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**Mainland and  
Insular Lacertid Lizards:  
a Mediterranean Perspective**

Edited by  
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# Introduction

Lacertid lizards have long been a fruitful field of scientific enquiry with many people working on them over the past couple of hundred years. The scope of the field has steadily increased, beginning with taxonomy and anatomy and gradually spreading so that it includes such topics as phylogenetics, behaviour, ecology, and conservation. All these now coexist side by side, resulting in a lot of reciprocal illumination. The series of symposia on lacertid lizards of the Mediterranean basin which have taken place every three years since 1992 have been an important forum for such interactions and a stimulus to further investigations. This volume, which stems from the 2004 meeting in the Aeolian Islands, fulfils both these roles.

One theme that has been developed over the years and was particularly emphasised in 2004 is the insular populations of lacertids in the Mediterranean. Large numbers of these exist and are important for a number of reasons. Island lizard communities are simpler than those on mainlands and at least appear to be more easily understood. Insular lacertids also provide a useful comparison with other more famous island lizard groups, like *Anolis* iguanids in the Caribbean. When these two sets of communities are considered, along with those on other archipelagos, such as the Canaries, Cape Verdes and Mascarenes, it is apparent that there is no simple repeating story. For example, Mediterranean lacertids lack the high species numbers found on the larger Caribbean islands. Whereas these sometimes have dozens of species of *Anolis*, it seems unlikely that even the largest Mediterranean islands had more than four or so species, even before they were colonised by people. Mediterranean islands also lack the giant lizards that occurred in many other archipelagos and sometimes survive today. The differences between these various island groups appears to a considerable extent to be the result of history, both of the islands and of the *taxa* concerned.

In the present volume a wide range of island topics are considered, including the systematics of the species concerned, from both morphological and molecular viewpoints, interaction with other *taxa*, and conservation. The last topic is especially important, as island lizards across the world have often been vulnerable to extinction, once they came into contact with people and the animals they introduced. In the Mediterranean area, classical examples are *Podarcis lilfordi* in the eastern Balearics, and *Podarcis raffonei* on the Aeolian Islands. The main apparent causes of these two partial extinction events have turned out to be different, respectively introduced predators and interaction with a competitor, *P. sicula*, which also hybridises with the threatened species. Understanding such factors is of course an essential first and necessary step to controlling them.

The volume also has papers on the more positive aspects of human influence, specifically the benign effects of traditional agriculture on at least some reptile species. Olive trees, cork oaks and the banks and walls of loose rocks that crisscross the Mediterranean scene all often contribute to elevated lizard populations.

Nor is more basic biology neglected and there are articles on morphology, reproduction, development and thermoregulation. Finally, it is good to see one paper on non-Mediterranean species is included. For, to fully understand the lacertids of this region, it is necessary to appreciate their close relatives in Africa, Asia and the archipelagos of the northeastern Atlantic Ocean.

An important additional point that should be clearly appreciated at this place is the role of the senior editor of this book, Claudia Corti. As well as to her primary editorship, a large task in itself she is coauthoring no less than five original papers out of 16 contained in this book. This demonstrates the continuing high degree of energy she is able to invest in this fascinating field of research. Moreover, it was she who started, by inviting a small number of colleagues to Florence, the first initiative that gave rise to the origin of this successful working group. The consequence was the first Mediterranean lacertid meeting on Lesbos Island, Greece, in 1992, followed by many others, all of them resulting in a nice proceedings volume like the present one. This activity which is not only dealing with European lizards but which is a truly European initiative, cannot be estimated highly enough.

Most promising is that the next Mediterranean lacertid meeting which is scheduled for June 2008 will be held again in Greece. It will certainly be as successful and prolific in terms of increasing our knowledge on Mediterranean lacertids in all their facets outlined above, as its many predecessors.

London & Bonn, October 2006

Nick Arnold & Wolfgang Böhme



# Preface

In the charming scenario of the Castle of Lipari Island – carrying traces of 7,000 years of Mediterranean history – the prestigious Archaeological Museum hosted the “5th Symposium on the Lacertids of the Mediterranean Basin”. The Sicilian Regional Council kindly offered us this unique venue which was the only one feasible for hosting an international meeting on the island. Indeed the Museum could well represent the site where Mediterranean natural history crosses human history. The history of the Mediterranean is based on this *crossroad* where landscape dynamics and structures, faunal and floristic composition, as well as changes in biodiversity have been smoothed over time.

The Aeolian Archipelago well embodies this metaphor. One of the most threatened species of the Mediterranean herpetofauna, *Podarcis raffonei*, occurs on these islands, where it survives in very small fragments of territory not suitable for exploitation. This species also represents a paradigm: even if the risk of an imminent extinction is well documented, it is not yet included in any annex of the EU Directive 43/92, as well as the islets where it occurs are still seriously unprotected. More work is still therefore needed to fill the gap between science and conservation.

Current trends in herpetological researches dedicated to conservation are gaining appropriate space by taxonomical, zoogeographical, biological and ecological studies, which have been improved since the first edition of the Symposiums: Greece (1992), Portugal (1995), Croatia (1998), Spain (2001), and Italy (2004). We hope that several other meetings will take place in the future and that an increasing number of colleagues will improve the knowledge on the Lacertids of the Mediterranean.

It is a pleasure for us to present “*Mainland and Insular Lacertid Lizards: a Mediterranean Perspective*”, which includes reviewed articles submitted to the 5th International Symposium on the Lacertids of the Mediterranean Basin. We would like to express our sincere gratitude to all the participants, as well as the institutions and/or associations which provided us with their support: Societas Herpetologica Italica, Presidenza della Regione Siciliana, Provincia Regionale di Messina, Museo Archeologico Regionale “L. Bernabò Brea”, Riserva Naturale Orientata “Le Montagne delle Felci e dei Porri”, Società Elettrica Liparese, Associazione Nesos. We would like also to thank Francesca Graziani, Giuseppe Allegrino, Emanuele Paggetti, for their help during the Symposium; Riccardo Gullo, for his warm hospitality; Maria Letizia Molino, for the great care shown under several circumstances; the referees which kindly reviews all the

papers, Baldo Conti and Riccardo Petrini, for their important help during the final editing of the volume and Cinzia Giuliani for the last graphic corrections.

Marta Biaggini, Claudia Corti & Pietro Lo Cascio

## Prefazione

*Nel suggestivo scenario del Castello dell'Isola di Lipari – le cui mura recano i segni di 7000 anni di storia mediterranea – un prestigioso museo archeologico ha ospitato per alcuni giorni il “5th Symposium on the Lacertids of the Mediterranean Basin”. La scelta di questa sede si deve alla gentile opportunità concessa dalla Regione Siciliana, che ha messo a disposizione l'unica struttura esistente nell'isola adatta a ospitare un congresso internazionale; ma si presta bene, se vogliamo, a incarnare la metafora della storia naturale che incontra la storia dell'uomo. E su questo incontro, sui suoi intensi interscambi, si basa la storia del Mediterraneo, la struttura e la dinamica dei suoi paesaggi, la composizione delle sue faune e delle sue flore. Talvolta, anche la perdita di biodiversità, forse qui apparentemente meno drammatica che in altri luoghi, perché attutita dal tempo e nel tempo.*

*In questo senso, anche la scelta di Lipari e dell'Arcipelago Eoliano incarna una metafora. Queste isole ospitano una delle specie più minacciate dell'erpetofauna mediterranea, che sopravvive in piccoli lembi di territorio mai sfruttati dall'uomo. E anche un paradigma: il rischio di imminente estinzione per Podarcis raffonei è documentato da seri contributi scientifici, e tuttavia la specie non è ancora stata inclusa in allegato alla Direttiva 43/92, né gli isolotti dove sono ancora presenti le sue poche popolazioni risultano soggetti a concrete misure di tutela. Questo ci racconta come molto lavoro ancora sia necessario per ottenere importanti risultati nel raccordo tra conoscenze scientifiche e applicazioni nella conservazione.*

*Parte delle attuali tendenze nelle ricerche erpetologiche, dedicate alla conservazione della specie e dei loro habitat, trova infatti oggi opportunamente spazio accanto agli studi tassonomici, zoogeografici, biologici ed ecologici, che hanno caratterizzato dall'inizio e con un crescente livello di interesse le ormai numerose edizioni di questo Symposium: dopo la Grecia (1992), il Portogallo (1995), la Croazia (1998), la Spagna (2001) e l'Italia (2004), ci auguriamo che molte altre occasioni ci vedano riuniti, insieme a un sempre maggiore numero di colleghi, per fare “il punto” delle conoscenze disponibili sui Lacertidi dell'area mediterranea.*

*Siamo dunque lieti di presentare i risultati in un volume dove, per precisa scelta editoriale, vengono pubblicati lavori che sono stati sottoposti a revisione e al quale abbiamo voluto dare il titolo di “Mainland and Insular Lacertid Lizards: a Mediterranean Perspective”. La nostra gratitudine va a tutti i partecipanti e a chi ha reso possibile, a vario titolo, l'organizzazione e lo svolgimento del 5th International Symposium on the Lacertids of the Mediterranean Basin: Societas Herpetologica Italica, Presidenza della Regione Siciliana, Provincia Regionale di Messina, Museo Archeologico Regionale “L. Bernabò Brea”, Riserva Naturale Orientata “Le Montagne delle Felci e dei Porri”, Società Elettrica Liparese, Associazione Nesos. Un sentito ringraziamento va ancora a Francesca Graziani, Giuseppe Allegrino, Emanuele Paggetti, per la loro collaborazione durante il simposio; a Riccardo Gullo, per la sua cordiale ospitalità; a Maria Letizia Molino, per la sensibilità e l'attenzione dimostrate in più di un'occasione; ai colleghi che hanno provveduto al controllo dei testi, a Baldo Conti e Riccardo Petrini, per il significativo contributo dato alla fase definitiva di editing e Cinzia Giuliani per le indispensabili correzioni apportate alle figure.*

Marta Biaggini, Claudia Corti & Pietro Lo Cascio



# Morphology, mitochondrial DNA and the relationships of the Zagros lizard, *Lacerta zagrosica* Rastegar-Pouyani and Nilson 1998 (Squamata: Lacertidae)

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*Lacerta zagrosica*, which inhabits rock exposures at altitudes of over 2000 m in the Zagros mountains, is described more fully, including skeletal, hemipenial and other internal features. It lacks the distinctive features of *Omanosaura* and other *Eremiinae*, and a phylogenetic analysis of morphological data of the *Lacertinae*, based on 64 characters (equivalent to 83 binary ones), fails to associate it with any of the other taxa. But 620 bp of mitochondrial DNA (fragments of the *cyt b* and 12S rRNA genes) place *L. zagrosica* firmly and convincingly with its geographical neighbour, the ground-dwelling *Lacerta brandtii*, although it shows a divergence of 10% from this species. Morphological differences from *L. brandtii* are considerable and may be largely related to the different functional demands of the habitats of the two species.

Keywords: morphology, mitochondrial DNA, relationship, *Lacerta zagrosica*.

## INTRODUCTION

The Zagros lizard, *Lacerta zagrosica* Rastegar-Pouyani & Nilson 1998, was described from high altitudes, above 2000 m, in the Zagros Mountains of western Iran and is also discussed by Nilson *et al.* (2003). Among its more distinctive external features are a flattened head and body, single postnasal scale, masseteric scale sometimes discernible, six or seven pairs of chin shields, maximum of 10 enlarged ventral scales across the mid-body, and digits strongly compressed laterally. Relationships of *L. zagrosica* are unclear. Its habitat and some aspects of its anatomy, including its flattened head and body, suggest it may related to other rock-dwelling *taxa* in the *Lacertinae*,

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of which the geographically closest are members of *Darevskia* (formerly termed the *Lacerta saxicola* group) in northern Iran and the Caucasus. Alternatively, it could possibly have affinities with *Omanosaura*, a member of the Eremiinae that is found in the northern Oman area of Arabia, to the south of the range of *L. zagrosica*, and also includes a rock-dwelling form, *O. cyanura*.

To provide a broader basis for assessing the relationships of *L. zagrosica*, its morphology has been investigated more thoroughly than before, particularly the internal anatomy. In addition, 620 base pairs of mitochondrial DNA (291 base pairs of cytochrome b and 329 base pairs of 12S rRNA) of *L. zagrosica* have been compared with homologous DNA sequence of a wide range of lacertid lizards including 56 species in the Lacertinae. Methods used for DNA extraction, amplification, and phylogenetic analysis, employing Maximum Parsimony and Maximum Likelihood approaches, are described by Carranza *et al.* (2004).

## MORPHOLOGY OF *LACERTA ZAGROSICA*

Many external features of *L. zagrosica* have already been described (Rastegar-Pouyani & Nilson 1998, Nilson *et al.* 2003). The anatomical terms used here are explained by Arnold (1973, 1989).

*Material examined.* 3 km northwest of Fereydun Sahr, about 140 km northwest of Esfahan city, Esfahan province, west-central Iran (32°58'N; 50°04'E). GNM Re.ex. 5614, female; GNM Re.ex 5615, male (GNM = Göteborg Natural History Museum, Sweden).

*Size and shape.* Up to about 68 mm from snout to vent; head and body strongly depressed; digits laterally compressed, and toes 3-5 on hind feet kinked in the vertical plane.

*Skull.* About seven premaxillary teeth in adults; pterygoid teeth apparently absent; nasal process of premaxilla slender; septomaxilla without anterior and posterior processes; postorbital and postfrontal bones separated and postorbital short; maxillary-jugal suture not stepped. Supraocular osteoderms incomplete and fenestrated in adults; no ossification of temporal scales.

*Postcranial skeleton.* 26 presacral vertebrae in male, 27 in female; 7 posterior presacral vertebrae with short ribs; medial loop of clavicle continuous or interrupted posteriorly; interclavicle cruciform with the arms more or less perpendicular to the sagittal axis; sternal fontanelle oval; a single pair of inscriptional ribs; 5 or 6 pygal vertebrae; pattern of tail vertebrae AB type, with one to three anterior autotomic vertebrae that have a large pair of transverse processes and a smaller posterior one, the two pairs being parallel to each other and perpendicular to the main axis of the tail.

*Scaling.* Rostral separated from frontonasal scale; row of supraciliary granules complete; outer edge of parietal scale not reaching the lateral border of the parietal table of skull. Nostril in contact with first upper labial scale; a single postnasal scale; no contact between supranasal and anterior loreal scales above nostril; five upper labial scales in front of subocular; no transparent window in lower eyelid; first upper temporal scale may be large; masseteric scale sometimes discernible. Six or seven chin shields on each side, if seven, the last two arranged laterally.

Dorsal scales small, flattish and smooth, 58-61 in a transverse row across mid-body. Collar smooth-edged; eight complete longitudinal rows of rectangular, non-imbricate, ventral scales, with sometimes a short additional row on each side at mid-body, raising the maximum number of ventral scales across belly to ten in at least some individuals; preanal scale broad and sometimes triangular, bordered in front by one or two semicircles of smaller scales; median longitudinal rows of scales under the tail not laterally expanded. Toes strongly laterally compressed, a single row of narrow tubercular scales ventrally and often a partial posterior row as well.

*Colouring.* Details are given elsewhere (Rastegar-Pouyani & Nilson 1998, Nilson *et al.* 2003). Distinctive features include: no narrow light stripes on dorsum, concolor morph absent; ocelli present in shoulder region and often along flanks, at least in males; underside with dark spots and often blue, the throat undifferentiated.

*Internal features.* Nasal vestibule not elongate. Primitive 'Lacertide' pattern of ulnar nerve, in which it is completely independent of the brachial trunk and the nerves originating from it, and follows a superficial course in the lower limb.

*Hemipenis.* Lobes with plicae, the apical section of each longer than the basal one, sulcal lips on lobe sulci small; no armature or folding of the lobes in the retracted hemipenis; microornamentation consisting of crown-shaped tubercles.

*Chromosomes.* Not yet studied.

*Reproduction.* Almost unknown. Scars on some females indicates that males bite their flanks during copulation; studied female contains three eggs.

### *Relationships*

*Lacerta zagrosica* lacks the derived state of the ulnar nerve and a hemipenial armature. As these are characteristic features of *Omanosaura* and nearly universal in other members of the Eremiinae (Arnold 1989, Harris *et al.* 1998), *L. zagrosica* is unlikely to belong to these groups. Nor does it have the characteristic features of the Gallotiinae, including fused postorbital and postfrontal bones, C-type tail vertebrae, patch of large soft spines on the hemipenis near the lobe bifurcation, and frequent vocalisation. *L. zagrosica* is therefore more likely to belong to the Lacertinae and has some features widespread in this group, including a polymorphic clavicle in which the

medial loop is sometimes interrupted posteriorly, tail vertebrae with an AB pattern and a sometimes a masseteric scale.

Within the Lacertinae, the combination of morphological features found in *L. zagrosica* does not obviously associate it with any other species or species group. It showed no close relationship with any of 21 such distinctive units, when it was included with them in a phylogenetic analysis based on 64 morphological characters (equivalent to 83 binary ones). This is perhaps not unexpected, as the tree produced by this analysis shows very little structure in the Lacertinae as a whole.

A separate phylogenetic analysis was carried out using mitochondrial DNA sequence of *Lacerta zagrosica* and members of the same 21 units including a total of 56 species and 138 individuals of Lacertinae. In contrast to the morphological analysis, *L. zagrosica* is firmly placed as the sister of *Lacerta brandtii*, with a high bootstrap value. This well supported relationship is likely to be valid, particularly as there is no strong conflicting morphological hypothesis. The association between *L. zagrosica* and *L. brandtii* is not unexpected, given the close geographical proximity of the two forms in West Iran. This case underlines the great lability of many morphological features in Lacertinae. No less than 15 out of the 64 features used in phylogenetic analysis show differences between the two species. These differences appear to be related to the disparate functional problems of living on often steep rock exposures and exploiting crevices, as *L. zagrosica* does, and dwelling mainly on the ground in the manner of *L. brandtii* (Arnold 1973, 1989, 1998a, 1998b).

## ACKNOWLEDGEMENTS

We are very grateful to Salvador Carranza (University of Barcelona) for arranging sequencing of the DNA of *Lacerta zagrosica* and incorporating it into a tree of lacertid relationships. Roberto Portela Mínguez (Natural History Museum, London) made radiographs of the studied material.

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# Distribution of lacertid lizards in a Tuscan agro-ecosystem (Central Italy)

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*The intensification of agricultural practices, during the last decades, has resulted in enormous changes in the landscape structure in Western Europe. The use of chemicals, the extension of the exploited area and the loss of many undisturbed environmental elements (such as woodlots, grass field margins, riparian strips) have brought to an impoverished wildlife, especially in arable landscapes. The protection of the remaining natural and semi-natural habitat patches or the creation and maintenance of new semi-natural areas may represent a promising way for conservation in agro-ecosystems.*

*In this paper we presented some aspects of a wider research mainly focused on arthropod fauna. The study was performed in Tuscany, Central Italy, during a whole year (from May 2003 to May 2004). Our aim was to determine if there are significant differences in lizard abundance and diversity in relation to ground cover features and land uses of six different micro-habitats, most of which typically occurring in Tuscan agro-ecosystems. In particular, two different agricultural managements were compared. We registered three lacertid species in the study area: Podarcis sicula, P. muralis and Lacerta bilineata. Their distribution resulted to be actually linked to the above mentioned characteristics and it stressed the importance of semi-natural habitats in order to enhance the presence of lacertid lizards in arable landscapes.*

Keywords: Lacertidae, agro-ecosystem, Italy.

## INTRODUCTION

During the last century, the intensification of land use has considerably changed the environment in Western Europe (Nentwig 2003). The traditional landscape characterized by a mosaic of pastures, prairies and woodlots has been replaced by an artifi-

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cial one in which the new agricultural practices, developed for large-scale production, have led to a great expansion of cultivated areas and to the loss of most undisturbed portions of land (Wilcove *et al.* 1986, Andr en 1994, Maisonneuve & Rioux 2001). Such processes have lead most often to landscape simplification and decreasing species richness (Meeus *et al.* 1990, Moser *et al.* 2002).

The protection of the remaining natural and semi-natural habitat patches or the creation and maintenance of new semi-natural areas may represent a promising way for conservation in agricultural landscapes (Greaves & Marshall 1987, Duelli & Obrist 2003, Pfiffner & Luka 2003, Woodcock *et al.* 2005). Indeed, the few areas which escaped from the intensification of farming management, provide a promising source of populations for the enhancement of biodiversity in depleted areas. Small uncultivated zones in form of grass borders, hedges or riparian strips can assure refuge for many animals and, even, the possibility of flow and exchange among individuals living in separated areas.

The present study, that is part of a wider research mainly focused on arthropod fauna, aimed at determining if there are significant differences in lizard abundance and diversity in relation to ground cover and land use characteristics of some microhabitats, most of which typically present in Tuscan agricultural landscapes. In particular, we tried to investigate the effects of undisturbed environmental elements (field margins, riparian strips and hedgerows) on the presence of lacertid lizards. Furthermore, we focused on the possible consequences of two different agricultural managements: intensive agriculture and experimental cultivation, with organic farming and presence of undisturbed grass strips within the cultivated area.

## MATERIALS AND METHODS

### *Study sites*

The study was carried out in the Valdera area, Central Tuscany (Italy). The region is subjected to high anthropic pressure and the landscape is characterised by wide intensively managed arable areas. Most of the land is destined to cereal production and the remaining areas at field margins are covered with woodlots, riparian strips and hedgerows.

The climate is mesothermal, humid, Mediterranean (Pinna 1977); the mean annual temperature is 12.7 °C (with extreme values of -10 °C and +40 °C in winter and summer, respectively) and the average annual rainfall is 678 mm, with maximum falls in autumn and spring (data derive from the meteorological station inside the experimental centre).

Sampling activity and observations were carried out in 6 different sites:

- an intensively managed wheat field (F) with a bramble bush hedge on one of its sides;
  - a woodlot (W) formed by an oak coppice, with trees reaching the height of 8-10 m. The underwood is mainly made by butcher's broom and thorn-bush;
- and 4 units with different land use and vegetation features inside the experimental centre "S. Elisabetta" (Vicarello di Volterra – Pisa), belonging to the Experimental Institute for Soil Study and Conservation of Florence. These sites have been classified as follow:
- grass strips (GS): inserted in the cultivated area. The strips (4.40 × 60 m) are overgrown with a continuous cover of herbaceous vegetation, growing up to 50 cm in spring;
  - cultivated strips (CS): alternated with the grasscovered strips, they are devoted to organic farming of cereals (17 × 60 m);
  - meadow (M): situated close to the strips (GS and CS) and a shrubby lot. The prevalent essence is couch grass, some small cement structures are also present;
  - riparian strip (R): the banks are overgrown with a thick vegetation, made up of herbaceous species and bramble bushes, which hides the ditch-bed. The ditch-bed is less than 1m wide and it dries out during summer.

### *Sampling procedures*

Data derive from animals occasionally fallen into pitfall traps set for arthropod fauna sampling. Sampling was carried out in 6 different sites (see above) during a whole year (from May 2003 to May 2004). We placed a total of 30 traps in the investigated area (4 traps in W, CS, GS and R, 6 in F, and 8 in M). In each site traps were located in grids of 30 × 10 m on average and their distribution was planned in order to obtain data which were representative of the whole detected area, considering factors such as closeness to semi-natural vegetation and presence of refuge function elements. Each trap consisted of a plastic tank ( $\varnothing = 8$  cm; h = 10 cm) filled with a solution of vinegar (with attractive function) and acetylsalicylic acid (with preservative function); every tank was buried in the ground so that its border was just at the ground level. A non-transparent plastic cover was placed 10 cm above each trap to prevent flooding from rainwater and evaporation of the inside solution. Traps were emptied and replaced once every 15 days.

Field observations of free ranging animals were performed, too. Moreover we registered individuals occasionally captured in water tanks set inside the experimental area and used for rainfall, runoff and sediment sampling. Collecting tanks were situated at the bottom of 15 cultivated experimental plots. Tanks checking and field observations were carried out contemporarily to pitfall sampling.

### Statistical analyses

In order to highlight possible differences in the lacertid lizard abundance of different land uses, we compared the number of lizards collected in the 6 surveyed sites using Kruskal-Wallis test. We employed the multiple comparison tests (post hoc test) to determine what sites differed significantly (Siegel & Castellan 1988). We verified with the Monte Carlo method (10000 iterations) as suggested by Mundry & Fischer (1998) for small samples.

Furthermore, focusing on the two examined agricultural managements, we compared the number of specimens captured in the traps of the intensive field (F) and in those of the experimentally cultivated area. For this purpose we put together the data collected in the grass strips and in the cultivated ones (GS+CS). We used a Mann-Whitney U-test verified with the Monte Carlo method (with 10000 iterations) for this analysis.

## RESULTS

During our study we recorded all the three lacertid species living in Tuscany (*Podarcis sicula*, *P. muralis* and *Lacerta bilineata*) (Vanni & Nistri 2006). Fig. 1 shows the species distribution obtained from the total number of individuals captured by pitfall traps in each of the six investigated sites. The most abundant species was *P. sicula*: it was captured especially in the meadow and in the grass strips, inside the

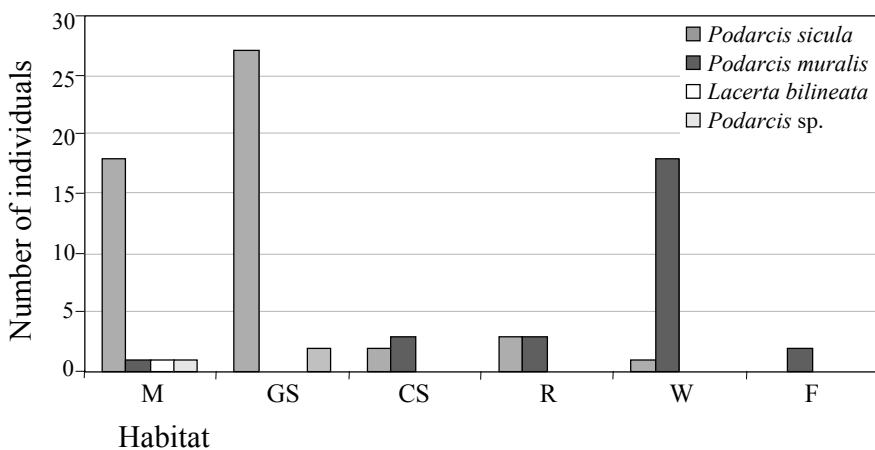


Fig. 1. Total number of individuals fallen in the pitfall traps, in each of the six investigated sites (M = meadow; GS = grass strips; CS = cultivated strips; R = riparian strip; W = woodlot; F = intensive wheat field).

experimentally cultivated area. *P. muralis* was found in five of the six surveyed habitats, too, but only in the traps close to shrubs or structures like walls. *P. muralis* was the only species found inside the intensive agriculture field where it was captured next to the field margin (bramble bushes); it was the most abundant species in the woodlot. *Lacerta bilineata*, as expected, was encountered much less frequently than the two other species. It was observed only one time between the meadow and the riparian strip, where dense vegetation is present; we found just a juvenile individual in one of the meadow traps. Such a low amount of captures may be due to the big body size of this species that could affect its capturability by pitfall traps. It has to be said that in few cases it was impossible to determine the specimens because of their bad state of conservation. As a matter of facts, the attractive solution we used for arthropod sampling resulted to be damaging for the vertebrates occasionally fallen into pitfall traps.

Kruskall-Wallis test performed on the number of individuals found in the traps revealed that there were differences in lacertid lizard abundance among the six investigated sites (Chi-square = 17.33, Monte Carlo sig.  $P = 0.001$ ) (Fig. 2). Post-hoc comparisons showed a significant difference only between GS and F.

The comparison between the two different agricultural managements showed that the experimental one, with presence of grass undisturbed strips, allowed a significantly higher presence of lacertids in the cultivated area (GS+CS vs F,  $n_1 = 8, n_2 = 6$ , Mann Whitney test:  $U = 9.000$ , Monte Carlo sig.  $P = 0.047$ ) (Fig. 3).

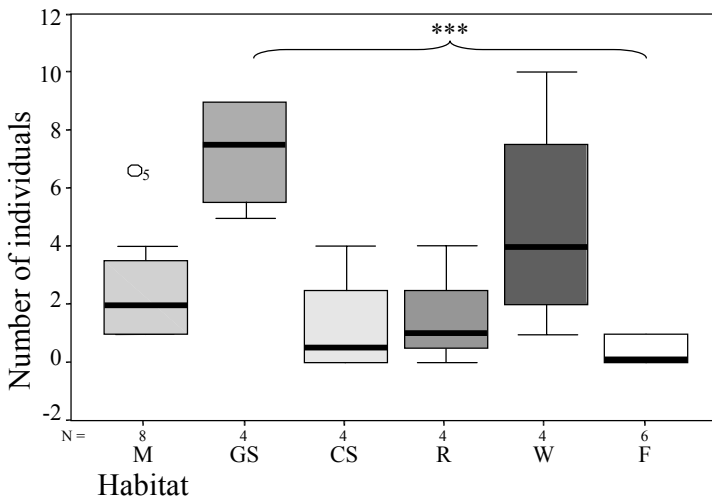


Fig. 2. Kruskal-Wallis test performed on the total number of individuals fallen into the pitfall-traps in the six investigated sites (M = meadow; GS = grass strips; CS = cultivated strips; R = riparian strip; W = woodlot; F = intensive wheat field). Boxplots show median, interquartile, and extreme values.

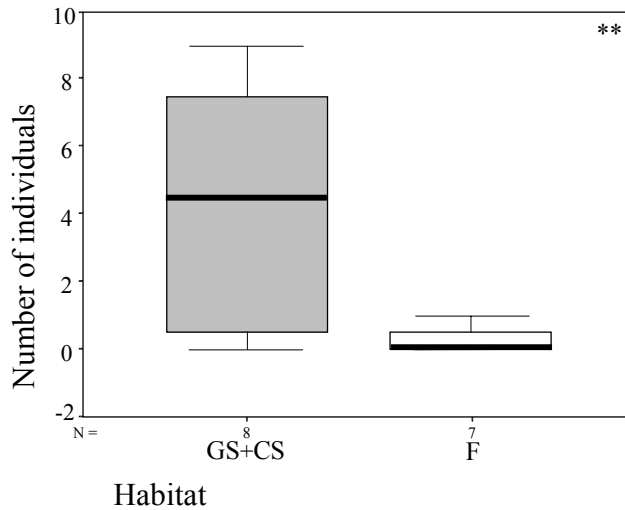


Fig. 3. Mann-Whitney U-test performed on the total number of individuals fallen into the pitfalltraps in the experimental managed field (GS+CS, in grey) and in the intensive wheat field (F, in white).

## DISCUSSION

Loss and degradation of natural habitats, unsustainable land use, increasing presence of invasive species, climatic changes and pollution are indicated as the main causes of the general decline of reptiles (Gibbons & Stangel 1999). Among these constrains, the loss of natural habitats can be considered the most serious threat for herpetofauna (Gibbons *et al.* 2000). However, anthropic activity is not always incompatible with the presence and conservation of these vertebrates. For example, it is well known that open areas, subjected to a moderate disturbance, can favour the presence of some reptiles (Hódar *et al.* 2000, Germaine & Wakeling 2001, Smart *et al.* 2005). In the same way, artificial elements such as dry-stone walls, typical of the Mediterranean agro-environments, and stone heaps due to land improvement, can represent important refuges for reptiles and their prey, sometimes allowing concentrations of individuals higher than in natural conditions (McNeely 1995, La Mantia 1997). Such situations, however, are typical of traditionally cultivated areas where moderate exploitation and conservation of natural and semi-natural zones are carried out. Our data seems to be in accordance with these evidences: inside the experimental centre, characterised by nonintensive management and presence of undisturbed habitats, it was possible to find all the three lacertid species living in Tuscany (*Podarcis sicula*, *P. muralis* and *Lacerta bilineata*) (Vanni & Nistri 2006). Moreover, the grass strips within the organically cultivated area were the place with the highest number of captures as well as the preferred habitat of *P. sicula*. On the other hand, the intensively cultivated



area sheltered only a few individuals of *P. muralis* and just very close to the vegetated field margin. Such results, even if they have to be considered preliminary, suggest the importance of maintaining semi-natural areas inside agricultural landscapes in order to favour the presence of natural populations of lacertid lizards.

Distribution and abundance of the three lizard species seemed to be strictly linked to the land use, vegetation and moisture features of the surveyed sites (Fig. 1). Our data seem to confirm that open spaces (the meadow and the grass strips) can be considered the most suitable habitats for *P. sicula*, according to its biological characteristics. On the contrary, *P. muralis* showed to prefer more moisten habitats (the woodlot), with a more complex vegetation structure (Rugiero 1993, Corti & Lo Cascio 2002). As already known, *L. bilineata* can be found in agricultural habitats too, but only near ecotonal zones with high grass soil cover and green vegetated hedgerows.

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# Population heterogeneity and conservation of the Aeolian wall lizard, *Podarcis raffonei*

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*The conservation status of Podarcis raffonei, native to the Aeolian Islands (north-east of Sicily, Tyrrhenian Sea), is assessed analyzing its genetic structure and evidencing factors affecting the survival of the species. Genetic variation in the species is quite low and genetic subdivision is high, as compared to other lacertid lizards. The four remaining populations of P. raffonei are under severe threat from the effects of habitat alteration, interspecific competition, collecting and trade, very low population density and inbreeding, population fragmentation, and apparent loss of genetic variation. Possible management and conservation measures are suggested.*

Keywords: *Podarcis raffonei*, Lacertidae, population heterogeneity, conservation, Aeolian Islands.

## INTRODUCTION

*Podarcis raffonei* (Mertens 1952) is a lacertid lizard endemic to the Aeolian Islands (north-east of Sicily, Tyrrhenian Sea) (Fig. 1). The species is genetically and morphologically related to *P. wagleriana* Gistel 1868, a lizard endemic to Sicily, Egadi Islands and Stagnone Islands (Capula 1994a, b; Capula *et al.* 2001). The Aeolian Islands are also inhabited by *Podarcis sicula* (Rafinesque 1810), but the occurrence of this species in the Archipelago is probably due to introduction by humans in proto-historical times (Capula 1994a, Corti & Lo Cascio 1999, Lo Cascio & Corti 2004). *P. sicula* occurs on all large islands as well as on some islets of the Aeolian Archipelago (Capula 1992, 1993, 1994a; Corti *et al.* 1998; Corti & Lo Cascio 1999), while *P. raffonei* is currently confined to one large island (Vulcano, 2120 ha), where it occurs sympatrically with *P. sicula*, and to three islets (Scoglio Faraglione, 0.5 ha, 0.3 km west of Salina; La Canna, 1 ha, 1.5 km west of Filicudi; Strombolicchio, 0.19 ha, 1.6 km north-east of Stromboli), where it is the only extant lacertid lizard (Capula



Fig. 1. Adult male *Podarcis raffonei antoninoi* (Vulcano Island).

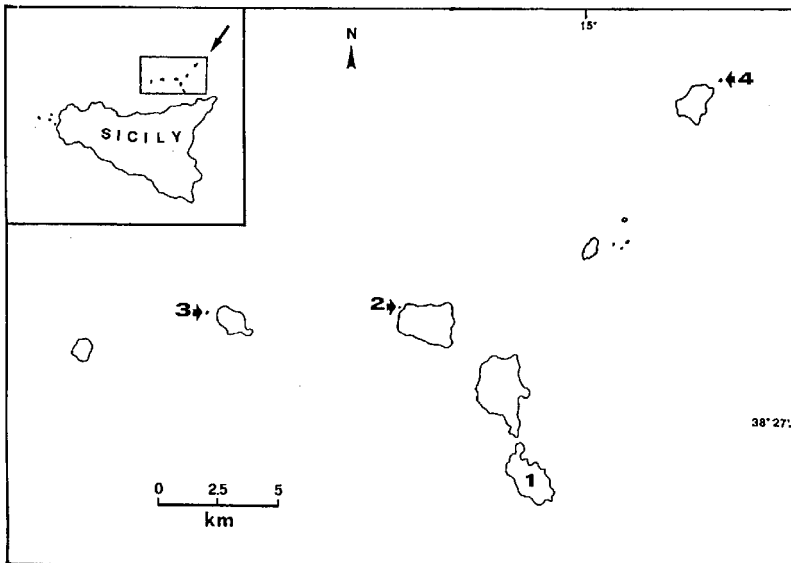


Fig. 2. Distribution of *Podarcis raffonei* in the Aeolian Islands. 1, Vulcano; 2, Scoglio Faraglione, near Salina; 3, La Canna, near Filicudi; 4, Strombolicchio, near Stromboli. Insert shows location of the Aeolian Islands.

*et al.* 2002) (Fig. 2). The following subspecies are currently recognized: *P. r. raffonei* (Strombolicchio), *P. r. antoninoi* (Vulcano), *P. r. alvearioi* (Scoglio Faraglione), *P. r. cucchiarae* (La Canna) (Capula 1994a, b; Turrisi & Vaccaro 1998).

Because of (i) the extremely low density of *P. raffonei* on the smallest islets of the Aeolian Archipelago (Scoglio Faraglione, La Canna, Strombolicchio, each with an area of  $2 < \text{ha}$ ) (Capula *et al.* 2002, Capula & Lo Cascio 2006), and (ii) its complete absence from the large islands – with the exception of Vulcano, where the species is at present extremely rare and nearly reaching the extinction – the Aeolian wall lizard was categorised as critically endangered (Capula *et al.* 2002).

In this paper, the status of *P. raffonei* is assessed by reviewing the existing information on the genetic and morphological structure of the species, and by providing management recommendations to ensure its conservation in the Aeolian Islands.

## MATERIALS AND METHODS

### *Population heterogeneity*

The genetic structure of *P. raffonei* populations occurring on Vulcano Island and on Scoglio Faraglione, La Canna and Strombolicchio islets was analyzed and discussed using allozyme variation data at 26 gene loci ( $\alpha\text{Gpd}$ , *Ldh-1*, *Ldh-2*, *Mdh-1*, *Mdh-2*, *Me-1*, *Me-2*, *Idh-1*, *Idh-2*, *6Pgd*, *Gapd*, *Sod-1*, *Np*, *Got-1*, *Got-2*, *Ck*, *Ak*, *Ada*, *Ca*, *Mpi*, *Gpi*, *Pgm-1*, *Pgm-2*, *Gp-1*, *Gp-2*, *Gp-4*) provided by Capula (1994a, 2004). The genetic variability for each population and for the species as a whole was estimated using the following parameters: mean number of alleles per locus (*A*), percentage of loci polymorphic (*P*, at the 99% level), observed mean heterozygosity (*H<sub>o</sub>*), expected mean heterozygosity (*H<sub>e</sub>*) in Hardy-Weinberg equilibrium (unbiased estimate; Nei 1978). The distribution of genetic variation within and among populations was assessed using Wright's *F*-statistics (Wright 1965, 1978). The genetic relationships among the populations studied were evaluated using Nei's (1972) standard genetic identity (*I*) and standard genetic distance (*D*). All genetic variability, *F*-statistics, and genetic distance measures were calculated by the computer program BIOSYS-1 (Swofford & Selander 1989).

### *Conservation status*

Information concerning the conservation status and the factors affecting the survival of *P. raffonei* is based on field investigations carried out on all large islands of the Aeolian Archipelago (Lipari, Salina, Vulcano, Stromboli, Filicudi, Alicudi, Panarea) and on six islets (Basiluzzo, Bottaro, Lisca Bianca, La Canna, Scoglio Faraglione,

Strombolicchio) during a 16-year research period (1989-2004). All observations were carried out during springtime (April, May) and at the end of summer (September). To describe the main causes affecting the survival of *P. raffonei*, data gathered from the literature concerning the species (Capula 1993, 1994a, 2004; Capula & Luiselli 1997; Capula *et al.* 2002; Corti & Lo Cascio 1999, 2002; Lo Cascio & Pasta 2004; Luiselli *et al.* 2004; Capula & Lo Cascio 2006) were also analyzed and discussed.

## RESULTS AND DISCUSSION

### *Population heterogeneity*

The small and geographically fragmented populations of *P. raffonei* are characterized by very low levels of genetic variability and by a genetic structure more subdivided than that of other *Podarcis* species studied to date. According to Capula (2004) 22 of the 26 presumptive gene loci scored (85%) were found to be monomorphic and fixed for the same allele in all the samples of *P. raffonei*, and four loci only (15 %) were found to be polymorphic (*6Pgd*, *Ca*, *Mpi*, *Pgm-2*).

All the analysed samples of *P. raffonei* show noticeably low values of polymorphism and heterozygosity (see Table 1). The overall mean number of alleles per locus was 1.05, and the proportion of polymorphic loci ( $P$ ) averaged 4.8 (Capula 2004). The observed heterozygosity ( $H_o$ ) showed a similar trend, ranging from 0.000 (La Canna) to 0.018 (Vulcano) and averaging 0.011. The Aeolian wall lizard shows values of polymorphism and heterozygosity noticeably lower than those detected in the phylogenetically related *P. wagleriana* from Sicily ( $P = 15$ ;  $H_o = 0.037$ ; Capula 1994a) and the average ones calculated by Capula (1990) for nine species of the genus *Podarcis* ( $P = 13$ ;  $H_o = 0.053$ ). This is probably because three out of four extant *P. raffonei* populations occur on tiny fringing islands, each characterized by a very limited area (< 2 ha) and separated by a short linear distance and shallow channel depth from the mother island; on the other hand, the populations occurring on Vulcano Island – which is

Table 1. Genetic variability parameters in *Podarcis raffonei* populations.  $A$ , mean number of alleles per locus;  $P$ , mean proportion of polymorphic loci;  $H_o$ , observed mean heterozygosity;  $H_e$ , expected mean heterozygosity; (SE), standard error.

Population	$A$	$P$	$H_o$	(SE)	$H_e$	(SE)
Vulcano	1.1	7.7	0.018	0.014	0.016	0.012
Scoglio Faraglione	1.0	3.8	0.008	0.008	0.008	0.008
La Canna	1.0	0.0	0.000	0.000	0.000	0.000
Strombolicchio	1.1	7.7	0.017	0.013	0.016	0.012



the only large Aeolian island inhabited by the species – are extremely fragmented and are characterized by a very small number of individuals (Capula 1994a, Capula *et al.* 2002). The severe reduction in genetic variability pointed out in *P. raffonei* was also evidenced by molecular analyses (Oliverio *et al.* 1998). However, it must be noted that the results by Oliverio *et al.* (1998) concerning the phylogenetic relationships of *P. raffonei* are in disagreement with those pointed out by allozyme (Capula 1994a, b), mtDNA (Harris & Arnold 1999), and morphometric analyses (Capula *et al.* 2001). According to Capula (1994a, b), Harris & Arnold (1999) and Capula *et al.* (2001) *P. raffonei* is phylogenetically related to *P. wagleriana*, while the results by Oliverio *et al.* (1998) would indicate a strict relationship between *P. raffonei* and *P. muralis* (Laurenti 1768). However, on a subsequent paper Oliverio *et al.* (2000) proposed a very different phylogenetic scenario, biogeographically not congruent neither with that reported by Oliverio *et al.* (1998) nor with that by Capula (1994a, b), Harris & Arnold (1999) and Capula *et al.* (2001), suggesting a close affinity between *P. raffonei* and *P. tiliguerta* (Gmelin 1789).

The values of standard genetic identity and genetic distance for each pairwise comparison are given in Table 2. Nei's standard genetic distance ( $D$ ) between the *P. raffonei* populations ranged from 0 to 0.041, averaging 0.020. Despite the large geographic distance and the wide sea channel separating the islands of Vulcano, Scoglio Faraglione and La Canna, the values of genetic distance between the samples from these islands were quite low (average  $D = 0.0003$ ). On the other hand, the comparison between the Strombolicchio sample and the other Aeolian samples gave the highest distances (average  $D = 0.040$ ). The individuals of this population are characterized by a unique electrophoretic allele (*Pgm-2<sup>105</sup>*) and are large-sized and morphologically recognizable from those occurring on the other Aeolian Islands (Capula *et al.* 2001, Capula 2004).

According to Capula (2004) the levels of genetic subdivision observed in *P. raffonei* exceeded values known for other lizard species (McKinney *et al.* 1972, Sites & Greenbaum 1983, Sarre *et al.* 1990). The estimated standardized variance in gene frequency ( $F_{ST}$ ) is highly significant (Capula 2004), with a value (0.610) much higher

Table 2. Values of Nei's (1972) standard genetic identity (above the diagonal) and standard genetic distance (below the diagonal) among populations of *Podarcis raffonei* (Vu= Vulcano, SF= Scoglio Faraglione, LC= La Canna, St= Strombolicchio).

Population	Vu	SF	LC	St
Vu	----	1.000	0.999	0.961
SF	0.000	----	1.000	0.961
LC	0.001	0.000	----	0.960
St	0.040	0.040	0.041	----

than those calculated by Capula (1994a, 1996) for the related insular lacertid lizards *P. wagleriana* ( $F_{ST} = 0.153$ ) and *P. tiliguerta* ( $F_{ST} = 0.460$ ), and very high for vertebrates in general (Gorman *et al.* 1975, Wright 1978, Raghianti & Wake 1986). These data are congruent with the results of the geometric morphometric analyses (Capula *et al.* 2001). According to the landmark based morphometric analyses of the dermal skulls of twentytwo adult males *Podarcis raffonei* from the four islands of the Aeolian Archipelago (Capula *et al.* 2001), the *P. raffonei* populations are morphologically highly differentiated to each other (see, *e.g.*, Fig. 3). This is probably due to geographic isolation of populations in different islands, and to genetic drift and effects.

Genetic and morphometric data (Capula *et al.* 2001, Capula 2004) indicate that geographic variation in *P. raffonei* is distributed into two population groups. The first group includes the very close populations from the southern (Vulcano) and western (La Canna, Scoglio Faraglione) Aeolian Islands; the second group includes the populations from Strombolicchio, off the north-eastern coast of Stromboli, i.e. the northernmost island of the Aeolian Archipelago. Genetic variability is very low, and genetic and morphometric subdivision is high. According to these results the subspecies *antoninoi* and *cucchiarai* should be synonymized with *alvearioi*. Therefore the following subspecies should be now recognized: *P. r. raffonei* (Strombolicchio), *P. r. alvearioi* (Vulcano, La Canna, Scoglio, Faraglione).

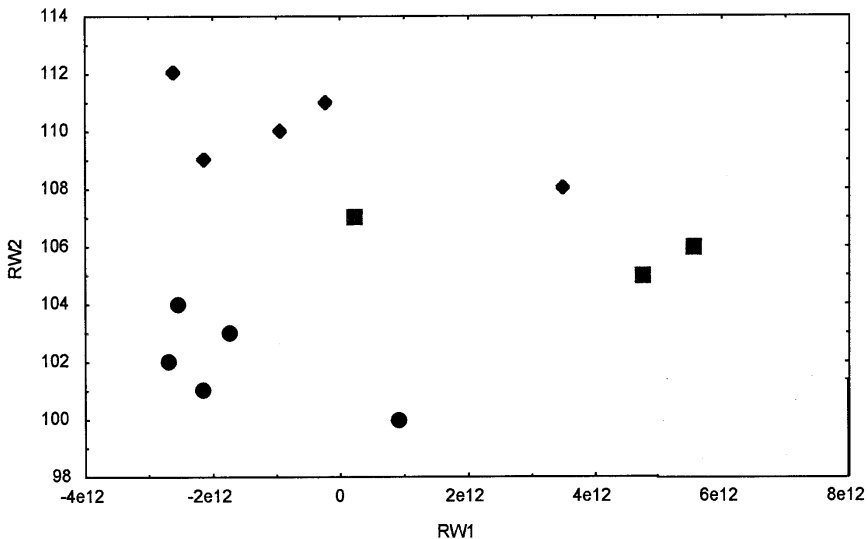


Fig. 3. Ordination of adult males *P. raffonei* (circle: Strombolicchio,  $n = 5$ ; square: Scoglio Faraglione,  $n = 3$ ; rhombus: Vulcano,  $n = 5$ ) along the first two relative warps (RW1, RW2; 31.07% and 25.50% of variation explained respectively) for the cranial dermal skulls (see Capula *et al.* 2001 for more information).

*Conservation status*

Investigations carried out by Capula *et al.* (2002) and Capula (2004) in the Aeolian Islands gathered evidence strongly indicating that the endemic *P. raffonei* is close to extinction. According to these authors, each of the three tiny islands inhabited only by *P. raffonei* supports a very small number of lizards (Strombolicchio and Scoglio Faraglione: 200-400 individuals; La Canna: 20-30 individuals; Capula & Lo Cascio 2006). On the other hand, on Vulcano the species occurs in two localities only, and it is nearly reaching extinction probably because of competitive exclusion by the very abundant lizard *P. sicula* (Capula 1993, Capula *et al.* 2002, Capula & Lo Cascio 2006, Capula unpublished personal observations). Direct evidence for the decline of the Aeolian wall lizard is scarce because there are no previous data (i.e. prior to 1994) on the original distribution and demography of the species, but the decline is indicated indirectly by several factors. According to Capula *et al.* (2002) and Capula (2004), six main pressures on the species can be listed as contributory factors to extinction: (1) habitat alteration (fire, over-grazing, agricultural practices, touristic activities); (2) competitive exclusion by the lacertid lizard *P. sicula*, introduced by humans in historical times; (3) collecting and trade; (4) very low population density and inbreeding; (5) very reduced gene flow between populations, due to high geographic fragmentation and isolation; (6) apparent decline in genetic variability. The amount of genetic variation is believed to be positively correlated to fitness (Lande 1988, Lynch 1996), and so the small populations of *P. raffonei* are genetically highly vulnerable to environmental changes and human impacts. All of the above factors probably affect the survival of this insular species and underline the urgent need for implementation of conservation measures (Reid & Miller 1989).

