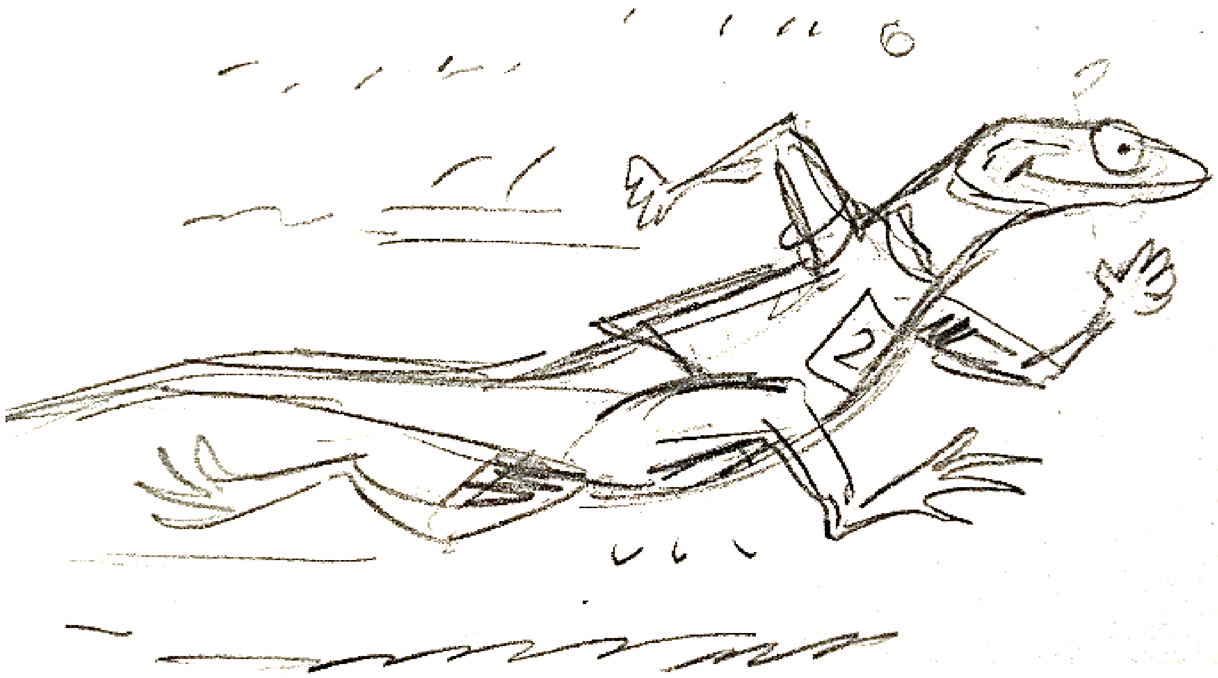

seppo.net – environmental cartoon

Does morphology matter? Unravelling the evolutionary significance of morphological variation in *Podarcis* wall lizards

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NOTA PRÉVIA

Na elaboração desta tese, e nos termos do número 2 do Artigo 4º do Regulamento Geral dos Terceiros Ciclos de Estudos da Universidade do Porto e do Artigo 31º do Decreto-Lei 74/2006, de 24 de Março, com a nova redação introduzida pelo Decreto-Lei 230/2009, de 14 de Setembro, foi efetuado o aproveitamento total de um conjunto coerente de trabalhos de investigação já publicados ou submetidos para publicação em revistas internacionais indexadas e com arbitragem científica, os quais integram alguns dos capítulos da presente tese. Tendo em conta que os referidos trabalhos foram realizados com a colaboração de outros autores, o candidato esclarece que, em todos eles, participou ativamente na sua conceção, na obtenção, análise e discussão de resultados, bem como na elaboração da sua forma publicada. A instituição de origem da candidata foi a Faculdade de Ciências da Universidade do Porto, tendo o trabalho sido realizado sob orientação da Doutora Antigoni Kaliontzopoulou, Investigadora Auxiliar do Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO-InBio) e sob co-orientação do Doutor Miguel Carretero, Professor Auxiliar Convidado no Departamento de Biologia da Faculdade de Ciências da Universidade do Porto e Investigador Principal do Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO-InBio).

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SUMMARY

How do performance, behaviour and ecology drive phenotypic diversity? Despite numerous interspecific studies identifying a relationship between morphology and ecology, we still lack an understanding of how selection moulds morphology-performance-behaviour associations at the intraspecific level. Lizards provide excellent models for such analysis in light of the ecomorphological paradigm, as their phenotypic traits are easily quantified. Studies with species exhibiting high levels of phenotypic variability, as *Podarcis* wall lizards, are relevant for identifying the microevolutionary mechanisms underlying variation in functional performance and that may in turn affect fitness.

As such, this thesis aimed to study the interplay between morphology, performance, and environmental variation in a functional morphology framework, using *Podarcis* sp. as a model. Locomotor and bite performance were investigated and their relationship with morphology analysed, also considering habitat use and behaviour. We examined how habitat use could mould morphology and performance in syntopic populations of different species or populations of a single species but from different habitats. We also examined how methodological choices can influence the quality of maximal locomotor performance data and the biological interpretations derived. Finally, we examined how different functional components of biting performance are optimized during different types of ecological and social behaviours.

Surprisingly, we did not detect a link between morphology and locomotion between populations of the same species, or when examining co-existing, closely related species. However, locomotion varied substantially across populations of the same species, suggesting a very fast evolutionary response to habitat type. In the context of these studies, we demonstrated that the method used for speed quantification influences the quality of performance descriptors, which can affect our capacity of detecting variation in functional traits. In terms of bite performance, we detected an association with head morphology and differences between animals from different habitats even at the intraspecific level. However, sexual differentiation was the main factor driving variation in head functional morphology, where male bite performance was unaffected by variation in head dimensions and shape caused as a response to habitat type. Indeed, bite performance is highlighted as a trait of major importance in both ecological and social tasks. Interestingly, different bite-performance functions (i.e. duration and maximal capacity) are optimized through different sets of head traits, which yields a morphological trade-off between them.

Put together, the results of this thesis shed more light on the complex interactions between different selective mechanisms that mould morphology-function associations, and sometimes confirm while other contradict the predictions of the ecomorphological paradigm. Overall, the conclusions drawn from the studies included here enhance our understanding of how functional responses induced to fulfil social and ecological requirements contribute to the morphological diversity observed in nature.

Keywords

Morphology, Locomotor performance, Bite performance, Habitat use, Behaviour, Natural selection, Sexual selection, Lab methods, Lizards

RESUMO

Como é que a performance, o comportamento e a ecologia impulsionam a diversidade fenotípica? Apesar dos inúmeros estudos interespecíficos que identificam uma relação entre morfologia e ecologia, a compreensão de como a selecção molda as associações entre morfologia-performance-comportamento ao nível intraespecífico ainda não existe. Os lagartos são excelentes modelos para tal análise como proposto pelo paradigma ecomorfológico, já que suas características fenotípicas são facilmente quantificáveis. Estudos com espécies que exibem altos níveis de variabilidade fenotípica, como as lagartixas do género *Podarcis*, são relevantes para identificar os mecanismos microevolutivos subjacentes à variação da performance funcional e que, por sua vez, podem afetar o fitness.

Assim sendo, esta tese teve como objetivo estudar a interação entre morfologia, performance e variação ambiental num contexto de morfologia funcional, utilizando *Podarcis* sp. como modelo. Investigamos a performance locomotora e de mordida e analisamos a sua relação com a morfologia, considerando também o uso do habitat e comportamento. Foi examinado como o uso do habitat poderia moldar a morfologia e a performance em populações sintopicas de diferentes espécies ou populações de uma única espécie, mas de diferentes habitats. Também examinamos como as escolhas metodológicas poderiam influenciar a qualidade dos dados de máxima performance locomotora e as interpretações biológicas derivadas. Por último, examinamos como as diferentes componentes funcionais da performance de mordida são optimizadas durante diferentes tipos de comportamento ecológico e social.

Surpreendentemente, não detectamos uma ligação entre morfologia e locomoção entre populações da mesma espécie, ou quando examinamos espécies coexistentes no mesmo local e intimamente relacionadas. No entanto, a locomoção variou substancialmente em populações da mesma espécie, sugerindo uma resposta evolutiva muito rápida ao tipo de habitat. No contexto desses estudos, demonstramos que o método utilizado para a quantificação da velocidade influência a qualidade dos descritores da performance, o que pode afetar a nossa capacidade de detectar variações em características funcionais. No que diz respeito à performance de mordida, detectamos uma associação com a morfologia da cabeça e detectamos diferenças entre animais de diferentes habitats, mesmo ao nível intraespecífico. No entanto, a diferenciação sexual foi o principal factor que determinou a variação na morfologia funcional da cabeça, onde a performance da mordida nos machos não foi afetada pela variação da forma e dimensões da cabeça causada como resposta ao tipo de habitat. De facto, é de destacar que a performance da mordida é uma característica de grande importância em tarefas ecológicas e sociais. Curiosamente, diferentes funções da performance da mordida (ou seja, duração e capacidade máxima) são optimizadas através de diferentes conjuntos de características da cabeça, o que produz um “trade-off” morfológico entre eles.

Em conjunto, os resultados desta tese ajudam a perceber as interacções complexas entre diferentes mecanismos selectivos que moldam as associações da função morfológica, e algumas vezes confirmam enquanto outras contradizem as previsões do paradigma ecomorfológico. No geral, as conclusões obtidas dos estudos incluídos nesta tese aumentam a nossa compreensão de como as respostas funcionais necessárias para cumprir os requisitos sociais e ecológicos contribuem para a diversidade morfológica observada na natureza.

Palavras-chave

Morfologia, Performance locomotora, Performance de mordida, Uso do habitat, Comportamento, Selecção natural, Selecção sexual, Metodos de laboratório, Lagartixas

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CF - Closed front
CL - Closed back
CLI - Climbing capacity
CLI60 - Cork-substrate racetrack tilted to an angle of 60º
CLI60s - Sandpaper-substrate racetrack tilted to an angle of 60º
CLI90 - Cork-substrate racetrack tilted to an angle of 90º
CV- Coefficient of variation
DEV – Deviance
F - Females
HAB - Habitat
HGR - Height from the ground
HH – Head height
HL – Head length
HLL – Hind limb kength
HS - Head size
HW – Head width
FLL – Fore limb length
INC – Inclination
LJL - Lower-jaw length
M - Males
MAN – Manoeuvrability
MO - Mouth opening
mSPEEDS - Multivariate set of maximal locomotor speeds
OF - Open front
OL - Open back
PBF - P. bocagei females
PBM - P. bocagei males
PGF - P. g. lusitanicus females
PGM - P. g. lusitanicus males
PLS - Partial least-squares regression
POP - Population
prjHH – SIZE corrected head height
prjHL – SIZE corrected head length
prjHLL – SIZE corrected hind limb kength

prjHW – SIZE corrected head width

prjFLL – SIZE corrected fore limb length

prjTRL – SIZE corrected Trunk length

R.DEV - Residual deviance

ROCKD - Diameter of the rock

SIZE – Isometric size

SP – Species

SPR - Sprint speed

SVL - Snout-vent length

SUBS - Substrate

TRL – Trunk length

Var - Variance

Veg - Vegetation

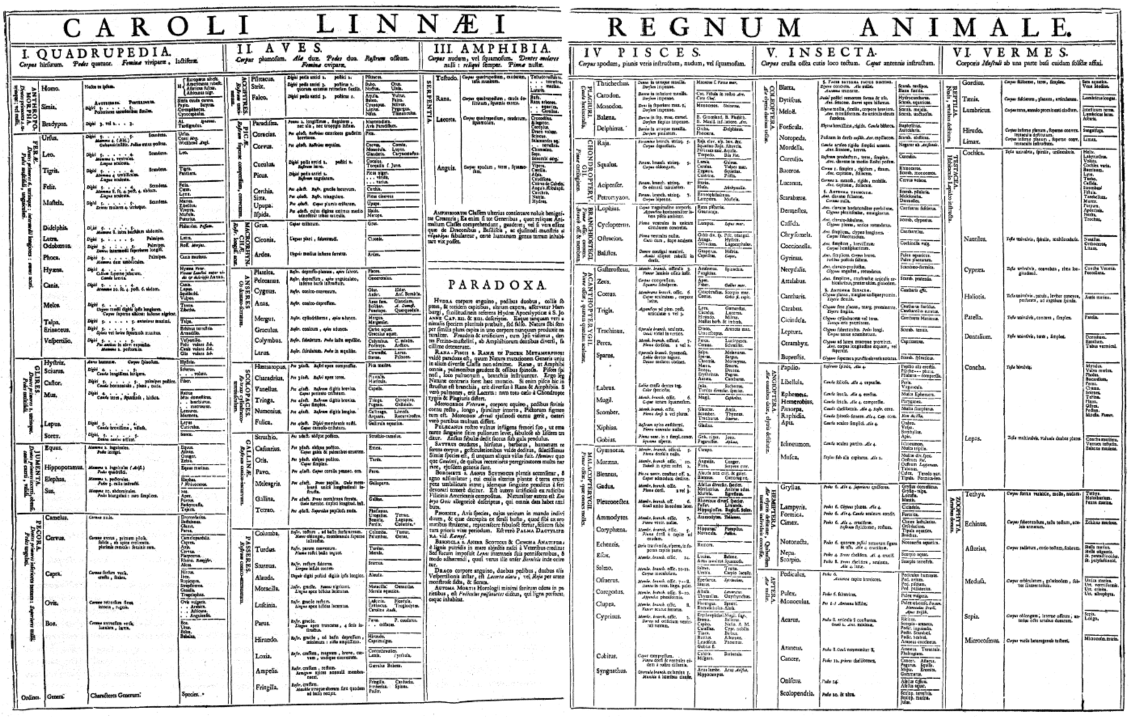
Chapter 1

General Introduction

Why Morphology

Form and function have always intrigued biologists and they are among the earliest records available regarding the study of animals. The understanding of the connection between the two – form and function – date back to Aristotle (Greece, 384-322 B.C., Russel 1916). He provided a detailed description of the structure of animals, that he collected, dissected, and classified using scientific methodologies. He not only described the structure but also tried to understand the relation between form and function and even the possibility of structural adaptation (Russel 1916). After Aristotle the study of form – anatomy – has been a central topic in the field of comparative morphology. Subsequently, the names of several researchers became a point of reference because they dedicated their lives to describe and classify biological diversity:

- Carl von Linné (Sweden, 1707-1778) – established the foundation of hierarchical structure of classification based on relative similarities of body form, which is still the base of the modern scheme of scientific classification and binomial nomenclature (Fig. 1.1) even if still deprived of evolutionary context;



CAROLI LINNÆI REGNUM ANIMALE.

I. QUADRUPEDIA. II. AVES. III. AMPHIBIA. IV. PISCES. V. INSECTA. VI. VERMES.

PARADOXA.

Figure 1.1 – Table of the Animal Kingdom - *Regnum Animale* - from *Systema Naturæ* (1735).

- Georges-Louis Leclerc, Comte de Buffon (France, 1707-1788) – he was no morphologist, but instead a naturalist that influenced next generations of zoological researchers (Lamarck and Cuvier). Considered by Charles Darwin "... the first author who in modern times has treated it [evolution] in a scientific spirit ..." (Darwin 1872);

- Jean-Baptiste Lamarck (France, 1744-1829) – the two main points in his biological work were that environment can lead to changes in animals and that life was structured in an orderly manner (Osborn 1905);
- Georges Cuvier (France, 1769-1832) – like Aristotle, he studied form and function together, even putting function before form. Cuvier was very critical to the theories of evolution proposed by Lamarck and Geoffroy Saint-Hillaire;
- Johann Wolfgang von Goethe (Germany, 1749-1832) – he was a poet but also a morphologist. He focused his work on homologies, used later by Darwin as evidence of common descent and laws of variation;
- Étienne Geoffroy Saint-Hillaire (France, 1772-1884) – he defended the same theory as Lamarck considering evolution. He believed in the underlying unity of organismal design, and the potential of the transmutation of species in time;
- Richard Owen (England, 1804-1892) – he was a comparative anatomist and palaeontologist. Owen accepted Darwin's theory in evolution but he believed that it was more complex than presented in Darwin's *On the Origin of Species* (1872);
- Francis Galton (England, 1822-1911) – he was a pioneer in biometrics. Galton was the first using statistical methodologies to study variation in human populations;
- Karl Gegenbaur (Germany, 1826-1903) – he was a German anatomist who demonstrated that comparative anatomy holds important information supporting the theory of evolution proposed by Darwin;
- Ernst Haeckel (Germany, 1834-1919) – he promoted and popularised Darwin's work and he is also known because of his recapitulation theory;
- Francis Maitland Balfour (England, 1851-1882) - a successor of Darwin and considered a great biologist. Balfour publish the book of Comparative Embryology which gave him recognition in the field of animal morphology.

How form and function are associated with the environment where organisms live was considered the work of a divine creator until Darwin (1859). Charles Darwin (1859) departing from the same observation - relation between form and function -, transformed the argument for the power of evolution by natural selection. In more recent years, the association between morphology and ecology has become one of the main topics of comparative biology (reviewed in Ricklefs and Miles 1994).

Ecomorphology

Differences in an organism's functional morphology should result in differences in performance which in turn translate into variation in fitness in a given environment (Arnold 1983; Emerson and Arnold 1989; Irschick 2002). The link between morphology, performance and fitness is usually addressed in the framework of the ecomorphological paradigm (Arnold 1983; Fig. 1.2). This framework can be divided in two parts: (i) investigating if functional capacities are influenced by morphological traits – i.e., describing the performance gradient – which can be studied in the laboratory, and (ii) studying how differences in functional capacities lead to variation in fitness – i.e., quantifying the fitness gradient – which can be studied in the field (Garland and Losos 1994; Irschick 2002; Kingsolver and Huey 2003).

Figure 1.2 – Original Arnold's paradigm (1983) – simplified version.

This paradigm has been extended to include not only ecomorphological associations, but also social functions (Garland and Losos 1994; Fig. 1.3). In this context, one may consider how natural and sexual selection mould morphology to optimize whole-organism performance by influencing the traits involved in ecological functions - feeding, escape from predators, habitat and refuge use - and social interactions - intraspecific antagonistic behaviour, territory and mate acquisition, competition with conspecifics. However, morphology-performance relationships are not linear, but are also modulated by behaviour and/or physiology. Therefore, performance and fitness could be (partially) uncoupled from morphological variation if organisms behaviourally control how they implement their performance capacities (Garland and Losos 1994; Fig. 1.3). This control can in turn be influenced by environmental effects or inter- and/or intraspecific interactions (Garland and Losos 1994; Fig. 1.3).

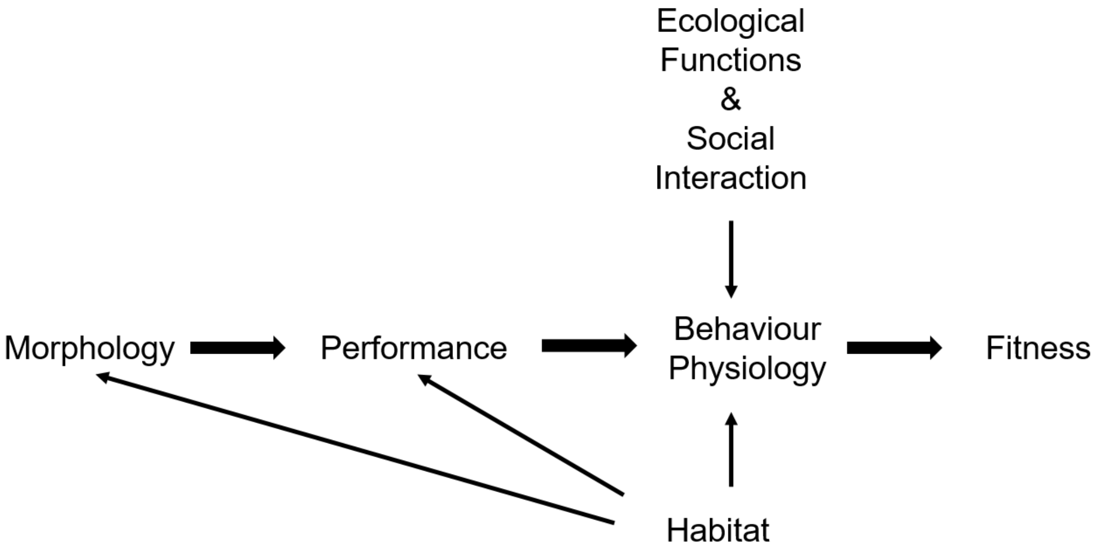


Figure 1.3 – Expansion of Arnold's paradigm - incorporation of factor that likely affect aspect of the paradigm. Redrawn from Figure 10.3 of Garland and Losos 1994.

Arnold’s conceptual framework was originally described for studying adaptation at the intraspecific level – i.e. “*the analysis of adaptation within populations of conspecifics*” (Arnold 1983: 348). However, this approach has been extensively used for interspecific analyses of ecomorphological associations (e.g. Dumont & Herrel 2003, Elstrott & Irschick 2004, Goodman et al 2008, Irschick & Losos 1999, Lailvaux & Irschick 2007, Losos 1990a,b, Santana et al 2010, Van der Meij & Bout 2004, Vanhooydonck & Van Damme 2001, 2003). A textbook example are *Anolis* lizards, where sympatric species that emerged through an adaptive radiation repeatedly evolved divergent morphologies, performance capacities and behaviours to be able to exploit different habitats, enhancing niche segregation between the so-called ecomorphs (Losos and Sinervo 1989). Despite the numerous studies revealing this association between morphology and performance in this model group, several other studies have failed to detect similar correlations in other cases, both at the intra- and interspecific levels (Brecko et al., 2008; Goodman et al., 2008; Van Damme et al., 1997; Vanhooydonck et al., 2000), challenging the generality of the ecomorphological paradigm. Even in *Anolis* lizards the relationship between morphology and ecology differs when comparing mainland species to those inhabiting the Caribbean (Irschick et al 1997). Of particular importance is to note that the magnitude of variation between species is indeed bigger and generally it is easier to detect differences among groups than when examining variation between individuals within a single species (Losos and Miles 1994; Kaliontzopoulou et al 2010). Nevertheless, studies at the intraspecific level are not so common although they are relevant for identifying microevolutionary mechanisms and linking them to the adaptive pressures that lead to fine-scale phenotypic differentiation (Irschick et al. 2005a).

Morphology-Performance relationships

Several studies have investigated whole-organism performance to better understand the relationships among phenotypic traits that culminate in differences in fitness in a given environment (Ghalambor et al. 2003; Miles 2004; Mowles et al. 2010; Santana et al. 2010; Herrel et al 2012; McGinley et al 2013; Irschick and Higham 2016). The goal of measuring animal performance in laboratory conditions is to measure maximum performance free of other constraints (e.g. predators, competitors, temperature fluctuations, etc.) so that comparisons among individuals are meaningful (Losos et al 2002). Remarkably, in terms of published articles, locomotor performance is the most measured performance trait, followed by bite performance (Lappin & Jones 2014).

Locomotor performance

It has been shown that locomotor performance is relevant for social and ecological tasks, as animals need to search for prey, escape from predators, find mates and defend territories (Irschick and Losos 1999, Miles et al 2000, Domenici 2001; Irschick et al 2002, Vásquez et al 2002; Blumstein et al 2004; Husak 2006; Husak et al 2006a; Husak et al 2008; Mowles et al 2010; Pruitt 2010; Irschick and Higham 2016). Different locomotor skills with relevance for different ecological and social tasks can be quantified in laboratory conditions, such as maximal sprint speed (Garland and Losos 1994, Punzo 2003; Blumstein et al. 2004; Miles 2004; Husak et al. 2006a; Husak 2006; Médoc and Beisel 2008; Tulli et al. 2012; Collins et al. 2013; McGinley et al. 2013; da Silva et al. 2014; Zamora-Camacho et al. 2015), endurance (Weinstein and Full 1999, Miles et al 2000, Irwin et al 2003, Vanhooydonck and Van Damme 2003, Mowles et al 2010, Herrel and Bonneaud 2012), climbing ability at different angles (Vanhooydonck and Van Damme 2001, 2003, Goodman et al 2008, Kaliontzopoulou et al 2013), manoeuvrability (Vanhooydonck and Van Damme 2003, Kaliontzopoulou te al 2013), jumping (Losos 1990a, Goodman et al 2008), sand-diving (Arnold 1995), gliding

(McGuire and Dudley 2005), and swimming speed (Drucker and Lauder 2000; Domenici 2001; Rivera et al. 2006).

Biomechanical models predict a specific set of morphological traits depending on the locomotor performance (Van Damme et al 2003, Fig. 1.4) or the other way around, where given a known locomotor performance a prediction of morphological traits can be made. For example, animals considered as runners (high sprint speed at horizontal surfaces) will benefit from long hind limbs that enable longer strides and with a ling floating phase (Van Damme et al 2003). Furthermore, the body should be laterally flattened to enable lateral body flexion and maximize stride length (Van Damme et al 2003).

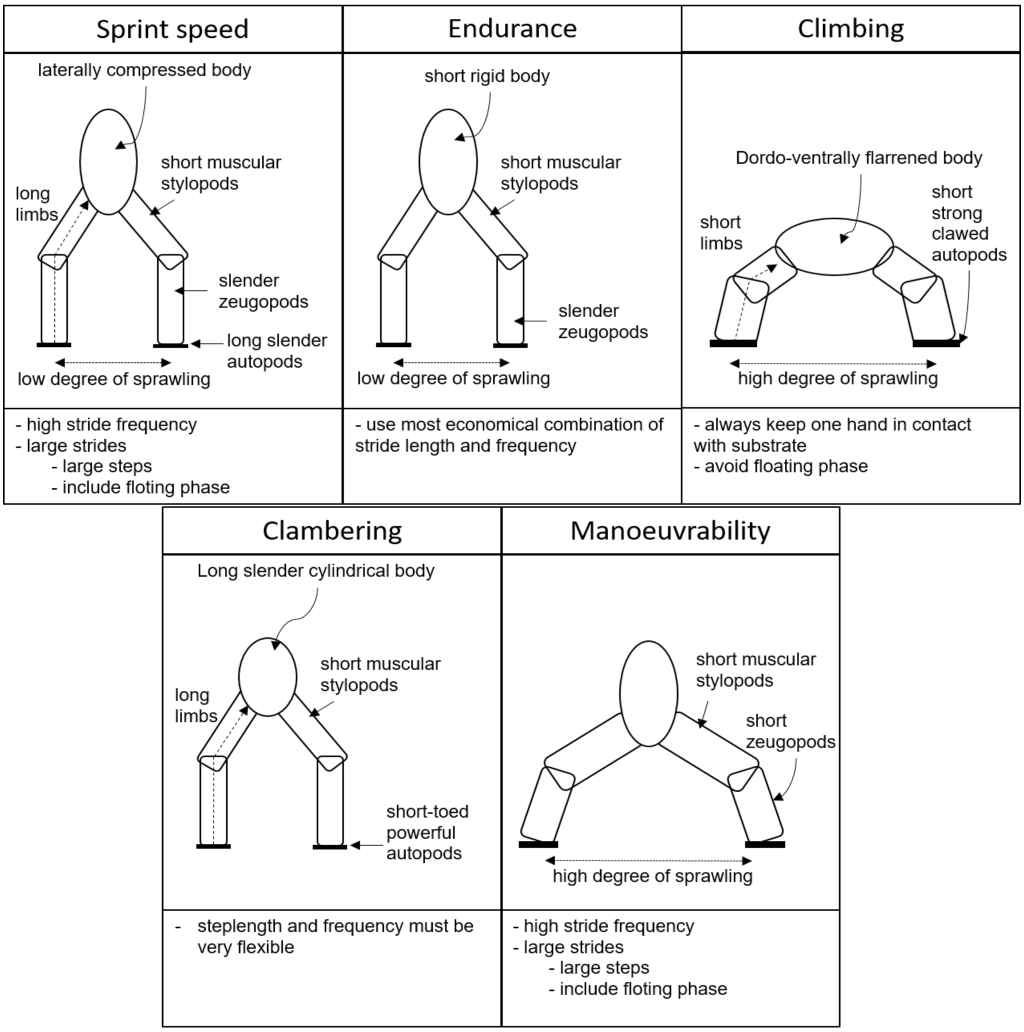


Figure 1.4 – Hypothesis of morphological traits, shape and gait characteristics in lacertids adapted to master a specific locomotor ability. Adapted from Figure 3 of Van Damme et al 2003.

Bite performance

Head morphology and its association with bite performance and ecology is one of the most studied animal functional systems (e.g. Dessem & Druzinsky 1992; Dumont & Herrel 2003; Erickson et al. 2004; Herrel et al. 1999; van der Meij and Bout 2004). Feeding is associated with head morphology and bite performance (Herrel et al. 1998a, 2001a; Metzger and Herrel 2005; Vincent and Herrel 2007), where natural selection may play a role in head shape and bite performance diversification. Head size and shape is a sexual dimorphic trait in several species (Cooper and Vitt 1989; Preest 1994; Braña 1996; Kratochvíl and Frynta 2002; Kaliontzopoulou et al. 2008). This pattern enhances male potential because of antagonist behaviours and territoriality (Stamps 1983; Andersson 1994; Braña 1996; Huyghe et al. 2005) where selection acts through its influence on bite force (Lappin and Husak 2005; Lappin et al. 2006, Husak et al. 2006b; Lailvaux and Irschick 2007; Husak et al. 2009; Herrel et al. 2010).

The investigation of the association between body size, head morphology and bite performance has been extensive in lizards (e.g. Herrel et al. 2001c; Verwaijen et al. 2002; Herrel and O'Reilly 2006; Lappin et al. 2006; Huyghe et al. 2009; Measey et al. 2009), where an association between bite force and external head traits has been demonstrated (Herrel et al. 1999, 2001b; Lappin and Husak 2005). However, different head traits can be responsible of this association depending on the species investigated (for example: Herrel et al. 2001c; Lappin and Husak 2005). These head properties influencing bite force can be size of the individual and more importantly head size, teeth shape, in-lever-out-lever proportions of the jaw, insertion points and orientation of the jaw musculature (Herrel et al. 1998, 2001d).

