Geographic patterns of genetic and morphological variation of the Sicilian wall lizard, *Podarcis wagleriana*

Viktoria Litsi Mizan
MSc in Biodiversity, Genetics and Evolution
Faculty of Sciences of Univesirsity of Porto
2015

Supervisor
Dr. James Harris, Researcher ,CIBIO

Co-supervisor
Dr. Antigoni Kaliontzopoulou, Researcher, CIBIO
Dr. Daniele Salvi, Researcher, CIBIO

MSc

U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

MSc

AKNOWLEDGMENTS

First of all I would like to thank my supervisor James for trusting me with this project and gave me the opportunity to work on something I love and be part of a great group of people.

I mostly thank Daniele and Antigoni, for their help and patience during these months, and for all the fun moments during the fieldwork in Sicily. I surely keep great memories from there. I could not have asked for better people to help me in this journey!

To everyone in Cibio, but especially the ecology lab, for all the fun, the laughs and the crazy dinners. I especially thank all the people that helped me in the analysis and the writing during the last months.

To Daniel, I thank you for always support me and being the most special person.

Finally, to my family thank you for always trusting me in every new step of my life; you have always been the best support and my most important people: Mom, Dad and Sam.

ABSTRACT

The unique conditions of island environments make them particularly interesting systems to study genetic and morphological variation and to assess the evolutionary processes that determine them. Sicily is the largest island of the Mediterranean basin that, considering its complex paleogeographic history and geographic features and its heterogeneous environments, provides an excellent setting to investigate microevolutionary processes that have led to present day diversity of its endemic taxa. In this context, we investigated the diversity, both at the genetic and morphological level, of the wall lizard *Podarcis wagleriana,* a species strictly endemic to Sicily and its satellite islands.

We used two mitochondrial markers (nd4 and cytb) to assess the genetic structure and investigate the phylogeographic patterns of the species based on samples from 24 different localities. This analysis revealed three distinct mitochondrial clades, located in the west, east and north of the range, likely originated in distinct glacial refugia during Pleistocene, thus conforming to the “refugia within refugia” model. Allopatric divergence in isolated refugia as well as demographic expansions followed by secondary contacts, probably played a major role in shaping the genetic diversity patterns of this species.

To assess the morphological variation of *P. wagleriana,* three morphological character sets (size, pholidosis, head shape) were analyzed in individuals from 37 different localities. Our results indicated that in most cases the variation was spatially structured and could be explained as the result of phenotypic plasticity or adaptations to local environmental and ecological conditions. However, some characters (head shape) showed also concordance with genetic patterns, indicating that the long persistence in Pleistocene refugia could have also triggered natural selection on certain characters.

KEYWORDS

Phylogeography, Morphology, Mediterranean island, Sicily, Biodiversity, Pleistocene oscillations, Scalation, Geometric Morphometrics

RESUMO

As condições únicas das ilhas torna-as modelos ideais para investigar a variação genética e morfológica e avaliar os processos evolutivos responsaveis pela sua formação. A Sicilia é a maior ilha da bacia Mediteranea e devido à sua historia paleogeográfica, caracteristicas geográficas complexas e ambiente heterogéneo, os seus taxa endémicos tornam-se excelentes modelos para investigar processos microevolutivos responsáveis pela diversidade atual. Nos investigámos a diversidade genética e morfologica da espécie *Podarcis wagleriana,* uma espécie endémica da Sicilia e das suas ilhas satélite.

Neste estudo, dois marcadores mitocondriais (nd4, cytb) foram amplificados para avaliar a estrutura genética da espécie com base em amostras de 24 localidades diferentes. Esta análise revelou três clados mitocondriais distintos, localizados no norte, no este e no oeste, os quais, podem representar três refúgios durante o Pleistoceno, suportando o modelo “refugia within refugia”. O isolamento prolongado da espécie dentro de cada um dos três refugios e a divergência alopátrica foram, provavelamente, cruciais na formação dos padrões da diversidade genética. Contudo, outros processos tais como expansões demográficas e contatos secundários entre populações de refúgios diferentes, ou processos estocásticos como deriva genética parecem ter contribuído para o resultado final da variação genética.

Para avaliar a variação morfológica de *P.wagleriana* foram usados diferentes caracteres morfológicos (SVL, pholidosis, forma da cabeça). Os resultados indicam que, na maioria dos casos, a variação foi espacialmente estruturada e formada sob diferentes pressões seletivas de condições ambientais e ecológicas, resultando em adaptações locais na forma de plasticidade fenotípica. No entanto, alguns caracteres (forma da cabeça) coincidem com os padrões genéticos, indicando que a longa persistência dentro dos refúgios no Pleistoceno podem também ter desencadeado a seleção natural nesses caracteres.

PALAVRAS-CHAVE

Filogeografia, Morfologia, Ilha Mediterânea, Sicilia, Biodiversidade, Oscilações do Pleistoceno, Escalação, Técnicas de Geometria Morfometria

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

Mya	Million years ago
Kya	Thousand years ago
DNA	Deoxyribonucleic acid
mtDNA	Mitochondrial Deoxyribonucleic acid
EC	Expansion- Contraction model
MRCA	Most recent common ancestor
TMRCA	Time from the most recent common ancestor
NJ	Neighbor joining
UPGMA	Unweighted pair group method with arithmetic mean
MCMC	Marcov chain monte carlo
SVL	Snout vent length
PCR	Polymerase chain reaction
dntp	Deoxynucleotide triphosphate
$Mgcl_2$	Magnesium chloride
BSA	Bovine Serum Albumin
IUCN	International Union for Conservation of Nature
NE	North-east
GM	Geometric morphometrics
bp	Base pair
tRNA	Transfer ribonucleic acid
AIC	Akaike’s information criteria
BIC	Bayesian information criteria
HKY	Hasegawa- Kishino-Yano model
HPD	Highest posterior density
nd4	NADH dehydrogenase subunit 4
cytb	Cytochrome b

GENERAL INTRODUCTION

WHY ISLANDS?

Since early times, insular systems have piqued the curiosity of biologists; even Darwin set the bases of the evolutionary theory while studying the populations of the Galapagos Islands. Insular systems are characterized by unique conditions that led to their characterization as natural laboratories for the study of microevolutionary processes (Darwin & Wallace 1858) and have rendered them as particularly interesting systems to study biodiversity in all levels. The biological interest around insular systems has emerged due to some of their main features such as: the lower biological complexity of island communities, when compared to mainland, and their clearly defined limits. Islands vary also in terms of area, age, altitude, latitude, species richness, geographical barriers both in micro- and macrogeographical scales, and some of them show heterogeneous habitats and different ecological conditions. However, one of the most crucial factors that determine the composition of their biota is their isolation.

The degree of an island's isolation determines its accessibly from a source of colonizers; due to their limited accessibility island systems are usually characterized by a poorer representation of different taxa, comparing to the mainland. This is really important as it can lead to the relaxation of interspecific competition and predation pressure and an increase of intraspecific competition, which may in turn result in higher population sizes and densities (density compensation; MacArthur *et al.*1972) of individual species. Under these conditions, individuals that can successfully exploit the new resources can gain advantages increasing the likelihood of the species to undergo ecological release and niche shift. The need for exploiting new habitats, the fragmented species ranges and the low dispersal capacities of island species makes them more prone to evolutionary processes like directional selection and genetic drift (Wright 1978, Barton 1996, Frankham 1997, Clegg *et al.* 2002). Furthermore, the size of an island plays a crucial role in shaping its diversity. Usually, smaller areas have less diversity due to a higher impact of stochastic processes like genetic drift due to past bottlenecks or stronger directional selections. Consequently, islands provide a particularly interesting system to study colonization processes, adaptive radiation, allopatric fragmentation and processes of genetic and morphological differentiation and divergence (Soule 1976, Thorpe & Baez 1987, Schluter 2000, Hewitt 2001, Salvi *et al*. 2009).

The above unique conditions of insular systems piqued our interest and led to the present investigation of an island species. Many studies have focused on the evolutionary mechanisms that have shaped insular ecosystems and intraspecific variation in many species (Selander & Whittman 1983, Barton & Charlesworth 1984, Gavrilets & Hastings 1996, Givnish *et al.* 2000, Stuessy *et al.* 2006, Mappes *et al.* 2008). Some studies have particularly focused on the Mediterranean basin which exhibits a wide variety of insular environments of different origins (continental, oceanic) as well as great biological diversity and ecological complexity. Some of these studies provide insight on the relationships between geographical patterns of biological variation and evolutionary processes (Capula 1994a; b, Harris *et al.* 2005, Brown *et al*. 2008, Salvi *et al.* 2009; 2011; 2014). In this framework, the present study focused on an insular Mediterranean species in an effort to describe its intraspecific diversity, both at the genetic and morphological level, the way this diversity is geographically distributed and the eco-evolutionary factors that may have shaped it.

STUDY REGION

Mediterranean islands hold a high proportion of the world's biodiversity and endemisms. The study region of this thesis included the island of Sicily and three of its satellite islands (Egadi Islands), namely Marettimo, Levanzo and Favignana. Among them, Sicily is the biggest one and it concentrates important reservoirs of biodiversity. Its considerable size, its complex but well- known paleogeographic history and its central position in the Mediterranean Sea makes it an ideal model system in which to study the evolutionary processes that shape genetic and morphological differentiation and led to the high levels of diversity observed nowadays.

Sicily is situated in the central Mediterranean where it extends from the tip of the Apennine Peninsula, from which it is separated only by the narrow Strait of Messina, towards the North African coast. Its most prominent landmark is Mount Etna; at 3,350 m it is the highest point of the island and the tallest active volcano in Europe (figure 1). Sicily has a typical Mediterranean climate with mild, wet winters and hot, dry summers. The Egadi Islands are situated on the northwest coast of Sicily, with Favignana being the largest of them.

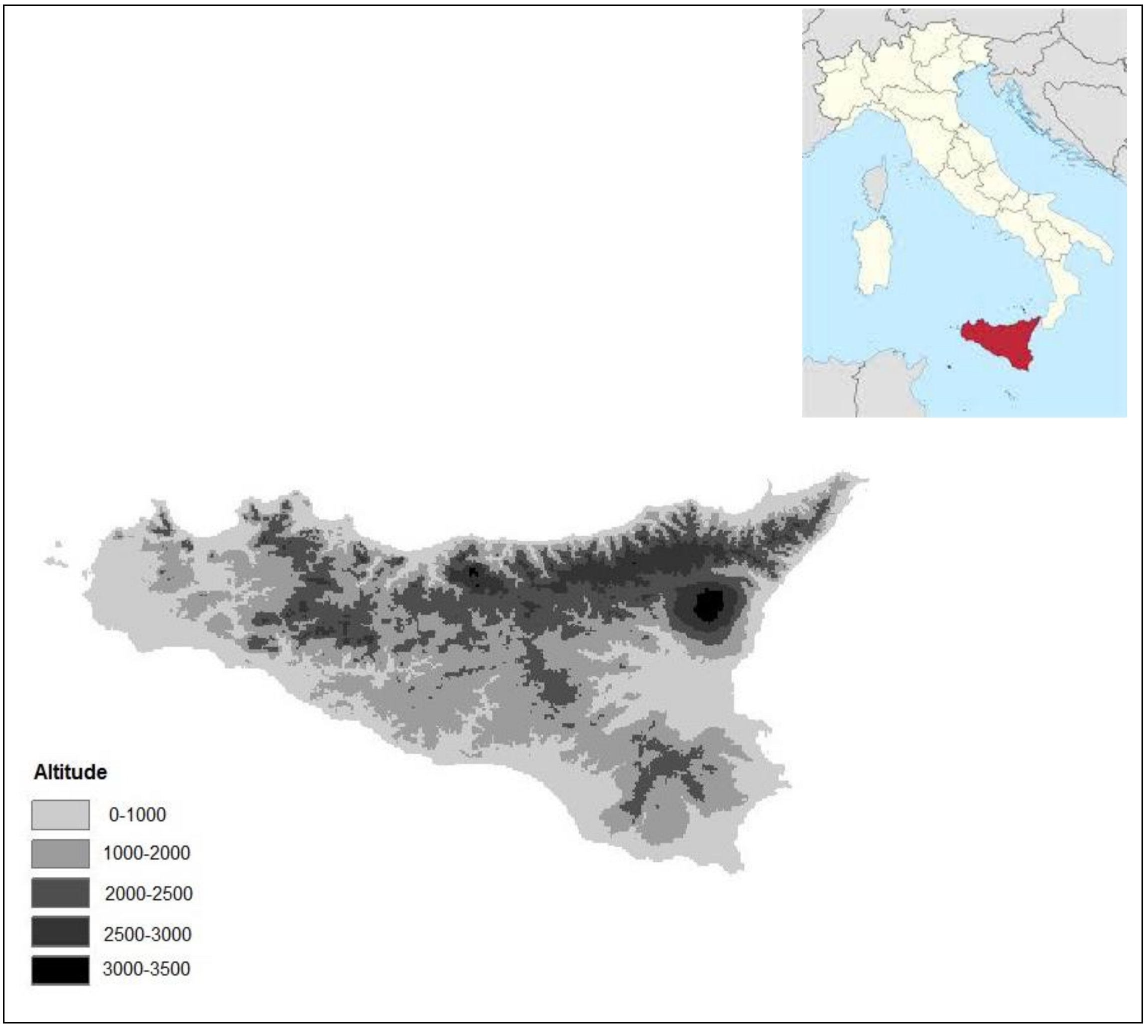


Figure 1: Map of Sicily and its satellite islands (Egadi Islands; Favignana, Marettimo, Levanzo).

Paleogeographic events have probably played a crucial role in the formation of the present biological diversity encountered in Sicily. In the Miocene (~23.03-5.33 Mya) and specifically during the Messinian period (~5.59-5.33 Mya) the Mediterranean Sea became isolated from the Atlantic Ocean resulting in a decrease of the Mediterranean Sea level up to its nearly complete dessication and the formation of land bridges between Europe, Africa and most of the Mediterranean islands (figure 2).

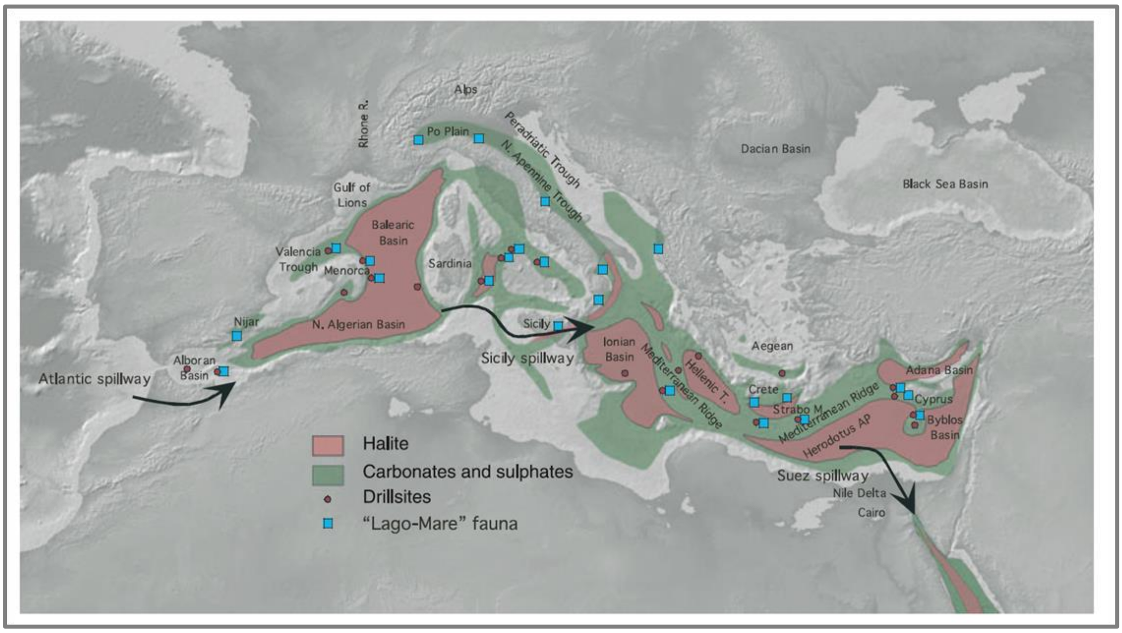


Figure 2: Representation of the Mediterranean basin during the Messinian salinity crisis when it was totally isolated from the Atlantic Ocean and almost entirely dessicated. Photo credit: Ryan 2009.

During this long period of desiccation called, the Messinian salinity crisis (Krijgsman *et al.* 1999, Pierre *et al.* 2006), the geographical set of the Mediterranean basin led to a series of dispersal events between landmasses, while, the reopening of the Strait of Gibraltar in the end of the Messinian salinity crisis (~5.3 Mya), at the Miocene/Pliocene boundary, promoted vicariance and speciation events. During this time, the Maltese Islands became isolated from Sicily and most probably occured the separation of our study species, *Podarcis wagleriana* from the closely related species, the Maltese *Podarcis filfolensis* (Harris *et al.* 2005). Furthermore, during the Early Pleistocene, Sicily and Calabria were fragmented in small islands, constituting an archipelago. Finally, in the course of the Middle Pleistocene, the uplift of inner and coastal plains led to a geographical setting more and more similar to the present day. The final separation of Sicily from southern Calabria, during the same period, resulted in the formation of the Messina strait. The Messina strait could have served as an intermittent filtering barrier, which probably controlled the processes and timing of the Late Middle Pleistocene - Late Glacial vertebrate faunal dispersal towards Sicily (Bonfigio *et al*. 2004).

As expected from an island with a long Plio-Pleistocene isolation (Bonfiglio *et al.* 2000), Sicily hosts a great number of well-differentiated, endemic intraspecific taxa in several animal groups including hedgehogs, *Erinaceus* (Seddon *et al.* 2001*)*; mice, *Apodemus* (Michaux *et al.* 2005*);* beetles, *Pachydemidae* (Sanmartin 2003*)*; skinks, *Chalcide*s, (Giovannotti *et al.* 2007), as well as endemic species such as shrews;

Crocidura sicula, turtles; *Emys trinacris,* toads; *Bufo siculus,* lizards; *Podarcis wagleriana*. Using molecular methods, some of those species have been shown to be sister taxa to populations from the Apennine Peninsula or to be of very recent Apennine peninsular origin (Shmitt 2007). Even though most Sicilian fauna probably originated in the Italian Peninsula, hypotheses have been made for an African origin of some species. For instance, Bonfiglio (2000) suggested an African origin of fossil elephants (*Elephas falconeri)* as an alternative hypothesis from Italian/European derivation from *E.antiquus* (Bonfiglio *et al.* 2004). However, post-Messinian land bridges to Africa are still debatable as a possible African origin of terrestrial fauna has scarcely been tested with molecular methods and has been inferred for only three terrestrial taxa; *Chalcides*, (Giovannotti *et al.* 2007), *Discoglossus*, (Zangari *et al.* 2006), and *Bufo*, (Stöck *et al*. 2008).

Podarcis wall lizards

The genus *Podarcis* (Wagler 1830) is a well differentiated group of lizards of the family Lacertidae endemic in the Mediterranean basin (Arnold 1973, Arnold *et al.* 2007). The genus was widely recognized only after the morphological revalidation by Arnold (1979). Its distribution ranges from Central Europe to the Sahara and from the Iberian Peninsula to the Crimea (figure 3). The highest endemism and diversity is found in the European southern peninsulas and respective archipelagos. The species *P. vaucheri* is found also in northwestern Africa, and only one species, *P. muralis*, is found in the northern parts of the species' range. According to the Reptile Database (http://www.reptile-database.org, accessed August 12, 2015) 23 species have been currently recognized as part of the genus and some of them have been listed by IUCN red list in threatened categories as vulnerable (*P.gaigeae, P.levendis, P.milensis),* endangered (*P. carbonelli*, *P.cretensis*, *P. lilfordi)* or critically endangered (*P. raffonei*).

Figure 3: Distribution map of the genus *Podarcis*.

Since the recognition of the genus morphological and molecular techniques have been used to delimit species within it. However, the taxonomy of the species within the genus is both complex and unstable, as they exhibit high morphological similarity, yet show subtantial intraspecific variability (Arnold & Ovenden 2002). Many attemps to reconstruct its phylogeny have led to poorly resolved nodes and different estimates from different studies (Harris & Arnold 1999, Oliveiro *et al.* 2000, Poulakakis *et al*. 2003). Partially these difficulties were due to the fact that some recognized species could be in fact species complexes (e.g *Podarcis hispanica* species complex ; Harris *et al.* 2002) with highly genetically distinct lineages some of which may deserve species status. Reexamination of earlier taxonomies has led to recognition of various new species. For example, *P.carbonelli* that was originally considered a subspecies of *P. bocagei* is now recognized as a full species (Sá-Sousa & Harris 2002). More species, formely included in *P. hispanica* complex, were also eleveted to the species status after rexamination; the south Iberian/West Mahgrebin *Podarcis vaucheri*, (Oliverio *et al*. 2000, Busack *et al*. 2005) and recently various other *Podarcis* species within the Iberian Peninsula were proposed (Geniez *et al.* 2014)

Despite ambiguities rearding within-species taxonomy, phylogenetic studies based on mtDNA (Arnold & Harris 1999, Oliverio *et al.* 2000, Harris *et al.* 2005, Poulakakis *et al*. 2005, Arnold *et al*. 2007), coincide in characterizing the genus as monophyletic. Even though the topology of the phylogenies is still unclear, and in cases they show poor nodal support, more recent studies (Harris *et al.* 2005) concord in the monophyletic origin of the species of the wider Sicilian area (*P.wagleriana, P.raffonei, P. filfolensis)*(Figure 4).

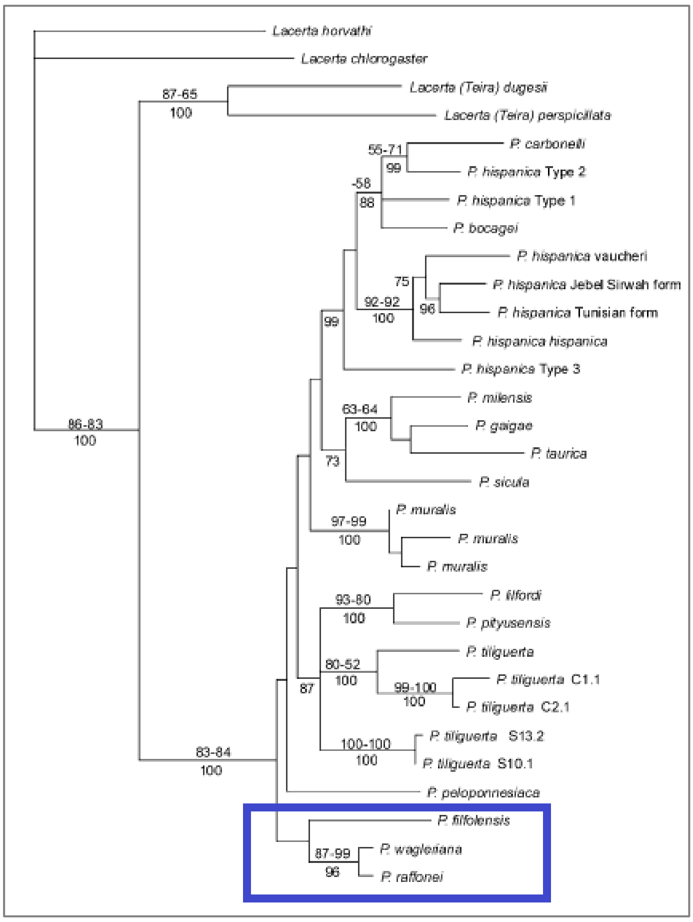


Figure 4: Phylogeny of the genus *Podarcis*. The three species highlighted are the ones met in the wider Sicilian area (*P.filfolensis, P.wagleriana, P. raffonei*). Photo credit: Harris *et al.* 2005.

Morphologically the genus is characterized by elevated variation (Arnold 1973, 2004), showing high levels of diversity in body size, size of different body parts, pholidotic traits and colour patterns. The investigation of their morphological variation in many cases can be necessary for correctly classifying and conserving biodiversity (Beheregaray & Caccone 2007) but also for understanding evolutionary mechanisms that led to morphological diversification (Adams *et al.* 2009). Especially, when dealing with species complexes with unsorted lineages, morphological characters can help in the diagnosis and delimitation of different species (Lymberakis *et al.* 2008, Kaliontzopoulou *et al.* 2012). Additionaly, morphological change can emerge wihout genetic basis, consequently morphological studies on *Podarcis* have led to the recognition of microevolutionary processes (for example local adaptations or phenotypic plasticity; DeWitt & Scheiner 2004) responsible for the high variation of the genus (Kaliontzopoulou *et al.* 2012).

STUDY SPECIES: *Podarcis wagleriana*

This study aims to unravel the genetic and morphological differentiation of the Sicilian wall lizard, *Podarcis wagleriana*. *P. wagleriana* (Gistel 1865) is a lacertid lizard, endemic to Sicily and its satellite islands, i.e the Egadi Islands (Marettimo, Levanzo, Favignana) and the Stagnone islands (Isola Grande and La Scuola; Arnold & Ovenden 2002, Corti & Lo Cascio 2002). A formerly considered subspecies of *P. wagleriana* from the Aeolian Islands (Vulcano, Filcudi, Salina and Strombolicchio) is currently recognized as a separate species (*P. raffonei*, Mertens 1952; Lanza 1973, Capula *et al.* 1987, Capula 1990). In Sicily, the species can be found all across the island with the exception of its NE part (figure 5).