*Podarcis raffonei* and its habitat are still not protected by the Italian law or by any international convention concerning conservation and protection of vertebrate fauna. The species requires urgent protection as it can be considered the most threatened lizard in Italy and possibly in the whole Europe (Capula *et al.* 2002, Corti & Lo Cascio 2002, Lo Cascio & Pasta 2004). *Podarcis raffonei* may yet be saved from extinction if the following recommendations are acted upon:

- The last wild populations should be officially protected as soon as possible by creating natural reserves.
- Collection and trade for commercial purposes should be forbidden by full legal protection to reduce the risk of depletion of wild populations.
- Habitat conservation measures should be instigated by monitoring and managing areas where the natural habitat of the species still persists.
- Control of the presence of alien fauna and prevention of intentional or accidental introductions of predators (e.g. *Hierophis viridiflavus*) and competitor species (e.g. *Podarcis sicula*) is required.

- Continuity of scientific studies on relict populations should be promoted in order to document annual fluctuations in population parameters.
- It is necessary to avoid habitat stress on the islands inhabited by the species and to provide for habitat restoration.
- It is necessary to prevent gene flow between islands.
- Since the existing wild populations are composed by a small number of individuals and thus are vulnerable to stochastic impacts, it is urgent to plan captive breeding programmes.
- Documented gene banks should be established adjacent to protected areas by creating effective *ex situ* populations (IUCN 1998).
- *Podarcis raffonei* should be included in the IUCN Red List of Threatened Species as “Critically Endangered” (see Capula *et al.* 2002; Capula & Lo Cascio 2006).

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# Reproductive cycles in Mediterranean lacertids: plasticity and constraints

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*Reproductive timing is one of the most critical issues for lacertids inhabiting temperate regions where favourable conditions are restricted seasonally. Cycles of gonads and associated lipid reserves represent the manifestation of this discontinuous reproductive mode. Although lacertids seem to use both temperature and photoperiod to adjust their reproductive clocks to the environmental conditions, these cues seem to act at different stages of the cycle. Within species, interindividual, interpopulational and interannual variation have been documented. Thermal seasonality, changing between sites and years, determines not only the length of the reproductive season but also the intensity of variation for several reproductive parameters. Except in those species/populations with extremely short reproductive activity, lacertids are rather asynchronous, large adults starting reproduction later than small ones. In contrast, the end of the breeding period is less variable and probably associated with photoperiod.*

*Prior to all these factors, lipid storage of excess energy is a necessary condition for beginning reproduction. Lipids peak in late summer or early autumn, are not depleted in winter and are consumed during the reproductive season. Males spend reserves earlier than females in the activities related with breeding and recover them soon after since mixed-type spermatogenesis distributes energy costs along a prolonged period. Only spermiogenesis is highly variable in time depending on the species/population although previous classifications based on it are simplistic. Females mainly behave as capital breeders investing lipids in developing a first (or unique) clutch but may act as income breeders for the subsequent clutches if any. The degree of iteroparity depends on the same factors but just within the species limits. Some of the patterns observed are, nevertheless, uncorrelated with abiotic environment and may reflect other pressures. Theoretically, any influence able to provoke food shortage would delay reproduction independently of climate conditions. In some cases, traits could be historical, deriving from pressures acting in the past. Long egg retention and viviparity are strong constraints since they prolong single reproductive events preventing its repetition even when environmental conditions would allow it. Moreover, thermophile species evolved under mild conditions are unable to start reproduction when and where other more cold-adapted species do. On the other hand, insular lacertids enlarge the reproductive period in comparison with their continental equivalents living under similar*

*climate regimes but with different demographic pressures. Finally, a biogeographic scenario for the evolution of reproductive cycles in the whole family is proposed.*

Keywords: reproduction, phenology, spermatogenesis, vitellogenesis, fat bodies, viviparity, insularity, Lacertidae.

## INTRODUCTION

Lacertids are among the most genuine Mediterranean vertebrates since the whole group originated around this area (Estes 1983, Arnold 1989) and radiated and diversified there tracking its complex geological history (Harris *et al.* 1998, Carretero 2004), extensively contributing for it to be considered a biodiversity hotspot at the global level (Myers *et al.* 2000). Since the Mediterranean Basin falls within the temperate region, seasonal restriction of favourable conditions for ectotherms, more or less intensified throughout the geological changes and climatic cycles, has undoubtedly modelled the evolution of lacertids since the beginning and still plays a prominent role on the lineages inhabiting this area. Under such environmental constraints, reproductive timing becomes one of the most critical issues in the lizard's biology (James & Shine 1985). Life cycles as well as the cyclical variations of gonads and associated lipidic reserves represent the expression of this discontinuous reproductive mode. Whereas life history traits in lacertids have been extensively reviewed (Bauwens & Díaz-Uriarte 1997, Bauwens 1999), no comprehensive general approach is available on how such biological events are allocated in time. This is the aim of this review.

## LIFE CYCLES

Regarding their life cycles, Mediterranean lacertids are conservative. Despite the number of phylogenetically diverse lineages present in this region (Harris *et al.* 1998) no strong variation of patterns is found. Cycles work on an annual basis (Fig. 1), breeding season taking place in spring-early summer, clutches in late spring-early summer and hatchling in summer-early autumn. Lacertids become sexually mature when attaining a minimum body size rather than an age (Marco *et al.* 1994, Galán 1996b, Olsson & Shine 1997, Bauwens 1999). Depending on the species and the



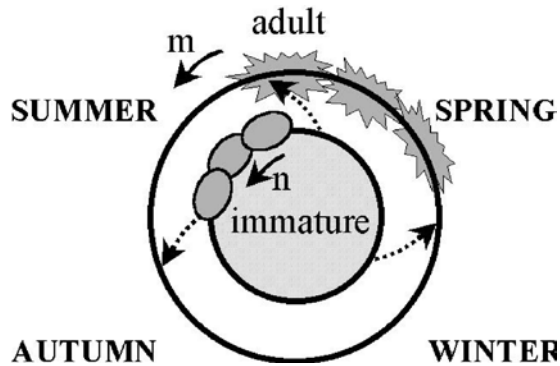


Fig. 1. Schematic representation of the life cycle in a Mediterranean lacertid. Stars: copulation period; ellipses: clutch period; dashed arrows: attainment of sexual maturity; n: number of cycles in immature stage; m: number of cycles in adult stage.

population, sexual maturity can be attained either during the first calendar year or one to several years later, then passing through a subadult stage (Carretero & Llorente 1993) simultaneously present with adults and juveniles. Such distinction is not irrelevant since the lack of autumnal reproduction prevents the existence of intermediate patterns. In fact, if the minimum size is not attained in a given season, individuals do not incorporate to the adult stage but continue growing and attain bigger adult sizes the following one (Carretero & Llorente 1997). At an intraspecific level, variation in the age of sexual maturity between sexes (Amat *et al.* 2000, Olsson & Shine 1997, Galán & Arribas 2005), populations (Bauwens & Verheyen 1987, Fig. 2) and years (Bauwens & Verheyen 1987, Heulin *et al.* 1994, Fig. 3) has been described. Usually, mild conditions tend to promote fast growth and early reproduction. This effect can be responsible for the adult size differences found between populations of the same species facing different climatic conditions (*sensu* Adolph & Porter 1993), especially for those undergoing deep winter diapause and may even contribute with other factors to sexual size dimorphism (Carretero & Mateos 2002, Roitberg & Smirina 2004).

In those species with a subadult stage, the number of years involved may vary between one, being the most common in small-sized species (Galán 1996b), to three-four in the large green lizards (Mateo & Castanet 1994, Elbing 2001). Nevertheless, some small species inhabiting mountains or northern habitats delay several years before attaining sexual maturity (Bauwens & Verheyen 1987, Arribas 2004, Galán & Arribas 2005) although it could be argued that the effective life (*i.e.* in activity) is not very different from lizards under less extreme conditions (Carretero & Mateos 2002). Sexual maturity and total longevity tend to be correlated at population and species level. As an exception, giant *Gallotia* species from the Canary Islands start reproduction relatively early but their growth does not decrease with sexual maturity and continues for a long period (Castanet & Báez 1988, 1991) resulting in a high maximum

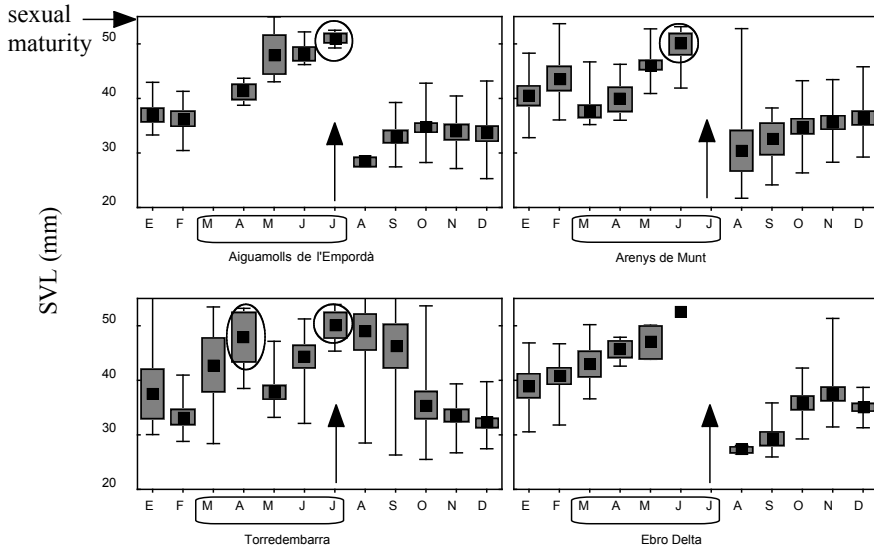


Fig. 2. Variation of the life history of the lacertid *Psammodromus algirus* between four coastal populations in NE Iberian (from north to south) studied during the same period. Distribution of immatures randomly sampled throughout the year: in the northernmost population (A. Empordà) no immatures reached sexual maturity within the first calendar year but all of them did in the southernmost one (Ebro Delta); the other two populations were intermediate, Torredembarra being especially asynchronous. Hatchlings started appearing in August. Rectangles: reproductive season; arrows: end of the reproduction. Box: mean  $\pm$  SE; whisker: mean  $\pm$  SD.

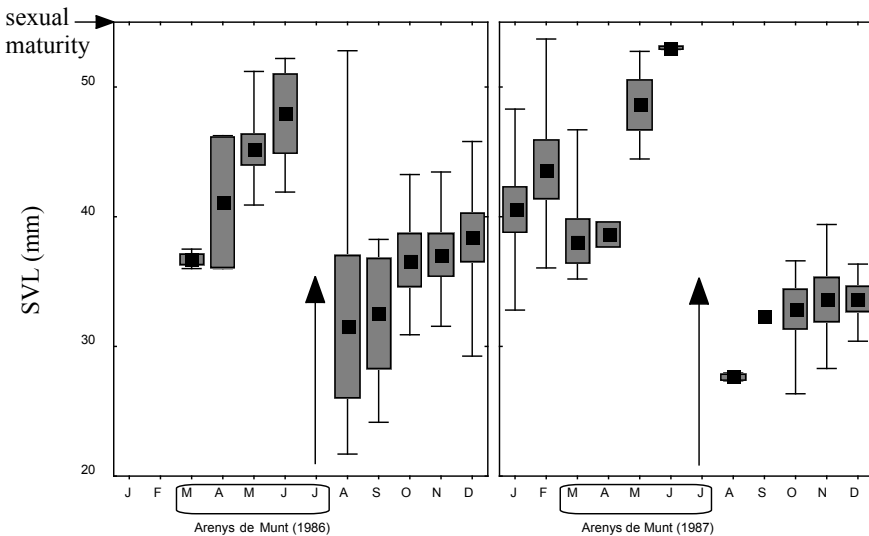


Fig. 3. Variation of the life history of the lacertid *Psammodromus algirus* between two consecutive years in the same locality. Distribution of immatures randomly sampled throughout the year: whereas only a part of the individuals attained sexual maturity before the end of the breeding season in 1986 (see increased variance in August), most of them had already reached the minimum size in June 1987. Rectangles: reproductive season; arrows: end of the reproduction. Box: mean  $\pm$  SE; whisker: mean  $\pm$  SD.

longevity with considerable size variation within adults. On the opposite extreme, the small-sized *Psammodromus hispanicus* may reach minimum adult size in less than three months and most individuals only breed during one season, all within less than one year of life span (Pascual & Pérez-Mellado 1989, Carretero & Llorente 1991, Carretero 1992). Members of other dwarf genera as *Ophisops* and *Mesalina* from N Africa, Levant and Asia Minor probably display similar patterns (Pérez-Mellado *et al.* 1993, Schleich *et al.* 1996). Olsson & Shine (2002) have experimentally evidenced negative relationships between the rates of early growth and late survivorship for one skink species and this may also be the case in lacertids.

## GONAD CYCLES: VARIABILITY AND REGULATION

Male and female cycles in lacertids are linked, which seems rather obvious but is not the rule in many other squamates (Fitch 1970). That means that neither sperm needs to wait for ovarian follicles to mature nor these become “frozen” waiting for spermiogenesis. In fact, no long-term sperm storage have been demonstrated in the whole family. Conversely, although copulatory plugs have been described (in den Bosch 1994), their effectiveness is limited (Moreira & Birkhead 2004a, b) and males mainly rely on mate guarding to ensure their reproductive success (Olsson *et al.* 1996, 1997a, b; Gullberg *et al.* 1997).

At a macroscopic level, male cycle consists of seasonal changes in the size of the sexual organs (Fig. 4). Essentially, testis suffers a strong decrease after the reproductive period and keeps its size throughout the rest of the year whereas epididymis enlarges in the reproductive period (or immediately before) and remains small out of it. Such changes are related, although not completely, with the seasonal variation in the abundance of the different sexual cells (see spermatogenesis section). In contrast, female cycle displays changes, affecting ovary and oviduct, exclusively concentrated in the reproductive period (Fig. 5).

Although lacertids seem to use both temperature and photoperiod to adjust their reproductive clocks to the environmental conditions, these cues seem to act at different stages of the cycle. Thus, experimental research has demonstrated that the spermatogenesis is thermodependent (Joly & Saint-Girons 1975; Angelini *et al.* 1976, 1979), the beginning of the reproductive activity thermally controlled in both sexes. In contrast, the end of the reproduction remains similar under different thermal regimes probably adjusted with the photoperiod and endogenous clocks (Angelini *et al.* 1976, Botte *et al.* 1976, Tosini *et al.* 2001). The environmental and internal set points for such events are expected not only to change between species (Saint-Girons & Saint-Girons 1956, Saint-Girons & Duguy 1970) but also to carry substantial phylogenetic inertia according to

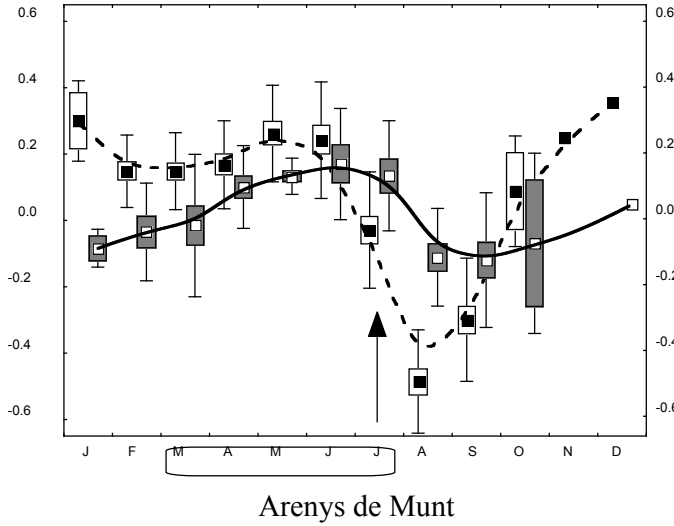


Fig. 4. Seasonal variation of testis (closed squares) and epididymis (open squares) sizes in the lacertid *Psammodromus algirus* from a coastal Mediterranean locality. The sizes of both organs have been standardised to the lizard size using regression residuals of log-transformed variables. Lines have been fitted by least-squares. Rectangles: reproductive season; arrows: end of the reproduction. Box: mean  $\pm$  SE; whisker: mean  $\pm$  SD.

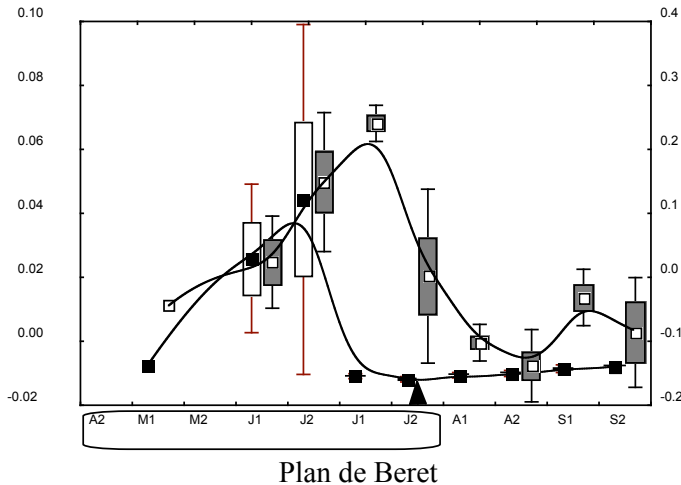


Fig. 5. Seasonal variation in 15-days periods of ovary (closed squares) and oviduct (open squares) sizes in the lacertid *Lacerta (Zootoca) vivipara* from the western Pyrenees. The sizes of both organs have been standardised to the lizard size using regression residuals of log-transformed variables. Lines have been fitted by least-squares. Rectangles: reproductive season; arrows: end of the reproduction. Box: mean  $\pm$  SE; whisker: mean  $\pm$  SD (from Roig *et al.* 2000, modified).

the past environmental conditions in the areas of origin of each group, which probably parallel preferred temperatures (see Carretero *et al.* 2005 and references therein). This means that groups evolved under mild conditions and recently colonising more extreme environments should display a more restricted reproductive period than those originated in such restrictive environments (Perez-Mellado 1982, Busack & Klostermann 1987, Pollo & Perez-Mellado 1990, Castilla *et al.* 1992, Carretero & Llorente 1995).

Within species, interindividual (Seva 1982, Braña 1986, Bauwens & Veheyen 1987, Marco *et al.* 1994, Castilla & Bauwens 1989, Carretero & Llorente 1997, Galán 1997, Olsson & Madsen 1996, Olsson & Shine 1996), interpopulational (Hraoui-Bloquet 1985, 1987; Hraoui-Bloquet & Bloquet 1988; Braña *et al.* 1990; Carretero & Llorente 1997) and interannual (Castilla *et al.* 1992, Carretero & Mateos 2002) variation in reproductive events have been documented. Thermal seasonality, changing according to locations and years, determines not only the length of the reproductive season but also the intensity of variation for several reproductive parameters (Gavaud 1991). The beginning of the reproductive season is usually more variable than the end, reproduction in warmer years or sites starting earlier (see previous references). Except in those species/populations with extremely short reproductive activity (Elvira & Vigal 1985, Argüello 1990, Carretero & Llorente 1995, Amat *et al.* 2000, Roig *et al.* 2000, Arribas 2004, Galán & Arribas 2005) lacertids are rather asynchronous, larger adults and specially females starting reproduction earlier than small ones (Olsson & Shine 1997, Galán 1997, Marco & Pérez-Mellado 1998). In contrast, the end of the breeding period does not show much variation and is probably associated with photoperiod. Nevertheless, the final part of the reproduction may be truncated when harsh summer conditions promote aestivation (Hraoui-Bloquet & Bloquet 1988, Pollo & Perez-Mellado 1990).

## SPERMATOGENETIC CYCLES

In males, the variation of cell types parallels macroscopic changes observed in the testis but just during the reproductive season (Roig *et al.* 2000, Carretero *et al.* 2006) whereas the rest of the year the abundance and proportion of the different cell types change without affecting the organ size.

Classic literature classifies reptile spermatogenic cycles according to the stage in which sexual cells become after the reproductive period (Saint-Girons 1963, 1984). Lacertids do not produce spermatozoa immediately after the breeding season (postnuptial spermatogenesis) as found in many snakes and tortoises (Bons & Saint-Girons 1982) but delay this process along a variable period, thus distributing the energetic costs associated (Olsson *et al.* 1997, Roig *et al.* 2000). Instead, two other types of spermatogenesis occur in Mediterranean lacertids: spermatogonia may rapidly develop into spermatocytes and

spermatides immediately after breeding and then the maturation to spermatozoa extends until the following season (mixed type) or the whole maturation from spermatogonia to spermatozoa is concentrated immediately before or even during breeding (prenuptial or vernal type). In theory, most mesic species (*Psammodromus*, *Podarcis*, *Lacerta* s.l.) fall within the first category whereas only those with desert affinities (*Acanthodactylus*, *Mesalina*) belong to the second (Bons & Saint-Girons 1982). Nevertheless, such dichotomy constitutes a simplification since the final part of the spermatogenesis (the spermiogenesis) is highly variable in time within species. For instance, some individuals of species ascribed to the mixed type are able to produce spermatozoa already in autumn (*Lacerta vivipara*, Roig *et al.* 2000; *Podarcis sicula*, Angelini *et al.* 1979; *P. bocagei*, Carretero *et al.* 2006). Whether this autumnal spermiogenesis is just abortive (Angelini *et al.* 1979) or represents a potential second reproductive season remains speculative (see below). Furthermore, advanced states of sexual cells are even found in autumn for typically prenuptial species (i.e. *Acanthodactylus erythrurus*, Bons 1969).

Whatever the case, even if spermatozoa have already been produced in testis, males become fertile only when these pass to the epididymis which is revealed externally by the enlargement of this organ (Roig *et al.* 2000, Carretero *et al.* 2006). Among the Mediterranean lacertids the anticipation of this enlargement to the breeding season is variable (Fig. 6) but is specially marked in some species inhabiting warm, mesic habitats (*Psammodromus hispanicus*, Carretero & Llorente 1991; *Lacerta laevis*, Hraoui-Bloquet & Bloquet 1988 and *Lacerta lepida*, Castilla & Bauwens 2000a, b). In those species undergoing a marked winter diapause, males usually emerge earlier than females (Saint-Girons 1976, Nuland & Strijbosch 1981, Salvador 1987) and this phase takes place when females are still inactive (Olsson & Madsen 1996, Roig *et al.* 2000, Carretero *et al.* 2006). It is considered that, in fact, females are avoiding copulation with these functionally infertile males at the beginning of the season (Olsson & Madsen 1996).

## VITELLOGENESIS

In comparison, the female cycle is relatively simple with all events restricted to the reproductive period. Ovary increases in size due to the maturation of vitellogenic follicles at the beginning of the reproductive season. After fecundation, eggs develop inside the oviducts which have previously increased their diameter and change their histology to receive them (Bons 1972, Roig *et al.* 2000). Minimum diameters of vitellogenic follicles (2-3 mm) have been described for different species (Carretero & Llorente 1991, 1995, 1997; Roig *et al.* 2000). Clutch and egg size usually correlate with body size and depend on population and species (see below) but follicle and egg numbers are equivalent in all cases (see previous references)

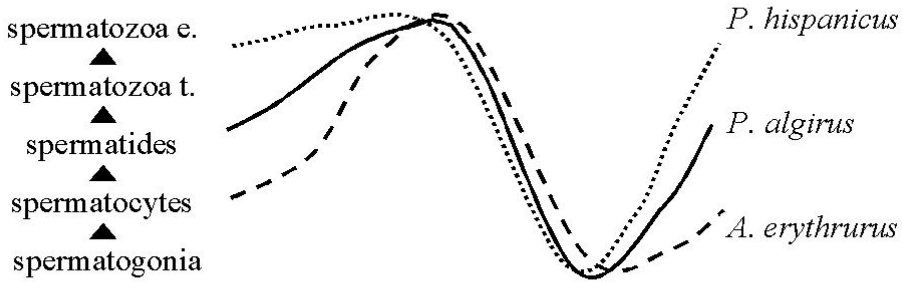


Fig. 6. Schematisation of the spermatogenic cycles in three species of Mediterranean lacertids from coastal areas of NW Iberia (data taken from Carretero & Llorente 1991, 1995, 1997).

which suggest that atresia may be rare in lacertids (but not in other groups, see Méndez de la Cruz *et al.* 1993). Egg retention inside the female changes not only with the reproductive modality (oviparous/viviparous, Heulin *et al.* 1991) but also between different oviparous species (Braña *et al.* 1991, Galán & Arribas 2005) and maybe between populations.

## CYCLES OF LIPIDIC RESERVES

Prior to climatic factors, lipid storage of excess energy is a necessary condition for beginning reproduction in reptiles (Derikson 1976). Such lipids are stored in abdominal fat bodies in lacertids (Fitch 1970) but other compartments such as the tail, liver and carcass may also be involved (Roig *et al.* 2000). Such reserves describe an annual cycle inverse to the gonadal: lipids peak in late summer or early autumn, are not depleted in winter and are consumed during the reproductive season (Braña 1983; Braña *et al.* 1990, 1992; Carretero & Llorente 1991, 1995, 1997; Amat *et al.* 2000; Roig *et al.* 2000). Nevertheless, male and female cycles differ in level, intensity and timing of this cycle. Males store less reserves and spend them earlier than females in the activities related with breeding (mate searching and guarding, agonistic interactions, copulation) and recover them soon after because sperm production extends along several months (see spermatogenesis). As a result, the cycles of gonads and reserves are uncorrelated in males.

In contrast, ovarian and lipidic cycles are strongly adjusted. Females mainly behave as capital breeders directly investing lipids stored in the previous season developing the first (or unique) clutch (Derikson 1976, Etheridge *et al.* 1986). In the iteroparous lacertids, this does not apply to the subsequent clutches since after the first egg laying females lack or almost lack fat bodies (Braña 1983; Braña *et al.* 1992; Galán 1996a; Carretero & Llorente 1991, 1997). This finding suggests that they apparently behave

as income breeders investing the matter and energy provided by the prey ingested during the breeding season. As in the case of gonadal cycles, the degree of iteroparity (clutch frequency, % females involved) also depends on the “window chance” provided by temperature, photoperiod and food availability but just within the species limits.

## OTHER FACTORS?

Some of the patterns observed are, nevertheless, uncorrelated with abiotic environment and may reflect other pressures. Theoretically, any influence able to provoke food shortage would delay reproduction independently of climate conditions. Carretero & Llorente (1997) detected a delay in the reproduction of *Psammodromus algirus* in comparison with other localities with similar temperature and photoperiod associated with low spring rainfall and arthropod availability (see Santos & Llorente 2001 for a similar case in snakes). Habitat fragmentation also reduced the clutch size and mass in this species (Díaz *et al.* 2005) but these authors do not provide evidence if that takes place via follicular atresia (Méndez de la Cruz *et al.* 1993). Similarly, Amat (1997) in a path analysis of factors determining the clutch traits in a Pyrenean population of *Lacerta agilis* concludes that, whereas egg number is controlled by female size, her body condition is reflected on egg size. Furthermore at present, the only studies specifically testing for competitive influences in reproductive cycle Carretero *et al.* (2006) produced negative results.

## HISTORICAL CONSTRAINTS

Some reproductive traits are not environmentally mediated but historical, deriving from pressures acting in the past. Viviparity and, in general, egg retention (evolved in extreme cold environments, Shine 1983) represent strong constraints for reproductive timing since they prolong single reproductive events preventing its repetition even when environmental conditions would allow it. This affects mainly females since viviparous species (Heulin *et al.* 1991) and those with long egg retention (Roig *et al.* 2000, Arribas 2004, Galán & Arribas 2005) are not iteroparous. The same applies to large-sized species which are more constrained thermally than small ones (Bauwens 1999). Paradoxically, the thermophile species evolved under mild conditions but living in colder environments also face similar problems because they are more selective when starting reproduction (see before). In all cases, males are also affected since opportunities for fecundating monoestrous females are then more restricted in time



(Roig *et al.* 2000, Carretero *et al.* 2006) although female promiscuity and multiparental clutches seem to be the rule in such cases (Olsson *et al.* 1994a, b; Laloï *et al.* 2004; Uller & Olsson 2005).

Another example of historical influences on reproductive timing is constituted by those lacertids evolving under insular conditions, essentially, low predation, high density and intraspecific competition and unpredictable prey availability (Fig. 7). In order to face such pressures, insular lacertids enlarge the reproductive period in comparison with their continental equivalents living under similar climate regimes (Carretero *et al.* 1995; Adamopoulou & Valakos 2000; Castilla & Bauwens 2000a, b; Perera & Pérez-Mellado 2002; Galán 2003; Galán & Vicente 2003). This strategy assures the exploitation of scarce resources available since the body condition of lizards of both sexes may be extremely variable throughout the year without following a clear cycle (Carretero *et al.* 2005). Thus, male spermiogenesis is probably forced to track an extremely irregular and asynchronous vitellogenesis in females; it could be even predicted strong sperm competition based on amount and not in mate guarding WHY Putting the eggs in several clutches would be a way to provide suitable conditions for at least

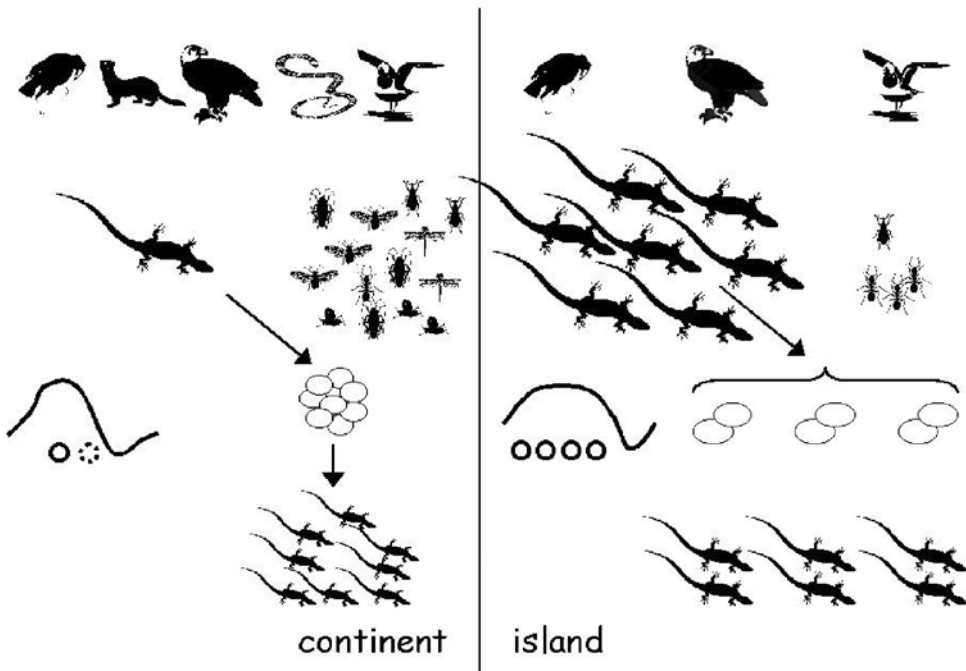


Fig. 7. Diagram of the reproductive strategy of insular lacertids. In islands, lizards have fewer terrestrial predators, attain high densities but face high intraspecific competition and low arthropod availability. Consequently, reproductive period is enlarged, males prolonging sperm production (lines) and females laying clutches (circles) of few, large eggs.

a part of the progeny and making them large would increase the survival possibilities of juveniles (Sinervo 1990) to face intraspecific aggression and cannibalism which are more common in dense island populations (Castilla & Dunlap 2001).

Such traits are shared by lacertids belonging to lineages with a long evolution in insularity (see previous references) and do not display much variation either between islands (Perera & Pérez-Mellado 2002) or when island species are introduced in the continent (Carretero *et al.* 1995). On the other hand, they do not appear in lacertids recently colonising islands which are more similar to continental forms (Bejakovic 1996a, c; Rúa & Galán 2003). Such conservativeness has also been evidenced for trophic ecology and provides additional support for the time-for-evolution hypothesis (Stephens & Wiens 2003, Carretero 2004).

One last example are lacertids with strong anatomical constraints, namely, those adapted to a strongly saxicolous life. In such cases, crevice dwelling limits not only the shape but also the number of eggs and such species usually lay elongated eggs in small, numerous clutches along a considerable period (Bejakovic *et al.* 1996b, Perera 2005), much as insular species do.

## AN EVOLUTIONARY PERSPECTIVE

Lacertid early evolution took place in the Western Palearctic (Estes 1983, Arnold 1989) where the most based branches of the family are still restricted (Harris *et al.* 1998). This suggest that reproductive cycles with “mixed-type” tendency in spermatogenesis and facultative iteroparity appeared as adaptations to temperate climate and can be considered plesiomorphic within the whole family (Fig. 8). As commented previously, the autumnal spermatogenesis and even vitellogenesis (Carretero & Llorente 1997) could represent the remains of a second reproductive season in the past under subtropical climates perhaps during the Miocene but more evidence on this should be provided.

In the context of conservativeness of lizard cycles (James & Shine 1985), adaptations to insularity and mountains or cold environments would just constitute derivations from such a primitive cycle. The same cycle has also been conserved in the lineages colonising central Asia and Far East living under temperate regimes (Telford 1997, Huang 1998, Ji *et al.* 1998, Szczerbak 2000). However, when the more advance members of the armatured clade colonised Africa (Arnold 1989) they had to face desertic and equatorial conditions of the Ethiopian region where temperature and photoperiod may be uninformative about environmental resources. Desert and savannah species in North Africa and the Middle East have probably shifted spermatogenesis to the breeding period (Perry & Dmi'el 1994, Schleich

*et al.* 1996) but tend to rely more on precipitation as an environmental cue for starting reproduction. Some members of the clade, namely *Acanthodactylus*, have secondarily recolonized Europe in the late Miocene (Harris *et al.* 2004) and still conserve this reproductive pattern (see previous references). However, equatorial species completely lost the seasonality in reproduction and display a continuous spermatogenesis, vitellogenesis and egg-laying solely constrained by food availability (Spawls *et al.* 2002, Fig. 8). Although temperate climate is also present in southern Africa where some species display patterns similar to those in Western Palearctic (Goldberg & Robinson 1979, Nkosi *et al.* 2004), it seems that crossing the equator has “released” some secondary temperate species from their phylogenetic constraints and more diversity is found. The aseasonal iteroparity of *Aporosaura achietae* from Namibia (Goldberg & Robinson 1979) and the inverse, autumnal cycle of *Ichnotropis capensis* from Botswana (Broadley 1967, Branch 1998, Fig. 8) can be interpreted in this sense. The subtropical members of the Asian genus *Takydromus* would be equally interesting to be investigated.

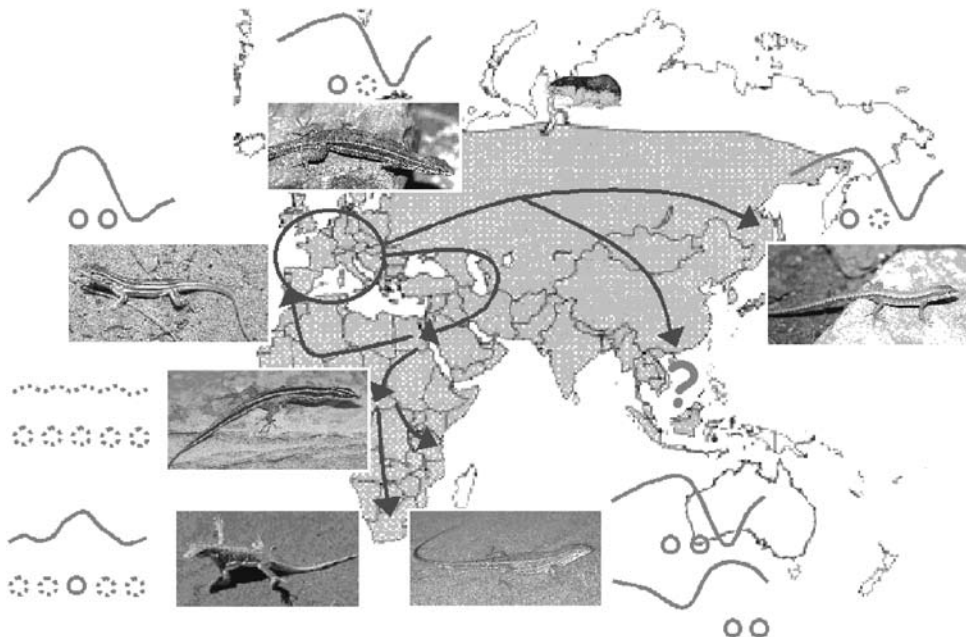


Fig. 8. Tentative scheme of the evolution of the reproductive cycles in lacertids according to the biogeography of this family. Sperm production (lines) and clutches (circles) are represented as in Figs 6 and 7.

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# Intraspecific variation of preferred temperatures in the NE form of *Podarcis hispanica*\*

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*Podarcis hispanica*\* is, in fact, a complex of several forms and ecophysiological traits previously investigated in one of them may be no longer generalisable to the others. Intraspecific variation of preferred temperatures ( $T_p$ ) was analysed in a population of the NE form of *P. hispanica*\* and compared with data on other forms coming from the literature. Adult lizards were collected in May, measured (SVL), weighed and exposed to a thermal gradient at nine time intervals.  $T_p$  changed with sex (males > females), size (positive correlation with body mass) and, more weakly, with time of day (early morning > mid morning and mid-day > afternoon). Two females decreased their  $T_p$  after egg-laying. Results corroborate previous studies demonstrating diel adaptability of  $T_p$  in lacertids and strong dependence on the individual's condition.  $T_p$  should be interpreted as a compromise between different pressures including gonadal changes, embryo development and social behaviour. Furthermore, in a comparison restricted to males, the NE form attained lower  $T_p$  than other Iberian *Podarcis* not directly related in the phylogeny, which may indicate changes at evolutionary level.

Keywords: thermal ecology, preferred temperatures, sex variation, diel variation, pregnancy, *Podarcis hispanica*\*, Iberian Peninsula.

## INTRODUCTION

Once considered a single species, *Podarcis hispanica*\* from the Iberian Peninsula, SE France and N Africa constitutes, in fact, a species complex, paraphyletic with *P. bocagei*

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and *P. carbonelli* (Harris & Sá-Sousa 2001, 2002; Harris *et al.* 2002; Pinho *et al.* 2003, 2006). Up to 9-10 forms of this complex, 6-7 of them in the Iberian Peninsula, have been characterised based on nuclear and mitochondrial markers (see previous references) and, some of them, are also supported by morphological (Geniez 2001, Sá-Sousa *et al.* 2002) and behavioural (Barbosa *et al.* 2005) evidence. Since considerable evolutionary divergence between them has been found (i.e. 10-12% in *cyt-b* mtDNA equivalent to 6-7 My, Harris *et al.* 2002), those ecophysiological traits previously investigated in one of these forms may be no longer generalisable to the others.

Preferred body temperature (Tp), in the absence of thermoregulatory constraints, correlates with several physiological optima in lizards (Huey & Bennet 1987, Bauwens *et al.* 1995). Two kinds of Tp variability should be considered (Castilla *et al.* 1999): within a population (short time scale) and among populations/species (evolutionary scale). On the other hand, individual lacertids are able to change their Tp in response to temporal variation within their life such as seasonal changes, reproductive condition and feeding status (Castilla *et al.* 1999, Carretero *et al.* 2005). In contrast, this trait appears to be evolutionarily conservative in lacertids responding slowly to directional selection, hence, Tp remaining similar between different populations of the same species (Patterson & Davies 1978; Van Damme *et al.* 1989, 1990; Gvozdík & Castilla 2001; Carretero *et al.* 2005; but see Scheers & Van Damme 2002) and only varying between species (Bauwens *et al.* 1995). Such variation could take place between the different Iberian forms of *Podarcis* since considerably variation is found in thermal environment of both their present ranges and their putative areas of origin (Sá-Sousa 2000, 2001; Harris *et al.* 2002).

Thus, in this paper we aim 1) to analyse the patterns of the intraspecific variation of Tp within a population of the NE form of *P. hispanica*\* and 2) to make a preliminary assessment of the conservativeness of Tp in the *P. hispanica*\* species complex by comparing the values obtained with those available for other forms in the literature.

## MATERIALS AND METHODS

Sixteen adult *P. hispanica*\* (9 males and 7 females) were collected in a thermomediterranean site from NE Iberia (Bellaterra, Cerdanyola del Vallés, UTM 31T DF2395, 150 m altitude) dominated by garrigue patching with *Pinus halepensis* and *Quercus ilex* forests in May 2000, coinciding with the peak of oviposition in this area (Llorente 1988, Kaliontzopoulou *et al.* 2004). Lizards were kept in individual 0.5 × 0.4 × 0.3 m terraria during not more than 2 weeks with food and water provided *ad libitum* and then released in the site of capture after the experiments. Immediately before each test, the lizard was measured (snout-vent length, SVL) to the nearest 0.05

mm with a digital calliper and weighed to the nearest 0.01 g with a digital balance. Each lizard was individually exposed to a photothermal gradient ( $\sim 20\text{--}45\text{ }^{\circ}\text{C}$ ,  $0.5 \times 0.5 \times 1.5\text{ m}$  length) produced by a 100-w reflector bulb fixed 15 cm above the *substratum*. The room of the experiments was fully exposed to sun light, hence, natural photoperiod was maintained.  $T_p$  was measured by inserting a k-termocouple probe associated with a digital thermometer (Digitron® 3208K, accuracy  $0.01\text{ }^{\circ}\text{C}$ ) in the cloaca. Body temperatures were recorded only for active lizards during a single day at nine consecutive intervals (Fig. 1, Table 1) distributed throughout the period of diel activity observed in the field (Llorente 1988). Time interval between two consecutive measurements was 1 hour. A limited subset of females ( $n = 2$ ) was analysed before and after egg-laying in terraria.

Data were not transformed since distributions did not deviate from normality (Kolmogorov-Smirnov tests,  $P > 0.05$  in all cases), were homocedastic (univariate Levene tests and multivariate Box M,  $P > 0.05$  in all cases) and variances and means were uncorrelated. Since measures were repeated for the same individual (for each interval and, in two females, before and after egg-laying) statistical analysis was based on Analysis of Variance for Repeated Measures (ANOVAR) of  $T_p$  with class (males, pregnant females, non-pregnant females) as between subject factor and time interval as within subject factor. Sphericity assumption of ANOVAR was tested by means of Mauchley's tests.

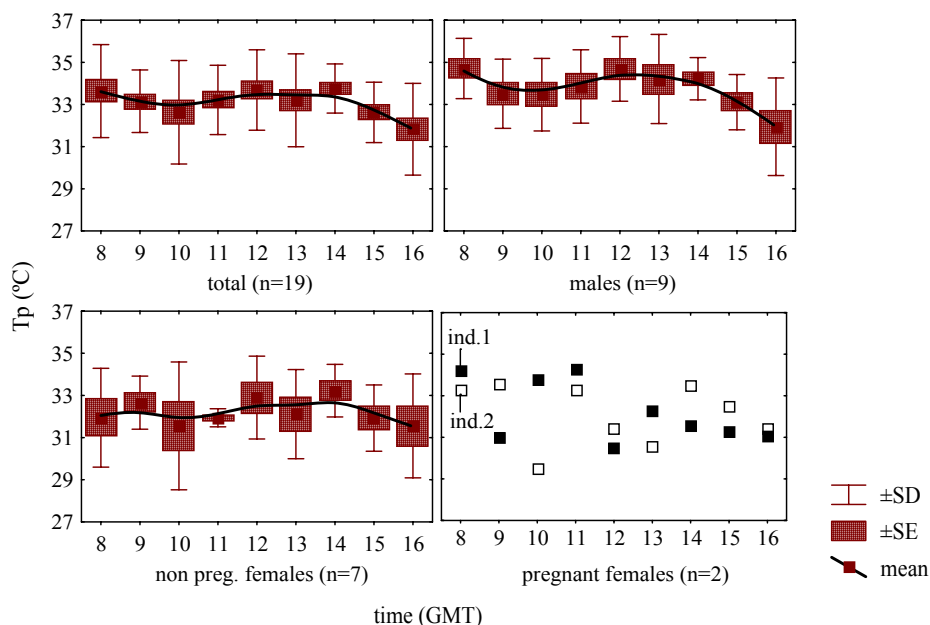


Fig. 1. Temporal variation of  $T_p$  considering the three classes and pooled.

Table 1. Descriptive statistics of  $T_p$  considering lizard classes and time intervals.  
\* means of 9 time intervals.

class	time	n	mean $\pm$ SE	median	min.	max.
males	8	9	34.71 $\pm$ 0.48	35.30	32.5	36.7
	9	9	33.51 $\pm$ 0.55	33.50	30.7	36.7
	10	9	33.47 $\pm$ 0.57	32.60	32.0	37.3
	11	9	33.86 $\pm$ 0.58	34.10	30.6	36.7
	12	9	34.69 $\pm$ 0.51	34.80	32.0	36.5
	13	9	34.21 $\pm$ 0.71	35.50	29.9	36.4
	14	9	34.22 $\pm$ 0.33	34.50	32.8	35.8
	15	9	33.11 $\pm$ 0.44	32.70	31.9	36.0
	16	9	31.94 $\pm$ 0.77	31.60	29.0	35.7
	total*	9	33.75 $\pm$ 0.24	33.85	29.0	37.3
non preg. females	8	7	31.94 $\pm$ 0.89	33.00	28.8	34.5
	9	7	32.66 $\pm$ 0.48	32.40	31.1	34.4
	10	7	31.56 $\pm$ 1.15	32.00	26.0	36.1
	11	7	31.94 $\pm$ 0.16	32.00	31.3	32.7
	12	7	32.90 $\pm$ 0.74	32.80	30.0	35.3
	13	7	32.11 $\pm$ 0.80	31.80	28.7	34.9
	14	7	33.23 $\pm$ 0.47	33.20	31.7	35.5
	15	7	31.93 $\pm$ 0.59	31.50	29.3	33.9
	16	7	31.56 $\pm$ 0.93	33.10	28.0	34.0
	total*	7	32.20 $\pm$ 0.31	32.12	26.0	36.1
pregnant females	8	2	34.75 $\pm$ 0.45	34.75	34.3	35.2
	9	2	33.30 $\pm$ 1.30	33.30	32.0	34.6
	10	2	32.65 $\pm$ 2.15	32.65	30.5	34.8
	11	2	34.80 $\pm$ 0.50	34.80	34.3	35.3
	12	2	31.95 $\pm$ 0.45	31.95	31.5	32.4
	13	2	32.45 $\pm$ 0.85	32.45	31.6	33.3
	14	2	33.55 $\pm$ 0.95	33.55	32.6	34.5
	15	2	32.90 $\pm$ 0.60	32.90	32.3	33.5
	16	2	32.25 $\pm$ 0.15	32.25	32.1	32.4
	total*	2	33.18 $\pm$ 0.06	33.18	30.5	35.3

## RESULTS

Table 1 shows the descriptive statistics by class and time interval. Overall  $T_p$  (means of 9 intervals, pregnant females excluded) was  $33.07 \pm 0.27$  °C (26.0-37.3). Nevertheless, ANOVAR revealed substantial variation with both class and time in-



Table 2. Results of the ANOVARs. Analysis restricted to females produced identical results with and without covariate.

2-way ANOVAR	F	d.f.	P
sex	15.91	1, 14	0.001
time (R)	2.16	8, 112	0.036
sex × time	0.71	8, 112	0.682
Mauchley's sphericity test	$\chi^2 = 37.10$	35	0.37
2-way ANOVAR (weight cov.)	F	d.f.	P
sex	8.20	1, 13	0.01
time (R)	2.16	8, 112	0.036
sex × time	0.71	8, 112	0.682
Mauchley's sphericity test	$\chi^2 = 37.10$	35	0.37
2-way ANOVAR (females)	F	d.f.	P
pregnancy (R)	3.30	8, 8	0.055
time (R)	0.47	1, 1	0.618
pregnancy × time	0.28	8, 8	0.183

terval (Fig. 1, Table 2).  $T_p$  values were high in the early morning, decreased in the mid-morning, rose in the early afternoon and decreased again in the late afternoon. Males selected for higher temperatures than females and, in the two cases analysed, females decreased their  $T_p$  after egg-laying, although such variation was only marginally significant (Fig. 2, Table 2).

Furthermore, partial correlation analysis with all individuals except pregnant females detected significant positive correlation of individual mean  $T_p$  with weight ( $R_{p_{13}} = 0.63$ ,  $P = 0.01$ ) but not with SVL ( $R_{p_{13}} = -0.49$ ,  $P = 0.07$ , n.s.) (Fig. 2). Similar results were obtained for minimum  $T_p$  reached by individual in the nine time intervals (SVL  $R_{p_{13}} = 0.55$ ,  $P = 0.03$ ; weight  $R_{p_{13}} = -0.25$ ,  $P = 0.37$ , n.s.). Thus, we repeated both ANOVARs using body weight as a covariate but the results of comparisons remained the same (Table 2).

## DISCUSSION

These results corroborate recent studies on other lacertids demonstrating strong dependence of  $T_p$  on the individual's condition as well as weaker but significant diel adaptability (Castilla *et al.* 1999 and references therein, Gvozdík & Castilla 2001, Carretero *et al.* 2005).