Relevance of Habitat use

Understanding how the environment affects function (i.e. performance) is a central goal for investigators of different areas as ecology, evolution and conservation biology (Aerts et al., 2000; Arnold, 1998; Collar et al., 2010; Elstrott and Irschick, 2004; Kaliontzopoulou et al., 2010). The habitat where an animal lives might be a crucial determinant of the morphology of individuals, populations and species (Losos, 1990a; Losos, 1990b; Losos et al., 1998; Van Damme et al., 1998; Goodman et al., 2008; Bergmann et al., 2009; Bergmann and Irschick, 2010; Vanhooydonck et al., 2005; Vanhooydonck et al., 2011). Convergent morphological traits in unrelated organisms from similar habitats has commonly been associated with natural selection and ultimately adaptation (e.g. Losos 2011).

> "*. . .we can clearly understand why analogical or adaptive characters,
> although of the utmost importance to the welfare of the
> being, are almost valueless to the systematist. For animals
> belonging to two most distinct lines of descent, may readily
> become adapted to similar conditions, and thus assume a close
> external resemblance*. . ." Charles Darwin (1859, p. 427).

In other words, a similar morphology is expected if the environment is similar even in distantly related taxa and different morphological traits should appear when selection act upon related individuals to became specialized in fulfilling a specific performance given the environment. Natural selection may operate on individuals in contrasting ways depending on the surrounding environment and may enhance evolution of substantial differences in locomotor function (Losos 2011).

Lizards as model organisms

The association between morphological traits and environment has been extensively investigated in squamate reptiles (lizards and snakes), which include a diverse range of taxa with a wide variety of ecologies, morphologies, behaviours and phylogenetic origins (e.g. Pianka 1986, Losos 1990, Miles 1994, Vanhooydonck and Van Damme 1999, Irschick and Vanhooydonck 2002). Lizards provide excellent models for ecomorphological studies (Losos 1990a, Arnold 1998, Herrel et al 1999, Irschick and Garland 2001, Dumont and Herrel 2003, Kingsolver and Huey 2003) because their morphology and performance are easy to quantify (Garland and Losos 1994). Also, lizards display a wide range of ecologies and microhabitat use, where closely related species or even populations can show contrasting microhabitat preferences. Finally, from a practical point of view, lizards are easy to spot, catch and manipulate and it is also easy to keep them in the laboratory.

The case Study – *Podarcis* spp.

In the Mediterranean Basin, wall lizards (*Podarcis* spp.) are intensively studied phylogenetically (e.g. Carretero 2008, Kaliontzopoulou et al 2011, Poulakakis et al 2005, Salvi et al 2013, Van Damme et al 1998), morphologically (e.g. Braña & Ji 2000, Huyghe et al 2007, Kaliontzopoulou et al 2012, Van Damme et al 1997), and ecologically (e.g. Huyghe et al 2007, Kaliontzopoulou et al 2010, 2013, Ortega et al 2017, Sannolo et al 2018, Van Damme et al 1997). They provide an intriguing model system for investigating the evolutionary meaning of phenotypic traits, as they exhibit generally preserved body plans, but also high inter- and intraspecific morphological variation (Arnold 1998, Kaliontzopoulou et al 2012). They are very widespread around the Mediterranean, where they are almost omnipresent in native and anthropized ecosystems. Thus, they are a good model for studying how environmental diversity drives the evolution of organismal diversity. Detailed knowledge exists on how morphology varies between the sexes (Herrel et al 1998, Kaliontzopoulou et al 2010, 2012, 2013, 2015), across different environments (Kaliontzopoulou et al 2010, 2015), and across species (Kaliontzopoulou et al 2012). Further, some evidence has been presented regarding the relationship between morphology and performance (Braña & Ji 2000, Huyghe et al 2007, Kaliontzopoulou et al 2013). Therefore, there is a solid basis on how morphology varies at different biological levels which allows to formulate specific prediction about performance and its relation with environment.

Thesis objectives and structure

The main goal of this PhD project was to examine the interplay between morphology, performance, and environmental variation in lizards in a functional morphology framework, using *Podarcis* spp. as a model organism.

This thesis is organised in six Chapters. The present chapter - Chapter 1 – is a General Introduction which provides a summary of the current knowledge to the subjects and questions developed in the following chapters. Chapter 2, 3, 4 and 5 include four scientific manuscripts developed with the purpose of investigating how natural and sexual selection, mould the interaction between morphology and performance in lizards, given their surrounding environment, and also taking into account the methodological aspects affecting data acquisition.

Chapter 2 presents a study entitled: “The relevance of morphology for habitat use and locomotion in two species of wall lizards” and it was published in *Acta Oecologica*. The main aim of this study was to investigate the three components of the ecomorphological paradigm, morphology, locomotor performance and habitat use, using a two-species system with *Podarcis bocagei* and *Podarcis guadarramae lusitanicus* as a case study to examine whether morphological variation is associated with habitat use and determine the potential relevance of locomotor performance for such an association.

In Chapter 3 a methodological work is presented where we explored how the method used to estimate maximum running speed can influence the statistical properties of the obtained data and, in consequence, biological interpretations. This chapter comprises a published paper entitled “Instantaneous versus interval speed estimates of maximum locomotor capacities for whole-organism performance studies” that has been published in the journal *Evolutionary Biology*.

The work developed in Chapter 4 aimed to investigate how evolutionary forces mold morphological traits and performance differently given the surrounding environment, at the intraspecific level. This chapter includes a paper entitled “Run for your life, but bite for your rights? How interactions between natural and sexual selection shape functional morphology across habitats”, that has been published in *The Science of Nature*.

The main aim of the work developed in Chapter 5 was to examine how different functional components of biting performance are optimized for different demands during ecologically and socially relevant tasks. This chapter includes a paper currently in preparation entitled “Morphological trade-offs underlie the duration and magnitude of bite force generation in lizards”.

Finally, Chapter 6 summarizes the main conclusions of this thesis and explores future challenges emerging as a direct result of the thesis´ findings.

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Chapter 2

Locomotor performance interspecific study

Article I - The relevance of morphology for habitat use and locomotion in two species of wall lizards

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ABSTRACT

Understanding if morphological differences between organisms that occupy different environments are associated to differences in functional performance can suggest a functional link between environmental and morphological variation. In this study we examined three components of the ecomorphological paradigm e morphology, locomotor performance and habitat use e using two syntopic wall lizards endemic to the Iberian Peninsula as a case study to establish whether morphological variation is associated with habitat use and determine the potential relevance of locomotor performance for such an association. Differences in habitat use between both lizards matched patterns of morphological variation. Indeed, individuals of *Podarcis guadarramae lusitanicus*, which are more flattened, used more rocky environments, whereas *Podarcis bocagei*, which have higher heads, used more vegetation than rocks. These patterns translated into a significant association between morphology and habitat use. Nevertheless, the two species were only differentiated in some of the functional traits quantified, and locomotor performance did not exhibit an association with morphological traits. Our results suggest that the link between morphology and habitat use is mediated by refuge use, rather than locomotor performance, in this system, and advise caution when extrapolating morphology-performance-environment associations across organisms.

KEYWORDS

Morphology, Habitat, Performance, Lizards

INTRODUCTION

Whether or not, and how, organisms adapt to their environment are two central questions in ecology, evolution and conservation biology (Aerts et al., 2000; Arnold, 1998; Collar et al., 2010; Elstrott and Irschick, 2004; Kaliontzopoulou et al., 2010a). Different climatic, structural and biotic characteristics of the environment may impose ecological pressures on organisms, and mould phenotypic evolution and morphological diversification (Irschick and Garland, 2001; Ricklefs et al., 1981; Vitt et al., 1997). To start understanding the potential evolutionary meaning of morphological variation, we need to evaluate whether specific traits increase the functional capability of an organism (Arnold, 1983; Garland and Losos, 1994). This is usually done in the framework of the ecomorphological paradigm, where differences in morphology are expected to be associated with different ecological performance capacities (e.g. maximum sprint speed), which in turn translate into variation in fitness among individuals in a given environment (Arnold, 1983). Here, selection acts on whole-organism performance to maximize the ability of the individuals to perform certain ecological and social tasks, guarantee their survivorship, and enhance their reproductive success, with correlated effects on morphological characters of biomechanical relevance for these functions (Irschick et al., 2008). Because the functional challenges that organisms face vary across environments, this type of microevolutionary process eventually leads to an association between morphological and environmental variation. As such, establishing whether morphological differences among organisms that occupy different environments are reflected into differences in functional performance can help us to disentangle the complex relationship between traits that culminate in differences in fitness and enhance our understanding of the underlying evolutionary mechanisms (Irschick and Garland, 2001; Irschick et al., 2005a; Miles, 2004; Vanhooydonck and Van Damme, 2001).

Lizards have been extensively used as model organisms for ecomorphological studies because they are present in a great variety of habitats, and they exhibit a wide range of morphologies and locomotor modes (Arnold, 1998; Garland and Losos, 1994; Irschick and Garland, 2001; Irschick, 2002). Numerous studies indicate that different aspects of locomotor performance are ecologically relevant in different structural habitats, as lizards need to move to capture prey, escape from predators, thermoregulate, find mates and defend territories (Garland and Losos, 1994). As such, those lizard species that live in open areas and use sprinting as their main antipredatory strategy are considered as runners, and they are expected to have long hind limbs to enable longer strides; relatively short forelimbs, to avoid interference with the cycling of the hind limbs; and laterally flattened bodies, to enhance lateral flexibility and maximise stride length (Da Silva et al., 2014; Kaliontzopoulou et al., 2010a; Losos and Sinervo, 1989; Melville and Swain, 2000; Vanhooydonck and Van Damme, 2001). In contrast, lizards living mostly in rocks are considered as climbers, and they are expected to have shorter limbs and dorsally flattened bodies, to lower the centre of gravity closer to the substrate (Da Silva et al., 2014; Kaliontzopoulou et al., 2010a; Melville and Swain, 2000; Sinervo and Losos, 1991; Van Damme et al., 1997; Vanhooydonck and Van Damme, 2001). Therefore, traits that are favoured in one microhabitat preference may conflict with those that are beneficial in another (Clemente et al., 2013).

Podarcis wall lizards from the Iberian Peninsula and North Africa form a monophyletic clade and they are considered a cryptic species complex (i.e. *Podarcis hispanica* species complex: Kaliontzopoulou et al., 2011; Kaliontzopoulou et al. 2012a). In NW Iberia, we encounter two endemic forms – *Podarcis bocagei* and *P. guadarramae lusitanicus* (Geniez et al., 2014; previously treated as *P. hispanica* type 1A, see Kaliontzopoulou et al., 2011). These two sister forms are particularly interesting from an ecomorphological perspective because, unlike most other members of the group, their distributions overlap widely, both at a wide and local geographic scale (Carretero, 2008; Kaliontzopoulou et al., 2011). Further, despite being sister taxa, both forms markedly differ in body shape, whereby *P. bocagei* has relatively longer limbs, and a higher and more rounded head compared to *P. g. lusitanicus* (Galán, 1986; Kaliontzopoulou et al., 2012b; Pérez-Mellado, 1980). It has been suggested that these differences in body shape reflect different habitat preferences, where *P. g. lusitanicus* is highly saxicolous (Galán, 1986; Gosá et al., 1986; Pérez-Mellado, 1980; Sá-Sousa et al., 2002), whereas *P. bocagei* is mostly ground-dwelling (Domínguez and Salvador, 1989; Galán, 1986, 1994; Kaliontzopoulou et al., 2012b; Párez-Mellado, 1980). However, differences in habitat use between the two species have never been formally quantified and the relevance of their potential ecological segregation for their morphological differentiation has never been examined.

In this study, we investigate the three components of the ecomorphological paradigm – morphology, locomotor performance and habitat use – using a community with *P. bocagei* and *P. g. lusitanicus* as a case study to examine whether morphological variation is associated with habitat use and determine the potential relevance of locomotor performance for such an association. For this purpose, we first examine if there are differences in habitat use between both forms, which would suggest that they exploit different ecological resources in terms of structural niche. Further, we test whether both forms differ in locomotor performance, as we would expect under predictions of the ecomorphological paradigm. Finally, we test if individual variation in morphology translates into variation in habitat use and locomotor performance, as suggested by studies in other lizard groups. Based on previous observations on the Morphology and ecology of the two species, and considering ecomorphological patterns in other lizards, we hypothesize that *P. g. lusitanicus*, which has been generally considered as saxicolous, will mainly use rocky environments. In addition, if morphological variation bears a functional meaning, we expect *P. g. lusitanicus*, which has a flattened head and shorter limbs, to perform better when climbing. On the other hand, *P. bocagei*, which is generally ground-dwelling, with a much higher and rounded head and longer limbs, is expected to be more flexible in terms of habitat use. Because this species uses different types of habitat including vegetation (Kaliontzopoulou et al., 2012b; Sá-Sousa, 2001), we predict that it might perform equally well under different locomotor conditions.

MATERIAL AND METHODS

Lizards for this study were captured in the coastal zone near the beach of Moledo (N Portugal coast e 41º500N, 8º520W), where *P. bocagei* and *P. g. lusitanicus* are found in syntopy across a sandy area with rocks, sparse vegetation, and agricultural fields delimited by human constructed stone walls. The total area sampled was about 6500 m2. We collected a total of 121 adult individuals by noosing (García-Muñoz and Sillero, 2010), including 65 animals for studying habitat use and 56 for locomotor performance experiments (see also below).

2.1. Morphological variables

In all individuals captured (i.e.,47 males and 32 females of *P. bocagei*, and 20 males and 22 females of *P. g. lusitanicus*), we measured the following linear biometric traits: trunk length (TRL), head length (HL), head width (HW), head height (HH), fore limb length (FLL) and hind limb length (HLL), using electronic calipers (precision 0.01 mm; see Kaliontzopoulou et al., 2007 for a detailed description of variables). In order to separate size and shape, we calculated the isometric size (SIZE) of each individual by projecting all log-transformed linear measurements on an isometric vector (Kaliontzopoulou et al., 2010a) and used it as a multivariate estimate of total body size. To obtain size-corrected variables representing shape variation, we regressed each linear trait on SIZE and retained the regression residuals (hereafter prjTRL, prjHL, prjHW, prjHH, prjFLL and prjHLL) (Kaliontzopoulou et al., 2010a). Animals used to quantify locomotor performance were also weighted using a digital balance (precision 0.0001 g).

2.2. Habitat use variables

In May 2011, when the activity of lizards is at its maximum, we captured a first set of 48 *P. bocagei* (30 males and 18 females) and 15 *P. g. lusitanicus* (five males and 10 females) in order to perform habitat and microhabitat use observations in the field. For each individual captured, we recorded the exact location of capture, using a high-precision GPS (Trimble GPS GeoExplorer 2008 HX). Each individual was measured to record morphological traits and marked with a unique colour code using non-toxic marker paint, so we could distinguish individuals in the field without capturing them. All the animals were released in the same location of capture. To record microhabitat and habitat use, we performed normalized transects during seven days and 10 h/day with favourable weather conditions. To ensure that all lizards were observed at different times of the day, and therefore capture the individual variability in habitat use, transects were performed in a random order and allowing at least 1 h and a half between repetitions of the same transect. During these transects, we collected a total of 197 observations for *P. bocagei* (114 for males and 83 for females) and 60 observations for *P. g. lusitanicus* (20 for males and 40 for females) and recorded their associated microhabitat variables. To quantify microhabitat use we recorded the type of substrate where the lizard was observed (SUBS: classified as either walls, rocks, vegetation or soil), the height from the ground at the point of observation (HGR), inclination quantified as the angle between the surface where the individual was observed and the horizontal plane (INC), and the diameter of the rock (ROCKD), when lizards were observed on rocks. We also considered habitat used in the 2 m-diameter area surrounding the point of observation by recording the percentage of bare soil, vegetation and rocks. Before subsequent statistical analyses, height from the ground and inclination were transformed as $y = \log10 (x + 0.5)$, rock diameter was logarithmically transformed and all percentages were arcsine-transformed.

2.3. Locomotor performance

In October 2011, we captured a second set of 30 *P. bocagei* (15 males and 15 females) and 26 *P. g. lusitanicus* (15 males and 11 females) in order to quantify locomotor performance. We performed locomotor experiments in the autumn to exclude any potential effects of reproduction (i.e. pregnancy) on female performance (Bauwens and Thoen, 1981). Lizards were placed in opaque cloth bags, transported to the laboratory and housed in individual terraria, where they were fed with live mealworms and provided with water ad libitum. All animals were allowed to rest for two days after arrival to the lab to ensure acclimation. All experiments were carried out at a room temperature of about 31ºC, which is approximately the selected body temperature of the two species (Amaral et al., 2012; Carretero et al., 2012). Prior to and in between all performance trials, lizards were placed for at least 1 h in an individual terrarium that was exposed to direct natural light, allowing them to thermoregulate and attain their preferred body temperature.

For all individuals, five types of locomotor performance were measured (Kaliontzopoulou et al., 2013): sprint speed (one type), climbing capacity (three types) and manoeuvrability (one type). Sprint speed (SPR) on a horizontal surface was measured by chasing animals along a 1 m-long and 15 cm-wide racetrack, on a cork substrate (Braña, 2003; Van Berkum et al., 1989). Climbing capacity was quantified by chasing animals up a similar racetrack, with varying substrates and inclination conditions. These included: (1) a cork-substrate racetrack tilted to an angle of 60º (CLI60), (2) a sandpaper-substrate racetrack tilted to an angle of 60º (CLI60s) and (3) a cork-substrate racetrack tilted to an angle of 90º (CLI90). Cork was used as a substrate because it provides very good traction (Van Damme et al.,1997), while the sandpaper provided a surface similar to a granite rock (Goodman, 2007). To quantify manoeuvrability (MAN), a 0.5 m-long and 15 cm-wide pinboard was placed on the racetrack. This pinboard was made of 8-mm diameter pins placed at equal distances of 35 mm (Vanhooydonck et al., 2000).

Locomotor speed in the five types of racetracks was measured on different days and the order in which animals were subjected to the tests was randomized. Each individual was tested three times in each type of racetrack to ensure that maximal locomotor capacity was recorded. Each run was scored as “bad” or “good” and the “bad” races, in which the animals completely stopped or turned around during the race, were eliminated from the analyses (sensu Tsuji et al., 1989; Van Berkum and Tsuji, 1987).

All trials were filmed with a digital camera (Casio EXILIM EX-F1) at a filming speed of 30 fps. The position of the lizard across each trial was digitized using MaxTRAQ 2D motion analysis software (Innovision Systems Inc., 2009). For each type of racetrack, the highest speed recorded over any 10-cm interval across all repetitions was taken as an estimate of each animal's maximum speed in that racetrack. All data of locomotor performance were logarithmically transformed prior to analyses. After the experiments, all individuals were released in the site of capture.

2.4. Data analyses

Because both species are sexually dimorphic, including the populations in the study site (Kaliontzopoulou et al., 2012a, 2012b, 2013) we included sex as a predictor variable in all statistical analyses conducted. To investigate if there were differences between species and sexes in morphology we used ANOVA procedures. All ANOVA models were run using morphological traits (i.e. SIZE and each size-corrected trait separately) as the response variable, and species, sex and their interaction as predictors.

Concerning microhabitat use, we fitted a log-linear model to examine whether species and sexes were found on different substrates with a different frequency. Additionally, for all continuously represented microhabitat (e.g. HGR, INC, ROCKD) and habitat variables (% of bare soil, vegetation and rocks) we performed permutation ANOVAs using species, sex and their interaction as predictor variables, to evaluate differences between SP × SEX groups. This analysis considered individual observations as independent and therefore encompassed variability within individuals in habitat use. In addition, because some individuals were observed more than once, the same analysis was repeated using a linear mixed-effects model as implemented in the lmer function of the lme4 R-package (Bates et al., 2014) and including individual as a random factor.

To examine whether species and sexes differed in locomotor performance, we performed a MANOVA on the multivariate set of maximal locomotor speeds, and also randomized ANOVA comparisons on each type of locomotor speed separately, with species, sex and their interaction as factors. Subsequently, we performed posthoc comparisons (Tukey's HSD) to test for differences between pairs of groups. We also performed ANCOVA comparisons on the multivariate set of maximal locomotor speeds to examine the effectof species, sex, and their interaction, while considering SIZE and weight as covariates. We always used (M)ANOVA based on permutations, because sample size varied across groups, which resulted in differences in variance. These were based on 1000 permutations of Euclidean distance matrices as implemented in the adonis function of the vegan R-package (Oksanen et al., 2012). Because multiple comparisons were evaluated simultaneously, we implemented the False Discovery Rate (FDR) procedure to adjust the p-values of statistical tests (Benjamini and Yekutieli 2001). To investigate the multivariate association between morphology and performance, and between morphology and habitat, we used a two-block partial least-squares regression as implemented in the plsr function of the pls R-package (Mevik et al., 2011). To investigate the association between morphology and locomotor performance, we only used limb and trunk variables, as these traits have been suggested to determine locomotor performance in these and other lizard species (Kaliontzopoulou et al., 2010a, 2013; Vanhooydonck and Van Damme, 2001). In the case of the association between morphology and habitat use, more than one habitat observation was available for several of the individuals sampled. To address this fact, while maximizing the use of the data available, we used the mean of each variable across observations of each individual and weighted the contribution of each individual to the pls regression by the number of habitat observations available for that individual. All statistical analyses were performed using R v. 2.14.1 (R Development Core Team, 2011).

RESULTS

3.1. Morphological traits

ANOVA comparisons of morphological traits indicated that *P. bocagei* and *P. g. lusitanicus* differed in total of body size, relative trunk length, relative head length and head height (Table 2.1, Table S_2.1 Supp. Inf.). ANOVA also confirmed significant differences between both sexes in SIZE and in all size-corrected traits in our sample, while some significant SP × SEX interactions were also observed. Generally, *P. bocagei* was larger in body size than *P. g. lusitanicus*, with higher but shorter heads. Males were larger in body size than females, with longer and higher heads, shorter trunks, and longer limbs.

Table 2.1 – Results of ANOVA comparisons performed on total body size (SIZE) and size corrected morphological traits to examine the effect of species (SP), sex and their interaction (SP*SEX). F: F-statistic, P: corresponding p-value, adjusted for multiple testing using the False Discovery Rate procedure. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SP		SEX		SP*SEX	
	F	P	F	P	F	P
SIZE	17.052	**0.002**	28.706	**0.002**	4.300	**0.049**
prjTRL	19.541	**0.002**	186.958	**0.002**	8.017	**0.017**
prjHW	0.139	0.766	27.922	**0.002**	2.596	0.161
prjHH	62.798	**0.002**	9.728	**0.002**	20.875	**0.002**
prjHL	43.318	**0.002**	34.996	**0.002**	0.043	0.871
prjFLL	0.027	0.871	11.540	**0.004**	8.405	**0.013**
prjHLL	2.070	0.168	27.830	**0.002**	0.229	0.740

3.2. Habitat use

The log-linear model fitted to investigate microhabitat use revealed differences in the type of substrate where each species was found (point of observation; Table 2.2, Fig. 2.1, Table S_2.2 Supp. Inf.), whereas no significant differences existed between sexes within each species. *P. bocagei* was found with a higher frequency on walls as compared to *P. g. lusitanicus*, which was more frequently encountered on isolated rocks (Fig. 2.1). ANOVA comparisons of microhabitat use indicated significant differences between species, but not between sexes, in rock diameter (Table 2.3). Specifically, *P. bocagei* was encountered in smaller rocks (Fig. 2.2) than *P. g. lusitanicus*. Linear mixed-effects models only showed significant differences between species in rock diameter (p = 0.004).

Table 2.2 – Results of log-linear model performed on microhabitat use variables. See DEV: Deviance, R.DEV: Residual Deviance, P: corresponding p-value. Significant effects are marked in bold letter. Material and Methods for variable abbreviations.

	DEV	R.DEV	P
SUBS	463.122	115.123	**4.68×10^{-100}**
SP	76.956	38.167	**1.75×10^{-18}**
SEX	0.471	37.696	0.493
SUBS*SP	23.408	14.288	**3.32×10^{-5}**
SUBS*SEX	4.937	9.351	0.176
SP*SEX	8.837	0.514	**0.003**
SUBS*SP*SEX	0.514	0.000	0.916

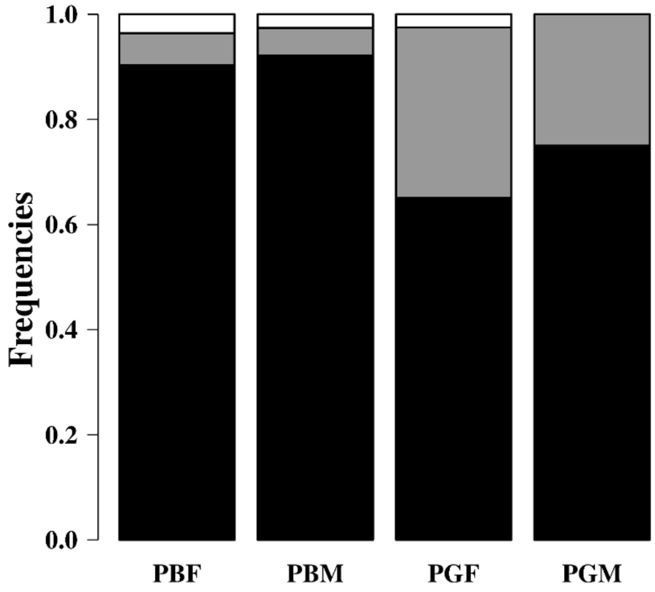

Figure 2.1 – Observed frequencies of each species and sex using each type of substrate. Black: walls; grey: rocks; white: others (vegetation and bare soil). PBF: *P. bocagei* females, PBM: *P. bocagei* males, PGF: *P. g. lusitanicus* females, PGM: *P. g. lusitanicus* males. See Material and Methods for variable abbreviations.

Table 2.3 – Results of ANOVA comparisons performed on all continuously represented microhabitat (HGR, INC, ROCKD) and habitat variables (% of bare soil, vegetation and rocks) to examine the effect of species (SP), sex and their interaction (SP*SEX). F: F-statistic, P: corresponding p-value, adjusted for multiple testing using the False Discovery Rate procedure. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SP		SEX		SP*SEX	
	F	P	F	P	F	P
HGR	5.783	0.086	0.803	0.687	0.097	0.818
INC	0.516	0.687	2.094	0.383	4.562	0.086
ROCKD	30.829	**0.018**	0.483	0.687	2.353	0.383
SOIL%	0.109	0.818	0.054	0.818	0.484	0.687
VEG%	6.922	0.086	0.297	0.740	0.995	0.678
ROCK%	5.947	0.086	0.386	0.687	1.760	0.459

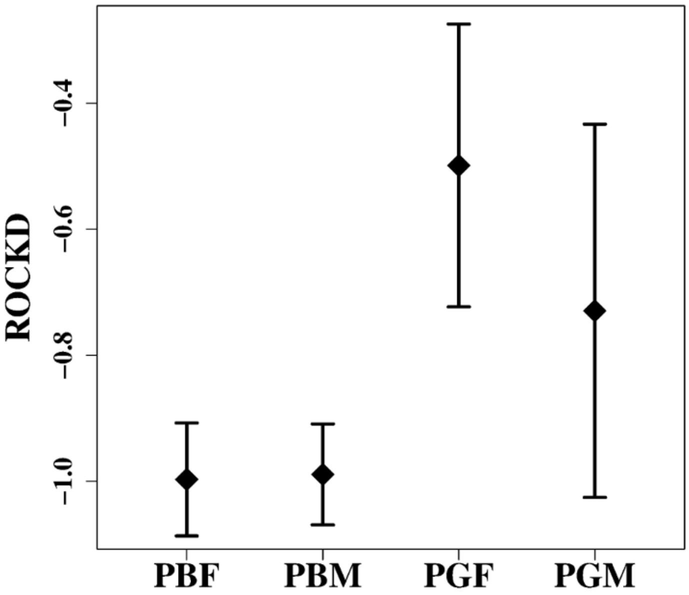

Figure 2.2 – Least-squares means for each species and sex for the diameter of the rock (ROCKD) at the point of observation. Vertical bars denote 95% confidence intervals. PBF: *P. bocagei* females, PBM: *P. bocagei* males, PGF: *P. g. lusitanicus* females, PGM: *P. g. lusitanicus* males. See Material and Methods for variable abbreviations.

3.3. Locomotor performance

MANOVA comparisons on locomotor performance indicated that *P. bocagei* and *P. g. lusitanicus* differed when considering the multivariate set of maximal locomotor speeds. Univariate analyses showed that *P. bocagei* attained higher speeds in the three climbing racetracks (CLI60, CLI90 and CLI60s; Table 2.4, Fig. 2.3, Table S_2.3 Supp. Inf.). Sex did not have a significant effect on locomotor performance. However, post-hoc comparisons indicated that differences between both species were only significant in males and not in females (Fig. 2.3). ANCOVA comparisons using SIZE and weight as covariates did not reveal a significant effect of body size and/or robustness on locomotor performance (Table S_2.4 Supp. Inf.).

Table 2.4 – Results of (M)ANOVA comparisons performed on multivariate set of maximal locomotor speeds (mSPEEDS) and each type of locomotor speed separately to examine the effect of species (SP), sex and their interaction (SP*SEX). F: F-statistic, P: corresponding p-value, adjusted for multiple testing using the False Discovery Rate procedure. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SP		SEX		SP*SEX	
	F	P	F	P	F	P
mSPEEDS	16.504	**0.005**	0.779	0.618	1.748	0.371
SPR	0.137	0.836	1.917	0.383	0.044	0.858
MAN	2.726	0.303	0.379	0.618	5.972	0.058
CLI60	32.446	**0.005**	1.610	0.383	2.659	0.303
CLI90	16.082	**0.005**	0.544	0.618	0.114	0.802
CLI60s	20.756	**0.005**	0.411	0.618	0.976	0.529

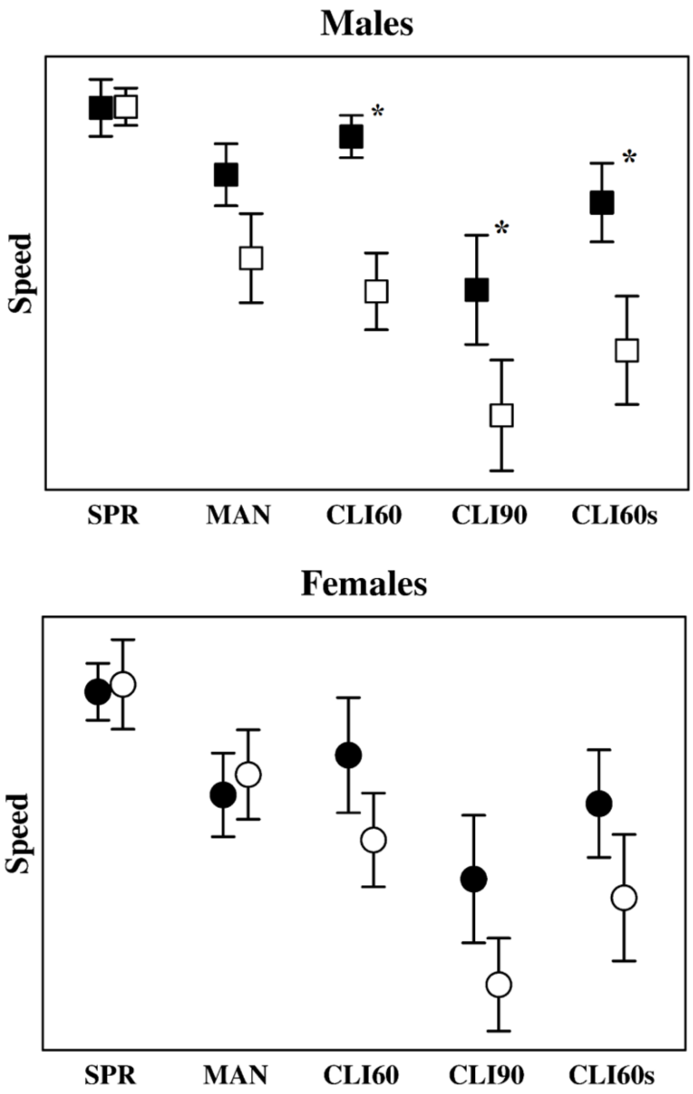

Figure 2.3 – Least-squares means observed in males and females of each species for the different locomotor variables quantified. Open squares, *P. g. lusitanicus*; closed squares, *P. bocagei*. Vertical bars denote 95% confidence intervals. * Significant post-hoc comparisons. See Material and Methods for variable abbreviations.

