlands with smaller crop areas intermingled with more naturalised patches are expected to have higher species abundance and diversity, making them crucial for maintaining biodiversity in agricultural landscapes (Fahrig et al. 2019). However, protecting individual- or sets of small habitat-patches, without accounting for the overall landscape that surrounds them (and largely creates the condition for their existence) will most likely lead to unsuccessful conservation outcomes. On the other hand, the protection of biodiversity embedded in agricultural land must involve landowners, thus promoting not only extensive and traditional agricultural practices without subtracting important income from farmers, but also engaging with local communities, addressing their concerns, and involving them in the protection of biodiversity.

Furthermore, patterns of survival, abundance, and connectivity of Cabrera voles in this system reveal that the threshold for the persistence of this metapopulation may change if drastic modifications in the landscape also occur. Indeed, current changes in farming activities across SW Portugal farmland landscapes already reflect a replacement of extensive pastures by other more intensive land uses, namely intensive pastures, intensive crops, and greenhouses. If, additionally, intensive farmland areas are to become more homogenous, connected, and widespread, as has been referred to for other vole species inhabiting farmlands (Renwick and Lambin 2011; Rodríguez-Pastor et al. 2016), it will have impactful consequences for a species highly dependent on extensive pastures and verge habitats to persist. There is thus a major threat to Cabrera voles long-term persistence in the region, adding to what has been also described for other sympatric species (Pita et al. 2009; Rosalino et al. 2009; Pereira et al. 2014; Pina et al. 2017).

Based on overall results and pressing threats for the species, some management practices may contribute to promote or maintain not only high-quality habitat patches within high natural value farmlands, but also habitats along crop edges and verges within more intensive farmlands. Conservationists and farmers alike should be interested in the preservation of this species, so the following guidelines are an important output of this study:

i. **Grasslands and extensive pastures**. Extensive pastures are the most important matrix land use for keeping the connectivity among Cabrera voles' local populations. As economic pressure increases, farmers often increase the amount of cattle or transition to irrigated farming, both of which could severely impact Cabrera vole metapopulations. Policymakers should thus promote extensive and low-density farming, keeping pastures or keeping areas with fallow periods. This could be achieved by developing agro-environmental schemes that stimulate such activities by financing farmers who keep parcels with specific conservation uses (Moreira et al. 2019; Aguilera et al. 2020);

ii. **Edge and verge preservation**. As mentioned in Chapter 2 (Proença-Ferreira et al. 2019) and Chapter 3, dirt-road verges are important as refuges and as corridors connecting high-quality habitat-patches, which is probably associated with a low degree of human intervention along those areas. Although not explicitly addressed here, it is likely that other undisturbed linear habitats, such as those occurring in hedgerows or ditches, could also provide breeding and dispersal opportunities for voles (Gelling et al. 2007; Rodríguez-Pastor et al. 2016; Feber et al. 2019; Barão et al. 2022). Therefore, to ensure the species' persistence in Mediterranean farmland, dirt road verges and edges should be maintained with minimal intervention, ensuring that herb vegetation cutting does not occur at heights below 30 cm (Pita et al. 2006);

iii. **Small-sized farms.** Results revealed that survival decreased with the proximity to the croplands present in the area. Several factors may be associated with this impact, all related to constant human presence and activities. A possible approach could be to have population persistence and connectivity of voles not dependent on the habitat-patches closer to those land uses, where such patches may be destroyed by farming actions. This would mean conservation efforts for habitat management might focus primarily on other, more distant areas, separated from agricultural lands by more than 150 meters, where human-vole conflicts are less likely to occur. At the same time, allowing for the subsistence of these smaller farms may also prevent conflicts with local communities (Henle et al. 2008). Following guideline (i), keeping a mosaic of small crop areas, intermingled with stable habitat-patches without human intervention, may also be enough for Cabrera vole persistence in the area;