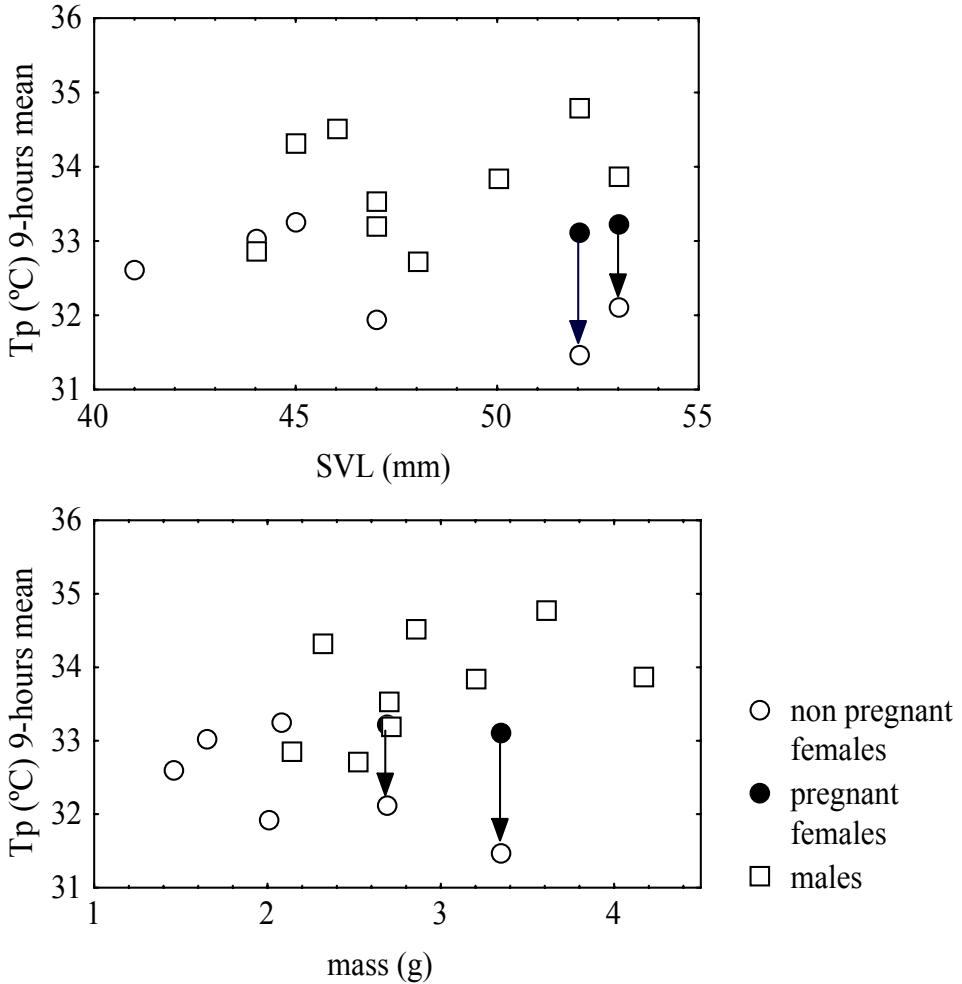


Fig. 2. Relationships between Tp and body size. Arrows indicate changes after egg-laying.

First, lacertids tend to raise Tp in the early morning when environmental temperatures are low and thermoregulation becomes a priority in relation to other requirements (Rismiller & Heldmaier 1982, Tosini & Avery 1994, Carretero *et al.* 2005). The results of this study indicate that the same does not apply to the late afternoon, at least in *P. hispanica*\*

Moreover, pregnant female Tp usually reflects the optimum for embryonic development rather than for the physiology of the female itself (Carretero *et al.* 2005). In contrast, with *L. vivipara* (Heulin 1987, Van Damme *et al.* 1987, Gvozdík & Castilla 2001, Carretero *et al.* 2005), *P. hispanica*\* females decreased

T<sub>p</sub> after egg-laying. Although low sample size does not allow a general picture to be drawn, gestation period is much shorter in *Podarcis* spp. than oviparous *L. vivipara* (Braña *et al.* 1991), suggesting lower constraints for embryo development. Nevertheless, other physiological processes may be involved since non-pregnant females still showed lower T<sub>p</sub> than males. For instance, it has been suggested that the spermatogenic cycle in males would need higher temperatures (Carretero *et al.* 2005), especially in the beginning of the reproductive season (Gavaud 1991, Carretero *et al.* submitted).

On the other hand, influence of relative body size also occurred within adults supporting previous suggestions that small individuals are thermally more opportunistic (Carretero & Llorente 1995, Carretero *et al.* 2005) and/or compensate for evaporative loss (Bowker 1993, Lorenzon *et al.* 1999). The fact that body mass and not body length itself is the main responsible for such variation does not exclude either of both hypotheses and further research is needed to distinguish between these two factors. Essentially, discrepancies between mass and length in lizards are due to regenerated tails and fat reserves. Both parameters involve changes in body condition which should be the putative proximal factor to be explored.

Obviously, after all this evidence any interpopulation analysis should avoid using pooled data but perform at least class-by-class comparisons (see Carretero *et al.* 2005). Male T<sub>p</sub>s observed here (mean  $\pm$  SE: 33.75  $\pm$  0.24, median: 33.85 °C) were lower than those published for other Iberian members of the complex (*P. carbonelli* median 35.15 °C; *P. hispanica*\* NW form: median 34.40 °C; *P. (h.) atrata* from Columbretes islands: median 34.50 °C; Castilla & Bauwens 1991, Bauwens *et al.* 1995) and even than the Middle European *P. muralis* (median 34.15 °C; Bauwens *et al.* 1995). Since lab methods were essentially the same, then assuming the comparativeness of the results, it should be concluded that T<sub>p</sub> differences arose from old, independent evolutionary trajectories under different thermal conditions. In fact, available phylogenies indicate that, whereas *P. carbonelli* and the NW form of *P. hispanica*\* are related groups originated in W Iberian Peninsula (Harris & Sá-Sousa 2001, 2002), the NE form of *P. hispanica*\* is deeply divergent form all other Iberian *Podarcis* (except those from the SE, Pinho *et al.* 2006) and occupies the northernmost range (including France, Catalonia, Ebro Valley, Basque Country and N Castilian Plateau, Geniez 2001 unpubl. data). Nevertheless, the same evidence indicates a weak separation between *P. (h.) atrata* and the NE form of *P. hispanica* (Harris & Sá-Sousa 2002). Further studies including the other forms and replicating the same methodology are currently being carried out (unpubl. data) to corroborate these preliminary conclusions.

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# Podarcis lizards in an agro-environment in Tuscany (Central Italy): preliminary data on the role of olive tree plantations

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*Both, Podarcis muralis and P. sicula are widespread in Tuscany (Central Italy). The first species is found along the coast and up to mountain localities while the second, in general more eurytopic, is mainly found at low and medium altitudes. The present study deals with micro-habitat preference of both species in a typical Tuscan agro-environment (olive tree plantations). Podarcis muralis resulted to prefer fields covered by vegetation, while P. sicula prefers bare soil fields. Both species have been most frequently observed on olive trees. The latter also resulted to be the preferred substratum for basking and resting activities for both lizards. The diet resulted to be mainly insectivorous in both species, though feeding spectrum of P. sicula is relatively wider than in P. muralis.*

Keywords: *Podarcis muralis*, *P. sicula*, agro-environment, Tuscany, Italy.

## INTRODUCTION

Olive tree plantations are one of the traditional cultivations in Tuscany. The fields located on hilly areas are mainly disposed on terraces supported by dry-stone walls; the latter are well known as one of the most suitable places for many lizard species.

The olive tree (*Olea europaea*) is an evergreen plant which belongs to the family *Oleaceae*. Its growth is very slow and this plant can live for centuries. As the tree gets older, the trunk becomes irregular and twisted, and the bark becomes thick and tends to crumble easily. These changes in external morphology make the trees very suitable to arthropods and small-sized vertebrates.

The lacertid lizards *Podarcis sicula* and *P. muralis* can be often observed on olive trees. In order to evaluate the role of these trees in the ecology of these lizards, we

started to monitor the behaviour of both species in two olive fields located on the western slope of a hilly area North off Florence (Tuscany, Central Italy).

*P. muralis* is distributed in the most part of the Italian peninsula. In the southern regions of its distribution range the species prefers more shady and moist habitats (Arnold & Burton 1995, Corti & Lo Cascio 1999). This is evident also in microhabitat selection: some studies on this matter (Capula *et al.* 1993, Rugiero 1993) revealed that *P. muralis* prefers more moist microhabitats, also richer in vegetation. *P. sicula* is often sympatric with *P. muralis* in many parts of Italy. *P. sicula* is characteristic of open fields, cultivations and coastal environments (Arnold & Burton 1995) and it is considered by many authors to be an opportunistic species. It prefers dryer and more open habitats when compared with *P. muralis*. Activity rhythms are bimodal in both lizards (Pianka 1973, Avery 1993) and their diet is mainly insectivorous (Pérez-Mellado & Corti 1993).

## MATERIALS AND METHODS

### *Study area*

The field study was carried out from May to November 2003 and from March to April 2004 within the lizard's activity period, in two separate olive tree plantations situated in the NW Florentine countryside. One was characterised by herbaceous soil cover ("Field 1") and one by ploughed soil ("Field 2"), the latter being ploughed several times, starting by early spring. Both fields were partially surrounded by dry-stone walls, made mainly by local sedimentary stones.

### *Olive tree plantation characteristics*

"Field 1": area 1700 m<sup>2</sup>, perimeter 228 m, covered by spontaneous herbaceous vegetation for the whole observation period. No. of olive trees = 35. Other trees present in the field: bushes and 2 peach trees (*Prunus persica*). Presence of a stone built water tank considered, in the present study, as stone wall habitat.

"Field 2": area 1300 m<sup>2</sup>, perimeter 150 m, periodically ploughed during the whole observation period therefore always without vegetation soil cover. No. of olive trees = 52. Other trees present in the field: 2 *Cupressus sempervirens*.

Both sites are located on the southern slope of the same hill at a distance of about 100 m. "Field 2" is placed at a higher position on the hill and is more exposed to sun; all these features make it, together with the absence of soil cover, a more open as well as, a dryer environment.



### Protocol

During the first study year, habitat and microhabitat selection were investigated for both species, studied in terms of number of sightings of individuals in the two fields and on different substrates. During the second year our attention was focused on some ethological aspects linked with the selection of microhabitat, by using the focal animal technique. We investigated also the diet of the two species.

We conducted 72 observation days, with an average observation time of 3 hours/day. Climate conditions, air and substrates temperatures were registered in all cases. In total, 140 hours of research were spent during the first year of study, and 100 hours during the second one.

Animals resident in the two sites were periodically captured (using a noose) and marked with nail polish (water ink marker and permanent water resistant ink pen used initially did not last enough). After about 20 days the markings had to be renewed, because of both the progressive signal's attenuation and epidermis sloughing. Data were collected by visiting study areas one or more times in a day for every day of work, walking all over in random directions (Rugiero 1993) in order to record the presence of individuals (marked or not) on the different substrates (classified in: soil, olive tree, walls, other trees), times of observations, sex, tail conditions, presence or absence of marking, as well as air and *substratum* temperature (using an electronic thermometer) and meteorological conditions. Habitat preference of the two species was observed using the "focal samples" as recording technique (Avery 1993, Pisani & Bulla 2000). We followed 3 adult *P. muralis* in "Field 1" and 4 *P. sicula* in "Field 2", for 65 hours (*P. muralis*: 31hr 50min; *P. sicula* 33hr 44min). Focal individuals were followed and observed for as long as possible using binoculars, recording each activity behaviour and its length. Behaviour were categorised as follows (see also Pérez-Mellado & Diego-Rasilla 2000):

- *Basking*: direct exposition to the sun to increase body temperature, by dorsoventral flattening of the body (Avery 1978);
- *Moving*: every single event of locomotory activity;
- *Resting*: inactivity periods in a cool retreat;
- *Vigilance*: usually defining bursts of movement with pauses during which lizards are immobile and scan the environment keeping their head high and their eyes rose to the maximum level above the ground. In the present research we defined as vigilance all behaviours performed by lizards to locate potential preys as well as predators, considering all movements as distinct;
- *Social interactions*: displays concerned with court-ship, mating, aggressions;
- *Predation*: prey capture and manipulation.

To reduce seasonality effects the two species were observed on alternate days.

When possible during the whole period of study the lizard faecal pellets were collected at the times of the capture (to avoid misidentification) in order to analyse their diet (Capula *et. al.* 1993, Peréz-Mellado & Corti 1993). Faeces were analyzed using a microscope, examining rests of consumed preys and classifying them by order or, when possible, by family. Trophic niche breadth was estimated by using Levins' formula (Levins 1968). Trophic niche overlap was calculated using Pianka's formula (Pianka 1973). Population density has been estimated with CMR method (Krebs 1999). G- and  $\chi^2$  tests were performed to analyse habitat and *substratum* preference. We used a cluster analysis (Unweighted Pair Group Method with Arithmetic mean, UPGMA) to test differences between focal individuals in the distribution of behaviour on the different substrates and a Friedman test to analyse the preference of *substratum* in the performing of the above mentioned behaviours. All data were analysed with Excel and SPSS 8.0 Statistical Package.

## RESULTS

### *Habitat preferences*

First year results showed that *P. muralis* was more abundant (in terms of number of sightings) than *P. sicula* in "Field 1" (*P. muralis*: n = 182, *P. sicula*: n = 49; G-Test,  $P = < 0.001$ ). *P. sicula* preferred, as expected, "Field 2", which was more open and arid (*P. muralis*: n = 39, *P. sicula*: n = 100; G-Test,  $P = < 0.001$ ).

Population density in the two fields was estimated as follows: 20 individuals of *P. muralis* and 6 of *P. sicula* every 1000 m<sup>2</sup> in "Field 1"; 10 individuals of *P. muralis* and 24 of *P. sicula* every 1000 m<sup>2</sup> in "Field 1".

### *Substratum preferences*

We calculated the frequencies of observation of the animals on the different substrates: both species were observed spending most of the times on olive trees. In "Field 1" *P. muralis* was seen on olive trees in 72% of the observation time, 27% on walls, 0.5% on soil, 0.5% on other trees ( $\chi^2 = 247.98$ ,  $P < 0.001$ ); *P. sicula* was observed on olive trees on 74.4% of the observation time, 20.5% on walls, 5.1% on soil ( $\chi^2 = 26.65$ ,  $P < 0.001$ ). In "Field 2" *P. muralis* was observed on olive trees in 83.3% of the observation time, on walls 8.3%, on soil 5.6% and on other trees 2.8% ( $\chi^2 = 65.89$ ,  $P < 0.001$ ); *P. sicula* was observed on trees in 73% of the observation time, on walls 11% and on soil 16% ( $\chi^2 = 71.18$ ,  $P < 0.001$ ) (Fig. 1). Dividing all the observations between Spring/Summer and early Autumn we observed that olive trees are the pre-

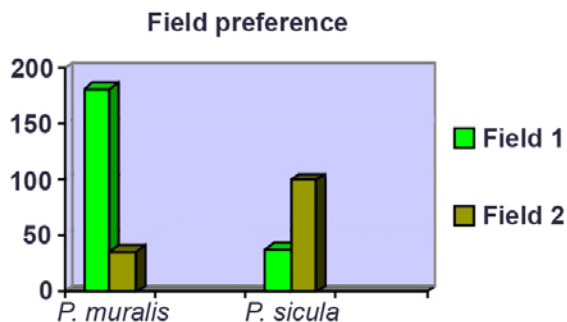


Fig.1. Field preference observed by the two lizard species.

ferred *substratum* in Spring/Summer for both species ( $\chi^2 = 24.97$ ,  $P < 0.001$ ), while in the cooler season data were not enough to be statistically significant (though trend seems to suggest that *P. muralis* prefers walls frequentation in “Field 1”).

*Ethological aspects*

Both lizard species spent most of the time basking (*P. muralis* 41.9%,  $\chi^2 = 18.16$ ,  $P < 0.001$ ; *P. sicula* 32.53%,  $\chi^2 = 9.3$ ,  $P < 0.005$ ). Resting was represented by 31.8% in *P. sicula* and by 25.7% in *P. muralis*, while movement represented by 19.6% in *P. sicula* and by 17.0% in *P. muralis*. The latter spent 15% of the time in vigilance activity while *P. sicula* 14.8%. Lastly, *P. muralis* spent respectively 0.6% and 0.1% of the observed time in predation acts and in social interactions, while *P. sicula* respectively 0.5% and 0.1%.

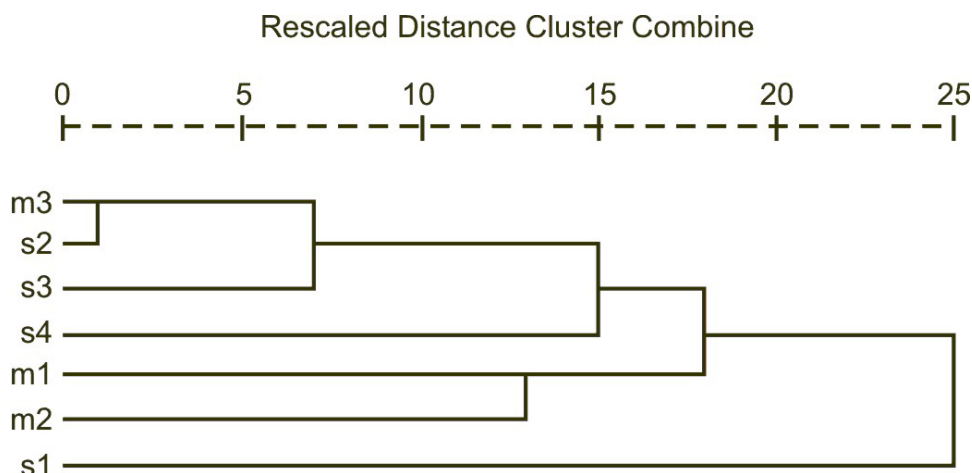


Fig 2. UPGMA Cluster Analysis of behavioural frequencies of *P muralis* (m1-m3) and *P. sicula* (s1-s4) individuals.

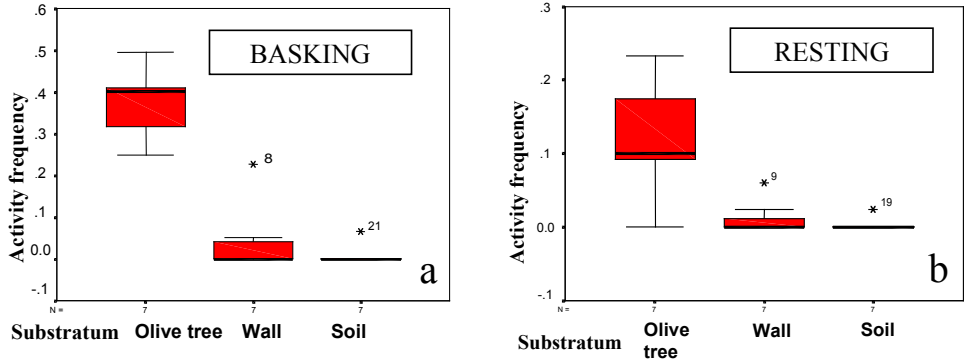


Fig. 3. Preferred *substratum* for basking (a) and resting (b).

In order to evaluate the eventual differences in the choice of *substratum* for the various behaviours between the focal individuals of both species (amount of time spent in every activity on every *substrata*), a cluster analysis was performed only on morning data as they were more abundant. Results showed that both species adopt comparable behavioural strategies (Fig. 2) and therefore a Friedman test has been performed on the cumulative data to see if any *substratum* was preferred by lizards for any behaviour. Olive trees resulted to be the preferred *substratum* for basking and resting (Fig. 3a, b), while movement and vigilance did not (social interactions and predation observation were not statistically sufficient).

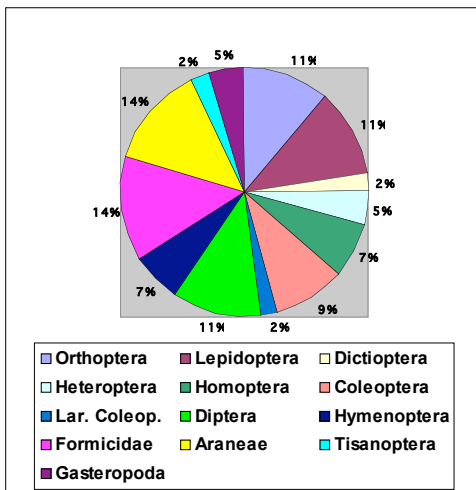


Fig. 4. Prey types consumed by *P. sicula*.

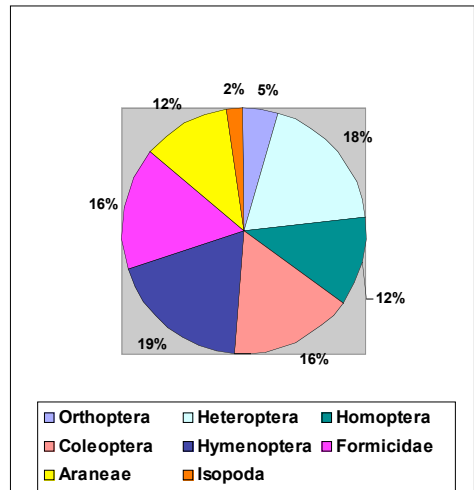


Fig. 5. Prey types consumed by *P. muralis*.

### Diet

Diet analysis was carried out only on a small sample size of faecal pellets collected (total prey  $n = 96$ , 44 from *P. sicula* and 51 from *P. muralis*). *Podarcis sicula* niche breadth (10.08) was higher than that of *P. muralis* (7.53). Trophic niche overlap was relatively high (0.79). *P. sicula* consumed preys that were absent in the diet of *P. muralis*. These prey are typical of an active forager hunting mainly on the ground, as *e.g.*, Gasteropoda (Figs 4-5) (Pérez-Mellado & Corti 1993).

## DISCUSSION

Olive trees represent a suitable microhabitat for both *P. muralis* and *P. sicula*. They are the most frequented *substratum* during the main activity period of these lizards.

Olive trees provide to lizards:

- optimal basking places in which easily sun exposed sites can be alternated with shaded ones,
- good observation places from which easily scan the surroundings,
- availability of shelters to escape predators,
- availability of food, because of the variable arthropod fauna detected on the trunk and surrounding of the trees.

The association olive trees/stone-wall, seem to be very important for the maintenance of both *Podarcis* species in the Tuscan agro-ecosystems. Therefore land use conversion, *i.e.*, eventual conversion of olive tree plantation into another kind of cultivation, *e.g.*, into vineyards, could severely affect the distribution of these species.

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# Patterns of shape and size sexual dimorphism in a population of *Podarcis hispanica*\* (Reptilia: Lacertidae) from NE Iberia

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*Size and shape sexual dimorphism have long been of great interest for animal biologists and herpetologists. Although general trends do exist, every species may represent a particular case and intraspecific variation in the degree and patterns of sexual dimorphism is not rare. In the case of P. hispanica\*, which presents a high morphological variability and whose intraspecific taxonomy is yet not well defined, interpopulational differences could help to elucidate the morphological patterns observed. The results of a morphological analysis of a population belonging the NE form of P. hispanica\* are here provided. A marked sexual dimorphism exists for the characters studied, both biometric and pholidotic, although sexual dimorphism in SVL is absent. Sexual differences in pholidotic characters, rarely examined, were marked, not only in femoral pores and ventral scales which are the characters usually studied in lizards, but also in gular scales and subdigital lamellae. The patterns of size and shape sexual dimorphism were in some cases also reflected in the analysis of static allometries. Influences of sexual and natural selection on those traits are discussed.*

Keywords: sexual dimorphism, size, shape, scaling, *Podarcis hispanica*\*, Iberian Peninsula.

## INTRODUCTION

Sexual dimorphism is a common trait in reptiles (Schoener 1977, Cooper & Vitt 1989) and it has been shown to be related to sexual selection (Shine 1978, Perry 1996, Berry & Shine 1980, Anderson & Vitt 1990, Olsson *et al.* 2002), fecundity selection (Bonnet *et al.* 1997, Shine *et al.* 1998, Olsson *et al.* 2002) and resource par-

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tioning between the two sexes (Schoener 1977, Shine 1991, Andersson 1994, Herrel *et al.* 1999, Shine *et al.* 2002, Baird *et al.* 2003). In lacertid lizards, males are usually larger than females (Böhme 1986; Pérez-Mellado 1998a, b). However, there are species or populations where the reverse situation can be found or, simply, no significant size differences between the sexes exist (Braña 1996). Nevertheless, as a general rule in lizards, males have relatively bigger heads (Braña 1996; Herrel *et al.* 1996, 1999; Molina-Borja 2003), a pattern usually attributed to sexual selection (Carothers 1984, Hews 1990, Andersson 1994, Braña 1996, Gvozdik & Van Damme 2003). Sexual dimorphism in body size and shape in lizards might reflect differences in age distribution between the sexes or differences in body size within age classes (Stamps 1993, Stamps & Krishnan 1997), as well as differences in strategies for allocation of energy during growth (Dunham 1978, Haenel & John-Alder 2002).

*Podarcis hispanica*\* (*sensu lato*) is distributed throughout the Iberian Peninsula, South France and North Africa. It was once considered a single species, presenting a very high intraspecific morphological variability and its subspecific taxonomy has been revised many times (Pérez-Mellado 1998a, b; Barbadillo *et al.* 1999; Salvador 1986). However, increasing evidence advocates for a species complex. Different authors have defined a variable number of forms (Pérez-Mellado 1998a, b; Geniez 2001) and recent molecular studies (Harris & Sá-Sousa 2001, 2002; Pinho *et al.* 2003, 2004) indicate the presence of five-six morphological/genetic forms in the Iberian Peninsula.

As described in its general form, *P. hispanica*\* is a medium-sized species, adult males being from 38 to 70 mm and adult females from 37 to 67 mm long. (Pérez-Mellado 1998a, b; Barbadillo *et al.* 1999). It usually lives in rocky environments where it climbs frequently, but it can also be found in trees or present ground-dwelling behaviour in lack of rocks (Pérez-Mellado 1998a, b; Barbadillo *et al.* 1999). Although sexual dimorphism is not extreme, males are slightly larger than females, their heads being broader and generally more robust and their extremities longer (Pérez-Mellado 1998a, b). Females of *P. hispanica*\* from the Cantabrian Mountains (Northwestern Spain) have been reported to have longer trunks than males (Braña 1996). However, there are marked interpopulational differences in the species' morphology (Geniez 2001), as well as in other aspects of its biology (Pérez-Mellado 1998a, b). Consequently, aspects previously investigated in one form are not to be generalized to others without previous investigation.

Geographic differences in the *P. hispanica* complex bring forward the need for local studies on morphology to test sexual variability. The subject of this study was to describe the morphology of a population of *P. hispanica*\* from the area of Barcelona (NE Spain), to investigate the morphological differences between the two sexes and to elucidate the proximal mechanisms responsible for these differences.



## MATERIALS AND METHODS

### *Study population*

Specimens for this study were collected during the years 1984-1986. The sampling site was located in the Experimental Area of the Faculty of Biology of the University of Barcelona (UTM 31TDF28) and the studied population is genetically typified as belonging to the NE type of *P. hispanica*\* (Harris & Sá-Sousa 2002, Pinho *et al.* 2003). Lizards were collected by hand approximately at a monthly basis and an effort was made for all size classes to be included in the sampling. As both sexes in the population reach sexual maturity within the first year after their birth (Llorente 1988), a distinction was made only between adult and immature individuals. Size class and sex of the individuals were determined after dissection by the presence of enlarged follicles or oviductal eggs in females, and the size and aspect of testes and epididymes in males (Llorente 1988). A total of 59 adult males, 52 females, 7 immature males and 12 immature females were examined.

### *Characters studied*

Eleven biometric characters and six pholidotic characters were recorded in the specimens analyzed. The following biometric characters were measured to the nearest 0.1 mm using an electronic caliper: snout-vent length (SVL), trunk length (TRL), head length (HL), head width (HW), head height (HH), mouth opening (MO) defined as the distance between the point of the snout and the end of the last supralabial scale, front foot length (FFL), femur length (FL), tibia length (TBL), total length of the 4th toe and the tarsus (4TL) and hind foot length (HFL). Bilateral characters were measured on the right side of the lizards' body when possible. Measurements were taken following the methodological recommendations on morphometry of the Lacertidae given by Pérez-Mellado & Gosá (1988). Six more pholidotic characters were studied: colar scale number (CSN), gular scale number (GSN), dorsal scales around midbody (DSN), number of transversal ventral scale lines (VSN), femoral pores at the left and right side of the body (FPNL and FPNR respectively) and subdigital lamellae at the left and right side of the body (SLNL and SLNR respectively).

### *Data analysis*

Pholidotic characters were not normally distributed (Kolmogorov-Smirnov test) and thus were examined for differences among the four size/sex classes using non-parametric statistics. In the case of bilateral characters, the mean of the two sides was

calculated, in order to include all the information available without pseudoreplicating the data. For biometric characters, we first conducted univariate two-way ANOVAs for each character to examine the effect of size class and sex on the biometry of the lizards and the interaction between them. When the interaction size\*sex was significant, Scheffé's *post-hoc* comparisons were applied to further investigate the differences between groups. The same procedure was followed using only measurements from the ten largest individuals of each sex, as to investigate for sexual size dimorphism, eliminating biases possibly introduced by differential sampling of the two sexes (Carothers 1984, Gibbons & Lovich 1990, Stamps & Andrews 1992, Smith & Nickel 2002). In this case the non-parametric Mann-Whitney U test was applied to compare between the two sexes. A discriminant and canonical variate analysis was conducted on raw data, in order to detect the variables responsible for the discrimination among the four size/sex classes.

In order to investigate differences in shape among the size/sex classes, univariate ANCOVAs were conducted for each character, using SVL as a covariate. The residuals of log-transformed biometric variables in their regression with log (SVL) were introduced in a discriminant and canonical variate analysis and the patterns of variation observed among the size/sex classes were examined.

Finally, the scaling of each of the biometric variables (log-transformed) with SVL was examined, treating separately the two sexes. Due to the presence of measurement error in both the independent and the dependent variable, ordinary least-squares regression would provide skewed values for the allometry equations (McArdle 1988, Sokal & Rohlf 1995). Thus, reduced major axis (RMA) regression was applied, using the software developed by Bohonak (2002) for this purpose. Deviations from isometry were tested using the formulas given in Clarke (1980) and homogeneity of slopes between groups was examined by inspection of the 95% confidence limits of the slopes' estimates.

## RESULTS

### *Pholidosis*

No differences were detected between immature and adult animals (Mann-Whitney U test,  $P > 0.1$  for both sexes and all the examined variables), thus subsequent analyses were conducted grouping immatures and adults of each sex. Sexes differed significantly in dorsal, ventral and gular scales, femoral pores and subdigital lamellae (see statistics in Table 1).

Table 1.

Descriptive statistics for the two sexes of the studied population and results of Mann-Whitney U tests; Numbers indicate mean  $\pm$  SE, range and sample size. See materials and methods section for variables' abbreviations.

Variable	Males	Females	Mann-Whitney Z	P-level
CSN	10.02 $\pm$ 0.13	9.72 $\pm$ 0.18		
	8-12	7-13	-1.233	0.217
	66	60		
GSN	27.06 $\pm$ 0.33	26.00 $\pm$ 0.27		
	22-33	21-33	-2.371	1.8*10 <sup>-2</sup>
	65	62		
DSN	59.02 $\pm$ 0.53	56.92 $\pm$ 0.43		
	51-74	50-68	-3.059	2*10 <sup>-3</sup>
	66	62		
VSN	25.47 $\pm$ 0.14	29.20 $\pm$ 0.20		
	22-29	26-34	9.196	3.7*10 <sup>-20</sup>
	66	60		
FPN	18.58 $\pm$ 0.18	17.64 $\pm$ 0.22		
	15.5-21	14-21.5	-3.139	1.7*10 <sup>-3</sup>
	60	61		
SLN	25.07 $\pm$ 0.25	23.76 $\pm$ 0.21		
	21-29.5	20-27.5	-3.713	2.05*10 <sup>-4</sup>
	61	54		

## Biometry

### Size variation

Table 2 shows the descriptive statistics of all the biometric variables studied for the four size/sex classes, as well as the results of the ANOVA comparisons. The *post-hoc* comparisons showed no significant differences in SVL between different sexes of the same size class. For the rest of the biometric characters studied, differences were significant among all size/sex classes (Scheffé's tests,  $P < 0.05$ ), except between the two sexes of immature animals, which only presented significant differences in trunk length (TRL). On the other hand, the two sexes of adult lizards were significantly different for the rest of biometric characters (Scheffé's tests,  $P < 0.05$ ), but not for TRL (Scheffé's tests,  $P = 0.28$ ). In contrast, when analyzing the ten largest adult individuals of each sex, differences between sexes were significant for all the biometric variables including SVL (Mann-Whitney tests,  $P < 0.01$  for all the variables) males being larger than females, except for TRL, which was not significantly different between the sexes (Mann-Whitney test,  $P = 0.52$ ).

Table 2.

Descriptive statistics of the biometric variables for the four age/sex classes in the population studied and ANOVA comparisons for age and sex. Numbers indicate mean  $\pm$  SE, range and sample size. See materials and methods section for variables' abbreviations.

Character	Adult	Adult	Immature	Immature	ANOVA (age, sex, age*sex)		
	males	females	males	females	F	d.f.	P-level
SVL	50.46 $\pm$ 0.68	48.83 $\pm$ 0.63	26.14 $\pm$ 1.53	32.57 $\pm$ 1.77	252.844	1, 125	<0.01
	40.50-61.10	41.00-56.30	22.10-33.20	22.50-38.60	3.525	1, 125	6.27*10 <sup>-2</sup>
	59	51	7	12	9.958	1, 125	2*10 <sup>-3</sup>
TRL	22.94 $\pm$ 0.38	24.07 $\pm$ 0.42	10.32 $\pm$ 0.92	14.62 $\pm$ 1.20	196.444	1, 124	<0.01
	17.58-29.59	18.47-29.37	7.38-14.74	7.93-20.19	11.923	1, 124	7.6*10 <sup>-4</sup>
	59	51	7	11	4.036	1, 124	4.7*10 <sup>-2</sup>
HL	13.79 $\pm$ 0.18	11.75 $\pm$ 0.11	7.83 $\pm$ 0.27	8.97 $\pm$ 0.33	221.449	1, 125	<0.01
	10.80-17.20	10.00-13.20	7.10-9.20	7.20-10.20	2.341	1, 125	0.13
	59	51	7	12	29.203	1, 125	3.2*10 <sup>-7</sup>
HW	8.23 $\pm$ 0.13	6.65 $\pm$ 0.73	4.59 $\pm$ 0.20	5.12 $\pm$ 0.25	166.047	1, 125	<0.01
	5.70-10.40	5.00-7.50	4.00-5.70	3.70-6.30	6.899	1, 125	9.7*10 <sup>-3</sup>
	59	51	7	12	27.790	1, 125	5.7*10 <sup>-7</sup>
HH	6.20 $\pm$ 0.10	4.98 $\pm$ 0.06	3.30 $\pm$ 0.11	3.50 $\pm$ 0.18	190.265	1, 125	<0.01
	4.30-7.90	4.20-5.70	3.00-3.80	2.50-4.20	10.407	1, 125	1.6*10 <sup>-3</sup>
	59	51	7	12	20.130	1, 125	1.6*10 <sup>-5</sup>
MO	11.05 $\pm$ 0.16	9.06 $\pm$ 0.11	5.58 $\pm$ 0.26	6.06 $\pm$ 0.39	239.438	1, 123	<0.01
	7.20-13.14	7.66-11.02	4.70-6.80	4.00-7.70	7.641	1, 123	6.6*10 <sup>-3</sup>
	59	50	7	11	20.354	1, 123	1.5*10 <sup>-5</sup>
FFL	17.81 $\pm$ 0.28	15.12 $\pm$ 0.19	9.22 $\pm$ 0.40	11.13 $\pm$ 0.50	183.221	1, 125	<0.01
	12.40-22.50	11.40-17.40	7.90-11.10	8.10-13.30	0.703	1, 125	0.40
	59	51	7	12	24.471	1, 125	2.4*10 <sup>-6</sup>
FL	8.21 $\pm$ 0.14	6.84 $\pm$ 0.10	3.82 $\pm$ 0.22	4.56 $\pm$ 0.36	188.112	1, 121	<0.01
	5.60-10.57	5.64-8.70	3.30-4.85	2.60-6.30	1.703	1, 121	0.19
	58	49	7	11	18.993	1, 121	2.8*10 <sup>-5</sup>
TBL	6.26 $\pm$ 0.12	5.06 $\pm$ 0.07	2.93 $\pm$ 0.12	3.61 $\pm$ 0.21	153.343	1, 120	<0.01
	4.20-9.67	3.98-6.41	2.40-3.40	2.30-4.50	1.898	1, 120	0.17
	58	48	7	11	23.803	1, 120	3*10 <sup>-6</sup>
4TL	13.78 $\pm$ 0.18	11.78 $\pm$ 0.14	7.56 $\pm$ 0.42	8.85 $\pm$ 0.49	201.934	1, 117	<0.01
	8.95-16.40	9.78-13.75	6.50-9.80	5.90-10.70	1.223	1, 117	0.27
	55	48	7	11	25.924	1, 117	1.4*10 <sup>-6</sup>
HFL	28.03 $\pm$ 0.33	23.59 $\pm$ 0.27	14.47 $\pm$ 0.65	16.88 $\pm$ 0.87	294.356	1, 125	<0.01
	21.00-33.80	20.10-28.40	6.50-9.80	12.40-20.10	2.945	1, 125	0.09
	59	51	7	12	33.657	1, 125	5.1*10 <sup>-8</sup>

Table 3.  
Summary of the discriminant analysis of the four size/sex classes based on raw data. See materials and methods section for variables' abbreviations.

	Wilks' Lambda	Partial Lambda	F-remove (3.106)	P-level	Tolerance	1-Toler. (R <sup>2</sup> )
SVL	0.064	0.680	16.66	<0.001	0.122	0.878
TRL	0.051	0.852	6.14	0.001	0.305	0.695
HL	0.053	0.821	7.72	0.000	0.158	0.842
HW	0.045	0.953	1.74	0.163	0.248	0.752
HH	0.044	0.975	0.92	0.435	0.347	0.653
MO	0.046	0.949	1.91	0.132	0.166	0.834
FFL	0.044	0.991	0.33	0.801	0.368	0.632
FL	0.044	0.991	0.32	0.811	0.358	0.642
TBL	0.044	0.979	0.77	0.512	0.481	0.519
4TL	0.046	0.948	1.96	0.125	0.503	0.497
HFL	0.045	0.958	1.56	0.202	0.241	0.759

	% correct	AF	AM	IF	IM
AF	95.83	46	1	0	1
AM	96.36	2	53	0	0
IM	85.71	0	0	6	1
IF	80.00	1	0	1	8
Total	94.17	49	54	7	10

The discriminant analysis conducted on raw data showed a good level of discrimination between the four size/sex classes, although percentages of correct classification were notably lower for immature lizards. The biometric variables significant for the discrimination among the four groups were SVL, TRL and HL. The summary of the discriminant analysis are shown in Table 3.

### Shape variation

The results of the univariate ANCOVAs for all the studied biometric characters, using SVL as a covariate, are presented in Table 4. The *post-hoc* comparisons revealed significant differences between animals of different size classes for all the variables and for both sexes. Adult individuals of different sexes were significantly different for all the characters studied (Scheffé's test,  $P < 0.05$ ), males presenting relatively larger characters, except for TRL which was relatively longer in female lizards. On the other hand, immature individuals only presented significant differences between the sexes for TRL, HL, FFL and HFL (Scheffé's test,  $P < 0.02$ ),

Table 4.

Results of the univariate ANCOVA comparisons between the four size/sex classes in the populations studied. See materials and methods section for variables' abbreviations.

Character	Factor	F	d.f.	P-value
TRL	age	2.000	1, 123	0.160
	sex	11.432	1, 123	9.7*10 <sup>-4</sup>
	age*sex	0.299	1, 123	0.585
HL	age	1.032	1, 124	0.312
	sex	44.774	1, 124	6.8*10 <sup>-10</sup>
	age*sex	28.678	1, 124	4*10 <sup>-7</sup>
HW	age	0.209	1, 124	0.648
	sex	41.010	1, 124	2.8*10 <sup>-9</sup>
	age*sex	18.494	1, 124	3.4*10 <sup>-5</sup>
HH	age	4.402	1, 124	0.038
	sex	39.541	1, 124	5*10 <sup>-9</sup>
	age*sex	9.346	1, 124	2.7*10 <sup>-3</sup>
MO	age	5.439	1, 122	0.021
	sex	59.452	1, 122	3.8*10 <sup>-12</sup>
	age*sex	12.411	1, 122	6*10 <sup>-4</sup>
FFL	age	2.288	1, 124	0.133
	sex	10.557	1, 124	1.5*10 <sup>-3</sup>
	age*sex	13.846	1, 124	3*10 <sup>-4</sup>
FL	age	5.064	1, 120	0.026
	sex	11.123	1, 120	1.1*10 <sup>-3</sup>
	age*sex	8.812	1, 120	3.6*10 <sup>-3</sup>
TBL	age	4.440	1, 119	0.037
	sex	8.478	1, 119	4.3*10 <sup>-3</sup>
	age*sex	13.219	1, 119	4.1*10 <sup>-4</sup>
4TL	age	16.154	1, 116	10 <sup>-4</sup>
	sex	5.425	1, 116	0.022
	age*sex	15.780	1, 116	1.2*10 <sup>-4</sup>
HFL	age	20.161	1, 124	1.6*10 <sup>-5</sup>
	sex	22.216	1, 124	6*10 <sup>-6</sup>
	age*sex	25.051	1, 124	2*10 <sup>-6</sup>

whereas there were no significant differences for the rest of the characters studied (Scheffé's test,  $P > 0.1$ ).

The discriminant analysis based on the regression residuals of log-transformed biometric variables against log (SVL) followed a similar pattern with that using raw data. In this case, the variables significant for the discrimination among the four size/sex classes

Table 5.

Summary of the discriminant analysis of the four size/sex classes based on residuals of the biometric variables in their regression with SVL (logarithmic scale). See materials and methods section for variables' abbreviations.

	Wilks' Lambda	Partial Lambda	F-remove (3.106)	P-level	Tolerance	1-Toler. (R <sup>2</sup> )
TRL	0.182	0.981	0.698	0.555	0.959	0.041
HL	0.219	0.816	8.024	<0.001	0.760	0.240
HW	0.193	0.927	2.806	0.043	0.660	0.340
HH	0.184	0.973	0.983	0.404	0.711	0.289
MO	0.198	0.905	3.754	0.013	0.552	0.448
FFL	0.181	0.987	0.464	0.708	0.795	0.205
FL	0.181	0.990	0.365	0.779	0.625	0.375
TBL	0.183	0.978	0.789	0.502	0.655	0.345
4TL	0.181	0.988	0.447	0.720	0.621	0.379
HFL	0.183	0.976	0.867	0.461	0.504	0.496

	% correct	AF	AM	IF	IM
AF	91.67	44	2	0	2
AM	92.73	0	51	1	3
IM	14.29	1	5	1	0
IF	20.00	6	2	0	2
Total	81.67	51	60	2	7

were HL, HW and MO. Correct classification was very high for adult animals, but on the contrary a very high percentage of immature individuals was classified erroneously. The summary of the discriminant analysis on residuals is shown in Table 5.

### Scaling

The regression parameters for biometric characters for each size/sex class are given in Table 6. The only character that presented significant deviations from isometry was head length (HL), which was negatively allometric (or hipometric) for the immature males and females. The examination of the slopes estimates revealed a significant deviation of adult males from the rest of the groups in some characters, namely head length (HL) and head height (HH). For head length and height, adult males presented a steeper slope than the rest of the groups. Adult males and females differed in all characters except for TRL, FL, 4TL and HFL. No differences were present in the slopes of immature animals of different sexes, nor between adult females and immature animals of either sex.

Table 6.

Intercept and slope estimates of RMA regression for biometric variables (log-transformed) with log(SVL). AF: Adult females, AM: Adult males, IF: Immature females, IM: Immature males. See materials and methods section for variables' abbreviations.

Class	Character	N	Intercept	Intercept Confidence Limits		Slope	Slope Confidence Limits		R <sup>2</sup>
AF	TRL	51	-0.86	-1.29	-0.44	1.33	1.08	1.58	0.58
	HL	51	-0.14	-0.33	0.05	0.72	0.61	0.83	0.71
	HW	50	-0.60	-0.92	-0.28	0.84	0.65	1.03	0.41
	HH	51	-0.76	-1.11	-0.28	0.86	0.65	1.07	0.27
	MO	50	-0.55	-0.81	-0.29	0.89	0.74	1.05	0.65
	FFL	50	-0.40	-0.66	-0.13	0.94	0.77	1.09	0.66
	FL	49	-1.01	-1.45	-0.57	1.09	0.83	1.35	0.35
	TBL	48	-0.95	-1.36	-0.53	0.98	0.73	1.23	0.28
	4TL	48	-0.39	-0.76	-0.02	0.87	0.65	1.09	0.28
AM	HFL	51	-0.09	-0.37	0.18	0.87	0.71	1.03	0.58
	TRL	59	-0.76	-1.11	-0.41	1.24	1.04	1.45	0.61
	HL	59	-0.56	-0.75	-0.38	1.00	0.89	1.11	0.83
	HW	59	-1.04	-1.34	-0.75	1.15	0.98	1.32	0.68
	HH	59	-1.31	-1.66	-0.95	1.23	1.02	1.44	0.60
	MO	59	-0.90	-1.17	-0.63	1.14	0.98	1.30	0.72
	FFL	57	-0.70	-1.04	-0.36	1.15	0.95	1.35	0.59
	FL	58	-1.31	-1.73	-0.90	1.31	1.06	1.55	0.51
	TBL	58	-1.61	-2.10	-1.12	1.41	1.13	1.70	0.43
IF	4TL	55	-0.58	-1.00	-0.15	1.01	0.76	1.26	0.19
	HFL	59	-0.09	-0.35	0.18	0.90	0.74	1.06	0.57
	TRL	11	-1.04	-1.68	-0.39	1.46	1.03	1.89	0.85
	HL	12	-0.03	-0.22	0.15	0.65	0.53	0.77	0.93
	HW	12	-0.63	-1.02	-0.23	0.88	0.62	1.15	0.82
	HH	12	-0.86	-1.20	-0.53	0.93	0.71	1.15	0.89
	MO	11	-0.87	-1.24	-0.51	1.10	0.86	1.34	0.91
	FFL	12	-0.20	-0.45	0.04	0.83	0.66	0.99	0.92
	FL	11	-1.35	-2.12	-0.58	1.33	0.82	1.84	0.74
IM	TBL	11	-0.93	-1.55	-0.31	0.99	0.57	1.40	0.69
	4TL	11	-0.52	-1.01	-0.03	0.97	0.65	1.30	0.80
	HFL	12	-0.18	-0.76	0.41	0.93	0.54	1.32	0.65
	TRL	7	-1.15	-1.65	-0.66	1.53	1.18	1.88	0.96
	HL	7	0.04	-0.14	0.23	0.60	0.47	0.73	0.96
	HW	7	-0.38	-1.09	0.32	0.74	0.24	1.24	0.65
	HH	7	-0.31	-1.20	0.57	0.59	-0.03	1.21	0.15



MO	7	-0.44	-0.97	0.09	0.84	0.47	1.21	0.85
FFL	7	-0.10	-0.91	0.71	0.75	0.18	1.32	0.56
FL	7	-0.81	-0.67	0.04	0.99	0.38	1.59	0.71
TBL	7	-0.62	-1.70	0.47	0.77	0.00	1.53	0.24
4TL	7	-0.46	-0.94	0.01	0.95	0.61	1.28	0.90
HFL	7	0.08	-0.30	0.46	0.76	0.49	1.03	0.91

## DISCUSSION

### *Pholidosis*

The results obtained on the pholidosis of the population studied are in accordance with previous studies of the species in Catalonia (Vives-Balmaña 1982, Carretero & Llorente 1993), as well as with the pholidotic patterns reported in the most recent morphological study on the NE type of *P. hispanica*\* (Geniez 2001). This makes this form one of the most distinctive in scale counts, since all of its pholidotic characters are close to the upper limit of the range given in the general description of the species (Pérez-Mellado 1998a, b). For example, when compared to the form present in the NW of the Iberian Peninsula (Galán 1986), the population studied presents less ventral scales and more femoral pores in both sexes.

Concerning the sexual dimorphism present in pholidotic characters, males have significantly more gular and dorsal scales, femoral pores and subdigital lamellae, while females have more ventral scales. Sexual dimorphism in pholidotic characters is not a rare feature in lacertid lizards, although to our knowledge it has never been studied extensively. A higher number of ventral scales in females has been reported in various cases for *P. hispanica*\* (Galán 1986, Carretero & Llorente 1993, Geniez 2001, Sá-Sousa *et al.* 2002) and it might be related to the presence of a longer trunk in females, constrained by the need of space for the allocation of eggs. On the other hand, the observed differences in the rest of the pholidotic characters are rarely studied. Concerning the higher number of gular scales in males, the same pattern has been observed in *P. pityusensis* (Carretero *et al.* 1999). The higher number of femoral pores observed in males has also been reported or can be deduced for *P. hispanica*\* (Galán 1986, Carretero & Llorente 1993, Geniez 2001, Sá-Sousa *et al.* 2002). A common feature for lacertid lizards is the different aspect of femoral pores in the two sexes, they are generally bigger and have higher secretory capacity in males and are involved in individual recognition related with social or reproductive behaviours (Cole 1966, Carretero & Llorente 1993, López & Martín 2004). Consequently, it is not surprising that a sexual dimorphism in the number of femoral pores exists, at least in some species.