3.4. Morphology traits vs locomotor performance

Two-block partial least-squares regression did not reveal a significant association between morphological traits and locomotor performance (r = 0.354 and p = 0.227).

3.5. Morphological traits vs habitat use

Two-block partial least-squares regression indicated a significant association between both sets of variables (r = 0.426 and p = 0.001). The morphological PLS vector was most highly correlated with trunk length (positive correlation) and head height (negative correlation) (Fig. 2.4). The vector representing microhabitat use was most highly correlated with the percentage and the diameter of rocks, and with the percentage of vegetation, this last variable showing an opposite loading to the first two (Fig. 2.4). As such, the association between morphology and habitat use was summarized by multivariate vectors where lizards with longer trunks and flatter heads were more frequently found in rocks of a large diameter than in spots with vegetation.

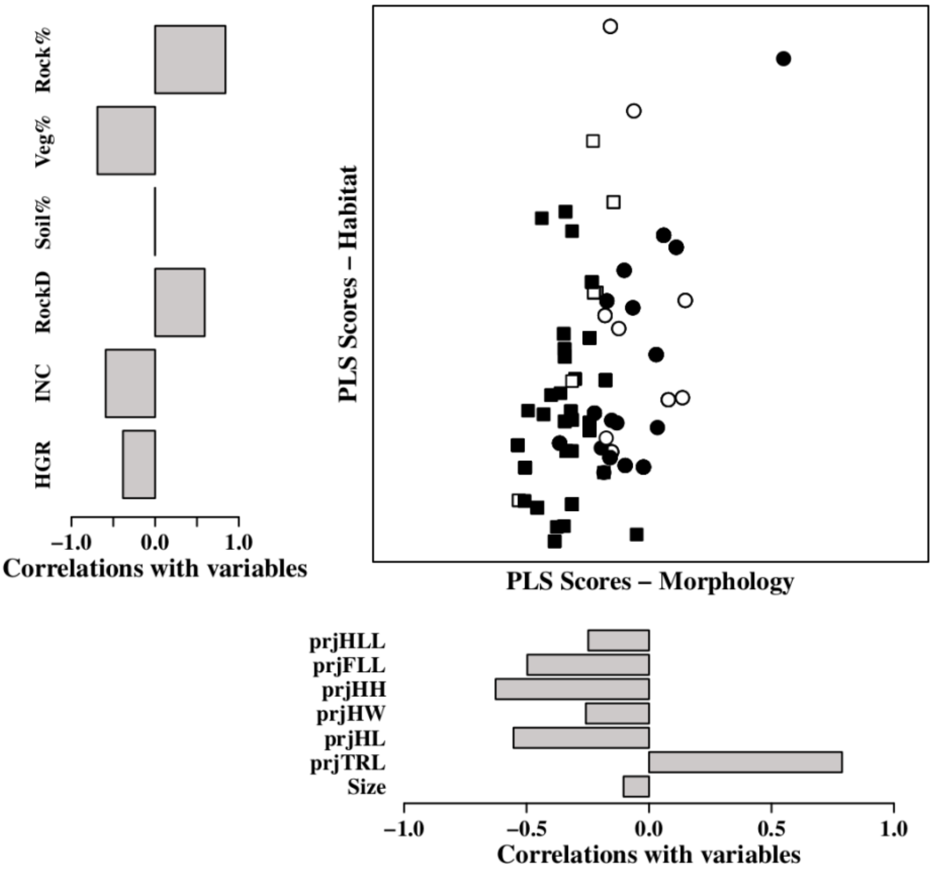

Figure 2.4 – Scatter-plot of individual scores of *P. bocagei* (black squares: males; black circles: females) and *P. g. lusitanicus* (white squares: males; white circles: females) obtained from partial least-squares analysis between morphological and continuous habitat variables. Bar-plots next to each axis represent the correlations observed between that axis and the variables included in each block. See Material and Methods for variable abbreviations.

DISCUSSION

Our investigation of the three components of the ecomorphological paradigm e morphology, locomotor performance and habitat use e in a syntopic population of *P. bocagei* and *P. g. lusitanicus* revealed an interesting pattern of ecomorphological variation, where the association between morphology and habitat is not accompanied by an association between morphology and locomotor performance. This deviates from what has been observed in numerous other lizard species (e.g. Goodman et al., 2008; Irschick et al., 2005b; Losos and Sinervo, 1989; Melville and Swain, 2000; Sinervo and Losos, 1991) and has potential implications for our understanding of the ecological and evolutionary mechanisms that underlie the high levels of morphological diversity observed in the *P. hispanica* species complex.

Although *P. bocagei* and *P. g. lusitanicus* live in strict syntopy in Moledo, our results indicate that these two forms exploit different habitat resources. Individuals of *P. g. lusitanicus* were most frequently observed on big rocks than on other substrates, while individuals of *P. bocagei* were found most frequently on human constructed walls and rocks of a smaller diameter (Fig. 2.1; Fig. 2.2), as already suggested by others studies (Galán, 1986; Sá-Sousa et al., 2002). These results provide the first quantitative account of a significant differentiation between the two forms in terms of habitat use.

Despite this differentiation, the observed ecological differences do not correspond to the predicted differences in locomotor requirements and associated morphological traits. Instead, our results indicate that the morphological and ecological divergence between *P. bocagei* and *P. g. lusitanicus* is most probably associated to refuge use, rather than mediated by locomotor performance. Indeed, individuals of the two species from this population do not differ significantly in either limb length (Table 2.1), or sprinting speed (Fig. 2.3), but we found that *P. bocagei* climbed faster than *P. g. lusitanicus* (Fig. 2.3). Several factors may explain these results. First, although generally characterised as a ground-dweller, *P. bocagei* is a relatively versatile species, that uses several different types of habitats across its distribution range (Kaliontzopoulou et al.,2010a). Indeed, in the studied population it extensively uses human-constructed walls (Fig. 2.1). As such, this species benefits not only from enhanced sprinting, but also climbing capacities, which may explain its very good locomotor performance in the climbing racetrack (Fig. 2.3). Whether this pattern of locomotor differentiation between these two forms extends to other populations with different habitat availability or, instead, locomotor performance is a plastic trait in these lizards, varying across populations depending on the habitat they use, would need to be further investigated. Further, it is also important to note that, although a general association between habitat use and locomotor performance was expected based on previous studies and ecomorphological predictions, the experimental design implemented here did not allow us to directly test this association. Indeed, because of practical limitations related to the activity of the study organisms (higher activity in the spring, that also coincides with the reproductive season), here we analysed data from two different seasons (i.e. habitat use was quantified in the spring and locomotor performance in the autumn). As such, seasonality and plasticity may also have an effect on this lack of association (e.g. Irschick et al., 2006; Irschick and Meyers, 2007), although the potential role of seasonality should be investigated in detail in the future.

A high spatial and temporal plasticity of locomotor capacities may, in fact, also explain why several studies have failed to demonstrate an evolutionary link between morphology and performance in lacertids. In this study system, we could not establish a significant association between morphology and locomotor performance. Likewise, several other studies have also failed to detect similar correlations in some cases, both at the intra- and interspecific levels (Brecko et al., 2008; Goodman et al., 2008; Van Damme et al., 1997; Vanhooydonck et al., 2000), challenging the generality of the ecomorphological paradigm. Furthermore, the lack of association between morphology and locomotor performance could also be explained by other factors like behaviour (Braña, 2003; Irschick et al., 2005b; Vervust et al., 2007; Žagar et al., 2015a), physiology (e.g. muscle composition; Adolph and Pickering, 2008; Van Damme et al., 1997), biochemistry (e.g. enzyme activity; Adolph and Pickering, 2008; Van Damme et al., 1997; Vervust et al., 2007; Žagar et al., 2015b) and/or other morphological traits not investigated in this study as orientation of the limbs, differences in the claws or even the distance between the body and an inclined surface to oppose the force of gravity (Jayne and Irschick, 1999; Revell et al., 2007). In addition, the ecological and morphological segregation between the two species could be reflected in specialization in other performance traits, not examined here, such as acceleration capacity (Vanhooydonck et al., 2005), endurance (Vanhooydonck et al., 2000; Vanhooydonck, Van Damme & Aerts, 2001) and/or agility (Van Damme and Vanhooydonck, 2002). Finally, locomotor performance is involved in several ecological (feeding, thermoregulation, escape from predators, habitat and refuge use) and social (antagonistic behaviour, territory and mate acquisition, competition with heterospecifics) functions. This means that a differentiation between both species could exist when performing different tasks in nature, which may not be observable in laboratory experiments (Irschick et al., 2005a). Such a hypothesis should be further investigated in the future by examining how individuals of the two species perform in natural conditions.

The patterns observed in relation to locomotor performance do not, however, invalidate the link observed between morphology and habitat. Several studies have demonstrated a significant evolutionary correlation between morphological and environmental variation in lizards (e.g. Goodman et al., 2008; Herrel et al., 2001; Irschick et al., 2005b; Kaliontzopoulou et al., 2010a; Melville and Swain, 2000; Vanhooydonck and Van Damme, 1999; Vitt et al., 1997). The analyses conducted here, indicate a significant correlation between habitat use and morphology in a syntopic population of *P. bocagei* and *P. g. lusitanicus* in northern Portugal. Examination of variable loadings on the morphological axis obtained through two-block partial least-squares regression with habitat use reveals that trunk length and head height are the traits with the highest contributions to this association (Fig. 2.4). Trunk length is linked to vertebral number in *Podarcis* (Arnold, 1973), it is known to enhance body flexibility for lateral bending (Arnold, 1998; Van Damme and Vanhooydonck, 2002), and it has been associated to habitat use in lacertids (Van Damme and Vanhooydonck, 2002; Kaliontzopoulou et al., 2010b). On the other hand, habitat use has been shown to drive macroevolutionary variation in head shape, but not in body size or relative limb length in wall lizards (Kaliontzopoulou et al., 2015). As already proposed by other studies, the dorso-ventral compression of the head in rocky environments, or in saxicolous species, can derive from a mechanical constraint related to refuge use (Edwards et al., 2012; Herrel et al., 2001; Kaliontzopoulou et al., 2010a; Vanhooydonck and Van Damme, 1999; Vitt et al., 1997). Indeed, in organisms like wall lizards, which rely more on crypsis or in hiding in refuges rather than on long-distance running for predator avoidance (Carretero et al., 2006; Martín et al., 2009), habitat use may represent an evolutionary constraint for head, but not for limb traits. If this were the case here, we may expect the two species to employ different antipredatory behavioural responses. Indeed, *P. bocagei* seems to use ground-level vegetation for hiding, while *P. g. lusitanicus* usually flees towards small crevices and cracks in agricultural walls (pers. obs. of the authors). However, a detailed study of escape tactics would be necessary to test this hypothesis. Finally, the association between habitat and head traits observed here could also be driven by differences in feeding ecology between animals using different structural niches. However, this does not seem to be the case in this system, as a previous study in the same study area demonstrated that these two species do not differ in bite force or in diet (Kaliontzopoulou et al., 2012b).

Put together, the results obtained in this study reveal a significant relationship between morphological traits and habitat use in *P. bocagei* and *P. g. lusitanicus*, but, unlike what happens in numerous other lizard groups, this relationship is not accompanied by an association between morphology and locomotor performance used for fleeing. The evidence at hand does not allow us to further investigate the precise mechanisms determining this pattern and, given that this study only includes two species, we can make no extrapolations to the rest of the group (Garland and Adolph, 1994). Instead, further studies would be necessary in order to elucidate the ecological significance and plasticity of locomotor performance and habitat use in these lizards. First, understanding how maximal locomotor capacities are used in nature (e.g. for prey capture, predator avoidance, social encounters) is essential for deciphering their true evolutionary potential (Irschick et al., 2005a; Husak, 2006). Further, it would be relevant to investigate how morphological and locomotor traits are involved in shaping individual fitness (survival, mate acquisition, outcome of antagonistic encounters) in this group of lizards, and how these effects may vary across different environments.

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Supporting Information Chapter 2

Table S_2.1 – Descriptive statistics for the raw biometric characters in males and females of *Podarcis bocagei* and *P. g. lusitanicus*. Values are given as mean ± standard deviation (top) and range (bottom). See Material and Methods for variable abbreviations.

	Podarcis bocagei		*P. g. lusitanicus*	
	Males	Females	Males	Females
TRL	24.42 ± 0.68	28.58 ± 0.66	25.69 ± 0.48	26.37 ± 0.67
	17.51 - 32.03	18.88 - 34.56	22.17 - 32.11	18.78 - 33.47
HW	7.4 ± 0.21	6.52 ± 0.07	7.09 ± 0.16	5.89 ± 0.08
	5.43 - 9.39	5.21 - 7.7	5.51 - 8.25	4.91 - 6.75
HH	5.43 ± 0.13	4.63 ± 0.08	4.53 ± 0.08	3.98 ± 0.08
	3.9 - 6.56	3.97 - 5.45	3.71 - 5.55	3.17 - 4.76
FLL	16.33 ± 0.27	15.07 ± 0.31	16.21 ± 0.16	13.24 ± 0.18
	11.27 - 20.17	12.35 - 17.7	13.97 - 18.72	11.56 - 14.63
HLL	27.11 ± 0.71	24.28 ± 0.40	26.12 ± 0.44	21.45 ± 0.24
	18.68 - 32.8	19.44 - 29.98	22 - 30.42	18.96 - 23.72
HL	17.59 ± 0.48	15.65 ± 0.20	17.99 ± 0.31	14.78 ± 0.18
	12.88 - 21.27	12.69 - 17.9	14.46 - 19.62	12.23 - 16.75

Table S_2.2 – Descriptive statistics for the microhabitat use traits (in the point of observation) in males and females of *Podarcis bocagei* and *P. g. lusitanicus*. Values are given as mean ± standard deviation (top) and range (bottom). See Material and Methods for variable abbreviations.

	Podarcis bocagei		*P. g. lusitanicus*	
	Males	Females	Males	Females
HGR	0.83 ± 0.05	0.8 ± 0.04	0.72 ± 0.06	0.71 ± 0.05
	0 - 1.4	0 - 1.6	0.1 - 1.2	0 - 2
INC	22.72 ± 2.15	30.90 ± 2.84	34.75 ± 5.47	26.75 ± 3.58
	0 - 90	0 - 90	0 - 90	0 - 90
ROCKD	0.41 ± 0.02	0.4 ± 0.02	0.63 ± 0.13	0.79 ± 0.11
	0.1 - 0.9	0.1 - 0.9	0.25 - 2.5	0.2 - 2.5
SOIL %	4.25 ± 1.10	3.49 ± 0.9	3.50 ± 1.82	4.88 ± 2.16
	0 - 60	0 - 30	0 - 30	0 - 70
VEG%	55.48 ± 1.40	53.55 ± 1.60	47.25 ± 2.98	49.63 ± 2.72
	20 - 90	20 - 80	20 - 70	5 - 80
ROCK %	40.26 ± 1.32	42.83 ± 1.67	49.25 ± 3.21	45.00 ± 2.70
	0 - 80	0 - 80	30 - 80	0 - 95

Table S_2.3 – Descriptive statistics for the locomotor performance in males and females of *Podarcis bocagei* and *P. g. lusitanicus*. Values are given as mean ± standard deviation (top) and range (bottom). See Material and Methods for variable abbreviations.

	Podarcis bocagei		*P. g. lusitanicus*	
	Males	Females	Males	Females
SPR.max	118.39 ± 10.02	99.83 ± 8.53	115.82 ± 8.53	110.32 ± 13.78
	37.09 - 211.74	31.17 - 152.48	81.33 - 195.13	34.15 - 184.46
MAN.max	72.97 ± 7.26	51.06 ± 6.05	44.73 ± 6.58	60.77 ± 8.59
	33.6 - 110.54	13.07 - 89.2	12.74 - 107.38	18.69 - 105.38
CLI60.max	93.77 ± 6.09	70.49 ± 10.07	34.2 ± 4.87	40.01 ± 7.56
	38.66 - 131.17	12.22 - 123.27	15.1 - 71.58	16.04 - 98.72
CLI90.max	38.28 ± 6.3	33.72 ± 6.66	14.88 ± 2.86	14.78 ± 4.01
	8.04 - 87.65	5.06 - 84.97	3.43 - 37.54	5.72 - 60.14
CLI60s.max	64.47 ± 8.54	52.44 ± 8.28	25.37 ± 4.82	28.3 ± 7.36
	21.96 - 119.97	8.39 - 105.57	6.01 - 63.47	7.77 - 76.16

Table S_2.4 – Results of ANCOVA comparisons performed on multivariate set of maximal locomotor speeds (mSPEEDS) to examine the effect of species (SP), sex, their interaction (SP*SEX) and SIZE and Weight as covariate. Df: Degrees of freedom, F: F-statistic, p: corresponding p-value. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	Df	F	p
(Intercept)	1	1430.12	**3.01E-49**
SP	1	7.79	**2.24E-05**
SEX	1	0.51	0.77
SIZE	1	0.76	0.58
Weight	1	2.89	**0.02**
SP:SEX	1	1.18	0.33
Residuals	50		

Chapter 3

Instantaneous vs Interval speed

Article II - Instantaneous Versus Interval Speed Estimates of Maximum Locomotor Capacities for Whole-Organism Performance Studies

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ABSTRACT

Numerous studies have demonstrated the ecological and social relevance of different aspects of animal locomotor performance, as locomotion is frequently required to capture prey, escape from predators, find mates and defend territories. Typically, maximal performance is quantified using two alternative methods: across distance intervals or across time intervals, the later by extracting the speed observed between a specified number of video frames. Here, we analyze how this choice may influence statistical inference and the derived biological interpretations. Our results indicate that data obtained using 10-cm intervals are categorized, not normally distributed, exhibit lower individual repeatabilities and have four times more variance compared to the data obtained by quantifying speeds across short time intervals. These results have important methodological implications, as they suggest that the choice of method of speed quantification substantially influences the quality and bias of maximal performance descriptors. This may in turn have a profound influence on ecomorphological inference, as it affects our capacity of detecting variation in performance within and across groups, and associations between locomotor performance and other traits.

KEYWORDS

Locomotion, Methodology, Performance, Sprint capacity

INTRODUCTION

Numerous studies have investigated whole-organism performance to disentangle the complex relationships among phenotypic traits that culminate in differences in fitness (Ghalambor et al. 2003; Miles 2004; Mowles et al. 2010; Santana et al. 2010; Herrel et al. 2012; McGinley et al. 2013; Irschick and Higham 2016). Locomotor performance is a function with a high ecological and social relevance, as animals need to search for prey, escape from predators, find mates and defend territories (Domenici 2001; Vásquez et al. 2002; Blumstein et al. 2004; Husak 2006; Husak et al. 2006; Husaket al. 2008; Mowles et al. 2010; Pruitt 2010; Irschick and Higham 2016). For this reason, several ecomorphological, physiological, genetic and parasitological studies have investigated how locomotor capacities vary across species, environments, and in relation to other organismal traits (Schall et al. 1982; Angilletta et al. 2002; Ghalambor et al. 2003; Goodman and Johnson 2011; Zamora-Camacho et al. 2015; Irschick and Higham 2016). For instance, locomotor performance has been used to establish links between morphological variation and habitat use. Indeed, different morphological properties are known to enhance specific locomotor capacities, which in turn match the ecological requirements of specific habitats (Irschick and Garland 2001), an evolutionary association which is studied under the framework of the ecomorphological paradigm (Arnold 1983).

To examine the ecological and evolutionary significance of locomotor performance in small animals, laboratory experiments are usually performed to quantify maximal sprinting capacity (see e.g. Punzo 2003; Blumstein et al. 2004; Miles 2004; Husak et al. 2006; Husak 2006; Médoc and Beisel 2008; Tulli et al. 2012; Collins et al. 2013; McGinley et al. 2013; Da Silva et al. 2014; recently, other types of locomotor performance have also been studied, such as climbing ability at different angles, maneuverability, endurance and acceleration capacity, in the case of terrestrial locomotion (Van Damme et al. 1997; Vanhooydonck and Van Damme 2003; Goodman et al. 2008; Herrel and Bonneaud 2012; Prenter et al. 2012; Tulli et al. 2012; Kaliontzopoulou et al. 2013; Gomes et al. 2016); or swimming speed, acceleration and maneuverability, in the case of fish and other aquatic organisms (Drucker and Lauder 2000; Domenici 2001; Rivera et al. 2006). In all such studies, the objective was to estimate the maximal performance capacity of individuals. This is because – setting behavior aside (i.e. Irschick et al. 2005) – biomechanical and physiological predictions establish links between maximal functional capacities and morphological traits such as skeletal elements, muscle morphology and composition, external morphology, or biochemical and hormonal traits, to mention just a few (Irschick and Higham 2016). These are then expected to translate into variation in how animals interact with the surrounding environment (Arnold 1983) or with each other (Husak and Fox 2008). However, some caution is necessary when recording maximum performance, as methodological procedures may also influence the results obtained and alter the biological conclusions extracted. This has been shown to be the case for biting performance – the second most frequently studied performance trait (e.g. Dessem and Druzinsky 1992; Dumont and Herrel 2003; Erickson et al. 2004; Herrel et al. 1999; van der; Meij and Bout 2004). Here, both biting substrate and bite out-lever have been shown to considerably influence the accuracy of bite force measurements (Dumont and Herrel 2003). With respect to locomotor performance, Losos et al. (2002) already suggested that the number of locomotor trials, or the inclusion of data from animals performing sub-maximally, could influence biological inferences. However, the influence of the method used to quantify individual speed, which is one of the most basic components of the experimental design, has not been previously explored.

In the case of terrestrial locomotion, maximal sprinting performance is typically measured by chasing small animals along a racetrack of 1–4 m long (Losos et al. 2002). To quantify individual functional performance one of two alternative methods is most frequently used: (1) recording the maximum speed observed across regular distance intervals; (2) extracting the instantaneous speed observed between a specified number of frames of video-recorded runs, corresponding to time intervals (Losos et al. 2002). Although both approaches are used quite frequently in the literature, they have never been compared. However, the equivalence between methods cannot be taken for granted, but rather needs to be verified, as the two approaches are conceptually expected to exhibit substantial differences. In principle, velocities obtained across distance intervals are thought to be prone to a higher influence of behaviour, as well as of the mode of locomotion of the species (Arnold 1998; Braña 2003; Kaliontzopoulou et al. 2013). From a more technical perspective, spatial precision and temporal resolution are the two characteristics that can lead to differences in the results obtained by using these two methodologies.

Here, we explored how the method used to estimate maximum running speed can influence the statistical properties of the obtained data and, in consequence, biological interpretations. In principle, these two methods are so different that estimates obtained from distance intervals or time intervals should not be comparable. However, since both are widely used in the literature, their statistical properties should be assessed when making inference on maximum running speed with organisms moving at non-constant velocity within a given spatial interval, in order to enhance experimental inference of functional hypotheses. For this purpose, we used locomotor performance data recorded for the lizard *Podarcis bocagei* (Seoane 1884). Lizards have been extensively used as models for ecomorphological studies (Arnold 1998; Vanhooydonck and Van Damme 2003; Huyghe et al. 2007; Brecko et al. 2008; Kaliontzopoulou et al. 2013; Gomes et al. 2016) because they exhibit a wide variety of locomotor modes (e.g. climbers, sprinters, matrix-dwellers, etc.), they explore many different habitat types, and their morphology and performance are easy to quantify (Garland and Losos 1994). Nevertheless, the conclusions drawn here are relevant for any study aiming to quantify animal locomotor capacities, at least when working with small, fast-moving animals, where spatial precision and temporal resolution can be an issue (e.g. swimming performance in amphibian larvae or fish, running performance in small mammals etc.).

MATERIAL AND METHODS

Podarcis bocagei is a lacertid lizard that occurs in the NW Iberian Peninsula and is found in a variety of habitats: sandy areas with rocks, sparse vegetation, and stone walls that delimit agricultural fields (see e.g. Kaliontzopoulou et al. 2010). Lizards for this study were captured in the coastal zone around the Metropolitan Area of Porto (Portugal). We collected a total of 156 adult individuals, including 76 females and 80 males, by noosing (García-Muñoz and Sillero 2010), in October of 2012. Lizards were placed in cloth bags, transported to the laboratory, and housed in individual terraria, where they were fed with live mealworms and provided with water ad libitum. Before performance experiments, animals were allowed to rest for 2 days to ensure acclimation. After the conclusion of laboratory trials, all individuals were released back to the site where they had been captured.

For all individuals, sprint speed (SPR) on a horizontal surface was measured by chasing animals along a 1 m-long and 15 cm-wide racetrack, on a cork substrate (Van Berkum et al. 1989; Braña 2003). Cork was used as a substrate because it provides very good traction (Van Damme et al. 1997). All animals were tested three times in order to estimate individual repeatability. This experimental procedure is typically used to ensure that maximal performance capacity is recorded (Losos et al. 2002; Adolph and Pickering 2008). Trials for each individual were randomized to ensure that all lizards were tested during different times of the day and to avoid sequential repeats. We allowed a rest of at least 2 h between trials to ensure physical recovery of the individuals. All experiments were carried out at a room temperature of about 31°C, which is approximately the selected body temperature of this species (Amaral et al. 2012). Prior to and in between trials, lizards were placed for at least 1 h in a terrarium exposed to an infrared light of 150 W to thermoregulate and attain their preferred body temperature (Veríssimo and Carretero 2009). All running trials were filmed with a digital camera (Canon EOS 60D) at a filming speed of 50 frames per second (fps). It is worth noting that although 50 fps could be a low filming speed for some species, *P. bocagei* is a small lizard and it is not a long-distance runner (Braña 2003); as such, this filming speed is adequate for the purposes of this study. Each run was scored as “bad” or “good”, and the “bad” races, in which the animals completely stopped or turned around during the race, were eliminated (sensu Van Berkum and Tsuji 1987; Tsuji et al. 1989). The position of the lizard across each run was digitized using MaxTRAQ 2D motion analysis software (Innovision Systems Inc. 2009). The maximum performance capacity of each individual in each run was then estimated using two alternative methods: (1) as the maximum speed observed across the whole trial for any interval of two sequential frames (hereafter referred to as ‘instantaneous speed’); and (2) as the maximum speed across any 10-cm interval. For each of the two methods, the highest speed recorded across the three trials was taken as an estimate of each animal’s maximum performing capacity. To investigate how different time intervals can influence the estimation of maximum speed, we also extracted frame interval speeds throughout the entire gradient between 2 and 50 frames (the minimum and maximum number possible with our video records), where we resampled the data to extract the maximum speed observed over complementary sets of longer sequences (e.g. for five frames we calculated speeds for frame sets 1-5, 6-10 etc.).

Based on these estimates, we performed several tests to explore the statistical properties of maximum speed values and compare them between methods. First, to visualize the distribution of speed estimates, we produced a bivariate graph and visually inspected the relationship between the data produced by the two methods. We then investigated if the two datasets were normally distributed using a Shapiro–Wilks normality test. To quantify the repeatability of our data, we examined the correlation between the mean and maximum speed recorded across the three repetitions of each individual (Huey and Dunham 1987; Van Berkum et al. 1989). To investigate if the two methods provided different estimates of maximum speed values, we performed a repeated-measures ANOVA on maximum speed, with method as a fixed factor. Finally, we used Levene’s test to compare the variance observed among individuals of the two datasets. Because we found differences in means across methods (see Results) that could influence variance estimates, we also visualized differences in the degree of variability across datasets using the coefficient of variation—i.e. the standard deviation divided by the mean (Sokal and Rohlf 1995, pp. 97–98). All data were logarithmically transformed prior to analyses. All analyses were implemented in R (version 3.2.0) using RStudio (0.99.447).