Figure 5: Distribution range of *Podarcis wagleriana*. Shapefile downloaded from IUCN website (http://www.iucnredlist.org) (accessed on June 2015).

The species is sympatric with the Italian Wall lizard, *Podarcis sicula*, (Capula 1990) across all its range with the exception of the islet La Scuola which is the smallest island where the species occurs and where *P.wagleriana* is the only lacertid lizard (Lo Valvo & Massa 1999). The morphological distinction between *P. wagleriana* and *P. sicula* can be quite difficult as they usually differ only in certain color patterns which show high variation (Arnold & Burton 1978) and in cases they can hybridize in the small islets (Capula 1993).

P. wagleriana can reach up to 7.5 cm in snout-vent length with males being usually slightly larger than females. Their dorsal color is usually green, but they can also be brownish, stripped or dotted (figure 6). Their ventral color is usually white, with a spotted throat and sometimes suffused with orange, usually in breeding males.

The habitat of *P.wagleriana* includes the occupancy of low vegetation areas on the edges of woodland, bushy slopes, pasture and cultivated land. It is often present in gardens, especially irrigated ones. In contrast to the Italian wall lizard (*P. sicula*) it does not climb much and occupies more densely vegetated areas. It is the commonest wall lizard inland while *P.sicula* is the more common near the coast.

Figure 6: *Podarcis wagleriana* from the main island of Sicily (left) and from Marettimo island (right). Photos were downloaded from www.alamy.com and www.faunamelitensis.devianart.com (accessed on June 2015).

PHYLOGEOGRAPHY

Coalescent theory

Phylogeography as an integrative field of study aims to relate evolutionary processes to spatial, temporal and environmental factors in order to understand past and present biodiversity (Avise *et al.* 1987, Hickerson *et al.* 2010; see also chapter 1). Advances in both laboratory methods that allowed easier sequencing of DNA and computational methods that make better use of the data (e.g. employing coalescent theory) have both greatly contributed in the advancement of phylogeographic inference.

The coalescence theory, first defined mathematically by Kingman 1982, introduced a new model when studying phylogenetic relationships. Coalescence is a retrospective stochastic model that traces back the time to ancestry of a lineage until all lineages coalesce to a common ancestor (MRCA). The basic idea, in the absence of selection, is that the sampled lineages can be viewed as randomly “picking” their parenting lineages as we go back in time. Whenever two lineages pick the same parent they coalesce until eventually they coalesce into a single lineage, the MCRA of the sample (Rosenberg & Nordborg 2002). The theory also predicts that, the rate at which lineages coalesce depends on the size of the population (Salemi & Vandamme 2003). Should only genetic drift be a factor, smaller populations will present a faster rate of drift, and, as a result, of coalescent lineages. When comparing similarity in more than one population historical processes can be assessed by the increased or decreased rate of genetic diversity (Salemi & Vandamme 2003). However, selection is always present and consequently, genetic diversity is not only stochastic but some genotypes can have greater fitness than others (Rosenberg & Nordborg 2002).

The coalescent theory has been applied to produce phylogenetic trees. When studying a species tree, the branch length represents the time that an ancestral form took to split into its descendants and is measured by the number of mutations that accumulated between that split. The mutations have a rate, which is logically defined by the number of mutations that are expected in each generation (Salemi & Vandamme 2003).

Molecular data

Modern phylogeography is based on the use of molecular data and typically uses both mitochondrial and nuclear DNA sequences as they both have been proven to be informative in different ways. Even though, the word phylogeography was first introduced in 1987 by Avise (Avise *et al.* 1987), scientists had already begun to use mitochondrial DNA data a decade earlier to describe how conspecific individuals are linked through common ancestors. Since then, mitochondrial DNA has become a very significant tool in phylogeography and is widely used to study evolution of species and populations (Ballard & Whitlock 2004). Its suitability was not without reasoning, as mitochondrial DNA offers considerable advantages.

Mitochondrial DNA is typically represented by 37 genes, in animal cells, linked in a circular molecule of about 17,000 base pairs in length. It is highly compacted and, in contrast with the nuclear DNA, is intron- free and normally does not have large intergenic stretches of non-coding regions. As first shown by Brown *et al.* (1979) this highly compact molecule accumulates nucleotide substitutions several times faster than a typical single-copy nuclear DNA. This can be due to: 1) insufficient mechanisms of DNA repair, 2) the oxygen rich environment in which the mtDNA molecules are exposed inside the mitochondria, 3) the relaxation of several functional constraints because of the low production of mtDNA proteins directly involved in its own mechanisms and, 4) the fact that mtDNA is naked from histone proteins that themselves are considered conservative and can constrain the evolutionary rates. Consequently, its rapid evolutionary rate makes it ideal for accumulation of sequence variation and use in phylogenetic analyses. Another property of mtDNA is its maternal inheritance that makes its transmission haploid, asexual and typically without recombination. The lack of recombination makes the nucleotide mutations the only source of variation in the mtDNA genome and thus simplifies the analyses. However, it has been suggested that the use of mtDNA alone is not enough to obtain a complete image of the evolutionary history of populations (Ballard & Whitlock 2004, Godinho *et al.* 2008). The fact that mtDNA provides knowledge only of a single locus can lead to underestimation of diversity and oversimplification of the evolutionary history (Zhang & Hewitt 2003). The nuclear genome is much larger in number of base pairs, includes recombination and has lower mutation rates. However, its use can be complicated due to the recombination and the heterozygosity and can be more difficult to amplify and sequence. Most importantly, nuclear sequences may have low information content for intraspecific studies because of the low subtitution rate and the relatively recent time frame of intraspecific evolution. However, phylogeographic studies that use both types of DNA are becoming more frequent, in an effort to obtain a more complete image on the evolutionary history of organisms (e.g. Lerner & Mindell 2005, Sheldon *et al.* 2005, Toews & Brelsford 2012, Salvi *et al*. 2013; 2014).

MORPHOLOGY

Traditional Morphology

For centuries the description of organisms' diversity and the assessment of their phylogenetic relationships depended on the use of morphological characters (Wiens 2007). Initially, the assessment of morphological diversity was mainly based on observation of external features but gradually a broad number of traits started to be used, for example, different anatomical features. Another approach has been to focus on characteristics that are relevant for addressing the biological questions of interest, like when focusing on functional morphology (Carlson 1989) or ecomorphology.

The great interest in morphological variation and its causes led to the advancement of statistical methods that gradually gave birth to the discipline of biometry (Sokal & Rolhf 1995, Slice 2005, Mitteroecker & Gunz 2009). The new morphological tools were focused on description and statistical analysis, using mainly linear distances, of shape variation among and within species. These measurements used traditionally include different measured distances of an organism, like widths and lengths or structures, distances between specified landmarks as well as angles and ratios. Different statistical methods and graphical representations are used to describe the results. The development of multivariate statistics to analyze the above measurements allowed the exploration of specific biological hypotheses concerning the origin of different phenotypes and led to their characterization as “traditional morphometrics” (Marcus 1990).

Scalation characters have also been used extensively in lizards for taxonomic purposes and field identification (Salvador 1998, Arnold & Ovenden 2002, Geniez *et al.* 2007). Many taxonomic keys are based or include information on pholidotic traits (Salvador 1997, Barbadillo *et al.* 1999, Ferrand de Almeida *et al.* 2001, Arnold & Ovenden 2002). Most commonly different scale counts, presence or absence of certain scales, scale shape and texture are used for this purpose. Especially at the interspecific level these traits can exhibit significant variation, as shown also for members of the genus *Podarcis* (Guillaume 1976, Carretero *et al*. 2003, Kaliontzopoulou *et al.* 2005). As such, when a large number of individuals and populations the usefulness of these characters can be overwhelmed by local variation (Kaliontzopoulou *et al.* 2012). Additionally, scalation characters may have an adaptive value as they frequently present geographical variation across different environmental and/or ecological conditions. Extensive work in different taxa, like *Anolis* lizards (Malhotra & Thorpe 1997), *Gallotia* lizards (Thorpe & Baez 1987; 1993) and *Chalcides* skinks (Brown & Thorpe 1991) have highlighted the possible ecogenetic origin of variation of pholidotic variation and how these characters can vary across different habitat types, climatic conditions and latitudinal or altitudinal gradients.

Geometric morphometric techniques

The introduction of geometric morphometric techniques (GM), relatively recently, (Bookstein 1991, Rolhf & Marcus 1993) revolutionized the way morphological diversity is investigated. The birth of GM methods was based on four considerations irrespectively linked to biological form: size, homology, shape description and visualization. Practical and theoretical problems accompanying the mathematical definition of size (Bookstein 1989, Slice 2005), the selection of quantities to use to measure homology (Bookstein 1982, Slice 2005), the selection of variables used for shape description (Bookstein 1982; 1996, Slice 2005) and the proper tools for shape visualization (Adams *et al.* 2004) led to the development of the GM methods. GM tools are based on the application of outlines and landmarks that, in combination with the development of a new statistical theory for shape analysis (Kendall 1984; 1985), set the base for the development of the geometrical study of organisms' shape (Bookstein 1986, Rolhf 1986). Today, the most frequent implementation of these techniques is through the use of two- and three- dimensional landmark-based GM techniques. When compared to traditional techniques that use linear measurements, GM are usually more powerful for the description and interpretation of patterns of variation (Valenzuela *et al.* 2004, Bonnan *et al.* 2008, Kaliontzopoulou *et al.* 2008, Arendt 2010) and have been shown to be particularly useful in studies below species level (Loy 1996, Zelditch *et al.* 2004).

CHAPTER 1

Phylogeographic patterns of *Podarcis wagleriana*

1.1. INTRODUCTION

Phylogeography, as an integrative field of science, uses genetic information to study the geographical arrangements of genetic lineages, in most cases focusing on the intraspecific level (Avise 2000) or on closely related species. Although scientists had previously used mtDNA to address how conspecific individuals can be linked through common ancestors, the term "phylogeography" first appeared only in 1987 (Avise *et al.* 1987). In this seminal work, Avise *et al.* (1987) also envisioned that the investigation of intraspecific microevolutionary processes could answer questions regarding macroevolutionary differences in higher taxa, giving a historical perspective to the analysis of the genetic data. This historical perspective introduced a new era to the field and has since developed quickly, passing from descriptive methods to coalescent models, estimation of demographic histories in an explicit geographical framework (Lemmon & Lemmon 2008) and methods testing geographic and temporal congruence between co-distributed taxa (Hickerson *et al.* 2006, Carnaval *et al.* 2009).

The advancement of the phylogeography has also led to the description of empirical patterns, such as the strong genealogical structure that seems to characterize low dispersal species (Avise 2009). Hundreds of studies have been conducted on low-vagility species and have shown that, frequently, these organisms are characterized by similar or identical mtDNA haplotypes that are spatially restricted, and by only a few haplotypes that are highly genealogically different (Avise 2009). These few divergent haplotypes could have been differentiated due to past condition of allopatry and their presence and geographic structure are usually linked to known historical events that could have obstructed dispersal. For example, according to the "expansion-contraction" model (EC; Hewitt 1996; 2004, Provan & Bennet 2008) the Pleistocene climate oscillations that occurred during the Quaternary forced temperate species to migrate to southern refugia during glacials and recolonize northwards during postglacial periods (Hewitt 1996; 2004, Provan & Bennet 2008), resulting in a cyclic contraction-expansion pattern of species demography and distribution ranges. These climate cycles with glacial periods followed by interglacial periods and related changes in habitat and sea level fluctuations associated with ice-volume changes, have had a great impact on species' geographical distribution and diversity. In the western Palearctic region the three southern Mediterranean peninsulas (Iberian, Balkan, Italian) have been identified as important refugia for many species during these oscillations (Hewitt 1996, 2000). A major attribute of these refugia is that they provided the conditions necessary for long-term stability and *in situ* diversification of temperate biota, thus playing an important role in the formation and maintenance of biodiversity. This is, for instance, in contrast to the populations that arose from northward expansion, which could have faced a series of bottlenecks (Hewitt 1996). However, recent studies have suggested that the richness pattern, observed in the southern peninsulas, could have also derived from multiple refugia that would allow distinct populations to differentiate in allopatry ("refugia-within-refugia" scenario, Gomez & Lunt 2006) with or without subsequent secondary admixture among lineages (Canestrelli & Nascetii 2008, Canestrelli *et al.* 2010). This multiple refugia scenario has been supported in various studies that showed strong differentiation among populations from the putative refugia range (e.g. Santucci *et al.* 1996, Podnar *et al.*2005, Canestrelli & Nascetti 2008). This scenario also entails that genetic diversity arises from microevolutionary processes other than the prolonged stability of populations in these refugia. These microevolutionary processes could have acted simultaneously with the allopatric differentiation or irrespectively of it. Local adaptations in special environmental/ecological conditions, natural barriers, bottlenecks during migrations and recolonizations or stochastic processes, like genetic drift, could have contributed to the presently observed genetic diversity.

Mediterranean islands constitute a very interesting setting for investigating the evolutionary consequences of Pleistocene climate oscillations because, in islands, climatic fluctuations could have been milder, thus allowing the survival of most insular biota. In addition, sea level fluctuations led to repeated cycles of land bridge formations which resulted to the recurrent merging and separation of islands, affecting the evolutionary history of species and their genetic structure (Canestrelli & Nascetti 2008, Bisconti *et al.* 2011, Salvi *et al.* 2014). Sea level fluctuations also resulted in great changes in species ranges; species could occupy a single island during glacial periods and multiple islands during interglacial periods, when, due to the rise of sea level, the islands would become fragmented thus creating a physical barrier to gene flow (surrounding sea) isolating populations from each other.

Among taxa from temperate areas the lizard genus *Podarcis,* Wagler 1830, constitutes an interesting case study. *Podarcis* wall lizards are locally abundant and widespread in Europe with many species restricted to the Mediterranean area that are characterized by high genetic variation both at the intra- and at the inter- specific level (Arnold & Ovenden 2002). Populations of the Mediterranean islands are particularly interesting to investigate evolutionary processes (see General introduction) due to the role of islands as key hotspots and glacial refugia for biodiversity (Medail & Quézel 1999, Medail 2009). *P. wagleriana* Gistel 1868, as an insular species, therefore represents an ideal model to investigate the processes that have led to present day variation: it is strictly endemic to Sicily, where its distribution range encompasses the entire island except for the NE part, and is also present in its satellite islands, including the Egadi (Levanzo, Favignana, and Maretitmo) and the Stagnone islands, and it is locally abundant. Very few studies have investigated geographic variation in *P. wagleriana*. Capula 1994b used allozymes to pinpoint some genetic differentiation between populations from Sicily and those from the satellite islands (Egadi Islands). On the other hand, Capula 1994b used allozyme electrophoresis in an effort to investigate the evolutionary relationship between *P. wagleriana*, *P. sicula, P. raffonei* and *P. filfolensis*. His results indicated a closer relationship between *P. wagleriana* and *P. raffonei* despite the apparent morphological similarity between *P. walgeriana* and *P. filfolensis*. However, no more extensive work has been done on this species.

1.2. OBJECTIVES

The main aim of this first part of the thesis was to study the genetic diversity and determine phylogeographic patterns within *P.wagleriana*, in an effort to enhance the limited knowledge that we have for this endemic species. In particular, the main objectives were: 1) to assess genetic variation and divergence across the species' distribution range based on a sampling of individuals from different localities, by using mtDNA markers in an explicit geographical framework; 2) to infer possible past evolutionary and demographic processes responsible for the observed phylogeographic pattern 3) assess the concordance of our results with the (EC) expansion-contraction model which predicts demographic/spatial contraction during glacials and long-term demographic stability in glacial refugia as the main cause of high levels of intraspecific diversity.

1.3. MATERIALS AND METHODS

1.3.1. Sampling and laboratory procedures

For the genetic analysis 92 specimens were used, belonging to 24 different localities across the distribution range of the species, from the main island of Sicily and two of the Egadi Islands, Marettimo and Favignana. Detailed information about the specimens and the localities is presented in figure 1.1 and table 1.1.

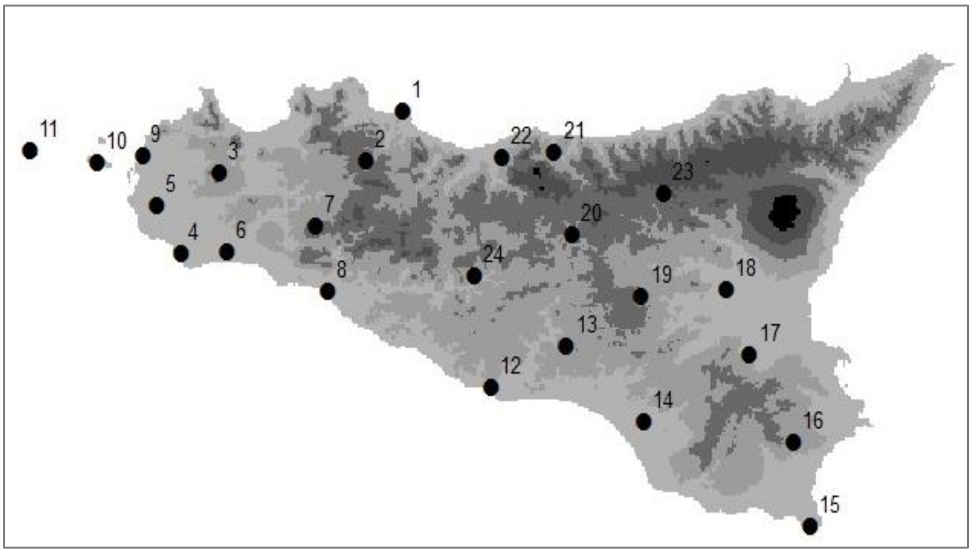


Figure 1.1: Map presenting the sampling localities in the main island of Sicily and the Egadi Islands (Marettimo and Favignana).

Table 1.1: Geographic locations, sample size and frequencies of the haplotypes found for the populations of *P.wagleriana*.

Name	Locality	Altitude (m)	Coordinates	Number of samples	Haplotypes
1	Monte Catalfano	290	38° 6' N 13° 30' E	5	h1(4), h2(1)
2	Luisa	542	37° 55' N 13° 22' E	6	h1(1), h3(4), h4(1)
3	Vita	429	37° 53' N 12° 48' E	1	h5(1)
4	Stazione di Santa Nicola di Mazara	34	37° 36' N 12° 38' E	4	h3(2), h6(1), h7(1),
5	Ciavolo	112	37° 46' N 12° 33' E	1	h3(1)
6	Santa Tereza	68	37° 37' N 12° 49' E	5	h3(2), h8(2), h9(1),
7	Abbazia di Santa	803	37° 42' N 13° 10' E	1	h10(1)

	Maria del Bosco				
8	Piana Grande	43	37° 28' N 13° 13' E	6	h11(2), h12(1), h13(1), h14(1), h15(1)
9	Arcieri-Isolotto	5	37° 57' N 12° 29' E	4	h3(2), h16(1), h17(1)
10	Favignana	7	37° 55' N 12° 19' E	3	h18(1), h19(1), h20(1)
11	Marettimo	7	37° 57' N 12° 4' E	9	h21(1), h22(2), h23(1), h24(2), h25(1), h26(1), h27(1)
12	Torre di Gaffe	46	37° 8' N 13° 51' E	5	h28(1), h29(1), h30(1), h31(1), h32(1)
13	Le Schiette	410	37° 17' N 14° 9' E	5	h33(1), h34(2), h35(1), h36(1)
14	Siriac Srl	60	37° 1' N 14° 27' E	4	h37(1), h38(1), h39(1), h40(1)
15	Maucini	14	36° 40' N 15° 5' E	6	h41(4), h42(1), h43(1)
16	Pieta San Giovanello	432	36° 57' N 15° 1'	3	h43(1), h44(1), h45(1)
17	Castagna	159	36° 15' N 14° 51' E	4	h36(1), h46(1), h47(1), h48(1), h49(1)
18	Sferro	65	37° 29' N 14° 46' E	2	h36(1), h50(1)
19	Cunazzo	538	37° 27'N 14° 26' E	4	h36(1), h47(1), h51(1), h52(1)
20	Alleri	419	37° 40' N 14° 10' E	2	h36(1), h53(1)
21	Castelbuono	236	37° 57' N 14° 6' E	3	h53(1), h54(2)
22	Sant'Agata	442	37° 56' N 13° 53' E	2	h53(1), h55(1)
23	Cerami	990	37° 49' N 14° 31' E	2	h34(1), h56(1)
24	Cozzo Reina	214	37° 33' N 13° 47' E	4	h36(1), h57(1), h58(1), h59(1)

All of the specimens were captured by noose in the field and a small piece of their tail was preserved in 96% alcohol. Fieldwork was carried out in Sicily during April 2014 and in the Egadi Islands during summer 2013. Total genomic DNA was extracted using a high-salt extraction method (Sambrook *et al.*1989). Two mitochondrial fragments that have already been used successfully for assessing variation within other lacertid lizards (Salvi *et al.*2010, Barata *et al.* 2012) were amplified using the polymerase chain reaction (PCR). The first fragment was 744 bp of the NADH dehydrogenase subunit 4 (nd4) gene and its flanking TRNAs Serine (tRNA^{Ser}) and Histidine (tRNA^{Hist}) using the primers ND4 and LEU (Arevalo *et al*. 1994). Amplifications were conducted in 25 µl volumes containing reaction buffer (4µl), Mg (0.5 µl), BSA (0.5 µl), the two primers (0.5 µl from each), the enzyme taq polymerase (0.1 µl) and 1 µl of DNA template. The second fragment of 979 bp of the cytochrome b (cytb) gene was amplified using primers CB1 (Kocher *et al.* 1989) and THR8 (Sprinks *et al.* 2004). Amplifications were conducted also in 25 µl volumes containing reaction buffer (5 µl), Mg (2 µl), dntps (0.5 µl), the two primers (0.5 µl of each), the enzyme polymerase Gotaq (0.2 µl) and 1 µl of DNA template. The amplification conditions are presented to the table 1.2. The PCR products were sent to external company for sequencing (Beckman Coulter Genomics).

Table 1.2: PCR cycling conditions and information on the primers used.

GENE	ND4			CytB		
Step	T (^{0} C)	TIME	X	T (^{0} C)	TIME	X
Initial	94	3'	1	94	3'	1
Denaturation	94	30''	34	94	30''	36
Annealing	49	45''		49	30''	
Extension	72	50''		72	50''	
Final Extension	72	10'	1	72	10'	
Primer Forward	ND4			CB1		
Primer Reverse	LEU			THR8		
Citation	Arevalo *et al.* 1994			Kocher *et al.* 1989, Sprinks *et al.* 2004		

1.3.2. Data analysis

Phylogenetic analysis, population genetic structure and molecular dating

Chromatographs were checked and the sequences were aligned in Geneious v5.6 (www.geneious.com) using the Geneious alignment algorithm. We used DnasP v.5.10.01 (Librado *et al.* 2009) in order to estimate the following summary statistics of genetic diversity for the concatenated dataset: nucleotide diversity, π, number of haplotypes, H, and haplotype diversity Hd.

We inferred the genealogical relationships between haplotypes using a median-joining network approach, as implemented in the software network (www.fluxus-engineering.com) .The network approach can be considered as the most appropriate when examining intraspecific gene evolution, particularly when few characters for phylogenetic analysis are available due to swallow divergence (Posada &Crandall 2001).

To define the population genetic structure of *P. wagleriana*, we performed a spatial analysis of molecular variance (SAMOVA) as implement in SAMOVA 2.0 (Dupanloup *et al.* 2002). SAMOVA analysis is used to define groups that are geographically homogeneous and maximally differentiated from each other without any prior assumption of population grouping. In order to estimate which evolutionary model is best fitting the concatenated dataset, we used jmodelTest2 (Posada & Crandall 1998) and the the Akaike's information criterion (AIC), for, testing 88 models. The best model chosen for the concatenated set of genes was the Hasegawa- Kishino-Yano (HKY) with a proportion of irreversible sites (p-inv=0.846) (Hasegawa *et.al* 1985). Based on this information, the Tamura- Nei model was used in SAMOVA as it was the closest model in the HKY that was implemented in SAMOVA2 software. The program run for 100 random initial conditions, test for grouping options varying from K=2 until K=23. The same software provided also the relative fixation indices for each grouping option, the F_{CT} (differentiation within groups), F_{SC} (differentiation among groups), and the F_{ST} value that is indicating the overall genetic distance between groups.

Based on the haplogroups identified by phylogenetic analysis we calculated the pairwise differences (p-distances) within each haplogroup, as well as between haplogroups using MEGA5 (Tamura *et al*. 2011), under the Tamura-Nei evolutionary model. Finally, we used MEGA 5 to produce a matrix of the net genetic distances between the different sampling localities (Tamura –Nei model) in order to perform mantel test comparing them with a matrix of morphological distances (see chapter 2).