Finally, the absence of pholidotic differences between immatures and adults is not surprising, since pholidotic characters do not vary with age (Klauber 1943, Arnold & Bennet 1988, Carretero & Llorente 1993).

### *Size and shape dimorphism*

In the population studied, immature animals were monomorphic, sexual dimorphism being restricted to the adult stage, as in the case of many lizard species (Cooper & Vitt 1989, Braña 1996). Concerning sexual dimorphism in adults, no significant differences in SVL were present between the sexes. This has been reported before for other populations of *P. hispanica*\* in NE Iberia (Vives-Balmaña 1982, Carretero & Llorente 1993). However, when the largest individuals of each sex were compared, males were found to be significantly larger than females, possibly indicating sexual differences in growth patterns or survival rates. For the rest of the characters studied, adult males presented absolutely (size) and relatively (shape) higher values than adult females, except for TRL which was found to be relatively longer in females. The shape variation observed is in accordance with sexual dimorphism patterns present in other lacertid lizards, males presenting more developed head characters, both in size and in shape, and females presenting relatively longer trunks (Braña 1996, Olsson *et al.* 2002). It has been stated elsewhere that a bigger head in males is advantageous both for male-to-male combats and for immobilisation of females during copulation (Hews 1990, Braña 1996, Olsson *et al.* 2002). Consequently, the head size and shape dimorphism observed, is probably the result of the effect of sexual selection for head dimensions in males. On the other hand, a longer trunk provides females with a reproductive advantage in terms of fecundity, since it would offer more space for egg allocation (Schoener 1977, Olsson *et al.* 2002), and would thus be an indication of natural selection.

It is interesting to note that, apart from longer trunks, females also present shorter limb lengths both in size and shape analysis. This phenomenon could be due to mechanical restrictions imposed to female lizards by the presence of a longer trunk (Carretero & Llorente 1993).

### *Character scaling*

The results on static allometry revealed an interesting pattern for the scaling of head length and height. No significant deviations from isometry were detected in any size/sex class. However, regression slopes for adult males differed from the rest of the classes for these characters. This apparent contradiction is probably attributable to statistical restrictions. In fact, examining deviations from isometry by inspection of

slope confidence limits, at least head length could be considered hypermetric. The pattern of dimorphism observed could be an indication of sexual selection for head dimensions in adult males. Head size is known to be sexually selected in numerous lizard species (Braña 1996, Olsson *et al.* 2002). It has been stated (Hews 1990) that head height could be related to male capability for immobilising females during copulation since bite force in lacertid lizards depends on the mass of the jaw adductor muscles which are fixed on the postorbital region of the cranium (Herrel *et al.* 1996, 1999). Although sexually selected characters are usually hypermetric (Green 1992, Petrie 1992), we were not able to detect such a pattern in the population studied. However, other factors that might affect head size should not be neglected, such as the habitat preferences of this species. In fact, head dimensions in rock lizards are restricted by natural selection due to habitat use (Carretero & Llorente 1993). Having in mind both selective forces acting on head dimensions, we consider that the pattern observed for head length and height could be a strong indication for sexual selection on head size which could be more constrained in the vertical dimension.

#### *Discrimination between classes*

Size and shape discriminant and canonical analyses showed a very good discrimination between the two sexes in adults. Although the variables significant for the discrimination differed in the two analyses, percentages of correct classification were very high, in accordance with the patterns of sexual dimorphism observed in the univariate analyses. These results reflect conclusions reported on pholidosis, as sexual differences in some characters are related to size and shape (*e.g.* ventral scales). Considering the immature lizards, discrimination between the sexes was also highly correct in both analyses but less marked. However, the discrimination of immature animals presented more difficulties when shape variables were used, many individuals being erroneously classified as adults. Obviously, since the criterion used to distinguish immatures from adults is exclusively body size (determining the gonad development), once this variable is statistically extracted from the analysis, this class becomes a poorly defined group because it is composed of animals of different ages. In contrast, in the size discriminant analysis, the discrimination between immatures and adults was very good, being based on variables directly related to size (SVL, TRL, HL).

Combining the results from univariate character analysis, examination of static allometry patterns and size and shape discrimination analyses, we can confirm that sexual dimorphism in the population studied seems to be driven by sexual and natural selection for some traits, as head size in males and trunk length in females, while for others, such as extremity length, it could be constrained by biomechanical limitations.

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# The micro-insular distribution of the genus *Podarcis* within the Aeolian Archipelago: historical vs. palaeogeographical interpretation

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*Several uninhabited islets of the Aeolian Archipelago harbour lacertid lizard populations, but only on three of these the autochthonous lizard *Podarcis raffonei* occurs. A detailed analysis of the relative palaeogeographical and historical features suggests that human exploitation in particular, represents one of the main causes which have influenced their present-day micro-insular distribution.*

Keywords: *Podarcis raffonei*, *Podarcis sicula*, palaeogeography, history, Aeolian Islands.

## INTRODUCTION

Two species of lizards of the genus *Podarcis* occur in the Aeolian Archipelago: the Italian Wall Lizard, *Podarcis sicula* (Rafinesque-Schmaltz 1810), and the threatened endemic Aeolian Wall Lizard, *P. raffonei* (Mertens 1952) (Fig. 1). A “micro-insular” pattern seems to characterise the present distribution of the latter species, which occurs on three islets (La Canna, Scoglio Faraglione, and Strombolicchio) and on some fragmented areas of Vulcano Island. According to Capula (1992) and Capula *et al.* (2002), the present-day absence of *P. raffonei* on the main islands is due to competitive exclusion and, extinction occurred as a consequence of the islands’ colonisation by *P. sicula*. This hypothesis is strongly supported by the finding in the genome of *P. sicula* population of Lipari of some alleles typical of the *P. raffonei* genome (Capula 1994, 2004), which suggests a past hybridization and introgression of the two species followed by the extinction of *P. raffonei* on Vulcano Island. Therefore, *P. raffonei* is strongly believed to be distributed in the past on the whole (or at least on the mostern

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Fig. 1. Adult male of *Podarcis raffonei* from Strombolicchio Islet.

part) of the archipelago. On the other hand, *P. sicula* is nowadays common and widespread on the main islands and on several islets of the archipelago. It is an anthropochorous and invasive species, whose high colonisation capability and the competitive capacities during the colonization process, are confirmed by new recent records out of its distribution range (see Corti & Lo Cascio 2002, and references therein) and by some recent experimental evidences (Downes & Bauwens 2004).

Despite the lack of fossil evidences, it is highly probable that *P. sicula* could have reached and colonised the Aeolian Islands after the human settlement, which started 7,000 yr BP. In this perspective, the “persistence” of *P. raffonei* on Vulcano agrees with the recent anthropic colonisation of this island, which was steadily occupied by humans only in the 18th century. It is more difficult to explain the apparently “stochastic” distribution of both species on several small islets of the Aeolian Archipelago. Why on some islets *P. raffonei* still occurs and on others *P. sicula* is found (could the latter having replaced the autochthonous one)?

The aim of this paper is to discuss the possible causes of the present distribution pattern of these lizards on each single islet, analyzing historical and palaeogeographical data.

## STUDY AREA

Apart from the seven main islands, the Aeolian Archipelago is composed by more than 30 “satellite” islets and rocks. Ten of these (Basiluzzo, Bottaro, Dattilo, Faraglione di Cala Fico, La Canna, La Nave, Lisca Bianca, Scoglio Faraglione, Spinazzola, and Strombolicchio) are inhabited by lacertid lizards (Corti *et al.* 1998, P. Lo Cascio, unpublished data). Furthermore, on Lisca Nera Islet lizards underwent extinction between the late 19th century and 1950s (Mertens 1955, Lanza & Corti 1996). The localisation of the islets and relative geographical data (surface and altitude) are given in Fig. 2.

Considering both geo-chemical characteristics and the age of formation, the islets are extremely diversified. Some of them are the remains of active volcanoes whose emersion occurred independently, while others got isolated due to erosive processes which definitively separate the islets from the main islands.

### *Geo-volcanological data*

Basiluzzo and Spinazzola (off the NE coast of Panarea Island) were a unique rhyolitic endogenous dome, emerged about 60,000 yr BP (Gabbianelli *et al.* 1990, Ca-

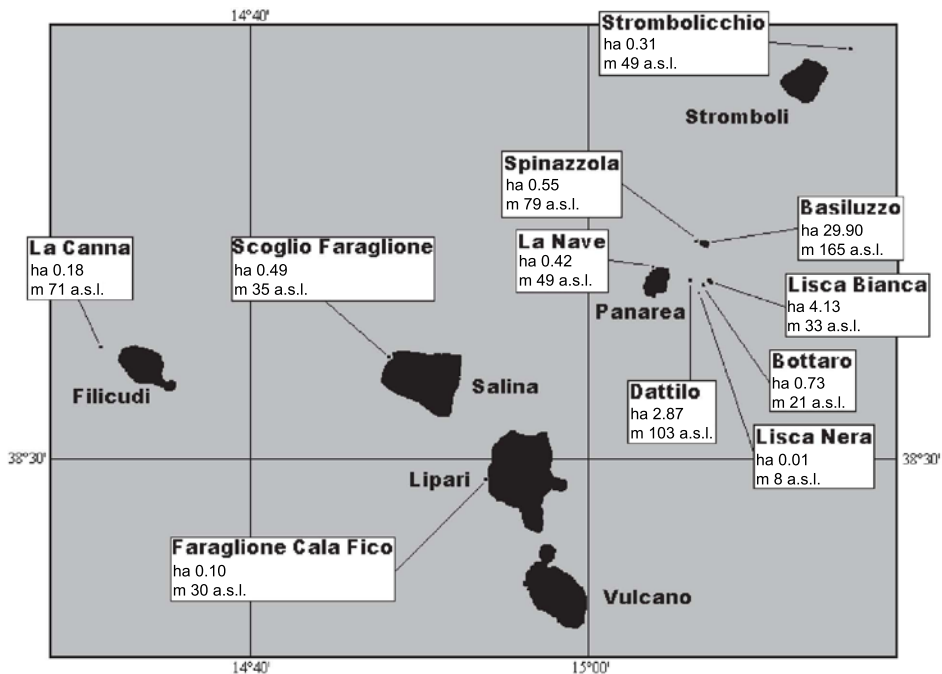


Fig. 2. Geographical data and localisation of the islets with lizards' populations.

lanchi *et al.* 1999) and successively fragmented due to erosive processes. The islets off the E coast of Panarea (Lisca Bianca, Dattilo, Bottaro, and Lisca Nera, Fig. 3) lie along a submerged crater rim, whose fumarolic activity had strongly altered their lavas. Lisca Bianca and Dattilo are mainly dacitic, while Bottaro and Lisca Nera are respectively andesitic and andesitic-dacitic. The emersion of this group (hereafter, “Lisca Bianca-group”) started about 130,000-124,000 yr BP (Calanchi *et al.* 1999, Lucchi *et al.* 1999), but this eruptive centre continued to have explosive phases even after the emersion of the islets (probably until 10,000 yr BP), as indicated by the deposition of an andesitic-basaltic layer on the flat top of Basiluzzo and Lisca Bianca. La Nave (off the NW coast of Panarea) is an andesitic-dacitic dome belonging to the “Palaeo-Panarea” Volcano (155,000-125,000 yr BP), from which it was separated due to collapse and following erosive processes (Calanchi *et al.* 1999). Scoglio Faraglione is a basaltic fragment belonging to the “Perciato” formation (Salina Island), whose age is estimable as 20,000-40,000 yr BP. About 13,000 yr BP the islet was involved in the sub-Plinian eruption of the “Pollara” Volcano (De Rosa *et al.* 1989, Calanchi *et al.* 1993) which surely represented a great problem for the surviving of any faunistic element. Its separation from the main island took place after this eruptive activity. Strombolicchio (off the NE coast of Stromboli Island) is formed by andesitic-basaltic lavas which represent the neck of a volcano, emerged about 200,000 yr BP (earlier than the emersion of Stromboli) and later destroyed by erosive processes (Gillot & Keller 1993, Hornig-Kjarsgaard *et al.* 1993). Similarly, La Canna (off the W coast of Filicudi Island) has been recognised as the



Fig. 3. The “Lisca Bianca-group”.

basaltic neck of an eruptive centre, active about 40,000 yr BP, and that successively underwent sea erosion (Santo *et al.* 1995, Tranne *et al.* 2002). Finally, Faraglione di Cala Fico represents a small part of the former W coast of Lipari Island, from which it probably separated relatively recently (Lucchi *et al.* 2004).

### *Vegetation*

The vegetation of these islets is characterised by a quite wide variety both in terms of species richness and floristic assemblages. The smallest islets, such as La Canna or Faraglione di Cala Fico, are poorly covered and only few species occur. Strombolicchio, Spinazzola, and La Nave, formed only by very steep cliffs, display sparse halochasmophyllous vegetation referred to the *Hyoseridetum taurinae-dianthetosum aeolici* or to the *Limonietum minutiflori*. The latter occurs also on the rocky cliffs of Scoglio Faraglione, whereas the top is covered by dense vegetation, referred to the *Senecioni bicoloris-Helichrysetum litorei*. Halo-nitrophilous formations dominated by chenopod species cover the plateaux of Lisca Bianca and Bottaro as well as most of Dattilo. Basiluzzo is characterized by a mosaic of xerophytic grassland referred to the *Hypparhenietum hirtopubescentis* and scattered spots of thermophilic shrubland dominated by *Pistacia lentiscus* and *Erica arborea* (Lo Cascio & Navarra 2003).

### *Palaeogeographical setting*

The reconstructions of sea level variations for Late Pleistocene and Holocene show that from 18,000 yr BP to around 6,000-5,000 yr BP, the glacio-eustatic component of the sea level raised up to 120 m (Pirazzoli 1991, 1996; Mörner 1996; Fleming *et al.* 1998). Many records indicate, during the last 6,000 years, a relative stabilization of the sea level related to the decreasing melting process of the major ice sheets, and the sea level rising up to about 2 m (Nakada & Lambeck 1988, Lambeck 1993). Nevertheless the sea-level changes exhibited considerable spatial and temporal variability along the Tyrrhenian coasts (Lambeck *et al.* 2004, and references therein).

Unfortunately, no detailed data are available for the last sea level variations occurred in the SE Tyrrhenian Sea. In particular, for the Aeolian Archipelago, most of the recent studies focus mainly on marine terraces deposited on present-day sub-aerial zones (Bernasconi & Ferrini 1988, 1989; Corselli & Travaini 1989; Lucchi 2000; Lucchi *et al.* 1999, 2004). As this archipelago is a tectonic active area, an accurate reconstruction of its former shorelines may be problematic because of many unpredictable and uncertain factors (*e.g.*, uplifts, local subsidence, collapses, local tectonic evolution, etc.). Nevertheless, modelling the separation events of the islets necessarily follows the isopleths curves, even if the present bathymetry not always corresponds

thoroughly to the past topography of the studied area. At the same time, we considered the volcanic events and/or vertical movements related to each islet and/or islets' group, in order to obtain a palaeogeographical setting as reliable as possible. The models were drawn out using the high-resolution electronic chart system of Cmapecs' software (version 4).

According to the present submarine morphology, three main "clusters" of isolation episodes can be recognised. The earliest concerns Basiluzzo and Spinazzola, included in Panarea isopleth of  $-80$  m. The other islets eastward of Panarea (the "Lisca Bianca-group") are located within the isopleth of  $-50$  m. The isolation from Panarea could have happened between 10,000 and 8,000-7,000 years ago, resulting therefore earlier than the human colonisation of the Aeolian Islands (Fig. 4). A similar isolation age could be considered also reliable for Strombolicchio and La Canna, which are located within the isopleth of  $-50$  m from Stromboli and Filicudi respectively. A third group, for which only a recent isolation age could be hypothesised, includes La Nave, Scoglio Faraglione, and Faraglione di Cala Fico, located within the isopleth of  $-10$  m, formerly owing to Panarea, Salina, and Lipari respectively.

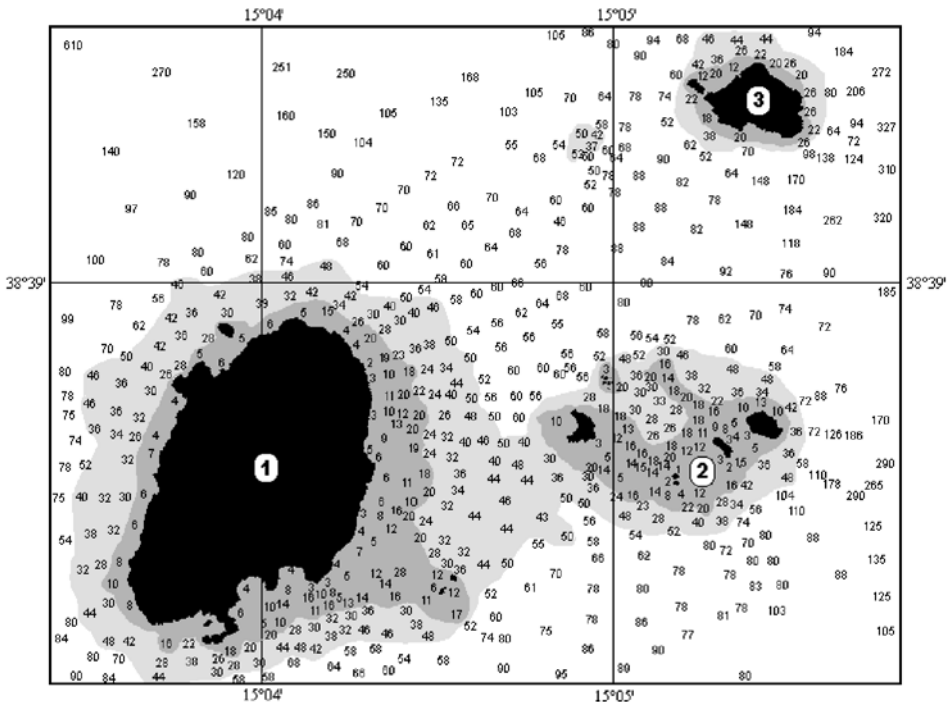


Fig. 4. Probable extension of the shorelines of Panarea and its satellite islets earlier than the human colonisation of the Aeolian Archipelago (along the isopleths of  $-50$  and  $-20$  m). 1) Panarea-La Nave; 2) "Lisca Bianca-group"; 3) Basiluzzo-Spinazzola.

*Historical setting*

The information on the historical scenario of the islets, on the contrary, is more detailed. The archaeological investigations carried out in 1920s by De Fiore (1921, 1925) showed that Basiluzzo and the “Lisca Bianca-group” were visited by fishermen and/or hunters since the Upper Neolithic (6,000 yr BP). On these islets, human settlements occurred during the Hellenistic and Roman ages (Bernabò Brea 1949; Cavalier 1985, 1991a, 1991b). Some of these may be related to the exploitation of local thermal springs, as suggested by the finding of probable thermal ruins submerged between the islets of Lisca Bianca and Dattilo (Bellia *et al.* 1987, Gabbianelli *et al.* 1990). The present localisation of these structures (at -14 m) suggests that a strong subsidence occurred in this area during almost the last 2,000 years, which associated to the erosive processes could have remarkably modified the former extension of the islets (see Fig. 4). Subsidence also involved the SE shore of Basiluzzo, where a presumably defensive building dating back to the Roman age is nowadays located at about -2 m (Bernabò Brea 1985, G. Kápitan pers. comm. 1993). This islet was almost certainly seasonally inhabited, as demonstrated by the occurrence of a villa of the II century A.D. (Libertini 1921, Bernabò Brea & Cavalier 1977). After a long period during which the supremacy of the North-African pirates in the Southern Tyrrhenian Sea constituted a severe limit for human settlement on the peripheral and vulnerable islands of the archipelago, the agricultural use of Basiluzzo in early 17th century is documented by La Rosa (1784). Some travellers, who visited this islet between late 18th and middle 19th centuries, reported the presence of few inhabitants in a small rural settlement, which probably was visited only seasonally (Spallanzani 1793, Smyth 1824, Dumas 1842). The agricultural use of Basiluzzo was extended until late 19th century (Habsburg Lothringen 1895), while the “Lisca Bianca-group” was exploited because of sulphur mine and grazing land for goats and sheep (Lojacono-Pojero 1878, Habsburg Lothringen 1895).

All the above data indicate that a prolonged human presence occurred on the islets off the NE and the E coasts of Panarea. Contrariwise, it did not happen on the inaccessible and smaller rocks, such as La Canna, La Nave, Scoglio Faraglione, and Strombolicchio. The latter is characterised by the following recent history: the top of this islet was demolished using dynamite to build a light-house at the end of the 19th century, and its elevation was reduced from 70 circa to 49 m a.s.l.

## DISCUSSION

It is strongly believed that uninhabited islets and rocks should have represented potential “refuges” where *Podarcis raffonei* could have been preserved from competition with

*P. sicula*. Palaeogeographical data show that some islets (La Canna, Strombolicchio) were separated from the main contiguous islands before the beginning of the human colonisation of the archipelago, while this is still doubtful for Scoglio Faraglione. However, the persistence of *P. raffonei* on this islet can be explained by the gradual spread over of *P. sicula* on each island, as demonstrated by the present sympatry of both species on Vulcano.

As the Aeolian Wall Lizard was probably distributed on the whole archipelago, populations of this species could have occurred also on the islets off the E and NE coast of Panarea. Palaeogeographical data (Fig. 4) indicate that the separation of the “Lisca Bianca-group” from the main island occurred approximately at the same time for La Canna and Strombolicchio, while for Basiluzzo-Spinazzola probably earlier. Anyway, the natural process of isolation did not represent a sufficient boundary to preserve the autochthonous species on these islets. The common historical traits shown by the satellite islands of Panarea suggest that the human presence has been the most prominent factor which has influenced the present-day faunal composition.

Thus, the occurrence of *P. sicula* on the Aeolian islets seems to coincide with the human settlement (or exploitation) on the micro-insular environments. As the unexploited or inaccessible islets, such as Scoglio Faraglione, La Canna and Strombolicchio (for the latter, almost until the building of a lighthouse in the late 19th century) are still occupied by the autochthonous *P. raffonei*, means that men is playnig the most important role in the present distribution of the Aeolian lizards.

These findings can be added to a more wider scenario already shown for many other Mediterranean islands (Corti *et al.* 1999a, 1999b). Once more, the lizard populations of Lisca Bianca and Bottaro show different characteristics which were used



Fig. 5. Adult male of *Podarcis sicula* from Lisca Bianca Islet.



to describe them as two distinct subspecies (Mertens 1952) (Fig. 5). Even if their taxonomical value is still under debate (see Corti & Lo Cascio 2002, and references therein), this leads to exclude a recent colonisation of these islets by *P. sicula* according to the differentiation time recorded for other lizards (Malhotra & Thorpe 1991) or for the genus *Podarcis* (Castilla 2000).

The very fragile equilibrium in which the last alive populations of *Podarcis raffonei* occur suggests the need of immediate protection measures to avoid further anthropic pressure, specially on the micro-insular environments, and accidental introductions of allochthonous lizards. The only possible survival of micro-insular populations can be assured by the constitution of strictly protected areas (*e.g.*, as in the case of the Aeolian Archipelago the constitution of a: Riserva Naturale Integrale).

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# Preliminary data on the ecology of *Podarcis filfolensis* of Lampione Islet (Pelagian Islands, Channel of Sicily, Italy)

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*Some preliminary data on Podarcis filfolensis of Lampione Islet are given. Estimated density was 75–80 individuals per 100 m<sup>2</sup>. Most part of the lizards had the tail broken or regenerated. The diet was mainly based on arthropods, and Formicidae (42%) and Coleoptera (23%) represented the main prey-groups. Vegetal matter was found in 61% of the examined faecal pellets, and plant consumption resulted widespread at all levels of this population.*

Keywords: *Podarcis filfolensis*, population density, trophic ecology, Lampione Islet, Pelagian Archipelago.

## INTRODUCTION

An extremely “isolated” population of the Maltese Wall Lizard, *Podarcis filfolensis* (Bedriaga 1876), occurs on Lampione Islet (Pelagic Archipelago), which is the westernmost site of the distribution range of the species. This population was referred by Lanza & Bruzzone (1961) to the ssp. *laurentiimuelleri* (Fejérváry 1924), which also occurs on Linosa Island. From a zoogeographical point of view, its occurrence on Lampione could be explained with a probable introduction by man. In fact, (1) Lampione, being the closest island to Lampedusa, is part of the African continental plate and never got palaeogeographical relation with the Maltese Islands; (2) no lizards of the genus *Podarcis* seem to have occurred on Lampedusa (except for recent introduced populations on Lampedusa village, see Lo Cascio *et al.* 2005).

Only scattered data on the ecology of *Podarcis filfolensis* are available (Sorci 1990, Di Palma 1991, Moravec 1993, Corti & Lo Cascio 2002, Scalera *et al.* 2004, Bombi

*et al.* 2005), but there are no studies concerning the Lampione population specifically, even if it seems particularly interesting due to the extreme geographical and ecological features of the islet. In this paper, we present preliminary data on the ecology of this population, focusing our interest especially on the diet and the population density.

## MATERIAL AND METHODS

### *Study area*

Lampione (35°33'00"N – 12°19'11"E Greenwich) (Fig. 1) is the smallest islet of the Pelagian Archipelago (Channel of Sicily), with a surface of about 2.10 ha, 700 m of coastal perimeter, and a maximum elevation of 36 m a.s.l. It is located 17 km off the W coast of Lampedusa Island. From a geographical point of view, Lampione belongs to the African plate. It was connected with North Africa during the last marine regression (about 18,000 yrs BP). The islet is entirely calcareous, with dolomitized carbonates composed by associated wackestone and packestone, referred to the "Halk al-Menzel formation" (Tunisian offshore, 46-34 Myrs BP) (Bonnefus & Bismuth 1982, Grasso *et al.* 1985). The average annual temperature is 19 °C and the average rainfall is less than 320 mm (measured on the close island of Lampedusa). Apart from the Maltese Wall Lizard, Lampione is inhabited by the



Fig. 1. Lampione Islet. Note on the upper plateau covered by nitrophilous high herbs, a high lizard density (approximately 75-80 individuals per 100 m<sup>2</sup>) was observed.

Ocellated Skink, *Chalcides ocellatus*. Colonies of Cory's Shearwater, *Calonectris diomedea* (40-50 pairs), and Eleanor's Falcon, *Falco eleonorae* (7-8 pairs) occur on the islet, while about 250 nesting pairs of Mediterranean Yellow-legged Gull, *Larus cachinnans*, were counted during our visit. Due to the intense erosive processes and the high concentration of gulls, the soil is scarce and characterised by high level of eutrophication, acidification, and nutrient imbalances. This features strongly influence the vegetation, dominated by alo-nitrophilous perennial shrubs (as *Arthrocnemum macrostachyum*) and high annual herbs (as *Lavatera arborea*) (Corti *et al.* 2002, and references therein). An early human presence, probably only seasonal, is documented by the ruins of a building, traced back to the late Roman age by Ashby & Litt (1912). During the 20th century a lighthouse, unguarded at present, was built on the islet.

### Methods

All data were gathered during a survey in April 2001. All the captured lizards were measured with a calliper "Mauser" (accuracy 0.1 mm) and released. Data on population density are based on preliminary surveys carried out using a "line transect method".

Faecal pellets were obtained by handling specimens or collected on surfaces where lizards' activity was previously observed, in order to avoid miss identification with those of *Chalcides ocellatus*. Faecal contents were examined in the laboratory using a stereoscope. When possible, remains were identified to the lowest taxonomic rank by comparison with the arthropods' specimens kept in the private collection of one of the authors (PLC). Statistical analyses were carried out using SPSS 11.5 (alpha set at 5% and all tests being two tailed).

## RESULTS

### *Population body size, density, and frequency of regenerated (broken) tails*

Adult male SVL ranged from 68 to 71 mm ( $n = 5$ ), while two adult female SVL were respectively 63 and 66 mm. Although male size probably exceeded female size, our sample size was too small to allow statistical comparisons. Population density was generally high, especially on the upper plateau covered by nitrophilous high herbs, where 75-80 individuals per 100 m<sup>2</sup> were estimated (i.e. approximately 7500 to 8000 individuals  $\times$  ha<sup>-1</sup>, SE =  $\pm$  1326; CV = 0. 172). Among the measured specimens, three out of five males, and one of the two females had the tail broken or regenerated.

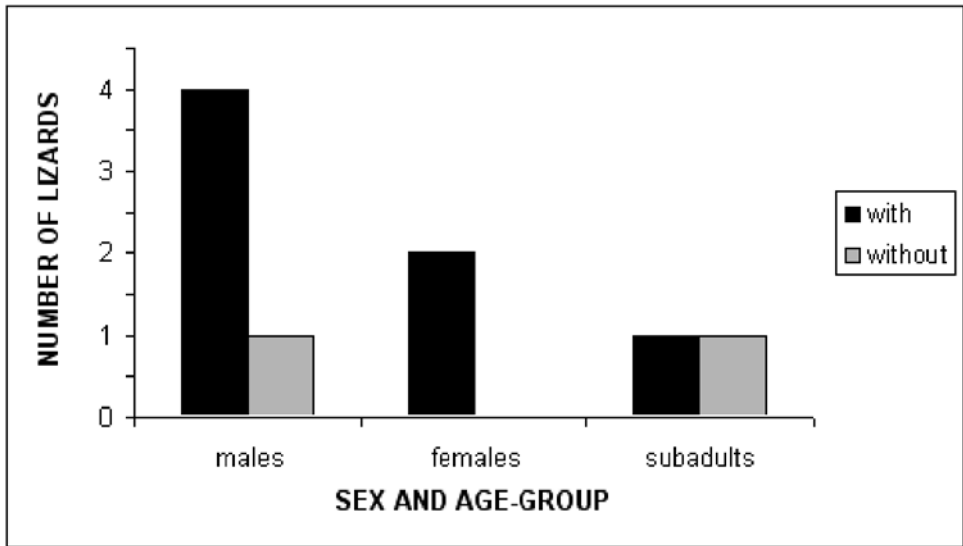


Fig. 2. Number of males, females, and subadults of *Podarcis filfolensis* whit faeces containing, or not containing plant matter residuals, analysis. Only faecal pellets collected from specimens with known identity were used for this.

### Diet

Vegetal matters were found in 60.9% of the examined faecal pellets (total  $n = 23$ ). Vegetal matters exceeded any other food category in terms of frequency of occurrence of the various prey items ( $\chi^2$  test,  $P < 0.04$ ). In particular, comparing the frequency of occurrence of vegetal matters in specimens with known sex, 4 out of 5 males, all females and 1 out of 2 subadults consumed plant matter (Fig. 2). The rest of the diet consisted mainly of arthropods (Table 1). Among these Formicidae (42.3%) and Coleoptera (22.9%) represented the main preys. Remains of a lizard (some tail scales) were found in one faecal pellet.

## DISCUSSION

The high population density observed on Lampione sounds particularly interesting when we consider i) the presumably scarce food availability and ii) the occurrence of a big yellow-footed seagull colony (see above). Gulls are doubtfully considered potential predators of lizards (Araujo *et al.* 1977, Martin & Lopez 1990, Carretero *et al.* 1993, Mayol 2004). During our investigations we never observed this bird preying lizards and no lizard remains have been found in the examined gull pellets. A similar situation was also observed by Pérez-Mellado (unpublished com-



Table 1. Dietary composition of *Podarcis filfolensis* at Lampione Islet, by number of items found in the faecal pellets.

Gastropoda	4
unidentified Arthropoda	9
Pseudoscorpiones	1
Araneae	5
Crustacea Isopoda	6
Collembola	6
Dictyoptera	1
Heteroptera	1
Coleoptera	4
Coleoptera larvae	3
Coleoptera Melolonthidae	1
Coleoptera Tenebrionidae	20
Coleoptera Cerambycidae	1
Coleoptera Curculionidae	7
Diptera	4
Lepidoptera larvae	2
Hymenoptera	1
Hymenoptera Apoidea	2
Hymenoptera Ichneumonidae	5
Hymenoptera Formicidae	61
Reptilia Lacertidae	1

munication to the authors) on the Balearic islands. On the contrary, it is plausible that the presence of seagulls could be a source of additional food remains, including carcasses and flying insects linked by the organic matter of the breeding colony. Therefore, it is possible that these birds positively affect the lizard abundance in this small islet rather than decrease it. Nevertheless, other potential predators could be kestrels (*Falco tinnunculus*) and/or other birds which occasionally visit the islet (Moltoni 1970, Fornasari & Zava 2001). On the other hand, the great percentage of lizards (both males and females) showing regenerated tails should suggest eventual high predation pressure as well as intra- and/or inter-specific competition. The remarkable plant consumption, widespread among both sexes and all age classes, exposes lizards to easy predation (Pérez-Mellado & Corti 1993, Van Damme 1999) and therefore seems to contradict the hypothesis of high predation pressure on this islet. Even if based on preliminary data, our results suggest that intra- and/or inter-specific competition, due to the small viable space and the presumably scarce food availability, can be considered the main explanation to this trait. Further investiga-

tions will be needed in order to assess if trophic niche overlap is also present on this islet between *P. filfolensis* and the syntopic skink *Chalcides ocellatus* (Capula & Luiselli 1994). Concerning the diet, mirmecophagy and herbivorous habits have been previously documented for other populations of *Podarcis filfolensis* (Sorci 1990, Bombi *et al.* 2005) but the rate observed was not so high as for the Lampione population. Analogous observations have been already reported for other Mediterranean small islands lizard populations (Pérez-Mellado 1989, Pérez-Mellado & Corti 1993).

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# How variable can be *Podarcis carbonelli berlengensis*? A study in comparative morphology

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*A study in comparative morphology between two different populations of Podarcis carbonelli berlengensis on the Berlenga's Archipelago was conducted and showed significant differences between them. The lizards found on the island of Farilhão Grande are larger, have relatively longer limbs and are more melanistic than the individuals living on the island of Berlenga Grande. Differences related to the head shape, scalation and chromatic pattern were also found. Such differences could be assigned to different selective pressures, founder effect or genetic drift.*

Keywords: *Podarcis*, Berlenga, island, morphology, comparative, variability.

## INTRODUCTION

Differences between insular populations should result from both random sample effects and selective pressures; environmental differences on islands should act as selective pressures promoting genetic differences (Vicente 1999).

Islands are ideal ecological contexts to interpret how selective pressures act and to test if they produce evolutionary changes in species inhabiting them. Lizards, due to their abundance, easy capture and handling, can be successfully used to measure morphological and behavioural trends in their populations, contributing to clarify how environmental influences are affecting them. *Podarcis carbonelli berlengensis* is a lacertid restricted to the Berlenga's Archipelago, off the western coast of Portugal, and to the adjacent continental coast, in the peninsula of Peniche (Sá-Sousa *et al.* 2000). Its taxonomic relation to other species in the genus has been subject of discussion in

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the last years (Sá-Sousa *et al.* 2000, 2002; Harris & Sá-Sousa 2001; Sá-Sousa 2001). However, this insular subspecies lacks population comparative studies of different islands of the archipelago.

*Podarcis carbonelli berlengensis* can be found in some of the islands of the archipelago of Berlengas, in particular in Berlenga Grande and Farilhão Grande, which offer contrasted habitats. While the former is the biggest (78 ha), with human settlements, granitic *substrata* and gently slopes, the latter is much smaller (7 ha), with no human access, basaltic origin and characterized by steep cliffs or precipices. The two islands have also important differences concerning vegetation and potential competitors and predators. Berlenga has diverse vegetation, mainly composed of Mediterranean and Atlantic shrub species, while Farilhão is completely dominated by the introduced *Carpobrotus edulis*. At Berlenga there is also present another lacertid species (*Lacerta lepida*) that competes with *P. c. berlengensis* by food resources (Vicente 1989). Insectivorous birds are also present at Berlenga, but absent at Farilhão. In addition, predation risk is possibly more intense at the Berlenga Island, where some birds of prey are common and where an important population of *Larus cachinnans* is biologically considered a pest (Morais *et al.* 1998) and is an occasional predator of this lizard (Morais & Vicente 1998). Therefore, our hypothesis is that the *P. c. berlengensis* populations may exhibit different morphological traits resulting in part from different selective pressures.

The aim of this study is to test morphological differences in two different insular populations of *P. c. berlengensis*. Once significant morphological differences are found, an evolutionary causality is approached.

## MATERIAL AND METHODS

Samples of adult males and females of *P. c. berlengensis* were collected in Portugal from the islands of Berlenga Grande (39°24'N, 9°30'W) and Farilhão Grande (39°28'N, 9°31'W). The lizards were hand captured or trapped with plastic pit fall jars with fruit, marked with colour paint, measured, photographed and released at the original point of capture.

Following Pérez-Mellado & Gosá (1988), Vicente (1989), Sá-Sousa & Harris (2002) and Sá-Sousa *et al.* (2000, 2002) 14 biometric characters were selected: SLV snout-vent length, VHL ventral head length, SD lateral distance of the left head-side, HL head length, HW head width, HD head depth, OW inter-orbital width, OD orbital depth, FW inter-frontal width, FD frontal depth, NW inter-nasal width, ND nasal depth, HDL left hand length and HLL left hind limb length. Scalation was assessed through four unilateral linear counts: GUL gular scales along the throat mid-

line, COL large scales in the collar, VTR inner ventral scales counted longitudinally and FPO left femoral pores. The record of chromatic pattern was made through three different classes of variables: type of dorsal patterns, body colour and colour intensity found on the BDS black dorsal stripes, LDS light dorsal stripes, MBZ mid-back zone and FKZ left flank zone (Sá-Sousa *et al.* 2002). The type of chromatic pattern was composed of eleven variables: three for BDS and LDS (uniform, irregular or diffused stripes) and eight for MBZ and FKZ (thick or thin ocellated, spotted, reticulated or speckled pattern; Sá-Sousa *et al.* 2002). The percentage of black pigmentation MEL on the body was also estimated. This estimation was made considering as referential model a complete melanistic lizard (100% of black pigmentation; see Ferreira *et al.* 2005); the estimation of the black colouration rate was made by comparing the referential model with the study individual (see Appendix 1 for details). All linear measurements were taken to the nearest 0.05 mm using a digital calliper. The record of chromatic pattern was made through three different classes of variables according to Sá-Sousa *et al.* (2002): type of dorsal patterns, body colouration and colour intensity found on the different zones of the body.

Each sex was analysed separately, due to *P. c. berlengensis* sexual dimorphism (Vicente 1985). To evaluate the distinctiveness of the samples, a Multivariate Analysis of Covariance (MANCOVA) was performed using the 14 biometric characters (SLV, VHL, SD, HL, HW, HD, OW, OD, FW, FD, NW, ND, HDL and HLL) plus scalation variables (GUL, COL, VTR, FPO) and melanistic percentage MEL, and having SLV as covariate in order to eliminate the effect of the absolute body size (Sokal & Rohlf 1995). Also based on the 14 biometric variables described upper, a Principal Component Analysis (PCA) was used to discriminate the two lizard forms (Sneath & Sokal 1973). UPGMA cluster analysis (based on Euclidean distances) was performed to detect phenetic relationships. Chi-square test or t-Student test (depending on assumptions) were used to test for significant differences of chromatic patterns between samples. In statistical tests the cut-off for significance was set at 0.05.

## RESULTS

Descriptive statistics of the biometric variables is given in Appendix 2. Both males and females from Farilhão Grande are larger, have relatively longer limbs and are more melanistic than the individuals from Berlenga Grande. The head shape also varies: it is longer, slightly flattened and less pointed in the Farilhão Grande males and in the females from Berlenga. MANCOVA revealed significant differences between the two lizard forms: males Wilks'  $\lambda = 0.3089$ ,  $P < 0.05$ ; females Wilks'  $\lambda = 0.2444$ ,  $P < 0.05$ . The variables that more contributed to this discrimination were SD, HL, HDL,

HLL, PF and MEL for both sexes, plus COL for males and GUL and VTR for females. The ordination of males and females in the Cartesian space defined by the first two components of variation (PC1, PC2) deduced from the biometric variables are shown in Figs 1 and 2. In males PC1 (68.3%) reflects general size and it is positively and strongly associated ( $> 0.70$ ) with all the characters, while in females (50.2%) it is particularly associated ( $> 0.70$ ) with those that globally expresses the body size and head and limbs lengths (SVL, VHL, SD, HL, OD, HDL and HLL). PC2 in males (7.45%) is not significantly correlated with any of the variables; in females (11.9%) just ND is determining the factor ( $> 0.70$ ; Appendix 3).

UPGMA clustering depicted in Figs 3 and 4 shows a clear separation between *P. c. berlengensis* from Farilhão Grande and Berlenga Grande.

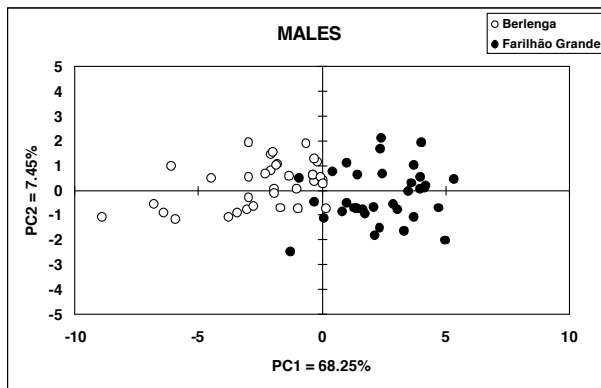


Fig. 1. Scatterplot resulting from PCA of males *P. c. berlengensis* from Berlenga Grande and Farilhão Grande on the first two component axes.

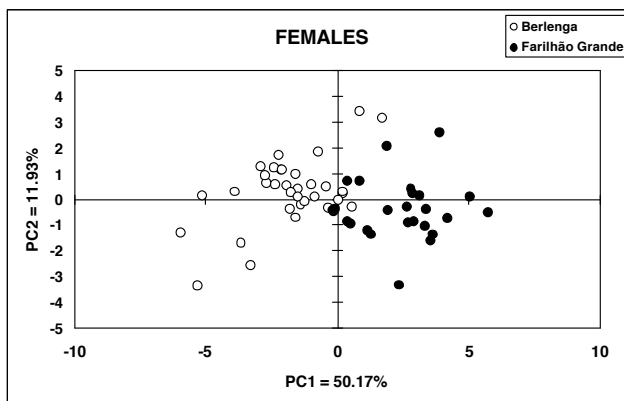


Fig. 2. Scatterplot resulting from PCA of females *P. c. berlengensis* from Berlenga Grande and Farilhão Grande on the first two component axes.



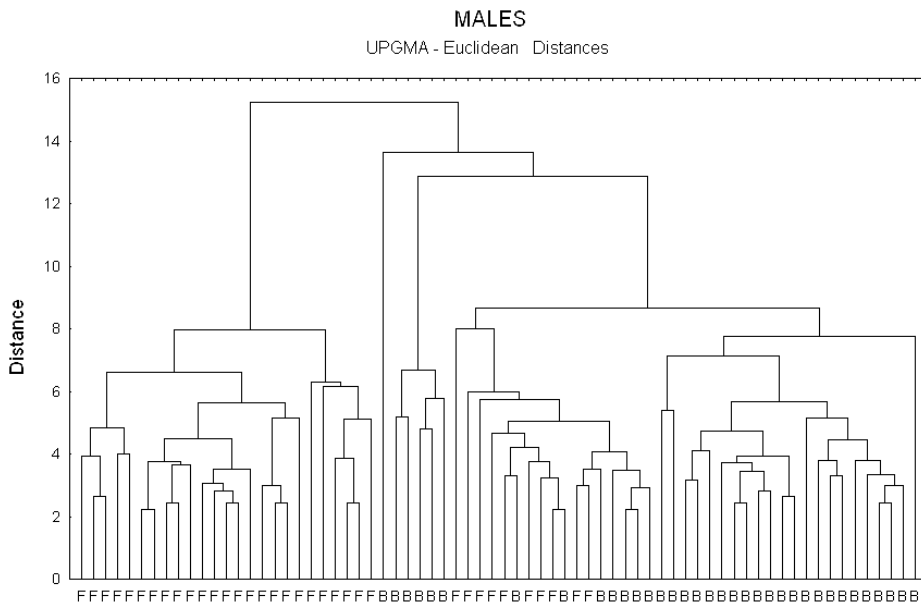


Fig. 3. UPGMA clusters of *P. c. berlengensis* samples from B Berlenga Grande and F Farilhão Grande (males).

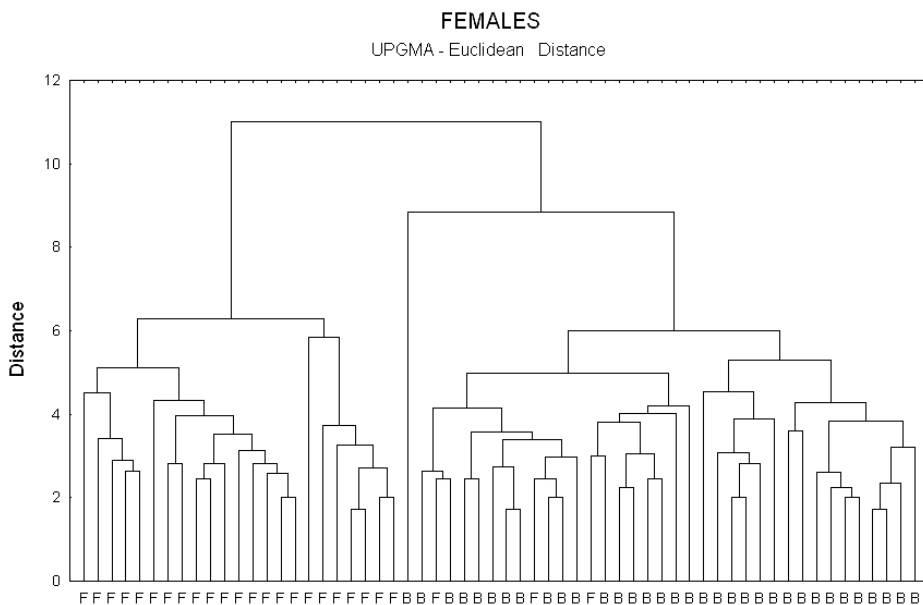


Fig. 4. UPGMA clusters of *P. c. berlengensis* samples from B Berlenga Grande and F Farilhão Grande (females).

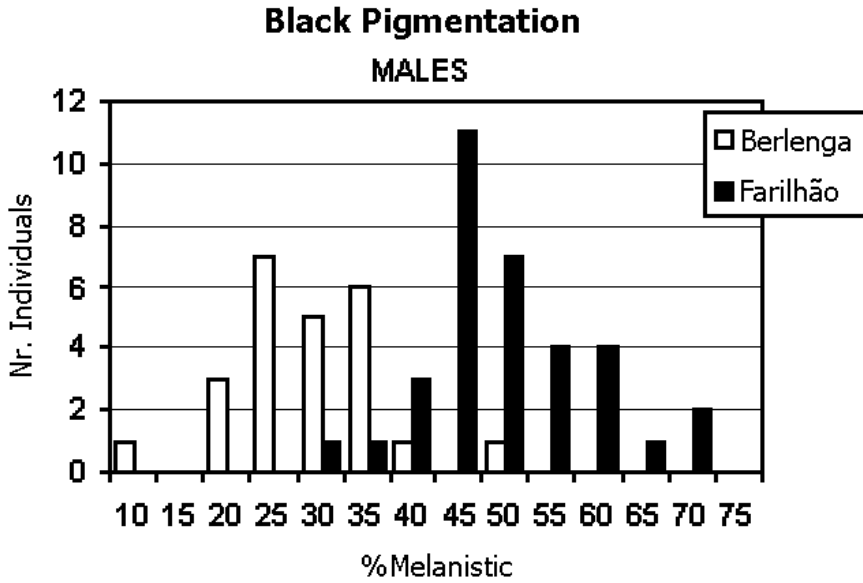


Fig. 5. Distribution of males from Berlenga Grande and Farilhão Grande, according to the percentage of black I pigmentation.

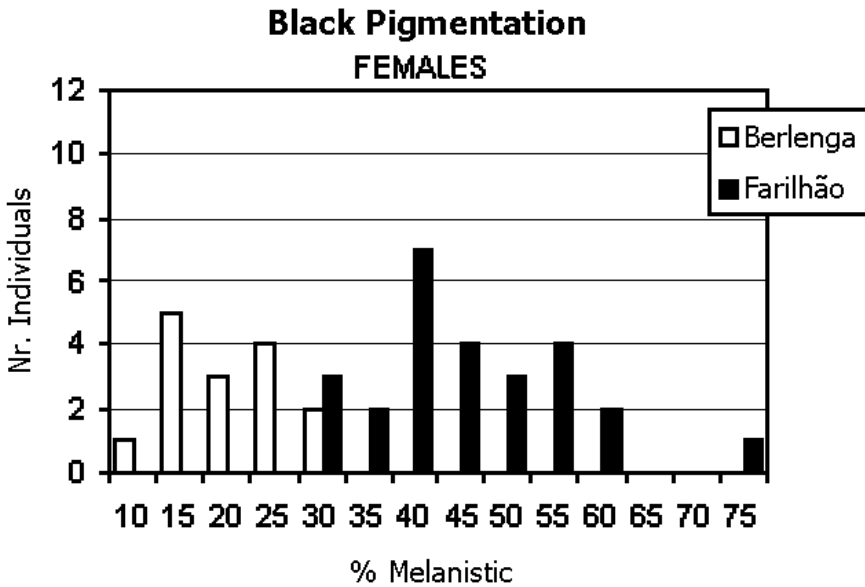


Fig. 6. Distribution of females from Berlenga Grande and Farilhão Grande, according to the percentage of black I pigmentation.