RESULTS

Data of instantaneous estimates of maximum speed followed a normal distribution (W = 0.991, p value = 0.459), but those obtained from 10 cm-intervals deviated from normality with a bias towards low speed values (W = 0.931, p value =

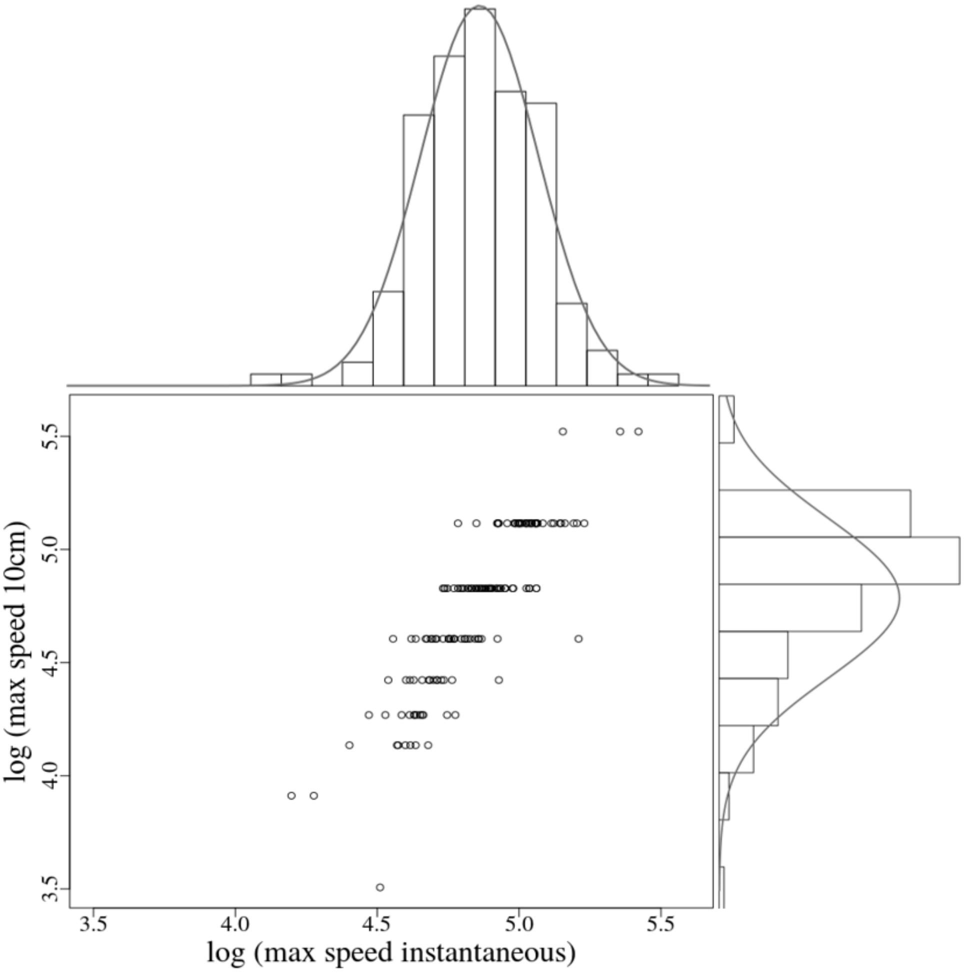

Figure 3.1 – Relationship between maximum speed from data of 10 cm-interval and instantaneous speed, with the representation of the histograms with the normal curve represented.

8.04×10^{-7}; Fig. 3.1). The time frame number corresponding to a 10 cm-interval was 17.69 (Fig. 3.2). Repeatability was higher in the speeds estimated using time-intervals than in the data obtained using 10 cm-intervals (Fig. 3.2a). The repeated-measures ANOVA showed that the two methods produced significantly different maximum speed estimates, where the 10 cm-interval approach provided lower values compared to the time-interval speeds estimated across two sequential frames (F = 11.98; p value < 0.001; Fig. 3.3). This was the result of a gradual decrease of maximum speed estimates with increasing

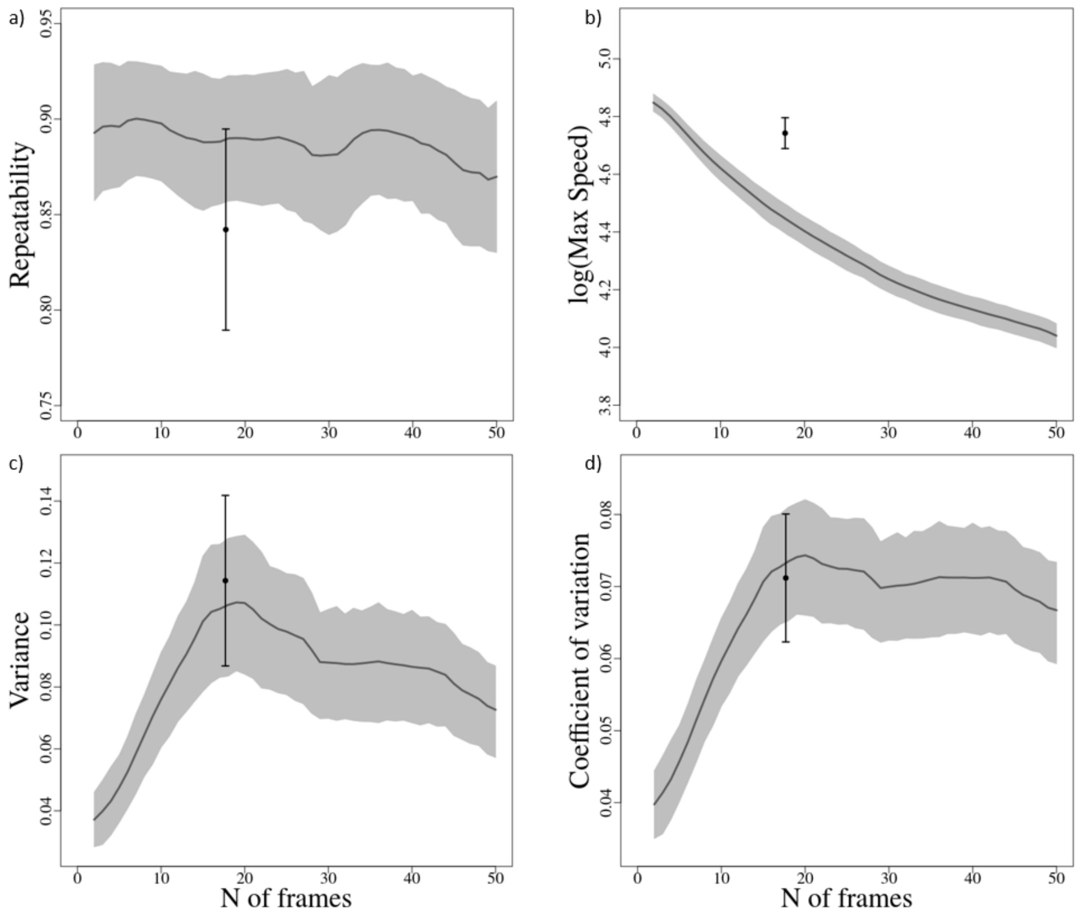

Figure 3.2 – Mean value for **a** repeatability – correlation between mean and maximum speed by individual, **b** maximum speed, **c** variance of maximum speed and **d** coefficient of variation of maximum speed. The x-axis represents the examined gradient of number of frames used to define time intervals for extracting speed estimates. The value of each descriptor estimated using 10 cm-interval data is placed at the mean corresponding number of frames (*black*). Confidence intervals around the mean value of each descriptor (*shaded grey area*) were calculated using bootstrapping with 1000 random samples.

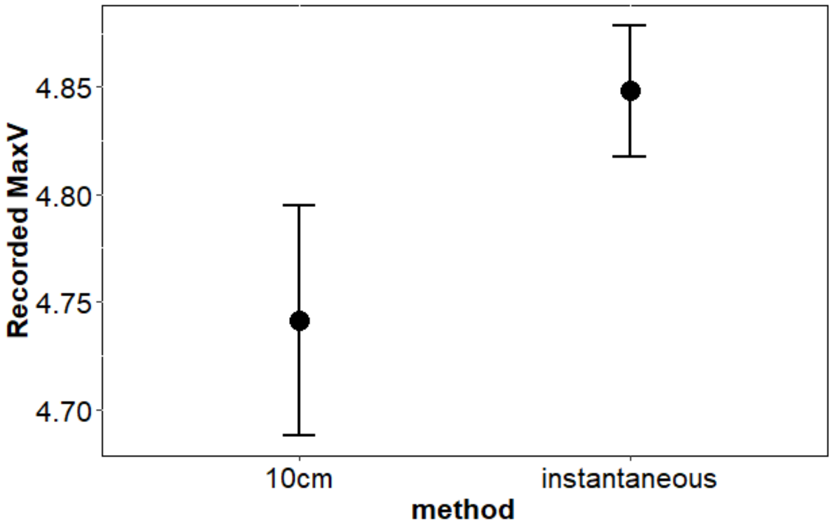

Figure 3.3 – Least-squares means for each method—maximum 10 cm interval speed and maximum instantaneous speed. Vertical bars denote 95% confidence intervals.

length of the time interval used to quantify speeds (Fig. 3.2b). Indeed, the speeds obtained using a time interval were lower than those from 10 cm intervals when time-interval speeds were obtained using five or more frames (Fig. 3.2b). Speed estimates obtained using 10 cm intervals exhibited approximately three times more total variance (F = 1.593; p value = 9.29×10^{-7}; Fig. 3.2c), and about double the coefficient of variation (Fig. 3.2d), as compared to instantaneous speeds.

DISCUSSION

Our comparison of the data obtained using two frequently implemented methodologies for quantifying locomotor performance in animals revealed substantial differences in the estimates of maximum speed, which exhibit different statistical properties. Previous observations had suggested that these two methodologies may differ in resolution and accuracy, where instantaneous speeds were expected to provide better estimates than those calculated across distance intervals (Kaliontzopoulou et al. 2013). Indeed, the comprehensive set of comparisons conducted here revealed that the data obtained across 10 cm-intervals deviated from normality (Fig. 3.1) and exhibited lower means (Figs. 3.2b, 3.3), lower individual repeatabilities (Fig. 3.2a), and higher variances among individuals (Fig. 3.2c) when compared to instantaneous speed estimates. These results suggest that the approach used for extracting estimates of maximal performance considerably influences the quality of the data obtained. As a result, our capacity for detecting differences in performance across groups of interest or for identifying associations between functional and other (e.g. anatomical, morphological, physiological, behavioural) traits may become compromised. Such methodological effects may entangle biological inference, and addressing them is of relevance for enhancing studies of functional performance.

Our results showed that data from 10 cm-intervals did not follow the normal distribution and exhibited a higher variance as compared to instantaneous speed data. These statistical properties are most likely a result of behavioural modulation of movement patterns. Indeed, *Podarcis* lizards are not long-distance runners but instead perform short bursts of running alternated with frequent pauses (Braña 2003). These pauses within distance intervals can lead to an underestimation of the maximum running potential of each individual, as shown by the lower mean velocity estimates obtained when using distance interval estimates. Even individuals that are highly motivated when performing locomotor experiments will exhibit this running behaviour. Furthermore, burst-pause running patterns will vary randomly among individuals, as well as across trials of the same individual and across intervals within trials (Avery et al. 1987). As a result, speed data obtained across intervals exhibit higher variance among individuals and lower individual repeatability compared with time-interval speed data. Note that this effect is already quite marked when considering sprint speed on a flat surface, but it is expected to have an even stronger effect when considering other types of locomotion, such as climbing or manoeuvring. Performance trials conducted on tilted surfaces or including obstacles are generally more challenging and, as a result, animals tend to stop more frequently than on a flat surface (pers. obs.), a fact that increases this behavioural effect of burst-pause movement on speed estimates. Similarly, we expect the effect of locomotor pattern to be much more prominent when investigating different species, possibly engaging in different running strategies. In such cases, this behavioural effect may vary more extensively than across individuals of the same species, as in the example dataset used here.

In addition, as highlighted by Losos et al. 2002, it is also important to always keep in mind the effect of motivation. To motivate an animal to run during laboratory trials, a predator attack is simulated where the animals escape from the researcher. Therefore, maximal functional performance measures obtained in the laboratory have been associated with escape performance in the field (e.g. Martín and López 1999; Diego-Rasilla 2003). When escaping from a predator, lizards evaluate the costs and benefits of fleeing, (Carretero et al. 2006; Cooper and Peréz-Mellado 2004; Martín and López 1999). Many lizards adopt different tactics when escaping from predators—e.g. they may not allow the predator to come very close (increasing approach distance), or they may stay close to their refuge (decreasing the distance fled). The last case is the most common in *Podarcis* wall lizards, where fleeing distance is usually quite short (Cooper et al. 2009; Diego-Rasilla 2003, pers. obs. of the authors). Comparing laboratory experiments and field tests is important to understand how behaviour may mould performance (Husak and Fox 2006; Irschick et al. 2005; Losos and Irschick 1996). When we try to link maximal performance measured in the laboratory with observations of behaviour in the field, the method used to estimate maximal performance becomes important. In this context, different methods may be more adequate depending on the ecological or social task of interest. In the case of escape or foraging behaviour, during which the animals normally run longer distances, interval estimates could be used. However, if faster tasks, as antagonistic behaviour, are being investigated, instantaneous estimates should be preferred to accurately capture individual variation at a short temporal scale.

Indeed, another related feature influencing the quality of maximal speed estimates is scale and its relationship with running mode. An inherent problem of speed analysis during animal locomotion is precisely the connection between temporal and spatial scale. Indeed, while trying to estimate maximum speed, one may choose to sample running trials using distance intervals, as traditionally done, or time intervals (in the form of number of frames). In either case, data collected at different temporal or spatial scales will not be comparable, as the resolution used for data recording will influence the estimated speeds (and their variance and repeatability, i.e. Fig. 3.2). The effect of scale on locomotor performance estimates is nicely illustrated by considering acceleration, instead of speed, and its variation. Acceleration is frequently examined in performance studies, as it is a critical feature of locomotion in many animals, particularly during escape from predators (Domenici 2001; Miles 2004; Vanhooydonck et al. 2006). However, it is also more sensitive to scale effects, as it occurs at a finer temporal scale and it therefore exhibits a higher dependence on the temporal resolution of the quantification approach. This inflation of variance estimates for speed measures artificially increases the overlap between the distributions of compared groups (e.g. sexes, habitat types, species). As such, the detectability of small differences among groups, or of the association of locomotion descriptors with other traits, will be more difficult when using a lower resolution.

Distance-interval estimates of maximal velocity have been used in several studies, where it was possible to detect differences between groups or correlate morphological traits with performance (Bauwens et al. 1995; Goodman et al. 2008; Gomes et al. 2016; Huyghe et al. 2007; Kaliontzopoulou et al. 2013; Vanhooydonck and

Van Damme 2001; Vanhooydonck et al. 2006; Žagar et al. 2017). Most of these studies examined locomotor variation at the interspecific, and sometimes at the family, level (see Table 3.1). In these cases, the magnitude of variation is larger and identifying differences among groups and associating them to other traits as morphology is easier than it is within species (Kaliontzopoulou et al. 2010a; Losos and Miles 1994). However, when interested in intraspecific functional variation, the magnitude of variation across individuals is much smaller, and a method with more resolution and accuracy will provide a higher capacity for identifying significant variation among groups in locomotor performance, or covariation with other traits. For instance, Kaliontzopoulou et al. (2013) found an association between morphological traits and performance at the intraspecific level using instantaneous speed data, but not considering 10 cm-interval estimates of the same data. To obtain a more global view of how the difference between methods could affect biological inferences, we reviewed locomotor speed data from available intraspecific and interspecific studies. We focused mainly on studies examining species of the family Lacertidae, which are – evolutionarily speaking – more associated to our dataset (Table 3.1). Based on the maximal velocities reported in each study, we calculate the coefficient of variation observed across the groups of interest compared by the authors (i.e. sexes, ages, or populations, for intraspecific studies; and species, for interspecific studies), to contrast these values with the variation between methods retrieved in this study. The coefficients of variation reported in intraspecific studies are quite smaller than those observed when considering variation at the genus or family level (Table 3.1). Putting the results obtained here in the context of those studies, the coefficient of variation obtained when comparing the two methods for data acquisition is similar to the values reported in intraspecific studies. This suggests that using lower-resolution estimates of maximal performance should not be problematic when examining highly differentiated groups (i.e. different species or genera), but it also reinforces the importance of using more accurate methods, as instantaneous speed estimates, when working at the intraspecific level.

Table 3.1 – Study system, compared groups, range, mean and coefficient of variation of maximum velocities, and method used to quantify locomotor capacity in different studies.

Study system	Compared groups	Range (cms^{-1})	Mean (cms^{-1})	CV	Method	Reference
Podarcis melisellensis	colour morphs	197.89 - 202.4	200.56	0.02	interval	Huyghe et al 2007
Podarcis melisellensis	age	142.69-181.8	163.56	0.24	interval	Brecko et al 2008
Podarcis bocagei	sexes	138.97 - 157.88	148.43	0.13	interval	Kaliontzopoulou et al 2013
Podarcis bocagei	sexes	139.56 - 161.21	150.39	0.14	instantaneous	Kaliontzopoulou et al 2013
Podarcis bocagei	sexes	99.83 - 118.39	109.11	0.17	interval	Gomes et al 2016
Podarcis guadarramae	sexes	110.32 - 115.82	113.07	0.05	interval	Gomes et al 2016
Podarcis sps.	species (2)	99.83 - 118.39	111.09	0.17	interval	Gomes et al 2016
Podarcis sps.	species (2)	108.88-202.68	155.78	0.6	interval	Van Damme et al 1997
Podarcis muralis	sexes	163.72 - 171.24	167.48	0.05	instantaneous	Žagar et al 2017
Iberolacerta horvathi	sexes	222.94 - 226.44	224.69	0.02	instantaneous	Žagar et al 2017
Podarcis muralis, Iberolacerta horvathi	species (2)	163.72 - 226.44	196.09	0.32	instantaneous	Žagar et al 2017
Podarcis sicula	sexes	173-175.6	174.3	0.01	interval	Vervust et al 2007
Podarcis sicula	sexes	182.8-184.9	183.85	0.01	interval	Vervust et al 2007
Podarcis sicula	islands	173-184.9	179.8	0.07	interval	Vervust et al 2007
Gallotia simonyi	age	171-253.4	212.2	0.39	interval	Cejudo & Márquez 2001
Gallotia stehlini	age	236-330.2	283.1	0.33	interval	Cejudo & Márquez 2001
Gallotia sps.	species (2)	171-330.2	247.65	0.64	interval	Cejudo & Márquez 2001
Psammodromus algirus	sexes	129.27-155.79	142.53	0.19	interval	Zamora-Camacho et al 2015
Psammodromus algirus	sexes	222.89-225.58	224.24	0.01	interval	Iraeta et al 2011
Psammodromus algirus	sexes	224.17-231	227.59	0.03	interval	Iraeta et al 2011
Psammodromus algirus	habitats	222.89-231	225.91	0.04	interval	Iraeta et al 2011
Lacertidae	species (13)	109 - 334	207.46	1.09	interval	Vanhooydonck & Van Damme 2001
Lacertidae	species (13)	90 - 313	188.02	1.19	interval	Bauwens et al 1995
Lacertidae	species (22)	87-298	213.96	0.99	interval	Verwaijen & Van Damme 2008
methods	this study	120.8 - 129.85	125.33	0.07	between both	this study

Another effect related to spatial scale is that of the body size. Most studies investigating locomotor performance use photocells placed at regular intervals, the length of which is usually defined taking the size of the animal into account (e.g. Van Damme et al. 1997; Brecko et al. 2008; Goodman et al. 2008). This has consequences for the methodological definition of the quantified measures, as larger animals need to take fewer strides than smaller ones to cover the same distance. Furthermore, variations across studies in the selection of interval length exist even within specific groups, potentially hindering comparisons across them. For instance, different published studies investigating locomotion in Podarcis lizards have used different intervals to obtain maximum speed (Van Damme et al. 1997 – 25 cm; Braña 2003 – 20 cm; Kaliontzopoulou et al. 2013 – 10 cm). Our results advise against comparisons across these studies, as the time or length interval used to record running performance considerably affects the mean and variance of maximum speed estimates. Most importantly, this has severe consequences for the posterior use of data recorded over different intervals for meta-analyses, which would be of interest e.g. for conducting comparative studies across different species (Kramer and McLaughlin 2001; Iriarte-Díaz 2002; Van Damme et al. 2008).

In conclusion, the methodology used to obtain maximal performance capability estimates substantially modifies the statistical properties of such estimates. This may have an influence on ecomorphological inference, as it affects our capacity for accurately describing variation in performance and associations between locomotor performance and other traits (e.g. morphology, ecology, fitness). For instance, the higher variance that characterizes interval speed estimates may entangle statistical inference by increasing the overlap between different groups (e.g. populations or species inhabiting different habitats), making the detection of eventual differences more difficult. This is the same effect as that caused by the inclusion of individuals performing submaximally, which has been previously demonstrated to hinder the detection of interspecific differences in locomotor performance (Losos et al. 2002). Such effects may be more pervasive in locomotor performance studies and could have a stronger effect on ecomorphological inference than previously thought. Taking our results into account, the use of instantaneous speed estimates should be preferred over distance-interval estimates when possible. Given the increasing accessibility to high resolution, high-speed filming equipment, and also to computer programs that allow the automatic detection of animal position, obtaining instantaneous maximum speed estimates is becoming increasingly easier, with evident advantages for researchers interested in understanding the evolution of animal locomotion.

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COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Chapter 4

Run for your life, but bite for your rights?

Article III - Run for your life, but bite for your rights? How interactions between natural and sexual selection shape functional morphology across habitats

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ABSTRACT

A central issue in evolutionary biology is how morphology, performance, and habitat use coevolve. If morphological variation is tightly associated with habitat use, then differences in morphology should affect fitness through their effect on performance within specific habitats. In this study, we investigate how evolutionary forces mold morphological traits and performance differently given the surrounding environment, at the intraspecific level. For this purpose, we selected populations of the lizard *Podarcis bocagei* from two different habitat types, agricultural walls and dunes, which we expected to reflect saxicolous vs ground-dwelling habits. In the laboratory, we recorded morphological traits as well as performance traits by measuring sprint speed, climbing capacity, maneuverability, and bite force. Our results revealed fast-evolving ecomorphological variation among populations of *P. bocagei*, where a direct association existed between head morphology and bite performance. However, we could not establish links between limb morphology and locomotor performance at the individual level. Lizards from walls were better climbers than those from dunes, suggesting a very fast evolutionary response. Interestingly, a significant interaction between habitat and sex was detected in climbing performance. In addition, lizards from dunes bit harder than those from walls, although sexual differentiation was definitely the main factor driving variation in head functional morphology. Taking into account all the results, we found a complex interaction between natural and sexual selection on whole-organism performance, which are, in some cases, reflected in morphological variation.

KEYWORDS

Morphological traits, Habitat use, Performance, Natural selection, Sexual selection

INTRODUCTION

A central theme in evolutionary biology is to understand how phenotypic variation evolves and how the phenotype is related with the environment. Morphological traits are a substantial part of the phenotype: they are the structural components with which organisms interact with their environment. As such, they are involved in very different and crucial ecological and social functions, and they are subject to strong selective influences. Selection can be described as the relationship between variation in phenotypic traits and variation in fitness across individuals (Kingsolver and Huey 2003). Differences in an organism's functional morphology should result in differences in performance that directly affect fitness in a given environment (Arnold 1983; Emerson and Arnold 1989; Irschick 2002), yielding links between morphology, performance, and fitness that have been studied under the framework of the ecomorphological paradigm (Arnold 1983).

Selection pressures act on individuals, maximizing their chances for survival and reproduction, which is why the approach described by Arnold (1983) was initially put forward at the intraspecific level, and in particular highlighting the relevance of "*the analysis of adaptation within populations of conspecifics*" (Arnold 1983: 348). However, similar approaches have been used extensively to study macroevolutionary associations between morphology, performance, and ecology across species. For instance, *Anolis* lizards are one of the most remarkable examples of an adaptive radiation where sympatric species have repeatedly evolved divergent morphologies, performance, and behaviors as means of exploiting different microhabitats and enhancing niche segregation (Losos and Sinervo 1989). This and other textbook ecomorphological examples have attracted attention in part due to the remarkable morphological diversity they encompass, which makes patterns of variation easier to detect. Indeed, because the magnitude of variation is big, it is generally easier to identify differences among groups and associate them to hypothesized explanatory factors at higher taxonomic levels than within species (Losos and Miles 1994; Kaliontzopoulou et al. 2010a). However, when examining species that encompass a wide evolutionary array, in many occasions, the detected phenotypic patterns and associations across traits can be the result of adaptation or exaptation (sensu Gould and Vrba 1982), evolutionary signal (Blomberg and Garland 2002), or phenotypic plasticity (Losos et al. 2000) potentially making the interpretation of the results difficult. Hence, studies at the intraspecific level are relevant for detecting microevolutionary mechanisms and linking them to adaptive pressures that lead to phenotypic differentiation (Irschick et al. 2005a).

To optimize whole-organism performance, natural and sexual selection mold morphological traits that are involved in ecological functions (e.g., feeding, escape from predators, habitat and refuge use, competition with heterospecifics) and social interactions (e.g., antagonistic behavior, territory and mate acquisition, mating), respectively. Natural selection may influence performance and morphology to enhance the survivorship of the individuals when performing different tasks (Husak and Fox 2006). For instance, when escaping from predators, a fast locomotor performance will be the best strategy to avoid predation, but the probability of survival can be further augmented through morphological adaptations that facilitate the use of specific refuges (Goodman 2009). On the other side, sexual selection may influence performance and morphology to provide individuals with an advantage in mating through intrasexual competition and mate choice (Husak and Fox 2008). For instance, increased locomotor and bite performance enhance the capacity of males for defending territories and increase their mating possibilities (Husak et al. 2006; Husak and Fox 2008). These processes occur simultaneously, and they can be parallel, when both natural and sexual selection act on performance and/or morphological traits in the same way to enhance fitness, or opposite, if ecological and social functions impose conflicting performance demands (Husak et al. 2006; Husak and Fox 2008). The balance between natural and sexual selection is complex, but it can be generally inferred when observing variation in the degree of sexual dimorphism depending on the ecological context (Butler et al. 2000; Butler and Losos 2002; Husak and Fox 2008; Kaliontzopoulou et al. 2010a, 2012).

Lizards provide excellent models for studies of functional morphology because of their wide range of morphologies and performance skills, and of their presence in a variety of habitats (Arnold 1998; Garland and Losos 1994; Irschick and Garland, 2001; Irschick 2002). Further, they are usually sexually dimorphic in body size and shape, where males are frequently larger, with larger heads and longer limbs, whereas females tend to have longer inter-limb length (Kaliontzopoulou et al. 2012, 2013; Gomes et al. 2016; Irschick and Higham 2016). These differences probably result from sexual and fecundity selection, and they occur in morphological traits with a high functional relevance (e.g., Kaliontzopoulou et al. 2010b, 2012, 2013). This provides the opportunity of combining morphological, functional, and ecological data to investigate how natural selection—e.g., with respect to habitat variation—and sexual selection are integrated to shape functional and morphological diversity, and to decipher the evolutionary meaning of morphological variation. In the Mediterranean Basin, wall lizards (*Podarcis* spp.)

provide a particularly interesting system for investigating phenotypic variation, with remarkable levels of intraspecific morphological variation and cryptic diversity. Several studies in this group have investigated variation in morphology and performance, and their relationship, both in relation to habitat and focusing on sexual dimorphism (Van Damme et al. 1997; Brecko et al. 2008; Kaliontzopoulou et al. 2012, 2013; Gomes et al. 2016). From an ecomorphological perspective, habitat use has been shown to be an important determinant of macroevolutionary phenotypic differentiation in head shape but not in body size or limb length in wall lizards, although patterns differed between the sexes (Kaliontzopoulou et al. 2015). At the intraspecific level, both limb and head morphology are known to differ across different habitat types of *P. bocagei*, but again the degree of differentiation between sexes varies between habitats, suggesting an interaction between natural and sexual selection (Kaliontzopoulou et al. 2010a). Interestingly, some of the morphological patterns retrieved in that study aligned with, while others contradicted, predictions of the ecomorphological paradigm, raising questions about the functional, and therefore evolutionary, significance of the observed variation.

Indeed, morphological differentiation does not always translate into functional variation, and the evidence available is frequently contradictory. In the context of sexual dimorphism, differences in morphology between males and females of *P. melisellensis* translate into different bite force capacities, but not different sprint speeds (Brecko et al. 2008). By contrast, two Iberian *Podarcis* wall lizards have been reported to translate into bite force capacity, suggesting that ecological variation between species was only reflected on morphology but not on functional capacities (Kaliontzopoulou et al. 2012). In the case of locomotor performance, some studies have provided evidence of an association between morphological traits and locomotor performance (Kaliontzopoulou et al. 2013), but others have failed to detect this association (Van Damme et al. 1997; Vanhooydonck et al. 2000; Gomes et al. 2016). Thereby, it is not clear whether morphological evolutionary responses to habitat and sexual dimorphism are mediated by functional performance and, if so, which traits are involved and which is the exact link between morphology and function (Irschick et al. 2008).

In this study, we investigate how natural and sexual selection may contribute in shaping morphological traits and performance differently given the surrounding environment, at the intraspecific level. For this purpose, we selected populations of *P. bocagei* from two contrasting habitat types, representing saxicolous and ground-dwelling ecological habits. We tested whether individuals from different habitats differ in locomotor and biting performance, as we would expect under predictions of the ecomorphological paradigm. Further, we investigated to what extent and how individual variation in morphology translates into variation in functional performance, as predicted by biomechanical rules and as suggested by studies in other lizard groups. Based on previous observations on the morphology and ecology of this species, and considering ecomorphological patterns in other lizards, we hypothesize that individuals from dunes, which are generally ground-dwelling, have little chance to climb, live in more open habitats, and have higher and more rounded heads and longer limbs, will exhibit stronger bites and will be better sprinters. On the other hand, we expect individuals from agricultural walls, which have been generally considered as saxicolous, frequently using vertical surfaces and have flattened head and shorter limbs, to perform better when climbing and possibly exhibit reduced biting performance, as a result of physical constraints on head height. In addition, considering the well-known existence of male-biased sexual dimorphism in these lizards, we expect that males will exhibit stronger biting and locomotor performance. Finally, given that the ground-dwelling populations studied here are known to be morphologically more dimorphic than saxicolous ones (Kaliontzopoulou et al. 2010a), we were interested in investigating if this variation also translates into differences in the degree of sexual dimorphism in whole-organism performance between habitats. The mechanical constraints imposed by a saxicolous life translated in a less pronounced sexual dimorphism than in the animals living in open environments (Kaliontzopoulou et al. 2010a), and may lead to differences in functional capacities.

MATERIAL AND METHODS

Study organism

Podarcis bocagei is a lacertid lizard endemic to the NW Iberian Peninsula, and it can be found in a variety of habitats: sandy areas with or without rocks, sparse vegetation, and stone walls that delimit agricultural fields (see e.g., Kaliontzopoulou et al. 2010a). Lizards for this study were captured in four localities: two dune areas (Madalena and Mindelo) and two sites with agricultural stone walls (Gião and São Mamede do Coronado). We collected a total of 156 adult individuals, including 76 females and 80 males (approximately 20 males and 20 females from each population), by noosing (García-Muñoz and Sillero 2010) in October 2012. Lizards were placed in cloth bags, transported to the laboratory, and housed in individual terraria, where they were fed with live mealworms and provided with water ad libitum. Before experiments, animals were allowed to rest for two days to ensure acclimation. After the conclusion of laboratory experiments, all individuals were released back to the site where they had been captured. We performed all experiments in autumn, that is, in the advanced post-reproductive season (Carretero et al. 2006), to exclude any potential effects of pregnancy on female performance (Bauwens and Thoen 1981) or due to low body condition immediately after reproduction on both sexes (Galán 1996).