Bayesian analysis were carried out using the BEAST v1.7.4 package in order to: 1) estimate a phylogenetic tree of the concatenated dataset based on Bayesian inference and 2) to obtain time estimates of the most recent common ancestor (TMRCA) of *P.wagleriana* and the clades detected within the species. As outgroup for the phylogenetic tree we chose the closely related species *P.filfolensis* (Harris *et al.* 2005). We implemented a model with a two-partition scheme defined by the specific model selected by Jmodel of the two gene fragments. The best evolutionary model was the HKY+ G (gamma shape=0.434) for the nd4 fragment and the HKY+ I (p-inv=0.646) for the cytb fragment. Each model was then partitioned into two codon positions ((1+2), 3).To infer the TMRCAs we assumed a relaxed lognormal clock for both genes. As calibration point we used the time of separation between the Sicilian landmass from the Maltese archipelago (Pedley & Clark 2002) that presumably led to the separation of *P.wagleriana* from *P.filfolensis*. This vicariant event took place 5.33 million years ago (Mya), after the reopening of the Gibraltar strait (see general introduction). We assumed a normal prior distribution for the tree root with mean 5.33 Mya and 95% of the probability distribution deviating by 0.01 Mya. In the case of nd4 as divergence rate we assumed 2.26% per million years which is the average between previously suggested rates for *Podarcis* (Pinho *et al.* 2007). We defined a normal prior distribution on the mutation rate with mean 1.15% per million years and 95% of the probability distribution ranging between the limits 0.0087 and 0.0139. Lastly, a constant population size was assumed under the coalescent model. BEAST analysis ran with 50 million iterations, sampling every 5000 steps. TRACER v1.5 (http://beast.bio.ed.ac.uk/TRACER) was then used to visualize the results and assess whether the estimated parameters were satisfactory. The initial 25% of the trees were discarded as burn-in.

Historical demography

Past demographic changes of the main haplogroups found previously were investigated using a mismatch distribution analysis (Rogers & Harpending 1992) in the software Arlequin 3.5.1.2 (Excoffier *et al.* 2005). In this analysis the observed distribution of nucleotide differences between haplotype pairs (mismatch distribution) is compared with the number expected under a model of demographic expansion (Rogers & Harpending 1992). We used the sum of squared deviations between the observed and estimated mismatch distributions as goodness-of-fit statistics; its significance was assessed using 1000 bootstrap replicates. Furthermore, we estimated the time of expansion as the time in generation t, given the parameter τ and the mutation rate per sequence and per generation u (τ=2ut). We used the substiution rate of the mitochondrial fragment (1.15%) considering the length of the sequence (1723 bp) and a mean generation time of 2.09 years for *Podarcis* (Barbault & Mu 1988, Galán 1996; 1999, Adamopoulou & Valakos 2000). Secondly, neutrality tests were performed using DnasP v.5.10.01 (Librado *et al.* 2009) to assess past demographic changes. Specifically, we calculated Tajima's D (Tajima 1989), the Fu's (Fu 1997) and R^2 statistics (Ramos- Onsins & Rozas 2002). Negative values of Tajima's D, high, significant and negative values of Fs and small, positive, significant values of R^2 are all indicative of past demographic expansions (Tajima 1989, Fu 1997, Ramos-Onsins & Rozas 2002). The significance of the neutrality indices was assessed through 1000 coalescent simulations under the hypothesis of population equilibrium and selective neutrality.

1.3. RESULTS

In the total of 92 concatenated sequences (1723 bp) we found 59 different haplotypes (hd= 0.976, π=0.003). Table 1.3 provides a summary of the estimates of number of haplotypes (h), haplotypic diversity (hd) and nucleotide diversity (π), for each sampling locality.

Table 1.3: number of haplotypes (H), haplotypic diversity (hd) and nucleotic diversity (π) for the different sampling localities. Sampling localities with only one individual are not presented.

POP	H	Hd (SD)	π ($x10^3$)
1	2	0.40 (0.23)	1
2	3	0.60 (0.21)	4
4	3	0.83 (0.22)	0.8
6	3	0.80 (0.16)	1
8	5	0.93 (0.12)	3
9	3	0.83 (0.22)	0.8
10	3	1.00 (0.27)	1
11	7	0.94 (0.07)	2
12	4	1.00 (0.12)	1
13	4	0.90 (0.16)	1
14	4	1.00 (0.17)	2
15	3	0.60 (0.21)	1
16	3	1.00 (0.27)	1
17	5	1.00 (0.12)	1
18	2	1.00 (0.50)	1
19	4	1.00 (0.17)	1
20	2	1.00 (0.50)	0.5
21	2	0.66(0.31)	0.7
22	2	1.00(0.50)	1
23	2	1.00 (0.50)	1
24	4	1.00 (0.17)	1

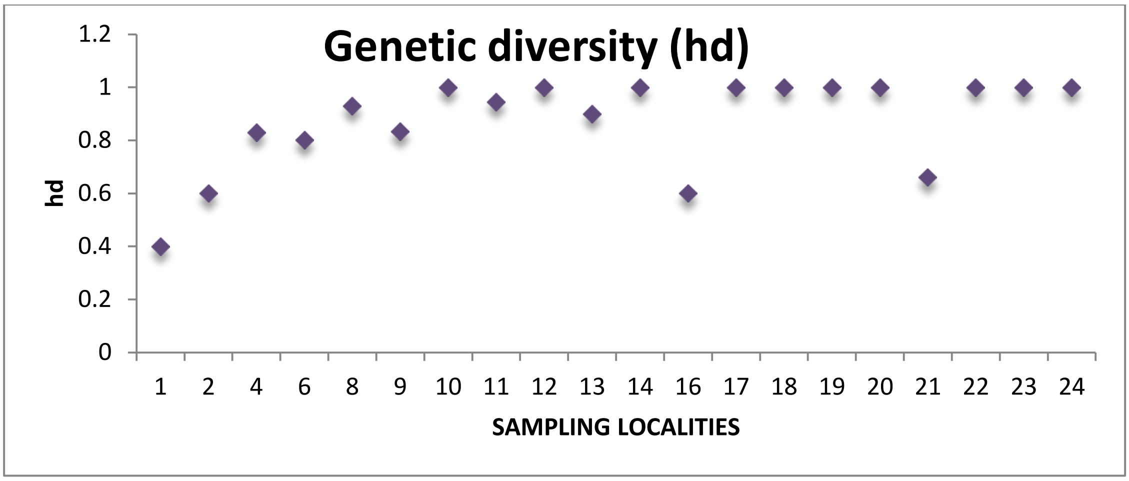


Figure 1.2: Genetic diversity (hd) for each sampling location. Sampling localities with only one individual are not presented.

The phylogenetic relationships between haplotypes, as inferred by the median-joining approach, revealed high haplotypic diversity (59 haplotypes) but low genetic divergence. The network showed a clear phylogeographic structure with 59 haplotypes were clustered in 3 main clades associated to three geographical areas: a clade including the eastern populations (clade E, purple color, figure 1.3); a clade of the western populations (clade W, blue color, figure 1.3) that includes the islets Favignana and Marettimo which appear as subgroup of the second clade (lighter blue color, figure 1.3); and a much more distinct clade of the northern population of Monte Catalfano (clade N, green color, figure 1.3) that is separated from the others by 8 mutational steps. Overall, haplotypes within clades are connected by a maximum of three mutational steps while a maximum of eight mutational steps separates the clades from each other.

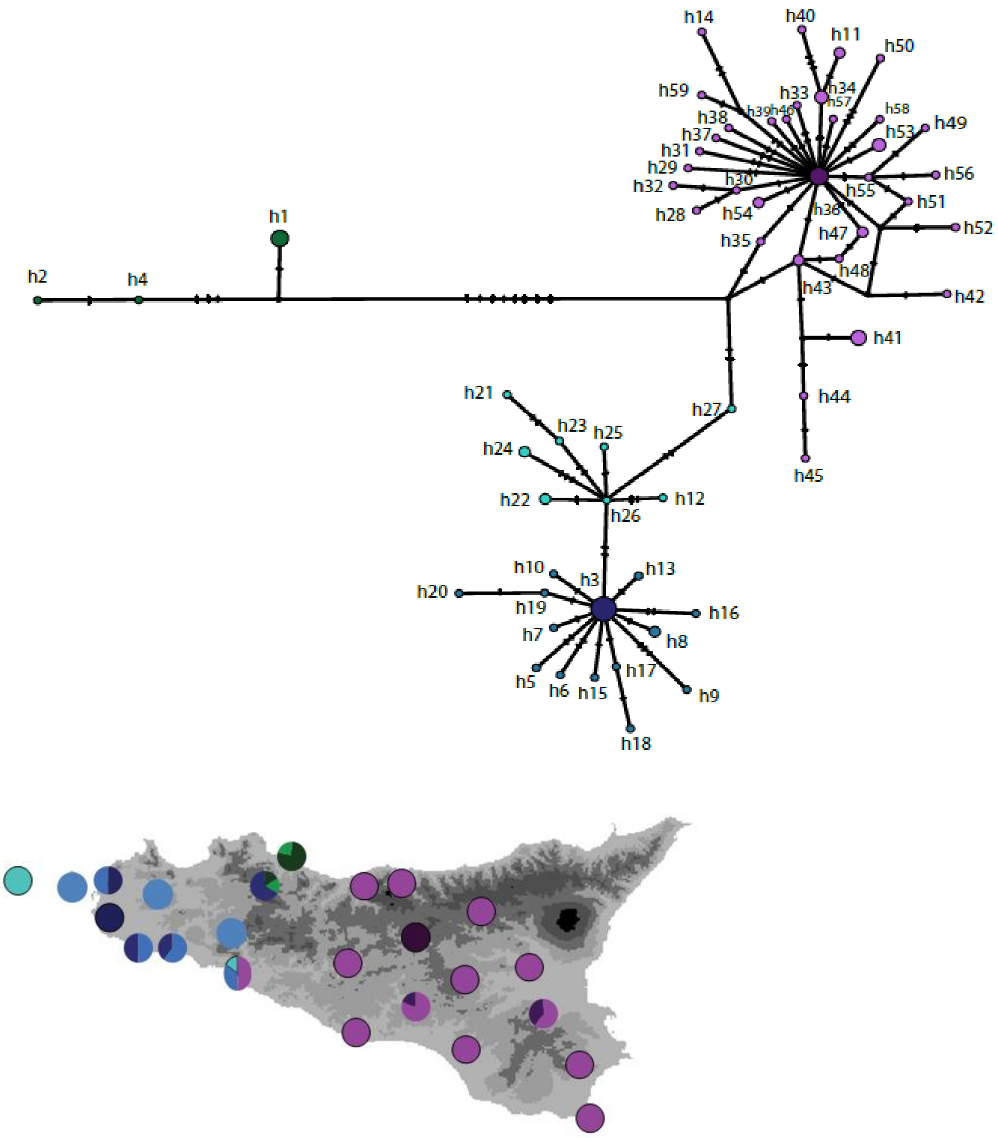
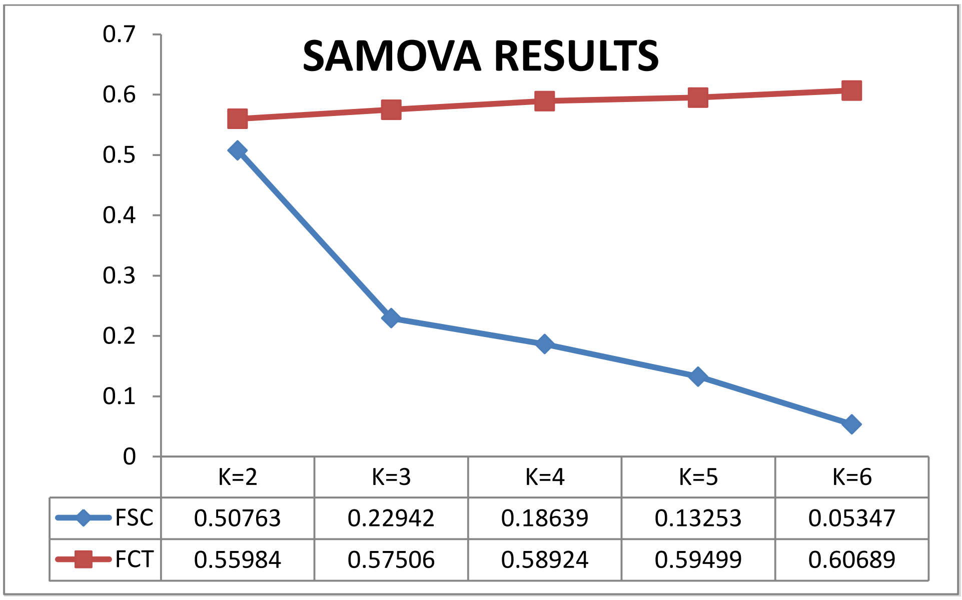


Figure 1.3: Genealogical relationships between haplotypes of the concatenated dataset of *P.wagleriana* and their geographical distribution. In the median joining network cycles represent haplotypes and their size is proportional to their frequencies. The pies of the map represent the frequency of haplotypes belonging to distinct haplogroups observed in each sampling location.

The best partitioning of genetic diversity by SAMOVA was obtained when samples were divided in three groups (K=3), which best explained the among group spatial partitioning of molecular variance (figure 1.4). Values higher than K=6 showed either non-significant or negative F_{SC} values. For the first six K grouping options F_{CT} values were quite uniform, but only for K=3 the F_{SC} value showed a dramatic decrease when comparing with the previous grouping options, thus it was considered as the most informative option. The three-group genetic structure explains 57.51% of total molecular variance (F_{CT}=0.575, P<0.05). Of this, 9.75% was explained by differences among populations but within groups (F_{SC}=0.229, P<0.05), and 32.75% was explained by differences within populations (F_{ST}=0.672, P<0.05). The partitioning of populations into three groups is shown in figure 1.5. The three-group genetic structure identified by SAMOVA analysis reflects the geographic partition of haplotype groups as identified by the network analysis.



	K=2	K=3	K=4	K=5	K=6
FSC	0.50763	0.22942	0.18639	0.13253	0.05347
FCT	0.55984	0.57506	0.58924	0.59499	0.60689

Figure 1.4: Summary of results of spatial analysis of mitochondrial molecular variance (mtDNA SAMOVA). For each predefined value of K (K=2 until K=6) the relative fixation indices, F_{SC} and F_{CT}, are shown. In the graph are shown the K values for which both fixation indices have p<0.05.

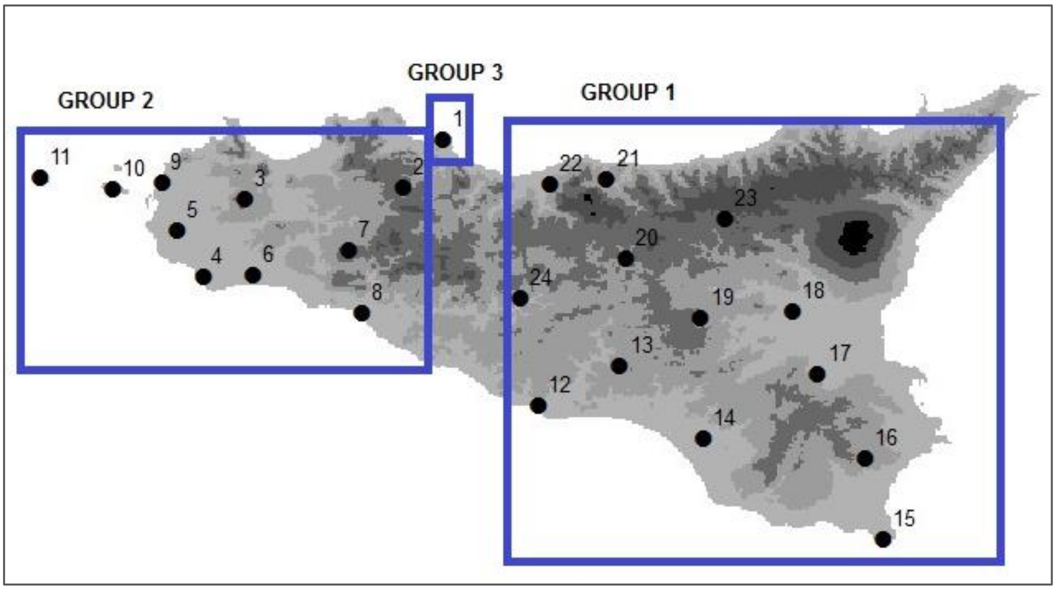


Figure 1.5: Map showing the partitioning of the populations in three groups based on the results obtained by SAMOVA2.

In the table 1.4 we can see the average genetic distances between the three haplogroups as well as within each one of them based on the results obtained in MEGA.

Table1.4: Average genetic distance between and within the three haplogroups based on Tamura-Nei (1993) distances.

	1	2	3
1	0.002		
2	0.005	0.003	
3	0.008	0.009	0.003

The time of split between *P.wagleriana* and *P.filfolensis* was defined as being at 5.33 Mya, coinciding with the Miocene-Pliocene boundary, while the MCRA of *P.wagleriana* was estimated at 0.37 Mya. The TMRCA of each clade was estimated within the Middle Pleistocene period (figure 1.6). Exact date estimates and their 95% HPD levels are shown in table 1.5.

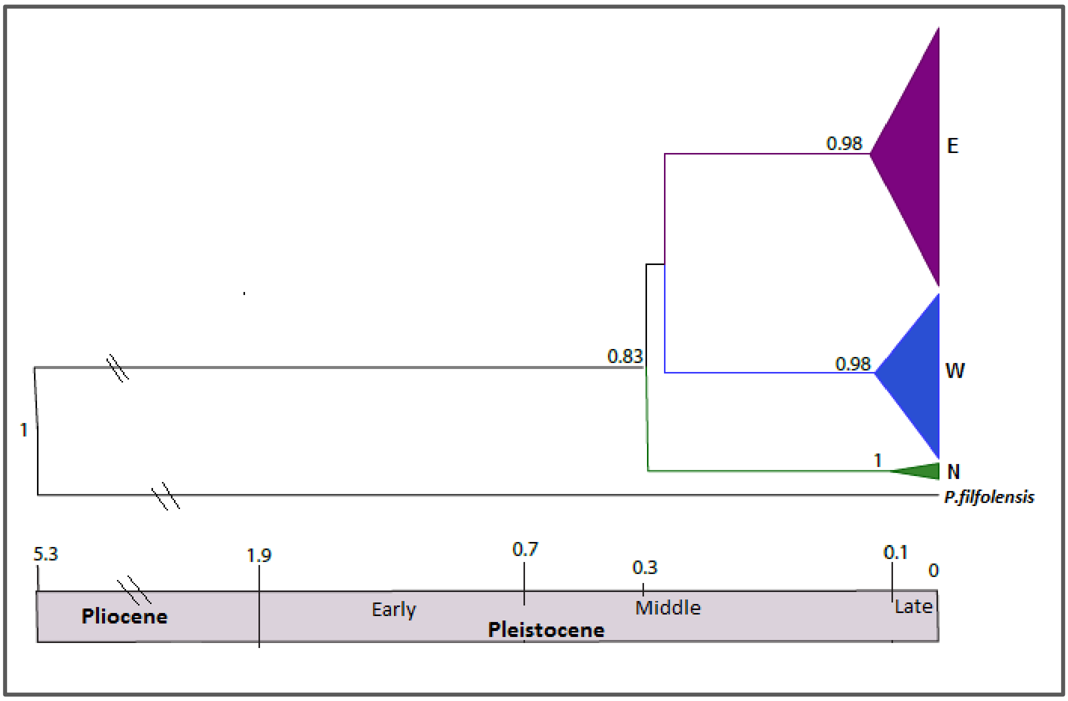


Figure 1.6: Chronogram showing the estimates of time to the most recent common ancestor (TMCRA) for the three mtDNA clades of *P.wagleriana* (E: eastern clade, W: western clade, N: northern clade). Terminal haplogroups were collapsed. The main historical epochs presented in the scale bar in million years. Nodal support for Bayesian posterior probabilities (BA) are represented for each of the main clades of the chronogram.

Table 1.5: Time of the most recent common (TMCRA) in million years for each clade and their 95% highest posterior density (HPD).

CLADE	TMCRA (years)	95% HPD(lower-upper)
Ingroup	370,000	0.2035 - 0.5631
E	182,000	0.0097 - 0.2773
W	178,000	0.0088 - 0.2853
N	100,000	0.0013 - 0.1196

Results from the mismatch distribution analysis and the values of demographic summary statistics for the two main haplogroups tested (western and eastern clades) are presented in figure 1.7. The northern clade was excluded from the analysis because of its low number of individuals. The observed mismatch distributions for both haplogroups were unimodal and not significantly different from those expected under a demographic expansion model (western clade: SSD= 0.00154, p=0.94; eastern clade: SSD=0.0078, p=0.07). Tajima's D, Fs and R^2 values were always statistically significant and their signs and magnitude were indicative of population expansion. The estimates of the time since the demographic expansion of both clades, based on the parameter τ, were not informative due to the large confidence intervals obtained (W clade: 221 kya; confidence intervals: 214 Kya- 1.6 Mya, E clade: 384 Kya; confidence intervals: 213 Kya- 530 Kya).

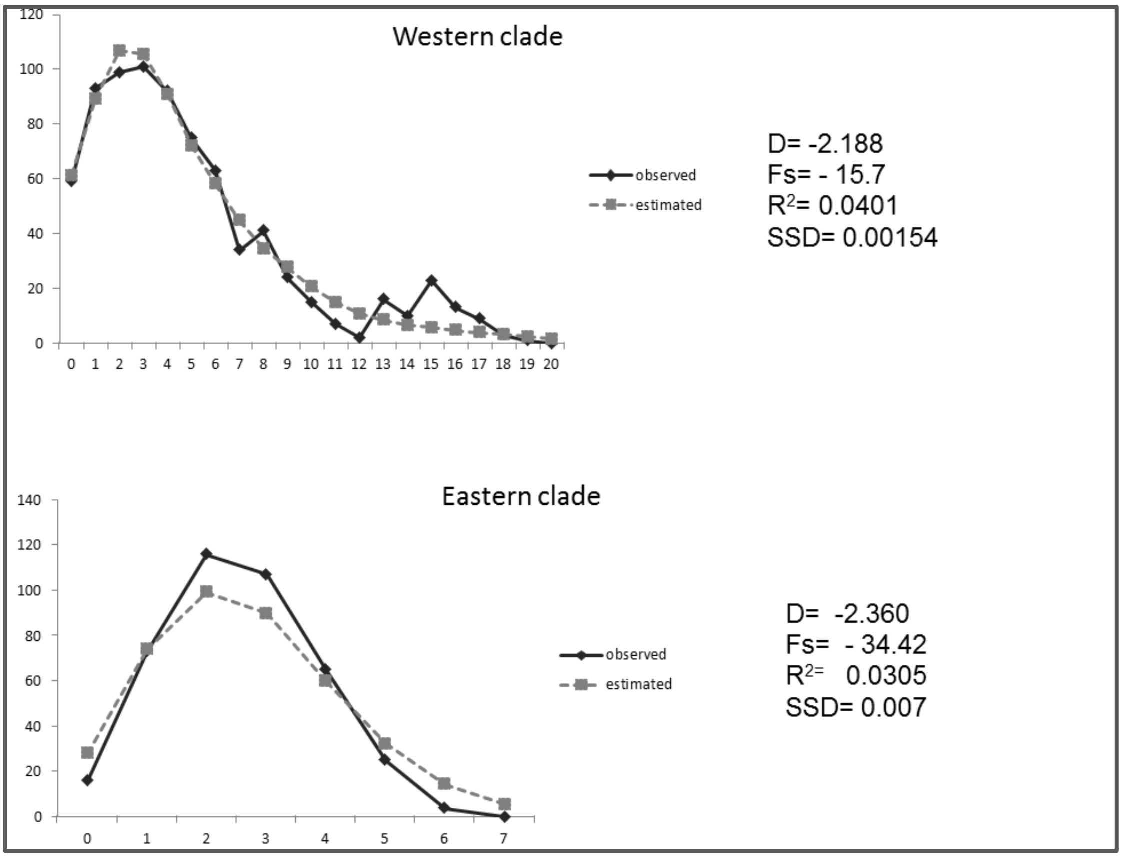


Figure 1.7: Mismatch distribution analysis and values of the demographic summary statistics for the western and eastern clade of *P.wagleriana*. Solid lines represented the observed distributions of pairwise differences; dotted lines represent the expected pairwise differences under a model of demographic expansion. SSD= sum of square deviations for the goodness-of-fit between observed and estimated mismatch distributions under a demographic expansion model. D= Tajima D statistics, F_S= Fu's statistic, R^2= Ramos-Onsin and Roza's test.

1.5. DISCUSSION

The phylogenetic network and the spatial analysis of molecular variance indicated a genetic structure with high intraspecific haplotypic diversity (59 haplotypes) but low genetic divergence. The haplotypic diversity is high (>0.5) in most of sampling populations, especially in most eastern populations (see table 1.3 and figure 1.1) where the haplotypic diversity is equal to 1 (hd =1). On the other hand, nucleotide diversity is relatively low in all cases (Fig.1.2). The low genetic divergence was also supported by the results of the phylogenetic network where the haplotype groups were differentiated from each other by a maximum of 8 mutational steps.

Podarcis wagleriana shows a clear phylogeographic structure with three distinct mtDNA clades*.* An eastern clade (E), including 13 sampling localities, a western clade (W) with 10 sampling localities, including the two islets of Marettimo and Favignana, and a third northern clade represented by one single sampling locality (N), Monte Catalfano (locality 1; table 1.1). Additionally, the phylogenetic network indicated a possible subgrouping within the eastern clade, where the islet of Marettimo appears to form a quite distinct subgroup within (figure 1.3; color light blue). Finally, two sampling localities (2, 8) were found to be admixed, carrying haplotypes from different clades (see map, fig 1.3).