iv. **Ponds**. The presence of water, even if from an artificial source, is particularly important in Mediterranean areas, where rain is scarce to absent for large stretches of time (Blondel et al. 2010). Ponds not only support the presence of green vegetation essential for providing food and shelter for many species, but also regulate the micro-climate of nearby areas. In the case of Cabrera voles, higher survival was found in individuals occupying habitat-patches associated to small ponds (Chapter 2 - Proença-Ferreira et al. 2019), highlighting their value in providing potential refuges for the species. Their presence also allows for small-scale irrigation of low-intensity crops, providing benefits for farmers (although agricultural areas have detrimental effects on vole survival). Allied with other management proposals, the building of small artificial ponds while keeping their edges with wet grasses at low management, may promote Cabrera vole persistence in farmlands.

6.5 Future research

There is no shortage of research routes for improving the conservation of biodiversity and advancing knowledge on species and ecosystems. Based on the main findings of the study, a few research topics could be addressed in future research.

In this thesis, landscape effects were predominant in explaining the population dynamics of the Cabrera vole. Their impact on this very restrict and specialist species may reflect similar effects on species with comparable requirements (Pita et al. 2010). In particular, both the water vole (*Arvicola sapidus*, Miller 1908) and the Portuguese field vole (*Microtus rozianus*, Bocage 1865), which are also species of conservation concern, recently evaluated as "Vulnerable" in the Red Book of Portuguese Mammals (Mathias et al. 2023), may show similar responses to land use intensification trends in agroecosystems across their distribution ranges. Thus, the application of similar approaches to improve our understanding of the ecology of the water voles or Portuguese field voles in other geographical regions would surely be of interest to improve the conservation of these communities and their ecosystems.

The replication of the approaches presented here to other areas with contrasting land use intensification levels should shed a light on the thresholds of habitat connectivity, turn-over and other ecological factors affecting Cabrera vole metapopulation persistence. In particular, the region south of the study area has been experiencing more profound changes in the landscape, mainly due to a wide irrigation permitter extracting water from the Mira river (located less than 2 km away from the study area) (Pereira et al. 2016). These have led to profound changes in the types of agricultural activities, with a shift towards intensive irrigated crops and the establishment of greenhouses, together with relevant modifications in the social fabric of the area (Pereira et al. 2016). The higher intensification of that area should have significantly impacted existing biodiversity, in particular Cabrera voles' populations. As shown in this work for more extensive areas, the use of verges, hedgerows, ditches, or other fringe habitats may play a role in keeping metapopulation persistence in more intensified areas, but very little is known about more intensified systems.

The influence of the landscape on population persistence and individual movement should have an effect not only at different spatial scales, but also at different temporal scales. While most landscape genetic studies look at the landscape and genetic structure as the same snapshots in time, landscape composition and configuration may change over time, even during the course of a few months, as human activities in farmlands vary depending on crops.

Therefore, the current spatial genetic structure may be a factor of how the landscape was, not at this moment, but before the breeding season or dispersal movements, or even a consequence of dispersal over multiple generations, a concept referred to as lag effect (Driscoll et al. 2014). Furthermore, in highly dynamic landscapes, both adults and juveniles may be forced to move, even to lower quality habitat-patches, and through otherwise less permeable land uses. How and if these dynamics affect populations of voles should be of interest for advancing the knowledge on their ecology and for informed actions towards their conservation.

Over the last 15 years, most studies addressing Cabrera voles have taken place in the dynamic farmland ecosystem of southwest Portugal (as exceptions see Garrido García et al. 2017 and Fernandes et al. 2022). The positive side is that our knowledge of this species has improved a lot in the past years, together with our ability to sample its population. Although we are now better suited to advise on conservation practices that should work, there are drawbacks to focusing only on certain areas. Less detail is known about Cabrera voles' ecology and behaviour in areas with fewer disturbances or with different climates. For instance, no studies exist on the dynamics of Cabrera voles' populations when in sympatry with the Portuguese field vole, in more northern areas of the Iberian Peninsula (Mathias et al. 2024). The interactions between these species may have important effects on how they occupy different landscapes and habitats, as suggested by the detected interaction between Cabrera voles and water voles (Pita et al. 2011), and thus be relevant for habitat conservation at regional scales.