Quantified parameters

In all individuals captured, we measured the following linear biometric traits: snout-vent length (SVL), trunk length (TRL), head length (HL), head width (HW), head height (HH), mouth opening (MO), forelimb length (FLL), and hind limb length (HLL), using electronic calipers (precision ± 0.01 mm; see Kaliontzopoulou et al. 2007 for a detailed description of variables). All morphological traits were ln-transformed for further analyses.

All experiments to quantify functional performance and examine microhabitat selection were carried out at a room temperature of about 31°C, which is approximately the selected body temperature of the species (Amaral et al. 2012). Prior to and in between trials, lizards were placed for at least 1 h in a terrarium exposed to an infrared lamp of 150 W, allowing them to thermoregulate and attain their preferred body temperatures (Veríssimo and Carretero 2009).

For all individuals, three types of locomotor performance were measured (Kaliontzopoulou et al. 2013): sprint speed (SPR), climbing capacity (CLI), and maneuverability (MAN). Sprint speed on a horizontal surface was measured by chasing animals along a 1-m-long and 15-cm-wide racetrack, with a cork substrate (Van Berkum et al. 1989; Braña 2003). Climbing capacity was quantified by chasing animals up a similar racetrack, tilted to an angle of 60°. Cork was used as a substrate because it provides very good traction (Van Damme et al. 1997). To quantify maneuverability, a 0.5-mlong and 15-cm-wide pinboard was placed on the racetrack. This pinboard was made of 8-mm diameter pins placed at equal distances of 35 mm (Vanhooydonck et al. 2000). We allowed a rest of at least 2 h between trials to ensure physical recovery of the individuals. All trials were filmed with a digital camera (Canon EOS 60D) at a filming speed of 50 frames per second. Locomotor speed in the three types of racetracks was measured on different days, and the order in which animals were subjected to the tests was randomized. Each individual was tested three times in each type of racetrack to ensure that maximal locomotor capacity was recorded. Each run was scored as “bad” or “good”, and the “bad” races, in which the animals turned around during the race, were eliminated (sensu Van Berkum and Tsuji 1987; Tsuji et al. 1989). The position of the lizard across each run was digitized using MaxTRAQ 2D motion analysis software (Innovision Systems Inc. 2009). The highest instantaneous speed (Gomes et al. 2017) recorded across the three trials was taken as an estimate of each animal’s maximum performing capacity in each of the three types of racetrack and log-transformed for further analyses.

Bite force was measured using an isometric Kistler force transducer (type 9203, Kistler Inc., Winterthur, Switzerland) mounted on a vertical holder and connected to a Kistler charge amplifier (type 5058A, Kistler Inc., Winterthur, Switzerland). Bite force measurements were obtained by provoking the lizard to bite a pair of thin metal plates connected to the force transducer (see Herrel et al. 2001a for a detailed description). The tip of the metal plates where the lizard bit was delimited with a marker to ensure all lizards bit at an equal distance from the revolving arms and thus standardize the point of force exertion. Each lizard was tested five times to ensure that the maximal individual bite force per individual was registered. The maximum bite force measure per individual was retained and log-transformed for further analyses.

Statistical analyses

To represent total head size (HS), we used the scores of the first principal component calculated using HL, HW, HH, and MO. For this purpose, principal components of the covariance matrix of head dimensions were calculated using the function prcomp of stats package (R Development Core Team 2016). The four head dimensions had similarly high loadings of the same sign (MO, 0.52; HH, 0.50; HW, 0.49; and HL, 0.49) on the first principal component, which explained 91% of total variance, and was therefore taken to represent HS. To investigate if there were differences between habitats and sexes in size, we used an ANOVA comparison. Next, ANCOVA models were run using each measured morphological trait as the response variable, and habitat (HAB), population nested within habitat (POP), sex, and interaction terms (HAB×SEX and POP×SEX) as predictors, and SVL as a covariate. To examine whether habitats and sexes differed in locomotor performance, we performed ANOVA comparisons on each type of locomotor speed and bite force performance separately, with the same design as for morphology. We also performed ANCOVA comparisons on each type of locomotor speed and bite force performance separately with the same design and considering SVL – in the case of locomotor performance – and HS – in the case of bite force – as covariates. Throughout, we always used permutation-based ANOVA procedures using 1000 randomizations of Euclidean distance matrices as implemented in the adonis function of the vegan R-package (Oksanen et al. 2012).

To investigate the multivariate association between morphology and performance, we used two-block partial least-squares regression (PLS) as implemented in the plsr function of the pls R-package (Mevik et al. 2011). To investigate the association between morphology and locomotor performance (three variables: SPR, CLI, MAN), we only used head size and limb and trunk variables, as these traits are known to determine locomotor performance in these and other lizard species (Kaliontzopoulou et al. 2010a, 2013; Vanhooydonck and Van Damme, 2001 Cameron et al. 2013; Gomes et al. 2016). We first performed the PLS with raw variables, and then we repeated the analysis after correcting all locomotor performance and morphological variables for size effects through a regression on SVL. In the case of the association between morphology and biting performance, we only have one performance variable; however, we used two-block partial least-squares regression to be concordant with the previous analysis of locomotor performance. We only used head dimensions (HL, HW, HH, and MO), which are the main morphological determinants of bite force (e.g., Herrel et al. 2001a; Kaliontzopoulou et al. 2012). Similar to the approach for locomotor performance, we first performed the PLS with raw variables and then we re-run the analysis after size-correcting both bite performance and morphological variables through a regression on HS.

All statistical analyses were performed using R v. 3.3.1 (R Development Core Team 2016).

RESULTS

ANOVA comparisons indicated that individuals from different habitats differed in size ($F = 8.135$, $p = 0.007$), where animals from dunes were larger in body size. ANCOVA comparisons using SVL as the covariate only revealed significant differences between habitats in relative head height, where individuals from dune environments had relatively higher heads than those from walls. ANCOVA also confirmed significant differences between the sexes in all morphological traits, where males had longer limbs and larger heads, but shorter trunks than females (Table 4.1).

Table 4.1 – Results of ANCOVA comparisons performed on each linear measurement separately with habitat (HAB), population nested within habitat (POP), and sex as predictors, and SVL as a covariate in Bocage's wall lizard – *P. bocagei*. Significant effects are marked in bold. See Material and methods for variable abbreviations F, F-statistic; p, corresponding p value.

		HL	HW	HH	MO	FLL	HLL
SVL	F	536.69	954.88	447.83	1130.57	508.69	758.89
	p	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
HAB	F	0.43	0.61	7.94	0.2	0.31	0.05
	p	0.503	0.432	**0.008**	0.643	0.551	0.826
SEX	F	209.35	349.7	112.12	462.35	193.41	412.58
	p	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
SVL×HAB	F	0.3	0.42	0.01	4.66	0.36	0.42
	p	0.6	0.518	0.907	0.034	0.532	0.528
SVL×SEX	F	6.75	23.13	12.83	24.54	11.73	22.54
	p	**0.014**	**0.001**	**0.001**	**0.001**	**0.002**	**0.001**
HAB×SEX	F	2.3	0.001	0.71	0.44	0.01	1.73
	p	0.137	0.979	0.386	0.482	0.922	0.169
SVL×POP	F	1.28	6.02	11.23	0.63	0.7	0.79
	p	0.273	**0.007**	**0.001**	0.544	0.487	0.458
SVL×HAB×SEX	F	1.77	0.12	0.23	0.47	0.01	0.02
	p	0.197	0.733	0.656	0.505	0.919	0.894
SVL×POP×SEX	F	4.38	2.92	1.77	0.53	0.8	3.34
	p	**0.013**	0.058	0.128	0.6	0.471	**0.038**

ANOVA comparisons on locomotor performance showed that individuals from walls exhibited higher climbing capacities (Table 4.2, Fig. 4.1). Sex did not have a significant effect on locomotor performance. We also detected significant differences between population in sprint capacity. Furthermore, a significant interaction between habitat and sex was identified, where individuals of both sexes from walls exhibited similar locomotor performance, whereas in dunes, males were better climbers then females. ANCOVA comparisons using SVL as the covariate revealed a significant effect of body size on locomotor performance, but differences between habitats and the interaction between habitat and sex remained significant for climbing after accounting for variation in body size (Table 4.3, Fig. 4.1). ANOVA comparisons also showed that lizards from dunes bit harder than those from walls. Bite force was also significantly different between the sexes, where males attained a higher maximal bite force than did females (Table 4.2, Fig. 4.2).

Table 4.2 – Results of ANOVA comparisons performed on each type of locomotor speed and bite force separately to examine the effect of habitat (HAB), population nested within habitat (POP), sex, and their interaction in Bocage's wall lizard – *P. bocagei*. Significant effects are marked in bold. See Material and methods for variable abbreviations F, F-statistic; p, corresponding p value

		SPR	MAN	CLI	Bite
HAB	F	0.689	1.773	9.783	6.49
	p	0.419	0.187	**0.003**	**0.018**
SEX	F	1.117	1.035	3.068	81.123
	p	0.304	0.291	0.07	**0.001**
POP	F	10.771	2.184	2.689	0.927
	p	**0.001**	0.127	0.071	0.381
HAB×SEX	F	2.562	1.131	4.485	0.113
	p	0.132	0.252	**0.03**	0.716
POP×SEX	F	1.451	0.216	1.987	0.791
	p	0.26	0.839	0.139	0.476

Finally, ANCOVA comparisons using HS as the covariate revealed a significant effect of head size on bite performance, and differences between habitats were still

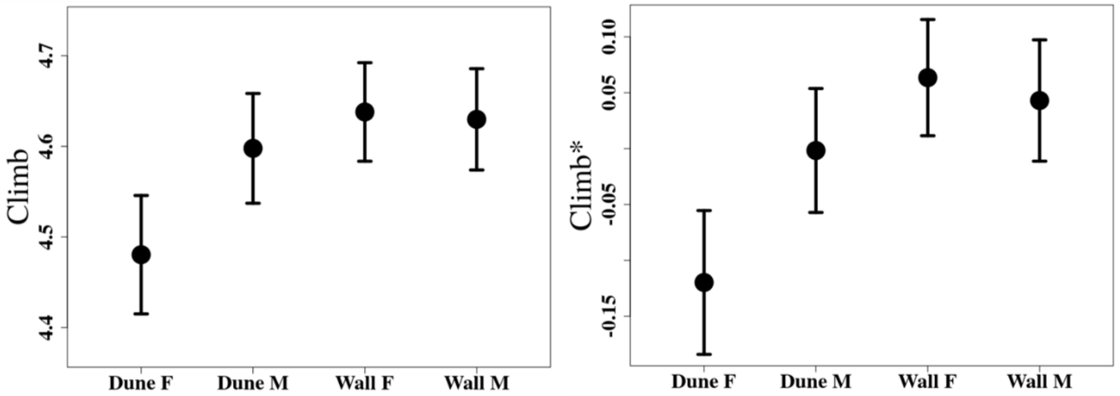

Figure 4.1 – Variation across groups of Bocage's wall lizard— P. bocagei—in climbing speed (left), and climbing speed corrected for SVL (right). Points represent means, and vertical bars denote 95% confidence intervals. Dune F, females from dunes; Dune M, males from dunes; Wall F, females from walls; and Wall M, males from walls.

Table 4.3 – Results of ANCOVA comparisons performed on each type of locomotor speed and bite force performance separately with habitat (HAB), population nested within habitat (POP), and sex as predictors, and SVL (in the case of locomotion) or HS (in the case of bite) as a covariate (represented by X) in Bocage's wall lizard – *P. bocagei*. Significant effects are marked in bold. See Material and methods for variable abbreviations F, F-statistic; p, corresponding p value

		SRP	MAN	CLI	Bite
X	F	14.911	2.308	8.908	2344.48
	p	**0.001**	0.124	**0.003**	**0.001**
HAB	F	3.182	2.969	16.134	3.867
	p	0.069	0.082	**0.001**	**0.048**
SEX	F	0.637	0.784	2.361	10.279
	p	0.414	0.368	0.118	**0.003**
X×HAB	F	1.939	0.328	0.25	0.572
	p	0.166	0.549	0.625	0.451
X×SEX	F	1.159	0.024	1.518	0.358
	p	0.259	0.882	0.23	0.548
HAB×SEX	F	2.828	1.343	5.085	0.686
	p	0.089	0.255	**0.024**	0.415
X×POP	F	9.409	1.814	2.348	1.085
	p	**0.001**	0.148	0.105	0.332
X×HAB×SEX	F	2.761	1.532	0.416	0.852
	p	0.123	0.234	0.508	0.37
X×POP×SEX	F	1.215	0.434	1.941	0.025
	p	0.305	0.606	0.132	0.974

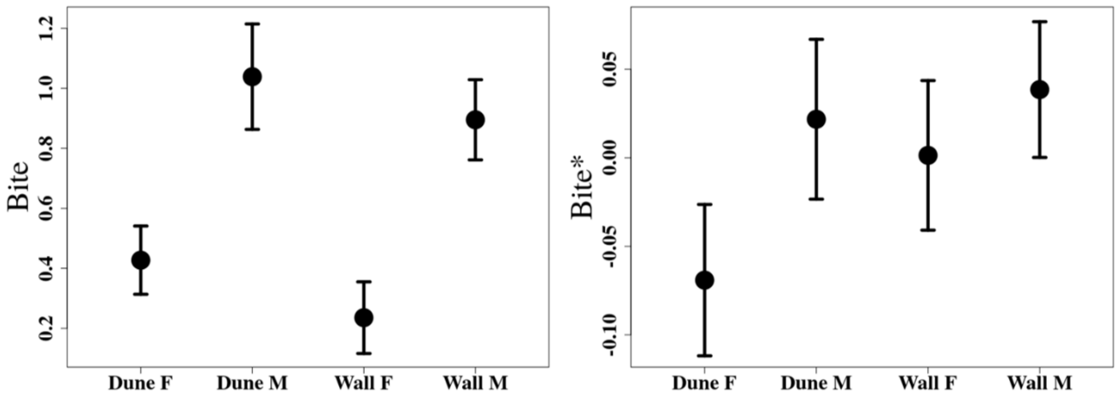

Figure 4.2 – Variation across groups of Bocage's wall lizard – P. bocagei – in bite force (left) and bite force corrected for HS (right). Points represent means, and vertical bars denote 95% confidence intervals. Dune F, females from dunes; Dune M, males from dunes; Wall F, females from walls; Wall M, males from walls

significant after accounting for variation in head size, and also differences between the sexes remained, at least in dunes (Table 4.3, Fig. 4.2).

Two-block partial least-squares regression revealed a significant association between trunk and limb morphology and locomotor performance (r = 0.305 and p = 0.001), where higher speeds are associated with longer trunks and limbs and smaller head size (Fig. 4.3).

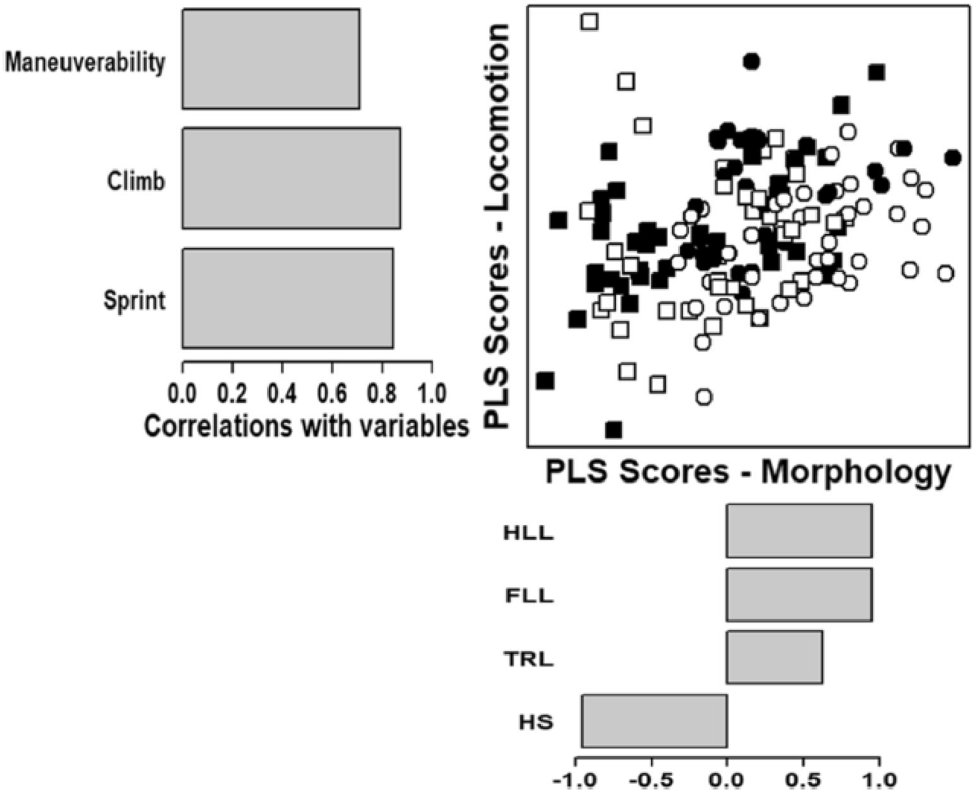

Figure 4.3 – Scatter-plot of individual Bocage's wall lizard – P. bocagei – scores of dune individuals (black squares, males; black circles, females) and wall individuals (white squares, males; white circles, females) obtained from partial least-squares (PLS) analysis between morphology (trunk length, TRL; forelimb length, FLL and hind limb length, HLL; and head size, HS) and locomotor performance (sprint, climb, maneuverability) – variables not corrected by size. Bar-plots next to morphology axis represent the correlations observed between that axis and locomotor performance

However, after size correcting all variables, the significant association between morphological traits and locomotor performance was lost (r = 0.135 and p = 0.565). Two-block partial least-squares regression also revealed a significant association between head morphology and bite performance (r = 0.968 and p = 0.001), where higher bite forces are associated with larger head dimensions (Fig. 4.4). This association remained significant after taking head size effects into account (r = 0.365 and p = 0.001), where relatively higher bite forces were associated with relatively wider and flatter heads, and with relatively longer jaws (Fig. 4.4).

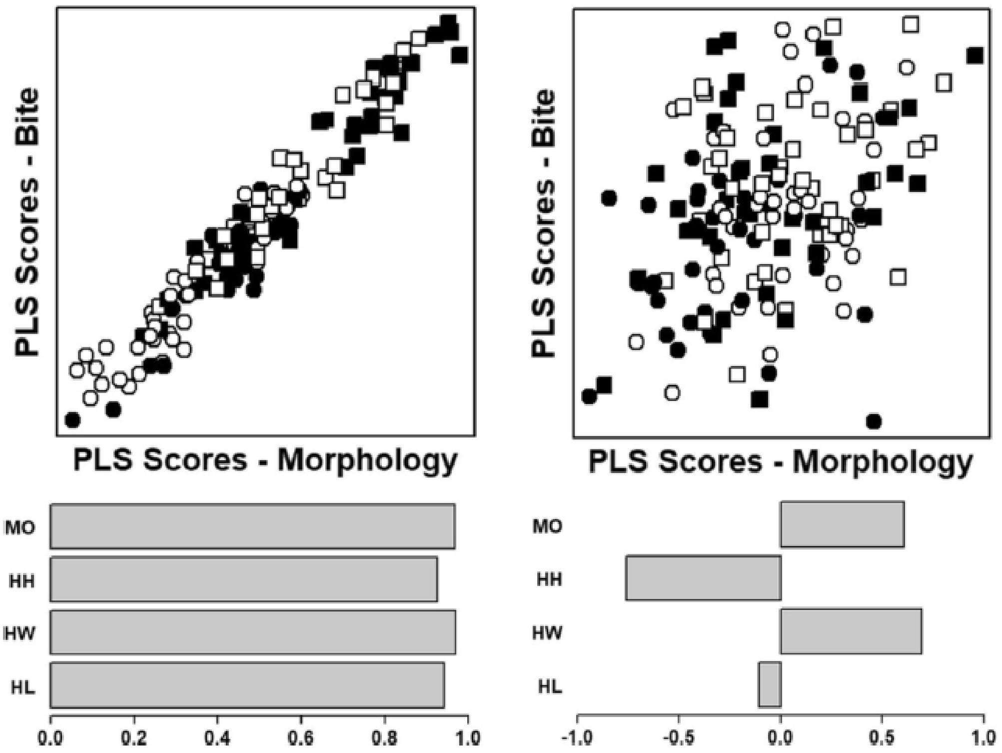

Figure 4.4 – Scatter-plot of individual Bocage's wall lizard – P. bocagei – scores of dune individuals (black squares, males; black circles, females) and wall individuals (white squares, males; white circles, females) obtained from partial least-squares (PLS) analysis between head morphology (head length, HL; head width, HW; head height, HH; mouth opening, MO) and bite force – left, variables not corrected by head size; right, variables corrected by head size. Bar-plots next to morphology axis represent the correlations observed between that axis and bite force

DISCUSSION

Our investigation of how functional performance mediates the evolutionary response of morphology to habitat revealed fast evolving ecomorphological variation among populations of *Podarcis bocagei*, where a direct association existed between head morphology and bite performance. However, we could not establish links between limb morphology and locomotor performance at the individual level after correcting for size effects. Nevertheless, significant differences in climbing capacity existed between habitats, where individuals from walls were better climbers than those from dunes, suggesting a very fast evolutionary response of functional performance to habitat use, identifiable at the intraspecific level. Interestingly, a significant interaction between habitat and sex was detected in climbing performance. The same was the case for biting performance, although sexual differentiation was definitely the main factor driving variation in head functional morphology. Taking into account all the results, we found a complex interaction between natural and sexual selection on whole-organism performance, which are, in some cases, reflected on morphological variation. This complex interaction between different types of selective mechanisms results in context dependent combined variation of morphological and performance traits, which sometimes confirms the ecomorphological paradigm and other times contradicts it.

Form-function associations

Indeed, under the ecomorphological paradigm, an association is predicted between morphology and performance as a response to different selective forces imposed by the environment, i.e., habitat structure in this study. However, here, we observe a complex pattern: we could detect an association between head morphology and bite force, but not between limb morphology and locomotor performance after correcting for size effects. Both pairs of functional morphology sets— head-biting and limbs-locomotion—are commonly associated as a response to environmental factors in lizard species (Losos and Sinervo 1989; Sinervo and Losos 1991; Melville and Swain 2000; Herrel et al. 2001b, Irschick et al. 2005b, Kaliontzopoulou et al. 2013), but cases where this association was not found are also known (Vanhooydonck et al. 2000; Goodman et al. 2008), including several examples in Podarcis lizards (Van Damme et al. 1997; Vanhooydonck et al. 2000; Brecko et al. 2008; Kaliontzopoulou et al. 2012; Gomes et al. 2016). This lack of the typical functional morphological pattern in the locomotor system could be explained by factors other than limb length regulating locomotor performance, such as physiology, behavior, or biochemistry, or even by other morphological and locomotor traits not examined in this study being more relevant (Van Damme et al. 1997; Jayne and Irschick 1999; Braña, 2003; Revell et al. 2007; Vervust et al. 2007).

In the case of bite force, our results suggest an association with head morphology, which is largely driven by size effects and sexual size dimorphism (Fig. 4.4, left). However, after taking size effects into account, we see that head height (negative correlation) and head width and mouth opening (positive correlation) are the traits that may constrain bite force (Fig. 4.4, right). Following biomechanical rules and a common pattern in lizards and other organisms (Herrel et al. 2001a, b, 2005; Thomas et al. 2015), two-block partial least-squares regression suggests that higher bite forces are associated with relatively wider heads (Fig. 4.4). However, we also observed a negative correlation between head height and bite force, where individuals with relatively flatter heads had higher bite forces than those with relatively higher heads, a pattern that disagrees with predictions of biomechanical models of biting in lizards. It has been demonstrating that high bite forces are associated with wider and taller heads in several organisms (e.g., Herrel et al. 2001a, b, 2005; Thomas et al. 2015), indicating that external head measures are not good predictors of the pattern observed and other factors as internal muscle composition should be studied. Our results are in accordance with the known pattern of sexual dimorphism in *Podarcis bocagei* and other lizards, where males are larger in total body size, with relatively larger heads and more robust head shapes (Kaliontzopoulou et al. 2008), a pattern also known to translate into higher bite forces (Herrel et al. 2001a, b; Kaliontzopoulou et al. 2012). Higher bite performance in males is known to be associated with male dominance (Herrel et al. 1999; Husak et al. 2006; Huyghe et al. 2009), an advantage in antagonistic behavior between males for territory defense and/or mate acquisition and/or an advantage during copulation (Lappin and Husak 2005). The morphology-performance axis aligns with sexual differentiation (Fig. 4.4), pointing to sexual selection as a major force driving the joint phenotypic differentiation of head morphology and function; however, natural selection related to habitat type also seems to play a role in shaping whole-organism performance.

Functional morphological responses to habitat variation

The investigation of performance across habitats revealed that lizards from dunes bite harder than those from walls (Table 4.2, Fig. 4.2), as is expected given the larger body size observed in dune populations, and provided that animals with larger heads bite harder (Herrel et al. 2001a, b). Interestingly, however, when body size effects were taken into account, we found that animals from walls bite harder relative to their head size than those from dunes (Table 4.3, Fig. 4.2). This result indicates that the small head size observed in walls does not have repercussions on bite performance. Thereby, the potential physical constraints imposed by saxicolous habits – i.e., smaller heads being favorable in rocky environments to facilitate the use of small holes and rock crevices for refuge (Vitt et al. 1997; Revell et al. 2007) – do not influence bite force performance. A potential explanation for this pattern is different osteology and/or different jaw muscle insertion, orientation, or even length across habitats (Herrel et al. 1996, 1998, 2001b; Lappin et al. 2006; Huyghe et al. 2009). Indeed, this is a very feasible explanation, which merits further attention in future studies, as the feeding apparatus is known to evolve very fast in wall lizards, exhibiting remarkable short-term responses to selection pressures (Herrel et al. 2008). In fact, this observation adds to the evidence suggesting that this flexibility in the relationship between head morphology and function to maintain biting performance is common in *Podarcis*, both within and across species (Herrel et al. 1996; Kaliontzopoulou et al. 2012). As such, a type of many-to-one mapping of morphology on function seems like a plausible mechanism for balancing the requirements posed by natural (habitat use) and sexual selection (Alfaro et al. 2005).

By contrast to this maintenance of biting functionality despite contrasting ecological demands across habitats, locomotor performance capacities seem to align more to expectations (Table 4.2, Table 4.3, Fig. 4.1), we found that animals from walls were faster climbers than those from dunes. Here, natural selection seems to act upon these animals increasing their ability to climb. In other words, lizards from agricultural walls adapt to their natural environment by increasing their climbing speed, which enhances their probability of survival, while escaping from predators in perpendicular surfaces. Several other studies have provided evidence of a clear and strong association between habitat use and performance capacities across species (e.g., *Anolis*: Losos 1990a, b; Irschick and Losos 1999; Elstrott and Irschick 2004, subfamily Lygosominae: Melville and Swain 2000; Goodman et al. 2008, or other systems: Vanhooydonck and Van Damme 2003; Vanhooydonck et al. 2005; Tulli et al. 2011). The results obtained here support the idea that this kind of association can evolve quite fast, being detectable at the microevolutionary level, i.e., among a set of geographically nearby, genetically quite uniform populations of the same species (Pinho et al. 2011). As such, the pattern of differentiation in climbing performance described here represents a very fast functional response to habitat type, occurring in a time frame of about 10,000 years (Pinho et al. 2011). Such a fast, fine-scale response could be facilitated by proximate mechanisms like phenotypic plasticity or differential growth in different habitats, as is known to occur in the locomotor apparatus of other lizard groups (Losos et al. 2000; Kolbe and Losos 2005). Given the high flexibility of ontogenetic trajectories observed across *Podarcis* wall lizards (e.g., Kaliontzopoulou et al. 2010b; Piras et al. 2011), this seems like a reasonable hypothesis, which would need to be further explored experimentally in future studies.

Despite this positive, short-frame association between habitat use and locomotor performance, we also found some unexpected results. Specifically, taking into account several interspecific studies that showed an association between morphology, performance, and habitat use to understand better how natural selection shape whole-organism (e.g., Losos 1990a, b; Irschick and Losos 1999: Melville and Swain 2000; Vanhooydonck and Van Damme 2003; Elstrott and Irschick 2004; Vanhooydonck et al. 2005; Goodman et al. 2008; Tulli et al. 2011), we had predicted that lizards from dunes would be better sprinters than those from walls. However, a differentiation between the two types of habitats in sprint speed was not identifiable in our data. This reduced functional differentiation between habitat types in locomotor performance, which is the "classical" trait expected to vary due to its direct connection to structural habitat, could be a matter of scale, as at the sampled populations are geographically very close, allowing extensive gene flow between them, and they are known to share the most important part of their evolutionary background, being genetically quite uniform (Pinho et al. 2011).

Alternatively, individuals from walls may adapt to moving on inclined surfaces by increasing their climbing capacity, but at the same time maintain their ability to sprint in horizontal surfaces, which is still beneficial given the generally ground-dwelling habits of this species. Indeed, horizontal sprinting is associated with escape from predators (Husak and Fox 2006; Miles 2004) and it should be still under selection in environments where agriculture walls are predominant. In fact, lizards in such environments also escape from predators by sprinting horizontally on top of the walls or by jumping to the ground and sprinting to hide within the adjacent vegetation cover (personal observation). Indeed, *P. bocagei* is quite generalistic in its ecological habits (i.e., sensu Kaliontzopoulou et al. 2015) and maintain a high degree of flexibility in terms of habitat preference. Despite the marked contrast of habitat structure in the localities of origin of the studied populations, individuals did not exhibit differences in the main aspects of microhabitat choice under experimental conditions (Supp. Inf.). This observation suggests that, although morphology and functional performance vary across populations, a global flexibility is also maintained, reinforcing the possibility of an important role for phenotypic plasticity in mediating the observed patterns. Particularly in view of the lack of an association between locomotor performance and morphology observed here, it seems that climbing performance can be enhanced in saxicolous populations through mechanisms (e.g., physiology: Braña, 2003; behavior: Van Damme et al. 1997) which do not influence the capacity of the lizards to also maintain their sprinting performance. In fact, this lack of a trade-off between sprinting and climbing seems to be a recurrent pattern, which occurs at several evolutionary levels in lacertids, including populations of the same species (i.e., results presented here), lineages of the same species complex (Van Damme et al. 1997), and deeply differentiated species at the family level (Vanhooydonck and Van Damme 2001).