The TMCRA of each clade falls into the Late Pleistocene period indicating that their current observed genetic diversity had a relatively recent evolutionary origin and probably explaining the low haplotypic divergence that we observed between the three clades. These three clades likely diverged from each other during the Middle Pleistocene (~370 Kya; figure 1.6). The phylogenetic pattern suggests that the splits between the clades occurred in the same time frame, likely due to the paleo-environmental conditions of Middle Pleistocene that may have triggered the fragmentation of the species into three geographically distinct clades.The transition between the Early and Middle Pleistocene (1.2-0.5 Mya; Head & Gibbard 2005) is also know as the “mid-Pleistocene revolution” (Berger & Jansen 1994) and it was characterized by an increase of the amplitude in the rhythm of climatic oscillations. This phsase was also characterized by an increase in global ice volume accompanied by cooler and drier atmospheric condtions with profound effects on species and paleo-landscapes, especially in the northern hemisphere biota (Head & Gibbart 2005, Hewitt 2011). It is also already known that the climatic changes during the Middle Pleistocene, namely the extreme aridification, led to substantial changes in the distribution and assemblages of various temperate species, as shown by pollen spectra and fossil data (Suc 1984, Bertini 2003, Kostopoulos *et al.* 2007, Kahlke *et al.* 2011).

Within Mediterranean islands climatic changes were likely attenuated during the Quartenary providing suitable habitats for the prolonged survival of populations in refugia throughout the glacial/interglacial cycles (Hewitt 2000, Medail & Diadema 2009). In our case, the phylogeographic pattern observed with three allopatric clades having a Pleistocene divergence suggests a long-term isolation into three distinct refugia. Therefore, the geographical distribution of the three lineages in Sicily is likely to reflect the refugial structure and the putative areas of genetic differentiation, with one refugium in the West (clade W; figure 1.6), one in the East (clade E; figure 1.6) and one in the North (clade N; figure 1.6). Given the lack of obvious geographical barriers across the west-east axis of the islands, a plausible scenario to explain the reduced gene flow between populations from these refugia could involve insuitable ecological condtions at mid altitudes in central-north Sicily during Middle-Late Pleistocene. On the other hand, the eastern edge of the E clade is delimited by Mount Etna that probably has acted as a strong barrier preventing further expansion of the species to the north-eastern part of the island. Etna, with a coverage of 1190 Km^{2,}, is the highest point of Sicily (3350 m), and harboring extreme climate conditions seems a hostile environment for a small reptile to cross.

A multiple refugia scenario has also been suggested for many continental species showing strong diversification among populations from putative refugia. (e.g. Santucci *et al.* 1996, Podnar *et al.*2005, Canestrelli & Nascetti 2008) and has recently pointed out also in several island species' populations (Salvi *et al.* 2010; 2014, Bisconti *et al.* 2013). A major consequence of this “refugia within refugia” scenario (Gómez & Lunt 2006) is that the genetic diversity found within the refugial areas could not have arisen only due to prolonged stability of populations within these areas but also due to microevolutionary processes, encompassing allopatric differentiation, post-glacial range expansions, secondary contacts and admixture among populations differentiated in separate refugia (Canestrelli *et al.* 2010, Salvi *et al.* 2014). The evidence from the demographic analyses (figure 1.7) suggests that both the W and the E clade underwent demographic expansion.of populations both from the W and E clade possibly occurred. The historical demography of the E clade clearly indicated a signal of demographic expansion.

Following the demographic expansion, secondary contacts between the E and W clades could explain the presence of an admixed population (8) that was found right in the “border” between them (figure 1.3). Following demographic expansion, secondary contacts between the N and W clades could also account for the admixed population 2 (figure 1.3). Secondary contact zones have been identified in several temperate species in midpoints between glacial refugia (Anderson 1949, Remington 1968, Swenson 2005) which seems to be the case for the admixed sampling localities 8 and 1 of our study. Secondary contact zones are considered hotspots of divergence and evolutionary potential (Canestrelli *et al.* 2012), thus they deserve further investigation.

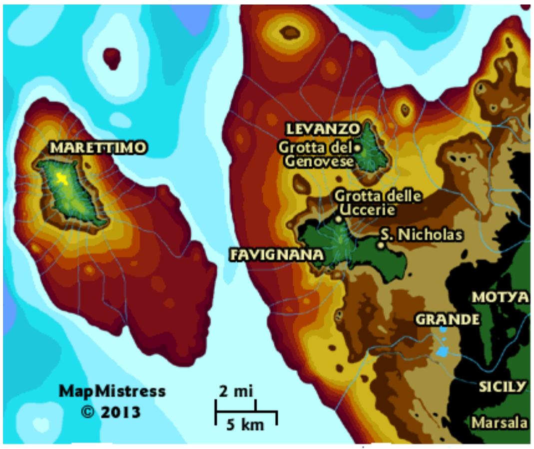


Figure 1.8: Position of the Egadi Islands during the Last Glacial Maximum (LGM). Photo downloaded from www.mapmistress.blog.com (accessed on June 2015)

To explain the subgroup of haplotypes of Marettimo and their position on the phylogenetic network, we need to consider the past connection of Egadi Islands with Sicily due to Pleistocene sea-level changes. During the Quartenary Sicily's geography was greatly impacted by, sea level fluctuations which may have played a crucial role shaping the diversity of Sicilian biota. Already during the middle Pleistocene the sea level had risen sufficiently, to separate the Marettimo islet from the main island of Sicily, and therefore act as a barrier to gene flow between the islet and the main island. However, the rest of the Egadi Islands were still attached to Sicily (Figure 1.8) even during the Last Glacial Maximum (20 Kya). Consequently, populations already inhabiting Marettimo islet were isolated and thus prone to allopatric divergence and diversification and more affected by evolutionary processes like increased genetic drift. This scenario may explain the presence of an endemic haplogroup of Marettimo in the network where it appears to form a quite separated subgroup within the W clade.

Sea-level changes during the glacial/interglacial periods may have also affected the timing of the demographic expansion observed in the clade E and W. While, according to the expansion-contraction model (EC) we expect temperate species to experience demographic/spatial contraction in refugia during glacials and expansions during interglacials, recent studies on temperate species from coastal areas and islands suggest an opposite scenario. For instance, demographic expansion during glacial periods have been inferred for the Maltese wall lizard *Podarcis filfolensis* (Salvi *et al.* 2014*)* and the tree frog *Hyla sarda* (Bisconti *et al*. 2011) endemic to Malta and the Sardinia and Corsica islands (see also Canestrelli *et al*. 2007; Canestrelli & Nascetti 2008). In these islands the climate conditions were milder and most likely habitat availability increased due to glacio-eustatic sea level lowering during glacials (Thiede 1978, Rholing *et al.* 1998). Consequently, the hypothesis of glacial expansion, during sea-level low-stands, would be equally plausible for an insular species like *P.wagleriana*. Additional nuclear data and multilocus demographical tools such as the Extended Bayesian Skyline analysis, can be used in the future to obtain an accurate estimate of the time of the expansions and clarify whether the species followed the pattern of other coastal temperate species or it conformed to the EC model predictions.

Finally, the EC model predicts prolonged stability in areas that acted as refugia during glacials as the main source of intraspecific diversity. In the case of *P. wagleriana*, the genetic diversity we observed likely survived in three distinct refugia and allopatric divergence between populations inhabiting distinct refugia was a major force shaping the genetic structure of the species. Additionally, secondary contacts following expansions of populations from these refugia further contributed to the observed present day diversity of populations of central Sicily. Therefore, the intraspecific diversity of *P. wagleriana,* rather than a direct outcome of the prolonged stability of a large population in a main refugia suggest a more complex scenario of isolation and divergence in multiple refugia and secondary contacts: a pattern already inferred for many species in the southern Mediterranean peninsulas (e.g. Martinez-Solano *et al.* 2006, Sotiropoulos *et al.* 2007, Ursenbancher *et al*. 2008, Vega *et al.* 2010, Canestrelli *et al.* 2012, Salvi *et al.* 2013).

In the future, the use of nuclear markers combined with the mitochondrial ones can give a more complete image of the overall variation as shown by an increased number of case studies that combine the two markers to investigate patterns of variation between populations or closely related species (Monsen & Bluin 2003, Pinho *et al.* 2007, Rato *et al.* 2010, Prado *et al.* 2012). Furthermore, ecological niche models could also be applied to test whether past ecological conditions can account for the diversification of each clade as well as predict responses to possible future climatic scenarios.

1.6. REFERENCES

Adamopoulou, C., Valakos, E. D., (2000). **Small clutch size in a Mediterranean endemic lacertid (*Podarcis milensis*)**. Copeia, 2000(2), 610-614.

Anderson, E., (1949). **Introgressive hybridization**. Wiley, New York.

Arevalo, E., Davis, S. K., Sites, J. W., (1994). **Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico.** Systematic Biology, 43, 387-418.

Arnold, E. N., Ovenden, D., (2002). **Reptiles and amphibians of Britain and Europe.** Hong-Kong: HarperCollins Publishers.

Avise, J. C., (2000). **Phylogeography: the history and formation of species**. Harvard University Press, Cambridge, MA.

Avice, J. C., (2009). **Phylogeography: Retrospect and prospect.** Journal of Biogeography, 36, 3-15.

Avise, J. C., Arnold, J., Ball, R. M., Jr., Berminham, E., Lamb, T., Neigel, J. E., Reeb C. A., Saunders, N. C., (1987). **Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics.** Annual Review of Ecology and Systematics, 18, 489-522.

Barata, M., Carranza, S., Harris, D. J., (2012). **Extreme genetic diversity in the lizard *Atlantolacerta andreanskyi* (Werner, 1929): A montane cryptic species complex.** BMC evolutionary biology, 12(1), 167.

Barbault, R., Mou, Y. P., (1988). **Population dynamics of the common wall lizard, *Podarcis muralis,* in southwestern France**. Herpetologica, 38-47.

Berger, W. H., Jansen, E., (1994)**. Mid-Pleistocene Climate Shift-The Nansen Connection.** The polar oceans and their role in shaping the global environment, 295-311.

Bertini, A., (2003). **Early to Middle Pleistocene changes of the Italian flora and vegetation in the light of a chronostratigraphic framework.** Il Quaternario, 16(1), 19-36.

Bisconti, R., Canestrelli, D., Colangelo, P., Nascetti, G., (2011). **Multiple lines of evidence for demographic and range expansion of a temperate species (*Hyla sarda*) during the last glaciation.** Molecular Ecology, 20(24), 5313-5327.

Bisconti, R., Canestrelli, D., Nascetti, G., Lalueza-Fox, C., (2013). **Has living on islands been so simple? Insights from the insular endemic frog *Discoglossus montalentii*.** PloS one, 8(2), e55735.

Canestrelli, D., Cimmaruta, R., Nascetti, G., (2007). **Phylogeography and historical demography of the Italian treefrog, *Hyla intermedia*, reveals multiple refugia, population expansions and secondary contacts within peninsular Italy.** Molecular Ecology, 16(22), 4808-4821.

Canestrelli, D., Nascetti, G., (2008). **Phylogeography of the pool frog *Rana (Pelophylax) lessonae* in the Italian peninsula and Sicily: multiple refugia, glacial expansions and nuclear–mitochondrial discordance**. Journal of Biogeography, 35(10), 1923-1936.

Canestrelli, D., Aloise, G., Cecchetti, S., Nascetti, G., (2010). **Birth of a hotspot of intraspecific genetic diversity: notes from the underground.** Molecular Ecology, 19(24), 5432-5451.

Canestrelli, D., Salvi, D., Maura, M., Bologna, M. A., Nascetti, G., (2012). **One species, three Pleistocene evolutionary histories: phylogeography of the Italian crested newt, *Triturus carnifex.*** PLoS One, 7(7), e41754.

Capula, M., (1994a). **Evolutionary relationships of *Podarcis* lizards from Sicily and the Maltese Islands.** Journal of zoological Systematics and evolutionary Research, 32(3), 180-192.

Capula, M., (1994b). **Genetic variation and differentiation in the lizard, *Podarcis wagleriana (Reptilia: Lacertidae).*** Biological Journal of the Linnean Society, 52(2), 177-196.

Carnaval, A., Hickerson, M. J., Haddad, C.F.B., Rodrigues, M. T., Moritz, C., (2009). **Stability predicts genetic diversity in the Brazilian Atlantic Forest Hotspot**. Science, 323, 785-789.

Dupanloup, I., Schneider, S., Excoffier, L., (2002). **A simulated annealing approach to define the genetic structure of populations.** Molecular Ecology, 11(12), 2571-2581.

Excoffier, L., Laval, G., Schneider, S., (2005). **Arlequin (version 3.0): an integrated software package for population genetics data analysis**. Evolutionary bioinformatics online, 1, 47.

Fu, Y. X., (1997). **Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection.** Genetics, 147(2), 915-925.

Galán, P., (1996). **Sexual maturity in a population of the lacertid lizard *Podarcis bocagei.*** Herpetological Journal, 6, 87-93.

Galán, P., (1999). **Demography and population dynamics of the lacertid lizard *Podarcis bocagei* in north-west Spain.** Journal of Zoology, 249(02), 203-218.

Gómez, A., Lunt, D. H., (2006). **Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula**. Phylogeography of southern European refugia, 155-188, Springer Netherlands.

Harris, D. J., Pinho, C., Carretero, M. A., Corti, C., Böhme, W., (2005). **Determination of genetic diversity within the insular lizard *Podarcis tiliguerta* using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*.** Amphibia-Reptilia, 26(3), 401-407.

Hasegawa, M., Kishino, H., Yano, T., (1985). **Dating of the human-ape splitting by a molecular clock of mitochondrial DNA**. Journal of Molecular Evolution, 22(2), 160-174.

Head, M. J., Gibbard, P. L., (2005). **Early-Middle Pleistocene transitions: the land-ocean evidence.** Geological Society of London.

Hewitt, G.M., (1996). **Some genetic consequences of ice ages, and their role in divergence and speciation**. Biological Journal of the Linnean Society, 58, 247-276.

Hewitt, G.M., (2000). **The genetic legacy of the Quaternary ice ages.** Nature, 405, 907-913.

Hewitt, G.M., (2004). **Genetic consequences of climatic oscillations in the Qauternary.** Transactions of the Royal Society of London B., 359, 183-195.

Hickerson, M. J., Dolman, G., Moritz, C., (2006). **Comparative phylogeographic summary statistics for testing simultaneous vicariance across taxon-pairs**. Molecular Ecology, 15, 209-224.

Kahlke, R. D., García, N., Kostopoulos, D. S., Lacombat, F., Lister, A. M., Mazza, P. P.A., Spassov, N., Titov, V. V., (2011). **Western Palaearctic palaeoenvironmental conditions during the Early and early Middle Pleistocene inferred from large mammal communities, and implications for hominin dispersal in Europe**. Quaternary Science Reviews, 30(11), 1368-1395.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., Wilson, A. C., (1989). **Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers**.Proceedings of the National Academy of Sciences, 86(16), 6196-6200.

Kostopoulos, D. S., Palombo, M. R., Alberdi, M. T., Valli, A. M., (2007). **Pliocene to Pleistocene large mammal diversity and turnover in North Mediterranean region: the Greek Peninsula with respect to the Iberian and Italian ones.** Geodiversitas, 29(3), 401-419.

Lemmon, A. R, Lemmon, E. M, (2008). **A likelihood framework for estimating phylogeographic history on a continuous landscape.** Systematic Biology, 57(4), 544-561.

Librado, P., Rozas, J., (2009). **A software for comprehensive analysis of DNA polymorphism data**. Bioinformatics, 25, 1451-1452.

Martinez-Solano, I., Teixeira, J., Buckley, D., Garcia-Paris, M., (2006). **Mitochondrial DNA phylogeography of *Lissotriton boscai* (Caudata, Salamandridae): evidence for old, multiple refugia in an Iberian endemic.** Molecular Ecology, 15(11), 3375-3388.

Médail, F., Quézel, P., (1999). **Biodiversity hotspots in the Mediterranean Basin: setting global conservation priorities**. Conservation biology, 13(6), 1510-1513.

Médail, F., Diadema, K., (2009). **Glacial refugia influence plant diversity patterns in the Mediterranean Basin.** Journal of Biogeography, 36(7), 1333-1345.

Monsen, K. J., Blouin, M. S., (2003). **Genetic structure in a montane ranid frog: restricted gene flow and nuclear–mitochondrial discordance**. Molecular Ecology, 12(12), 3275-3286.

Pedley, M., Clarke, M. H., (2002). **Limestone Isles in a Crystal Sea: the geology of the Maltese Islands.** Publishers Enterprises Group, San Gwann, Malta.

Pinho, C., Harris, D. J., Ferrand, N., (2007). **Contrasting patterns of population subdivision and historical demography in the three western Mediterrenean lizard species inferred from mitochondrial DNA variation**. Molecular Ecology, 16, 1191-1205.

Podnar, M., Mayer, W., Tvrtkovic, N., (2005). **Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences**. Molecular Ecology, 14(2), 575-588.

Prado, C., Haddad, C. F., Zamudio, K. R., (2012). **Cryptic lineages and Pleistocene population expansion in a Brazilian Cerrado frog**. Molecular Ecology, 21(4), 921-941.

Posada, D., Crandall, A., (1998). **MODELTEST: testing the model of DNA substitution**. Bioinformatics, 14, 817-818.

Posada, D., Crandall, A., (2001). **Intraspecific gene genealogies: trees grafting into networks**. Trends in Ecology and Evolution, 16(1), 37-45.

Provan, J., Bennet, K. D., (2008)**. Phylogeographic insights into cryptic glacial refugia.** Trends in Ecology and Evolution, 23, 564-571.

Ramos-Onsins, S. E., Rozas, J., (2002). **Statistical properties of new neutrality tests against population growth.** Molecular biology and evolution,19(12), 2092-2100.

Rato, C., Carranza, S., Perera, A., Carretero, M. A., Harris, D. J., (2010). **Conflicting patterns of nucleotide diversity between mtDNA and nDNA in the Moorish gecko, *Tarentola mauritanica***. Molecular Phylogenetics and Evolution, 56(3), 962-971.

Remington, C. L., (1968). **Suture-zones of hybrid interaction between recently joined biotas**. Evolutionary biology, Springer US, 321-428.

Rogers, A. R., Harpending, H., (1992). **Population growth makes waves in the distribution of pairwise genetic differences**. Molecular biology and evolution, 9(3), 552-569.

Rohling, E. J., Fenton, M., Jorissen, F. J., Bertrand, P., Ganssen, G., Caulet, J. P., (1998). **Magnitudes of sea-level lowstands of the past 500,000 years.** Nature, 394(6689), 162-165.

Salvi, D., Harris, D. J., Bombi, P., Carretero, M. A., Bologna, M. A., (2010). **Mitochondrial phylogeography of the Bedriaga's rock lizard, *Archaeolacerta bedriagae (Reptilia: Lacertidae*) endemic to Corsica and Sardinia**. Molecular Phylogenetics and Evolution, 56(2), 690-697.

Salvi, D., Schembri, P. J., Sciberras, A., Harris, D. J., (2014)**. Evolutionary history of the Maltese wall lizard *Podarcis filfolensis*: insights on the 'Expansion–Contraction'model of Pleistocene biogeography**. Molecular ecology, 23(5), 1167-1187.

Sambrook, J., Fritsch, E. F., Maniatis, T., (1989). **Molecular cloning: a laboratory manual**. Second ed. Cold Spring Harbor Press, New York.

Santucci, F., Nascetti, G., Bullini, L., (1996). **Hybrid zones between two genetically differentiated forms of the pond frog *Rana lessonae* in southern Italy**. Journal of Evolutionary Biology, 9(4), 429-450.

Sotiropoulos, K., Eleftherakos, K., Džukić, G., Kalezić, M. L., Legakis, A., Polymeni, R. M., (2007). **Phylogeny and biogeography of the alpine newt *Mesotriton alpestris* (Salamandridae, Caudata), inferred from mtDNA sequences**. Molecular phylogenetics and evolution, 45(1), 211-226.

Spinks, P. Q., Shaffer, H. B., Iverson, J. B., McCord, W. P., (2004). **Phylogenetic hypotheses for the turtle family Geoemydidae.** Molecular phylogenetics and evolution, 32(1), 164-182.

Suc, J. P., (1984). **Origin and evolution of the Mediterranean vegetation and climate in Europe.** Nature, 307(5950), 429-432.

Swenson, N. G., Howard, D. J., (2005). **Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America**. The American Naturalist, 166(5), 581-591.

Tajima, F., (1989). **Statistical method for testing the neutral mutation hypothesis by DNA polymorphism.** Genetics, 123(3), 585-59

Tamura, K., Peterson, D., Peterson, N., Stetcher, G., Nei, M., Kumar, S., (2011). **MEGA5: molecular evolutionary genetic data analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods.** Molecular Biology and Evolution, 28, 2731- 2739.

Thiede, J., (1978). **A glacial Mediterranean**. Nature, 276, 680-683.

Ursenbacher, S., Schweiger, S., Tomović, L., Crnobrnja-Isailović, J., Fumagalli, L., Mayer, W., (2008). **Molecular phylogeography of the nose-horned viper *(Vipera ammodytes*, Linnaeus (1758): evidence for high genetic diversity and multiple refugia in the Balkan Peninsula.** Molecular Phylogenetics and Evolution, 46(3), 1116-1128.

Vega, R., Amori, G., Aloise, G., Cellini, S., Loy, A., Searle, J. B., (2010). **Genetic and morphological variation in a Mediterranean glacial refugium: evidence from Italian pygmy shrews, *Sorex minutus* (Mammalia: Soricomorpha).** Biological Journal of the Linnean Society, 100(4), 774-787.

CHAPTER 2

Morphological Patterns of *Podarcis wagleriana*

2.1. INTRODUCTION

Morphology is one of the main features of any organism's phenotype. Since early times, morphological characters have been used as a basic tool in various biological fields like taxonomy and systematics (Arnold 1973; 1989, Archie 1985, Sá-Sousa *et al.* 2002), conservation (Wayne 1992, Taylor *et al.* 2011), ecology (Pianka 1986, Losos 1990a; 2009), biogeography (Arnold 1989, Stone *et al*. 2003, Nazari *et al.* 2007) and evolution (Arnold 1983, Kingsolver 2001). Organisms were classified into groups based on their morphological similarities and efforts were made to describe spatial and temporal patterns of morphological variation. Based on these patterns, explanations were sought on the processes that have shaped them, and hypotheses were formed on the origin of species and on their evolutionary history. Nowadays, the application of molecular techniques has brought a new era on the way organismic diversity is studied and described. However, even though the new techniques have given a clearer and more accurate insight into organisms' diversity, the morphological techniques are still of great interest. For instance, studying morphological diversity can aid us in elucidating microevolutionary mechanisms that cannot be revealed only with the use of genetic tools. Most morphological studies take place at the interspecific level, as evolutionary trends are easier to detect in higher taxonomic levels. However, intraspecific studies can prove to be more adequate for detecting microevolutionary processes that led to phenotypic diversity (Rieseberg et *al.* 2002, Kingsolver *et al.* 2007).

In many cases morphological and genetic differentiation may exhibit concordant patterns, as a result of evolutionary processes like natural selection, gene flow or stochastic processes like genetic drift. As already described in detail in chapter 1, the above processes can be related with past historical events. This is, for instance, the case of Pleistocene climate oscillations and glacial refugia in the Quaternary ice ages that may explain current species-rich areas and intra-specific diversity in the Iberian, Balkan and Italian peninsulas (Bilton *et al.* 1998, Hewitt 2000, Petit *et al*. 2003). However, morphological patterns do not always accompany genetic ones, as morphological evolution can be the result of the combination of many different microevolutionary processes that interact to shape the observed variation.

First, local adaptation can be a powerful driving force shaping intraspecific morphological differentiation. Ecomorphological studies have shown that adaptation to different habitats can lead to habitat-related morphological patterns (Arnold 1983, Pianka 1986, Losos 1990a, b, Sinervo *et al.* 1991, Irschick *et al.* 1997, Losos *et al.* 1998, Kohlsdorf *et al.* 2001, Butler *et al.* 2007, Kaliontzopoulou *et al.* 2010a), which many times can also be related to local environmental differences, like in altitude or temperature. Morphological variation matching the specific needs for survival in different environments is also inextricably linked with performance traits and fitness (Arnold 1983, Kingsolver 2003). Habitat-related differentiation can vary between the sexes, highlighting interactions between sexual and natural selection (Kaliontzopoulou *et al.* 2010a).

Natural and sexual selection underlie a great part of the variation observed in different morphological traits across different ecological and environmental conditions (Losos *et al.* 2003, Butler *et al.* 2007, Kaliontzopoulou *et al.* 2010a). In lizards, males 0tend to exhibit larger body size and head dimensions than females (Cooper & Vitt 1989, Anderson & Vitt 1990, Mouton & Van Wyk, 1993, Herrel *et al.* 1996; 1999; 2001a). Sexual differences have also been observed in different body parts like abdomen length or limb length. In addition to differences related to sex, ontogenetic differences have been shown between juveniles and adults. For instance, ontogenetic differences have been reported in limb and head dimensions (Brecko *et al.* 2008), head shape and bite force (Herrel *et al.* 2006), habitat use (Stamps 1983b, Keren- Rotem *et al*. 2006) and locomotor performance (Brecko *et al.* 2008).