The chromatic data is summarized in Appendix 4. The patterns found concerning BDS and MBZ types in males, and MBZ type in females are significantly different (Appendix 5). More males of both samples have diffused black dorsal stripes, while females have them in an irregular way. Males from Berlenga Grande show higher variety of patterns than the ones from Farilhão Grande. Patterns such as speckled or thick and thin spotted with green/brown, green or brown/yellow colours were found, while the majority of males from Farilhão Grande have thick green/brown spotted dorsal patterns and thick brown ocelli in the flanks zone. Females from both islands show typically a brown thick speckled dorsal pattern with brown-eyed flanks. Individuals from Berlenga have significantly less black pigmentation respectively to Farilhão Grande (Appendix 5, Figs 5 and 6).

## DISCUSSION

The populations of *P. c. berlengensis* from Berlenga Grande and Farilhão Grande are morphologically distinct. Data analysis showed a clear separation of the samples between the two populations. Diagnostically, the Berlenga Grande form is smaller, less robust and with proportionally shorter limbs. The head shape varies on the islands: males from Farilhão Grande have a longer flatted head than males from Berlenga Grande; females show an opposite pattern. Differences in scalation were also significant and allowed the differentiation of both populations, especially in female lizards. Chromatic patterns also vary between the populations and particularly among males. The higher percentage of black pigmentation was one of the most distinctive characters of the population of Farilhão Grande.

In Farilhão Grande the reduction of predator pressure and the simultaneous occurrence of a higher population density may possibly lead to a shortage of resources and to an increase of competition linked with agonistic behaviour, thus creating a trend towards gigantism (Vicente 1989, Cirer & Martínez-Rica 1990). Furthermore, on smaller islands (like Farilhão Grande), lizards tend to show a size increase and a superior morphological homogeneity (Cirer & Martínez-Rica 1990), what is corroborated by data. In fact, Farilhão is much smaller than the island Berlenga, what seems to accentuate the insular phenomena. Moreover, limb size can reflect locomotion and habitat. According to the microhabitat use, the locomotion strategy can vary (Pounds 1988, Herrel *et al.* 2001). Differences founded on limb size can be a result of divergent habitat use of the lizard populations, according to the characteristic *substrata* and vegetation cover of each island. The evolution of long limbs is usually related to living on non-horizontal surfaces while species that occupy flat areas with densely vegetated

microhabitats have short limbs (Melville & Swain 2000). These two types follow the pattern found in both islands.

Usually chromatic patterns can be explained by means of sexual selection, social structure, specific recognition, ecological background crypsis, etc. (Cirer & Martínez-Rica 1990, Galán 1995). Differences on the melanistic percentage may result from natural selection due to the geological characteristics of the islands and/or to the degree of reflection of the sea, but also from genetic drift. Considering that Farilhão Grande is an all-dark basaltic island, while Berlenga Grande is a light rose granitic one with no dark rocks, *substrata* colour could constrain the selection of more melanistic forms at Farilhão Grande.

In summary, morphological differences are possibly related to the characteristic environment of each island (different geology, vegetation cover, food resources) and to the different isolation degrees and selective pressures. Genetic drift may always be a causal explanation of some morphological features on both populations.

## ACKNOWLEDGEMENTS

The authors are very indebted to the director of the Reserva Natural das Berlengas and also to the Portuguese Navy for facilities and permissions.

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## Appendix 1. Data used on colour pattern evaluation.

	CODE		DISCRIPTION		
		Zone	Type	Color	Intensity
CHROMATIC PATTERN	<b>BDS</b>	Black Dorsal Stripes	Uniform irregular or diffused stripes	Cream yellow green/yellow	1 (lighter) 2
	<b>LDS</b>	Light Dorsal Stripes			
	<b>MBZ</b>	Mid-Back Zone	Thick ocellated thin ocellated thick spotted thin spotted thick reticulated thin reticulated thick speckled thin speckled pattern	green green/brown	3
	<b>FKZ</b>	Left Flank Zone		brown/yellow	4
				brown or black	5 (darker)
<b>MEL</b>	<i>Melanistic Percentage</i> Relative percentage of black pigmentation at the dorsal side of the body				

Appendix 2. Descriptive statistics (mean, standard deviation and coefficient of variation) for the morphological characters in *P. c. berlengensis*. Body measures are in mm, scalation in units and melanistic pigmentation in percentage. Abbreviations are shown as in text.

	MALES						FEMALES					
	BERLENGA n = 35			FARILHÃO GRANDE n = 35			BERLENGA n = 34			FARILHÃO GRANDE n = 26		
	mean	SD	CV	mean	SD	CV	mean	SD	CV	mean	SD	CV
<i>SVL</i>	58.60	3.42	0.06	68.06	4.35	0.06	55.47	2.42	0.04	63.00	2.93	0.05
<i>VHL</i>	20.11	2.14	0.11	24.4	1.82	0.07	16.71	1.36	0.08	19.58	1.24	0.06
<i>SD</i>	14.69	1.37	0.09	17.46	1.12	0.06	11.74	0.75	0.06	13.46	0.95	0.07
<i>HL</i>	13.94	1.26	0.09	16.66	0.94	0.06	11.21	0.73	0.07	12.88	0.65	0.05
<i>HW</i>	7.71	0.99	0.13	9.23	0.81	0.09	6.32	0.59	0.09	6.92	0.48	0.07
<i>HD</i>	7.37	0.84	0.11	8.74	0.89	0.10	5.85	0.56	0.10	6.42	0.50	0.08
<i>OW</i>	6.57	1.06	0.16	7.60	0.88	0.12	5.56	0.79	0.14	6.08	0.74	0.12
<i>OD</i>	6.26	0.82	0.13	7.37	0.81	0.11	5.03	0.52	0.10	5.50	0.51	0.09
<i>FW</i>	4.71	0.62	0.13	5.46	0.65	0.12	4.03	0.39	0.10	4.46	0.51	0.11
<i>FD</i>	4.94	0.73	0.15	5.91	0.82	0.14	4.24	0.55	0.13	4.58	0.50	0.11
<i>NW</i>	3.20	0.63	0.20	3.80	0.41	0.11	2.85	0.44	0.15	3.00	0.28	0.09
<i>ND</i>	3.34	0.68	0.20	4.09	0.56	0.14	2.91	0.51	0.18	3.04	0.45	0.15
<i>HDL</i>	7.71	0.96	0.12	9.54	0.78	0.08	6.38	0.74	0.12	7.77	0.76	0.10
<i>HLL</i>	26.00	3.33	0.13	32.86	2.57	0.08	21.76	1.99	0.09	26.27	2.07	0.08
<i>GUL</i>	11.06	1.08	0.10	10.74	0.86	0.08	10.65	1.01	0.09	9.77	0.91	0.09
<i>COL</i>	8.46	0.85	0.10	9.77	1.00	0.10	8.68	0.77	0.09	9.15	1.08	0.12
<i>VTL</i>	27.18	1.62	0.06	26.06	1.14	0.04	29.85	1.48	0.05	28.31	2.66	0.09
<i>FP</i>	17.71	1.53	0.09	19.74	1.15	0.06	16.44	1.73	0.11	18.15	1.43	0.08
<i>MEL</i>	28.95	8.07	0.28	49.85	9.09	0.18	20.33	6.11	0.30	45.58	10.71	0.23

## Appendix 3. Factor loadings for the principal components axes (PCA).

	MALES		FEMALES	
	PC1	PC2	PC1	PC2
SVL	0.894	0.232	0.891	-0.204
VHL	0.855	0.282	0.868	-0.099
SD	0.908	0.191	0.796	-0.360
HL	0.912	0.205	0.894	-0.172
HW	0.839	0.144	0.647	-0.266
HD	0.831	0.223	0.666	-0.404
OW	0.772	0.004	0.685	0.273
OD	0.887	-0.054	0.773	0.279
FW	0.772	-0.503	0.561	0.264
FD	0.777	-0.402	0.552	0.371
NW	0.777	-0.280	0.406	0.573
ND	0.765	-0.464	0.425	0.716
HDL	0.739	0.217	0.775	-0.038
HLL	0.810	0.052	0.741	-0.153
Eigenvalues	9,555	1,043	7,024	1,670
Explained Variance %	68,248	7,450	50,170	11,927
Cumulative Variance %	68,248	75,698	50,170	62,096

Appendix 4. Observations of the categorical chromatic variables studied on the population. Abbreviations are shown as in text.

VARIABLES	MALES				FEMALES			
	BERLENGA		FARILHÃO		BERLENGA		FARILHÃO	
	n = 35		n = 35		n = 34		n = 26	
<b>BDS</b>	type	uniform	1	0	5	0		
		irregular	14	7	21	14		
		diffused	20	28	8	12		
<b>LDL</b>	type	uniform	8	0	21	1		
		irregular	21	3	10	4		
		diffused	6	32	3	21		
	color	Cream	3	1	19	11		
		Yellow	0	0	1	1		
		Green/Yellow	15	14	2	1		
		Green	17	7	9	6		
		Green/Brown	0	4	1	0		
		Brown/Yellow	0	5	0	0		
		Brown	0	4	2	7		
intensity	1	3	3	12	15			
	2	24	26	17	10			
	3	7	4	4	1			
	4	1	0	0	0			
<b>MBZ</b>	type	Thick ocelado	0	3	0	0		
		Thick spotted	8	15	2	3		
		Thin spotted	7	4	1	8		
		Thick reticulated	6	4	2	0		
		Thin reticulated	0	2	0	0		
		Thick speckled	11	6	18	11		
		Thin speckle	3	1	11	4		
		color	Green/Yellow	2	8	0	0	
	Green		9	5	1	0		
	Green/Brown		15	13	2	5		
	Brown/Yellow		0	2	0	0		
	Brown		9	7	31	21		
	intensity		1	1	0	1	1	
		2	6	2	14	8		
		3	17	16	18	11		
		4	11	16	2	6		
	<b>MBZ</b>	type	Thick ocelado	9	29	6	15	
			Thin ocelado	1	1	2	1	
			Thick spotted	15	4	8	4	
Thin spotted			8	1	4	4		
Thick reticulated			1	0	3	2		
Thick speckled			1	0	8	0		
Thin speckle			0	0	3	0		
color		Cream	1	1	2	0		
		Green/Yellow	3	5	1	0		
		Green	13	3	0	0		
		Green/Brown	7	8	2	5		
		Brown/Yellow	0	6	0	0		
		Brown	11	12	29	21		
		intensity	1	3	3	1	2	
			2	16	20	10	8	
			3	12	11	15	11	
4	4		0	8	5			



Appendix 5. Comparison of chromatic patterns (Chi-square test and t-Student test) between *P. c. berlengensis* males and females from Berlenga and Farilhão Grande.  $\chi^2$  value of the test, t – value of t-Student test, P – significance level, ns – not significant, \*  $P < 0.05$ .

VARIABLES	DIFFERENCES BETWEEN SAMPLES			
	$\chi^2$ test			
	MALES		FEMALES	
	$\chi^2$	p	$\chi^2$	p
BDS type	4.67	*	6.24	ns
LDS type	39.3	ns	33.8	ns
LDS color	47.2	ns	36.8	ns
LDS intensity	13.6	ns	16.0	ns
MBZ type	10.8	*	11.7	*
MBZ color	37.8	ns	30.7	ns
MBZ intensity	11.2	ns	11.5	ns
FKZ type	24.3	ns	15.9	ns
FKZ color	42.1	ns	24.9	ns
FKZ intensity	7.73	ns	6.79	ns
	t test			
	t	p	t	p
% MEL	-9,0248	*	-8,3511	*



# Differentiation within Syrian populations of the lizard *Mesalina brevirostris*

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*To estimate phylogenetic relationships among the Syrian morphotypes of Mesalina brevirostris we analysed a stretch of 834 bp of mitochondrial cytochrome b of samples from four Syrian and two Jordan localities. The analysis reveals two main clades: the first one comprises the big part of samples from Syria and Jordan, the second one an undescribed cryptic form from central part of western Syria. Subdivisions within the first clade correspond to (1) the Lowland form occupying central and eastern Syria and traditionally associated with the name M. b. brevirostris (Blanford 1874), (2) the form from Jabal al Arab in southern Syria which can be assigned to the name M. b. microlepis (Angel 1936) and, finally, (3) an additional form of uncertain taxonomic status in eastern Jordan.*

Keywords: *Mesalina brevirostris*, mitochondrial DNA sequences, taxonomy, Syria, Jordan.

## INTRODUCTION

*Mesalina brevirostris* (Blanford 1874) is considered morphologically variable polytypic species (or group of species) widely distributed in the Near and Middle East (e.g., Arnold 1986, Anderson 1999, in den Bosch 2001). Traditionally, two morphotypes are distinguished within Syrian populations, usually associated with two names: *M. b. brevirostris* comprising eastern and central Syrian populations, and *M. b. microlepis* (Angel 1936), comprising western Syrian populations (e.g. Angel 1936; Haas & Werner 1969; Werner 1971; Disi 1991, 1996).

In a previous study (Moravec 2004), the re-evaluation of the distribution and morphological variation of Syrian *M. brevirostris* revealed the existence of three rather differentiated morphotypes within the Syrian borders: (1) Lowland form – mostly lower altitudes (up to 500-600 m) of the desert and desert-steppe regions of central and eastern Syria; (2) Western (intermediate) form – higher altitudes (600-800 m)

of the western part of Syria; (3) Jabal al Arab form – eastern slopes of Jabal al Arab (J. Duruz) in southern Syria (ca 1000 m a.s.l.). These morphotypes tend to differ in pholidotic characters, body shape and colouration. However, the morphological differences are not prominent enough to allow easy distinction of the individual forms in the field.

Therefore, the aim of this study was to investigate the phylogenetic relatedness among the specified morphotypes on the molecular level.

## MATERIAL AND METHODS

The localities of samples are given in Table 1 and Fig. 1. Total genomic DNA was extracted from deep-frozen or ethanol preserved soft tissues, tongues and tail tips following a standard phenol-chloroform procedure (Sambrook *et al.* 1989). Preserved specimens are deposited in the National Museum Prague (NMP) and the Naturhistorisches Museum Wien (NHMW).

Amplifications of all PCR fragments were performed in 25 µl reaction mixtures containing PCR buffer with 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.4 µM of each PCR primer, 0.5 units of Taq polymerase (Amersham). Reaction conditions comprised an initial denaturation step of 2 min at 94 °C, 35 cycles of 10 s at 95 °C, 20 s

Table 1. Samples of *Mesalina brevirostris* used for molecular analysis (NHMW: Natural History Museum, Vienna; NMP: National Museum, Prague).

Specimen-no.	Locality	Coord	Clade
NMP 70305/1	SYR: Palmyra	3433/3817	1
NMP 70305/2	SYR: Palmyra	-"	1
NMP 70211/2	SYR: J. Arab	32/36	1
NMP 70211/3	SYR: J. Arab	-"	1
NMP 70439/3	SYR: Sadat	3416/3704	1
NMP 70439/4	SYR: Sadat	-"	2
NMP 70439/5	SYR: Sadat	-"	2
NMP 70440/7	SYR: Hawarin	-"	1
NMP 70440/8	SYR: Hawarin	-"	1
NMP 70224/1	JOR: Azraq	3150/3649	1
NMP 70224/3	JOR: Azraq	-"	1
NMP 70629/1	JOR: Amman	3158/3558	1
NMP 70629/2	JOR: Amman	-"	1
NHMW 32326:10	UAE: Abu Dhabi		3

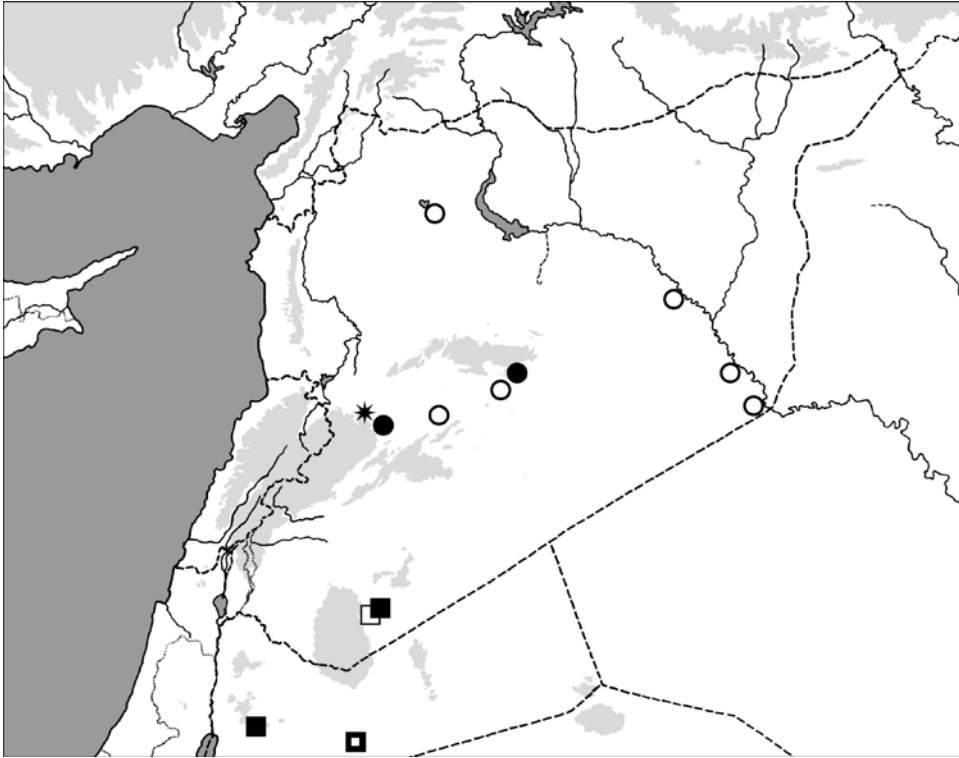


Fig. 1. Distribution of the individual forms of *Mesalina brevirostris* in Syria and northern Jordan according to examined samples. Open circles = not sequenced Lowland form samples; closed circles = sequenced Lowland form samples (Hawarin, Palmyra); asterisk = sequenced sample (Sadat) involving both Lowland and Cryptic form; open square = not sequenced sample of the J. Arab form (Jabal al Arab); closed squares = sequenced samples of the J. Arab form (Jabal al Arab, Amman); square with a light centre = sequenced sample of the desert population from eastern Jordan (Azraq).

Table 2. Primers used in the study.

Primer	Sequence	Use	Reference
L15050	5'-tat cta cat att gga cga ggc c-3'	PCR	this paper
L14775	5'-cag aca aaa ttc cat tcc acc-3'	PCR, Seq	this paper
H15425	5'-ggt tta caa gac cag tgc ttt-3'	PCR	Podnar <i>et al.</i> (2005)
H15465	5'-ttg ctg ggg tga agt ttt ctg ggt c-3'	PCR, Seq	this paper

at annealing temperature, 90 s at 72 °C, and a final extension step of 7 min at 72 °C. Negative and positive PCR controls were included in all PCR amplifications. Primers used in the study are given in Table 2. Two overlapping sections of *cytb* gene were amplified with the primers L15050 and H15465, and L14775 and H15150, respectively. Sequencing was performed by MWG (Ebersberg, Germany).

## RESULTS

MrBayes (Huelsenbeck & Ronquist 2001) and Neighbor-Joining (Saitou & Nei 1987) analyses resulted in trees of the same topology revealing three main clades with divergence of about 10% (Fig. 2). The first clade includes the representatives of all three morphotypes (*sensu* Moravec 2004) and is further divided into weakly divergent subclades: (i) Lowland form extending far to the west; (ii) J. Arab form of southern Syria and northern Jordan; (iii) Desert populations from the eastern Jordan (Azraq area). The second main clade includes a morphologically cryptic, so far undescribed form occurring syntopically with the Lowland form. The sample from Abu Dhabi represents a third main clade.

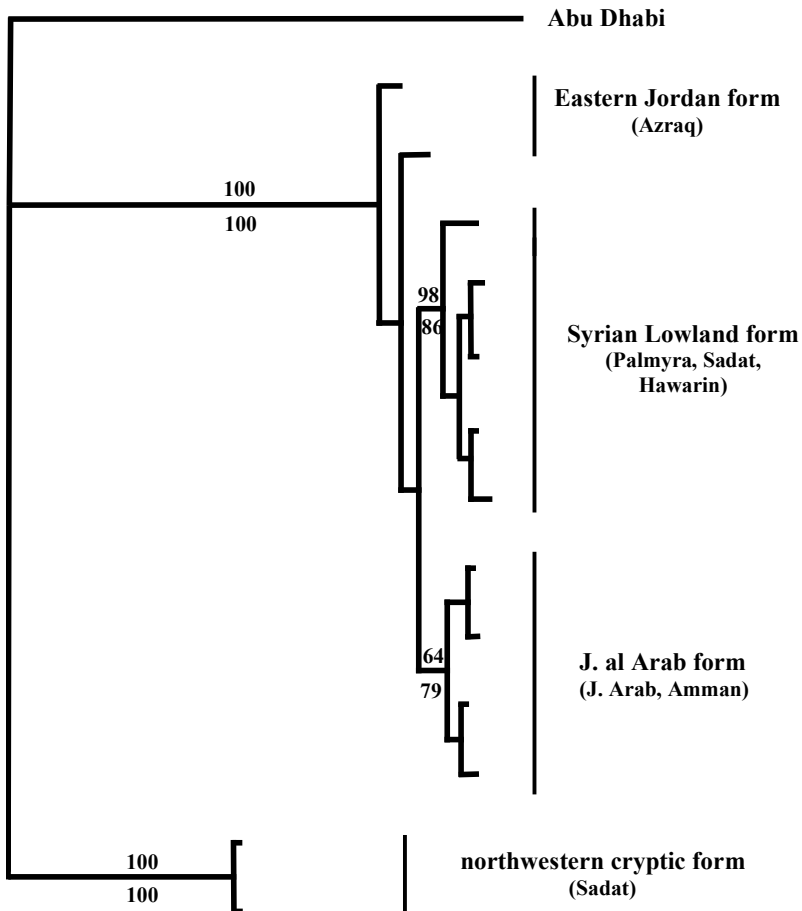


Fig. 2. Bayesian tree. Bayesian posterior probabilities are given above branches and NJ bootstrap values below branches, respectively).

## DISCUSSION

The results obtained suggest the following:

(1) The Syrian Lowland and J. Arab morphotypes may represent two different taxonomic units of *Mesalina brevirostris*, which evolved in relatively short time. The former can be associated with the traditional name *M. b. brevirostris*. The latter can be assigned to the name *M. b. microlepis*. These forms differ in size, number of gular, dorsal and preanal scales, as well as in body shape (Moravec 2004). However, this notion is not consistent with the type locality of *M. b. microlepis* (town of Hawarin ca.

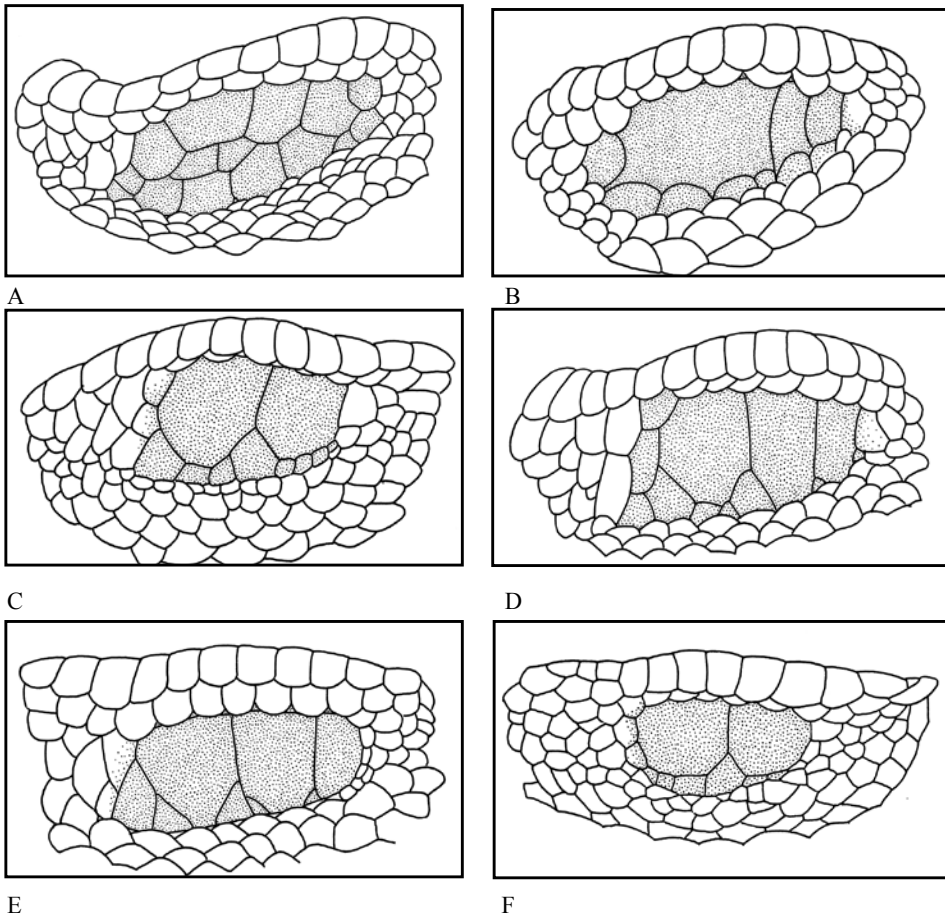


Fig. 3. Structure of the semitransparent window in different Syrian populations of *Mesalina brevirostris*. (A) Sadat, W Syria (Cryptic form); (B) Sadat, W Syria (Lowland form syntopic with the Cryptic form); (C) Palmyra, central Syria (Lowland form); (D) Hawarin, W Syria (present topotype of *M. b. microlepis*); (E) Jabal al Arab, S Syria (J. Arab form); (F) Azraq, E Jordan (Azraq form).

55 km SE of Homs, western Syria [“Haouarine – à 55 kilomètres au S.-E. de Homs”; Angel 1936]). Although the holotype of *microlepis* morphologically resembles J. Arab form rather than any other morphotype, the molecular data of the present topotypes show that the type locality of *microlepis* lies within the range of the Lowland form (*M. b. brevirostris*).

Nevertheless, this situation could be attributed to the changes in the geographical distribution of the respective forms due to current aridisation of the Near East. The expansion of the Lowland (desert) form to higher elevations of western Syria could lead to replacement or assimilation of the original population, which the type belonged to.

(2) The “Western form” is an artificial unit consisting of two sympatric and morphologically very similar forms. The first one belongs to the Lowland form (*M. b. brevirostris*). The second one represents an undescribed *taxon* (“Sadat form”), which differs morphologically in being slightly larger in size, displaying clearly ocellated colour pattern and in having semitransparent window of the lower lid consisting of several roughly equal scales (Fig. 3). The last characteristic seems to distinguish this otherwise cryptic form from all other morphotypes examined in this study (the window consists of 1-3 larger scales).

Future investigation of nuclear markers, extended sample size and intensified sampling of the presumptive contact areas between suggested population groups is necessary to test whether the “Sadat form” and the “western Lowland form” hybridize, i.e., whether they represent different biological species or not.

(3) The desert population from eastern Jordan (samples from Azraq) is divergent from the J. Arab form inhabiting the Jordan Irano-Turanian steppe zone. Morphological comparison shows difference in size and colouration (Disi *et al.* 2001). The data is thus indicative of yet another undescribed *taxon*.

## ACKNOWLEDGMENTS

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# A complex case of interaction between lizards and plants. The dead horse arum (*Dracunculus muscivorus*) and the Balearic lizard (*Podarcis lilfordi*)

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*In this work we describe the interaction of the dead horse arum and the Balearic lizard in Aire (Balearic Islands, Spain). In Aire Island, the dead horse arum, *Dracunculus muscivorus*, is particularly abundant. From 1999 to 2005 we studied the population of this plant species and its relationship with the Balearic lizard, *Podarcis lilfordi*. During blooming period, several lizards exhibited an intense foraging behaviour focused on open inflorescences. Lizards were able to capture flies attracted by the plants and those trapped in tubules as pollinators. The exploitation of pollinators was principally made by largest males of the population that actively excluded females and smaller males from plants, skewing lizard sex-ratio in areas of maximum plant density.*

*Lizards consumed fruits following the progressive fruit maturation. In two germination experiments, we detected an enhanced germination of seeds from lizards' faeces, in comparison with those directly taken from ripening fruits. Thus, the Balearic lizard is the main legitimate disperser of the dead horse arum.*

*From 1999 to 2005, the density of the dead horse arum increased from 4800 ind./ha to more than 25.000 ind./ha in some optimal areas of the islet, indicating a very positive balance of the interaction between plants and lizards.*

Keywords: mutualism, interaction, Balearic lizard, islands.

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## INTRODUCTION

Plants are the basis for animal radiation on earth and the dominance of flowering plants is directly linked with mutualistic relationships of plants, their pollinators and seeds dispersers (Price 2002). Hence, it is not surprising that a myriad of different interactions between plants and animals arose during biota evolution. At Mediterranean ecosystems these interactions are widespread. Even within lizards, several interactions are described (Pérez-Mellado & Traveset 1999, Olesen & Valido 2003).

Insular ecosystems of the Mediterranean basin are characterized by a low trophic availability and, consequently, a reduced number of terrestrial vertebrates. In addition, Mediterranean islands have a small number of autochthonous predators, at least, prior to human arrival during the Holocenic period (Corti *et al.* 2000, Pérez-Mellado 2002). It is the case of Balearic Islands (Spain). At several coastal islets, the Balearic lizard, *Podarcis lilfordi* (Squamata, Lacertidae), is the only terrestrial vertebrate, inhabiting islets without significant interferences from predators and/or competitors. This situation promotes the raise of a varied set of demographic and natural history traits, in some cases unique of one or more populations. Among these traits, we can mention a very high population density, generally higher than observed densities for continental lacertid lizards of similar body size (Pérez-Mellado 1989, Brown & Pérez-Mellado 1994 and a summary in Pérez-Mellado 1998). Another characteristic is a sharp sexual dimorphism, with bigger males than females, as well as an eclectic foraging behaviour (Pérez-Mellado & Corti 1993, Pérez-Mellado 1998, Pérez-Mellado & Traveset 1999).

Several examples of interaction between the Balearic lizard and different plant species has been already described, including the role of the lizard as the main pollinator of *Crithmum maritimum* and *Euphorbia dendroides* (Pérez-Mellado & Casas 1997, Traveset & Sáez 1997, respectively), as well as several cases of seed dispersal (see Pérez-Mellado & Traveset 1999 for a review). However, from 1997 a much more complex interaction between the dead horse arum and the Balearic lizard was detected at Aire Island (Menorca, Balearic Islands, Spain). The consumption of pollinators arriving to blooming plants, as well as an intense fruit consumption during the ripening period was shortly described in a preliminary paper (Pérez-Mellado *et al.* 2000). In the present work we summarize the findings of a deeper study made during the spring and summer 2003 (see also Pérez-Mellado *et al.* in press and Pérez-Mellado *et al.* in prep.). Starting from previous observations, we checked the role of the Balearic lizard in the pollination of the dead horse arum and the pattern of flower visitation by lizards. In addition, we studied the fruit consumption during the ripening period, the characteristics of fruits produced by the plant and the main cues used by lizards on fruit selection. Finally, we estimated the extent of seed dispersal and its influence on plant density.

## MATERIAL AND METHODS

### *Study area and period*

The study was carried out during the spring and summer 2003, with additional observations in 2002, 2004 and 2005. In 2003, intensive field work started on the first week of February till September.

Aire Island is located off the south-eastern coast of Menorca (Balearic Islands, Spain). The island has a surface of around 342500 m<sup>2</sup>. It is probably the oldest and most remote of the islands that surround the coastline of Menorca. It is a fairly flat island, with a slow gradient rising to 15 m above sea level in the north to south axis. A jetty, suitable for dockings of small to medium boats, is located in the central zone of the northern coast. The constructions of the island are all in ruin with the exception of a small hut by the jetty and the lighthouse, which was maintained by a resident lighthouse keeper up until the 1960s and is now automatic (Fig. 1). A colony of the yellow-legged gull (*Larus michaellis*) is located in the western end of the island (Fig. 6); other nesting birds are the peregrine (one breeding pair, *Falco peregrinus*), storm petrel (*Hydrobates pelagicus*), Cory's Shearwater (*Calonectris di-*

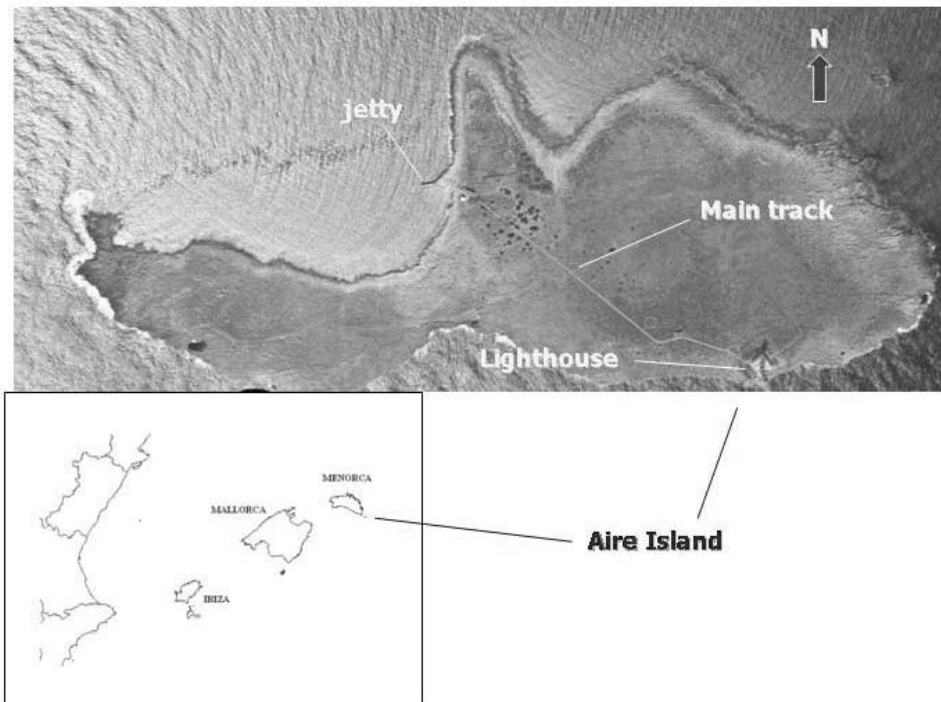


Fig. 1. Aire Island and its geographical situation. See the location of the main artificial track that divides the central area of the island in two distinct parts, with lower vegetal cover to the west of the track.

*omedea*), Balearic shearwater (*Puffinus mauretanicus*) and European shag (*Phalacrocorax aristotelis*).

Menorca, and therefore also Aire Island, has a mesomediterranean climate, characterised by mild temperatures with very seasonal precipitations, abundant rains in winter and very dry summers. There are frequent and strong winds principally from the north and the east. Aire Island is located in the driest zone of Menorca. Between 1945 and 1975 an average rainfall of 435 mm was recorded half yearly (lighthouse rain station from Aire Island). Yearly average temperature is 17.2 °C with a maximum of 27.5 °C in July and a minimum of 9.5 °C in January (weather station at Sant Lluís, the closest to the island, Jansà i Clar 1979).

Given its flatness and its relative distance from the coast, the whole surface of the island is subjected, from the point of view of the vegetation, to the effect of the sea. Consequently, with the exception of communities of shallow rooting plants, virtually all of the vegetation is hallophyllous. In a straight transect along the islet can be seen various patches of vegetation. First, nearest to the sea, plant communities with marked hallophyllous characteristics appear. Typical of places strongly affected by the sea, species such as *Crithmum maritimum* and *Limonium* spp. can be found. A little further from the coast, the vegetation opens out into shrub communities dominated by *Suaeda vera*, typical of saline soils. On sites protected from the wind and sea by barriers of human origin (remains of walls and plantations of *Tamarix africana*), species of typical Mediterranean vegetation dominated by *Pistacia lentiscus* appear. Furthermore, *Carlina corymbosa* forms dense patches in the least saline areas. Beyond the communities previously mentioned unfold the communities of annual plants including especially abundant species such as *Bellis annua*, *Asteriscus maritimus* and *Valantia muralis*.

Between the dock and the lighthouse there is a narrow track of 465 m long that divides the island in two well differentiated areas. To the west of the track rock *substrata* is dominant and the vegetation is formed by coastal species, being the sea fern, *Crithmum maritimum* the dominant plant species. The eastern area can be divided in two portions, the westernmost part, with a shrub vegetation of *Suaeda vera* in the south and *Carlina corymbosa* in the north, together with some shrubs of *Pistacia lentiscus*. The easternmost area is covered by rocks and a scarce vegetation of sea ferns. We made a preliminary division of the island surface in different areas according with these differences in vegetation cover (Fig. 2).

### *Species under study*

The dead horse arum, *Dracunculus muscivorus* Engler 1879 (Araceae, Aroideae), is a plant of Thyrranian distribution, that is, an endemic species only present on West-

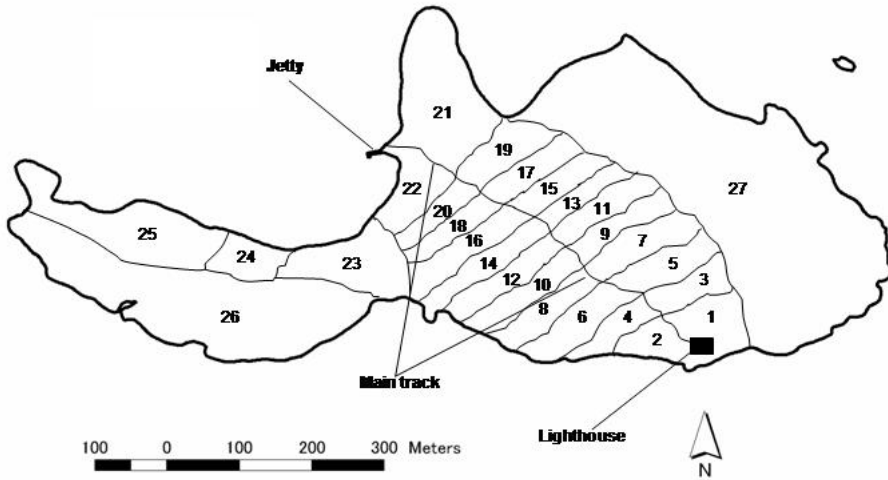


Fig. 2. Division of Aire Island in 27 different areas from which we made line transects of the dead horse arums and lizards.

ern Mediterranean islands, Corsica, Sardinia and the Balearic archipelago. This “very striking sinister-looking plant” (Polunin & Huxley 1981, Fig. 3), is considered the less common Araceae of Balearic Islands (Bonner 1994). Its natural habitat is formed by rocky areas with some degree of humidity (Collu & Dardani 1999). Some authors consider that differences on inflorescences, pollen grains and other morphological characteristics are sufficient to separate the dead horse arum in a different monotypic genus, *Helicodiceros* (Schott 1853).

Flowers are arranged around the floral axis or spadix, where male and female flowers are separated by a sterile portion of the spadix (Fig. 4). The spadix is surrounded by the basal portion of the spathe, a highly modified leaf that forms a narrow tubule, flanked by several filaments that can close the tubule above male or female flowers. The inner surface of the spathe is covered by short hairs and pigmented on red, pale pink or whitish. The outer portion of the spadix is yellowish with dark filaments and lacks flowers.

The dead horse arum has a highly sophisticated pollination mechanism. It produces an intense decaying meat odour, attracting flies (Diptera), especially from the family Calliphoridae. Some species are identified as pollinators, as *Lucilia caesar* and *Calliphora vicina* (Stensmyr *et al.* 2002). Female flies are attracted by the odour because they use carcasses as optimal laying sites. Recently it has been demonstrated that chemical composition of odours from *D. muscivorus* is extremely similar to the odour produced by decaying meat, eliciting almost identical neural responses on flies (Stensmyr *et al.* 2002). The plant odour, produced at the spadix, attracts flies coming from



Fig. 3. A blooming dead horse arum.

another plant, where they loaded pollen. Flies enter into the tubule and are trapped by a rosette of stiff hairs into the lower chamber with receptive female flowers. Flies' movements trying to escape sprinkles pollen grains on female flowers that close after pollination. Then, male flowers open and start pollen production to cover trapped flies. Pollen production takes place at night, followed by a fall on plants' temperatures, higher during diurnal period of female receptive flowers. In addition, the opening of male flowers stops the production of foetid odour by spadix (Bonner 1994). Finally, after two to several hours trapped, flies are released. This mechanism, with an asynchronous maturation of male and female flowers, precludes autopollination (Collu & Dardani 1999). *D. muscivorus* flowers remains open during no more than two days, while odour production is highest on the first day, when more pollinating flies are attracted (Stensmyr *et al.* 2002). Probably, the dead horse arum employs additional lures to attract flies as heat, because the plant is able to raise its temperature over more than 14 °C above air temperature (Pignatti 1982). Also the flashy colour of spathes can attract flies and other potential pollinators.

The Balearic lizard, *Podarcis lilfordi* (Günther 1874) (Lacertidae, Squamata) is a medium-sized lacertid lizard endemic to Balearic Islands. In some of the islands,



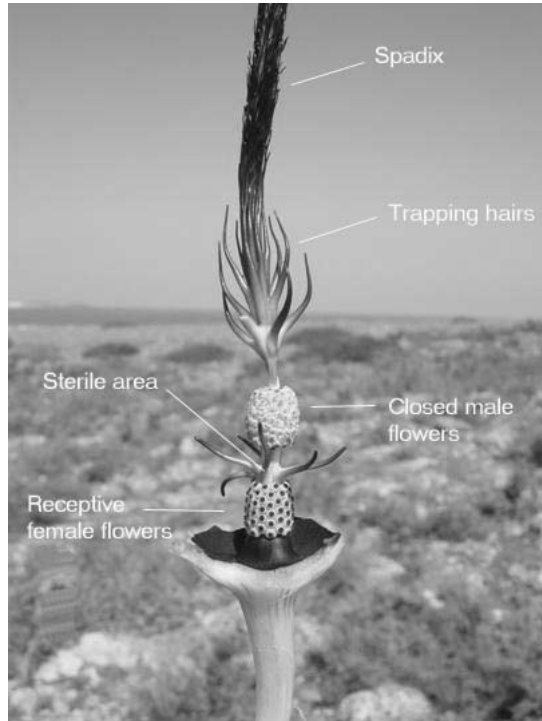


Fig. 4. Internal structure of a blooming dead horse arum after leaving the spathe.

extremely high population densities are found while in others the effective population has reduced to as little as just a few individuals (Pérez-Mellado 1989, 1998). Twenty-eight subspecies of *P. lilfordi* have been described, based on differences in morphometrics, design, colouration and scalation, but not all are accepted. In the most recent revisions (Pérez-Mellado 1997, 1998) 23 subspecies were recognised, with eight of them off the coast of Menorca, six off Mallorca and the rest present in Cabrera archipelago. The subspecies that occupies Aire Island is the nominal one, *Podarcis lilfordi lilfordi*. Along with the extinct population of Ratas Island, it is the most obviously differentiated of all the Menorcan populations (Pérez-Mellado & Salvador 1988, Pérez-Mellado 1998). *P. lilfordi lilfordi* is a melanistic lizard with a larger body size than any other Menorcan population and with characteristic biometrical and scalation traits (Fig. 5). It is a robust subspecies; the males have a head to tail length with an average of 69.98 mm and an average weight of 9.57 g, meanwhile the females are smaller with an average head to tail length of 61.73 mm and a weight of 6.34 g (Pérez-Mellado & Salvador 1988, Pérez-Mellado 1998). Lizards of Aire Island are active all year round. They are distributed throughout the island. The density reaches extremely high levels in certain areas around the moor and in the group of tamarinds situated in the eastern zone (Pérez-Mellado 1989, Brown &



Fig. 5. The Balearic lizard basking on a blooming dead horse arum.

Pérez-Mellado 1994 and pers. obs.). These densities fluctuate notably from one year to the next.

### *Density estimations and zonal stratification*

Plant and lizard densities were estimated with line transects (Buckland *et al.* 2001). For plants, line transects had a length of 70 m with a width of 1 m at both sides of the line. Prior to plant line transects, we made lizard density estimations with transects on the same areas. In this case the width of transect was determined by the outermost individual recorded at each transect (Buckland *et al.* 1993).

Aire Island was divided in different sectors to make density estimations of plants and lizards. Within the nucleus of previously observed highest plant density (Pérez-Mellado *et al.* 2000) we made narrow strips each 35 m from lighthouse enclosure to northern coast, taking as a reference the middle track from dock to lighthouse (Fig. 2). This artificial track marks a clear limit of vegetation. East to the track, the dominant plant is *Suaeda vera*, while at the western side the dominance corresponds to more coastal species as *Crithmum maritimum* and *Limonium* spp. Western area is also the main site of seagull breeding colonies (Fig. 6). Only three breeding pairs of *L.*

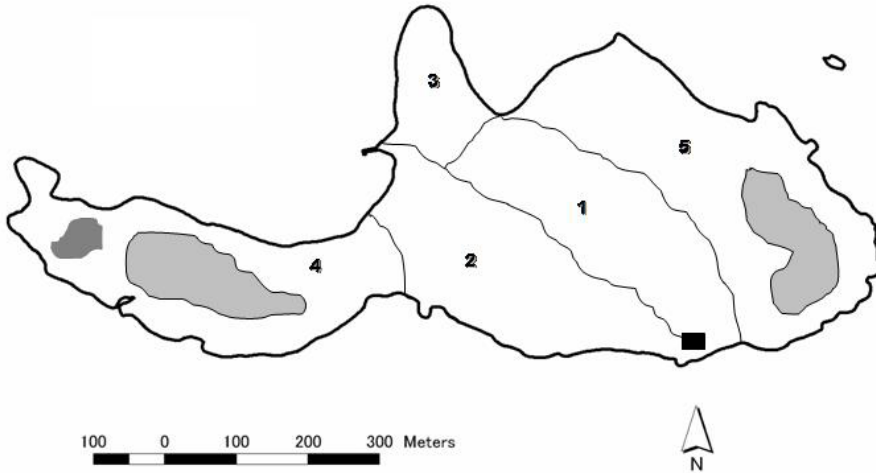


Fig. 6. The results of line transects allow us to divide Aire Island in five main areas with different plant and lizard density. Pale grey spots at areas 4 and 5 indicate the location of yellow-footed gull (*Larus michaellis*) colonies during 2003. Darker grey spot in area 4 was the site of Audouin's gull (*Larus audouinii*) colony.

*michaellis* were present close to the eastern side of the track during spring 2003. Then, another group of seagulls breed at the easternmost peninsula of islet. From the main track, parallel censuses of lizards and plants were made to both eastern and western sides heading to 220° and 40° compass courses respectively. In this way, 20 sectors of plant and lizard densities were defined. Then, four censuses covered the northeastern peninsula of the island (sector 21 of Fig. 2) were made. Finally, the western peninsula, a large rocky area with the largest seagull colony, was surveyed with four additional censuses (sectors 22 to 25 of Fig. 2) as well as the easternmost corner of the island. Thus, density of lizards and plants was estimated in the whole surface of the island, excepting from sector 27 for plants, with an extremely low density of *D. muscivorus*.

In addition, on 13th April 2003 and on 28th April 2005, we made four line transects of 25 m long and 100 cm to both sides of the line of transect each, at the same site of maximal plant density surveyed in 1999 (Pérez-Mellado *et al.* 2000 and Fig. 8) to make a direct comparison of plant density between 1999, 2003 and 2005.