Interactions between natural and sexual selection

We have seen how sexual and natural selection shape functional and morphological diversity separately. However, the balance between both selective forces is complex and in this study result in differences in the degree of functional sexual dimorphism between habitats (Table 4.1, Table 4.2, and Table 4.3). Our results on climbing performance showed that individuals from dune habitats were more dimorphic than those inhabiting agriculture walls (Fig. 4.1). This is in accordance with the variation in the degree of morphological sexual dimorphism between different habitats previously reported for *P. bocagei* (Kaliontzopoulou et al. 2010a). While the same morphological pattern was not detected here, most probably due to the lower number of populations and individuals examined, we did identify differences between habitats in the degree of sexual differentiation in climbing capacity. The fact that individuals of the two sexes living in walls had similar climbing performance could be explained by the necessity of both sexes to escape from predators in inclined surfaces. By contrast, individuals from dunes (ground-dwelling) have less of a selective pressure in this direction, and exhibit a more pronounced difference between sexes in their climbing capacities, possibly associated to behavioral differences related to their social roles. Here, males, which normally have larger home ranges to patrol (Stamps 1983; Perry and Garland 2002; Diego-Rasilla and Perez-Mellado 2003), and move more extensively in search of mating opportunities, may benefit more by enhanced climbing capacities, while females seem more limited in this respect (Fig. 4.1). Nevertheless, such a hypothesis should be further investigated in the future.

Put together, the results obtained here highlight the potential for remarkable flexibility in morphology-function associations, and their potential for responding to environmental variation in very short evolutionary time intervals. This means that establishing whether morphological variation is associated with habitat use through influences on whole-organism performance, as dictated by the ecomorphological paradigm, is not necessarily straightforward, even using data on individuals at the intraspecific level. Interestingly, both the head-bite and limbs-locomotion functional systems seem to be quite flexible in wall lizards, but in different directions. In one case – bite performance – the same whole-organism performance capacities can be maintained despite varying external morphological properties. In the second case –

locomotor performance – different performance capacities are achieved by individuals with similar morphological properties. This suggests that these lizards exhibit a particularly high complexity of the morphology-performance association, through the interference of other regulatory mechanisms such as muscle orientation, physiology, behavior, etc. Such complexity may uncouple performance and morphology and provide an explanation for success of this species in the ecosystems of NW Iberia, and also explain their potential for exhibiting extreme levels of intraspecific morphological variability. In this sense, morphology does not seem to be canalized through its integration with functional performance (sensu Klingenberg 2014), and as functionality can be maintained through other properties, external morphology is relatively free to vary. Further studies investigating the ecological significance of performance are required to fully understand how, and at which hierarchical level, these organisms' phenotypic traits respond to environmental variation. For this, investigating how lizards implement their maximal performance to perform different ecological and social tasks is important to understand their true evolutionary potential (Irschick et al. 2005c; Husak 2006).

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Supporting Information Chapter 4

Supporting Information - Microhabitat Use Experiment

Methods

To investigate microhabitat selection under controlled conditions, we performed focal observations in specially designed mesocosms of 1 m^2, made of transparent acrylic. The bottom of each mesocosm was covered by a layer of sand. In the center of each mesocosm we placed two clay vases one on top of the other and surrounded by small rocks, to imitate a stony microenvironment. In each mesocosm we placed two males and two females from the same population for two days. The first day the experiment started at 9h and we allow the animals to get used to the mesocosm during all day, and we only used the data collected in the second day for analyses. To record the substrate selected (vase, rocks, sand) by each individual we visited the mesocosm and made focal observations of the position of each individual at 30-minute intervals from 10h to 17h30 to allow the individuals to recover after each visit. A total of 10 males and 10 females of each habitat performed this experiment, this means 5 mesocosms per habitat type.

To quantify individual microhabitat selection, we calculated the percentage of use of each substrate (vase, rock or sand) throughout all the observations recorded for each lizard. To investigate if there were differences in substrate selected in the mesocosm between individuals from the different habitats and sexes we used a permutational ANOVA with habitat (HAB), population nested within habitat (POP), sex and all interaction terms as predictors. Because permutational ANOVA as implemented in the adonis function uses Euclidean distances, and since percentage data are not Euclidean, we first transformed our data by calculating the matrix of pairwise Bray-Curtis dissimilarities among individuals and then performing a Principal Coordinates Analysis on this matrix to obtain individual scores.

Results

ANOVA comparisons of habitat use in mesocosms did not reveal any differences in the selection of substrate between individuals from different environments however we detect a significant difference between populations.

Table S_4.1 – Results of ANOVA comparisons performed on the individual scores obtained from substrate percentages to examine the effect of habitat (HAB), population nested within habitat (POP), sex and their interaction. F: F-statistic, P: corresponding p-value. Significant effects are marked in bold letter.

	HAB		SEX		POP		HAB×SEX		POP×SEX	
	F	p	F	p	F	p	F	p	F	p
Substrate	2.391	0.087	1.024	0.310	4.364	**0.005**	1.615	0.182	1.221	0.274

Chapter 5

Morphological trade-offs – Bite Performance

Article IV - Morphological trade-offs underlie the duration and magnitude of bite force generation in lizards

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ABSTRACT

Biting performance is important for feeding, territory defense and mating in many animals. One of the most studied functional traits is maximal bite force, but other aspects of biting are rarely considered. Here we examined maximal biting capacity and bite duration in Podarcis bocagei, under different conditions designed to simulate feeding and antagonistic behavior, to investigate how different functional components are optimized for different ecological and social demands. For this purpose, we 1) measured bite duration and investigated its relationship with head morphology, 2) investigated how bite performance varies across ecological and social tasks using four different experimental setups, and 3) tested possible trade-offs between bite force and bite duration and its association with morphology. Our results reinforce the well-known pattern of higher bite force in males, both during frontal and posterior biting. We did not identify differences between the sexes in bite duration but bite performance only varied across experimental setups in males, suggesting a higher functional flexibility. We found no trade-off between bite force and duration when examining raw performance data, but the investigation of form-function associations using the F-matrix revealed that trade-offs, facilitations, and one-to-one relationships are simultaneously involved in the morphological optimization of bite force and duration. Put together, our findings demonstrate the importance of bite performance in ecological and social tasks in males. Remarkably, they also suggest the existence of trade-offs in the morphological optimization of maximal capacity and bite duration, possibly due to physiological constraints on muscle composition, insertion, and orientation.

KEYWORDS

Head morphology, Bite force, Bite duration, Behaviour

INTRODUCTION

The study of how morphology-function associations vary among animals when performing ecologically relevant tasks (e.g. capture prey or escape predators) can help us understand the selective pressures molding morphological diversity (Arnold, 1983; Irschick, 2002). Natural and sexual selection shape phenotypic variation across individuals (Vincent and Herrel, 2007), but the relative contribution of each is not always easy to determine (Herrel et al., 1999a; Lopez-Darias et al., 2015). Considering the balance between the evolutionary mechanisms involved, different functional needs may be difficult to optimize simultaneously, because of conflicting demands within the same phenotypic trait. Individuals may perform several tasks fairly well, but with none of them especially optimized. This trade-off between functional flexibility and performance excellence has been termed the “Jack-of-all-trades-master-of-none” hypothesis (Tulli et al., 2012; Irschick and Higham, 2016). Alternatively, individuals may become specialized in one task at the cost of performing sub-optimally in another, which leads to functional specialization (Arnold, 1992; Herrel et al., 2009; Huey and Hertz, 1984; Irschick and Higham, 2016; Komuna and Chiba, 2007). A well-known example is the trade-off between speed and endurance in athletes, which exhibit a marked negative correlation when comparing professional sprinters and marathoners (Heinrich, 1985). Another well-known trade-off occurs between velocity and force. Fiddler crabs, Uca pugnax, use their claws both for fighting rivals and for feeding (Levinton and Allen, 2005), and crabs with longer claws increase closing speed but exhibit lower pinching forces. In other words, crabs with one specific claw morphology cannot be quick and strong at the same time. The same is the case with the lever system of scorpion chela, where a functional trade-off between speed and force has been identified across species (Simone and van der Meijden, 2017). Likewise, a trade-off between bite force and jaw velocity exists in Darwin’s finches, where higher bite forces have been associated with slow beak movements (Herrel et al., 2009). Darwin’s finches that have strong and robust beaks, to crush harder seeds, may be constrained in their vocal tract resonance function (Herrel et al., 2009). In this case, finch species can have robust beaks to crush harder seeds or instead have gracile beaks allowing them to produce complex song types.

Head morphology and its association with bite performance and ecology is one of the most studied animal functional systems, due to its central role in many basic activities (e.g. Dessem and Druzinsky, 1992; Dumont and Herrel, 2003; Erickson et al., 2004; Herrel et al., 1999a,b; van der Meij and Bout, 2004). In lizards, which are probably the most studied model organisms, head morphology and bite performance are associated with highly relevant social and ecological tasks, including feeding, mating, antipredator defense, male-male combat and territorial contests. On one hand, sexual selection acts on bite force, which increases male potential for territorial and antagonistic behavior, yielding sexual dimorphism in biting performance and related morphological traits (Herrel et al., 1999a; Husak et al., 2006, 2009). On the other hand, natural selection may act to promote resource partitioning, where larger individuals may have the capacity to eat larger and/or harder prey as a result of larger body size, wider gapes and higher bite forces (Pérez-Mellado and Riva, 1993; Herrel et al., 1996, 1999a; Santos et al., 2000) or could lead only to an increase of the dietary spectrum in larger individuals. While the role of bite force in both a sexual and natural selection context is well understood, bite duration has never been thoroughly investigated, despite its importance for both social and ecological tasks. How long individuals can sustain their bite, and how this interacts with maximal biting capacity, are relevant for antagonistic contests, where males frequently “head lock” in fighting position, biting the head of their opponents for prolonged intervals producing injuries (Lappin and Husak, 2005; Lailvaux et al., 2004) and increase predation risk (Cooper and Vitt, 2002). The same is the case during mating: in several lizard species, males bite the females to immobilize them and copulate with them (Galán, 1997). Therefore, it is reasonable to predict that sexual selection may act to enhance biting endurance, where males able to sustain their bites for longer times would have a reproductive advantage. Similarly, biting duration may also be under the influence of natural selection and also potentially contribute to resource segregation both across individuals in general and among specific age and sex groups (e.g. males being able to consume larger or harder prey than females, or resulting in ontogenetic variations in diet) through its influence on prey manipulation (Herrel et al., 2001a). Despite its strong potential for being a sexual and natural selection agent, bite duration has never been examined in light of sexual differentiation, and the possible proximate determinants of such dimorphism, or its relation to other head sexually dimorphic traits, remains unexplored.

Indeed, the different functions of the head may easily yield conflicting demands and promote the appearance of trade-offs among different performance components. In this sense, maximal bite force and bite duration are traits that may be predicted to trade-off due to both physiological and biomechanical reasons. First, a trade-off between force and duration can be expected when considering muscle composition. Animals may differ in muscle contractile properties depending on whether biting is optimized for force or for duration. Maximum force will require more fast-twitch muscle fibers – white muscle –

capable to produce higher forces but easily exhausted. On the other hand, if an animal needs to optimize duration, it will need slow-twitch muscle fibers – red muscle – not capable to produce such high forces, but that are less easily fatigued (Herrel et al., 1999b; Wilson et al., 2002). Second, biomechanical conditions, such as gape angle and biting position, may influence bite force and bite duration (Anderson et al., 2008; Curtis et al., 2010; Dumont and Herrel, 2003). Based on simple lever-system functioning an increase in gape angle decreases bite force (Curtis et al., 2010; Dumont and Herrel, 2003); and bite force increases as the point of bite exertion is shifted to the posterior part of the jaw (Anderson et al., 2008; Curtis et al., 2010; Dumont and Herrel, 2003). Therefore, the combination of gape angle, biting position and morphological traits may lead to the optimization for bite force magnitude or duration, depending on the functional demands – antagonistic contests, copulation events and even prey consumption.

In this study, we examine how different functional components of biting performance are optimized for different demands during ecologically and socially relevant tasks. We examine bite duration to understand the relationship between this functional trait and head morphology, and to investigate if males and females differ in bite duration as is the case for bite force. We hypothesize that bite duration will have a similar pattern as bite force - where an association with head size and relative dimensions yields higher performance capabilities in males. Further, to test how different biting positions (i.e. with respect to gape angle and anteroposterior position, which are known to influence bite force; Dumont and Herrel, 2003) may lead to differences in performance across ecological and social tasks we used four different experimental setups – a combination of two gape angles (closed versus open) and two biting positions (frontal versus back of the tooth row). Based on biomechanical rules, we expect bite force to be higher when animals bite with the back of the tooth row than when biting frontally (Meyers et al., 2018), and at closed as compared to open gape angles (Dumont and Herrel, 2003; Meyers et al., 2018). However, how biting duration may respond to these different setups is not easy to predict, as it will depend on its relation with both bite force and morphological traits. Finally, given that optimal functional capacities may require opposing morphological, biomechanical or physiological adaptations (Stearns, 1992), we investigate possible trade-offs between force and duration in bite performance and in its association with morphological traits. As different morphological configurations may contribute to optimize different functional demands, we tested for the existence of many-to-many mapping using the F-matrix approach to relate several phenotypic traits to several performance variables at the intraspecific level (Ghalambor et al., 2003; Walker, 2007; Bergmann and McElroy, 2014).

MATERIAL AND METHODS

To investigate sexual dimorphism in bite duration, its relation to morphology, and the possible existence of trade-offs with bite force, we focused on the wall lizard Podarcis bocagei, a lacertid endemic to the NW Iberian Peninsula. This species is highly dimorphic in body size, relative head size and head shape (see e.g. Kaliontzopoulou et al., 2010). Lizards for this study were captured in a single population from northern coastal Portugal, in the coastal dunes of Madalena (N41.10□, W8.66□). We selected a coastal dune population because it exhibits more marked sexual dimorphism than those from other habitats (Kaliontzopoulou et al., 2010). We collected a total of 43 adult individuals, including 17 females and 26 males, by noose (García-Muñoz and Sillero, 2010) in April 2016. Lizards were placed in cloth bags, transported to the laboratory, and housed in individual terraria, where they were fed with live mealworms and provided with water ad libitum.

In all individuals captured, we measured snout-vent length (SVL), head length (HL), head width (HW), head height (HH), and lower-jaw length (LJL), using electronic calipers (precision ± 0.01 mm). These head traits were selected because they are the main morphological determinants of bite force (e.g. Herrel et al., 2001a; Kaliontzopoulou et al., 2012). Bite force was measured using an isometric Kistler force transducer (type 9203, Kistler Inc., Winterthur, Switzerland) mounted on a vertical holder and connected to a Kistler charge amplifier (type 5058A, Kistler Inc., Winterthur, Switzerland). Bite force measurements were obtained by provoking the lizard to bite a pair of thin metal plates connected to the force transducer (see Herrel et al., 2001a for a detailed description). Bite performance was recorded and viewed in a laptop using the DATAQ Instruments DI-149 data acquisition device and WinDaq Waveform Browser software (DATAQ Instruments, Akron, Ohio, USA). The tip of the metal plates where the lizard bit was delimited with a marker to ensure all lizards bit at an equal distance from the fulcrum and thus standardize the point of force exertion.

We examined two gape angles – closed (approx. 33º) and open (approx. 44º) – and two bite positions – front and back of the tooth row, which resulted in four experimental setups: closed front (CF), closed back (CL), open front (OF) and open back (OL). These conditions were designed and optimized before the beginning of the experiments for data collection to simulate biomechanical alternatives, but also functionally and ecologically relevant situations. Frontal biting is typically used in social interactions, where during mating males sustain their biting of the flanks of females at closed gape angles, while male antagonistic encounters rather involves wide-gape biting of the opponent for variable time intervals. On the other hand, frontal, closed gape, fast biting is possibly more relevant for prey capture, but lateral biting is also known to be used for prey manipulation, particularly when dealing with long and hard prey items. Each lizard was tested five times in each setup to ensure that the maximal bite force per individual was registered and retained for further analyses. We allowed a rest of at least 30 min between trials to ensure physical recovery of the individuals. Bite duration was then obtained using the maximal bite force curve profile (see e.g. Herrel et al., 2001a) recorded for each individual. To obtain bite duration the onset and offset of biting was determined by the time at which bite force curve crossed the 50% of its maximal value. Because of gape constraints, females only performed in the closed gape angle experiments.

Statistical Analyses

All morphological and bite performance variables were log-transformed before analyses. To represent total head size (HS) we used Mosimann's (1970) geometric mean approach and calculated HS as the third root of the product of the logarithms of HL, HW and HH.

We first investigated sexual dimorphism in bite performance using (M)ANOVA comparisons, with each bite performance trait (bite force or bite duration), under different gape angles (closed vs. open), separately as the response variable, and sex as the predictor. We also performed the same analysis with HS as a covariate. We used permutation-based (M)ANOVA with 1000 randomizations of Euclidean distance matrices, as implemented in the adonis function of the vegan R-package (Oksanen et al., 2017). Because bite duration has never been investigated before, we also performed (M)ANCOVA comparisons with each bite duration at different bite positions (frontal and lateral) separately as the response variable, each head dimension and sex as predictors, and HS has a covariate, to explore size-free dependence of bite duration on head morphology. To investigate how biting setup influenced biting performance, we performed repeated-measures ANOVA on maximum bite force and bite duration of each sex separately, with the experimental setup as a fixed factor and individual as a random factor.

To test for a trade-off between raw force and duration of biting at the individual level, we examined the fit of a linear model with bite duration (CF) as the response variable and bite force (CF) as the predictor. We used the closed frontal setup for this test, because this is the experimental setup most commonly used for measuring variation in bite force in the literature (Herrel et al., 2001a).

To understand the relationship between morphology and function and investigate many-to-many mapping (sensu Bergmann and McElroy, 2014), we used F-matrix statistics to explore the association between head dimensions and biting performance, and test for possible morphological trade-offs between force and duration. This method allows us to disentangle the complex relationship between phenotypic traits and several performance variables. By interpreting the values in the F-matrix we can infer: 1) trade-off relationships, where an inverse relationship exists between two performance measures, leading to a conflicting functional demand on a phenotypic trait; 2) facilitation, where similar functional demands are imposed on a phenotypic trait (i.e. the inverse of trade-off); and 3) one-to-one relationships, where a performance trait is influenced by a single phenotypic trait only.

SVL and head linear measurements were used as phenotype variables and the two recorded bite functional traits were used as performance variables. To obtain the intraspecific F-matrix we used the fmat function for R (Bergmann and McElroy, 2014). We performed this analysis with both sexes together and for each sex separately. Because morphology-function relationships may differ between the sexes due to other factors (e.g. muscle physiology, behavior etc.), we then used a Mantel test of matrix association with 1000 randomizations (as implemented by Bergmann and McElroy, 2014) to compare the male vs. female F-matrices. In the case of males, we also performed a more integrative analysis with performance measurements for all four experimental setups. Throughout, we used effect sizes to determine which values to consider interpretable, considering effects of 0.3-0.5 as “medium” and effects >0.5 as “large” (Cohen 1988).

All statistical analyses were performed using R v. 3.4.3 (R Development Core Team, 2017).

RESULTS

ANOVA comparisons revealed significant differences between the sexes in maximum bite force for closed gape with different bite positions (CF and CL), where males attained a higher maximal bite force than did females (Table 5.1, Fig. 5.1), and these differences remained significant after accounting for variation in head size (Table 5.1).

Table 5.1 – Results of ANOVA comparisons performed on each type of biting performance separately, with sex as a predictor (left) and including HS as a covariate (right). F: F-statistic, p: p-value. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SEX		HS		SEX	
	F	p	F	p	F	p
Maximum force CF	240.560	**0.001**	704.03	**0.001**	7.09	**0.018**
Maximum force CL	197.910	**0.001**	360.86	**0.001**	5.59	**0.019**
Duration CF	0.836	0.369	1.185	0.28	0.307	0.586
Duration CL	5.958	**0.019**	5.766	**0.023**	0.067	0.771

Table 5.2 – Results of ANCOVAs performed on bite duration for frontal and lateral bite positions separately with different head dimensions (X) and sex as predictors, and with HS as a covariate. F: F-statistic, p: p-value. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	HL		HW		HH		LJL	
				Duration CF				
	F	p	F	p	F	p	F	p
HS	0.861	0.369	0.839	0.398	0.844	0.376	0.948	0.341
X	1.717	0.195	0.485	0.480	0.973	0.343	4.341	0.044
SEX	0.010	0.925	0.046	0.839	0.034	0.865	0.007	0.935
X:SEX	0.055	0.800	0.245	0.600	0.001	0.994	1.322	0.282
				Duration CL				
	F	p	F	p	F	p	F	p
HS	5.432	0.028	5.503	0.024	6.002	0.026	0.948	0.333
X	0.765	0.393	0.576	0.459	4.918	0.043	4.341	0.039
SEX	0.065	0.817	0.130	0.719	0.216	0.676	0.007	0.945
X:SEX	0.782	0.401	1.413	0.255	0.527	0.499	1.322	0.259

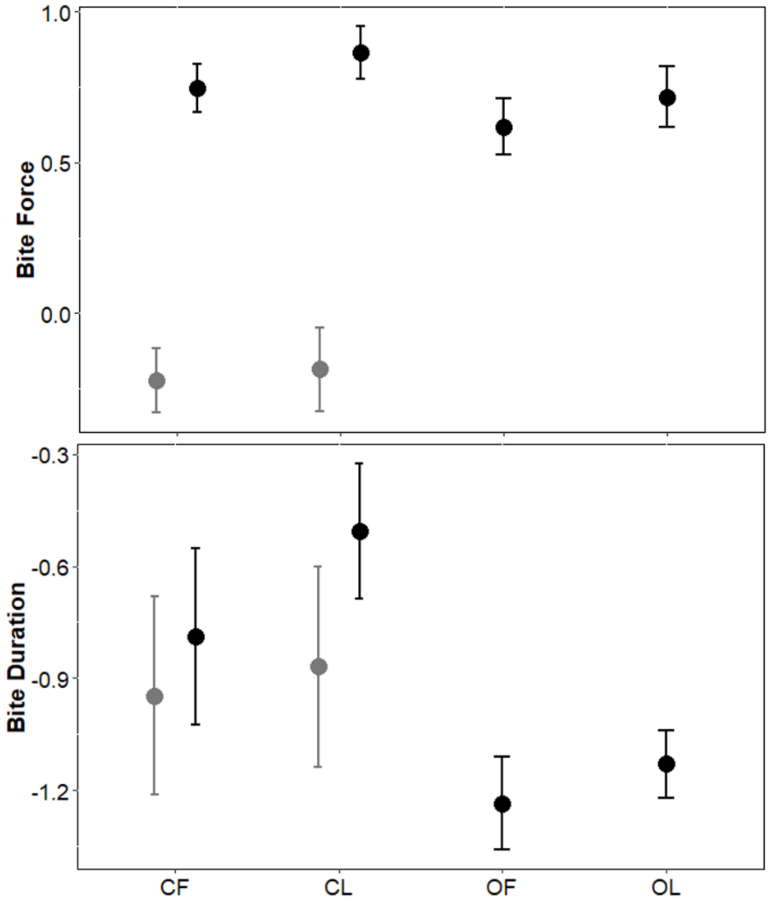

Figure 5.1 – Least-squares means observed in males (black) and females (grey) for maximum bite force (top) and bite duration (bottom). Vertical bars denote 95% confidence intervals. See Material and Methods for variable abbreviations.

Significant differences also existed in the duration of bite performance for closed lateral (CL) bites, where males sustained their bites longer than females (Table 5.1, Fig. 5.1). However, this effect was rendered non-significant after accounting for head size effects (Table 5.1). ANCOVA tests in the frontal biting setup indicated that only LJL had a significant effect on bite duration after size effects were taken into account (Table 5.2). In the case of lateral biting, both HH and LJL had a significant effect (Table 5.2). Repeated-measures ANOVA indicated that males attained higher bite forces when biting at the back of the tooth row compared to when biting at the front, and this was the case at both gape angles examined (Table 5.3, Fig. 5.1). Bite duration at closed gape angle was significantly higher during lateral compared to frontal biting in males (Table 5.3, Fig. 5.1). In the case of females, we did not detect significant differences between the different setups (Table 5.2, Fig. 5.1).

The linear model used to investigate the trade-off between raw values of bite duration and bite force (CF setup) was not significant (F=0.94, p=0.314).

Table 5.3 – Results of repeated measures ANOVA performed between different types of biting performance in each sex separately. F: F-statistic, p: p-value. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

		Males		Females	
		F	p-value	F	p-value
Maximum force	CF vs CL	8.527	**7.3×10^{-3}**	2.453	0.137
	OF vs OL	21.840	**8.7×10^{-5}**		
Duration	CF vs CL	6.798	**0.015**	0.321	0.579
	OF vs OL	2.720	0.112		

The examination of F-matrix statistics considering both sexes together (Table 5.4) revealed that HL is involved in one-to-one relationships, where animals with longer heads exhibit higher durations at closed gapes and frontal bite position. By contrast, HH was involved in facilitation where higher heads were associated to higher bite forces and longer durations in both closed and frontal bites. Moreover, we detected a trade-off that involved LJL, where longer lower jaws increased bite force but decreased duration in closed and frontal bites. When analyzing males and females separately, we identified one-to-one, facilitation and trade-off relationships that varied between the sexes (Supplementary material).

Table 5.4 – F-matrix for both sexes of *P. bocagei* with cells filled with standardized partial multiple regression coefficients. Row sums and variances, column sums and variances of the absolute values, and grand sums of absolute values for columns (before backslash) and rows (after backslash) can be found in the F-matrix. F-matrix cells with values of medium and large effect size are indicated by shading (0.3-0.5 light grey, >0.5 dark grey).

	CF	Duration CF	CL	Duration CL	Sum	Var
SVL	-0.018	-0.214	-0.029	-0.221	-0.481	0.013
HL	0.118	0.862	0.151	-0.083	1.049	0.171
HW	0.177	-0.105	0.070	-0.227	-0.086	0.032
HH	0.311	0.569	0.115	0.971	1.966	0.137
LJL	0.413	-1.005	0.668	-0.143	-0.067	0.549
Sum	1.036	2.756	1.034	1.646	6.471/3.65	
Var	0.024	0.154	0.069	0.132		

However, the comparison of the two F-matrices did not identify significant differences (Mantel r=0.109, p-value=0.612), and as such we renounced further interpretation of sex-specific matrices and focused on the pooled-sexes analysis (Table

5.4). When examining the extended dataset including all four biting setups available for males, we detected additional trade-offs and facilitations (Table 5.5). Three morphological variables were involved in trade-offs of biting performance: 1) higher SVLs were associated to longer bites in CL but shorter bites in OF and decreased bite forces in OL; 2) longer heads increased biting duration in CF, OF and OL, but decreased bite force in OL; and finally 3) longer lower jaws caused higher bite forces in all experimental setups, but a decrease in duration in three of the four setups (CF, OF and OL). Moreover, HH was again involved in facilitation, where higher heads were associated to higher bite forces in CF, OF and OL, and also to higher biting duration in CL.

Table 5.5 – F-matrix for males *P. bocagei*, and considering all four experimental setups, with cells filled with standardized partial multiple regression coefficients. Row sums and variances, column sums and variances of the absolute values, and grand sums of absolute values for columns (before backslash) and rows (after backslash) can be found in the F-matrix. F-matrix cells with values of medium and large effect size are indicated by shading (0.3-0.5 light grey, >0.5 dark grey).

	CF	Duration CF	CL	Duration CL	OF	Duration OF	OL	Duration OL	Sum	Var
SVL	0.245	-0.165	0.002	-0.815	0.288	0.320	0.391	-0.141	0.125	0.158
HL	-0.138	0.780	-0.194	0.197	-0.171	0.457	-0.432	0.423	0.923	0.171
HW	0.140	0.031	0.243	0.254	0.262	-0.126	0.012	-0.164	0.652	0.029
HH	0.361	0.080	-0.074	0.501	0.300	0.096	0.438	0.246	1.948	0.039
LJL	0.338	-0.757	0.731	0.225	0.321	-1.013	0.551	-0.419	-0.013	0.409
Sum	1.222	1.802	1.243	1.993	1.341	2.011	1.824	1.394	12.832/3.660	
Var	0.011	0.138	0.082	0.069	0.003	0.138	0.042	0.018		

DISCUSSION

Our investigation of the different functional components of bite performance revealed that trade-offs, facilitation, and one-to-one relationships are simultaneously involved in the morphological optimization of bite force and bite duration (Table 4, Table S_5.1 Supp. Inf.). Our results suggest that opposing selective pressures acting on bite performance underlie variation in some morphological traits, which cause bite force to increase at the cost of a reduction in bite duration, or the other way around. In terms of the selective forces driving this functional system, though, bite force seems to hold more weight than duration: while our results reinforce the well-established pattern of higher bite forces in males, both during frontal and posterior biting, we did not identify remarkable differences between the sexes in terms of bite duration (but see also below). The comparison of within-sex variation of experimental setups sheds more light to the possible causes of bite performance variation, where only males vary, a fact that suggests an important role of mainly social behaviors, but also with potential consequences for ecological functions.

To complement previous knowledge on the biting functional system, we investigated for the first time bite duration and its relationship with head morphology with particular attention on sexual dimorphism, which is known to be the major direction of phenotypic variation for head traits in lizards and other model organisms. We found that, contrary to the marked sexual differentiation observed for bite force, bite duration was similar between the sexes (Table 1). While males attained higher bite forces than females even when correcting for head size, a pattern typical of many lizard species (e.g. Herrel et al., 1999a, 2001a; Husak et al., 2006; Kaliontzopoulou et al., 2012), bite duration was only significantly different between the sexes in the CL setup, and this difference was mainly driven by head size, contradicting our starting hypothesis. This similarity between the sexes in biting duration could be explained by the risk of predation that affects both sexes equally, because the longer an animal takes to handle the prey (long bite duration) the more exposed it is to predation (Herrel et al., 2007). In addition, muscle architecture (presence/absence and position) are known to exhibit few differences between males and females of other Podarcis species, although total adductor mass is much higher in males (Herrel et al., 1996). Therefore, while muscle characteristics more associated to biting endurance, like the type and position of muscles, do not seem to be different between the sexes, the increase in total adductor muscle mass in males can presumably result in a higher bite force (Herrel et al., 2001b) as we observed in our data.