Finally, some characteristics can vary due to phenotypic plasticity related to specific habitat requirements, as is the case of hindlimb length in *Anolis* lizards (Losos *et al*. 2000).

Natural selection can also act as a response to environmental conditions. Especially, ectotherms, like reptiles, are expected to show morphological adaptability to local conditions, due to their increased dependence on climatic conditions, like temperature or rainfall. Differen morphological patterns are known to emrge as response to environmental conditions, such as selection on body size to maintain heat (Bergmann’s rule; Asthon & Feldman 2003) or adjustments in the number of scales to prevent water loss (Sanders *et al.* 2004).

The different factors that shape morphology have been studied in lizards by assessing variation in different traits. Snout-vent length, used as a representation of total body size (Hews 1996, Butler & Losos 2002, Kratochvil *et al.* 2003), and the relative size and shape of various body parts are frequently reported to respond to natural and sexual selection. For instance, lizards from different habitats, like saxicolous or ground-dwelling lizards, appear to have different total body size and shape of body parts, such as the femur, tibia and hind feet length or the head shape, depending on each habitat’s mechanical constraints that can be related with microhabitat and refuge use (Vanhooydonck & Van Damme 1999, Herrel *et al*. 2001b, Kaliontzopoulou 2010b).The high dimorphism in both total body size and head size and shape can derive from sexual selection driven by male antagonistic behavior and mating (Kaliontzopoulou 2008, 2010b). Larger male heads are believed to offer advantage in male-male rivalry, whereas bigger male size can offer dominance over access to females (Tokarz 1985, Vitt & Cooper 1985, Anderson & Vitt 1990, Olsson 1992) or increased number of copulations and offspring achieved (Anderson & Vitt 1990, Abell 1997, Wikelsi & Trillmich 1997, Haenel *et al.* 2003). On the other hand, larger female size can result from longer trunks related to fecundity selection for increased space to hold the eggs or embryos (Andersson 1994, Olsson *et al.* 2002, Cox *et al*. 2003).

Head shape is particularly interesting when studying lizard morphological evolution due to its use in different functions related with performance. These different functions provide opportunities for the action of different evolutionary forces resulting in variation in head size and shape. In most lizards the head is involved in different activities such as bite performance (Herrel *et al.* 2001a, Verwaijen *et al*. 2002, Herrel & O'Reilly 2006, Kaliontzopoulou 2011), feeding (Herrel *et al*. 1996, 1999), refuge use (Arnold 1988a, b), mating, male aggressive interactions (Hews 1990, Gvozdík, & Damme 2003), territory acquisition and defense (Stamps 1983a, Heulin 1988). The advancement of geometric morphometrics methods (GM; Rohlf & Marucs 1994, Adams *et al.* 2004) enhanced the study of different body structures. The head of lacertid lizards, which is covered with relatively large scales, facilitates the definition of landmarks that could be evolutionary and functionally informative (Kaliontzopoulou *et al.* 2007).

Finally, pholidotic characters have also been useful when studying morphological variation in lizards, as patterns of variation in these traits can reveal adaptation and evolutionary history. Variation in pholidosis can be related to habitat use (Glossip & Lossos 1997), reproductive signaling and territory acquisition (Cole 1966, Carretetro & Llorente 1993) and intra and inter-specific recognition (Gómez *et al.* 1993, Cooper 2004, Barbosa *et al.* 2006). Scalation characters, being less prone to selective pressures, have been extensively used in the taxonomy of *Podarcis* (Geniez *et al.* 2007) and lacertids in general (Arnold 1989, Arnold & Ovenden 2002). Pholidotic characters have also been used in reconstructing phylogenetic relationships (Arlond 1989, Harris *et al*. 1998) as they can exhibit important interspecific variation.

Populations inhabiting islands, are expected to show variation in all of the above morphological characteristics. Due to their unique characteristics and ecological conditions insular populations are more affected by various evolutionary pressures, compared to their mainland counterparts and they have been recognized as particularly interesting systems to study variation at all levels, including the morphological one (see general introduction). According to the island rule (Van Valen 1973) insular species or populations can undergo conspicuous changes, for example in body size, resulting in phenomena of dwarfism or gigantism (Lomolino 2005) in different taxa. Smaller animals, like lizards, frequently shift toward gigantic forms on islands due to a shift of available resources usually exploited by larger animals in the continent, or due to release from predation. The head shape of lizards is inextricably connected with various activities, mentioned above, and is subject to natural selection; it is expected to show variation in islands due to the heterogeneity in habitats, peculiar ecological conditions (e.g low preadation pressure), climatic conditions and reduced food availability. As mentioned earlier, pholidotic characters are less prone to respond to adaptive processes, which may lead us to predict less diversification in these traits in insular populations where adaptation is a crucial evolutionary force.

Podarcis wall lizards have been extensively used as model species for studying the microevolutionary processes involved in shaping morphological diversity. Their wide distribution across Europe and their taxonomic and ecological variation makes them useful models for investigating microevolutionary processes of divergence and adaptation (Heywood 2000). Yet, even though the genetic variation of the genus is widely studied, morphological studies have focused more in specific aspects like species delimitation (Pérez-Mellado 1981, Sá-Sousa & Harris 2002), ecomorphology (Arnold 1983; 1998, Brecko *et al.* 2008, Kaliontzopoulou *et al*. 2010a), sexual dimorphism (Kaliontzopoulou *et al.* 2007, 2008, 2010b, 2012a), ontogeny (Piras *et al.* 2011) and functional performance (Brana 2003, Huyghe *et al.* 2007, Kaliontzopoulou *et al.* 2012b). However, even though insular morphological variation is a particularly intriguing subject when taking into account the biological diversity and unique conditions of islands, only during the last years has research started to focus on morphological variation of insular populations (Lo Cascio & Pasta 2006, Capula 2009, Pafilis *et al.* 2009, Runemark *et al*.2010; 2014; Runemark & Svensson 2012). Mediterranean insular species are particularly interesting since they show high inter- and intra- specific morphological variation (Arnold & Ovenden 2002). *Podarcis wagleriana* is a strictly insular endemic species that is very abundant locally. Its unique presence on Sicily and its satellite islands, in combination with the complex paleogeographic history of the island, its role as a glacial refugium during the Pleistocene, and the unique ecological conditions (see General Introduction), make *P. wagleriana* a great model for studying the microevolutionary processes that could have been responsible for the observed morphological variation. However, the knowledge on the morphology of *P. wagleriana* is very scarce, as very few studies have addressed the subject. Previous studies have focused on sexual dimorphism of total body size (Lo Cascio & Pasta 2006), and on head shape differences between *P.wagleriana*, *P.raffonei* and *P.sicula* in a comparative study that used geometric morphometric techniques (Capula *et al.* 2009) that revealed morphological variation both at the intra- and inter- specific level.

2.2. OBJECTIVES

The main aim of the second part of the thesis was to assess the morphological variation within *P.wagleriana* and investigate its relationship to genetic patterns across the distribution of the species. To do this, we examined variation in snout-vent length (SVL), as a general representation of body size; we quantified pholidotic characters previously used in lacertid taxonomy; and we used geometric morphometrics (GM) to study variation in head shape. Furthermore, in addition to describing overall morphological patterns, the analysis focused on the variation observed across different sampling localities and among phylogeographic groups (Chapter 1).

By examining intraspecific morphological differentiation at different structural levels the main goals were: 1) to provide an overall description of morphological patterns in this endemic species in the main islands of Sicily and its satellite islets; 2) to investigate if morphological variation is geographically structured; and 3) to assess whether and at which structural levels morphological patterns are in concordance with the genetic ones, or whether –alternatively – they originate from microevolutionary processes that could be related to environmental or ecological factors.

2.3. MATERIALS AND METHODS

2.3.1. Sampling

For the morphological analyses, 323 adult specimens were used, 199 of which were males and 124 were females. These specimens came from 37 different localities from across the distribution range of the species, namely the main island of Sicily and three of its satellite islands: Marettimo, Favignana and Levanzo (figure 2.1). Of these specimens we collect, data on SVL for 252, data on pholidotic traits for 258 and data for head shape for 293, due to missing data. Most of them were captured by noose in the field (fieldwork in Sicily and the Egadi Islands 2014) while others originated from the museum collections of the Zoological Museum of Palermo, the National History Museum of London and the Museum of Natural History of the University of Florence. Detailed information on the sampling localities and the specimens where already presented in chapter 1 (table 1.1). Here there are only presented the details for the localities and specimens that were not used in the genetics section (table 2.1).

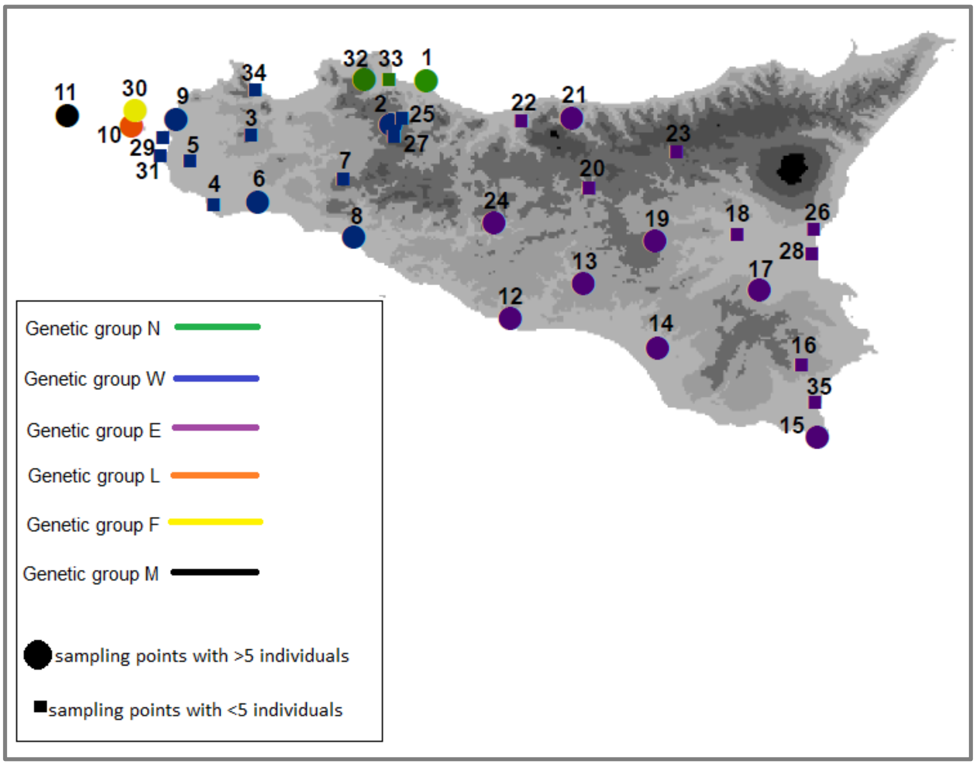


Figure 2.1: Sampling localities across the main island of Sicily and its satellite islands (Marettimo, Levanzo, Favignana). Different colors represent different phylogeographic groups(N:north, W: west, E: east, L: Levanzo, F: Favignana, M: Marettimo).

Table 2.1: Geographic locations of the sampling points that were used only in the morphological analysis. For the complete dataset see chapter 1, table 1.1.

Name	Locality	Altitude (m)	Coordinates
25	Bosco di Marineo	409	37° 57' N 13° 24' E
26	Catania	61	37° 30' N 15° 04' E
27	Ficuzza	910	37° 52' N 13° 22' E
28	Foce Simeto	3	37° 24' N 15° 04' E
29	Isola Grande dello Stagnone	0	37° 52' N 12° 26' E
30	Levanzo	259	37° 59' N 12° 19' E
31	Marsala	17	37° 48' N 12° 26' E
32	Monte Cuccio	597	38° 06' N 13° 15' E
33	Palermo	54	38° 06' N 13° 21' E
34	Scopello	121	38° 04' N 12° 49' E
35	Vendicari	33	36° 48' N 15° 05' E

2.3.2. Morphological characters

Morphological variation was examined taking into account body size and pholidotic characters, as well as using geometric morphometric techniques (GM) to study head shape. The only biometric character that was measured was snout-vent length (SVL) and which was recorded using an electronic caliper with accuracy of 0.01 mm. The pholidotic characters that were studied are classic traits of common use in lacertid taxonomy and morphological variation studies (Arnold 1973, Perez-Mellado *et al.* 1988, Kaliontzopoulou *et al.* 2012a). Specifically, a total of 6 continuous pholidotic characters were quantified including the number of collar scales (CSN), gular scales (GSN), transversal rows of ventral scales (VSN), supraciliary granules (SGN), femoral pores (FPN) and subdigital lamellae under the fourth toe (SDN) from digital photos that were taken in the field and from the museum collections (Fig 2.2). For all bilateral pholidotic characters, we always considered the average value of the two sides of the body.

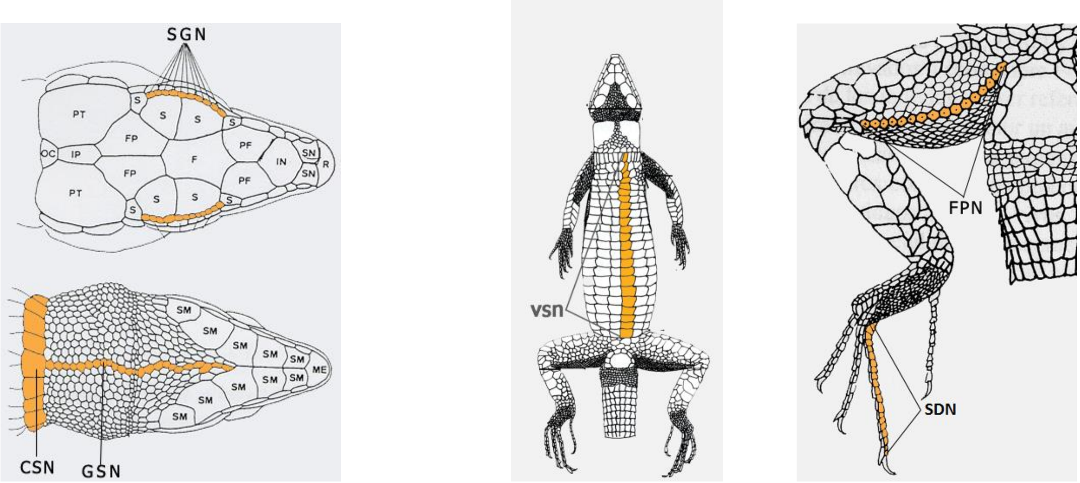


Figure 2.2: Pholidotic characters that were recorded

Finally, head shape variation was examined using geometric morphometric techniques (GM). In order to study head shape, we made digital photos of the dorsal view of each specimen's head in the field and in museums. All images were assembled in a tps file using tpsUtil software (Rohlf 2004). Twenty landmarks previously used to quantify dorsal head shape in *Podarcis* (Kaliontzopoulou *et al.*2007, 2008) were recorded using tpsDig2 (Rohlf 2005; Fig 2.3). Specimens with malformations in which any of the landmarks could not be placed correctly, were excluded from the analysis.

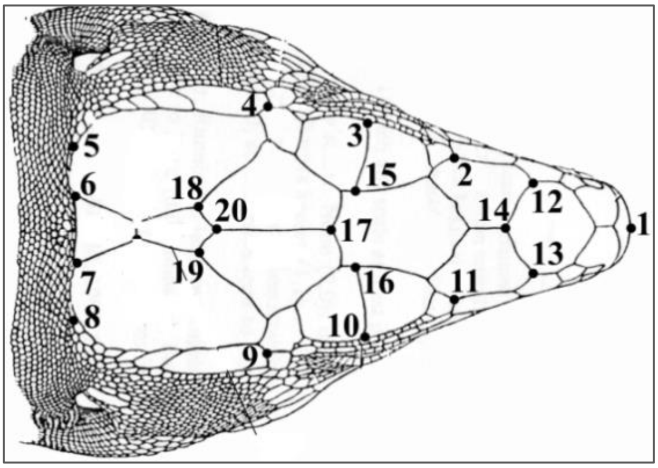


Figure 2.3: Landmarks recorded on the dorsal view of the lizards' head.

2.3.3. Statistical analyses

All statistical analyses were performed using the R programming language (http://www.R-project.org) and were conducted separately for SVL, pholidotic characters and head shape.

In order to examine intraspecific morphological differentiation, all analyses were performed considering different sampling localities (SITES) as well as different phylogeographic groups (GR). Sampling localities were examined in order to verify if morphological variation exhibits a geographic structure. Phylogeographic groups were considered in order to evaluate whether morphological variation is in general concordance with genetic patterns or whether it is shaped by different factors. The genetic groups considered were the three main groups previously defined in the SAMOVA analysis (Chapter 1; N: north, W: west, E: east). However, samples from the Egadi Islands (F: Favignana, M: Marettimo, L: Levanzo) were each assigned to a different group for these analyses in order to test if they show significant differentiation from the main island, taking into account the unique conditions of micro-insular populations. Museum specimens were assigned to a phylogeographic group based on their gps coordinates (figure 2.1). To ensure that this classification did not influence our inferences, analyses were also conducted excluding museum specimens. Because the results obtained did not vary, only analyses including all specimens are presented here.

All variables were log-transformed prior to analysis to ensure normality. A preliminary ANOVA was conducted separately for SVL, pholidotic measures and head shape variables to evaluate the effects of SEX. Previous studies have shown that lacertids show sexual variation in body size (Herrel *et al.* 1996, Kaliontzopoulou *et al*. 2007a, 2008, 2010b) head dimensions and shape (Kaliontzopoulou *et al.* 2007b) as well as in the scalation characters (Kaliontzopoulou *et. al* 2012a). Since sexual dimorphism is not the main focus of this study and most of the variables exhibited significant sexual dimorphism (see Results), we mean-centered the data for sex effects.

For GM data a Generalized Procrustes Analysis (GPA; Rolhf & Slice 1990a, b, Rohlf 1999) was performed to standardize size, translate and rotate landmark configurations, and obtain shape variables. To exclude asymmetry effects (Corti & Rohlf 2001), symmetric landmarks along the mid-line were averaged and all statistical analyses were performed based on this symmetric configuration. After the above preliminary analyses, the effect of SITE and GR was tested, separately for SVL, pholidosis and head shape, using a nested ANOVA where SITES was nested in GR. For pholidotic data, the differences of each pholidotic character among phylogeographic groups were visualized using plots of mean values and 95% confidence intervals.

A principal component analysis (PCA) was then performed for the pholidotic and head shape data in order to further explore intraspecific morphological differentiation and investigate the main source of variation in the sample. The head shapes that correspond to the minimum and maximum extreme of each axis were presented as vectors in order to visualize how the head shape is differentiated.

In order to investigate whether sampling localities and phylogeographic groups account for the structure of morphological variation, we performed a cluster analysis. For this purpose, we first calculated univariate (for SVL) or multivariate (considering all pholidotic traits and landmarks simultaneously) Euclidean distances. Then, based on these distances we implemented the unweight pair group method using arithmetic averages (UPGMA) to obtain dendrograms that represent similarity relationships among groups. Both for the PCA and cluster analyses we used a reduced dataset, where the localities with less than five individuals were excluded from the sample as this low sample size may not be sufficient to represent the morphological properties of the populations under investigation (see figure 2.1).

In the last part of the analysis, we compared the genetic with the morphological results in order to test if variation patterns coincide, and to assess if they appear to be influenced by geographical structure or they are irrespectively related. For this purpose, we used Mantel tests of matrix association to evaluate the Pearson correlation between pairwise distances for morphological traits, genetic differentiation and geographic distances. For morphological traits, we considered the Euclidean distances (ED) for SVL, pholidotic and GM data mentioned above. Genetic differentiation was represented through matrices of net genetic distances (see Chapter 1), and geographic distance was calculated as the straight distance between the GPS coordinates of the sampling points. Mantel tests were performed firstly pairwise testing if the genetic matrix is associated with the morphological matrix and if each one of those is associated with the matrix of geographical coordinates. Lastly, we performed a three-way Mantel test that took into account all three matrices in order to test how morphological and genetic distances are related when we account for the geographic structure of each of them.

2.4. RESULTS

2.4.1. Effect of SEX

The effect of SEX was significant for SVL, head shape and most of the pholidotic traits except for SGN (table 2.2). Since sexual dimorphism was not an objective of the study, sex effects were removed for all subsequent analyses.

Table 2.2: Results of ANOVA performed to assess the effects of SEX for SVL, pholidotic traits and head shape. df: degrees of freedom, F: value of F statistic, P : corresponding P-value. Significant values (α=0.05) are indicated in italics and bold.

	Df	**Sums of Squares**	**F**	**P**
SVL	1	0.131	26.857	***0.001***
SCALATION				
CSN	2	0.176	7.650	***0.006***
GSN	2	0.047	3.263	***0.049***
VSN	2	0.713	113.612	***0.001***
SGN	2	0.069	1.151	0.289
FPN	2	0.239	17.295	***0.001***
SGN	2	0.045	3.711	***0.024***
HEAD SHAPE				
	2	0.036	12.457	***0.001***

2.4.2. SVL, pholidotic and head shape patterns

Examination of ANOVA Sums of Squares indicates that the differences among phylogeographic groups have a stronger influence in explaining variability in SVL, and most of the pholidotic characters; however, in some pholidotic characters, i.e. CSN and SDN, the differences between localities seem to have a stronger effect (Table 2.3). On the other hand the differences on head shape seem to be influenced more by the different localities. The variation of SVL and scalation characters for the different phylogeographic groups can be seen with the help of plots (figure 2.4). In the plot of SVL no striking pattern can be observed. However, in most of the pholidotic characters, i.e. the CSN, GSN, SGN and FPN, the islets seem to have very similar values more distinct than the rest of the groups.

Table 2.3: Nested ANOVA where SITES is nested in GR. df= degrees of freedom, SS=sum of squares, F=value of the statistic, P: corresponding P-value. Significant values (α=0.05) are indicated in italics and bold.

	GR				**GR(SITES)**			
	Df	SS	F	P	Df	SS	F	P
SVL	33	0.420	2.975	***0.001***	32	0.064	12.131	***0.002***
SCALATION								
CSN	1	0.002	0.216	0.657	32	0.699	2.031	***0.004***
GSN	1	0.049	7.153	***0.011***	32	0.298	1.336	0.113
VSN	1	0.036	6.146	***0.009***	32	0.155	0.824	0.732
SGN	1	0.461	16.741	***0.001***	32	1.097	1.243	0.175
FPN	1	0.232	33.787	***0.001***	32	0.226	1.029	0.429
SDN	1	0.112	21.345	***0.001***	32	0.311	1.843	***0.004***
HEAD SHAPE	1	0.047	3.255	***0.011***	35	0.078	1.529	***0.001***

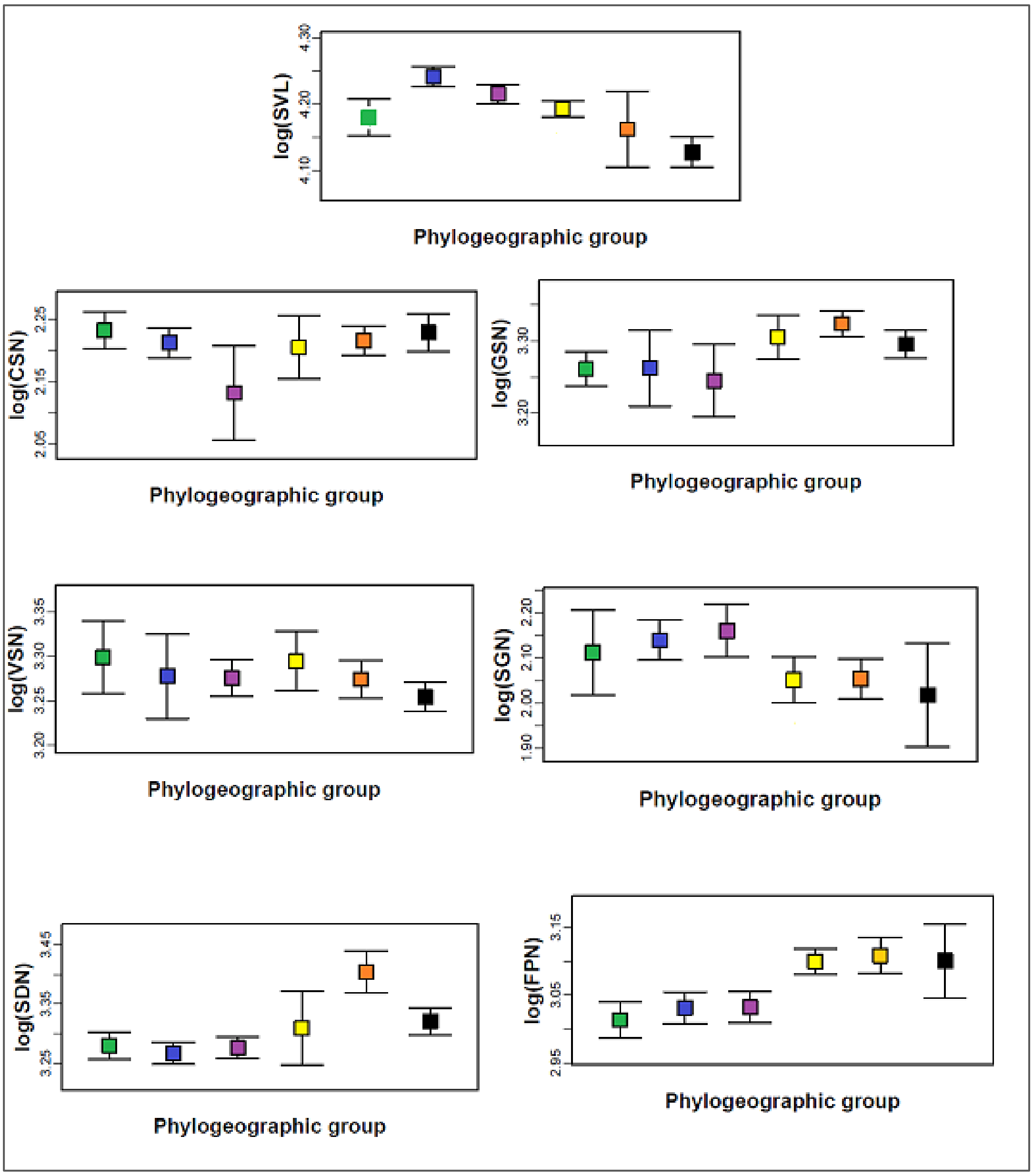


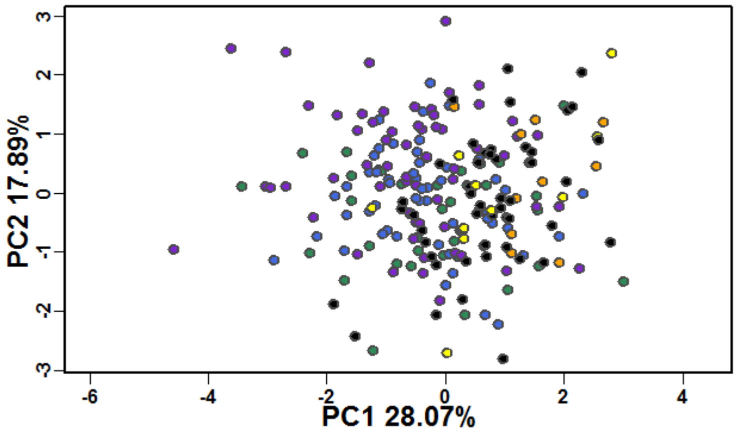
Figure 2.4: Means and 95% confidence intervals of snout-vent length (SVL) and scalation characters (see text) in the six phylogeographic groups. Each colored dot represents a different group (see map 2.1). green: northern group, blue: western group, purple: eastern group, yellow: Favignana, orange: Levanzo, black: Marettimo

PCA results on pholidotic characters showed that the first principal component explained 28.07% of total morphological variation in the sample, and it represents variation mostly in FPN, SDN and GSN. The second principal component explained 17.89% and it represents variation mostly in SGN and CSN. As for head shape, the first principal component explained 27.57% while the second explained 19.29% of the total variation (table 2.4, fig 2.5).