Within the first 20 narrow sectors, where plant density is higher (see below), we made an additional estimation of rock, open ground and plant covers. We made 25 m censuses on each strip, recording the percentage covered by each shrub species, that is, by each vascular plant that can be used by lizards as a refuge or perching site. Plant cover was recorded in this way for *Asteriscus aquaticus*, *Carlina corymbosa*, *Crithmum maritimum*, *Euphorbia sagetalis*, *Pistacia lentiscus* and *Suaeda vera*. Rock cover was recorded in three categories, rocks of more than 50 cm high, less of 50 cm and rocky

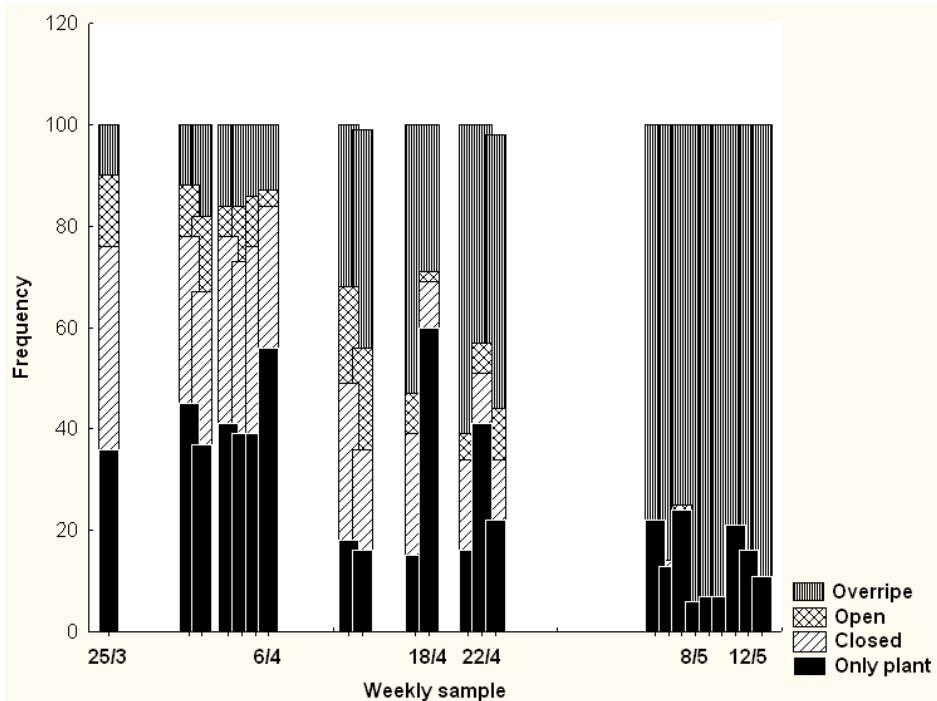


Fig. 7. Proportion of blooming *D. muscivorus* (open spathes), closed plants, plants after blooming period (overripe) and only plants (prior to blooming) during weekly sampling of spring 2003 at Aire Island. See the staggering of open spathes over a long period of almost two months.

ground. A comparative analysis of cover and its diversity was made at the first twenty areas surveyed. Diversity of vegetal and rock cover was estimated with Levins' niche breadth (Levins 1968), as well as its equitability according to Pielou's modification (Krebs 1989).

#### *Plant characteristics, availability and lizard behaviour*

Availability of open spathes of *D. muscivorus* was studied from 17th April till 12th May 2003. Sixty spathes were numbered with plastic labels recording their colour (whitish, pale pink, pink or reddish). The presence of flies as potential pollinators on spathes or trapped into the tubule, as well as the presence of lizards were also recorded. On additional 83 randomly selected open spathes the maximum height, spathe length and width, spathe orientation and distance to the nearest plant of the same species and to the nearest individual of *Suaeda vera* were measured.

Lizards on spathes were noosed. SVL and tail length was measured with a steel rule. Lizards were weighted with a spring balance and cloacal temperature was recorded with a digital thermometer (Pérez-Mellado *et al.* in prep.).

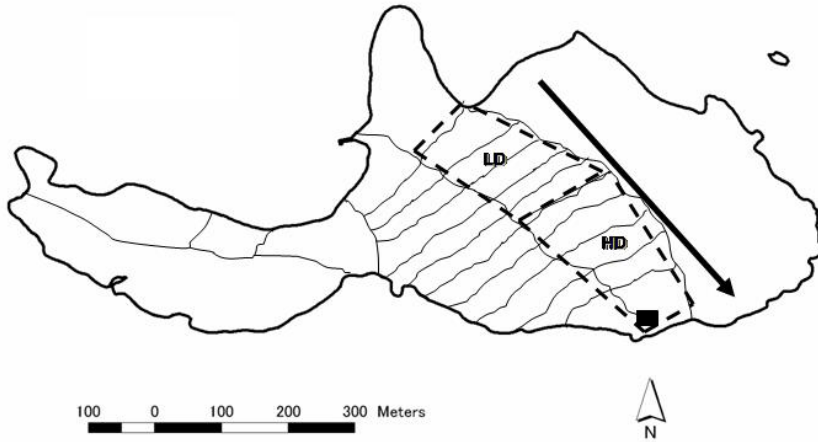


Fig. 8. Areas of low and high plant density to the east of the main track. The arrow indicates the general trend to higher plant and lizard density from north to south. A cross within high density area marks the point of identical line transects carried out in 1999, 2003 and 2005.

#### *Potential role of lizards as pollinators*

Potential role of *P. lilfordi* as a pollinator of *D. muscivorus* was studied with an exclusion experiment. From marked spathes (see above), we randomly selected six spathes, prior to their opening. Three spathes, to exclude flying pollinators (Calliphoridae flies), were covered with a cubic plastic mesh. Remaining three spathes were surrounded with a plastic enclosure open from the top. In this way, we precluded lizard access and allowed the access of flying pollinators. Ten of the remaining marked spathes acted as control, with a free access to flies and/or lizards. In addition, we evaluated the capacity of pollen load of this plant species by the lizard. Pollen grains of *D. muscivorus* are easily distinguishable for their spheroidal shape and spiny outer surface (Thanikaimoni 1969). Pollen load capacity was estimated using the same protocol of a previous study of the Balearic lizard and other plant species (see details in: Pérez-Mellado *et al.* 2000), extracting pollen grains from belly, dorsum and throat with cello-tape stripes. From April 2003 we obtained data of pollen load from 24 adult lizards. We added a sample of 10 lizards surveyed on May 1999.

#### *Seed dispersal*

Results of seed dispersal presented here are summarized from another work on this subject (Pérez-Mellado *et al.* in prep.). The study of available fruits was done from 500 ripening fruits obtained from 20 mature and intact infructescences in two different areas of Aire Island characterized by a very different plant density (see be-

low). Thus, 10 infructescences were randomly collected for each density area. For each infructescence we obtained its total fruit number, fruit colour, that reflects its maturation state, and, from a random subsample of 25 fruits of each infructescence, fruit length, width and weight. In the same way, we recorded the number of seeds per fruit and their weight, length and width from a random subsample of 25 seeds from each infructescence.

The intensity of dispersal was estimated from weekly samples of 100 faeces of *P. lilfordi* collected at the areas of low and high plant density (50 faeces for each area). This sample took place from 18th May till 13th June 2003. Then, we obtained two additional samples on 28th June (eighth week) and 19th July (11th week). We also made an analysis of three consecutive years, 2002, 2003 and 2004 with additional samples of faeces from June 2002 and 2004 collected at both areas of high and low plant density (see below). We counted dead horse arum seeds, as well as the presence of elaiosomes on seeds.

Viability tests were performed in October 2003 on three samples of seeds from wild plants randomly selected that had free access to potential pollinators, another sample from plants isolated from flying pollinators (see Methods) and a third group from lizard faeces. We used tetrazolium test to check the viability of seeds. Seeds were cut so that the embryo is bisected. Then, seeds were placed in a 0.1% solution of 2, 3, 5-triphenyl-2H-tetrazolium chloride (TTC). Viable embryos release hydrogen ions during respiration, which combine with TTC, causing it to turn reddish (ISTA 1999).

We made two experiments on seed germination. First experiment was performed on 1999 and the second experiment, with a larger sample size on 2002. Seeds from faeces and plants were collected at Aire Island. In 1999 seeds were collected on 17th

May 1999 and stored at 4 °C during two weeks. Prior to plantation, seeds were dried at room temperature during two days. The second experiment started on 29th June 2002 and finished after 182 days. In this case seeds were collected during June and May (see above). Seeds were buried on universal *substrata* and maintained during the whole duration of both experiments in open air at Menorca Island. Outdoor experiments on seed germination are better to detect effects (Traveset & Verdú 2002).

### *Seed predation*

After fruiting period, we did an experiment to test post-dispersal seed predation intensity at Aire Island. We arranged two randomly selected samples of *D. muscivorus* seeds from lizard's faeces and ripening fruits, respectively. We checked seeds from faeces to assure the absence of elaiosomes, while in all seeds from ripening fruits elaiosomes were present. Both samples of seeds came from the two areas of high and low plant density (see above).

Lizards were placed in two series of 20 plastic Petri dishes of 9 cm of diameter at both areas of high and low plant density from 18th July 2003, when ripening fruits of *D. muscivorus* were not anymore available. In each series, we placed two parallel lines of Petri dishes 7 m apart each other and with 3.5 m between lines, one line with dishes covered with a wire mesh (6 per 6 mm) that precluded the access of vertebrates (lizards, granivorous birds or rabbits) to seeds and another line of uncovered dishes. On each Petri dish we placed 10 seeds. For each Petri dish, seeds were obtained from the high or the low plant density areas. A careful inspection of the 40 Petri dishes was done each three days during a trial of 30 days.

### *Data analysis*

Data were analyzed with Statistica 6.0 package or with a handheld calculator for some likelihood tests. We employed parametric tests when data were normal and variances homogeneous or data log transformed. If data did not meet requirements for parametric tests after transformation, non-parametric alternative tests were employed. Frequency tables were analyzed with non-parametric test as G, McNemar or binomial tests (Zar 1999) or with log-linear models (Quinn & Keough 2002). In all cases we give the arithmetic mean ( $\bar{x}$ ) followed by its standard error ( $\pm$  SE), maximum and minimum values of the distribution and sample size (n).

## RESULTS

### *General characteristics of Dracunculus muscivorus*

The phenology of the dead horse arum can vary between years. In 2003, blooming period started earlier than in 1999 (Pérez-Mellado *et al.* 2000). During the first week of February we found several green plants at the central area of Aire Island. In March, several spathes were open, with the highest proportion during the four week of this month and the first two weeks of April (Fig. 7). Open spathes were present during a long period of more than five weeks, lacking synchronization of blooming plants.

Closed spathes of *D. muscivorus* are dark green and brownish spotted. Open spathes are white, yellowish, pink or reddish. Average length of spathes was  $\bar{x} = 223.0123 \pm 3.8941$  mm (n = 81). We did not find significant differences between spathe lengths of different colours (one-way ANOVA,  $F_{2,78} = 1.1899$ ,  $P = 0.3097$ , homogeneous variances, Levene's test,  $F = 0.5495$ ,  $P = 0.5794$ ). Maximal width of spathes was  $\bar{x} = 184.0617 \pm 3.2268$  mm, range: 119-255 mm, n = 81, again without significant differences between colours (one-way ANOVA,  $F_{2,78} = 1.1569$ ,  $P = 0.3198$ , homogeneous variances, Levene's test,  $F = 0.3860$ ,  $P = 0.6810$ ).

### *Density of lizards and plants*

The dead horse arum is distributed over the whole Aire Island surface, but it is rare at sectors 26 and 27 from the eastern part of the island and was not detected at sectors 20, 21, 22 and 25 (Fig. 2) during line transects. These four sectors were during 2003 the sites of seagull breeding colonies.

Including sectors where the presence of plants was not detected, the average plant density for Aire Island is:  $7186.54 \pm 1979.71$  plants/hectare, for  $n = 26$  line transects (ranging from 0 to 32146 plants/ha, 95% confidence limits: 3109.25-11263.83).

These results allow us to group sectors in larger areas of similar plant density. Thus, we consider four large areas: 1: Central area east from the track to lighthouse, 2: Central area west from the track to the lighthouse, 3: Northeastern peninsula, 4: Western peninsula and 5: Eastern part of the island (Fig. 6).

For all line transects, the average density of lizards during spring 2003 was:  $2188.89 \pm 213.44$  lizards/hectare (range: 398.67-5816.70,  $n = 35$  line transects). Area 3, showed the highest lizard density (Table 1). Areas 2 and 4 are occupied by seagull breeding colonies (Fig. 6) and showed lower lizard densities, as well as the area 5, with a poor vegetal cover, very low density of dead horse arums (see below) and also a small seagull colony. Finally, area 1 has a high lizard density. We found significant differences between the density of these areas (one way ANOVA,  $F_{4,30} = 3.9355$ ,  $P = 0.011$ , homogeneous variances, Levene test,  $F_{4,30} = 2.5017$ ,  $P = 0.0633$ ). *A posteriori* Duncan tests indicate significant differences between areas 5 and 1 ( $P = 0.03$ ), with lowest and highest lizard density respectively, and between areas 3 and 4 ( $P = 0.0247$ ), between

Table 1. Lizard density at five different zones of Aire Island.

Zone	$\bar{x}$	$\pm$ SE	Range	n
1	2825.88	346.22	2114.21-3537.55	10
2	1624.17	346.22	912.5-2335.84	10
3	3706.27	632.11	2406.94-5005.59	3
4	2110.01	413.81	1259.4-2960.62	7
5	1244.22	468.55	398.67-3002.10	5

Table 2. Dead horse arum density at different zones of Aire Island

Zone	$\bar{x}$	$\pm$ SE	n
1	10924.84	3846.21	10
2	6921.76	3206.91	9
4	5095.25	4385.73	3



3 and 2 ( $P = 0.005$ ) and between 3 and 5 ( $P = 0.001$ ). Thus, both island areas with breeding colonies of seagulls showed significantly lower lizard densities. It is also the case of area 5, with a smaller seagull colony and, probably, a suboptimal plant cover.

Regarding the dead horse arum, we restricted our analysis to areas 1, 2 and 4, because at the north-eastern peninsula (area 3) and area 5 we did not detect plants during transects, even if some individuals were present (pers. obs.). Densities are clearly higher at area 1 (Table 2), even if we did not detect significant differences among the three areas (one way ANOVA,  $F_{2,19} = 0.5042$ ,  $P = 0.61$ , homogeneous variances, Levene's test,  $F_{2,19} = 0.8164$ ,  $P = 0.4569$ ).

Taking into account all sectors of the island, we did not find any significant correlation between dead horse arum and lizard density (Spearman rank correlation,  $R_s = 0.00123$ ,  $P = 0.99$ ,  $n = 30$ ). If we consider only those sectors with detected plants, the correlation is still non significant ( $R_s = 0.2377$ ,  $P = 0.2866$ ,  $n = 25$ ).

However, at the central area of maximal plant density, east from the main track to the lighthouse (see above and Fig. 8), we found a significant correlation between plant and lizard density ( $R_s = 0.6322$ ,  $P = 0.0498$ ,  $n = 10$ ) with a decreasing density gradient from south to north. This correlation does not exist at the western side of the main track ( $R_s = -0.358$ ,  $P = 0.3096$ ,  $n = 9$ ) probably because these areas enter, at least partially, within the range of the breeding colony of seagulls.

Results from the central area of maximal plant density showed a clear north-south density gradient, suggesting an additional analysis. We grouped the twenty narrow areas east and west to the main track into four groups, southeast, southwest, northeast and northwest (Fig. 8) to compare these larger areas with fruit consumption and seed dispersal intensity. Densities of plants and lizards were again estimated for these four groups.

In the case of plants, we did not find significant differences in density between south-western and north-western groups (one way ANOVA for log-transformed data,  $F_{1,7} = 1.504$ ,  $P = 0.2598$ , homogeneous variances, Levene's test,  $F_{1,8} = 0.2584$ ,  $P = 0.6268$ ). However, we found significant differences between south-eastern and north-eastern groups, with a higher plant density at south-eastern group (density =  $19134.64 \pm 4055.004$  plants/hectare,  $n = 5$  line transects) than at north-eastern groups (density =  $2729.05 \pm 4055.004$ ,  $n = 5$  line transects, one way ANOVA,  $F_{1,7} = 10.90$ ,  $P = 0.011$ , homogeneous variances, Levene's test,  $F_{1,8} = 0.0766$ , g.l. = 1,8,  $P = 0.7889$ , Fig. 8). In next analyses we compare fruit availability and dispersal intensity by lizards between those south-eastern and north-eastern areas (Fig. 8). In addition, at the south-eastern area, where the four line transects of 1999 were done, 2003 and 2005 transects showed a constant increase of plant density (Fig. 9).

Regarding lizard density, again we did not find significant differences between south-western and north-western areas (one-way ANOVA of log-transformed da-

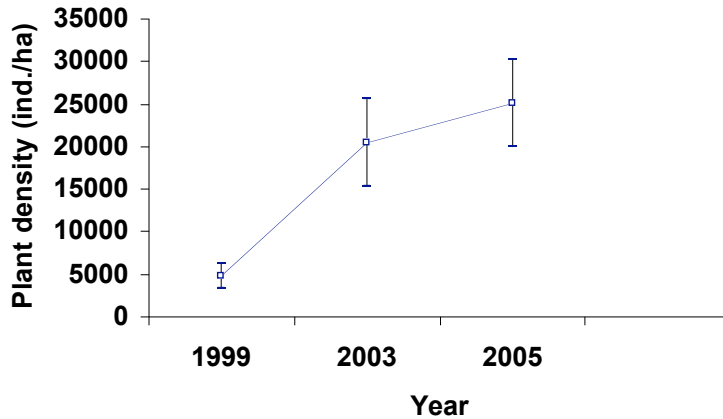


Fig. 9. Increase of plant density over a period of six years at the central area of Aire Island (average number of plant per hectare  $\pm$  SE).

ta,  $F_{1,7} = 0.082$ ,  $P = 0.782$ , homogeneous variances, Levene's test,  $F = 3.4380$ ,  $P = 0.1061$ ). A similar result was obtained in the comparison of south-eastern and north-eastern areas (Mann-Whitney U-test,  $Z = 1.1489$ ,  $P = 0.2506$ ).

At the twenty central sectors of density estimation, rocks and open grounds cover 59.62% of the surface and the remaining 40.38% corresponds to plant cover, with *Suaeda vera* as the main plant species (14.51%). We did not find significant differences between south-western, north-western from one side and north-eastern and south-eastern groups from the other side, excepting for *Suaeda vera* cover, higher at south-western area (Mann-Whitney U-test,  $Z = 2.2045$ ,  $P = 0.0275$ ; south-western =  $33.30 \pm 4.8191\%$ , range: 17.14–42.10%, north-western =  $6.68 \pm 4.2748\%$ , range: 0–19.17%). In fact, taking into account all cover plant species and substrates, we found only a significant correlation between the density of *D. muscivorus* and the percentage of *S. vera* cover (Spearman rank correlation,  $R_s = 0.4851$ ,  $P = 0.04$ ,  $n = 19$ ).

### *Lizard population structure*

During the morning hours of highest lizard activity, we made line censuses over total length of the twenty central areas of maximal densities of *D. muscivorus* counting the number of adult males, adult females and juveniles of *P. lilfordi*. Then, we compared sex and age ratios with dead horse arum and lizard densities. We did not find any significant correlation between lizard densities and lizard sex or age ratios. However, we found a significant correlation between age ratios and plant densities (Spearman rank correlation,  $R_s = 0.6042$ , d.f. = 19,  $P = 0.00614$ ). Hence, areas of maximum plant density had a significantly lower proportion of juvenile lizards.

*Lizard activity during the blooming period*

During the blooming period visits of lizards to open spathes of *D. muscivorus* were regular. In some cases only the outer surface of spathe was explored, basking during a long time there. In other cases, tubules were explored and trapped flies captured (see also Pérez-Mellado *et al.* 2000). We did at least four isolated observations of lizards basking on adjacent rocks, close to open spathes, that rapidly turned their heads after a clear trapped flies' noise inside the tubule. Then, lizards inspected the tubules and captured trapped flies.

Visits to spathes were done mainly by adult individuals. From 24 recorded visits, 22 were from adult lizards and 2 from juveniles. In addition, during censuses of age and sex ratios (see above), the proportion of juveniles basking on spathes (8.33%) was significantly lower than the total proportion of juveniles recorded during censuses (24.39%, G test,  $G = 3.946$ ,  $P = 0.0497$ ).

Moreover, the proportion of adult males on spathes was also significantly higher than observed adult males proportion during censuses (G test,  $G = 5.04$ ,  $P = 0.0276$ ). Adult males visiting spathes had significantly larger body sizes than a random sample of adult males from Aire island (Mann-Whitney U test,  $U = 1230$ ,  $P = 0.0107$ ; SVL for adult males observer on spathes:  $\bar{x} = 71.1471 \pm 0.6165$  mm, range: 68-76 mm,  $n = 17$ ; SVL of adult males of Aire Island,  $\bar{x} = 68.2591 \pm 0.3122$  mm, range: 51.50-80 mm,  $n = 230$ ).

*Potential role of lizards as pollinators*

Two of the three spathes isolated from flying pollinators were totally dry on 18th May 2003. In addition, from the third spathe only one seed of 66 was viable. Thus, the proportion of viable seeds was significantly lower than from control sample ( $G = 57.858$ ,  $P < 0.001$ ). These results confirm the absence of autopollination in *D. muscivorus*.

Regarding pollen transport capacity of *P. lilfordi*, Table 3 summarize the results from 1999 and 2003 samples. For ventral and dorsal regions, pollen load was significantly different on 1999 and 2003 samples (dorsal pollen, U Mann-Whitney test,

Table 3. Pollen load of *Dracunculus muscivorus* at dorsal, ventral y gular regions of *Podarcis lilfordi* individuals during April 1999 and May 2003 (see more details in the text).

	1999			2003		
Dorsal	$\bar{x} = 113 \pm 21.58$	39-234	10	$\bar{x} = 10.04 \pm 2.13$	0-40	24
Ventral	$\bar{x} = 36.20 \pm 9.66$	4-93	10	$\bar{x} = 7.15 \pm 1.72$	0-28	24
Gular	$\bar{x} = 17.00 \pm 5.03$	2-58	10	$\bar{x} = 15.83 \pm 7.10$	0-171	24

U = 1.0, Z = -4.4978,  $P = 0.000007$ , ventral pollen: U = 30.50, Z = -3.3828,  $P = 0.000686$ ). We did not find differences for gular pollen between both samples (one way ANOVA,  $F = 0.0102$ , d.f. = 1, 32,  $P = 0.9201$ , homogeneous variances, Levene test,  $F = 0.5967$ ,  $P = 0.4455$ ).

### *Fruit availability and lizard's access*

Fruits of *D. muscivorus* are approximately pyriform or spheroidal. They are arranged in a helicoidal way around the spadix. According to maturation stage, fruits changed from an initial green colour to whitish or yellowish and, finally, to intense orange or reddish colour in ripening fruits. Maturation is clearly enhanced by sun exposure. Thus, last ripening is reached after the opening of the infructescence and direct solar incidence on fruits. Infructescence opening can be produced by the plant itself, from apical zone of the infructescence or along the spathe' closing line. But in Aire Island, the opening is frequently the result of lizards' activity. Lizards bite the outer surface of apical zone or dig a lateral hole in the infructescence (Pérez-Mellado *et al.* in prep.). In this way, maturation was probably accelerated, allowing an earlier sun exposure. Then, foraging behaviour of lizards is quite similar in most of the observed cases. Lizards approach ripening infructescences, exploring them and eating only fully mature fruits of orange or reddish colour. We never observed the consumption of green or whitish fruits. Additional experiments confirmed these observations (Pérez-Mellado *et al.* in prep.).

From a random sample of 20 infructescences, we found an average of  $\bar{x} = 92.35 \pm 4.15$  fruits per infructescence, varying between 45 and 132 fruits ( $n = 20$ , 95% limits of confidence: 83.65-101.04). Their dimensions were:  $\bar{x} = 9.3608 \pm 0.0824$  mm of maximal width (range between 4.92 and 14.97 mm,  $n = 500$ ) and  $\bar{x} = 14.4224 \pm 0.1122$  mm of maximal length (range between 9.12 and 22.32 mm,  $n = 500$ ). Average fruit weight was estimated for a random subsample of 100 fruits from the area of high plant density ( $\bar{x} = 0.2097 \pm 0.015$  g, range: 0.025-0.7089 g,  $n = 100$ ).

Because the dead horse arum was not uniformly distributed over Aire island (see above), we made a comparative analysis of fruit availability between the areas of high and low plant density at the eastern part of the main track. We did not find significant differences in the average number of fruits per infructescence between these two areas (Mann-Whitney test, U = 48, Z = 0.1511,  $P = 0.8798$ ). Nor in the fruit weight (Mann-Whitney test, U = 29267.50, Z = 1.2272,  $P = 0.2197$ ), fruit length (one way ANOVA,  $F = 0.2780$ , d.f. = 1, 498,  $P = 0.5982$ , homogeneous variances, Levene test,  $F = 1.6898$ ,  $P = 0.1942$ ), or fruit width (one way ANOVA,  $F = 0.5054$ ,  $P = 0.4774$ , homogeneous variances, Levene's test,  $F = 0.5598$ ,  $P = 0.4546$ ).

The average number of seeds per fruit varies between 1 and 8 ( $\bar{x} = 2.4809 \pm$

0.0609,  $n = 499$  fruits). It is significantly larger at the area of high plant density (one way ANOVA,  $F = 16.1812$ ; d.f. = 1, 497;  $P = 0.000067$ ; high plant density area:  $\bar{x} = 2.7229 \pm 0.0926$ , range: 1-8,  $n = 249$ ; low plant density area:  $\bar{x} = 2.24 \pm 0.0764$ , range: 1-6,  $n = 250$ ).

However, even if the number of seeds per fruit is significantly higher at the area of high plant density, the average number of seeds per infructescence is similar in both areas (one way ANOVA,  $F = 0.6724$ , d.f. = 1, 15;  $P = 0.4250$ , homogeneous variances, Levene's test,  $F = 0.0076$ ,  $P = 0.9316$ ). That is, seeds from high plant density area are smaller than in low plant density area (for seed length: one way ANOVA,  $F = 39.1377$ , d.f. = 1, 1236,  $P < 0.00001$ , homogeneous variances, Levene test,  $F = 0.6307$ ,  $P = 0.4272$ ; high plant density area,  $\bar{x} = 4.7459 \pm 0.0235$  mm, range: 2.92-6.34 mm,  $n = 678$ ; low plant density area,  $\bar{x} = 4.9618 \pm 0.0251$  mm, range: 3.18-6.53 mm,  $n = 560$ ; for the whole sample:  $\bar{x} = 4.8436 \pm 0.0174$  mm, range: 2.92-6.53 mm,  $n = 1238$ , and for seed width, Mann-Whitney test,  $U = 104728.5$ ,  $Z = -13.5936$ ,  $P < 0.001$ ; high plant density area:  $\bar{x} = 3.6589 \pm 0.0213$  mm, range: 1.21-5.50 mm,  $n = 678$ ; low plant density area:  $\bar{x} = 4.0275 \pm 0.0162$  mm, range: 2.68-4.88 mm,  $n = 560$ ; for the whole sample:  $\bar{x} = 3.8256 \pm 0.0147$  mm, range: 1.21-5.50 mm,  $n = 1238$ ).

Fruits' water weights were in average:  $\bar{x} = 0.1599 \pm 0.011$  g, range: 0.0231-0.5306,  $n = 100$ ) that corresponds to an average percentage of the total fruit weight of:  $\bar{x} = 77.88 \pm 0.46\%$  (range: 63.76-91.97% of water,  $n = 100$ ). We did not find significant different in water content of fruits of different colour. That is, of fruits with a different maturation state (one way ANOVA,  $F = 1.4302$ , d.f. = 2,96,  $P = 0.2443$ , homogeneous variances, Levene test,  $F = 1.4769$ ,  $P = 0.2335$ ).

## Seed dispersal

### Timing and intensity of seed dispersal

In the three years surveyed (2002, 2003 and 2004), faeces from high plant density zone contained significantly more seeds than faeces from low plant density zone (Table 4). In 2003, we studied seed dispersal intensity throughout eight weekly samples. We

Table 4. Percentage of lizard faeces with seeds of *D. muscivorus* in May 2002 and June 2002 and 2004. Sample size within parentheses.

	May 2002		June 2002	
High density	37.05% (170)	$G = 5.54$	49.79% (492)	$G = 64.04$
Low density	15.78% (57)	$P = 0.018$	11.45% (262)	$P < 0.001$
			June 2004	
High density			92.30% (65)	$G = 29.77$
Low density			50% (62)	$P < 0.001$

examined the frequency of faeces with or without seeds in relation with two factors: the weekly sample and plant density zone; thus, considering the absence or presence of seeds in the faecal sample as the response variable (Quinn & Keough 2002). All tested models included the interaction between the two variables (week of sample and plant density). Sample week was variable 1 and plant density variable 2. The presence or absence of seeds in the faeces (response variable) was variable 3. Model 1 included the two interactions, 12 and 13, so we tested the null hypothesis of the independence of the presence/absence of seeds in relation to variable 2 (plant density zone). For this model we obtained a likelihood  $\chi^2 = 248.30$ , d.f. = 8,  $P < 0.001$ . Thus, we rejected the null hypothesis of an independence of dispersal intensity and plant density zone. With model 2, including the interactions 12 and 23, we explored the null hypothesis of the independence of seed presence/absence and the week of sample. In this case, likelihood  $\chi^2 = 227.62$ , d.f. = 14,  $P < 0.001$ . Thus, also in this case we rejected the null hypothesis of independence. Finally, model 3 of three way interaction: 12, 13 and 23 gave a  $\chi^2 = 15.326$ ,  $P = 0.03204$ , indicating a significant interaction between the three variables.

Figs 10 and 11 summarize the results of seed dispersal intensity during 2003 fruiting period. Seed dispersal started during the first half of May at the area of high plant density and around one or two weeks later at the area of low plant density.

In the area of high plant density dispersal finished almost completely between the second and the third week of July, while at the area of low plant density it was earlier.

Thus, as the results from log-linear analyses indicate, the intensity of seed dispersal was radically different between the areas for high and low plant density. In fact, in the area of high plant density, lizards include fruits in their diet from the beginning of the ripening period. Thus, the proportion of faeces with seeds was always higher than those without seeds. In addition, the analysis of three consecutive years indicates that the importance of *D. muscivorus* fruits in the diet of *P. lilfordi* had a continuous increase for both plant density areas.

#### Seed viability and seed germination

We detected 27 viable seeds from 30 seeds coming from lizard faeces, while from the control sample, coming from wild infructescences, 79 of 91 seeds were viable. Thus, we did not detect significant differences between seed from plants and faeces (G test,  $G = 0.014$ ,  $P = 0.9064$ ).

Regarding germination tests, in 2002 experiment we did not find significant differences between collected in May (33 germinated of 66 seeds) or June (125 germinated of 227 seeds,  $G = 0.162$ ,  $P = 0.687$ ). We found significant differences in the proportion of germinated seeds between those from plants and those from lizard faeces in both experiments of 1999 and 2002 (Table 5).

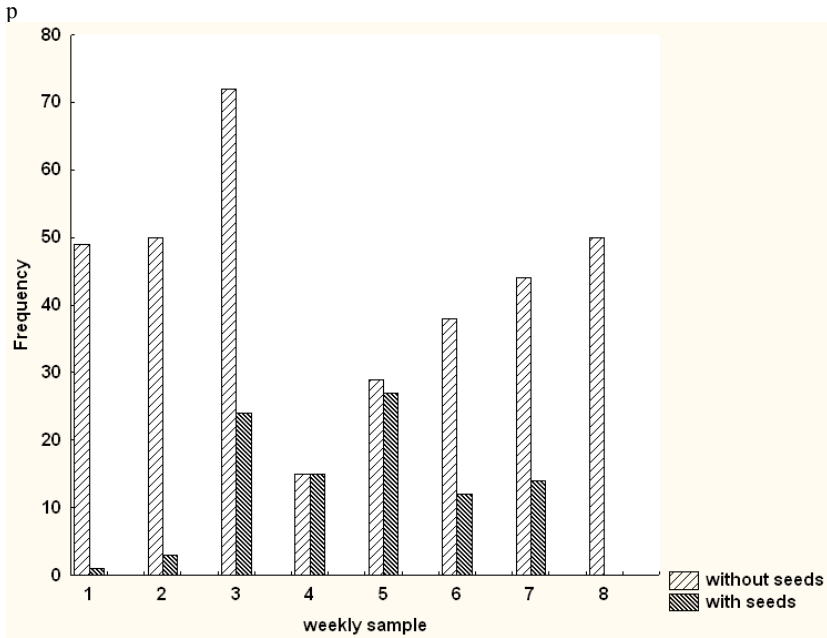


Fig. 10. Proportion of lizard's faeces with and without seeds of *D. muscivorus* at the area of low plant density.

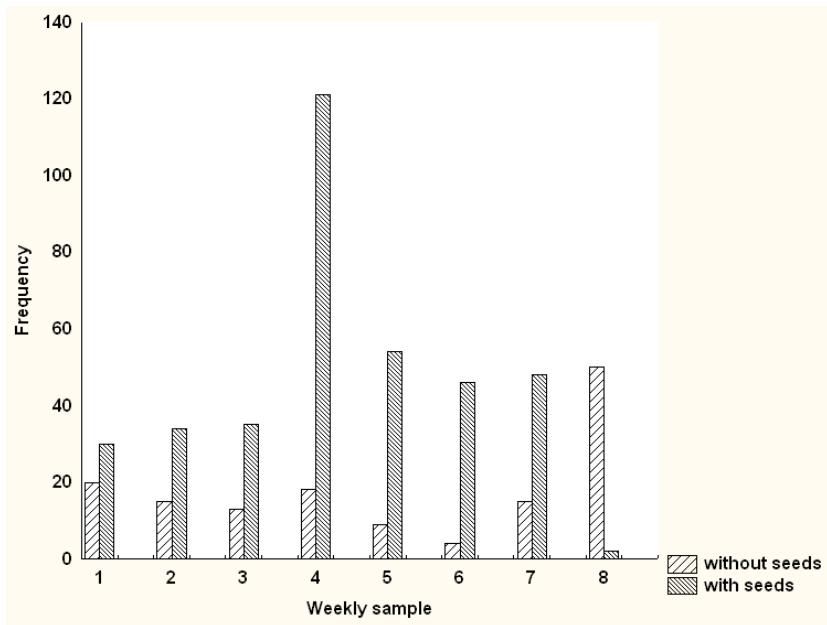


Fig. 11. Proportion of lizard's faeces with and without seeds of *D. muscivorus* at the area of high plant density.

Table 5. Germination experiments carried out in 1999 and 2002. Sample size of each group within parentheses.

Seed origin	1999			2002		
	% germinated	G test	<i>P</i>	% germinated	G test	<i>P</i>
Faeces	63.63 (66)	15,14	< 0.001	55.06 (227)	34,67	< 0.001
Plants	33 (100)			19.38 (294)		

### Seed predation

The results of seed predation experiments were similar at areas of high and low plant density. In both cases we found significant differences in the number of seed predated from covered or uncovered Petri dishes. Seeds from covered dishes were scarcely predated, while those from uncovered dishes were intensively consumed (for high plant density area: McNemar test,  $\chi^2 = 89.48$ ,  $P < 0.001$  and for low plant density area,  $\chi^2 = 95.09$ ,  $P < 0.001$ ).

Seeds with or without elaiosomes disappeared in a similar way from uncovered dishes. However, the rate of disappearance was significantly higher from the high

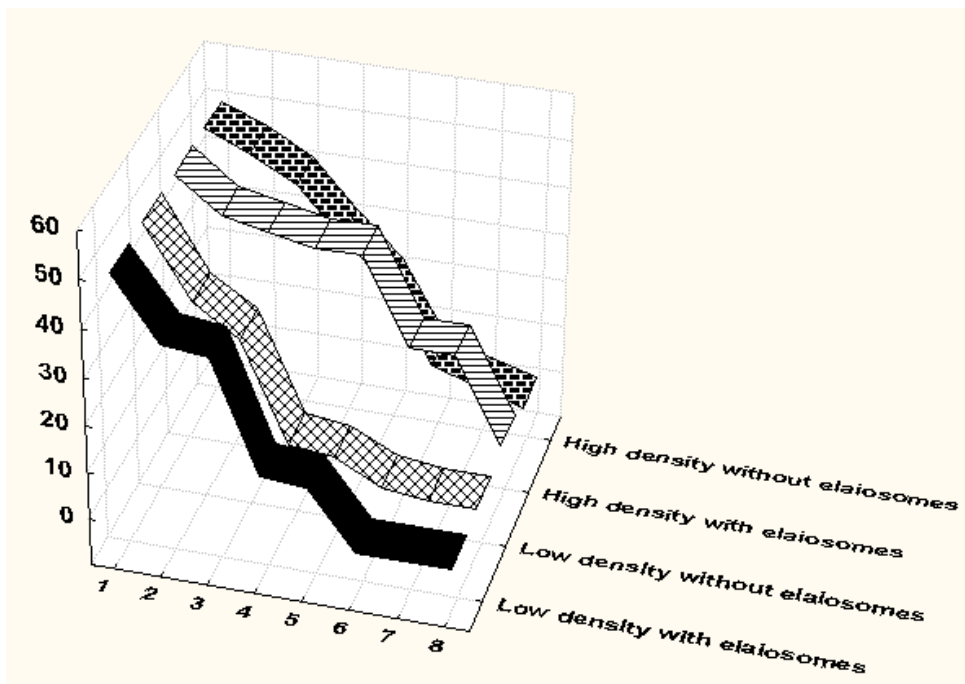


Fig. 12. Number of predated seeds with or without elaiosomes during the experiments performed at areas of low and high plant density (see more details in the text).



plant density area in the case of seeds without elaiosomes ( $\chi^2 = 160.89$ ,  $P < 0.001$ , see Fig. 12). We did not detect such differences at low plant density area ( $\chi^2 = 12.50$ ,  $P = 0.0852$ ).

## DISCUSSION

Lizard densities estimated during this study at Aire Island are similar to those recorded from previous years (Pérez-Mellado 1989, Pérez-Mellado *et al.* 2000 and unpub. data). But it is interesting to observe local variations of these values.

From one side, we detected very high densities at the north-eastern peninsula, with sandy substrates, lacking *Suaeda vera* and with the presence of Liliaceae and sea ferns as dominant plants. It is noteworthy that *D. muscivorus* is very rare at this zone and was not recorded during line transects. In addition, the high lizard density was linked with the presence of a large number of adult females and juveniles, indicating that probably this area is employed as a main laying site (pers. obs.) of Aire Island.

From another side, lizard density is lower at the area of breeding colonies of seagulls (western peninsula, some central areas and the easternmost area), probably as a consequence of seagull aggressiveness during breeding season and the sporadic capture of lizards (pers. obs.).

The existence of island zones with a high lizard density and almost absence of dead-horse arums precludes an overall correlation of lizard and plant density in the whole island. For this reason, only at central area of maximal plant density, east to the main track, we observed a significant correlation, in the part of the island densely covered by *Suaeda vera*, that permits optimal conditions for the development of *D. muscivorus*.

At the western peninsula we observed a much lower plant density than in the central zone, probably due to the rocky nature of the ground and a lower density of *Suaeda vera*. This western peninsula is also the site of the main breeding seagull colony. Thus, within Aire Island, we cannot confirm the supposed correlation of seagull breeding colonies and the presence of *D. muscivorus* (Meeuse & Morris 1984), apparently due to the abundance of calliphorid flies, attracted by organic matter and carcasses.

In a similar way, the analysis of correlation between vegetal cover, rock cover, lizard density and dead-horse density at the 20 central zones of the island (Fig. 8) revealed that only the percentage of *Suaeda vera* cover was significantly correlated with dead-horse arum density. This correlation is particularly important at the area to the west of the main track, where the overall density of *S. vera* is lower and, consequently, its presence is even more important for the development of *D. muscivorus*. Thus, we confirm the important role of *Suaeda vera* at Aire Island, probably as a protective shield for the development of the dead-horse arum.

In summary, lizard density is probably related to multiple factors as suitable laying sites for females, proximity of food resources as human visitors around the northern jetty, and the scarcity or absence of breeding seagulls, as occasional predators. On the other hand, dead-horse arum density depends on factors as the presence of protective plants against wind and sea influence, as *Suaeda vera*, as well as a certain distance from the shore, that mitigates that influence.

However, at better areas for lizards and plants, as the central zone, east from the main track, densities of both species were significantly correlated, indicating an interaction between the lizards and the plants, with a clear north-south gradient. In the case of lizards, such gradient is weaker, without significant differences, while for plants the gradient marks significant differences between higher densities in the south and lower in the north part of the zone.

The density of *Dracunculus muscivorus* at Aire Island can be viewed as extraordinary. In some particular spots it reaches more than 30,000 individuals per hectare! Within its reduced range, the dead-horse arum is considered a rare species (Corrias 1981) and, according to Boyce (2000), *Dracunculus muscivorus* is restricted to a few sites in Balearics, Corsica and Sardinia. Even if we lack estimations from other locations, probably Aire Island exhibits the highest known densities for the entire distribution range of this plant species. Moreover, identical censuses performed in 1999 (Pérez-Mellado *et al.* 2000), 2003 and 2005 indicate an amazing increase of density (Fig. 9), obviously as a result of the strong interaction with the Balearic lizard as its main seed disperser.

The activity of lizard on open spathes was intense during the whole blooming period. The peak of highest availability of open spathes took place during the fourth week of March and the two first weeks of April. However, open spathes were staggered during more than one month (Fig. 7). One of the most interesting results is the negative correlation between juvenile abundance and plant density. That is, the zones of maximal plant density have a higher proportion of juvenile lizards, probably indicating the active exclusion of juveniles from blooming spathes by adult lizards. Moreover, it was a higher proportion of adult males observed on spathes. In fact, the proportions of juveniles and adult males recorded on spathes were significantly lower and higher, respectively, to proportions recorded during ground visual censuses at the same areas. This result could indicate a displacement from this optimal zone of dead horse arums of adult females and juveniles by adult males. Thus, the access to open spathes, as a site for food (arrival of potential pollinators) and, probably, as an optimal thermoregulation site (Pérez-Mellado *et al.* 2000) is different for each sex and age class. Age and sex classes distribution at the zones of maximal plant density indicate that adult males actively preclude the access of juveniles to open spathes. Moreover, those adult males have significantly larger body sizes than the average of adult males from Aire Island. These results confirm the impor-

tance of open spathes as optimal sites from which big adult males exclude the access to smaller males, juveniles and most of the adult females.

Regarding pollination, our results demonstrate a negligible role of lizards as pollinators of the dead horse arum. In fact, the number of pollen grains loaded by lizards is clearly lower than in the cases of a proved role in pollination (Pérez-Mellado & Casas 1997, Pérez-Mellado *et al.* 2000). Also we can confirm the apparent absence of autopollination in this plant species (Collu & Dardani 1999).

The size of available fruits is similar at zones of high and low plant density. In addition, at the area of high plant density we detected fruits with more and smaller seeds. It is extremely difficult to construct a reliable hypothesis to explain such differences, also present on dispersed seeds. Apparently, plant density is the most relevant factor to explain dispersal intensity. Thus, dispersal is significantly higher at the area of high plant density, where ripening fruits of *D. muscivorus* is the major food resource of many lizards during fruiting period of the dead horse arum. In addition, studying a larger sample size, we were unable to confirm the selection of fruits with larger seeds by the Balearic lizard (Pérez-Mellado *et al.* 2000). There is a continuous increase of seed dispersal intensity during the three consecutive years under study, indicating a rapid spread of foraging behaviour on *D. muscivorus* fruits within lizard population.

Germination of fruits was significantly higher from those coming from lizard faeces indicating that the Balearic lizard is, not only a legitimate seed disperser of *D. muscivorus*, but also a disperser that increase germination probabilities of the seeds passing throughout its digestive tract.

Regarding seed predation, our results apparently indicate a very low predation pressure from arthropods, as well as a poor dispersal by members of this group as ants, that normally represent a coevolutionary unit with plants bearing elaiosomes on seeds, as it is the case for *D. muscivorus*. In addition, the high proportion of disappearance of seeds from uncovered Petri dishes probably indicates a strong predation pressure from granivorous or omnivorous vertebrates. At Aire Island, we can mention, among birds, the linnet, *Acanthis cannabina* and the rock dove, *Columba livia* or the rabbit, *Oryctolagus cuniculus* as the only introduced mammal present in the islet. We cannot discard also the consumption of seeds by non breeding granivorous birds that land time to time on the island. The practical absence of seed dispersal by ants increases the significance of lizards as seed dispersers. Moreover, the strong seed predation by other vertebrates was counterbalanced by seed dispersal within lizards' faeces, where seeds probably have a higher survival probability, remaining undetected by seed predators during some time.

Mutualism exists when two or more species enhance each other's fitnesses (Howe & Westley 1988). But, in the case of the Balearic lizard and the dead horse arum, we are in front of a more complex interaction where mutualistic relationship is mixed with an asymmetrical interaction during the blooming period, with the sole benefit

of lizards, consuming potential pollinators. Anyhow, benefit for plants seems to be higher than detrimental effects, according to the spectacular increase of plant density during a period of six years. As in other mutualistic relations (Thompson 1982), the interaction of these two species probably began as an antagonistic relationship where lizards acted only as pollinator's predators. Later, the use of blooming plants and optimal sites for thermoregulation (Pérez-Mellado *et al.* 2000) and/or foraging sites became general very rapidly among lizard population allowing the next step, the discovery of ripening fruits as a suitable food.

Even if plant-animal seed dispersal systems are characterized by the absence of obligate partnership and a weak mutual dependence between animals and plants (Herrera 2002), the case of the dead horse arum and the Balearic lizard is surprising in the sense of a strong effect of lizard' foraging behaviour on plant density and its extremely rapid spread over the island. Even if it is clear that a reduced population of the dead horse arum can be maintained without any interaction with lizards, the emergence of the strong interaction observed at Aire Island could be the only explanation for the extraordinary density reached by plants during a short period of six years.

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# Aspects of the evolutionary history of *Podarcis taurica* (Pallas 1814), *P. gaigeae* (Werner 1930) and *P. milensis* (Bedriaga 1882) in Greece

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*Wall lizards of the genus Podarcis (Sauria, Lacertidae) comprise 17 currently recognized species in southern Europe, where they are the predominant reptile group. The taxonomy of Podarcis is complex and unstable. Based on DNA sequence data, the species of Podarcis fall into four main groups that have substantial geographic coherence (Western Island group, Southwestern group, Italian group and Balkan group). The Balkan species, are divided in two subgroups, the subgroup of P. taurica, P. milensis, P. gaigeae, P. melisellensis and the subgroup of P. erhardii and P. peloponnesiaca, which are highly diversified and present great morphological and ecological plasticity, inhabiting many different ecotypes. We address the question of phylogenetic relations among the species of the P. taurica subgroup encountered in Greece, as they can be inferred from partial mtDNA (cyt b and 16S) and nuclear (c-mos) sequences. Our data suggest that P. gaigeae is closely related to P. milensis and both P. gaigeae and P. milensis to P. taurica. However the specimens of P. taurica are subdivided in two different groups. The first includes the specimens from Crimea (Ukraine) and Northeastern Greece and the other the specimens from the rest of continental Greece and Ionian Islands. This result suggests that the evolutionary history of P. taurica in Greece is more complex than a single evolutionary invasion. The data analyzed stress the need for a reconsideration of the evolutionary history of Greek Podarcis species and help to overcome difficulties that classical taxonomy has encountered at both the specific but mostly the subspecific level of this genus.*

Keywords: *Podarcis taurica*, *Podarcis gaigeae*, *Podarcis milensis*, molecular phylogeny, mtDNA and nuclear markers, evolution, Greece.

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## INTRODUCTION

The reconstruction of phylogenies is of primary importance in the understating of the dynamic patterns of evolution (the biogeography of a group, the bases of its biological diversity at any level). Although the phylogeny of the genus *Podarcis* has been the subject of much discussion, the relationship among the species are still unclear.

Wall lizards of the genus *Podarcis* (Sauria, Lacertidae) comprises 17 [or 18 if we consider that *P. carbonelli* is a distinct species (Sá-Souza & Harris 2002)] currently recognized species in southern Europe, where they are the predominant reptile group, but their taxonomy is complex and continuously revised, largely because the species are morphologically very similar but exhibit substantial levels of intraspecific variation (Arnold 2002). *Podarcis* is a complex of species, diffused in central Europe and in the circum-Mediterranean regions, with the exception of the xeric south-eastern areas and of Anatolia, and includes several endemic insular species (Oliverio *et al.* 1998, 2000).

There is substantial morphological evidence that *Podarcis* is a clade (Arnold 1973, 1989). Morphology also suggests that the closest relatives are the Moroccan *Lacerta andreanskyi* which in turn are sister to *L. persicillata* and *L. dugesii* (Arnold 1973). The same (the monophyly of *Podarcis*) is extrapolated by several molecular studies that have been done in the last 5-6 years (Oliverio *et al.* 1998, 2000; Harris & Arnold 1999; Fu 2000; Poulakakis *et al.* 2003).

However, within *Podarcis* relationships are poorly understood. Because morphology is so uniform, it provides few characters for phylogenetic analysis, and these tend to conflict (Arnold 1973, 1989). Various karyological, immunological and protein electrophoretic studies have been made, but these usually involve only a minority of species, and results from different species combinations often conflict (Lanza & Cei 1977; Tiedemann & Mayer 1980; Mayer & Tiedemann 1980, 1981; Olmo *et al.* 1986, 1987; Capula 1994, 1996, 1997, Chondropoulos *et al.* 2000).