To shed more light on the determinants of bite performance, and what might be causing sexual variation in this functional system, we examined how the magnitude and duration of biting vary potentially associated to social and ecological behaviors, simulated through different experimental configurations. We found that bite performance only varies in males (Table 3), which provides evidence for a previously unexplored functional differentiation between the sexes, suggesting an important role for social pressures, but also baring potential ecological implications. The role of bite force in both social and ecological tasks is well understood in lizards: higher bite force allows males to have larger territories (Lappin and Husak, 2005), win fights (Lailvaux et al., 2004; Huyghe et al., 2005), have access to more females (Lappin and Husak, 2005) and consume larger and harder preys (Stamps, 1977; Preest, 1994; Herrel et al., 1999a).We found that males have higher bite performance – both force and duration – when biting with a closed gape than at a wide gape (Table 3, Fig 1). Similarly, the results obtained through the comparison of frontal vs. posterior biting suggest that males – but not females – may further enhance their already higher bite forces when biting at the posterior region of their tooth raw. This kind of behavior is displayed during prey manipulation, when consuming long and hard prey (Herrel et al., 1999a; Meyers et al., 2018), and our results suggest that increased posterior biting performance in only one sex could contribute to the differences is prey size spectrum between sexes, as males may use this tactic to gain access to larger and/or larger prey (Herrel et al., 1999a; Meyers et al., 2018). From a previous study, we have little evidence of strong dietary divergence between sexes in P. bocagei, but males consumed relatively harder prey than females (Kaliontzopoulou et al., 2012).

Based on mating behavior, it is reasonable to predict that bite duration may be differentially important in both sexes, potentially yielding sexual differences in this trait. Males of P. bocagei bite the female in the flanks during copulation, which can last from 22 to 30 minutes (Galán, 1997), a behavior which is known to occur in several other lizard species (e.g. Algyroides sp., Iberolacerta sp, Lacerta sp., Timon sp. or Zootoca sp. in Arnold et al., 2007). This type of social pressure on biting performance was investigated here through the closed-frontal (CF) experimental setting, as males hold females by biting them with a relatively small gape and in a frontal position. However, our results do not support the prediction that sexual selection related to mating behavior may drive sexual dimorphism in bite duration, as males and females did not differ in bite duration in CF (Table 1). However, these results need to be taken with some caution, as this might be due to methodological aspects or even behaviour. First, bite duration, as measured here, may not directly reflect this behavior. In our study, we used the bite force curve to extract bite duration and we obtained the time at which the bite force curve crossed the 50% of its maximal value. However, during copulation males are not expected to perform at their highest force capacity, as this could cause serious injuries to females, a strategy which would not be evolutionarily favorable at the species level. To definitely evaluate the relevance of biting endurance for male reproductive fitness, one would need to uncouple maximal bite force from the capacity to sustain the bite, but such a behavior is particularly difficult to simulate under laboratory conditions. Furthermore, bite performance used in fighting behavior between males was simulated with the open gape setup, where males with high bite forces are expected to win more combats. Males, because they have bigger heads than females, performed at wide gapes but bite performance decreased in force and also in duration compared to bite performance at closed gape. Bite performance is important in antagonistic interaction but from our results this behavior is not the most important selective force on bite performance. However, simulating male antagonistic encounters in laboratory conditions could help us understand if not only bite force (Abalos et al., 2016; Huyghe et al., 2005) but also bite duration may predict the outcome of combats.

Theoretically, and based on the behavior of this and other lizard species, all the aforementioned social and ecological tasks would benefit if bite force and bite duration could be optimized at the same time. Head height seems to contribute in this direction in the biting functional system of lizards: our analyses of the F-matrix of performance-morphology associations suggests that this is a facilitation trait increasing both bite force and bite duration (in both sexes or in males separately). This adds to previous knowledge about the role of head height in driving bite performance and extends our understanding of why this trait is one of the main dimorphic characters in many lizard species (e.g. Herrel et al., 2001b; Husak et al., 2006; Kaliontzopoulou et al., 2012). Indeed, it seems that the augmentation of internal muscle mass enhanced through an increase of head height (e.g. Herrel et al., 2001a, b; Thomas et al., 2015) not only contributes to higher bite forces, but also amplifies the capacity to sustain a bite for longer intervals. Since maximum head height was measured at the posterior, higher region of the head, as is typically done in lizard biometrics, we can hypothesize that this may be the result of increased space for jaw adductor muscles. Yet, this remains to be investigated based on morphology and physiology of head muscle in males and females of P. bocagei.

Despite this pattern of morphological facilitation of functional performance, the production and maintenance of phenotypic traits may depend on evolutionary and biomechanical constrains, as well as on physical space and correlations with other traits (e.g. muscle physiology) (Irschick and Higham, 2016). As such, optimization of one trait usually occurs at the cost of another, due to mechanical or physiological trade-offs (Lailvaux and Husak, 2014; Irschick and Higham, 2016). Our results support this idea, as we found that trade-offs occurred at a much higher frequency in the biting functional system of P. bocagei, as compared to the single aforementioned case of facilitation. Indeed, while a trade-off between bite force and bite duration was not evident when examining raw traits, an evolutionary trade-off was clearly suggested through the analysis of morphology-function relationships. Our results indicate that an increase of LJL leads to an increase in bite force but a decrease in bite duration (Table 4). Supporting the same reasoning of a morphological trade-off, we also found that – when analyzing males separately – an increase in HL was associated to an increase of bite duration and a decrease of bite force. A trade-off between force and duration can be expected taking into account head morphology and associated muscle physiology. Animals with longer lower jaws will have more space in the posterior part of the jaw where the external adductors of the jaw attach – i.e. the musculus adductor mandibulae externus superficialis (MAMES). This muscle is composed of more fast fibers which could then insert onto the lateral side of the lower jaw and/or increase pennate muscle (Herrel et al., 1996, 1999b), potentially explaining the observed increase in bite force. On the other side, the increase of bite duration in males associated to longer heads (in total) may be associated to an increase of the musculus pterygoideus (MPt), which is composed of slower fibers (Herrel et al., 1996, 1999b). The use of specific muscles for certain performance traits (LJL[MAMES]-bite force, HL[MPt]-bite duration) and/or with different physiology (LJL[fast fibers]-bite force, HL[slow fibers]-bite duration) can lie at the origin of the trade-off detected within our data. However, these hypotheses are largely based on studies of muscle physiology in other, phylogenetically distant, lizard species (i.e. lizards of the genus Uromastix and Plocederma stellio, Herrel et al., 1999b), and as such a comprehensive investigation of the physiology of head muscles in P. bocagei would be necessary to test them.

In summary, our results indicate that bite force holds more importance than bite duration because little differences were detected in bite duration. However, duration is constrained by predation risk when performing social and ecological tasks and this affects both sexes. The use of different experimental setups allowed us to detect possible causes of bite performance variation, demonstrating the importance of bite performance in ecological tasks, and in social behavior in males. Remarkably, our results also suggest the existence of trade-offs in the morphological optimization of maximal capacity and bite duration, possibly due to physiological constraints on muscle composition, insertion, and orientation. This study is the first potentially needed to understand a very relevant performance used by several organisms not only lizards. Future work is needed to help us understand the relationship between internal morphology and bite performance and to include not only bite force and duration but also bite endurance which will allow us to associate with mating behavior and potentially with fitness. Further, it would be relevant to investigate if the trade-off found would be maintain in other species, populations or animals using other habitat types since it is known to influence social and ecological behaviors.

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Supporting Information Chapter 5

Table S_5.1 – F-matrices for males and females of *P. bocagei* separately with cells filled with standardized partial multiple regression coefficients. Row sums and variances, column sums and variances of the absolute values, and grand sums of absolute values for columns (before backslash) and rows (after backslash) can be found in the F-matrix. F-matrix cells with values of medium and large effect size are indicated by shading (0.3-0.5 light grey, >0.5 dark grey).

	CF	Duration CF	CL	Duration CL	Sum	Var
			Females			
SVL	0.364	-0.525	0.331	-0.234	-0.064	0.19
HL	0.106	0.414	0.231	-0.166	0.584	0.059
HW	-0.016	-0.586	-0.218	-0.447	-1.266	0.063
HH	0.269	-0.023	0.272	0.409	0.926	0.033
LJL	0.318	0.872	0.412	0.329	1.931	0.069
Sum	1.072	2.419	1.463	1.584	6.539/4.771	
Var	0.022	0.095	0.006	0.014		
			Males			
SVL	0.245	-0.165	0.002	-0.815	-0.733	0.206
HL	-0.138	0.780	-0.194	0.197	0.645	0.200
HW	0.140	0.031	0.243	0.254	0.668	0.010
HH	0.361	0.080	-0.074	0.501	0.868	0.068
LJL	0.338	-0.747	0.731	0.225	0.547	0.394
Sum	1.222	1.802	1.243	1.993	6.261/3.461	
Var	0.206	0.138	0.082	0.069		

Chapter 6

General Discussion

My study sought to investigate the interplay between morphology, performance, and environmental variation in lizards, using *Podarcis* spp. as a model organism. For this purpose, I followed Arnold's paradigm (1983) to investigate the relationship between external morphological traits and performance variables – locomotion and bite capacity. However, from the results obtained in this thesis for this model organism, it has become apparent that finding direct associations between these two set of traits is not always straightforward, but rather requires the incorporation of other variables such as habitat use and behaviour. The main predictions addressed in this thesis were:

i. morphological variation in syntopic populations of different species was expected to be associated to habitat use and this link was predicted to be established through locomotion – Chapter 2;

ii. the methodology used to quantify maximal performance capability (maximum sprint speed) could potentially influence the statistical properties of the collected data and the derived biological interpretations – Chapter 3;

iii. morphological traits and performance of different populations of a single species were expected to be mould by natural and sexual selection differently depending on the surrounding environment – Chapter 4;

iv. different demands during ecologically and socially relevant tasks were expected to result in different optimized functional components of biting performance – Chapter 5

This approach provided insights into the evolutionary importance and consequences of morphological variation. The results of the case studies that comprise this thesis were discussed in detail in each chapter. The purpose of this last chapter is to synthesize on the main findings and discuss their contribution in extending current knowledge in ecomorphology.

MORPHOLOGY – PERFORMANCE

In this study, an association between external morphology and locomotor performance was not detected at the interspecific or even at the intraspecific level

(Chapter 2 and 4). These results and, also other studies that failed to detect similar correlations both within and between species of wall lizards (Brecko et al., 2008; Goodman et al., 2008; Van Damme et al., 1997; Vanhooydonck et al., 2000), suggest that predictions formulated under the ecomorphological paradigm is not necessarily always fulfilled. One aspect that all these studies, including this thesis, had in common was the use of lacertid lizards and most commonly of wall lizards (*Podarcis* spp.) as model organisms. *Podarcis* wall lizards provide an intriguing model system for investigating the evolutionary meaning of phenotypic traits, as they exhibit generally preserved body plans, but also high inter- and intraspecific morphological variation (e.g. Arnold, 1998, Kaliontzopoulou et al., 2012a). However, this variation can lead to overlap between groups, not allowing the differentiation between lineages (Kaliontzopoulou et al., 2012a), which could make it harder to establish a link between morphology and functional traits. Nevertheless, the association between performance and design can actually operate through other phenotypic traits as physiology, internal anatomy, biochemical characteristics or behaviour (e.g. Adolph and Pickering, 2008; Van Damme et al., 1997; Vervust et al., 2007; Žagar et al., 2015) – but investigating all possible phenotypic traits at the same time is both time- and resource-demanding. Firstly, the decision of using external morphological traits was made because it is the part of the phenotype that interacts with the environment and understanding how external morphology varies is essential for understanding how the diversity of the organism evolves. Furthermore, external morphological traits are involved when performing a wide array of crucial ecological and social functions, and they are subject to strong selective influences (Garland and Losos 1994). Finally, external morphology is the easiest phenotypic trait to quantify in the field, which facilitates extensive data collection, essential when aiming to identify ecomorphological and functional morphology associations at the interspecific but even more at the intraspecific level.

An association was detected between bite performance and head morphology at the intraspecific level in this thesis (Chapter 4, Fig. 6.1). This association was mainly driven by size effects and sexual size dimorphism. It is well known that, in wall lizards, males tend to be larger in body size and normally have relatively larger heads and robust head shapes (Kaliontzopoulou et al., 2008), which translate into stronger bite forces (Herrel et al 2001a, b; Kaliontzopoulou el al., 2012b). It has also been demonstrated in previous studies that high bite forces are associated with wider and taller heads (e.g., Herrel et al., 2001a, b, 2005). Curiously, from the results obtained in this thesis a negative correlation between head height and bite force was observed, where individuals with relatively flatter heads had higher bite forces than those with relatively higher heads

(Chapter 4, Fig 6.1), a pattern that disagrees with predictions of biomechanical models of biting in lizards. External head measures are expected to be informative of bite capacities where high bite forces are associated with wider and taller heads in several organisms (e.g. Herrel et al. 2001a, b, 2005; Thomas et al. 2015). However, the result obtained in this thesis as already demonstrated by another study investigating bite force capacity in male collared lizards (Lappin and Husak 2005) suggest that some caution should be taken regarding the association of external morphology and bite capacities.

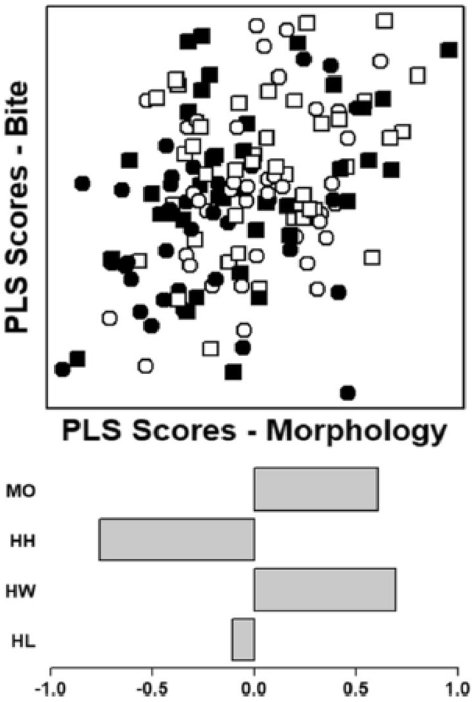

Figure 6.1 – Scatter-plot of individual Bocage's wall lizard – *P. bocagei* – scores of dune individuals (black squares, males; black circles, females) and wall individuals (white squares, males; white circles, females) obtained from partial least-squares (PLS) analysis between head morphology (head length, HL; head width, HW; head height, HH; mouth opening, MO) and bite force –variables corrected by head size. Bar-plots next to morphology axis represent the correlations observed between that axis and bite force.

Taking into account the results obtained during this thesis about morphology – performance relationships, and in order to shed further light into the detailed mechanisms that drive them, future work could examine other phenotypic traits such as muscle architecture – fibre composition and muscle insertion and orientation – as small differences in these features could substantially influence performance capacities (Bonine et al., 2005; Herrel et al., 1999b; Zaaf et al., 1999). In addition, it would be interesting to investigate the morphology of specific accessory structures, such as claws and toes, which may increase e.g. the ability to climb on vertical substrates (e.g. Arnold 1998; Rubolini et al 2006; Van Damme et al 2015; Zani 2000). Moreover, it may be relevant to investigate other aspects of performance in the species included in this thesis (e.g. locomotor endurance, acceleration or agility – Van Damme and Vanhooydonck, 2002; Vanhooydonck et al., 2000, 2001, Vanhooydonck and Van Damme, 2005; or bite endurance) and investigate their relationship with morphology. Future work could also examine if the pattern observed in Chapter 2 between two species – saxicolous and ground-dwelling lizards living in syntopy had few locomotor performance differences and no association with morphological traits – would be the same with other species pairs. More generally, it would be of relevance to investigate phylogenetically close species (i.e. of the same genus) living together in the same habitat – sympatry – and compare to the results when they are alone – allopatry –, as such a study would elucidate if and how the relationships between morphology and performance are modified due to species interactions (e.g. Žagar et al 2017). Furthermore, extending this framework, replicating this kind of study with several pairs would allow to test if the intensity and type of change due to species co-existence is the same depending on the environment and species selected.

HABITAT USE – ASSOCIATION WITH MORPHOLOGY AND PERFORMANCE

Investigating the link between an organism´s morphology, performance and its environment can provide useful insights into adaptation (Arnold 1983; Garland and Losos 1994; Wainwright and Reilly 1994). At the interspecific level (Chapter 2), a significant association between morphology and habitat use was found, where trunk length and head height were the traits with the highest contributions. Habitat use has been shown to drive macroevolutionary variation in head shape in wall lizards (Kaliontzopoulou et al., 2015; Urošević et al., 2012; Vanhooydonck and Van Damme, 1999). In fact, saxicolous lizards can be distinguished because of the dorso-ventral compression of the head, a

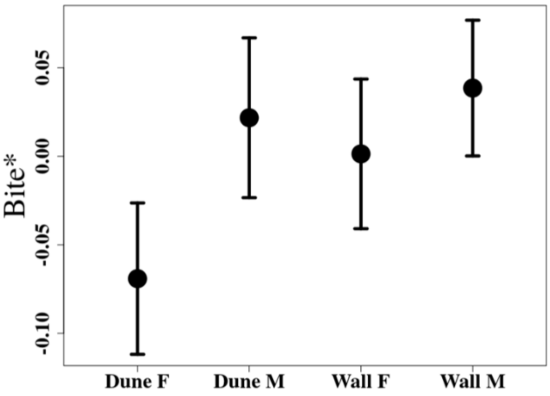

Figure 6.2 – Variation across groups of Bocage's wall lizard – *P. bocagei* – in bite force corrected for head size. Points represent means, and vertical bars denote 95% confidence intervals. Dune F, females from dunes; Dune M, males from dunes; Wall F, females from walls; Wall M, males from walls (data from Chapter 4).

mechanical constrain related to refuge use (Edwards et al., 2012; Herrel et al., 2001c; Kaliontzopoulou et al., 2010; Vanhooydonck and Van Damme, 1999; Vitt et al., 1997). We found that this mechanical constraint is also observed at the intraspecific level (Chapter 4), where animals from walls had relatively smaller heads than those from dunes. Surprisingly, however, this constraint did not translate into a disadvantage in terms of bite performance, as, when head size was taken into account, animals from walls bit harder than those from dunes (Fig 6.2). In other words, when habitat type imposed physical constraints, variation in head morphology occurred in a direction that facilitated the maintenance of biting performance.

In the same study (Chapter 4), the patterns observed for locomotor performance partially aligned to our starting predictions, where animals from walls were faster climbers than those from dunes (Fig. 6.3). Several studies had shown a strong association between habitat use and performance capacities (e.g., *Anolis*: Losos, 1990a, b; Irschick and Losos, 1999; Elstrott and Irschick, 2004, lygosomine geckos: Melville and Swain, 2000; Goodman et al., 2008, or other systems: Vanhooydonck and Van Damme, 2003; Vanhooydonck et al. 2005; Tulli et al., 2011). The enhanced climbing capacity of lizards from walls increase their probability of escaping from predators in perpendicular surfaces hence also increasing their chances of survival. Interestingly, then, we found variation in locomotor performance, but still could not identify differences in limb morphology between individuals from different habitats, which suggests a faster response of performance as compared to morphology. Furthermore, predation pressure may vary

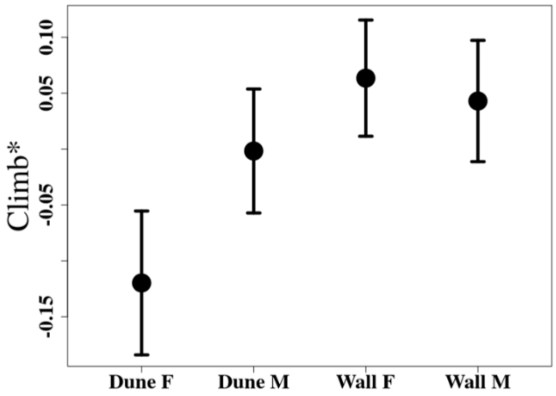

Figure 6.3 – Variation across groups of Bocage's wall lizard— *P. bocagei*—in climbing corrected for SVL. Points represent means, and vertical bars denote 95% confidence intervals. Dune F, females from dunes; Dune M, males from dunes; Wall F, females from walls; and Wall M, males from walls (data from Chapter 4).

across habitats (Cortada, 2016) and natural selection may be more intense in wall populations than in those inhabiting dunes - enhancing their climbing capacities.

Since differences in habitat use were related to morphological or performance differences, potentially interesting to investigate in future work is how habitat disturbance (fires, floods, etc.) may affect morphology and performance as this type of events can destroy or open the environment and individuals will need to adapt. Similarly, occurrences of translocation of individuals – invasive species – may also expose individuals to new habitats and result in the evolution of ecomorphological adaptations. Such research could provide an implementation of functional morphology and ecomorphology for conservation purposes, adding an important practical dimension to the results of this thesis.

THE RELEVANCE OF BEHAVIOUR

In many cases, behaviour can act as a "filter" on the association between morphology – performance – habitat use (Garland and Losos, 1994, Irschick et al 2005, Husak and Fox 2006). When using the ecomorphological paradigm, as I use in this thesis, it is essential to interpret the results taking into account the behaviour of organisms in their natural environment. With the extension of the ecomorphological paradigm to include not only ecological functions (e.g. feeding, escape from predators, habitat and refuge use) but also social interactions (Irschick 2007; Isrchick et al 2008;

Husak and Fox 2008), several behaviours used daily by the organisms were added to the paradigm – inter- and intraspecific antagonistic behaviour, territoriality and mating behaviour, or competition with conspecifics. This inclusion affects all our studies, from the choice of measured traits to the interpretation of the results.

For instance, in the interspecific study presented in Chapter 2, the variables used to quantify habitat and microhabitat use, and locomotor performance were selected because of their relevance in the everyday routine of the lizards in that environment. With this in mind, the results presented in this thesis showed that, even if considered a ground-dweller, *P. bocagei* was a better climber then *P. guadarramae lusitanicus*. This result can be explained because *P. bocagei*, although generally mentioned as a ground-dweller, is a generalist species, that uses several different types of habitats (Kaliontzopoulou et al., 2010) as agriculture walls – the main habitat used by this species in this syntopic place. These human-constructed walls are very steep so *P. bocagei*, benefits from being a sprinter, but also from enhanced climbing capacities. Had the distinction between agriculture walls and big rocks not been explicit in the protocol used for collecting habitat data, the interpretation of the results would have been difficult. This case exemplifies the importance of a good knowledge on the model organism´s behavioural ecology when designing study protocols and interpreting the observed statistical patterns.

Indeed, our knowledge about *P. bocagei* ecology allowed a more direct investigation of the association between performance and behaviour in the last study presented in this thesis – Chapter 5. Four different experimental setups were designed to simulate biomechanical alternatives, but also functionally and ecologically relevant situations common in the everyday routine of the lizards. Examining the results of this study, it was possible to demonstrate the importance of bite performance in ecological and social tasks in males, including feeding (Stamps, 1977; Preest, 1994; Herrel et al., 1999a), mating (Lappin and Husak, 2005), male-male combat (Lailvaux et al., 2004; Huyghe et al., 2005) and territorial contests (Lappin and Husak, 2005). Most of these behaviours are performed only by males, which can explain the variation observed in the study of Chapter 5 between experimental setups in males and not in females.

In order to better understand the role of behaviour in the ecomorphological paradigm, it would be relevant to complement the data presented here with behavioural observations in the field. Studying animals in their natural conditions can link performance traits and fitness – survival and reproductive success. While in this thesis all the performance traits were measured in the laboratory - where maximum performance free of other constraints can be obtained - understanding how animals optimize their performance in different ecological and social functions would help us understand in which behaviours animals use higher percentages of maximal capacities and in which ecological and social contexts selection pressures are acting (Husak and Fox 2006). Furthermore, with the use of mesocosm experiments where a small population of animals (females and males) can be maintained and followed, it may be possible to examine if individuals with better performance have bigger or better quality home-ranges (Lappin and Husak, 2005), increase possibility of chase rivals out of their territory and escape from predators (Husak and Fox, 2006), win more fights (Lailvaux et al., 2004; Huyghe et al., 2005) and have access to more females (Lappin and Husak, 2005). Another potentially interesting subject to investigate is seasonality (Husak et al 2006; Irschick and Meyers 2007), where a long-term study may help understand if individuals differ in strategies during the different seasons or even if they have a peak performance during one time of year (i.e., beginning of breeding season), whereas other individuals may show other patterns such as latter peaks in the breeding season.

ADVANCE IN METHODOLOGIES

With recent advances in filming and image analysis it is now possible to record locomotor performance at higher resolution with high-speed equipment. Furthermore, software that allows automated image or video analysis to detect animal position are increasing in number and also ease of access and use (e.g. MaxTRAQ 2D motion analysis software (Innovision Systems Inc. 2009), ToxTrac – Rodriguez et al., 2018, TRACKDEM – Bruijning et al., 2018). Taking into account these advances, it is important to understand the influence of statistical inferences when data is obtained across distance-intervals – older method, widely used in literature – or time intervals – more recent method, starting to appear more often in literature – but, more important, to investigate how the choice of method used would affect biological interpretations (Chapter 3). The results presented in this thesis suggested that the use of instantaneous speed – time interval – estimates should be preferred over distance-interval estimates when possible because they improve our capacity for accurately describing variation in performance and associations between locomotor performance and other traits (e.g. morphology, ecology, fitness). While it is impossible to go back to the past and reanalyse those data, it is now obvious that comparisons between studies using different methodologies are problematic.

Furthermore, in Chapter 5 of this thesis we examined a new bite-performance trait – bite duration. How long individuals sustain their bite, and how this interacts with maximal biting capacity, are relevant questions because bite duration is important in many ecological and social behaviours as our results showed mainly in males. Although this improved our knowledge of bite performance, in the future it will be important to uncouple maximal bite force from the capacity to sustain the bite and obtain a measure of bite endurance, which would help us to better understand the importance of bite performance in male antagonistic and mating behaviour. A way to obtain this bite performance trait could be using artificial muscular stimulation – electromyography (Herrel et al 2008).

Put together, the studies presented in this thesis highlighted the complex interaction between morphological and performance variables. Identifying associations between these two set of traits was straightforward for the biting functional system, but not for locomotion. Indeed, it was difficult to establish a direct link between morphology and locomotor performance, but it was possible to detect variation in performance as a response to habitat type, which suggests that short-time evolutionary responses are occurring due to environmental effects. This seems to suggest that, due to the very short evolutionary frames examined here, the two set of traits are not covarying together. Hopefully, this thesis contributed to increase the available knowledge on morphology – performance – habitat relationships but it is also a starting point for future studies.

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Appendix A – Article proofs

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Original article

The relevance of morphology for habitat use and locomotion in two species of wall lizards

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Understanding if morphological differences between organisms that occupy different environments are associated to differences in functional performance can suggest a functional link between environmental and morphological variation. In this study we examined three components of the ecomorphological paradigm — morphology, locomotor performance and habitat use — using two syntopic wall lizards endemic to the Iberian Peninsula as a case study to establish whether morphological variation is associated with habitat use and determine the potential relevance of locomotor performance for such an association. Differences in habitat use between both lizards matched patterns of morphological variation. Indeed, individuals of *Podarcis guadarramae lusitanicus*, which are more flattened, used more rocky environments, whereas *Podarcis bocagei*, which have higher heads, used more vegetation than rocks. These patterns translated into a significant association between morphology and habitat use. Nevertheless, the two species were only differentiated in some of the functional traits quantified, and locomotor performance did not exhibit an association with morphological traits. Our results suggest that the link between morphology and habitat use is mediated by refuge use, rather than locomotor performance, in this system, and advise caution when extrapolating morphology-performance-environment associations across organisms.

1. Introduction

Whether or not, and how, organisms adapt to their environment are two central questions in ecology, evolution and conservation biology (Aerts et al., 2000; Arnold, 1998; Collar et al., 2010; Elstrott and Irschick, 2004; Kaliontzopoulou et al., 2010a). Different climatic, structural and biotic characteristics of the environment may impose ecological pressures on organisms, and mould phenotypic evolution and morphological diversification (Irschick and Garland, 2001; Ricklefs et al., 1981; Vitt et al., 1997). To start understanding the potential evolutionary meaning of morphological variation, we need to evaluate whether specific traits increase the functional capability of an organism (Arnold, 1983; Garland and Losos, 1994). This is usually done in the framework of the ecomorphological paradigm, where differences in morphology are expected to be associated with different ecological performance capacities (e.g. maximum sprint speed), which in turn translate into variation in fitness among individuals in a given environment (Arnold, 1983). Here, selection acts on whole-organism performance to maximise the ability of the individuals to perform certain ecological and social tasks, guarantee their survivorship, and enhance their reproductive success, with correlated effects on morphological characters of biomechanical relevance for these functions (Irschick et al., 2008). Because the functional challenges that organisms face vary across environments, this type of microevolutionary process eventually leads to an association between morphological and environmental variation. As such, establishing whether morphological differences among organisms that occupy different environments are reflected into differences in functional performance can help us to disentangle the complex relationship between traits that culminate in differences in fitness and enhance our understanding of the underlying evolutionary mechanisms (Irschick and Garland, 2001; Irschick et al., 2005a; Miles, 2004; Vanhooydonck and Van Damme, 2001).

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Lizards have been extensively used as model organisms for ecomorphological studies because they are present in a great variety of habitats, and they exhibit a wide range of morphologies and locomotor modes (Arnold, 1998; Garland and Losos, 1994; Irschick and Garland, 2001; Irschick, 2002). Numerous studies indicate that different aspects of locomotor performance are ecologically relevant in different structural habitats, as lizards need to move to capture prey, escape from predators, thermoregulate, find mates and defend territories (Garland and Losos, 1994). As such, those lizard species that live in open areas and use sprinting as their main antipredatory strategy are considered as runners, and they are expected to have long hind limbs to enable longer strides; relatively short forelimbs, to avoid interference with the cycling of the hind limbs; and laterally flattened bodies, to enhance lateral flexibility and maximise stride length (Da Silva et al., 2014; Kaliontzopoulou et al., 2010a; Losos and Sinervo, 1989; Melville and Swain, 2000; Vanhooydonck and Van Damme, 2001). In contrast, lizards living mostly in rocks are considered as climbers, and they are expected to have shorter limbs and dorsally flattened bodies, to lower the centre of gravity closer to the substrate (Da Silva et al., 2014; Kaliontzopoulou et al., 2010a; Melville and Swain, 2000; Sinervo and Losos, 1991; Van Damme et al., 1997; Vanhooydonck and Van Damme, 2001). Therefore, traits that are favoured in one microhabitat preference may conflict with those that are beneficial in another (Clemente et al., 2013).