Table 2.4 Results of the first three principal components on pholidotic characters and scalation. %exp.: percentage of variation explained, %cum.: % cumulative percentage of variation explained.

SCALATION								
			CORRELATION					
PC	**% exp**	**% cum**	**CSN**	**GSN**	**VSN**	**SGN**	**FPN**	**SDN**
1	28.07	28.07	0.466	0.662	0.185	-0.149	0.701	0.69236
2	17.89	45.96	-0.563	0.044	-0.314	0.702	0.309	0.25805
3	16.59	62.55	0.022	0.139	0.824	0.476	-0.004	-0.26211
HEAD SHAPE								
1	27.57	27.57						
2	19.29	46.87						
3	9.70	57.57						

When examining individual variation in the space of the first two principal components in relation to phylogeographic group, it becomes evident that there is high morphological variation that is distributed among the groups, however, without a clear association to genetic variation.



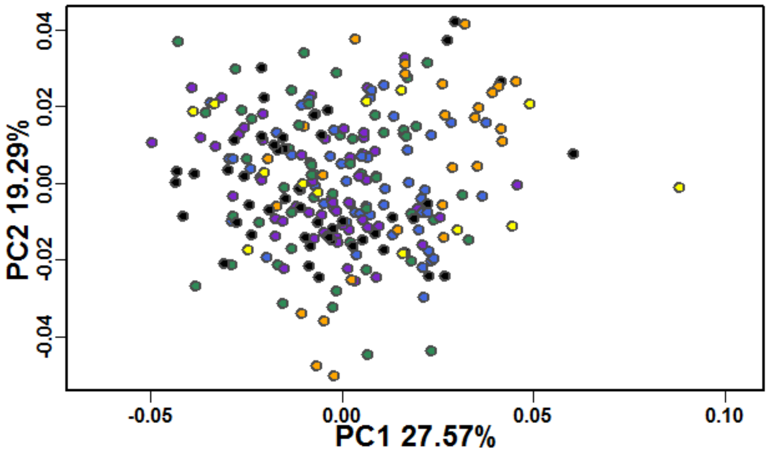


Figure 2.5: Graphic representation of the two principal components (up: scalation, down: head shape) in relation to phylogeographic groups. Each group is represented by a different color (see map 2.1). green: northern group, blue: western group, purple: eastern group, yellow: Favignana, orange: Levanzo, black: Marettimo

The visualization of head shape in the minimum and maximum of the two principal component axes revealed that the main source of head shape variation is related to a shortening of the snout and anterior head region, and an amplification of the posterior region of the head (Figure 2.6).

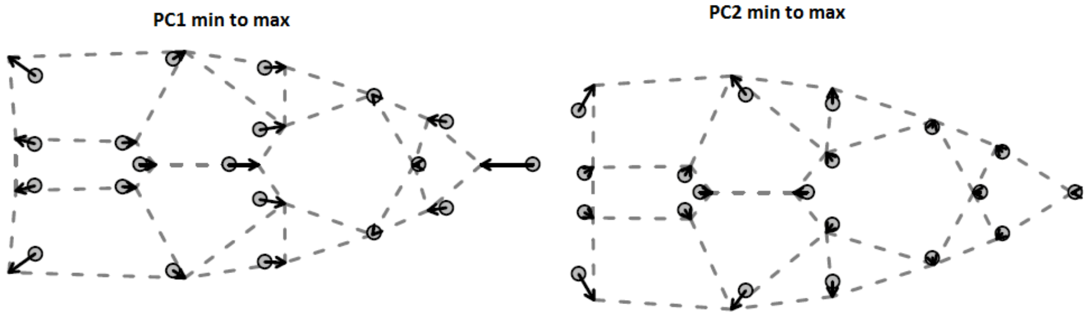


Figure 2.6 Vectors representing the two extremes of PC1 and PC2 respectively. Images are exaggerated two times.

As far as pholidotic variation among capture localities is concerned, the UPGMA analysis based on ED distances showed some geographical structure. The most striking pattern observed was that the three islets of Marettimo, Favignana and Levanzo appear clustered, being more similar to each other and differentiated from “mainland” Sicilian populations (Figure 2.5). Other populations sampled within Sicily do not exhibit a clear geographic pattern. When the same dendrogram is colored based on the genetic groups again the different localities do not show a clear pattern that would indicate concordance with phylogeographic groups. Clustering based on body size (SVL) did not show any clear geographic pattern either across localities or among phylogeographic groups (Figure 2.7). Finally, the cluster analysis of multivariate Euclidean distances based on head shape variables did not indicate any clear pattern except for some eastern localities (genetic group 2/purple) that seem to have more similarities than the rest of the groups in the case odf head shape. Contrary to what was observed for the pholidotic data, specimens from islets do not appear differentiated from the main island (Figure 2.7).

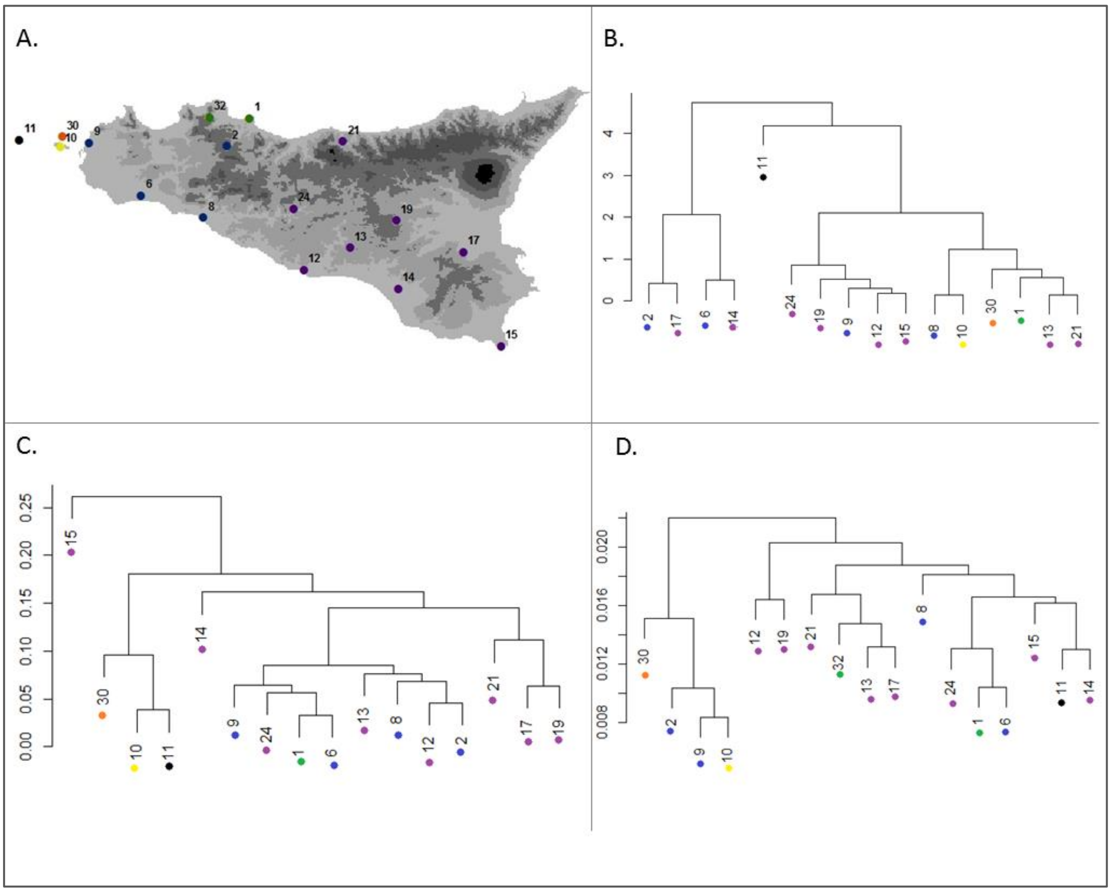


Figure 2.7: Cluster dendrograms representing similarity among localities in SVL (B), pholidotic characters (C), head shape (D) and respective map indicating the localities used for the cluster analysis (see map 2.1). Bullets of different colors represent different phylogeographic groups (see map 2.1). green: northern group, blue: western group, purple: eastern group, yellow: Favignana, orange: Levanzo, black: Marettimo

2.4.3. Mantel tests

Mantel tests for SVL revealed that size differentiation across sampling localities is associated to neither genetic differentiation nor geographic distance (Table 2.5).
We found no significant association between net genetic distances and ED distances calculated for pholidotic traits, indicating that morphological and genetic differentiation across populations do not follow the same pattern. However, both genetic and pholidotic differentiation were associated to geographic distance, reinforcing the view that both exhibit a geographic structure. When a partial mantel test was performed in an attempt to account for the geographical structure for the comparison of morphological and genetic patterns of differentiation, a non- significant association between the two distance matrices was found (r=-0.042, P=0.506). On the other hand, head shape variation was associated with the genetic distances but not with the geographical distances. When we accounted for the geographical structure in a partial mantel test, the relationship between the three matrices was not significant (r=-0.042, p=0.532).

Table 2.5: Results of mantel test comparisons. P: corresponding P-value. Significant values (α=0.05) are indicated in italics and bold.

	Net genetic distance		Geographic distance	
	r	P	R	P
SVL	-0.077	0.726	-0.003	0.452
Pholidotic	0.095	0.200	0.265	***0.005***
GM	0.127	***0.037***	-0.035	0.724
Net genetic distances	-	-	0.497	***0.001***

2.5. DISCUSSION

SVL, pholidotic and GM data revealed significant morphological differentiation across the species' distribution range. However, each type of trait revealed a different structure in its variability. Both SVL and scalation characters appear to be mostly affected at the level of the phylogeographic group. However, while SVL does not show any specific structure, pholidotic traits seem to form a more differentiated morphological group in the islets than in the "mainland" populations (figure 2.7a). Head shape, on the other hand, seems to be more affected by variations in the level of different sampling localities where a stronger differentiation of some eastern localities was observed (figure 2.7b).

Interestingly, body size did not exhibit a geographic structure or any striking pattern of variation. This observation could be considered unexpected, especially for the smaller islets where larger body size would be expected as a result of the insularity effect (Lomolino 2005, Meiri 2007; see also introduction). In fact, cases of gigantism in islands have been reported in several vertebrate species, including small mammals (Lomolino 1985, Angerbjörn 1986, Millien 2006) and reptiles (Barahona 2000, Pafilis *et al.* 2009, Jaffe 2011). The evolution of gigantism is usually linked with environments of low predation and interspecific competition, and an increase in food availability (Meiri *et al*. 2004, Dayan & Simberloff 2005, Meiri 2008). These phenomena can lead to higher population densities ("density compensation") that can prompt the emergence of the island syndrome (Adler & Levins, 1994, Raia *et al*., 2010, Pafilis *et al*. 2011). However, when examining Sicily and especially the Egadi islands, such conditions are not met. The main factor for the lack of increase in body size could be the presence of a strong competitor, like *Podarcis sicula*. *P. sicula* is an efficient colonizer that has been successfully introduced and acclimatized to several extralimital areas (see Capula 1994, Corti & Lo Cascio 2002 for an updated list of localities). In fact, the introduction of *P. sicula* has even led to the extinction of local populations of other lacertid lizards; this is the case with *P. melisellensis*, which has been reported to have gone extinct after the arrival of the competitively superior *P. sicula* (Radovanoviç 1959, 1960). Consequently, a strong adversary like this is competing with *P. wagleriana* for food resources and could possibly prevent the growth of its population size. Another factor that may have contributed to the lack of gigantism in *P.wagleriana* from the small Egadi islets is the presence of the snake *Hierophis viridiflavus,* a well-known lacertid lizard predator (Rugiero & Luiselli 1995, Capizzi & Luiselli 1996), a fact that precludes the predator-free condition for development of giant forms.

Examination of the scalation patterns reveals that most of the variation can be accounted for by differences in the number of femoral pores (FPN), subdigital lamellae under the fourth toe (SDN) and gular scales (GSN). According to Mantel test results, variation in pholidotic traits seems to be spatially structured but it does not exhibit any obvious correlation with genetic variation. Consequently, its origin should be sought in microevolutionary processes correlated with environmental and ecological conditions, rather than historical population connectivity and differentiation. In some cases, climate affects scalation through desiccation; many small scales can be selected in dry environments as large scales tend to have more surface area and thus increased desiccation potential (Soule & Kerfoot 1972). Furthermore, studies in the lacertid lizards *Gallotia stelhini* (Thorpe & Baez 1993) and *Gallotia galloti* (Thorpe & Baez 1987) showed altitudinal decrease in the number of gular scales and femoral pores, being also in concordance with the results found in the skink *Chalcides sexlineatus* (Brown & Thorpe 1991). In *Anolis* lizards the number of subdigital lamellae has been shown to be related to habitat use (Malhotra & Thorpe 1997, Glossip & Losos 1997) suggesting a relationship between perching and number of lamellae (Irschick *et al.* 1996, Moermond 1979). However, the most striking geographic pattern emerging in relation to pholidotic traits was that the populations of the Egadi islets seem to form a distinct morphological group, separated from the rest. The morphological distinctiveness of the islets can be attributed to several factors; the isolation of the islets from the “mainland” Sicily, in combination with the inability of the species for over-sea dispersal could have acted as a geographical barrier, leading to isolation by distance (less gene flow than in mainland populations). Furthermore, the dramatic changes in natural and sexual pressures in small islets, (i.e. decreased predation, high densities, intraspecific competition for food and sexual partners) can lead to a striking differentiation of local populations. Previous studies have shown the differentiation of *Podarcis* populations inhabiting small islets (Castilla & Bauwens 1997, Sá-Sousa *et al.* 2000, Arntzen & Sá-Sousa 2007). These patterns include some pholidotic characters, suggesting that the origin of geographic variation is mainly ecogenetic (Thorpe 1987; 1991, Thorpe *et al.* 1991). This means that different characters have responded to different selective factors, confirming the adaptive value of scalation characters and suggesting that the taxonomic value of these characters is mainly restricted to the interspecific level. The fact that our results did not indicate concordance with genetic patterns further reinforces the view for an ecogenetic origin of the differentiation; there have been suggestions that ecogenetically caused geographic variation is showing less congruence between characters than phylogenetic caused geographic variation (Thorpe 1987; 1991, Thorpe *et al.* 1991). Consequently, if the origin of the observed variation were phylogenetic, resulting from historical events like the ones shaping the genetic variation of *P.wagleriana* (see chapter 1), one would expect all characters to be influenced by the same processes; however in our case different characters respond to different selective factors. However, in order to test the ecogenetic origin of our results further analysis should be done testing for potential causal factors.

Head shape variation was found to be mainly differentiated at the level of different localities, indicating phenotypic plasticity or local adaptation as the main source of the observed variation. In *Podarcis*, head shape varies in different habitat types (Kaliontzopoulou *et al.* 2010a), offering biomechanical advantages; for instance, flatter heads can provide an advantage when seeking refuge in crevices (Arnold 1973). Harmon *et al.* 2005 suggested that head shape variation in *Anolis* lizards can be due to differences in diet, aggressive or antipredatory behavior in different habitat types. Since ecology and performance traits are linked (Arnold 1983, Kingsolver & Huey 2003) it is possible that local responses in the environmental and ecological conditions can trigger variations in head shape in order to enhance performance. Based on Mantel test results, head shape variation was found to be correlated with genetic variation. However when geographic distances were taken into account, this relationship ceased to exist. These results imply that head shape and genetic variation exhibit a similar spatial structure, which results in a correlation between them. For instance, some patterns, like the morphological grouping of some eastern populations (figure 2.6b), could have been the result of the same processes that shaped the eastern mitochondrial clade (see chapter 1). As mentioned in chapter 1, our results showed that during glacial periods of the Pleistocene the eastern population may have maintained viable populations in a distinct glacial refugium. Long persistence and isolation in this refugium probably led to the genetic distinctiveness of this clade. In addition, it is possible that such isolation may have triggered the action of natural selection on certain characters, like head shape. The fact that only the localities of this clade exhibit this kind of morphological distinctiveness may be related to their geographical position within Sicily. The high altitude of some of these localities, in combination with their proximity to Mount Etna, where climatic conditions are more extreme than in the rest of the island, can account for the distinctiveness of these populations. Future investigation testing for morphological variation under different climate and altitude conditions can confirm or reject the above hypothesis.

The origin of present-day morphological variation of *P.wagleriana* is certainly more complex and multifactorial than the reasoning proposed above. Our results indicated that the observed variation is mainly a consequence of different responses to local environmental and ecological conditions; however, the cases of congruence between morphological and genetic patterns (chapter 1) cannot be ignored. The most striking observation was the genetic and morphological distinctiveness of the populations from the Egadi islets. Although the islets do not form a distinct mtDNA clade, they are all characterized by unique haplotypes and one of them (Marettimo) forms a subgroup quite distinct from the rest (chapter 1; figure 1.3). This distinctiveness of the islets was also observed in some of the morphological patterns, including mainly a visible clustering of their populations in terms of pholidotic traits. In this context, the differentiation of the Egadi islets' populations renders them a particularly interesting case for future investigation and even for conservation purposes. Furthermore, the eastern populations present a certain morphological distinctiveness from the rest, being in concordance with the genetic results where they form a separate mitochondrial clade. It is possible that a more thorough sampling of the western and northern populations could provide a more complete image on the occurring morphological patterns as well as possible concordance of morphological patterns with the genetic ones.

This study provided a first view of the patterns of morphological variation of this poorly studied species across its distribution range. Our results indicated some geographical structure and local differentiation that in some cases seem to be in agreement with the genetic patterns described through mtDNA analyses. Future analyses can expand these results to provide more insights on the factors shaping morphological differentiation among populations of *P. wagleriana*. In order to pinpoint how and which different conditions have shaped intraspecific variation, a further analysis should be conducted taking into account different types of habitat, climatic conditions, variance of latitude and altitude. Geographic information systems (GIS) can be a really useful tool to map both genetic and morphological patterns of variation (Cesaroni *et al.* 1997, Kidd & Rithie 2000) and obtain continuous maps of variation for different conditions (climate, precipitation, altitude, longitude) across space. This spattialy- explicit approach can be used to test the correlation of morphological variation with environmental factors.

2.6. REFERENCES

Abell, A. J., (1997). **Estimating paternity with spatial behaviour and DNA fingerprinting in the striped plateau lizard, *Sceloporus virgatus* (Phrynosomatidae).** Behavioral Ecology and Sociobiology, 41(4), 217-226.

Adams, D. C., Rohlf, F. J., Slice, D. E., (2004). **Geometric morphometrics: ten years of progress following the 'revolution'**. Italian Journal of Zoology, 71(1), 5-16.

Adler, G. H., Levins, R., (1994). **The island syndrome in rodent populations.** Quarterly Review of Biology, 473-490.

Anderson, R. A., Vitt, L. J. (1990). **Sexual selection versus alternative causes of sexual dimorphism in teiid lizards.** Oecologia, 84(2), 145-157.

Andersson, M. B., (1994). **Sexual selection.** Princeton University Press.

Angerbjörn, A., (1986). **Gigantism in island populations of wood mice (*Apodemus*) in Europe**. Oikos, 47-56.

Archie, J. W., (1985). **Methods for coding variable morphological features for numerical taxonomic analysis.** Systematic Biology, 34(3), 326-345.

Arnold, E. N., (1973). **Relationships of the Palearctic lizards assigned to the genera *Lacerta, Algyroides and Psammodromus* (Reptilia: Lacertidae)**. Bulletin of the British Museum of NaturaL History (Zoology), 25, 289-366.

Arnold, E. N., (1988a). **Structural niche, limb morphology and locomotion in lacertid lizards (Squamata, Lacertidae): a preliminary survey.** Bulletin of the British Museum of Natural History (Zoology), 64, 63-89.

Arnold, E. N., (1988b). **Cranial kinesis in lizards, variations, uses and origins.** Evolutionary Biology, 30, 323-357.

Arnold, E. N., (1989). **Towards a phylogeny and biogeography of the Lacertidae*:* relationships within an Old-World family of lizards derived from morphology**. Bulletin of the British Museum of Natural History (Zoology), 55(2), 209-257.

Arnold, E. N., (1998). **Structural niche, limb morphology and locomotion in lacertid lizards (Squamata, Lacertidae); a preliminary survey**. Bulletin of the British Museum, Natural History (Zoology), 64, 63-90.

Arnold, E. N., Ovenden D., (2002). **Reptiles and amphibians of Britain and Europe.** Hong-Kong: HarperCollins Publishers.

Arnold, S. J., (1983). **Morphology, performance and fitness**. American Zoologist, 23, 347-361.

Arntzen, J. W., Sá-Sousa, P., (2007). **Morphological and genetical differentiation of lizards (*Podarcis bocagei* and *P. hispanica*) in the Ria de Arosa Archipelago (Galicia, Spain) resulting from vicariance and occasional dispersal.** Biogeography, time, and place: distributions, barriers and islands, Springer Netherlands, 365-401.

Ashton, K. G., Feldman, C. R., (2003). **Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it.** Evolution, 57(5), 1151-1163.

Barahona, F., Evans, S. E., Mateo, J. A., García-Márquez, M., López-Jurado, L. F., (2000). **Endemism, gigantism and extinction in island lizards: the genus *Gallotia* on the Canary Islands**. Journal of Zoology, 250(03), 373-388.

Barbosa, D., Font, E., Desfilis, E., Carretero, M. A., (2006). **Chemically mediated species recognition in closely related *Podarcis* wall lizards.** Journal of chemical ecology, 32(7), 1587-1598.

Bilton, D. T., Mirol P. M., Mascheretti S., Fredga K., Zima J., Searle J. B., (1998). **Mediterranean Europe as an area of endemism for small mammals rather than a source for nortwards postglacial colonization.** Proceeding of the Royal Society of London Series B, Biological Sciences, 265, 1219-1226.

Brana, F., (2003). **Morphological correlates of burst speed and field movement patterns: the behavioural adjustment of locomotion in wall *lizards (Podarcis muralis*).** Biological Journal of the Linnean Society, 80(1), 135-146.

Brecko, J., Huyghe, K., Vanhooydonck, B., Herrel, A., Grbac, I., Van Damme, R., (2008). **Functional and ecological relevance of intraspecific variation in body size and shape in the lizard *Podarcis melisellensis* (Lacertidae).** Biological Journal of the Linnean Society, 94(2), 251-264.