Chapter 2 (Proença-Ferreira et al. 2019) adds further evidence for monogamous behaviour in the Cabrera vole. As mentioned, higher apparent survival in males as compared to females has been described in monogamous species (Clutton-Brock and Lukas 2012; Lukas and Clutton-Brock 2013). However, monogamous mating systems in wild-living populations do not mean exclusive mating with a single partner. Therefore, further studies are needed to clarify whether Cabrera voles exhibit genetic or social monogamy, or whether the species may adopt alternative mating strategies according to environmental characteristics and how eventual variations impact population dynamics. At the same time, since the analysis was not able to separate between true survival and migration, further studies might find interest in understanding if these biases occur specifically in either survival or dispersal. With only 6% of rodents classified as socially monogamous (Lukas and Clutton-Brock 2013), the rarity of this behaviour makes it biologically intriguing, warranting further study.

6.6 Working in science – lessons for the future

The team I was included in has been working in the same area and with the same species since 2006, encompassing a period of almost 20 years, which in some places could be considered a long-term ecological research (Lindenmayer et al. 2012). We have been able to collect and analyse data for this and previous studies due to the funding secured over the years. The ability to keep advancing our understanding on the ecology of this ecosystem and this species is dependent on funds, which are randomly assigned in the Portuguese reality and eventually come to an end. Unfortunately, this has become the reality in the past 5 years, when the last project for the study of this ecosystem finished. It is not possible to conserve and protect a species without knowing it, without understanding its environment, how it interacts with it, with other species, and with human presence. But to increase the knowledge of species and ecosystems, we still need scientists and we still need money (OECD 2021). It is paramount that more stable funding for Portuguese science and better working conditions for researchers become the rule rather than the exception.

6.7 References

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Appendix A

Paper Proofs

Drivers of survival in a small mammal of conservation concern: An assessment using extensive genetic non-invasive sampling in fragmented farmland.

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Drivers of survival in a small mammal of conservation concern: An assessment using extensive genetic non-invasive sampling in fragmented farmland

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ABSTRACT

Although important to guide conservation management, detailed demographic studies on rare or elusive species inhabiting fragmented, human-dominated landscapes are often hampered by the species' low densities, and the logistic and ethical constraints in obtaining reliable information covering large areas. Genetic non-invasive sampling (gNIS) provides cost-effective access to demographic information, though its application to small mammals is still scarce. We used gNIS to infer on the demography of an endemic small mammal, the Cabrera vole (*Microtus cabrerae*), occurring as a spatially-structured population in a 462-ha Mediterranean farmland landscape. We intensively sampled fresh vole feces in four seasons, extracted the DNA, and performed individual identification based on genotypes built using nine microsatellites. We then estimated population size and individual survival relative to environmental variables, controlling for heterogeneity in capture probabilities using capture-mark-recapture modelling. Population size increased during the wet season and decreased during the dry season, while survival remained constant across the study period. Individuals captured along road-verges and around water-bodies survived longer than those captured near agricultural fields. The use of gNIS on a heterogeneous landscape such as our study area allowed us to demonstrate that human land-use activities affect Cabrera vole demographic parameters in Mediterranean farmland, with implications for conservation planning towards its long-term persistence. Our approach can be widely applied to other elusive small mammals of conservation concern, but for which informative demographic data are still scarce.

1. Introduction

Estimating large-scale demographic patterns (e.g. abundance, population growth, survival) of animal species in relation to both individual traits (e.g. sex, age, weight) and environmental factors (e.g. climate or land-use change) is a difficult but necessary goal to understand species ecology and sustain conservation policies (Smallwood and Schonewald, 1998; Williams et al., 2002). This is particularly true for species occurring in agricultural landscapes where major declines in biodiversity due to agricultural intensification have been reported

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dispfit: An R package to estimate species dispersal kernels.