Recent studies on the lacertid genus *Gallotia* (Thorpe *et al.* 1993, 1994) and on the iguanid genus *Anolis* (Losos *et al.* 1997, Jackmann *et al.* 1999), among others, confirmed the general power of DNA sequencing as a means to reconstruct phylogenies and zoogeography. Based on DNA sequence data (cytochrome b, *cyt-b* and 12S rRNA genes) the species of *Podarcis* fall into four main groups with substantial geographic coherence: (1) Western Island group (*P. filfolensis*, *P. pityusensis*, *P. tiliguerta*, and *P. lilfordi*), (2) Southwestern group (*P. atrata*, *P. bocagei*, *P. hispanica*, and perhaps *P. carbonelli*), (3) Italian group (*P. muralis*, *P. raffonei*, and *P. sicula*) and (4) Balkan group (*P. taurica*, *P. gageae*, *P. milensis*, *P. melisellensis* and perhaps *P. peloponnesiaca*, *P. erhardii*, and *P. wagleriana* (Harris & Arnold 1999). Using partial mitochondrial DNA (mtDNA) sequences, Harris (1999), Harris & Arnold (1999) and Oliverio *et*



*al.* (2000) concluded that the relationships among *Podarcis* species cannot be definitively resolved with the data sets they used. Nevertheless both these studies support the monophyly of a Balkan group of *Podarcis*, which includes *P. gaigeae*, *P. milensis*, *P. melisellensis*, *P. taurica*, and perhaps *P. wagleriana*, *P. erhardii*, and *P. peloponnesiaca*. However, Oliverio *et al.* (2000) do not agree with Harris & Arnold (1999) that *P. wagleriana* is part of the “Balkan” clade. The *Podarcis* species of Iberian Peninsula have been studied by Harris & Sá-Souza (2001, 2002), using partial 12S rRNA and *cyt-b* mitochondrial DNA sequences. Oliverio *et al.* (1998, 2000), Pinho *et al.* (2004), and Pretus *et al.* (2004) used partial mtDNA sequences (*cyt-b*, 12S rRNA) in order to explore the phylogenetic relationships of Italian and western islands species. So, the least studied group is the last one, the Balkan group, for which the only thing we know is that *P. erhardii*, which is closely related to *P. peloponnesiaca*, is probably a species complex (Poulakakis *et al.* 2003).

The Balkan group of species is probably divided, on the basis of the preliminary results of the molecular study of Poulakakis *et al.* (2003), in two subgroups, the subgroup of *P. taurica*, *P. milensis*, and *P. gaigeae* and the subgroup of *P. erhardii* and *P. peloponnesiaca*, which are highly diversified and present great morphological and ecological plasticity, inhabiting many different ecotypes. Within the former subgroup, protein electrophoresis indicates that *P. gaigeae* is closely related to *P. milensis* and both *P. gaigeae* and *P. milensis* to *P. taurica* (Tiedemann & Mayer 1980; Mayer & Tiedemann 1980, 1981), and that *P. taurica* has closer relation to *P. milensis* than to *P. peloponnesiaca* (Chondropoulos *et al.* 2000).

The Balkan wall lizard, *P. taurica*, is distributed in a large area of the Balkans, as well as in Hungary, Crimean Peninsula and NW Anatolia (Gasc *et al.* 1997). It exhibits a notable geographic variation in colour, pattern size. So far three subspecies have been described on the basis of coloration, patterning, and relative leg length: *P. t. taurica* (the largest part of species’ range), *P. t. thasopoulae* (Thasopoula isl.), *P. t. ionica* (west part of the Greek mainland and Ionian islands) (Chondropoulos *et al.* 1993).

The complex geological history of Hellenic area during the late Tertiary has influenced the distribution of all *Podarcis* species in the region (i.e., *P. erhardii*, *P. peloponnesiaca*, *P. muralis*, *P. taurica*, *P. milensis*, and *P. gaigeae*) and contributed to the diversification of each species. This diversity is thought to reflect the submergence and re-emergence of landmasses, due to tectonic, volcanic and eustatic events.

In the present study we examine the phylogenetic relationships of several populations of *P. taurica*, *P. gaigeae*, and *P. milensis* across Greece, using partial mtDNA (*cyt b* and 16S rRNA) and nuclear (*c-mos*) sequences. We combine this information with previously published sequences and use the results to produce a historical interpretation of the species’ distribution and morphological diversification.

## MATERIALS AND METHODS

Total genomic DNA was extracted from 40 specimens of *Podarcis* (Table 1, Fig. 1). Three target genes were selected for molecular phylogenetic analysis: (1) a partial sequence (425 bp) of the mitochondrial protein encoding cytochrome b gene (*cyt b*), (2) a partial sequence of the non-protein coding mitochondrial 16S rRNA (16S), and (3) a partial sequence (397bp) of the nuclear proto-oncogene that encodes a kinase expressed in germ cells. Sequencing was done on a PE-ABI377 automated sequencer.

Individuals from two closely related species of the same family (Lacertidae) were used as outgroup *taxa*: *L. andreaskyi* (*cyt b* – AF206537: Fu 2000, 16S rRNA – AF206603: Fu 2000, *c-mos* – AF211203: Brehm *et al.* unpub. data) and *Gallotia stehlini* (*cyt b* – AF439949: Rando *et al.* unpub. data, 16S rRNA – AF149936: Beyerlein & Mayer 1999, *c-mos* – AF435108: Maca-Mayer *et al.* unpub. data).

The alignment of the concatenated *cyt b* and 16S rRNA sequences was performed with Clustal X (Thompson *et al.* 1997) and corrected by eye. Sequence divergences were estimated in MEGA computer package (v.2, Kumar *et al.* 2001) using the Tamura – Nei model of evolution (Tamura & Nei 1993) to adjust for differences in nucleotide frequencies and substitution-rate heterogeneity.

Table 1.

List of the examined specimens of *Podarcis*, with *taxon* name, geographic origins, population map codes (see Fig. 1), and number of samples. Individuals from two closely related species were used as outgroup *taxa*: *Lacerta andreaskyi* and *Gallotia stehlini*. Note: Asterisk (\*) indicates gene regions previously published. For the specimen 37 there is not information about the *c-mos* gene.

Map Code	Species	Samples	Locality
1-9	<i>P. gaigeae gaigeae</i>	9	Skyros island and islets around it
10-11	<i>P. gaigeae weigandi</i>	2	Piperi island
12-16	<i>P. milensis milensis</i>	5	Milos island
17-20	<i>P. taurica ionica</i>	4	Ionian islands
21-27	<i>P. taurica taurica</i>	7	Peloponnesus
28-30	<i>P. taurica taurica</i>	3	Central Greece
31-34	<i>P. taurica taurica</i>	4	North Greece
35-36	<i>P. taurica thasopoulae</i>	2	Thasopoula island
37	<i>P. taurica taurica</i>	1	Out of Greece*
38-40	<i>P. muralis</i>	3	Continental Greece
41-42	<i>P. erhardii</i>	2	Crete and Cyclades
43	<i>P. peloponnesiaca</i>	1	Peloponnesus
44-45	Outgroup	2	Out of Greece*



Fig. 1. Map showing the sampling localities of the 42 specimens used for the DNA analysis. 21 specimens of *P. taurica*, 11 specimens of *P. gaigeae*, 5 specimens of *P. milensis*, 3 specimens of *P. muralis*, 2 specimens of *P. erhardii*, and 1 specimen of *P. peloponnesiaca*. Individuals from two closely related species were used as outgroup *taxa*: *Lacerta andreanskyi* and *Gallotia stehlini*.

Analyses for phylogenetic inference were conducted using three methods: maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Nucleotides were used as discrete, unordered characters. To examine whether the sequences from the three genes should be combined in a single analysis, a partition homogeneity test which was described as the incongruence-length difference test by Farris *et al.* (1995), was run in PAUP (v.4.0b10, Swofford 2002), and significance was estimated by 1000 repartitions. Maximum parsimony analysis was performed with PAUP 4.0b10, with heuristic searches using stepwise addition and performing tree-bisection reconnection (TBR) branch swapping (Swofford *et al.* 1996). Confidence in the nodes was assessed by 1000 bootstrap replicates (Felsenstein 1985) with random addition of *taxa*.

For maximum likelihood (ML) analysis (Felsenstein 1981), the best-fit model of DNA substitution and the parameter estimates used for tree construction were chosen by performing hierarchical likelihood-ratio tests (Huelsenbeck & Crandall 1997) in Modeltest (v.3.06, Posada & Crandall 1998). Likelihood-ratio tests and Akaike Information Criterion (AIC; Akaike 1973) indicated that the Tamura-Nei (TrN) model+I+G had the lowest likelihood score and showed a significantly better fit than

the other less complicated models. Heuristic ML searches were performed with 10 replicates of random sequence addition and TBR branch swapping. ML bootstraps employed only 100 iterations.

We performed Bayesian analysis with the program MrBayes (v3.0B, Huelsenbeck & Ronquist 2001) using the TrN model of substitution with rate heterogeneity set to a gamma distribution, hence applying the fewest possible number of constraints to the dataset. The analysis was run with four chains for  $10^7$  generations and the current tree was saved to file every 100 generations. This generated an output of  $10^5$  trees. The  $-\ln L$  stabilized after approximately  $10^5$  generations and the first  $10^4$  trees (10% “burn-in” in Bayesian terms, chain had not become stationary) were discarded as a conservative measure to avoid the possibility of including random, sub-optimal trees. The percentage of samples recovering any particular clade in a Bayesian analysis represents that clade’s posterior probability (Huelsenbeck & Ronquist 2001). We used one of the methods of Leaché & Reeder (2002) to assure that our analyses were not trapped on local optima. In particular, the posterior probabilities for individual clades obtained from separate analyses (4 runs) were compared for congruence (Huelsenbeck & Imennov 2002), given the possibility that two analyses could appear to converge on the same  $\ln$ -likelihood value while actually supporting incongruent phylogenetic trees.

## RESULTS

Of the 1330 sites examined, there were 295 variable sites, 221 of which were parsimony informative (403 and 251 respectively when the outgroups were included in the analysis). Pairwise sequence divergence in *Podarcis* species (Tamura & Nei 1993) ranged from 0 to 13.2% (Table 2).

A partition homogeneity test indicated no conflicting phylogenetic signals between the datasets ( $P = 0.971$ ) and the mtDNA and nuclear genes were analyzed together.

Tree length distribution, determined from random sampling of 106 unweighted trees, was significantly skewed to the left ( $g_1 = -0.49$ ), suggesting a strong phylogenetic signal in the data ( $P < 0.01$ ; Hillis & Huelsenbeck 1992).

The heuristic parsimony analysis produced 3 equally parsimonious trees of 705 steps (CI = 0.583, RI = 0.809). Maximum likelihood analysis under the TrN+I+G model resulted in a topology with  $\ln L = -5046.9971$ . For the Bayesian inference method, identical topologies were recovered for each of the 4 runs with the full dataset (Fig. 2).

Table 2.

(A) Sequence divergences (%) among the main clades/lineages of *Podarcis*. Values in diagonal are within clade sequence divergences. (B) Sequence divergences (%) among the major clades of *P. taurica* subgroup (3 clades of *P. taurica*, 1 clade of *P. gaigeae* and 1 clade of *P. milensis*).

A. Species	1	2	3	4	5	6
1. <i>P. taurica</i> (A)	(3.6)					
2. <i>P. milensis</i> (B)	6.4	(0.5)				
3. <i>P. gaigeae</i> (C)	6.5	6.2	(0.6)			
4. <i>P. erhardii</i> (D)	8.6	8.8	8.9	(3.7)		
5. <i>P. peloponnesiaca</i> (D)	8.9	9.1	9.2	3.4	(1.1)	
6. <i>P. muralis</i> (E)	8.3	8.6	8.9	9.7	10.3	(6.2)

B. Species	1	2	3	4
1. <i>P. taurica</i> (A1)				
2. <i>P. taurica</i> (A2)	2.8			
3. <i>P. taurica</i> (A3)	4.8	5.7		
4. <i>P. milensis</i> (B)	6.4	6.4	6.6	
5. <i>P. gaigeae</i> (C)	6.4	6.3	6.9	6.2

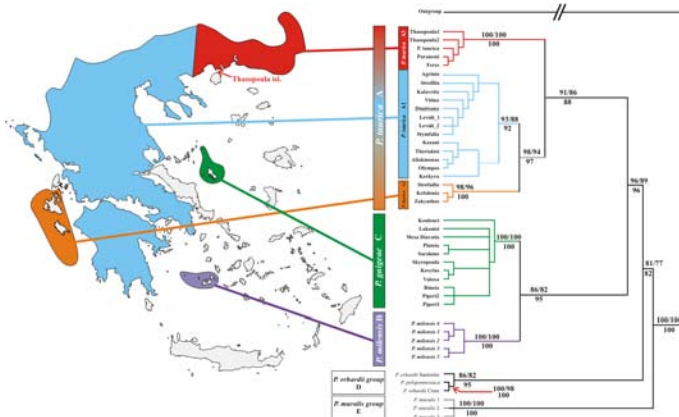


Fig. 2. Phylogenetic relationships among the 45 specimens of the *Podarcis* spp. and outgroups. Phylogenetic analyses (all the methods used: MP, ML, and BI) produced trees with the same topology. Only the Maximum Parsimony tree is presented. Numbers above branches indicate nonparametric bootstrap values (1000 pseudoreplicates) on MP and ML analysis respectively (MP/ML). Numbers below branches indicate posterior probabilities of Bayesian analysis (BI).

## DISCUSSION

The results of this study reveal a well-resolved phylogeny and identify a number of haplotype clades which, on the basis of the observed levels of sequence divergence (Table 2), represent long-separated lineages and diverse evolutionary histories within

*Podarcis*. All outgroups used in this analysis indicate that the genus is a monophyletic group (bootstrap value 100%), fact that comes in agreement with previous mtDNA studies (Harris & Arnold 1999, Oliverio *et al.* 2000). Concerning the *taxa* of the area of Greece (6 species in total), three phylogenetic lineages can be distinguished. The first includes *P. taurica*, *P. gaigeae*, and *P. milensis* (and perhaps *P. melisellensis*, but this species is not included in this study). The second includes *P. erhardii*, *P. peloponnesiaca* and the third includes *P. muralis*.

Within the first clade (the subgroup of *P. taurica*), our results recognize the three species (*P. taurica*, *P. gaigeae*, and *P. milensis*) as separate phylogenetic clades. In addition, *P. taurica* is the first species that diverges from the other two species, while *P. gaigeae* and *P. milensis* form a closely related pair, an observation that agrees with previously published results (Mayer & Tiedemann 1980, 1981; Tiedemann & Mayer 1980; Harris & Arnold 1999; Oliverio *et al.* 2000).

Within the clade of *P. taurica* (A), there are three groups of haplotypes. A1 includes populations from continental Greece and Peloponnisos. A2 corresponds to populations confined in the Ionian Islands, and A3 represents the haplotypes from Thasopoula Isl. and NE Greece. Harris & Sá-Souza (2002) and Poulakakis *et al.* (2003) have already referred to the inconsistencies between the molecular and morphological groupings of the various species within the genus *Podarcis*. With regard to morphological subspecies of *P. taurica*, it is clear that they do not represent monophyletic units and should be reevaluated in the light of new evidences. An interesting example supporting this claim is the assignment of the population from northern Greece (Paranesti, Feres) to *P. t. taurica*. In our analysis these individuals were assigned to the subclade A3 (Fig. 2), together with specimens from Thasopoula Island, which belong to a different subspecies (*P. t. thassopoulae*), whereas the other *P. t. taurica* individuals (from the rest continental Greece) were assigned to subclades A1. Constraining *P. taurica* subspecies to be monophyletic, performing a heuristic search to find the shortest tree with this constraint, and comparing this to the optimal tree using the Shimodaira & Hasegawa (SH) (1999) test shows that the difference is significant (SH test using 1000 RELL bootstraps,  $P > 0.001$ ). This brings into question the practice of subspecies recognition and subsequent assignment of local populations into these subspecies on the basis of an exclusive or limited collection of characters, be that morphological, behavioural or molecular. It is already known that the current taxonomy of *Podarcis* species in the Balkan Peninsula needs revision (Poulakakis *et al.* 2003). Given that the borders between the ranges of the currently recognized subspecies of *P. taurica* are not evident, the above subclades could correspond to the described subspecies of *P. taurica*.

In addition to providing a means for evaluating the validity of morphological taxonomy, the molecular data may provide insights concerning the biogeography of the

genus. The topology of the producing phylogenetic trees and the genetic distances among the clades of *P. taurica* subgroup may discriminate the biogeographic hypothesis of these species in the area of Greece. Given that the molecular clock hypothesis cannot be rejected (the likelihood-ratio test did not reject the null hypothesis of a homogeneous clocklike rate,  $LRT = 33.34$ ,  $d.f. = 34$ ,  $P\text{-value} = 0.49$ ), we consider that there is a homogeneous clocklike rate for the tree produced by the *Podarcis* sequences from Greece. Based on the Table 2A, the genetic distances among the species of the subgroup of *P. taurica* are similar. In particular, the genetic distance between *P. taurica* and *P. gaigeae* is 6.5, the distance between *P. taurica* and *P. milensis* is 6.4 and between *P. gaigeae* and *P. milensis* is 6.2. However, the intraspecific variation differs from species to species. For example in *P. gaigeae* and *P. milensis* is 0.6% and 0.5% respectively, whereas in *P. taurica* is 3.6%. This intriguing result led us to seek the genetic distances among the major clades of *P. taurica* species (namely A1, A2, and A3). As shown in Table 2B, the genetic distances between clade A3, which includes specimens from north-eastern Greece and Thassopoula Island, and the other clades (A1 and A2) of the same species (*P. taurica*), are 4.8 and 5.7 respectively. They are close to the distances between clade A3 and the species *P. gaigeae* and *P. milensis* (6.6 and 6.9 respectively), whereas the corresponding distance between clade A1 (specimens from continental Greece) and A2 (specimens from Ionian islands, except Kerkyra) is only 2.8%. Furthermore, the sequence divergence between *P. gaigeae* and *P. milensis* is 6%, which is almost equal to the intraspecific variation of *P. taurica* that we mentioned before (A3 vs A2 is 5.7%).

If we consider that *P. taurica* is a single species then the above genetic distances suggest that the evolutionary history of *P. taurica* in the area of Greece is not a simple case of one invasion during the past. The distribution of *P. taurica* subgroup (*P. taurica*, *P. gaigeae*, *P. milensis*, and perhaps *P. melisellensis*) mainly in Balkan Peninsula and its absence in the rest of Europe, suggests that the ancestral species of this group originated somewhere in the Balkan peninsula and expanded to this area. A historical fact, probably the arrival of the ancestral form of *P. erhardii* (Poulakakis *et al.* 2003) from northwest, following the eastward path of Dinaric Alps and Hellenides, led to the restriction of the distribution of the ancestral form of *P. taurica* subgroup in few small populations. Two of them (one south-east and the other central-east of Greece) produced the *taxa* we recognize today as *P. milensis* and *P. gaigeae*, which are distributed on the corresponding archipelagos of Milos and Skyros respectively.

Concerning the third species of this subgroup (*P. taurica*), there are two possible scenarios to explain its distribution. The first, the most parsimonious one, suggests the remaining of one population in northeast Greece or Balkan peninsula (clade A3), and, when the situation calmed down after the arrival of the ancestral form of *P. erhardii*, its recolonization of the area of Greece, producing clade A1 (continental Greece)

and A2 [Ionian islands – except Kerkyra – that according to the paleogeographic maps was a paleogeographic unit Dermitzakis (1990)]. The second scenario suggests the remaining of two populations, one in the northeast which produced clade A3 and the other in southwest Greece (clade A1), which produced by colonization clade A2 in the Ionian Islands. Considering the observed genetic distances mentioned above, if we accept the second scenario as the true one, since there is a homogeneous clock-like rate in the producing tree, we would expect the genetic distance between clade A1 (continental Greece) and clade A3 to be equal to the genetic distances of clade A1 and that of the species *P. gaigeae* and *P. milensis*. However, this distance (A1 vs. A3) is much smaller (4.8%) than the distances between the clade A1 and the species *P. gaigeae* and *P. milensis* (6.4% and 6.5% respectively).

From this evidence, we favor the first scenario, by which the colonization of Greece derived from the ancestral population of *P. taurica* of northeast area of Greece, following the dispersal route we mentioned above.

The results presented in this study stress the need for a reconsideration of the evolutionary history of Greek *Podarcis* species and help overcome difficulties that classical taxonomy has encountered at both the specific and mostly at the subspecific level of this genus.

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# Adult body length and sexual size dimorphism in *Lacerta agilis boemica* (Reptilia, Lacertidae): between-year and interlocality variation

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*Lacerta agilis boemica* occupies a wide range of altitudes in the eastern North Caucasus (Russia). Snout-vent length (SVL) of 713 individuals of *L. a. boemica* from 9 localities was studied for inter-annual and microgeographic variation in adult body size and sexual size dimorphism (SSD). Means and 80th percentiles of SVL distributions were used in our comparisons and showed strongly correlated patterns of interlocality variation. The largest differences between samples from different years within populations were comparable in magnitude with those among localities. Among localities, adult SVL of males and females exhibited no increase with altitude. Moreover, males from the lowland localities reach a larger body length than those from the other sites. SSD was strongly associated with altitude, with the SSD index of Lovich & Gibbons (1992) varying from  $-0.07$ – $-0.09$  in the lowland sites to  $-0.03$ / $0.02$  in the mountains. Higher growth costs of reproduction in females and a stronger intrasexual selection in males in the lowland sites relative to their counterparts from higher elevations were proposed to explain the altitudinal variation in SSD in *L. a. boemica*.

Keywords: *Lacerta agilis boemica*, lizards, sexual size dimorphism, altitudinal variation.

## INTRODUCTION

Differences between males and females in characteristic body size, so-called Sexual Size Dimorphism (SSD), are widespread among reptiles. During last decades, SSD has been a target of many herpetological studies dealing with ecological morphol-

ogy, life-history, and behaviour (*e.g.*, Braña 1996, Stamps *et al.* 1997, Wikelski & Trillmich 1997, Butler *et al.* 2000, Rutherford 2004). The primary adaptive hypotheses for the ultimate causes of SSD in lizards are (1) sexual selection for large male size (success in male combat) and (2) natural selection for large female size (advantage in fecundity) (see Cox *et al.* 2003 for a review). Both hypotheses were supported in quite many studies involving different lizard *taxa* (*e.g.*, Anderson & Vitt 1990, Olson 1992, Wikelski & Trillmich 1997). However, recent comparative phylogenetic analysis by Cox *et al.* (2003) found only a weak support for these two hypotheses as general explanations of SSD in lizards. The authors noted the importance of investigating proximate causation as had been previously stressed by other SSD students (*e.g.*, Shine 1990, Stamps 1993, Watkins 1996).

Lacertid lizards exhibit noticeable variation in the extent and direction of SSD (Fitch 1981). However, only few papers addressed this variation, mostly at the level of inter-specific differences (Braña 1996, Gvozdik & Boukal 1998, Molina-Borja 2003, Molina-Borja & Rodríguez-Domínguez 2004).

Recently we have initiated a study of intraspecific variation in SSD in the sand lizard, *Lacerta agilis* which occupies a larger part of the temperate Palearctic from southern England and the Pyrenees in the west to the Baikal Lake in the east (Bischoff 1988).

Centre of the origin and diversification of *L. agilis* is the Caucasus region where five of the nine broadly recognized subspecies occur (Darevsky *et al.* 1976, Kalyabina-Hauf 2003). The south-eastern North Caucasus (Russia) is occupied by *L. a. boemica* (Suchow 1929). This subspecies occurs in a wide range of altitudes from 0-2000 m above sea level, often in high population densities (Roitberg *et al.* 2000). Such a distribution provides an opportunity to comparatively study populations from geographically proximate but climatically contrasting localities. The present paper addresses the variation of adult body size and SSD in *L. a. boemica* on the temporal (over years within populations) and microgeographic scale. This report supplements our previous paper (Roitberg & Smirina 2006) in that it includes much larger study material and operates with traditional (rather than growth-based) estimates of body size. Our aims were (1) to present extensive body size data for this particular subspecies; (2) to estimate the extent of variation of adult body length and SSD in a large data set in which the effect of phylogeny is obviously negligible; (3) to test concordance of different statistics for adult body size and SSD. The latter two points were particularly important for future analysis of the macrogeographic variation in adult body length and SSD in *L. agilis*.

## MATERIALS AND METHODS

### *Study material*

The study material – total of 713 presumably adult individuals – originates from nine localities (sites) which are mostly concentrated in the south-eastern half of the *L. a. boemica* range (Table 1). These include four lowland sites (up to 250 m above sea level), three submontane sites (550-600 m a.s.l.), and two mountain sites (960, 1900 m a.s.l.). All but one site were repeatedly sampled in different years. In Site 4, which was sampled most intensively, the lizards captured in 1982-1984 were examined alive and then released in the capture site. In the other study sites the collected lizards were preserved and subsequently examined for many different aspects of morphological variation (Roitberg 1982, 1991, 1994; Roitberg & Rostova 1999) and skeletochronology (Roitberg & Smirina 1995, 2005). No animals were sacrificed for the present study.

In each study site, all specimens were caught from an area of a few dozen hectares. The only exception was Site 2 where we pooled small samples collected in several sites in and around Groznyy (these data are courtesy of K.Yu. Lotiev).

Snout-vent length (SVL), used as conventional measure of overall body size, was measured to the nearest 1 mm.

### *Definition of adult samples*

In a sample collected in the first half of the activity season the SVL distribution is clearly bimodal. The smaller-size class is represented by yearlings – individuals experienced one hibernation only, while the larger-size class is built by older animals experienced two or more hibernations (Roitberg & Smirina 1995, 2006). These classes

Table 1. Geographic characteristics of the study sites.

Locality	Geographical coordinates	Altitude, m
1. Novokurskiy	43°39'N, 43°54'E	240
2. Grosnyy	43°15'N, 45°41'E	200
3. Kostek	43°20'N, 46°46'E	50
4. Makhachkala	42°59'N, 47°30'E	– 10
5. Buinaksk	42°45'N, 47°04'E	600
6. Termenlik	42°25'N, 47°00'E	960
7. Sergokala	42°28'N, 47°42'E	550
8. Khuchni	41°57'N, 47°57'E	600
9. Kuli	42°01'N, 47°15'E	1900

generally correspond to subadults and adults. The yearlings tend to grow much faster than the adults, and later in the season their size distributions can overlap. In this study we treated as adults all animals with SVL > 70 mm. Following considerations led us to this criterion. 1. In virtually all investigated lizard species the onset of maturity is size- rather than age-dependent (*e.g.*, Galán 1996, Tracy 1999). 2. Females with SVL of 71-75 mm quite often had oviductal eggs, whereas those of 66-70 mm wore eggs exceptionally rare (no direct data were available for the breeding state of the males). 3. In most study samples, the SVL of 71 mm fall in the gap between body length distributions of adults and subadults; so a shift of the separation point from 70 to 73 mm resulted in only minor (if any) changes of our sample statistics, with the pattern of variation remaining virtually the same.

### *Estimating characteristic body size*

An important methodological problem in comparative studies is the choice of appropriate statistics for adult body size. Average and extreme values are the most widely used statistics and they are often the only parameters available in publications. The average size is statistically powerful thus providing reasonable estimates even by small sample sizes. However, in animals which exhibit substantial postmaturation growth, the mean adult size can be influenced by plenty of proximate factors affecting the size distribution. These are local and temporal variation in size at maturity, growth rates, adult mortality, and so forth (Stamps & Andrews 1992, Stamps 1993, Watkins 1996).

In lizards and other ectotherms, body growth after maturity is usually asymptotic. That is, it slows progressively with size and virtually ceases at advanced size and age (see *e.g.*, Olsson & Shine 1996 for *L. agilis* growth curves). The mean (typical) growth curve and its asymptote ( $A$ ) can be developed from individual growth increments or body sizes of aged individuals (*e.g.*, Andrews 1982, Brown *et al.* 1999). For comparative studies focusing on differences among populations or between sexes, asymptotic size ( $A$ ) is a preferable statistic because it is affected by a much shorter list of proximate factors than average size (Stamps & Andrews 1992, Stamps 1993, Brown *et al.* 1999). As growth curves are often not available, some other simple statistics were suggested to estimate asymptotic size. Largest individual method (Stamps & Andrews 1992) clearly overestimates  $A$  and it is highly dependent on sample size (Brown *et al.* 1999). Instead, the 80th or other higher percentiles have been recommended for theoretical reasons (Brown *et al.* 1999) and exhibited a good conformity with the growth-based estimates in several sets of lizard data (Brown *et al.* 1999, Kratochvíl & Frynta 2002, Roitberg & Smirina 2006). Both the means and 80th percentiles of adult samples were used in this study.



*Estimating sexual size dimorphism*

We quantified SSD with the index:  $SSD = (\text{size of larger sex} / \text{size of smaller sex}) - 1$ , arbitrarily expressed as positive if females are larger and negative if males are larger (Lovich & Gibbons 1992). We chose this index because it generates values that are intuitive, directional, properly scaled, and symmetrical around zero (Lovich & Gibbons 1992, Smith 1999). For each study sample, two SSD values, one based on means ( $SSD_x$ ) and the other on 80th percentiles ( $SSD_{p80}$ ), were computed.

*Statistical analysis*

For analysis of interannual variation, operational units of statistical comparisons were samples of specimens of the same sex, collected from the same locality in the same year. The interannual variation was not examined for localities where the operational units were too small ( $n < 10$ ). For analyses of interlocality variation, samples of different years were pooled for each combination of sex and locality. As the assumptions of normality (Shapiro-Wilk  $W$ -test test) or homogeneity of variances (Levene test, Liliefors) were often violated in our study samples, even if SVL was log-transformed, we used only nonparametric tests. By multiple comparisons, we considered only those individual differences, which remained significant ( $P < 0.05$ ) after the sequential Bonferroni adjustment (Rice 1989).

We used SPSS 11.5 for all the analyses.

**RESULTS***Temporal variation in adult SVL and sexual size dimorphism*

Summary statistics for male and female adult SVL and SSD in our study samples are given in Table 2 (rows with single years). A Kruskal-Wallis test was made for each of the 10 sex  $\times$  locality combinations with adequate sample sizes. The following interannual differences for mean SVL remained significant after the sequential Bonferroni adjustment: Site 4, males ( $\gamma^2 = 24.9$ , d.f. = 4,  $P < 0.001$ ); Site 5, females ( $\gamma^2 = 10.5$ , d.f. = 2,  $P = 0.005$ ). We could reveal no regular trend in this variation. In Site 4, the males collected in 1970 were clearly larger (means, 80th percentiles) than those of 1980, 1982, and 1983, but the males of 1984 were nearly as large as the males of 1970. In Site 5, females of 1982 were larger than those collected in 1970 and 1979 (Table 2). One regular trend was found in the temporal dynamics of SSD. In sites 4, 5, and 7, each of which provided samples of 1970, 1979 (1980), and 1982, the SSD for means was stronger in the samples of 1970 than in the samples of the other years (Table 2). This has a  $(1/3)^3 \approx 0.0027$  chance of occurring at random that significantly differs

Table 2. Male and female SVL and sexual size dimorphism (SSD) in adult samples of *Lacerta agilis boemica*. N – sample size; Min, Max – extreme values; X – mean; SD – standard deviation; P80 – 80th percentile;  $SSD_x$  and  $SSD_{P80}$  – Lovich & Gibbons (1992) SSD index calculated for means and percentiles, correspondingly. See Table 1 for geographic characteristics of the study sites.

Sites	Years	Males						Females						SSD_m	SSD_p80
		N	Min	Max	X	SD	P80	N	Min	Max	X	SD	P80		
1. Novokurskiy	1970	<b>34</b>	<b>73</b>	<b>106</b>	<b>92.5</b>	<b>7.39</b>	<b>100.0</b>	<b>34</b>	<b>74</b>	<b>98</b>	<b>85.2</b>	<b>6.63</b>	<b>92.0</b>	-0.09	-0.09
2. Groznyy	total	<b>14</b>	<b>77</b>	<b>114</b>	<b>93.7</b>	<b>10.84</b>	<b>101.0</b>	<b>14</b>	<b>73</b>	<b>104</b>	<b>87.2</b>	<b>9.28</b>	<b>96.0</b>	-0.07	-0.05
3. Kostek	1984	14	71	103	88.9	10.55	100.0	10	71	95	84.8	6.97	88.8	-0.05	-0.13
	1985	11	71	106	88.5	11.89	100.2	18	71	90	79.8	5.38	84.2	-0.11	-0.19
3. Kostek	total	<b>25</b>	<b>71</b>	<b>106</b>	<b>88.7</b>	<b>10.92</b>	<b>99.8</b>	<b>28</b>	<b>71</b>	<b>95</b>	<b>81.6</b>	<b>6.35</b>	<b>87.2</b>	-0.08	-0.14
4. Makhachkala	1970	36	80	109	94.0	7.15	100.0	24	71	95	83.8	6.44	90.0	-0.12	-0.11
	1980	25	71	102	83.8	9.47	96.6	24	73	97	81.1	6.48	86.0	-0.03	-0.12
	1982	18	71	99	86.1	7.53	91.6	23	72	97	83.3	6.79	90.4	-0.03	-0.01
	1983	14	72	104	82.9	10.08	95.0	20	73	97	80.3	6.37	86.6	-0.03	-0.10
	1984	13	76	106	90.5	10.85	102.0	17	75	95	82.2	6.73	90.0	-0.10	-0.13
4. Makhachkala	total	<b>106</b>	<b>71</b>	<b>109</b>	<b>88.3</b>	<b>9.89</b>	<b>98.0</b>	<b>108</b>	<b>71</b>	<b>97</b>	<b>82.2</b>	<b>6.57</b>	<b>88.0</b>	-0.07	-0.11
5. Bulnask	1970	27	72	97	85.8	6.83	93.4	26	72	88	79.4	4.78	86.0	-0.08	-0.09
	1979	18	73	97	81.9	7.43	90.0	23	71	88	78.8	4.83	83.4	-0.04	-0.08
	1982	16	75	99	85.3	6.27	90.0	16	76	92	84.4	5.45	90.6	-0.01	0.01
5. Bulnask	total	<b>61</b>	<b>72</b>	<b>99</b>	<b>84.5</b>	<b>6.97</b>	<b>91.0</b>	<b>65</b>	<b>71</b>	<b>92</b>	<b>80.4</b>	<b>5.42</b>	<b>86.0</b>	-0.05	-0.06
6. Termenlik	total	<b>14</b>	<b>79</b>	<b>100</b>	<b>90.4</b>	<b>7.02</b>	<b>97.0</b>	<b>16</b>	<b>75</b>	<b>99</b>	<b>87.7</b>	<b>6.55</b>	<b>93.4</b>	-0.03	-0.04
7. Sergokala	1970	20	78	94	85.2	4.87	89.8	21	71	87	77.8	4.81	83.2	-0.09	-0.08
	1979	21	72	96	84.0	7.36	92.6	23	71	90	79.3	5.98	85.4	-0.06	-0.08
	1982	17	75	97	84.2	5.80	90.0	16	76	88	80.9	3.57	83.8	-0.04	-0.07
7. Sergokala	total	<b>58</b>	<b>72</b>	<b>97</b>	<b>84.5</b>	<b>6.06</b>	<b>90.0</b>	<b>60</b>	<b>71</b>	<b>90</b>	<b>79.2</b>	<b>5.09</b>	<b>84.0</b>	-0.07	-0.07
8. Khuchni	1985	12	73	94	83.9	8.02	92.2	17	71	96	80.2	8.21	89.4	-0.05	-0.03
	1986	13	76	103	87.3	7.78	94.6	12	77	91	86.5	4.58	91.0	-0.01	-0.04
8. Khuchni	total	<b>25</b>	<b>73</b>	<b>103</b>	<b>85.7</b>	<b>7.92</b>	<b>93.4</b>	<b>29</b>	<b>71</b>	<b>98</b>	<b>82.8</b>	<b>7.53</b>	<b>90.0</b>	-0.03	-0.04
9. Kull	total	11	73	100	89.9	8.07	97.0	11	78	100	91.6	6.96	97.0	0.02	0.00

from 0.5 (binomial test,  $P < 0.001$ ). For comparisons between 1970 and 1982, the SSD for 80th percentiles showed a similar pattern (Table 2).

### *Interlocality variation in adult SVL and sexual size dimorphism*

Summary statistics for male and female adult SVL and SSD in 9 localities (years combined) are given in Table 2 (bold figures). Interlocality variation was highly significant in both sexes (Kruskal-Wallis test: males,  $\chi^2 = 35.2$ , d.f. = 8,  $P < 0.001$ ; females,  $\chi^2 = 49.1$ , d.f. = 8,  $P < 0.001$ ). Population means ranged from 85–93 mm in males and 79–92 mm in females (Table 2). In nearly all localities, sex differences were male-biased (males were the larger sex, SSD index negative), being significant for sites 1, 3–5, 7 ( $P < 0.001$ –0.01).

Table 3 shows geographic co-variation among the different statistics for male and female SVL, SSD, and altitude. Strong correlations were found within each pair of different statistics of the same biological variables: the mean and the 80th percentile of adult SVL in males ( $r_s = 0.86$ ,  $P < 0.003$ ) and females ( $r_s = 0.98$ ,  $P < 0.001$ ) as well as the figures of the SSD index calculated for the two SVL statistics ( $r_s = 0.92$ ,  $P <$

Table 3. Spearman rank correlation coefficients ( $r_s$ ) between different statistics for male and female SVL, sexual size dimorphism, and altitude. Interlocality variation. Statistics:  $X_m$  and  $X_f$  – sample means,  $P80_m$  and  $P80_f$  – 80th percentiles for males and females;  $SSD_x$  and  $SSD_{p80}$  – sexual size dimorphism index of Lovich & Gibbons (1992) calculated for means and 80th percentiles, correspondingly. Bold figures designate  $r_s$  values with  $P < 0.05$ .

Variables		$SSD_x$	$SSD_{p80}$	$X_m$	$P80_m$	$X_f$	$P80_f$
Elevation	$r_s$	<b>0.812</b>	<b>0.795</b>	-0.326	-0.664	0.176	0.142
	$P$	0.008	0.010	0.391	0.051	0.651	0.715
$SSD_x$	$r_s$		<b>0.917</b>	-0.133	-0.561	0.450	0.383
	$P$		0.001	0.732	0.116	0.224	0.308
$SSD_{p80}$	$r_s$			0.117	-0.301	0.617	0.600
	$P$			0.765	0.431	0.077	0.088
$X_m$	$r_s$				<b>0.862</b>	<b>0.783</b>	<b>0.817</b>
	$P$				0.003	0.013	0.007
$P80_m$	$r_s$					0.460	0.536
	$P$					0.213	0.137
$X_f$	$r_s$						<b>0.983</b>
	$P$						0.000

0.001). The SSD figures calculated for the means and 80th percentiles were also similar in their absolute values: the average difference was  $0.013 \pm 0.023$  ( $n = 9$ ).

Both SSD indices exhibited a strong positive correlation with altitude ( $r_s = 0.80$ – $0.81$ ,  $P < 0.01$ ). In contrast, among the SVL statistics for single sexes, only the 80th percentiles of male SVL showed a marginally significant correlation with altitude (Table 3). The two statistics of male SVL correlated moderately with those of females: only two of the four correlations were significant (Table 3).

## DISCUSSION

### *Temporal variation in adult SVL and sexual size dimorphism*

The largest of the interannual differences in adult SVL and SSD revealed in the study sites were comparable in magnitude with those among localities. One regular trend, a stronger male-biased SSD in the samples of 1970 relative to the samples of 1979–82 within three study sites, was revealed. Along with temporal, environmentally induced variation in maturation pattern, growth rates, and adult mortality (Stamps 1993 and references therein), seasonal changes in proportion of younger (newly matured) adults and behavioural exclusion (Andrews & Stamps 1994, Watkins 1996) can affect the size distribution of males and/or females and SSD in a particular sample. Lack of detailed data on population dynamics, environmental fluctuations, and spatial distribution of old and younger adults makes it difficult to identify sources of the revealed interannual variation in adult SVL and sexual size dimorphism in *L. a.*

*boemica*. In any event, the presented data show that differences in adult SVL or SSD observed between single-year samples from few localities may be irrelevant to geographic variation, and samples of several years and/or a considerable number of the study sites are necessary to discover the actual geographic or altitudinal pattern.

### *Patterns of altitudinal variation in adult SVL in lizards*

Unlike comparable studies on three Australian skink species (Rohr 1997, Qualls & Shine 1998, Wapstra *et al.* 2001) which showed a pronounced increase in maximum, asymptotic and mean adult SVL (along with a higher longevity) at high elevations, our study revealed no clear trend for adult SVL in males or females. This is despite the facts that the altitudinal range of our study sites (over 1900 m, Table 1) was even higher than in the investigations cited above, and the adult lizards from higher altitudes (960, 1900 m) exhibited clearly higher mean and maximum ages than those from elevations of 50-600 m (Roitberg & Smirina 1995, 2006). A comparison between a low-elevation (Ararat valley, 850 m a.s.l.) and a high-elevation (Sevan coast, 1900 m) populations of *L. strigata* in Armenia (Melkumyan 1983) exhibited the same pattern as *L. a. boemica*: relatively small difference in mean adult SVL (91.9 mm and 93.8 mm) and substantial difference in longevity (3-4 years *vs* 6-7 years) (Melkumyan 1983). Even related species from the same mountain system can have quite different patterns of altitudinal variation for adult SVL: in the Chiricahua Mountains (Arizona), reproducing females of *Sceloporus jarrovi* from a high elevation were larger than their lower-elevation counterparts (Ballinger 1979), while the opposite pattern was reported for *S. scalaris* (Mathies & Andrews 1995). Both patterns were, however, not as strong as those by skinks and did not involve substantial differences in maximum SVL (fig. 1 in: Ballinger 1979; fig. 5 in: Mathies & Andrews 1995). Further data on conspecific populations from geographically proximate but climatically contrasting localities, involving various lizard *taxa* and different environmental gradients should help to interpret the observed diversity of patterns of geographic variation in adult body length.

### *Altitudinal variation in sexual size dimorphism in L. a. boemica*

SSD in *L. a. boemica* is predominantly male-biased (Table 2). Interlocality variation of this SSD exhibited a strong correlation with altitude (Table 3), varying from -0.07/-0.09 in the lowland localities to -0.03/-0.02 in the mountain sites (Table 2). Our skeletochronological investigation of 5 of the 9 populations of this study (Roitberg & Smirina 2006) provided some insight to proximate causation of the observed SSD pattern. Sex-biased adult mortality does not seem to be an important factor as we did not find any consistent sex differences in the age composition of adults (*op. cit.*).

Instead, the SSD in *L. a. boemica* apparently results from sex differences in growth trajectories, mainly due to reduced growth in females in the year of their first reproduction (op. cit.). Based on the models by Adolph & Porter (1993, 1996) and some original data we hypothesized that the growth costs of reproduction should be higher in the lowland females than in females from higher elevations because the former are expected to mature at earlier age and smaller size, and produce more eggs per year. Thus we viewed the higher male-biased SSD in the lowland populations as a by-product of the interaction between the growth and maturation patterns (Roitberg & Smirina 2006). Sex differences in growth curves were reported as the primary proximate factor of SSD in some other lizard species (Watkins 1996, Rutherford 2004).

Unlike the interannual differences which should mainly reflect the proximate level processes (phenotypic plasticity or demographic dynamics), the geographic variation in adult body length and SSD can also involve a genetic component. The 80th percentiles of the SVL distributions of adult males showed a marginally significant negative correlation with altitude (Table 3). Moreover, for this statistic, as well as for maximum values, the males from four lowland localities are clearly larger than those from the higher elevation sites (Mann-Whitney U test,  $Z = -2.46$ ,  $P < 0.01$ ). We hypothesize the larger male size (and partly the stronger sexual size dimorphism) in the lowland populations of *L. a. boemica* to result from a stronger intrasexual selection due to a high population density (Stamps *et al.* 1997). Indeed, whereas in our lowland and submontane localities an observer could meet several dozen individuals during 3-5 hours, at most 10-15 animals were recorded during a comparable excursion in the mountain study sites (E.S. Roitberg unpublished data). Furthermore, the lowland populations of *L. a. boemica*, at least in the eastern part of its distribution, are rather dense but spatially limited (Roitberg *et al.* 2000). That is, in some respects these populations are comparable with the populations of small islands. A tendency of island populations to exhibit a stronger male-biased SSD as compared to their mainland relatives is widespread in reptiles (Fitch 1981). For lacertid lizards, a rather strong male-biased SSD was also reported just for the island forms with high population densities (Salvador 1984, Pérez-Mellado & Salvador 1988). Further studies should test our hypotheses on proximate and ultimate factors of variation in adult body size and sexual size dimorphism in *L. a. boemica*.

## FINAL COMMENTS

The mean and 80th percentile, two different summary statistics for adult body length, exhibited closely concordant patterns of geographic variation. Even stronger interlocality correlation was found between the figures of the Lovich & Gibbons

(1992) SSD index calculated for the two statistics; the figures were also similar in their absolute values. These findings provide support for an analysis of geographic variation of SSD in *L. agilis* by use of numerous published data on mean SVL. Such a study is now in progress (Roitberg 2005).

A comparison of mean and maximum values of adult body length in the study populations with those for the other *L. agilis* populations suggest that *L. a. boemica* belongs to the largest forms of this species. The largest male of the Site 2 was 114 mm SVL (Table 2). Like another *L. a. boemica* male individual of unknown origin which SVL was 115 mm (Warnecke 2000) it equals the maximum body length thus far reported for *L. agilis* (Blanke 2004).

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# Distribution and ecological preferences of lacertids in Calabria

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*There are three species of Lacertids present in Calabria: Podarcis sicula, P. muralis and Lacerta bilineata. On the basis of the collected data the first one is certainly the most abundant and ecologically interesting. This species can be considered eurytopous and basically thermophilous, as it is more common at middle and low altitudes. Podarcis muralis and Lacerta bilineata are more poorly distributed: the first one reaches in Calabria the southernmost limit of its Italian distribution. It is common at middle and high altitudes (over 800 m a.s.l.) and prefers mountain environments or humid areas situated at the foot of mountains (above all woods of mesophilous deciduous trees and mountain grasslands). The second displays a wide altitudinal distribution being very common in sclerophyllous or riparian woods and in Mediterranean shrubs. For all these species a spring activity peak is observed, followed by a summer decline and by a later smaller peak in autumn coinciding with hatching time. Winter activity of lacertids can be occasionally observed at low altitudes.*

Keywords: Lacertids, altitudinal distribution, ecological distribution, annual activity, Calabria.

## INTRODUCTION

Calabria is the southernmost tip of the Italian Peninsula. It is placed in the middle of the Mediterranean Sea and because of its particular geographical position it represents an important and interesting naturalistic study area. It occupies an area of 15,080 km<sup>2</sup> with a mainly mountainous (42%) and hilly (49%) territory and an average elevation of about 556 m a.s.l. Plains occupy a small part (about 9%) of the region. The climate is Mediterranean, although it differs throughout the region according to exposure and altitude: generally a mild winter is followed by a hot and

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sultry summer. Indeed, there are some important climatic differences between the two sides of Calabria: the Tyrrhenian side is more exposed to humid winds and is characterised by an oceanic-like climate with abundant rainfalls; on the contrary, the Ionian side is exposed to African influences and has high annual temperatures and short and intense rainfalls. On average rainfalls exceed 1000 mm/year with a peak of 181 mm in December and a minimum of 16 mm in July (Versace *et al.* 1989, Caloiro *et al.* 1990). Temperatures are highly variable. However, the lowest temperatures are recorded in January and the highest temperatures are recorded in August. Annual mean temperature is included within the 10 and 16 °C.

With regard to vegetation, Calabria can be divided into four vegetation belts (Pignatti 1979, Bernardo 1995):

- *Mediterranean vegetation belt* is characterized by the presence of *Erica arborea*, *Cistus* sp., *Rosmarinus officinalis*, *Juniperus oxicedrus*, *Quercus ilex*, *Arbutus unedo* and *Nerium oleander* and it extends from 0 to 800-850 m a.s.l.;
- *Supramediterranean vegetation belt* (850-1100 m a.s.l.) is dominated by woods of deciduous trees like oaks (*Quercus virgiliana*, *Quercus frainetto*, *Quercus cerris*, *Quercus pubescens*), maples (*Acer monspessulanum*, *Acer campestre*), chestnut (*Castanea sativa*) and alder (*Alnus cordata*);
- *Mountain vegetation belt* (1100-2000 m a.s.l.) is dominated by pine woods (*Pinus laricio*) and by beech woods (*Fagus sylvatica*) sometimes mixed with silver fir (*Abies alba*);
- *High-mountain vegetation belt* (over 2000 m a.s.l.) represents the timberline and is dominated by grasslands.

## MATERIALS AND METHODS

Data reported in this work were collected in the field from 1983 to 2003 for a total of 1002 observations. Each observation was considered as a sampling station. Environmental information was collected too and recorded in the Herpetological Database of the Department of Ecology, University of Calabria (Rossi *et al.* 1991).

On the basis of the altitudinal, ecological and temporal distribution of our observations we also made an analysis on the ecology and phenology of the species (according to Andreone & Sindaco 1999).

Percentage values were obtained by making the ratio of sampling reports about altitudinal, ecological or temporal information to all sampling reports for each species. We made an equivalent sampling effort for the tree species under study, for each habitat type and for each altitudinal category. Habitat categories were defined using Corine habitat list 1991.

I.G.M. maps to the scale of 1:25.000 were used to calculate terrestrial coordinates. Cartographical analysis was processed by vector-based GIS-software MAP-INFO, using Apple Macintosh computers.