Brown, R. P., Thorpe, R. S., (1991). **Within-island microgeographic variation in body dimensions and scalation of the skink *Chalcides sexlineatus*, with testing of causal hypotheses.** Biological Journal of the Linnean Society, 44(1), 47-64.

Butler, M. A., Losos, J. B., (2002). **Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards**. Ecological Monographs, 72(4), 541-559.

Butler, M. A., Sawyer, S. A., Losos, J. B., (2007). **Sexual dimorphism and adaptive radiation in *Anolis* lizards.** Nature, 447, 202-205.

Capizzi, D., Luiselli, L., (1996). **Feeding relationships and competitive interactions between phylogenetically unrelated predators (owls and snakes).** Acta Oecologica, 17(4), 265-284.

Capula, M., (1994). **Population genetics of a colonizing lizard: Loss of variability in introduced populations of *Podarcis sicula***. Experientia, 50(7), 691-696.

Capula, M., Chiantini, S., Luiselli, L., Loy, A., (2009). **Size and shape in Mediterranean insular lizards: patterns of variation in *Podarcis raffonei, P. sicula and P. wagleriana* (*Reptilia: Squamata: Lacertidae*).** Aldrovandia, *5*, 217-227.

Carretero, M. A., Llorente, G. A., (1993). **Morfometría en una comunidad de lacértidos mediterráneos, y su relación con la ecología**. Historia Animalium, *2*, 77-79.

Castilla, A. M., Bauwens, D., (1997). **La lagartija de las Islas Columbretes: biologia y conservacion.** Generalitat Valencia, Valencia.

Cesaroni, D., Matarazzo, P., Allegrucci, G., Sbordoni, V., (1997). **Comparing patterns of geographic variation in cave crickets by combining geostatistic methods and Mantel tests.** Journal of Biogeography, 24(4), 419-431.

Cole, C. J., (1966). **Femoral glands in lizards: a review**. Herpetologica, 199-206.

Corti, M., Rohlf, F. J., (2001). **Chromosomal speciation and phenotypic evolution in the house mouse**. Biological Journal of the Linnean Society of London, 73, 99-112.

Cooper, W. E., Vitt, L. J., (1989). **Sexual dimorphism of head and body size in an iguanid lizard: paradoxical results.** American Naturalist, 729-735.

Cooper Jr, W. E., (2004). **Adaptive chemosensory behavior by lacertid lizards. The Biology of Lacertid lizards**. Evolutionary and Ecological Perspectives. Menorca: Institut Menorquı d'Estudis, Recerca, 83-118.

Corti, C., Cascio, P. L., (2002). **The lizards of Italy and adjacent areas** (Vol. 14). Edition Chimaira

Cox, R. M., Skelly, S. L., John-Alder, H. B., (2003). **A comparative test of adaptive hypotheses for sexual size dimorphism in lizards.** Evolution, 57(7), 1653-1669.

Dayan, T., Simberloff, D., (2005). **Ecological and community-wide character displacement: the next generation.** Ecology Letters, 8(8), 875-894.

Geniez, P., Cluchier, A., Sá-Sousa, P., Guillaume, C. P., Crochet, P. A., (2007). **Systematics of the *Podarcis hispanicus*-complex (Sauria, Lacertidae) I: redefinition, morphology and distribution of the nominotypical taxon**. The Herpetological Journal, 17(2), 69-80.

Glossip, D., Losos, J. B., (1997). **Ecological correlates of number of subdigital lamellae in anoles.** Herpetologica, 192-199.

Gómez, A., Font, E., Desfilis, E., (1993). **Chemoreception in the Lacertidae: exploration and conspecific discrimination in the Spanish wall lizard, *Podarcis hispanica.*** Lacertids of the Mediterranean Region, 213-230.

Gvozdík, L., Damme, R., (2003). **Evolutionary maintenance of sexual dimorphism in head size in the lizard *Zootoca vivipara*: a test of two hypotheses**. Journal of Zoology, 259(1), 7-13.

Haenel, G. J., Smith, L. C., John-Alder, H. B., (2003). **Home-range analysis in *Sceloporus undulatus.* II. A test of spatial relationships and reproductive success.** Copeia, 2003(1), 113-123.

Harmon, L. J., Kolbe, J. J., Cheverud, J. M., Losos, J. B., (2005). **Convergence and the multidimensional niche.** Evolution, 59(2), 409-421.

Harris, D. J., Arnold, E. N., Thomas, R. H., (1998). **Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology.** Proceedings of the Royal Society of London B: Biological Sciences, 265(1409), 1939-1948.

Herrel, A., Spithoven, L., Van Damme, R., De Vree, F., (1996). **Sexual dimorphism of head size in *Podarcis hispanica atrata:* Testing the dietary divergence hypothesis by bite force analysis.** Netherlands Journal of Zoology, 46, 253-262.

Herrel, A., Spithoven, L., Van Damme, R., De Vree, F., (1999). **Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analyses.** Functional Ecology, 13(3), 289-297.

Herrel, A., De Grauw, E. D., Lemos-Espinal, J. A., (2001a). **Head shape and bite performance in xenosaurid lizards.** Journal of Experimental Zoology, 290(2), 101-107.

Herrel, A., Meyers, J. J., Vanhooydonck, B., (2001b). **Correlations between habitat use and body shape in a phrynosomatid lizard (*Urosaurus ornatus*): a population-level analysis**. Biological Journal of the Linnean Society, 74(3), 305-314.

Herrel, A., O'Reilly, J. C., (2006)**. Ontogenetic Scaling of Bite Force in Lizards and Turtles.** Physiological and Biochemical Zoology, 79(1), 31-42.

Heulin, B., (1988). **Observations sur l'organisation de la reproduction et sur les comportements sexuels et agonistiques chez *Lacerta vivipara***. Vie Milieu, 38, 177-187.

Heywood, V. H., (2000). **Global biodiversity Assesment**. UNEP c/o Cambridge University Press, 1140 pp.

Hewitt, G. M., (2000). **The genetic legacy of the Quaternary ice ages**. Nature, 405, 907-913.

Hews, D. K., (1990). **Examining hypotheses generated by field measures of sexual selection on male lizards, *Uta palmeri*.** Evolution, 1956-1966.

Hews, D. K., (1996). **Size and scaling of sexually-selected traits in the lizard, *Uta palmeri.*** Journal of Zoology, 238(4), 743-758.

Huyghe, K., Vanhooydonck, B., Herrel, A., Tadić, Z., Van Damme, R., (2007). **Morphology, performance, behavior and ecology of three color morphs in males of the lizard *Podarcis melisellensis***. Integrative and Comparative Biology, 47(2), 211-220.

Irschick, D. J., Austin, C. C., Petren, K., Fisher, R. N., Losos, J. B., Ellers, O., (1996). **A comparative analysis of clinging ability among pad-bearing lizards.** Biological Journal of the Linnean Society, 59(1), 21-35.

Irschick, D. J., Vitt, I. J., Zani, P. A., Losos, J. B., (1997). **A comparison of evolutionary radiations in mainland and Caribbean *Anolis* lizards.** Ecology, 78, 2191-2203.

Jaffe, A. L., Slater, G. J., Alfaro, M. E., (2011). **The evolution of island gigantism and body size variation in tortoises and turtles.** Biology letters, rsbl20101084.

Kaliontzopolou, A., Carretero, M., Llorente, G. A., (2007). **Multivariate and Geometric Morphometrics in the Analysis of Sexual Dimorphism Variation in *Podarcis* lizards.** Journal of Morphology, 268, 152-165.

Kaliontzopoulou, A., Carettero, M. A., Llorente, G. A., (2008). **Head shape allometry and proximate causes of head shape dimorphism in *Podarcis* lizards: joining linear and geometric morphometrics.** Biological Journal of the Linnean Society, 93, 111-124.

Kaliontzopoulou, A., Carettero, M. A., Llorente, G. A., (2010a). **Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat-related patterns within *Podarcis bocagei* wall lizards**. J. Evol. Biol, 23, 1234-1244.

Kaliontzopoulou, A., Carretero, M. A., Llorente, G. A., (2010b). **Sexual dimorphism in traits related to locomotion: ontogenetic patterns of variation in *Podarcis* wall lizards.** Biological Journal of the Linnean Society, 99(3), 530-543.

Kaliontzopoulou, A., (2011). **Geometric morphometrics in herpetology: modern tools for enhancing the study of morphological variation in amphibians and reptiles.** Basic and Applied Herpetology, 25, 5-32.

Kaliontzopolou, A., Carretero, M., Llorente, G. A., (2012a). **Morphology of the *Podarcis* wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex**. Zoological Journal of the Linnean Society, 164, 173-193.

Kaliontzopoulou, A., Adams, D. C., van der Meijden, A., Perera, A., Carretero, M. A., (2012b). **Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards.** Evolutionary Ecology, 26(4), 825-845.

Keren-Rotem, T. A. Bouskila, Geffen, E., (2006): **Ontogenetic habitat shift and risk of cannibalism in the common chameleon (*Chamaeleo chamaeleon*).** Behavioral Ecology and Sociobiology, 59, 723-731.

Kidd, D. M., Ritchie, M. G, (2000). **Inferring the patterns and causes of geographic variation in *Ephippiger ephippiger* (Orthoptera, Tettigoniidae) using geographical information systems (GIS).** Biological Journal of the Linnean Society, 71(2), 269-295.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P., Beerli, P., (2001). **The strength of phenotypic selection in natural populations.** The American Naturalist, 157(3), 245-261.

Kingsolver, J. G., Huey, R., B., (2003). **Introduction: The Evolution of Morphology, Performance and Fitness.** Intergrative and Comparative Biology, 43, 361-366.

Kingsolver, J., Pfenning, D. W., (2007). **Patterns and power of phenotypic selection in nature**. Bioscience, 57, 561-572.

Kohlsdorf, T., Garland T. Jr., Navas, C. A., (2001). **Limb and tail lenghts in relation to substrate usage in *Tripidurus* lizards**. Journal of Morphlogy., 248, 151-164.

Kratochvíl, L., Fokt, M., Rehák, I., Frynta, D., (2003). **Misinterpretation of character scaling: a tale of sexual dimorphism in body shape of common lizards.** Canadian Journal of Zoology, 81(6), 1112-1117.

Lo Cascio, P., Pasta, S., (2006). **Preliminary data on the biometry and the diet of a micro-insular population of *P.wagleriana* (Reptilia: Lacertidae).** Acta Herpetologica, 1(2), 147-152.

Lomolino, M. V., (1985). **Body size of mammals on islands: the island rule reexamined.** American Naturalist, 310-316.

Lomolino, M. V., (2005). **Body size evolution in insular vertebrates: generality of the island rule.** Journal of Biogeography, 32(10), 1683-1699.

Losos, J. B., (1990a). **Ecomorphology, performance capability and scaling of West Indian *Anolis* lizards: an evolutionary analysis**. Ecological Monographs, 60, 369-388.

Losos, J. B., (1990b). **The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards.** Evolution, 44, 1189-1203.

Losos, J. B, Jackman, T. R., Larson, A., de Queiroz, K., Rodriguez-Schetitino, L., (1998). **Contingency and determinism in replicated adaptive radiation of island lizards**. Science, 279, 2115-2118.

Losos, J. B., Creer, D. A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Ettling, J., (2000). **Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*.** Evolution, 54(1), 301-305.

Losos, J. B., Butler, M., Schoener, T. W., (2003). **Sexual dimorphism in body size and shape in relation to habitat use among species of Caribbean *Anolis* lizards**. Lizard social behaviour. John Hopkins University Press, Baltimore, 356-380.

Losos, J. B., (2009). **Lizards in an evolutionary tree: ecology and adaptive radiation of anoles (Vol. 10).** Univeristy of California Press.

Malhotra, A., Thorpe, R. S., (1997). **Microgeographic variation in scalation of *Anolis oculatus* (Dominica, West Indies): a multivariate analysis.** Herpetologica, 49-62.

Meiri, S., (2007). **Size evolution in island lizards**. Global Ecology and Biogeography, 16(6), 702-708.

Meiri, S., (2008). **Evolution and ecology of lizard body sizes.** Global Ecology and Biogeography, 17(6), 724-734.

Meiri, S., Dayan, T., Simberloff, D., (2004). **Body size of insular carnivores: little support for the island rule.** The American Naturalist, 163(3), 469-479.

Millien, V., (2006). **Morphological evolution is accelerated among island mammals**. PLoS Biology, 4(10), e32.

Moermond, T. C., (1979). **The Influence of Habitat Structure On *Anolis* Foraging Behavior.** Behaviour, 70(1), 147-167.

Mouton, P., L. F., Wyk, J. V., (1993). **Sexual dimorphism in cordylid lizards: a case study of the Drakensberg crag lizard, *Pseudocordylus melanotus*.** Canadian Journal of Zoology, 71(9), 1715-1723.

Nazari, V., Zakharov, E. V., Sperling, F. A., (2007). **Phylogeny, historical biogeography, and taxonomic ranking of Parnassiinae (Lepidoptera, Papilionidae) based on morphology and seven genes.** Molecular phylogenetics and evolution, 42(1), 131-156.

Olsson, M., (1992). **Contest success in relation to size and residency in male sand lizards, *Lacerta agilis***. Animal behaviour, 44, 386-388.

Olsson, M., Shine, R., Wapstra, E., Ujvari, B., Madsen, T., (2002). **Sexual dimorphism in lizard body shape: the roles of sexual selection and fecundity selection**. Evolution, 56(7), 1538-1542.

Pafilis, P., Meiri, S., Foufopoulos, J., Valakos, E., (2009). **Intraspecific competition and high food availability are associated with insular gigantism in a lizard.** Naturwissenschaften, 96(9), 1107-1113.

Pafilis, P., Foufopoulos, J., Sagonas, K., Runemark, A., Svensson, E., Valakos, E. D., (2011). **Reproductive biology of insular reptiles: marine subsidies modulate expression of the "island syndrome**". Copeia, 2011(4), 545-552.

Pianka, E. R., (1986). **Ecology and natural history of desert lizards: analyses of the ecological niche and community structure**. Princeton University Press, Princeton NJ.

Piras, P., Salvi, D., Ferrara, G., Maiorino, L., Delfino, M., Pedde, L., Kotsakis, T., (2011). **The role of post-natal ontogeny in the evolution of phenotypic diversity in *Podarcis* lizards.** Journal of evolutionary biology,24(12), 2705-2720.

Pérez-Mellado V., (1981). **Nuevos datos sobre la sistemática y distribución de Podarcis bocagei (Seoane, 1884)(Sauria, Lacertidae) en la Península Ibérica.** Amphibia-Reptilia, 2(3), 259-265.

Pérez-Mellado, V., (1998). **Género Podarcis (Wagler, 1830).** Reptiles, 242-243.

Petit, R. J., Aguinagalde, I., de Baulieu, J. L., Bittkau C., Brewer S., Cheddali, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Muller-Starck, G., Demesure-Musch, B., Palme, A., Martin, J. P., Rendell S., Vendramin, G. G., (2003). **Glacial refugia: hotspots but not meting pots of genetic diversity**. Science, 300, 1563-1565.

Pianka, E. R., (1986). **Ecology and Natural History of Desert Lizards**. Princeton University Press, Princeston, NJ.

Radovanoviç, M., (1959): **Zur Problem der Speziation bei Inseleidechsen**. Zool. Ib. Syst. 86 (4/5), 395–436.

Radovanoviç M., 1960**: Rezultati ispitivanja na Jadranskim ostrvima u svetlosti evolucionizma**. Glas SANU, Beograd, 243 (20), 93–140.

Raia, P., Guarino, F. M., Turano, M., Polese, G., Rippa, D., Carotenuto, F., Monti, D. M., Cardi, M., Fulgione, D., (2010). The blue lizard spandrel and the island syndrome. BMC evolutionary biology, 10(1), 289.

Rieseber, L. H, Wildmer, A., Arntz, A. M., Burke, J. M., (2002). **Directional selection is the primary cause of phenotypic diversification.** Proceedings of the National Academy of Sciences USA, 99, 12242-12245.

Rohlf, F. J., Marcus, L. F., (1993). **A revolution morphometrics**. Trends in Ecology and Evolution, 8(4), 129-132.

Rohlf, F.J., Slice D. E., (1990a). **Extensions of the Procrustes method for the optimal superimposition of landmark**. Systematic Zoology, 39, 40-59.

Rohlf, F.J., Slice D. E., (1990b). **Morphometrics.** Annual Review of Ecology and Systematics, 21, 299-316.

Rohlf, F.J., (1999). **Shape statistics: Procrustes superimpositions and tangent spaces.** Journal of Classification, 16, 197-223.

Rohlf, F.J., (2004). **tpsUtil, file utility program, version 1.26.** Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J., (2005). **tpsDig, digitize landmarks and outlines, version 2.04.** Department of Ecology and Evolution, State University of New York at Stony Brook.

Rugiero, L., Luiselli, L., (1995). **Food habits of the snake *Coluber viridiflavus* in relation to prey availability.** Amphibia- Reptilia, 16, 407-411.

Runemark, A., Hansson, B., Pafilis, P., Valakos, E. D., Svensson, E. I., (2010). **Island biology and morphological divergence of the Skyros wall lizard *Podarcis gaigeae:* a combined role for local selection and genetic drift on color morph frequency divergence?** BMC evolutionary biology, 10(1), 269.

Runemark, A., Svensson, E. I., (2012). **Sexual selection as a promoter of population divergence in male phenotypic characters: a study on mainland and islet lizard populations.** Biological Journal of the Linnean Society, 106(2), 374-389.

Runemark, A., Brydegaard, M., Svensson, E. I., (2014). **Does relaxed predation drive phenotypic divergence among insular populations?** Journal of evolutionary biology, 27(8), 1676-1690.

Sanders, K. L., Malhotra, A., Thorpe, R. S., (2004). **Ecological diversification in a group of *Indomalayan pitvipers* (Trimeresurus): convergence in taxonomically important traits has implications for species identification**.Journal of Evolutionary Biology, 17(4), 721-731.

Sá-Sousa, P., Almeida, A. P., Rosa, H., Vicente, L., Crespo, E. G., (2000). **Genetic and morphological relationships of the Berlenga wall lizard (*Podarcis bocagei berlengensis:* Lacertidae)**. Journal of Zoological Systematics and Evolutionary Research, 38(2), 95-102.

Sá-Sousa, P., Harris, D. J., (2002). ***Podarcis carbonelli* Pérez-Mellado, 1981 is a distinct species.** Amphibia-Reptilia, 23(4), 459-468.

Sinervo, B., Losos, J. B., (1991). **Walking the tight rope: arboreal sprint performance among *Sceloporus occidentalis* lizard populations.** Ecology, 72, 1225-1233.

Soulé, M., Kerfoot, W. C., (1972). **On the climatic determination of scale size in a lizard.** Systematic Biology, 21(1), 97-105.

Stamps, J., (1983a). **Territoriality and the defence of predator-refuges in juvenile lizards.** Animal Behaviour, 31(3), 857-870.

Stamps, J. A., (1983b). **The relationship between ontogenetic habitat shifts, competition and predator avoidance in a juvenile lizard** *(Anolis aeneus).*Behavioral Ecology and Sociobiology, 12(1), 19-33.

Stone, P. A., Snell, H. L., Snell, H. M., (2003). **Island biogeography of morphology and social behavior in the lava lizards of the Galápagos Islands. Lizard social behavior**. Johns Hopkins University Press, Baltimore, MD, 190-239.

Taylor, E. B., Tamkee, P., Keeley, E. R., Parkinson, E. A., (2011). **Conservation prioritization in widespread species: the use of genetic and morphological data to assess population distinctiveness in rainbow trout (*Oncorhynchus mykiss*) from British Columbia, Canada**. Evolutionary applications, 4(1), 100-115.

Thorpe, R. S., (1987). **Geographic variation: a synthesis of cause, data, pattern and congruence in relation to subspecies, multivariate analysis and phylogenesis**. Italian Journal of Zoology, 54(1), 3-11.

Thorpe, R. S., (1991). **Clines and cause: microgeographic variation in the Tenerife gecko (*Tarentola delalandii*).** Systematic Biology, 40(2), 172-187.

Thorpe, R. S., Baez, M., (1987). **Geographic variation within an island: univariate and multivariate contouring of scalation, size, and shape of the lizard *Gallotia galloti***. Evolution, 256-268.

Thorpe, R. S., Baez, M., (1993). **Geographic variation in scalation of the lizard *Gallotia stehlini* within the island of Gran Canaria.** Biological Journal of the Linnean Society, 48(1), 75-87.

Thorpe, R. S., Brown, R. P., Malhotra, A., Wuster, W., (1991). **Geographic variation and population systematics: distinguishing between ecogenetics and phylogenetics.** Italian Journal of Zoology, 58(4), 329-335

Tokarz, R. R., (1985). **Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*)**. Animal Behaviour, 33(3), 746-753.

Vanhooydonck, B., Van Damme, R., (1999). **Evolutionary relationships between body shape and habitat use in lacertid lizards**. Evolutionary Ecology Research, 1(7), 785-805.

Van Valen, L. (1973). **Body size and numbers of plants and animals.** Evolution, 27-35.

Verwaijen, D., Van Damme, R., Herrel, A., (2002). **Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards.** Functional Ecology, 16(6), 842-850.

Vitt, L. J., Cooper Jr, W. E., (1985). **The evolution of sexual dimorphism in the skink *Eumeces laticeps*: an example of sexual selection**. Canadian Journal of Zoology, 63(5), 995-1002.

Wayne, R. K., (1992). **On the use of morphologic and molecular genetic characters to investigate species status**. Conservation Biology, 590-592.

GENERAL DISCUSSION

The Mediterranean basin has always piqued the investigators' interest due to its composition of species-rich areas that exhibit remarkably high intraspecific diversity (Bilton *et al.*1998, Hewitt 2000, Myers *et al.* 2000, Petit *et al.* 2003). Additionally, the role of the southern Mediterranean peninsulas (Iberian, Balkan, Italian) as glacial refugia during the Pleistocene oscillations has further provoked the investigation leading to considerable knowledge of the Plio-Pleistocene biogeography of these areas including the locations of glacial refugia, hotspots of diversity, post-glacial recolonization routes, patterns of island occupancy and gene exchange between islands and continent (Hewitt 1996; 2000; 2004; 2011, Taberlet *et al.* 1998, Thopson 2005, Nieberding 2006, Schmitt 2007, Stock *et al.* 2008a, Stroscio *et al.* 2011). However, many questions concerning the way Pleistocene historical and microevolutionary events shaped the current biological variation and which mechanisms contributed to the survival of the species during these oscillations still remain unanswered. These questions are particularly interesting because understanding the way the diversity has been molded can assist evolutionary, ecological and conservation prospectives (Hampe & Petit 2005, Canestrelli *et al.* 2010, Feliner 2011) for those areas that may be "threatened" by current climate or human induced environmental changes (Hampe & Petit 2005, Araujo *et al.* 2006, Kier *et al.* 2009). Hewitt (2000) extensively studied the effect of Pleistocene climate oscillations and pinpointed their role in shaping the diversity in many different taxa. Recently, the investigation on coastal refugia and insland species led to the extension of previous "Expansion- Contraction" models intergrating the effect on habitat availability mediated by sea-level changes during glacial/interglacial cycles. Temperate species inhabiting these areas could have maintained stable populations that during glacials may have experienced range or demographic expansions due to increased availability of habitat caused by sea level drops and mild coastal microclimates (Canestrelli *et al.* 2007, Canestrelli & Nascetti 2008, Bisconti *et al.* 2011, Salvi *et al*. 2014).

Our study species, *P.wagleriana*, as a temperate insular species of the Mediterranean basin, endemic to Sicily and therefore subjected to the unique conditions that accompany insular ecosystems, was a particularly interesting case to investigate causal factors of its genetic and morphological variation. The present study revealed high variation both at the genetic and morphological level. Furthermore, the variation appeared to be geographically structured in the genetic level (three distinct genetic clades) and in most of the morphological characters.

The three genetic clades and their dating indicated the existence of three separate Pleistocene glacial refugia, concordant with the “refugia within refugia” paradigm (Gomez & Lunt 2006). The long persistence, historical isolation and allopatric divergence of distinct populations in these refugia probably shaped their genetic structure and played a major role in the formation of intraspecific variation. Demographic expansions and secondary contacts among them led to the observed admixed populations. Under these conditions of isolation in separate refugia natural selection could have acted on certain morphological characters although adaptations and phenotypic plasticity under different selective pressures of the ecological conditions and environmental gradients (temperature, altitude, latitude) seem to have played the most influential role in our case.

The present study demonstrates the value of combining information both from genetic and morphological data to obtain a complete view of the intraspecific variation and of the different evolutionary processes that have shaped it. The phylogeographic approach is certainly useful for exploring the historical factors that generated the geographical distribution of genealogical clades (Avise 2000). However, analysis of morphological variation adds great value in the description and interpretation of patterns of variation as it can detect microevolutionary processes, including local adaptation, that do not always have an historical basis.

Environmental niche modelling can be applied in the future to further determine the conditions under which the genetic and morphological variation of *P. wagleriana* was shaped. In addition to giving insights to past microevolutionary processes, understanding the ecological and environmental requirements of the species can be really powerful for future predictions. Especially, for the smaller, geographically separated populations of the Egadi islets this can be particularly important for a possible conservation assessment.