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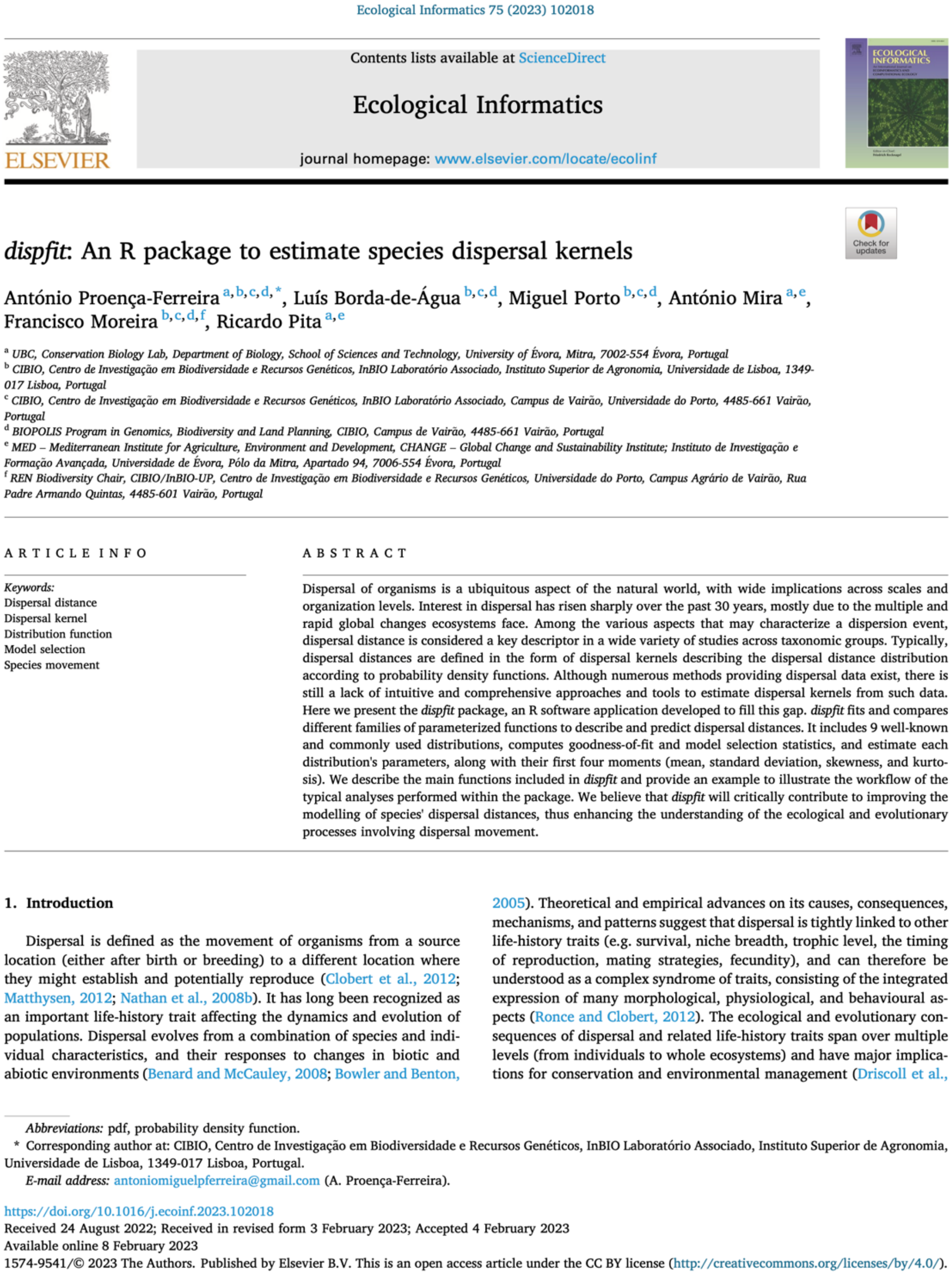