## RESULTS AND DISCUSSION

Of the three species of lacertids occurring in Calabria: *Podarcis sicula*, *P. muralis* and *Lacerta bilineata*, the first one is certainly the most abundant species: in fact it was found in 493 sampling stations with a quite uniform distribution throughout the entire area (Fig. 1). This lizard shows the major ecological value: it was found indeed in all the sampled stations with a slight dominance for areas without vegetation cover and grassy places; it was often found in towns, gardens and parks (Fig. 2). From an altitudinal point of view *P. sicula* was found from the sea level up to 1625 m a.s.l. (Sila Massif), although its occurrence decreases with increasing altitude (Fig. 3). On the basis of the collected data, in Calabria this species can be considered eurytopous and basically thermophylous and heliothermic (Avery 1982, Tosini *et al.* 1992), as it is more common at middle and low altitudes. According to other authors (Arnold & Ovenden 2002, Corti & Lo Cascio 2002, Capula & Ceccarelli 2003) this species seems to be a successful colonizer, a vigorous and opportunistic lizard.

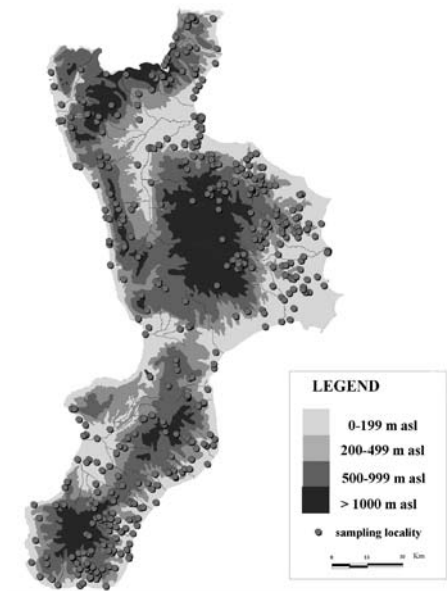


Fig. 1. Distribution of sampling localities of *Podarcis sicula* in the study area.

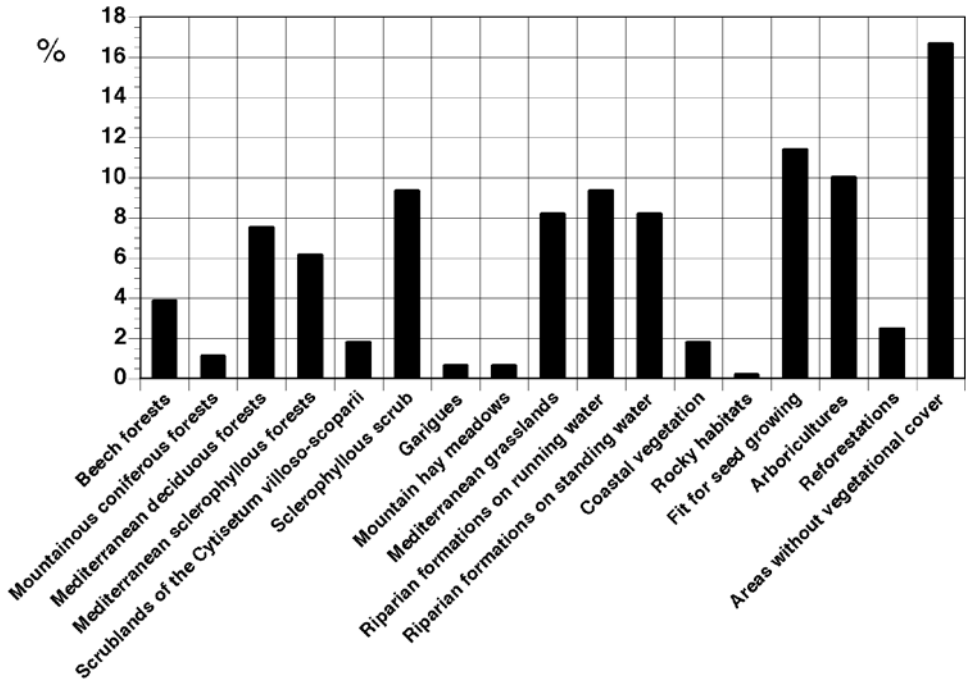


Fig. 2. Habitat preferences of *Podarcis sicula* in the study area.

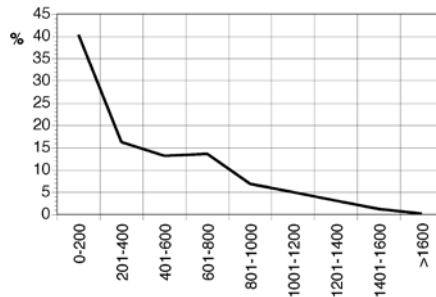


Fig. 3. Altitudinal preferences of *Podarcis sicula* in the study area.

*P. muralis* and *L. bilineata* are more poorly distributed. The first one, which in Calabria reaches the southernmost limit of its Italian distribution (S.H.I. 1996), was found in 179 sampling stations (Fig. 4) and it is common at middle and high altitudes (above 800 m a.s.l.); stations placed beneath 400 m a.s.l. are extremely rare (Fig. 5). This is a common condition for species in southern distributional area, whereas data collected in northern Italian areas show a strongly different pattern. In Piemonte, Valle d'Aosta, Veneto, Liguria, Emilia Romagna and northern Lazio (Mazzotti &

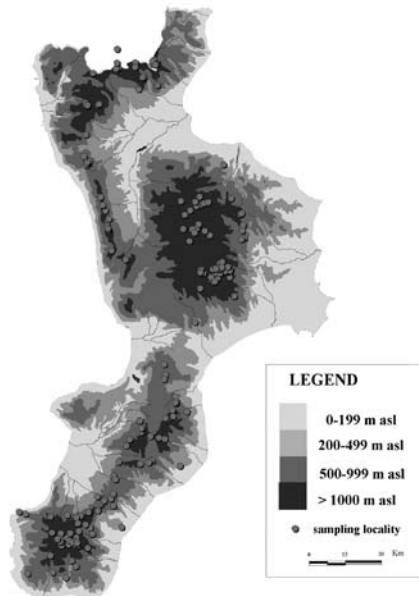


Fig. 4. Distribution of sampling localities of *Podarcis muralis* in the study area.

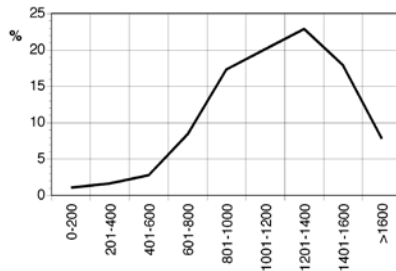


Fig. 5. Altitudinal preferences of *Podarcis muralis* in the study area.

Stagni 1993; Borgo 1994; Capula 1999, 2000; Pegoraro & Fioretto 2000) the occurrence of this species spreads from the sea level to high altitudes, but mostly it is found in flat and hilly areas, usually underneath 500 m a.s.l.. In southern Italy, as observed in southern Lazio and in Puglia (Garavelli 1996, Capula 2000), *P. muralis* moves towards altitudes above 500-600 m a.s.l., showing to be ecologically more similar to the Calabrian populations (Fig. 6).

If we compare data reported from other areas, the boundary position of the Calabrian populations could also explain this restricted niche.

In Calabria *L. bilineata* was found in 330 sampling stations (Fig. 7) and has a

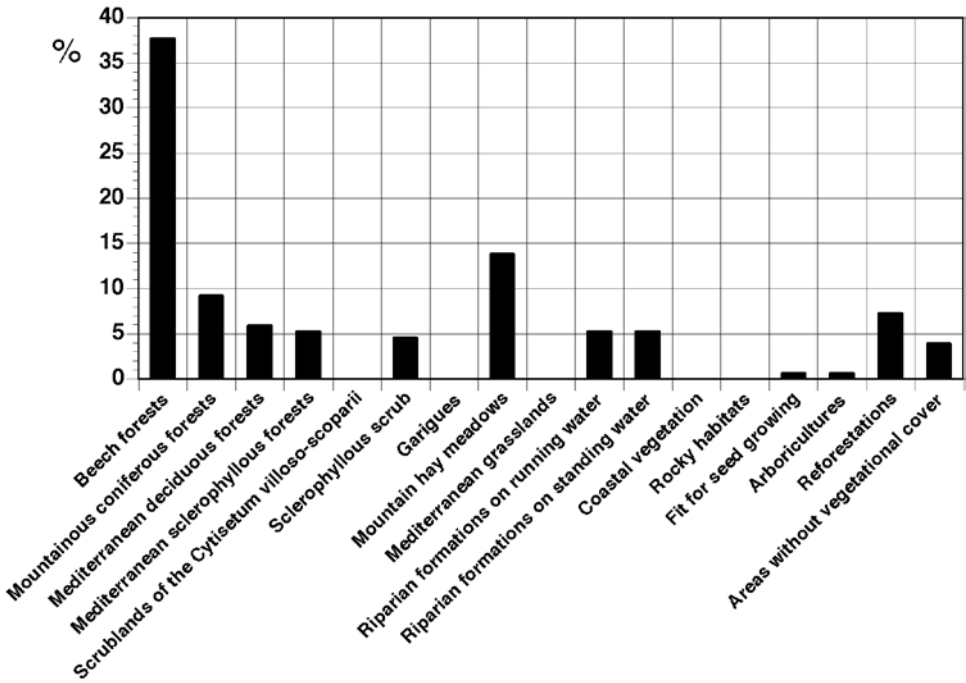


Fig. 6. Habitat preferences of *Podarcis muralis* in the study area.

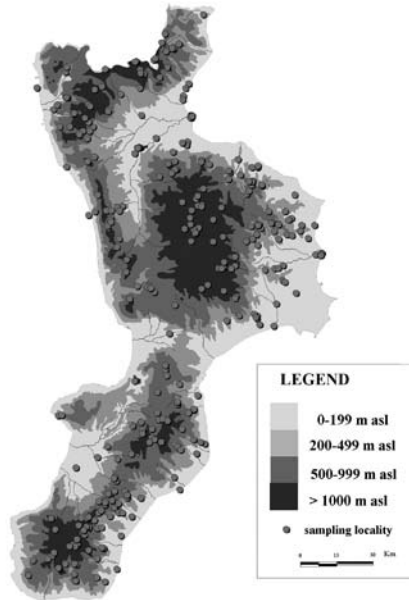


Fig. 7. Distribution of sampling localities of *Lacerta bilineata* in the study area.



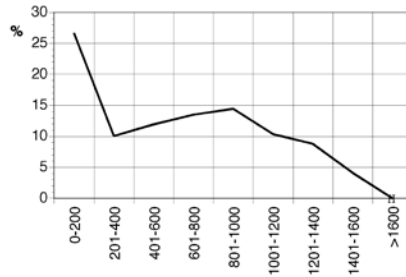


Fig. 8. Altitudinal preferences of *Lacerta bilineata* in the study area.

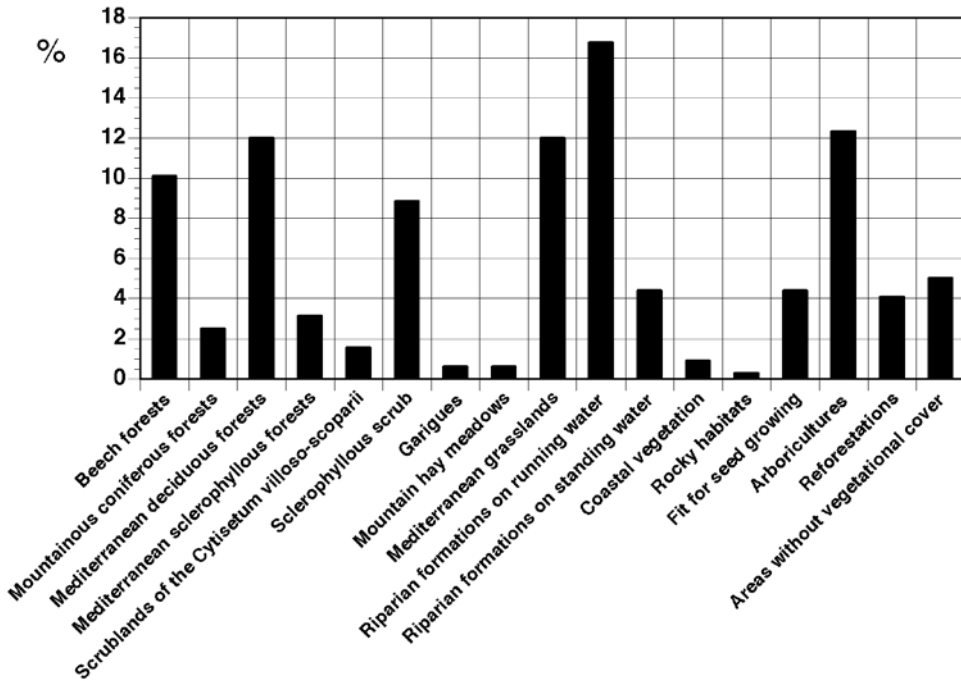


Fig. 9. Habitat preferences of *Lacerta bilineata* in the study area.

wide altitudinal distribution (Fig. 8); the species is very common in sclerophyllous or riparian woods, in arboricultures and in Mediterranean shrubs (Fig. 9). The ecology of the Calabrian populations seems not to be different from that of other Italian populations, except for the Venetian and Ligurian ones, which have an altitudinal distribution restricted only to middle and low altitudes, and for populations of Puglia, which are absolutely absent in anthropic areas.

In all these species a spring activity peak is observed, followed by a summer fall off and by a later smaller peak in autumn coinciding with hatching time. Winter activ-

ity of lacertids was occasionally observed at low altitudes, especially close to mouths of large seasonal streams (“*fiumare*”). Only *P. muralis* seems to be going towards a rigorous latent period. Occasional winter activity of *L. bilineata* has been observed in Calabria while other Italian populations seem not to be active during this season. If we compare our data with those reported in literature (Carretero & Llorente 1991) regarding *P. sicula* and *L. bilineata* we cannot exclude the possibility of a double and even triple egg-laying throughout the year.

## ACKNOWLEDGEMENTS

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# Genetic diversity within Corsican and Sardinian specimens of the Tyrrhenian Wall Lizard, *Podarcis tiliguerta*, estimated using mtDNA sequences

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*Podarcis tiliguerta*, a Wall Lizard endemic to Corsica and Sardinia, has recently been shown to harbour highly genetically distinct mitochondrial lineages, that may indicate it is in fact a species complex. Here we combine 12S rRNA mtDNA sequences from previous studies with 17 newly generated sequences to better understand genetic diversity within this group. In particular we include samples from the Cerbicale islands, which were quite distinct in an earlier assessment of protein electrophoretic variation. Results confirm that distinct lineages exist on Corsica and Sardinia. The Cerbicale islands appear as part of the Corsican group. A third lineage exists, although at present it is known only from a single specimen from Sardinia. Further morphological and molecular data is needed to revise the taxonomy of this apparent species complex.

Keywords: *Podarcis tiliguerta*, evolution, systematics, 12S rRNA, Corsica and Sardinia.

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## INTRODUCTION

Although wall lizards of the genus *Podarcis* are a dominant part of the herpetofauna of much of Mediterranean Europe, exact delimitations of species is often difficult. Recent applications of molecular methods to some forms indicate that they might be species complexes. For example, *P. hispanica* contains several highly genetically distinct lineages all of which may deserve species status (Harris *et al.* 2002, Pinho *et al.* 2006). *Podarcis erhardii* is also probably a species complex (Poulakakis *et al.* 2003), while *P. sicula*, *P. melisellensis* and *P. taurica* exhibit considerable intraspecific variation (Podnar *et al.* 2004, 2005; Poulakakis *et al.* 2005). On the other hand, the many insular subspecies described for species such as *P. lilfordi*, *P. pityusensis* and *P. melisellensis* often showed little or no genetic differentiation (Terrassa *et al.* 2004, Podnar *et al.* 2004). Thus there is a need for further phylogeographic analyses of remaining *Podarcis* species, especially the insular ones.

*Podarcis tiliguerta* is endemic to Corsica, Sardinia and many neighbouring small islands, with at least ten recognized subspecies (Arnold & Ovenden 2002). Using allozyme electrophoresis, Capula (1996) reported that genetic variation was distributed into three geographically coherent population groups: Corsica, the small islands off the south-eastern coast of Corsica (Cerbicale and Lavezzi) and Sardinia and Meli Island. A preliminary analysis of mtDNA sequences (Harris *et al.* 2005) indicated high levels of diversification between individuals from Corsica and Sardinia, at a level more typically observed between species. Another preliminary study, including four new 12S rRNA mtDNA sequences of *P. tiliguerta* (Podnar & Mayer 2005) obtained a similar result, with one distinct lineage on Corsica and two on Sardinia. However, few subspecies were included in either study, and in particular samples from the Cerbicale islands were lacking.

The aim of this work was to complement the earlier phylogeographic analysis of *P. tiliguerta* by extending the sampling area in order to cover more of its distribution range, including islets off Northeast Sardinian – Molaro, Molarotto and Tavolara – and Corsica – Finocchiarola island, and especially Toro Grande islet, one of the Cerbicale islands. Further, the data from the previous studies (Harris *et al.* 2005, Podnar & Mayer 2005) is combined for the first time to gain insight into the distribution of the mtDNA lineages.

## MATERIALS AND METHODS

*Podarcis tiliguerta* specimens were collected in the field from Corsica and Sardinia (Fig. 1) and released after a small tail clip, tissue samples being taken and stored in

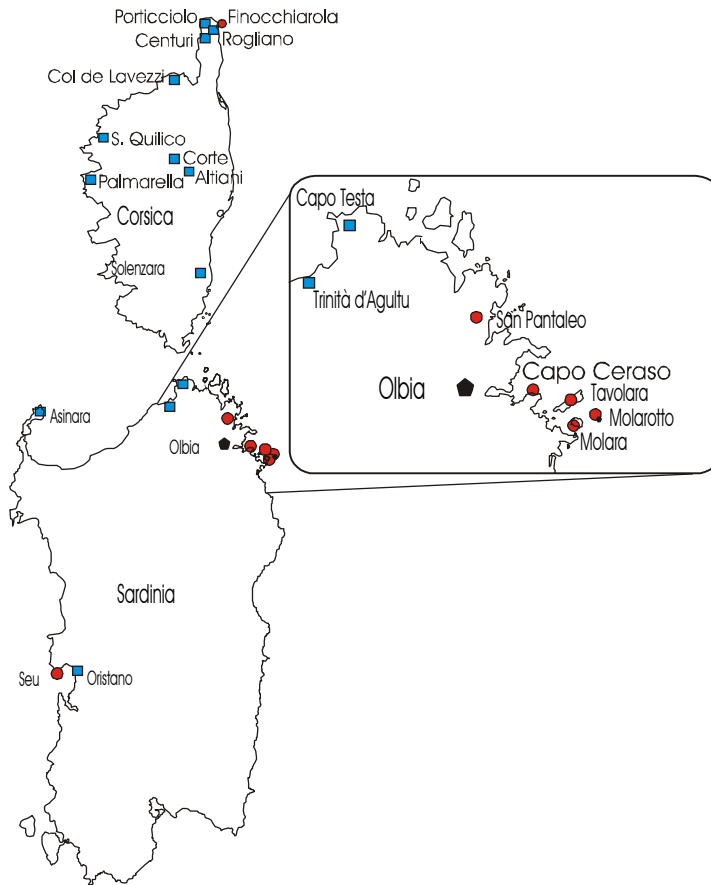


Fig. 1. Map showing sampling localities of *Podarcis tiliguerta* sequenced. Circles refer to the new localities sampled in this study and squares to the previous ones sampled by Harris *et al.* (2005).

ethanol. Genomic DNA was extracted following a standard high-salt protocol. Part of the 12S gene was amplified by PCR using 12Sa and 12Sb (Kocher *et al.* 1989) and conditions described by Harris (2001). This region was chosen to be amplified in order to include previous published sequences from Harris *et al.* (2005). The amplified products were sequenced on an automated sequencer (ABI 310 by Amersham Biosciences) and the 17 new sequences – 10 from Sardinia and seven from Corsica – were checked by eye and aligned against others previously published using BioEdit (Hall 1999) – 12 from Corsica and six from Sardinia, analysed by Harris *et al.* (2005) and four samples from Podnar & Mayer (2005). Details of the new samples and sequences used in this study are presented in the Table 1. The new sequences were deposited on GenBank, accession numbers EF 165017 to EF 165024.

Table 1. Location and sample codes of the new samples used in this study.

Location	Code
Sardinia, Molara island	SM1
Sardinia, Molara island	SM2
Sardinia, Tavolara island	ST
Sardinia, Capo Ceraso	Sc1
Sardinia, Capo Ceraso	Sc2
Sardinia, Capo Ceraso	Sc3
Sardinia, San Pantaleo	Ssp
Sardinia, Molarotto island	SMo1
Sardinia, Molarotto island	SMo2
Sardinia, Seu	Ss
Corsica, Finocchiarola island	CF1
Corsica Finocchiarola island	CF2
Corsica, Finocchiarola island	CF3
Corsica, Toro Grande islet	CTg1
Corsica, Toro Grande islet	CTg2
Corsica, Toro Grande islet	CTg3
Corsica, Toro Grande islet	CTg4

### *Analytical methods*

To assess the relationships of the lineages of *P. tiliguerta* to other *Podarcis* and to each other, 37 12S rRNA sequences from a wide variety of *Podarcis* were aligned, including representatives of the three known lineages within *P. tiliguerta*. Both Maximum Parsimony and Neighbour Joining were used to estimate relationships, and support for nodes was estimated using the Bootstrap technique. To analyse the low level of variation within each group of *P. tiliguerta* for which multiple samples were available – one consisting of the specimens from the Corsican archipelago and another of the Sardinian archipelago – the haplotypes of these groups were joined in two median-joining networks (Bandelt *et al.* 1999). In total 34 sequences of 343 base pairs length – 16 from Corsica and 18 from Sardinia – were studied.

## RESULTS

The level of divergence found within 12S sequences of *Podarcis tiliguerta* from Corsica and Sardinia was high as previously reported (Harris *et al.* 2005), with



# Developmental stability of sand lizard (*Lacerta agilis* L.) under optimal and non-optimal conditions

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*In this study we analyze developmental stability (DS) of *Lacerta agilis* under different experimental and natural conditions. Thirteen morphological characters (the number of scales on different parts of the head and the number of femoral pores) were used for assessing fluctuating asymmetry (FA) in 364 new-born laboratory juveniles hatched from eggs incubated at various temperatures, and in 805 wild specimens from 32 European populations.*

*FA is inversely related to DS and is believed to be lowest under optimal developmental conditions. In the experiment optimum incubation temperatures for lizards originating from populations from different parts of the range proved to be the same. Relationship between FA and incubation temperature was U-shaped, the minimum of the curve corresponding to optimum  $t$ . In natural populations DS decreased from the central part of the range towards its periphery, this decrease being most pronounced northwards. Thus the geographical pattern followed the experimentally established dependence of DS from incubation temperature.*

Keywords: developmental stability, population variability, developmental conditions, *Lacerta agilis*.

## INTRODUCTION

Developmental stability is the ability of an organism to form the phenotype without ontogenetic disturbances (Van Valen 1962). Developmental stability serves as the most general description of the developing organism *status*. High developmental stability is maintained on the basis of genetic coadaptation under optimal developmental conditions.

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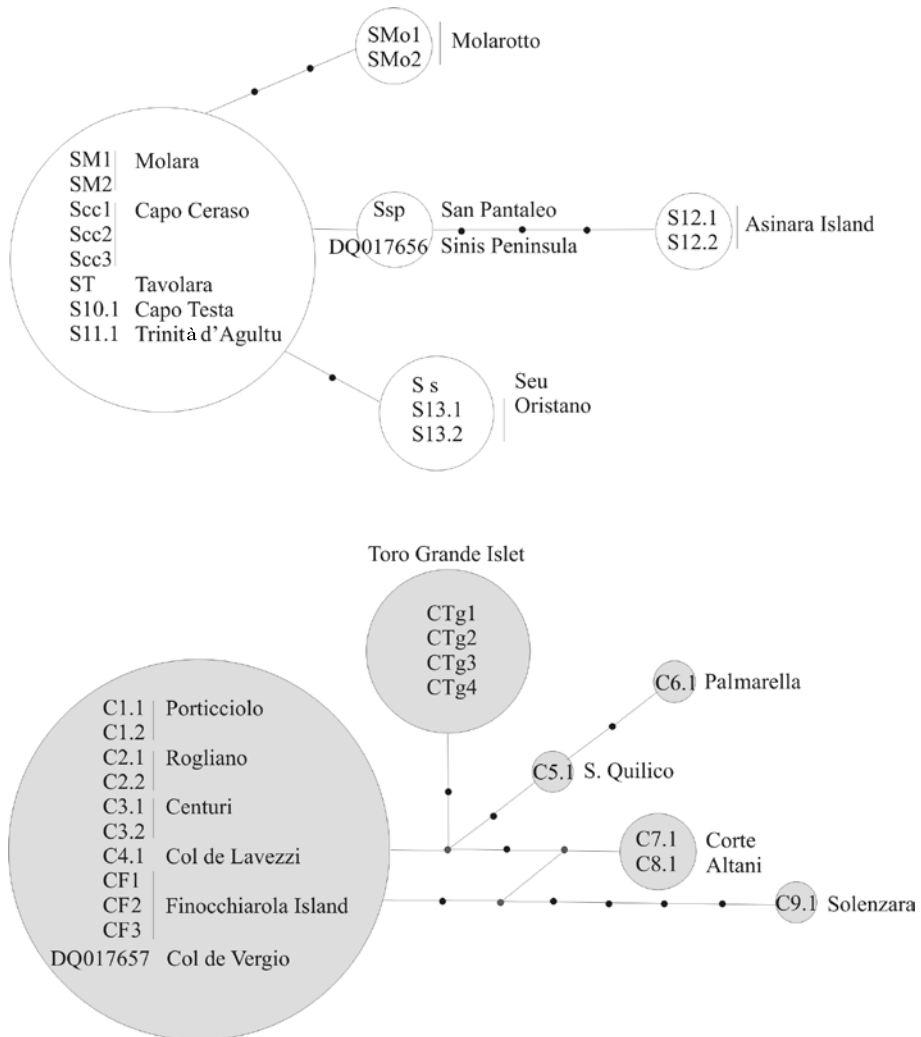


Fig. 3. Median-joining network of the 12 S rRNA sequences for *Podarcis tiliguerta*. The Corsican samples are in grey and the Sardinian ones in white. Filled circles indicate presumed missing haplotypes. Codes for new sequences are given in table 1; all others are from Harris *et al.* (2005) and Podnar & Mayer (2005).

from San Pantaleo one. Interestingly, in Corsica most genetic variation seems to occur in the southern populations. However, individuals from the Cerbicale island of Toro Grande, that are considered a distinct subspecies (*P. t. maresi*) and that formed a distinct group based on analysis of electrophoretic data (Fig. 4; Capula 1996), are not especially different from other Corsican populations.

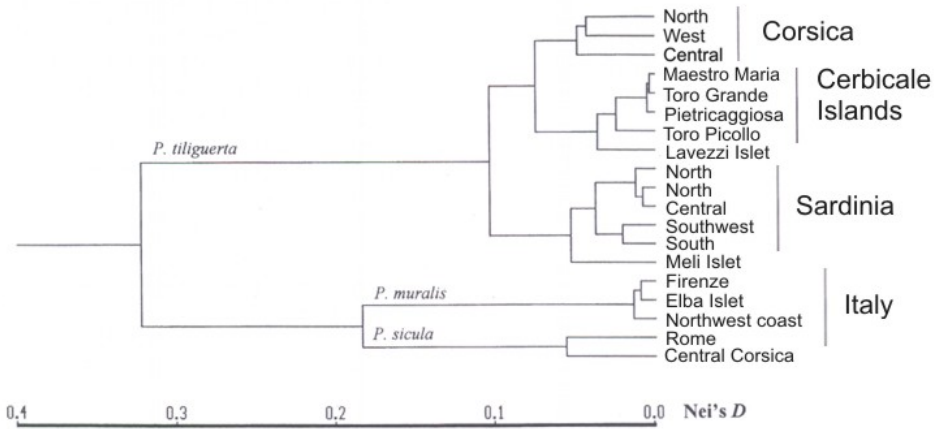


Fig. 4. Estimate of relationships derived from protein electrophoretic analysis, adapted from Capula (1996).

## DISCUSSION

These results generally support the conclusions of Harris *et al.* (2005) and Podnar & Mayer (2005). The high genetic variation (5-6%) between *Podarcis tiliguerta* sequences from Corsica and Sardinia does not conflict with the hypothesis of separate species in each archipelago. The Cerbicale islands appear to form part of the Corsican clade. A third lineage also exists in Sardinia, although at present only one sample is known so few conclusions can be drawn from this. The diversity of *P. tiliguerta* in Sardinia and in South Corsica is, however, clearly underestimated, as shown by the appearance of several new haplotypes. On the other hand, newly sequenced subspecies, such as *P. t. maresi*, do not appear to be very distinct. Thus the present taxonomy is clearly inadequate. Further sampling in broader areas, especially in the west of Sardinia and in the rest of the Southeastern Corsican Cerbicale islands, is needed. This, followed by morphological and molecular studies with nuclear markers with these specimens, will help to clarify its phylogeny and also its evolutionary and geological history and eventually to a revised taxonomy. Since the geology of the Corso-Sardinia microplate is well known, the area also promises to be an excellent region for calibrating molecular clocks and for other comparative phylogeographic studies. In particular, assessment of genetic variation within the other endemic lacertids, *Archedacerta bedriagae* and *Algyroides fitzingeri*, will be especially interesting.

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# Developmental stability of sand lizard (*Lacerta agilis* L.) under optimal and non-optimal conditions

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*In this study we analyze developmental stability (DS) of *Lacerta agilis* under different experimental and natural conditions. Thirteen morphological characters (the number of scales on different parts of the head and the number of femoral pores) were used for assessing fluctuating asymmetry (FA) in 364 new-born laboratory juveniles hatched from eggs incubated at various temperatures, and in 805 wild specimens from 32 European populations.*

*FA is inversely related to DS and is believed to be lowest under optimal developmental conditions. In the experiment optimum incubation temperatures for lizards originating from populations from different parts of the range proved to be the same. Relationship between FA and incubation temperature was U-shaped, the minimum of the curve corresponding to optimum  $t$ . In natural populations DS decreased from the central part of the range towards its periphery, this decrease being most pronounced northwards. Thus the geographical pattern followed the experimentally established dependence of DS from incubation temperature.*

Keywords: developmental stability, population variability, developmental conditions, *Lacerta agilis*.

## INTRODUCTION

Developmental stability is the ability of an organism to form the phenotype without ontogenetic disturbances (Van Valen 1962). Developmental stability serves as the most general description of the developing organism *status*. High developmental stability is maintained on the basis of genetic coadaptation under optimal developmental conditions.

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The most common manifestation of developmental stability is fluctuating asymmetry of bilateral characters (uncorrelated differences between paired characters on either side of a bilaterally symmetrical organism) (Thoday 1953, Leary *et al.* 1992).

Coordinated changes in developmental stability and other homeostatic indices, including genetic, physiological, biochemical and immune indices suggest that we consider the general status of the organism by evaluating its developmental stability. Evaluation of developmental stability as a measure of environmental stress, apparently, is the most promising. In practical terms, this allows us to evaluate and monitor developmental stability under both natural and various anthropogenic conditions.

It is considered that developmental stability could be regarded as the characteristic of optimal developmental conditions. Decreased developmental stability has been marked for some species at the periphery of the range (Soule & Baker 1968, Kat 1982, Shaikin 1992, Jenkins & Hoffmann 2000, Zakharov *et al.* 2001). Thus, this parameter is considered to be perspective for identification of optimal and non-optimal developmental conditions.

The purpose of the present study was to analyze the variation of morphological traits under different developmental conditions using natural and laboratory populations of sand lizards (*Lacerta agilis* L.).

The following objectives were set: (1) to analyze variation of morphological traits under several temperature regimes of incubation in laboratory conditions; (2) to analyze variation of the same traits in natural populations from different geographic locations; (3) to compare the results of natural populations research with the results of laboratory experiment.

## MATERIALS AND METHODS

As a model object of this study we regarded one of the most common Palearctic reptile species – sand lizard (*Lacerta agilis* L.). Sand lizards are a favourable subject of inquiry because of their small activity range and ease of capturing as they form local colonies. This species is also convenient for experimental incubation of egg-laying. Pholidosis of the sand lizard is suitable for description of developmental stability because of its regularity and clearness. We examined 476 neonatal sand lizards born during experimental incubation of eggs laid by females captured in natural populations. The experiment was performed in laboratory of postnatal ontogenesis in Koltzov Institute of Developmental Biology RAS in the eighties, but we were the first to conduct such an analysis. When studying natural populations we examined 805 adult and 62 immature sand lizards from 32 populations in the European part of the geographic range. We also used for analysis author's collections and collection sets of



Zoological Museum of Moscow State University. Sample size was 15-30 individuals for each population.

Thirteen meristic characters of pholidosis (number of scales on different areas of the body: postnasal and antejagular, preorbital, upper labial in front of suborbital, upper labial behind suborbital, lower labial, mandibular, supraorbital, upper ciliary, granules, lower ciliary, orbitotemporal, upper temporal, femoral pores) were used for assessing developmental stability. Directional asymmetry as well as antisymmetry was not registered for used characters (Zakharov 1987 [in Russian]) so we considered all manifestations of asymmetry as fluctuating asymmetry. It was stated earlier that there were no sexual distinctions in the values of these parameters (Zakharov 1987 [in Russian]), so aggregated samples were examined. Neither characters *per se* nor their asymmetry proved to correlate with each other.

Several integrated indexes were used to estimate developmental stability:

1. Average frequency of asymmetrical expression per character was calculated from the arithmetic average of the number of asymmetrical characters in each specimen related to the number of used characters (Leary *et al.* 1983, Zakharov *et al.* 2001).
2. Average level of asymmetry per character was calculated from the difference between sides of the body (Palmer & Strobek 1986). This index takes into account the intraindividual variation.
3. Generalized variance of asymmetry was calculated from the variance of the difference between sides of the body.

Statistical significance of intergroup differences in generalized variance was determined using Fisher's F-test (Zakharov 1987 [in Russian]). Statistical significance of differences between sample means traditionally was determined using Student's t-test. Friedman's nonparametric test was used to estimate statistical significance of concordant changes in the studied characters (Sokal & Rohlf 1981).

## RESULTS AND DISCUSSION

### *Experimental assessment of developmental stability*

The basic material was collected at Voronezh oblast (338 newborn individuals). Experimental incubation of eggs laid by females captured in natural populations was conducted under constant temperature modes: 20 °C, 22 °C, 25 °C, 27 °C, 30 °C, 32 °C. The values of developmental stability index – average frequency of asymmetrical expression per character in lizards incubated under different temperature modes differed significantly ( $P < 0,05$  according to Friedman's test). The index was at 20

°C  $-0.51 \pm 0.02$ ; 22 °C  $-0.33 \pm 0.06$ ; 25 °C  $-0.21 \pm 0.01$ ; 27 °C  $-0.23 \pm 0.09$ ; 30 °C  $-0.29 \pm 0.01$ ; 32 °C  $-0.38 \pm 0.02$ . The curve of dependence of average frequency of asymmetrical expression per character upon incubation temperature was U-shaped. Minimal values were registered at the temperature of incubation 25-27 °C ( $0.21 \pm 0.01$ ;  $0.23 \pm 0.09$ ). The difference between groups incubated at extreme and medium temperatures was statistically significant (30 °C and 32 °C ( $P < 0.01$ ); 27 °C and 32 °C ( $P < 0.05$ ); 20 °C and 25 °C ( $P < 0.001$ ); 20 °C and 22 °C ( $P < 0.01$ ); 22 °C and 25 °C ( $P < 0.05$ ) according to Student's test). The growth of this index was more pronounced at low temperatures of development.

The dependence of average frequency of asymmetrical expression per character upon incubation temperature was similar in populations from other parts of species' geographic range (Mahachkala oblast, Astrekhan oblast, Kaluga oblast). Incubation was conducted under three temperature modes: 20, 25 and 30 °C. The value of index was at 20 °C (Kaluga  $-0.46 \pm 0.08$ ; Mahachkala  $-0.37 \pm 0.08$ ; Astrakhan  $-0.42 \pm 0.05$ ), at 25 °C (Kaluga  $-0.18 \pm 0.05$ ; Mahachkala  $-0.21 \pm 0.04$ ; Astrakhan  $-0.15 \pm 0.04$ ), at 30 °C (Kaluga  $-0.32 \pm 0.03$ ; Mahachkala  $-0.31 \pm 0.02$ ; Astrakhan  $-0.3 \pm 0.03$ ) The difference between the groups that were incubated under different temperature modes was statistically non-significant.

The level of fluctuating asymmetry was lower in lizards that were incubated under 25 °C than at extreme temperatures (20 °C and 30 °C), this difference was statistically significant ( $P < 0.05$ , Student's test).

All the indexes analyzed proved to be independent from the size of the characters. As indicated above the difference between index values from different samples was non-significant. It allowed us to combine all the samples into one body of data. The frequency of asymmetrical expression per character was minimal in the group that was incubated under 25-27 °C. The difference between this group and the others was statistically significant ( $P < 0.001$ ) (Fig. 1A). When we examined asymmetry with regard to the degree of the difference between sides we obtained a similar result – the difference between groups incubated at extreme and medium temperatures was statistically significant ( $P < 0.001$ ) (Fig. 1B). Similar dependence of asymmetry variance upon the incubation temperature was registered (Fig. 1C). Statistically significant differences were revealed between asymmetry variance values in the group incubated under 25 °C and in the groups that were incubated at extreme temperatures ( $P < 0.01$  according to Fisher's F-test).

Thus, several indexes of developmental stability in the populations from different parts of natural area displayed similar dependence upon temperatures of incubation. This fact allowed us to suggest the range of optimal for development temperatures (approximately 25-27 °C). Accidental development variation was minimal when development occurred at these temperatures. The rate of hatchlings was maximal at

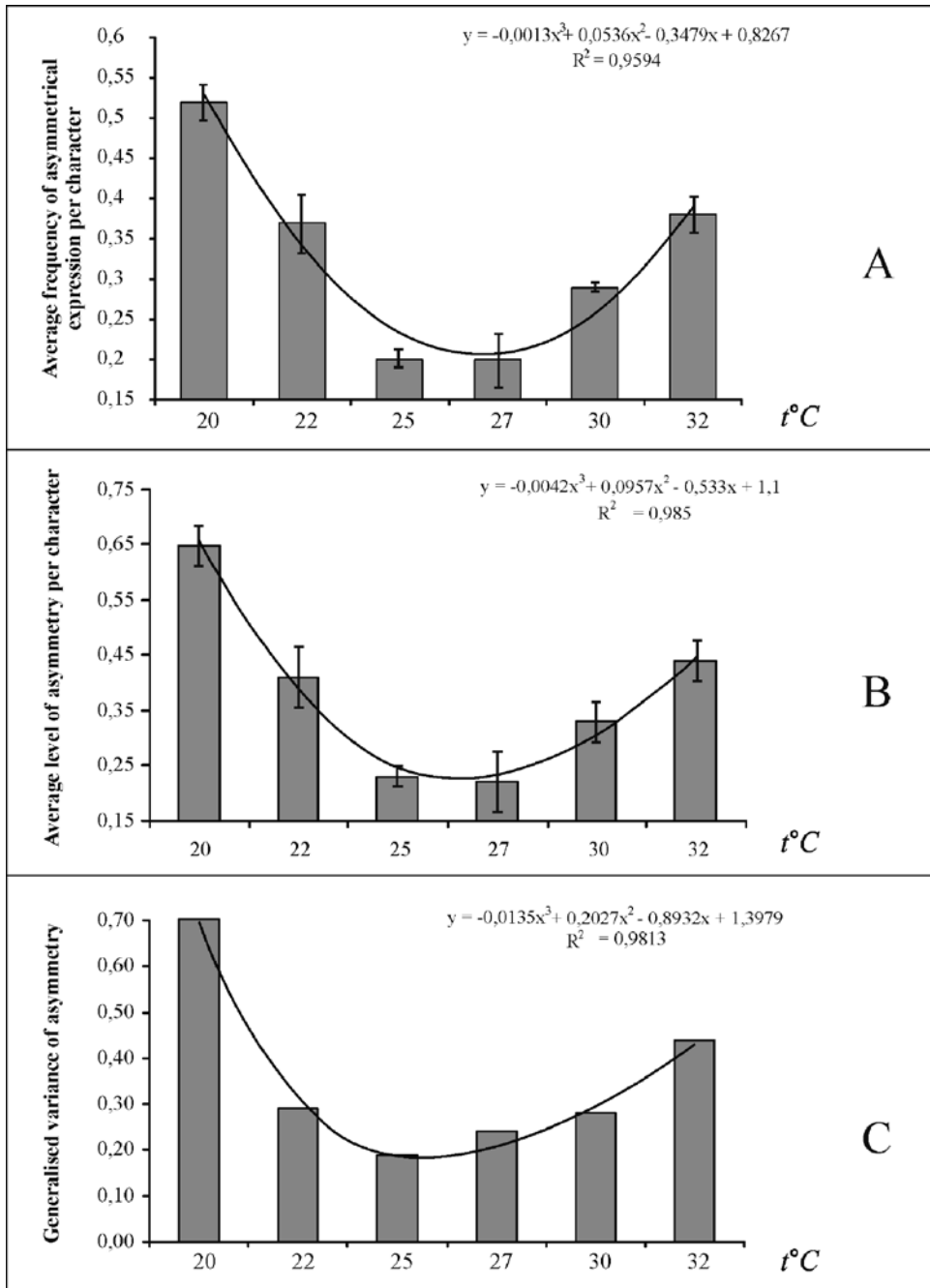


Fig. 1. Values of the indexes of developmental stability (A – average frequency of asymmetrical expression per character; B – average level of asymmetry per character; C – generalised variance of asymmetry) in the samples of sand lizard (*Lacerta agilis*) at different incubation temperatures ( $R^2$  here and below represents significance of trend approximation).

these temperatures (Zakharov *et al.* 1982 [in Russian]). Accidental development variation increased as the temperature of incubation diverged from these values; this was the highest at low temperature (20 °C). It should be noted that only few indexes allow defining the range of optimal development conditions. Physiological parameters as well as developmental time usually display linear (direct or inverse) dependence upon incubation temperature. It was demonstrated in lizards that rhythmic contractive activity of amnion displayed direct dependence upon incubation temperature (Nechaeva *et al.* 2003). It is substantial for assessment of this parameter over certain temperature range but there are no grounds to define the range of optimal conditions.

### *Assessment of developmental stability in natural populations*

We examined the samples of sand lizards taken from populations in the north, centre and south of its geographic range in the European part of Russia (Fig. 2). This territory is considered homogeneous and free from large-scale insulating barriers.

It was shown that the values of fluctuating asymmetry were minimal in natural populations at the central part of geographic range and increased with increasing distance from the centre of the species' geographic range. The growth of fluctuating asymmetry was statistically significant ( $P < 0.05$  according to Friedman's test) (Fig. 3). Peak values were registered at the northern periphery of the geographic range. All the three used indexes of stochastic developmental variation displayed similar intraspecific changes.

To estimate reliability of the applied indexes, we compared these indexes in the samples of juvenile individuals taken from one population at the northern periphery of the geographic range (the vicinity of Novgorod) over three years. The level of fluctuating asymmetry (average frequency of asymmetrical expression per character) registered over this period was high (0.35-0.37). It described the increment of fluctuating asymmetry at the periphery of the geographic range and indicated the significance of the applied indexes.

### *Comparison of the results of the study of natural populations and the laboratory experiment*

Laboratory and natural population analysis was carried out to attain the goals of the study.

It should be noted that several indexes of developmental stability that were estimated from uncorrelated morphological characters showed concordant changes at different growth temperatures in laboratory experiment and in natural populations from several locations over the geographic range. These changes were statistically sig-



Fig. 2. A sketch-map of sampling sites location.

nificant ( $P < 0.05$  according to Friedman's test). As the range of optimal growth temperatures was the same in all the populations over the geographic range it was possible to reveal these changes.

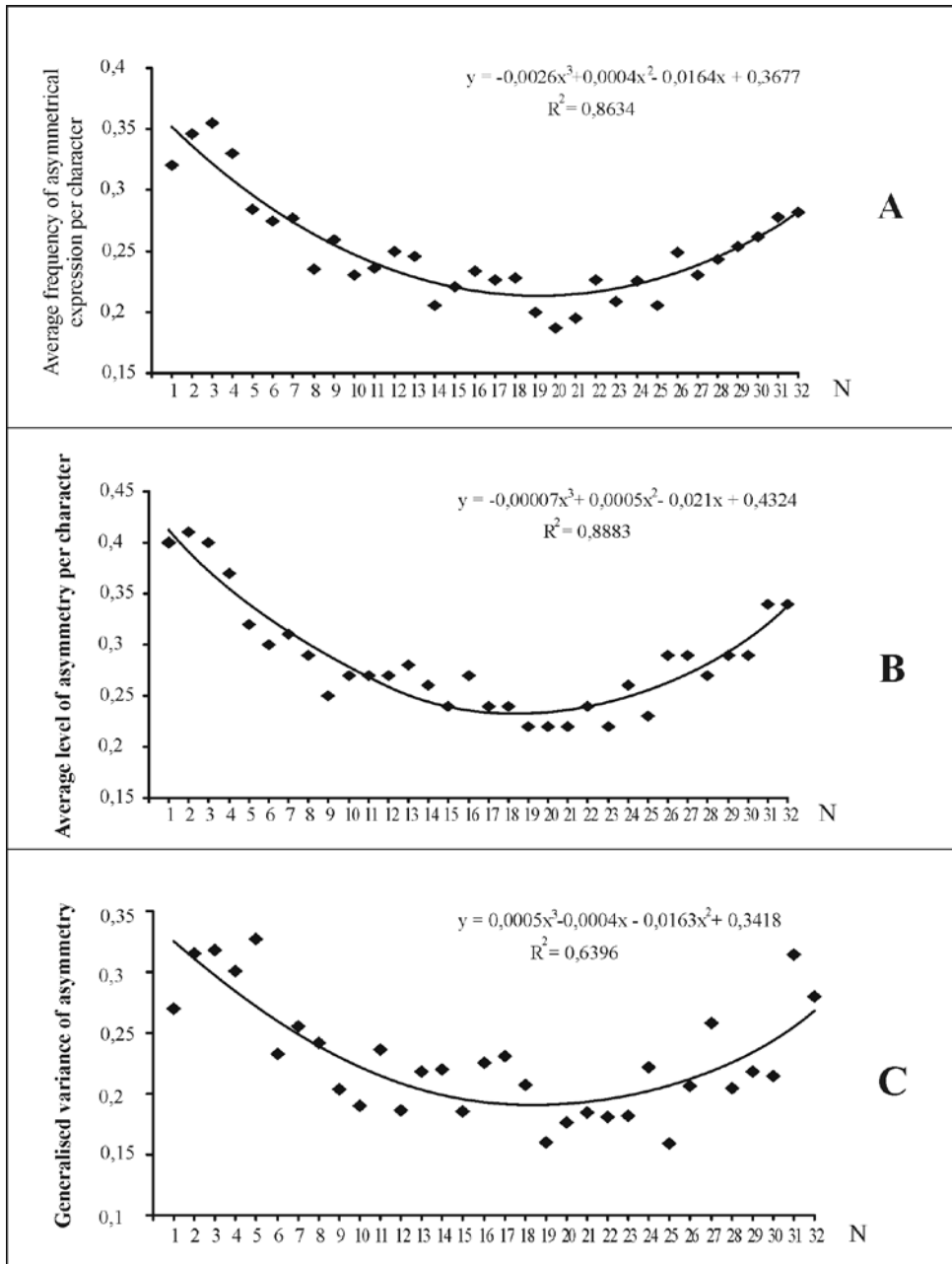


Fig. 3. Values of the indexes of developmental stability (A – average frequency of asymmetrical expression per character; B – average level of asymmetry per character; C – generalised variance of asymmetry) in the samples of sand lizard (*Lacerta agilis*) in the European part of Russia. N – the sample serial number (the samples are arranged in geographical order).

The similarity of data obtained in laboratory experiment and in natural population studies is evident (Figs 1 and 3). All the indexes showed similar patterns of changes and even proved to have similar absolute values. It was more pronounced for minimal values that were almost identical in laboratory and natural populations. The higher increment rate of the values at low growth temperatures in laboratory experiment corresponded to the higher rate of fluctuating asymmetry at the northern periphery of the geographic range than at the southern one.

Maximal values of fluctuating asymmetry in natural populations were at the same time lower than those in laboratory populations at the extreme growth temperatures. We regard the following as the possible causes: (1) the range of growth temperatures in the experiment was wider than in natural conditions; (2) not constant but fluctuating temperature regimes can be considered as optimal for the development; and (3) a laying period shift allows avoiding the effect of non-optimal temperature in natural conditions.

In this way, the patterns of developmental stability indexes over growth temperature range in laboratory and natural populations proved to be similar. This provided the basis for the following conclusions: (1) the values of developmental stability indexes in experimental group under optimal developmental conditions were the same as in natural populations from the central part of the geographic range where developmental conditions were also considered to be optimal; and (2) the lack of intraspecific differences in optimal developmental conditions and the growth of fluctuating asymmetry both at low and high experimental temperatures as well as in the northern and southern marginal populations assumed that not all the populations existed under optimal conditions. It presumes that developmental conditions affect phenotypic diversity which is sufficient to enable the state of populations to be described from the point of view of ontogeny.

Thus, the value of developmental stability was maximal under optimal conditions at the central part of the geographic range and decreased under non-optimal conditions in marginal populations.

On the whole, the results of this study show the applicability of developmental stability for identification of optimal developmental conditions in the framework of natural populations studies.

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