GENERAL REFERENCES

Adams, D. C., (2004). **Character displacement via aggressive interference in Appalachian salamanders**. Ecology, 85(10), 2664-2670.

Adams, D. C., Berns, C. M., Kozak, K. H., Wiens, J. J., (2009). **Are rates of species diversification correlated with rates of morphological evolution?** Proceedings of the Royal Society of London B: Biological Sciences, doi: 101.1098, rspb-2009.0543.

Araújo, M. B., Thuiller, W., Pearson, R. G., (2006). **Climate warming and the decline of amphibians and reptiles in Europe.** Journal of biogeography, 33(10), 1712-1728.

Arendt, J., (2010). **Morphological correlates of sprint swimming speed in five species of spadefoot toad tadpoles: comparison of morphometric methods**. Journal of morphology, 271(9), 1044-1052.

Arnold, E. N., (1973). **Relationships of the Palaearctic lizards assigned to the genera Lacerta*, Algyroides* and *Psammodromus (Reptilia: Lacertidae)*.** Bulletin of the British Museum (Natural History), 25(8), 289-366.

Arnold, E. N., (2004). **Overview of morphological evolution and radiation in the Lacertidae.** The Biology of Lacertid Lizards. Evolutionary and Ecological Perspectives, 11-36.

Arnold, E. N., Ovenden, D., (2002). **Reptiles and amphibians of Britain and Europe.** Hong-Kong: HarperCollins Publishers.

Arnold, E. N., Arribas, O., Carranza, S., (2007). **Systematics of the Palaearctic and Oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera.** Magnolia Press, Auckland, New Zealand.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C.A., Saunders, N. C., (1987). **Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics**. Annual review of ecology and systematics, 489-522.

Avise, J.C., (2000). **Phylogeography: the history and formation of species**. Harvard University Press, Cambridge, MA.

Ballard, J. W. O., Whitlock, M. C., (2004). **The incomplete natural history of mitochondria**. Molecular ecology, 13(4), 729-744.

Barbadillo, L. J., Lacomba J.I., Perez- Mellado, V., Sancho, V., Lopez-Jurado, L.F., (1999). **Anfibios y reptiles de la Península Ibérica, Baleares y Canarias**. Editorial GeoPlaneta, S.A., Barcelona.

Barton, N. H., Charlesworth, B., (1984). **Genetic revolutions, founder effects, and speciation**. Annual Review of Ecology and Systematics, 133-164.

Barton, N. H., Mallet, J., (1996). **Natural selection and random genetic drift as causes of evolution on islands [and discussion**]. Philosophical Transactions of the Royal Society B: Biological Sciences, 351(1341), 785-795**.**

Beheregaray, L. B., Caccone, A.,(2007). **Cryptic biodiversity in a changing world.** Journal of Biology, 6(9), 5.

Bisconti, R., Canestrelli, D., Colangelo, P., Nascetti, G., (2011**). Multiple lines of evidence for demographic and range expansion of a temperate species (*Hyla sarda*) during the last glaciation.** Molecular Ecology, 20(24), 5313-5327.

Bilton, D. T., Mirol, P. M., Mascheretti, S., Fredga, K., Zima, J., Searle, J. B., (1998). **Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization**. Proceedings of the Royal Society of London B: Biological Sciences, 265(1402), 1219-1226.

Bonfiglio, L., Marra, A. C., Masini, F., (2000). **The contribution of Quaternary vertebrates to palaeoenvironmental and palaeoclimatological reconstructions in Sicily.** Geological Society, London, Special Publications, 181(1), 171-184.

Bonfiglio, L., Agnesi, V., Masini, F., Di Maggio, C., (2004)**. Quaternary eustatic fluctuations and biochronology of vertebrate-bearing deposits correlated with marine terraces in Sicily**. In 32nd International Geological Congress, Field trip Guide Book-B07, Pre-Congress, Italy.

Bonnan, M. F., Farlow, J. O., Masters, S. L., (2008). **Using linear and geometric morphometrics to detect intraspecific variability and sexual dimorphism in femoral shape in *Alligator mississippiensis* and its implications for sexing fossil archosaurs.** Journal of Vertebrate Paleontology, 28(2), 422-431.

Bookstein, F. L., (1982). **Foundations of morphometrics**. Annual Review of Ecology and Systematics, 451-470.

Bookstein, F. L., (1986). **Size and shape spaces for landmark data in two dimensions.** Statistical Science, 181-222.

Bookstein, F. L., (1989). “**Size and shape”: a comment on semantics**.Systematic Biology, 38(2), 173-180.

Bookstein, F. L., (1991). **Morphometric tools for landmark data: geometry and biology.** Cambridge University Press.

Bookstein, F. L., (1996). **Biometrics, biomathematics and the morphometric synthesis.** Bulletin of mathematical biology, 58(2), 313-365.

Brown, W. M., George, M., Wilson, A. C., (1979). **Rapid evolution of animal mitochondrial DNA.** Proceedings of the National Academy of Sciences,76(4), 1967-1971.

Brown, R. P., Thorpe, R. S., (1991). **Within-island microgeographic variation in body dimensions and scalation of the skink *Chalcides sexlineatus,* with testing of causal hypotheses*.*** Biological Journal of the Linnean Society, 44(1), 47-64.

Brown, R. P., Terrasa, B., Pérez-Mellado, V., Castro, J. A., Hoskisson, P. A., Picornell, A., Ramon, M. M., (2008). **Bayesian estimation of post-Messinian divergence times in Balearic Island lizards**. Molecular Phylogenetics and Evolution, 48(1), 350-358.

Busack, S. D., Lawson, R., Arjo, W. M., (2005). **Mitochondrial DNA, allozymes, morphology and historical biogeography in the *Podarcis vaucheri* (Lacertidae) species complex**. Amphibia-Reptilia, 26(2), 239-256.

Canestrelli, D., Cimmaruta, R., Nascetti, G., (2007). **Phylogeography and historical demography of the Italian treefrog, *Hyla intermedia*, reveals multiple refugia,**

population expansions and secondary contacts within peninsular Italy. Molecular Ecology, 16(22), 4808-4821.

Canestrelli, D., Nascetti, G., (2008). **Phylogeography of the pool frog *Rana (Pelophylax) lessonae* in the Italian peninsula and Sicily: multiple refugia, glacial expansions and nuclear–mitochondrial discordance**. Journal of Biogeography, 35(10), 1923-1936.

Canestrelli, D., Aloise, G., Cecchetti, S., Nascetti, G., (2010). **Birth of a hotspot of intraspecific genetic diversity: notes from the underground.** Molecular Ecology, 19(24), 5432-5451.

Capula, M., Nascetti, G., Lanza, B., Bullini, L., (1987). ***Podarcis sicula* and *P. wagleriana* in the Aeolian Archipelago (Sicily): preliminary data (Reptilia: Lacertidae).**Museo regionale di Scienze Naturali Bolettino (Torino), 5(1), 35-43.

Capula, M., (1990). **Ricerche sulla struttura genetica di *Podarcis sicula, P. wagleriana* e *P. filfolensis* (Reptilia, Lacertidae): aspetti tassonomici ed evolutiv*i* (**Doctoral dissertation, PhD Thesis, University of Bologna).

Capula, M., (1993). **Natural hybridization in *Podarcis sicula* and *P. wagleriana* (Reptilia: Lacertidae).** Biochemical systematics and ecology, 21(3), 373-380.

Capula, M., (1994a). **Evolutionary relationships of *Podarcis l*izards from Sicily and the Maltese Islands.** Journal of zoological Systematics and evolutionary Research, 32(3), 180-192.

Capula, M., (1994b). **Genetic variation and differentiation in the lizard, *Podarcis wagleriana* (Reptilia: Lacertidae*).*** Biological Journal of the Linnean Society, 52(2), 177-196.

Carretero, M. A., Barbosa, D., Ribeiro, R., Sá-Sousa, P., Harris, D. J., (2003). **Assessing competition by means of fluctuating asymmetry in *Podarcis*.** In12th Ordinary General Meeting, Societas Europaea Herpetologica. St. Petersburg, Russia, 12-16 August 2003.

Carlson, S. J., (1989). **The articulate brachiopod hinge mechanism: morphological and functional variation.** Paleobiology, 364-386.

Carretero, M. A., (2008). **An integrated assessment of a group with complex systematics: the Iberomaghrebian lizard genus *Podarcis* (Squamata, Lacertidae).** Integrative Zoology, 4, 247-266.

Corti, C., Cascio, P. L., (2002). **The lizards of Italy and adjacent areas.** (Vol. 14). Edition Chimaira.

Clegg, S. M., Degnan, S. M., Moritz, C., Estoup, A., Kikkawa, J., Owens, I. P., (2002). **Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird.** Evolution, 56(10), 2090-2099.

Darwin, C., Wallace, A., (1858). **On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection.** Journal of the proceedings of the Linnean Society of London. Zoology, 3(9), 45-62.

DeWitt, T. J., Scheiner, S. M. (Eds.)., (2004). **Phenotypic plasticity: functional and conceptual approaches.** Oxford: Oxford University Press, 98-111.

Feliner, G.N., (2011). **Southern European glacial refugia: a tale of tales**. Taxon, 365-372.

Ferrand de Almeida, N., Ferrand de Almeida, P., Goncales, H., Sequeira, F., Teixeira, J., Ferrand de Almeida, F., (2001). **Anfíbios e répteis de Portugal**. FAPAS, Fundo para a proteccao dos Animais Selvagens, Porto.

Frankham, R., (1997). **Do island populations have less genetic variation than mainland populations?** Heredity, 78(3), 311-327.

Gavrilets, S., Hastings, A., (1996). **Founder effect speciation: a theoretical reassessment.** American Naturalist, 466-491.

Geniez, P., Cluchier, A., Sá-Sousa, P., Guillaume, C. P., Crochet, P. A., (2007). **Systematics of the *Podarcis hispanicus*-complex (Sauria, Lacertidae) I: redefinition, morphology and distribution of the nominotypical taxon.** The Herpetological Journal, 17(2), 69-80.

Geniez, P., Sa-Sousa, P., Guillaume, C.P., Cluchier, A., Crochet, P., (2014). **Systematics of the *Podarcis hispanicus* complex (Sauria, Lacertidae) III: valid nomina of the western and central Iberian forms.** Zootaxa, 3794(1), 001-051.

Giovannotti, M., Cerioni, P.N., Kalboussi, M., Aprea, G., Caputo, V., (2007). **Phylogeographic Inferences from the mtDNA Variation of the Three-Toed Skink.** Journal of Experimental Zoology (Mol Dev Evol), 308, 297-307.

Givnish, T. J., Evans, T.M., Zjhra, M. L., Patterson, T. B., Berry, P. E.,Sytsma, K. J., (2000). **Molecular evolution, adaptive radiation, and geographic diversification in the amphiatlantic family Rapateaceae: evidence from ndh F sequences and morphology**. Evolution, 54(6), 1915-1937.

Godinho, R., Crespo, E. G., Ferrand, N., (2008). **The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers.** Molecular Ecology, 17(21), 4670-4683.

Gómez, A., Lunt, D. H., (2006**). Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula**. Phylogeography of southern European refugia, Springer Netherlands, 155-188.

Guillaume, C. P., (1976). **Etude biométrique des espèces *Lacerta hispanica* Steindachner 1870 et Lacerta muralis Laurenti 1768.** Bulletin de la Société Zoologique da France, 101(3), 489-502.

Hampe, A., Petit, R. J., (2005). **Conserving biodiversity under climate change: the rear edge matters**. Ecology letters, 8(5), 461-467.

Harris, D. J., Arnold, E. N., (1999). **Relationships of wall lizards, *Podarcis* (Reptilia: Lacertidae) based on mitochondrial DNA sequences**. Copeia, 749-754.

Harris, D. J., Carranza, S., Arnold, E. N., Pinho, C., Ferrand, N., (2002). **Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar**. Journal of Biogeography, 29(9), 1257-1262.

Harris, D. J., Pinho, C., Carretero, M. A., Corti, C., Böhme, W., (2005). **Determination of genetic diversity within the insular lizard *Podarcis tiliguerta***

using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*. Amphibia-Reptilia, 26(3), 401-407.

Hewitt, G. M., (1996). **Some genetic consequences of ice ages, and their role in divergence and speciation**. Biological Journal of the Linnean Society, 58, 247-276.

Hewitt, G. M., (2000). **The genetic legacy of the Quaternary ice ages.** Nature, 405, 907-913

Hewitt, G. M., (2001). **Speciation, hybrid zones and phylogeography—or seeing genes in space and time**. Molecular ecology, 10(3), 537-549.

Hewitt, G. M., (2004). **Genetic consequences of climatic oscillations in the Qauternary.** Transaction of the Royal Society of London: B., 359, 183-195.

Hewitt, G. M., (2011). **Quaternary phylogeography: the roots of hybrid zones**. Genetica, 139(5), 617-638.

Hickerson, M. J., Carstens, B. C., Cavender-Bares, J., Crandall, K. A., Graham, C. H., Johnson, J. B., Rissler, L., Victoriano, P. F., Yoder, A. D., (2010). **Phylogeography's past, present, and future: 10 years after.** Molecular Phylogenetics and Evolution, 54(1), 291-301.

Kaliontzopoulou, A., Carretero, M. A., Llorente, G. A., (2005). **Differences in the pholidotic patterns of *Podarcis bocagei* and *P. carbonelli* and their implications for species determination**. Revista Española de Herpetología,19,71-86.

Kaliontzopoulou, A., Carretero, M. A., Llorente, G.A., (2008). **Head shape allometry and proximate causes of head sexual dimorphism in *Podarcis* lizards: joining linear and geometric morphometrics**. Biological Journal of the Linnean Society, 93(1), 111-124.

Kaliontzopoulou, A., Carretero, M. A., Llorente, G. A., (2012). **Morphology of the *Podarcis w*all lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex**. Zoological Journal of the Linnean Society, 164(1), 173-193.

Kendall, D. G., (1984). **Shape manifolds, procrustean metrics, and complex projective spaces.** Bulletin of the London Mathematical Society, 16(2), 81-121.

Kendall, D. G., (1985). **Exact distributions for shapes of random triangles in convex sets.** Advances in applied probability, 308-329.

Kier, G., Kreft, H., Lee, T. M., Jetz, W., Ibisch, P. L., Nowicki, C., Mutke, J., Barthlott, W., (2009)**. A global assessment of endemism and species richness across island and mainland regions**. Proceedings of the National Academy of Sciences, 106(23), 9322-9327.

Kingman, J. F., (1982). **On the genealogy of large populations**. Journal of Applied Probability, 27-43

Krijgsman, W., Hilgen, F. J., Raffi, I., Sierro, F. J., Wilson, D. S., (1999). **Chronology, causes and progression of the Messinian salinity crisis**. Nature, 400(6745), 652-655.

Lanza, B., (1973). **Gli Anfibi ei Rettili delle isole circumsiciliane.** Lavori de la Societa Italiana di Biogeografia, 3, 755-804.

Lerner, H. R., Mindell, D. P., (2005). **Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA**. Molecular phylogenetics and evolution, 37(2), 327-346.

Loy, A., Ciccotti, E., Ferrucci, L., Cataudella, S., (1996). **An application of automated feature extraction and geometric morphometrics: temperature-related changes in body form of *Cyprinus carpio* juveniles.** Aquacultural Engineering, 15(4), 301-311.

Lo Valvo, F., Massa, B., (1999). **Lista commentata dei vertebrati terrestri della Riserva Naturale Orientata "Isole dello Stagnone" (Sicilia). Naturalista Sicil**. 23 ,(4),(3-4), 419-266.

Lymberakis, P., Poulakakis, N., Kaliontzopoulou, A., Valakos, E., Mylonas, M., (2008). **Two new species of Podarcis (Squamata; Lacertidae) from Greece.** Systematics and Biodiversity, 6(3), 307-318.

MacArthur, R. H., Diamond, J. M., Karr, J. R., (1972). **Density compensation in island faunas**. Ecology, 330-34.

Malhotra, A.,Thorpe, R. S., (1997). **Microgeographic variation in scalation of *Anolis oculatus* (Dominica, West Indies): a multivariate analysis**. Herpetologica, 49-62.

Marcus, L. F., (1990). **Traditional morphometrics**. In Proceedings of the Michigan morphometrics workshop (Vol. 2, p. 77ą122). Special Publication.

Mappes, T., Grapputo, A., Hakkarainen, H., Huhta, E., Koskela, E., Saunanen, R., Suorsa, P., (2008). **Island selection on mammalian life-histories: genetic differentiation in offspring size**. BMC evolutionary biology, 8(1), 296.

Maura, M., Salvi, D., Bologna, M. A., Nascetti, G., Canestrelli, D., (2014). **Northern richness and cryptic refugia: phylogeography of the Italian smooth newt Lissotriton *vulgaris meridionalis***. Biological Journal of the Linnean Society, 113(2), 590-603.

Michaux, J. R., Libois, R., Filippucci, M. G., (2005). **So close and so different: comparative phylogeography of two small mammal species, the Yellow-necked fieldmouse *(Apodemus flavicollis*) and the Woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region.** Heredity, 94(1), 52-63.

Mitteroecker, P., Gunz, P., (2009). **Advances in geometric morphometrics.** Evolutionary Biology, 36(2), 235-247.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., Kent, J., (2000). **Biodiversity hotspots for conservation priorities**. Nature, 403(6772), 853-858.

Nieberding, C., Morand, S., Libois, R., Michaux, J. R., (2006). **Parasites and the island syndrome: the colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845).** Journal of Biogeography, 33(7), 1212-1222.

Oliverio, M., Bologna, M. A., Mariottini, P., (2000). **Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae).** Journal of Biogeography, 27(6), 1403-1420.

Petit, R. J., Aguinagalde, I., de Beaulieu, J. L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Muller-Starck, G., Demesure-Musch, B., Palme, A., Martin, J. P., Rendell, S., Vendramin, G. G., (2003). **Glacial refugia: hotspots but not melting pots of genetic diversity.** Science, 300(5625), 1563-1565.

Pierre, C., Caruso, A., Blanc-Valleron, M. M., Rouchy, J. M., Orzsag-Sperber, F., (2006). **Reconstruction of the paleoenvironmental changes around the Miocene–Pliocene boundary along a West–East transect across the Mediterranean.** Sedimentary Geology, 188, 319-340.

Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E., (2003). **Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae).** Molecular Phylogenetics and Evolution, 28(1), 38-46.

Poulakakis, N., Lymberakis, P., Valakos, E., Zouros, E., Mylonas, M., (2005). **Phylogenetic relationships and biogeography of *Podarcis* species from the Balkan Peninsula by Bayesian and maximum likelihood analyses of mitochondrial DNA sequences**. Molecular Phylogenetics and Evolution, 37(3), 845-857.

Rohlf, F. J., (1986). **Relationships among eigenshape analysis, Fourier analysis, and analysis of coordinates.** Mathematical Geology, 18(8), 845-854.

Rohlf, F. J., Marcus, L. F., (1993)**. A revolution morphometrics**. Trends in Ecology and Evolution, 8(4), 129-132.

Rosenberg, N. A., Nordborg, M., (2002). **Genealogical trees, coalescent theory and the analysis of genetic polymorphisms**. Nature Reviews Genetics, 3(5), 380-390.

Ryan, W. B. (2009). **Decodin the Mediterranean salinity crisis.** Sedimentology, 56(1), 95-136.

Salemi, M., Vandamme, A. M., (2003). **The phylogenetic handbook: a practical approach to DNA and protein phylogeny.** Cambridge University Press.

Salvador, A., (coord.)(1997). **Reptiles.** In: Fauna Iberica, Vol.10, Ramos, M.A. *et al.* (eds.). Museo Nacional de Ciencias Naturales, CSIC, Madrid.

Salvi, D., Capula, M., Bombi, P., Bologna, M. A., (2009). **Genetic variation and its evolutionary implications in a Mediterranean island endemic lizard**. Biological Journal of the Linnean Society, 98(3), 661-676.

Salvi, D., Harris, D. J., Perera, A., Bologna, M. A., Carretero, M. A., (2011). **Preliminary survey on genetic variation within the *Pygmy Algyroides, Algyroides fitzingeri,* across Corsica and Sardinia.** Amphibia-Reptilia, 32(2), 281-286.

Salvi, D., Harris, D. J., Kaliontzopoulou, A., Carretero, M. A., Pinho, C., (2013). **Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard.** BMC, evolutionary biology, 13(1), 147.

Salvi, D., Schembri, P. J., Sciberras, A., Harris, D. J., (2014)**. Evolutionary history of the Maltese wall lizard *Podarcis filfolensis*: insights on the 'Expansion–**

Contraction'model of Pleistocene biogeography. Molecular ecology, 23 (5), 1167-1187.

Sanmartín, I., (2003). **Dispersal vs. vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea**).Journal of Biogeography, 30(12), 1883-1897.

Sá-Sousa, P., Harris, D. J., (2002). ***Podarcis carbonelli*** **Pérez-Mellado, 1981 is a distinct species**. Amphibia-Reptilia, 23(4), 459-468.

Schluter, D., (2000). **The ecology of adaptive radiation**. Oxford University Press.

Schmitt, T., (2007). **Molecular biogeography of Europe: Pleistocene cycles and postglacial trends.** Frontiers in zoology, 4(11), 1-13.

Seddon, J. M., Santucci, F., Reeve, N. J., Hewitt, G. M., (2001). **DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes.** Molecular Ecology, 10(9), 2187-2198.

Selander, R. K., Whittam, T. S., (1983). **Protein polymorphism and the genetic structure of populations.** Evolution of genes and protein. Sunderland, MA: Massachussets Sinauer Associates Inc, 89-114.

Sheldon, F. H., Whittingham, L. A., Moyle, R. G., Slikas, B., Winkler, D. W., (2005). **Phylogeny of swallows (Aves: Hirundinidae) estimated from nuclear and mitochondrial DNA sequences**. Molecular phylogenetics and evolution, 35(1), 254-270.

Slice, D. E., (2005). **Modern morphometrics in physical anthropology** (Vol. 6). Springer Science & Business Media.

Sokal, R. R., Rohlf, F. J., (1995). **Biometry: the principles andpractice of statistics in biological research**. WH. Freeman & Co., San Francisco. SokalBiometry: the principles and practice of statistics in biological research1995.

Soulé, M., (1976). **Allozyme variation: its determinants in space and time**. Molecular evolution, 60-77.

Stöck, M., Dubey, S., Klütsch, C., Litvinchuk, S. N., Scheidt, U., Perrin, N., (2008). **Mitochondrial and nuclear phylogeny of circum-Mediterranean tree frogs from the *Hyla arborea* group.** Molecular Phylogenetics and Evolution,49(3), 1019-1024.

Stroscio, S., Baviera, C., Frati, F., Lo Paro, G., Nardi, F., (2011**). Colonization of the Aeolian Islands by *Pimelia rugulosa rugulosa* Germar, 1824 (Coleoptera: Tenebrionidae) inferred from the genetic structure of populations: geological and environmental relations**. Biological Journal of the Linnean Society, 104(1), 29-37.

Stuessy, T. F., Jakubowsky, G., Gómez, R. S., Pfosser, M., Schlüter, P. M., Fer, T., Sun, B., Kato, H., (2006). **Anagenetic evolution in island plants.** Journal of Biogeography, 33(7), 1259-1265.

Taberlet, P, Fumagalli, L., Wust-Saucy, A.G., Cosson, J. F., (1998). **Comparative phylogeography and postglacial colonization routes in Europe.** Molecular ecology, 7(4), 453-464.

Thompson, J. D., (2005). **Plant evolution in the Mediterranean.** Oxford: Oxford University Press.

Thorpe, R. S., Baez, M., (1987). **Geographic variation within an island: univariate and multivariate contouring of scalation, size, and shape of the lizard *Gallotia galloti*.** Evolution, 256-268.

Thorpe, R. S., Baez, M., (1993). **Geographic variation in scalation of the lizard *Gallotia stehlini* within the island of Gran Canaria**. Biological Journal of the Linnean Society, 48(1), 75-87.

Toews, D. P., Brelsford, A., (2012). **The biogeography of mitochondrial and nuclear discordance in animals.** Molecular Ecology, 21(16), 3907-3930.

Valenzuela, N., Adams, D.C., Bowden, R. M., Gauger, A.C., (2004). **Geometric morphometric sex estimation for hatchling turtles: a powerful alternative for detecting subtle sexual shape dimorphism.** Copeia, 2004(4), 735-742.

Wiens, J. J., (2007). **Species delimitation: new approaches for discovering diversity.** Systematic Biology, 56(6), 875-878.

Wright, S., (1978). **Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations**. Chicago, IL: University of Chicago Press.

Zangari, F., Cimmurata, R.,Nascetti, G., (2006). **Genetic relationships of the western Mediterranean painted frogs based on allozymes and mitochondrial markers: evolutionary and taxonomic inferences (Amphibia, Anura, Discoglossidae).** Biological Journal of the Linnean Society, 87(4), 515-536.

Zelditch, M. L., Swiderski, D. L., Sheets, H. D., (2012). **Geometric morphometrics for biologists: a primer.** Academic Press.

Zhang, D. X., Hewitt, G. M., (2003). **Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects.** Molecular ecology, 12(3), 563-584.