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dispfit: An R package to estimate species dispersal kernels

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ABSTRACT

Dispersal of organisms is a ubiquitous aspect of the natural world, with wide implications across scales and organization levels. Interest in dispersal has risen sharply over the past 30 years, mostly due to the multiple and rapid global changes ecosystems face. Among the various aspects that may characterize a dispersion event, dispersal distance is considered a key descriptor in a wide variety of studies across taxonomic groups. Typically, dispersal distances are defined in the form of dispersal kernels describing the dispersal distance distribution according to probability density functions. Although numerous methods providing dispersal data exist, there is still a lack of intuitive and comprehensive approaches and tools to estimate dispersal kernels from such data. Here we present the *dispfit* package, an R software application developed to fill this gap. *dispfit* fits and compares different families of parameterized functions to describe and predict dispersal distances. It includes 9 well-known and commonly used distributions, computes goodness-of-fit and model selection statistics, and estimate each distribution's parameters, along with their first four moments (mean, standard deviation, skewness, and kurtosis). We describe the main functions included in *dispfit* and provide an example to illustrate the workflow of the typical analyses performed within the package. We believe that *dispfit* will critically contribute to improving the modelling of species' dispersal distances, thus enhancing the understanding of the ecological and evolutionary processes involving dispersal movement.

1. Introduction

Dispersal is defined as the movement of organisms from a source location (either after birth or breeding) to a different location where they might establish and potentially reproduce (Clobert et al., 2012; Matthysen, 2012; Nathan et al., 2008b). It has long been recognized as an important life-history trait affecting the dynamics and evolution of populations. Dispersal evolves from a combination of species and individual characteristics, and their responses to changes in biotic and abiotic environments (Benard and McCauley, 2008; Bowler and Benton, 2005). Theoretical and empirical advances on its causes, consequences, mechanisms, and patterns suggest that dispersal is tightly linked to other life-history traits (e.g. survival, niche breadth, trophic level, the timing of reproduction, mating strategies, fecundity), and can therefore be understood as a complex syndrome of traits, consisting of the integrated expression of many morphological, physiological, and behavioural aspects (Ronce and Clobert, 2012). The ecological and evolutionary consequences of dispersal and related life-history traits span over multiple levels (from individuals to whole ecosystems) and have major implications for conservation and environmental management (Driscoll et al.,

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Abbreviations: pdf, probability density function.

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Appendix B

Other paper published during the PhD

Editors are biased too: an extension of Fox et al. (2023)'s analysis makes the case for triple-blind review.

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FORUM

Editors are biased too: An extension of Fox et al. (2023)'s analysis makes the case for triple-blind review

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Abstract

1. Functional Ecology conducted a randomised trial comparing single- and double-blind peer review; a recent analysis of this data found substantial evidence for bias by reviewers.
2. We show that this dataset can also be analysed for editor bias, after controlling for both reviewer bias and paper quality.
3. Our analysis shows that editors tend to be more likely to invite high-scoring manuscripts for revision or resubmission when the first author is a man from a country with a very high Human Development Index (HDI); first authors who were women or not from very high HDI countries were more likely to be rejected at this stage.
4. We propose that journals consider a triple-blind review process where neither editors nor reviewers know the identity of authors, and authors do not know the identity of reviewers nor editors.

KEYWORDS
equity in science, gender, nationality, publishing ethics, scientific review process

1 | INTRODUCTION

Science has an equity problem. Despite the mantra of scientific objectivity, there is accumulating evidence of systemic bias based on the gender, race, first language or nationality of scientists. Such bias affects not only access to and representation within science (Hughes et al., 2023; Kozlowski et al., 2022) but also the publishing process (Bancroft et al., 2022; Lee et al., 2013). However, the data compiled to demonstrate such bias can also help us evaluate potential solutions. Such is the case in the recent study by Fox et al. (2023) which used a large-scale randomised trial to both test for bias in the manuscript review process at Functional Ecology