Podarcis wall lizards from the Iberian Peninsula and North Africa form a monophyletic clade and they are considered a cryptic species complex (i.e. *Podarcis hispanica* species complex: Kaliontzopoulou et al., 2011; Kaliontzopoulou et al. 2012a). In NW Iberia, we encounter two endemic forms – *Podarcis bocagei* and *P. guadarramae lusitanicus* (Geniez et al., 2014; previously treated as *P. hispanica* type 1A, see Kaliontzopoulou et al., 2011). These two sister forms are particularly interesting from an ecomorphological perspective because, unlike most other members of the group, their distributions overlap widely, both at a wide and local geographic scale (Carretero, 2008; Kaliontzopoulou et al., 2011). Further, despite being sister taxa, both forms markedly differ in body shape, whereby *P. bocagei* has relatively longer limbs, and a higher and more rounded head compared to *P. g. lusitanicus* (Galán, 1986; Kaliontzopoulou et al., 2012b; Pérez-Mellado, 1980). It has been suggested that these differences in body shape reflect different habitat preferences, where *P. g. lusitanicus* is highly saxicolous (Galán, 1986; Gosá et al., 1986; Pérez-Mellado, 1980; Sá-Sousa et al., 2002), whereas *P. bocagei* is mostly ground-dwelling (Domínguez and Salvador, 1989; Galán, 1986, 1994; Kaliontzopoulou et al., 2012b; Pérez-Mellado, 1980). However, differences in habitat use between the two species have never been formally quantified and the relevance of their potential ecological segregation for their morphological differentiation has never been examined.

In this study, we investigate the three components of the ecomorphological paradigm – morphology, locomotor performance and habitat use – using a community with *P. bocagei* and *P. g. lusitanicus* as a case study to examine whether morphological variation is associated with habitat use and determine the potential relevance of locomotor performance for such an association. For this purpose, we first examine if there are differences in habitat use between both forms, which would suggest that they exploit different ecological resources in terms of structural niche. Further, we test whether both forms differ in locomotor performance, as we would expect under predictions of the ecomorphological paradigm. Finally, we test if individual variation in morphology translates into variation in habitat use and locomotor performance, as suggested by studies in other lizard groups. Based on previous observations on the morphology and ecology of the two species, and considering ecomorphological patterns in other lizards, we hypothesize that *P. g. lusitanicus*, which has been generally considered as saxicolous, will mainly use rocky environments. In addition, if morphological variation bears a functional meaning, we expect *P. g. lusitanicus*, which has a flattened head and shorter limbs, to perform better when climbing. On the other hand, *P. bocagei*, which is generally ground-dwelling, with a much higher and rounded head and longer limbs, is expected to be more flexible in terms of habitat use. Because this species uses different types of habitat including vegetation (Kaliontzopoulou et al., 2012b; Sá-Sousa, 2001), we predict that it might perform equally well under different locomotor conditions.

2. Material and methods

Lizards for this study were captured in the coastal zone near the beach of Moledo (N Portugal coast – 41°50′N, 8°52′W), where *P. bocagei* and *P. g. lusitanicus* are found in syntopy across a sandy area with rocks, sparse vegetation, and agricultural fields delimited by human constructed stone walls. The total area sampled was about 6500 m^2. We collected a total of 121 adult individuals by noosing (García-Muñoz and Sillero, 2010), including 65 animals for studying habitat use and 56 for locomotor performance experiments (see also below).

2.1. Morphological variables

In all individuals captured (i.e.,47 males and 32 females of *P. bocagei*, and 20 males and 22 females of *P. g. lusitanicus*), we measured the following linear biometric traits: trunk length (TRL), head length (HL), head width (HW), head height (HH), fore limb length (FLL) and hind limb length (HLL), using electronic callipers (precision 0.01 mm; see Kaliontzopoulou et al., 2007 for a detailed description of variables). In order to separate size and shape, we calculated the isometric size (SIZE) of each individual by projecting all log-transformed linear measurements on an isometric vector (Kaliontzopoulou et al., 2010a) and used it as a multivariate estimate of total body size. To obtain size-corrected variables representing shape variation, we regressed each linear trait on SIZE and retained the regression residuals (hereafter prjTRL, prjHL, prjHW, prjHH, prjFLL and prjHLL) (Kaliontzopoulou et al., 2010a). Animals used to quantify locomotor performance were also weighted using a digital balance (precision 0.0001 g).

2.2. Habitat use variables

In May 2011, when the activity of lizards is at its maximum, we captured a first set of 48 *P. bocagei* (30 males and 18 females) and 15 *P. g. lusitanicus* (five males and 10 females) in order to perform habitat and microhabitat use observations in the field. For each individual captured, we recorded the exact location of capture, using a high-precision GPS (Trimble GPS GeoExplorer 2008 HX). Each individual was measured to record morphological traits and marked with a unique colour code using non-toxic marker paint, so we could distinguish individuals in the field without capturing them. All the animals were released in the same location of capture.

To record microhabitat and habitat use, we performed normalised transects during seven days and 10 h/day with favourable weather conditions. To ensure that all lizards were observed at different times of the day, and therefore capture the individual variability in habitat use, transects were performed in a random order and allowing at least 1 h and a half between repetitions of the same transect. During these transects, we collected a total of 197 observations for *P. bocagei* (114 for males and 83 for females) and 60 observations for *P. g. lusitanicus* (20 for males and 40 for females) and recorded their associated microhabitat variables. To quantify microhabitat use we recorded the type of substrate where the lizard was observed (SUBS: classified as either walls, rocks, vegetation or soil), the height from the ground at the point of observation (HGR), inclination quantified as the angle between the surface where the individual was observed and the horizontal plane (INC), and the diameter of the rock (ROCKD), when lizards were observed on rocks. We also considered habitat used in the 2 m-diameter area surrounding the point of observation by recording the percentage of bare soil, vegetation and rocks.

Before subsequent statistical analyses, height from the ground and inclination were transformed as $y = \log10\ (x + 0.5)$, rock diameter was logarithmically transformed and all percentages were arcsine-transformed.

2.3. Locomotor performance

In October 2011, we captured a second set of 30 *P. bocagei* (15 males and 15 females) and 26 *P. g. lusitanicus* (15 males and 11 females) in order to quantify locomotor performance. We performed locomotor experiments in the autumn to exclude any potential effects of reproduction (i.e. pregnancy) on female performance (Bauwens and Thoen, 1981). Lizards were placed in opaque cloth bags, transported to the laboratory and housed in individual terraria, where they were fed with live mealworms and provided with water *ad libitum*. All animals were allowed to rest for two days after arrival to the lab to ensure acclimation. All experiments were carried out at a room temperature of about 31 °C, which is approximately the selected body temperature of the two species (Amaral et al., 2012; Carretero et al., 2012). Prior to and in between all performance trials, lizards were placed for at least 1 h in an individual terrarium that was exposed to direct natural light, allowing them to thermoregulate and attain their preferred body temperature.

For all individuals, five types of locomotor performance were measured (Kaliontzopoulou et al., 2013): sprint speed (one type), climbing capacity (three types) and manoeuvrability (one type). Sprint speed (SPR) on a horizontal surface was measured by chasing animals along a 1 m-long and 15 cm-wide racetrack, on a cork substrate (Braña, 2003; Van Berkum et al., 1989). Climbing capacity was quantified by chasing animals up a similar racetrack, with varying substrates and inclination conditions. These included: (1) a cork-substrate racetrack tilted to an angle of 60° (CLI60), (2) a sandpaper-substrate racetrack tilted to an angle of 60° (CLI60s) and (3) a cork-substrate racetrack tilted to an angle of 90° (CLI90). Cork was used as a substrate because it provides very good traction (Van Damme et al., 1997), while the sandpaper provided a surface similar to a granite rock (Goodman, 2007). To quantify manoeuvrability (MAN), a 0.5 m-long and 15 cm-wide pinboard was placed on the racetrack. This pinboard was made of 8-mm diameter pins placed at equal distances of 35 mm (Vanhooydonck et al., 2000).

Locomotor speed in the five types of racetracks was measured on different days and the order in which animals were subjected to the tests was randomized. Each individual was tested three times in each type of racetrack to ensure that maximal locomotor capacity was recorded. Each run was scored as "bad" or "good" and the "bad" races, in which the animals completely stopped or turned around during the race, were eliminated from the analyses (sensu Tsuji et al., 1989; Van Berkum and Tsuji, 1987).

All trials were filmed with a digital camera (Casio EXILIM EX-F1) at a filming speed of 30 fps. The position of the lizard across each trial was digitized using MaxTRAQ 2D motion analysis software (Innovision Systems Inc., 2009). For each type of racetrack, the highest speed recorded over any 10-cm interval across all repetitions was taken as an estimate of each animal's maximum speed in that racetrack. All data of locomotor performance were logarithmically transformed prior to analyses. After the experiments, all individuals were released in the site of capture.

2.4. Data analyses

Because both species are sexually dimorphic, including the populations in the study site (Kaliontzopoulou et al., 2012a, 2012b, 2013) we included sex as a predictor variable in all statistical analyses conducted. To investigate if there were differences between species and sexes in morphology we used ANOVA procedures. All ANOVA models were run using morphological traits (i.e. SIZE and each size-corrected trait separately) as the response variable, and species, sex and their interaction as predictors.

Concerning microhabitat use, we fitted a log-linear model to examine whether species and sexes were found on different substrates with a different frequency. Additionally, for all continuously represented microhabitat (e.g. HGR, INC, ROCKD) and habitat variables (% of bare soil, vegetation and rocks) we performed permutation ANOVAs using species, sex and their interaction as predictor variables, to evaluate differences between SP × SEX groups. This analysis considered individual observations as independent and therefore encompassed variability within individuals in habitat use. In addition, because some individuals were observed more than once, the same analysis was repeated using a linear mixed-effects model as implemented in the lmer function of the lme4 R-package (Bates et al., 2014) and including individual as a random factor.

To examine whether species and sexes differed in locomotor performance, we performed a MANOVA on the multivariate set of maximal locomotor speeds, and also randomized ANOVA comparisons on each type of locomotor speed separately, with species, sex and their interaction as factors. Subsequently, we performed post-hoc comparisons (Tukey's HSD) to test for differences between pairs of groups. We also performed ANCOVA comparisons on the multivariate set of maximal locomotor speeds to examine the effect of species, sex, and their interaction, while considering SIZE and weight as covariates. We always used (M)ANOVA based on permutations, because sample size varied across groups, which resulted in differences in variance. These were based on 1000 permutations of Euclidean distance matrices as implemented in the adonis function of the vegan R-package (Oksanen et al., 2012). Because multiple comparisons were evaluated simultaneously, we implemented the False Discovery Rate (FDR) procedure to adjust the p-values of statistical tests (Benjamini and Yekutieli 2001). To investigate the multivariate association between morphology and performance, and between morphology and habitat, we used a two-block partial least-squares regression as implemented in the plsr function of the pls R-package (Mevik et al., 2011). To investigate the association between morphology and locomotor performance, we only used limb and trunk variables, as these traits have been suggested to determine locomotor performance in these and other lizard species (Kaliontzopoulou et al., 2010a, 2013; Vanhooydonck and Van Damme, 2001). In the case of the association between morphology and habitat use, more than one habitat observation was available for several of the individuals sampled. To address this fact, while maximizing the use of the data available, we used the mean of each variable across observations of each individual and weighted the contribution of each individual to the pls regression by the number of habitat observations available for that individual. All statistical analyses were performed using R v. 2.14.1 (R Development Core Team, 2011).

3. Results

3.1. Morphological traits

ANOVA comparisons of morphological traits indicated that

P. bocagei and *P. g. lusitanicus* differed in total of body size, relative trunk length, relative head length and head height (Table 1, Appendix S1). ANOVA also confirmed significant differences between both sexes in SIZE and in all size-corrected traits in our sample, while some significant SP × SEX interactions were also observed. Generally, *P. bocagei* was larger in body size than *P. g. lusitanicus*, with higher but shorter heads. Males were larger in body size than females, with longer and higher heads, shorter trunks, and longer limbs.

3.2. Habitat use

The log-linear model fitted to investigate microhabitat use revealed differences in the type of substrate where each species was found (point of observation; Table 2, Fig. 1, Appendix S2), whereas no significant differences existed between sexes within each species. *P. bocagei* was found with a higher frequency on walls as compared to *P. g. lusitanicus*, which was more frequently encountered on isolated rocks (Fig. 1). ANOVA comparisons of microhabitat use indicated significant differences between species, but not between sexes, in rock diameter (Table 3). Specifically, *P. bocagei* was encountered in smaller rocks (Fig. 2) than *P. g. lusitanicus*. Linear mixed-effects models only showed significant differences between species in rock diameter ($p = 0.004$).

3.3. Locomotor performance

MANOVA comparisons on locomotor performance indicated that *P. bocagei* and *P. g. lusitanicus* differed when considering the multivariate set of maximal locomotor speeds. Univariate analyses showed that *P. bocagei* attained higher speeds in the three climbing racetracks (CLI60, CLI90 and CLI60s; Table 4, Fig. 3, Appendix S3). Sex did not have a significant effect on locomotor performance. However, post-hoc comparisons indicated that differences between both species were only significant in males and not in females (Fig. 3). ANCOVA comparisons using SIZE and weight as covariates did not reveal a significant effect of body size and/or robustness on locomotor performance (Appendix S4).

3.4. Morphology traits vs locomotor performance

Two-block partial least-squares regression did not reveal a significant association between morphological traits and locomotor performance ($r = 0.354$ and $p = 0.227$).

3.5. Morphological traits vs habitat use

Two-block partial least-squares regression indicated a significant association between both sets of variables ($r = 0.426$ and $p = 0.001$). The morphological PLS vector was most highly correlated with trunk length (positive correlation) and head height (negative correlation) (Fig. 4). The vector representing microhabitat use was most highly correlated with the percentage and the diameter of rocks, and with the percentage of vegetation, this last variable showing an opposite loading to the first two (Fig. 4). As such, the association between morphology and habitat use was summarized by multivariate vectors where lizards with longer trunks and flatter heads were more frequently found in rocks of a large diameter than in spots with vegetation.

Table 1
Results of ANOVA comparisons performed on total body size (SIZE) and size-corrected morphological traits to examine the effect of species (SP), sex and their interaction (SP*SEX). F: F-statistic, P: corresponding p-value, adjusted for multiple testing using the False Discovery Rate procedure. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SP		SEX		SP*SEX	
	F	P	F	P	F	P
SIZE	17.052	**0.002**	28.706	**0.002**	4.300	**0.049**
prjTRL	19.541	**0.002**	186.958	**0.002**	8.017	**0.017**
prjHW	0.139	0.766	27.922	**0.002**	2.596	0.161
prjHH	62.798	**0.002**	9.728	**0.002**	20.875	**0.002**
prjHL	43.318	**0.002**	34.996	**0.002**	0.043	0.871
prjFLL	0.027	0.871	11.540	**0.004**	8.405	**0.013**
prjHLL	2.070	0.168	27.830	**0.002**	0.229	0.740

Table 2
Results of log-linear model performed on microhabitat use variables. See DEV: Deviance, R.DEV: Residual Deviance, P: corresponding p-value. Significant effects are marked in bold letter. Material and Methods for variable abbreviations.

	DEV	R.DEV	P
SUBS	463.122	115.123	$\mathbf{4.68 \times 10^{-100}}$
SP	76.956	38.167	$\mathbf{1.75 \times 10^{-18}}$
SEX	0.471	37.696	0.493
SUBS*SP	23.408	14.288	$\mathbf{3.32 \times 10^{-5}}$
SUBS*SEX	4.937	9.351	0.176
SP*SEX	8.837	0.514	**0.003**
SUBS*SP*SEX	0.514	0.000	0.916

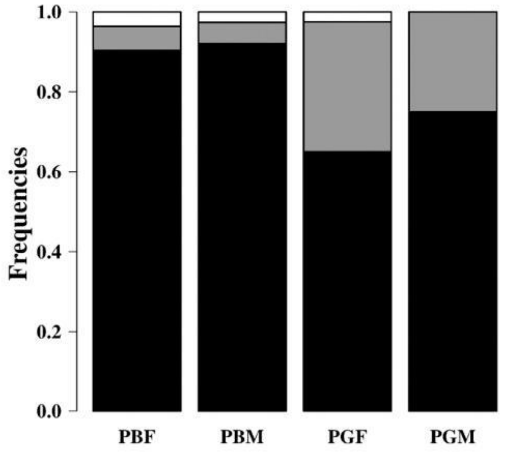

Fig. 1. Observed frequencies of each species and sex using each type of substrate. Black: walls; grey: rocks; white: others (vegetation and bare soil). PBF: *P. bocagei* females, PBM: *P. bocagei* males, PGF: *P. g. lusitanicus* females, PGM: *P. g. lusitanicus* males. See Material and Methods for variable abbreviations.

Table 3
Results of ANOVA comparisons performed on all continuously represented microhabitat (HGR, INC, ROCKD) and habitat variables (% of bare soil, vegetation and rocks) to examine the effect of species (SP), sex and their interaction (SP*SEX). F: F-statistic, P: corresponding p-value, adjusted for multiple testing using the False Discovery Rate procedure. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SP		SEX		SP*SEX	
	F	P	F	P	F	P
HGR	5.783	0.086	0.803	0.687	0.097	0.818
INC	0.516	0.687	2.094	0.383	4.562	0.086
ROCKD	30.829	**0.018**	0.483	0.687	2.353	0.383
SOIL%	0.109	0.818	0.054	0.818	0.484	0.687
VEG%	6.922	0.086	0.297	0.740	0.995	0.678
ROCK%	5.947	0.086	0.386	0.687	1.760	0.459

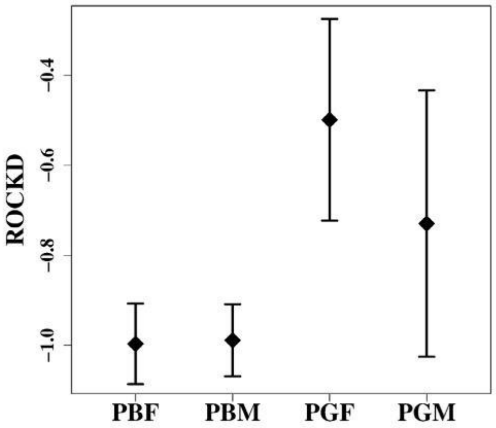

Fig. 2. Least-squares means for each species and sex for the diameter of the rock (ROCKD) at the point of observation. Vertical bars denote 95% confidence intervals. PBF: *P. bocagei* females, PBM: *P. bocagei* males, PGF: *P. g. lusitanicus* females, PGM: *P. g. lusitanicus* males. See Material and Methods for variable abbreviations.

Table 4
Results of (M)ANOVA comparisons performed on multivariate set of maximal locomotor speeds (mSPEEDS) and each type of locomotor speed separately to examine the effect of species (SP), sex and their interaction (SP*SEX). F: F-statistic, P: corresponding p-value, adjusted for multiple testing using the False Discovery Rate procedure. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	SP		SEX		SP*SEX	
	F	P	F	P	F	P
mSPEEDS	16.504	**0.005**	0.779	0.618	1.748	0.371
SPR	0.137	0.836	1.917	0.383	0.044	0.858
MAN	2.726	0.303	0.379	0.618	5.972	0.058
CLI60	32.446	**0.005**	1.610	0.383	2.659	0.303
CLI90	16.082	**0.005**	0.544	0.618	0.114	0.802
CLI60s	20.756	**0.005**	0.411	0.618	0.976	0.529

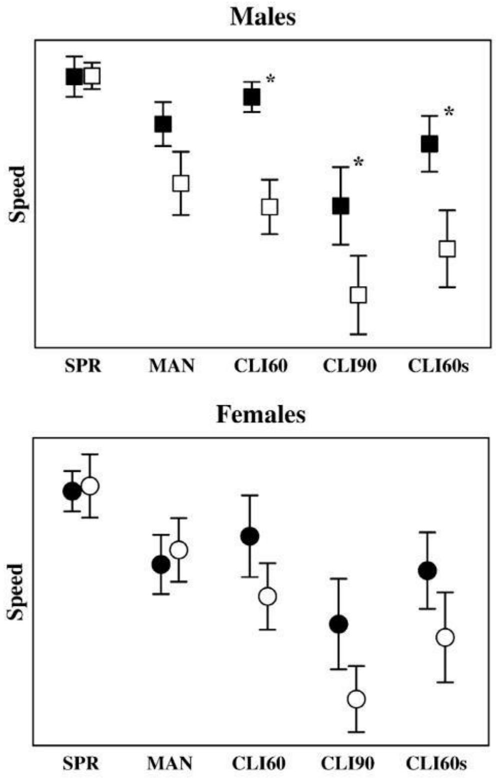

Fig. 3. Least-squares means observed in males and females of each species for the different locomotor variables quantified. Open squares, *P. g. lusitanicus*; closed squares, *P. bocagei*. Vertical bars denote 95% confidence intervals. * Significant post-hoc comparisons. See Material and Methods for variable abbreviations.

4. Discussion

Our investigation of the three components of the ecomorphological paradigm — morphology, locomotor performance and habitat use — in a syntopic population of *P. bocagei* and *P. g. lusitanicus* revealed an interesting pattern of ecomorphological variation, where the association between morphology and habitat is not accompanied by an association between morphology and locomotor performance. This deviates from what has been observed in numerous other lizard species (e.g. Goodman et al., 2008; Irschick et al., 2005b; Losos and Sinervo, 1989; Melville and Swain, 2000; Sinervo and Losos, 1991) and has potential implications for our understanding of the ecological and evolutionary mechanisms that underlie the high levels of morphological diversity observed in the *P. hispanica* species complex.

Although *P. bocagei* and *P. g. lusitanicus* live in strict syntopy in Moledo, our results indicate that these two forms exploit different habitat resources. Individuals of *P. g. lusitanicus* were most frequently observed on big rocks than on other substrates, while individuals of *P. bocagei* were found most frequently on human-constructed walls and rocks of a smaller diameter (Fig. 1; Fig. 2), as already suggested by others studies (Galán, 1986; Sá-Sousa et al., 2002). These results provide the first quantitative account of a significant differentiation between the two forms in terms of habitat use.

Despite this differentiation, the observed ecological differences do not correspond to the predicted differences in locomotor requirements and associated morphological traits. Instead, our results indicate that the morphological and ecological divergence between *P. bocagei* and *P. g. lusitanicus* is most probably associated to refuge use, rather than mediated by locomotor performance. Indeed, individuals of the two species from this population do not differ significantly in either limb length (Table 1), or sprinting speed (Fig. 3), but we found that *P. bocagei* climbed faster than *P. g. lusitanicus* (Fig. 3). Several factors may explain these results. First, although generally characterised as a ground-dweller, *P. bocagei* is a relatively versatile species, that uses several different types of habitats across its distribution range (Kaliontzopoulou et al., 2010a). Indeed, in the studied population it extensively uses human-constructed walls (Fig. 1). As such, this species benefits not only from enhanced sprinting, but also climbing capacities, which may explain its very good locomotor performance in the climbing racetrack (Fig. 3). Whether this pattern of locomotor differentiation between these two forms extends to other populations with different habitat availability or, instead, locomotor performance is a plastic trait in these lizards, varying across populations depending on the habitat they use, would need to be further investigated. Further, it is also important to note that, although a general

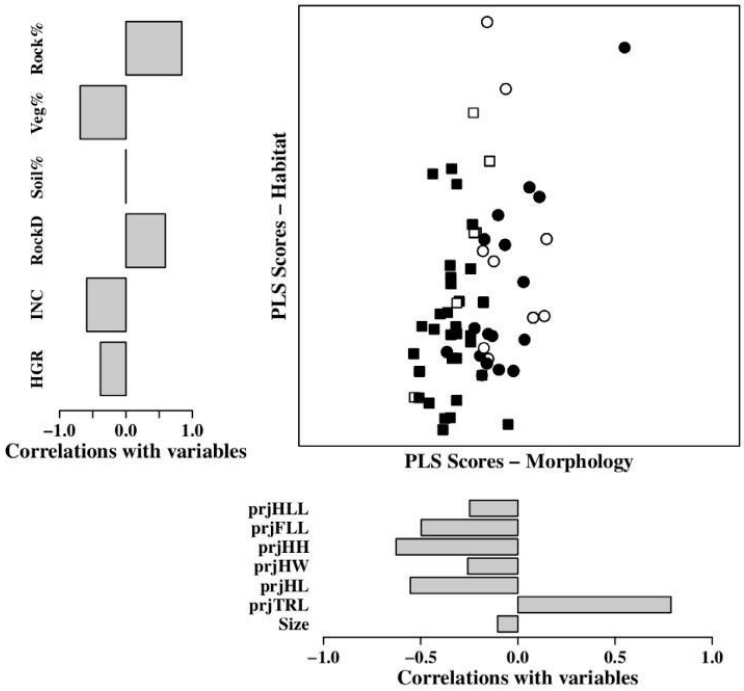

Fig. 4. Scatter-plot of individual scores of *P. bocagei* (black squares: males; black circles: females) and *P. g. lusitanicus* (white squares: males; white circles: females) obtained from partial least-squares analysis between morphological and continuous habitat variables. Bar-plots next to each axis represent the correlations observed between that axis and the variables included in each block. See Material and Methods for variable abbreviations.

association between habitat use and locomotor performance was expected based on previous studies and ecomorphological predictions, the experimental design implemented here did not allow us to directly test this association. Indeed, because of practical limitations related to the activity of the study organisms (higher activity in the spring, that also coincides with the reproductive season), here we analysed data from two different seasons (i.e. habitat use was quantified in the spring and locomotor performance in the autumn). As such, seasonality and plasticity may also have an effect on this lack of association (e.g. Irschick et al., 2006; Irschick and Meyers, 2007), although the potential role of seasonality should be investigated in detail in the future.

A high spatial and temporal plasticity of locomotor capacities may, in fact, also explain why several studies have failed to demonstrate an evolutionary link between morphology and performance in lacertids. In this study system, we could not establish a significant association between morphology and locomotor performance. Likewise, several other studies have also failed to detect similar correlations in some cases, both at the intra- and interspecific levels (Brecko et al., 2008; Goodman et al., 2008; Van Damme et al., 1997; Vanhooydonck et al., 2000), challenging the generality of the ecomorphological paradigm. Furthermore, the lack of association between morphology and locomotor performance could also be explained by other factors like behaviour (Braña, 2003; Irschick et al., 2005b; Vervust et al., 2007; Žagar et al., 2015a), physiology (e.g. muscle composition; Adolph and Pickering, 2008; Van Damme et al., 1997), biochemistry (e.g. enzyme activity; Adolph and Pickering, 2008; Van Damme et al., 1997; Vervust et al., 2007; Žagar et al., 2015b) and/or other morphological traits not investigated in this study as orientation of the limbs, differences in the claws or even the distance between the body and an inclined surface to oppose the force of gravity (Jayne and Irschick, 1999; Revell et al., 2007). In addition, the ecological and morphological segregation between the two species could be reflected in specialization in other performance traits, not examined here, such as acceleration capacity (Vanhooydonck et al., 2005), endurance (Vanhooydonck et al., 2000; Vanhooydonck, Van Damme & Aerts, 2001) and/or agility (Van Damme and Vanhooydonck, 2002). Finally, locomotor performance is involved in several ecological (feeding, thermoregulation, escape from predators, habitat and refuge use) and social (antagonistic behaviour, territory and mate acquisition, competition with heterospecifics) functions. This means that a differentiation between both species could exist when performing different tasks in nature, which may not be observable in laboratory experiments (Irschick et al., 2005a). Such a hypothesis should be further investigated in the future by examining how individuals of the two species perform in natural conditions.

The patterns observed in relation to locomotor performance do not, however, invalidate the link observed between morphology and habitat. Several studies have demonstrated a significant evolutionary correlation between morphological and environmental variation in lizards (e.g. Goodman et al., 2008; Herrel et al., 2001; Irschick et al., 2005b; Kaliontzopoulou et al., 2010a; Melville and Swain, 2000; Vanhooydonck and Van Damme, 1999; Vitt et al., 1997). The analyses conducted here, indicate a significant correlation between habitat use and morphology in a syntopic population of *P. bocagei* and *P. g. lusitanicus* in northern Portugal. Examination of variable loadings on the morphological axis obtained through two-block partial least-squares regression with habitat use reveals that trunk length and head height are the traits with the highest contributions to this association (Fig. 4). Trunk length is linked to vertebral number in *Podarcis* (Arnold, 1973), it is known to enhance body flexibility for lateral bending (Arnold, 1998; Van Damme and Vanhooydonck, 2002), and it has been associated to habitat use in lacertids (Van Damme and Vanhooydonck, 2002; Kaliontzopoulou et al., 2010b). On the other hand, habitat use has been shown to drive macroevolutionary variation in head shape, but not in body size or relative limb length in wall lizards (Kaliontzopoulou et al., 2015). As already proposed by other studies, the dorso-ventral compression of the head in rocky environments, or in saxicolous species, can derive from a mechanical constraint related to refuge use (Edwards et al., 2012; Herrel et al., 2001; Kaliontzopoulou et al., 2010a; Vanhooydonck and Van Damme, 1999; Vitt et al., 1997). Indeed, in organisms like wall lizards, which rely more on crypsis or in hiding in refuges rather than on long-distance running for predator avoidance (Carretero et al., 2006; Martín et al., 2009), habitat use may represent an evolutionary constraint for head, but not for limb traits. If this were the case here, we may expect the two species to employ different antipredatory behavioural responses. Indeed, *P. bocagei* seems to use ground-level vegetation for hiding, while *P. g. lusitanicus* usually flees towards small crevices and cracks in agricultural walls (pers. obs. of the authors). However, a detailed study of escape tactics would be necessary to test this hypothesis. Finally, the association between habitat and head traits observed here could also be driven by differences in feeding ecology between animals using different structural niches. However, this does not seem to be the case in this system, as a previous study in the same study area demonstrated that these two species do not differ in bite force or in diet (Kaliontzopoulou et al., 2012b).

Put together, the results obtained in this study reveal a significant relationship between morphological traits and habitat use in *P. bocagei* and *P. g. lusitanicus*, but, unlike what happens in numerous other lizard groups, this relationship is not accompanied by an association between morphology and locomotor performance used for fleeing. The evidence at hand does not allow us to further investigate the precise mechanisms determining this pattern and, given that this study only includes two species, we can make no extrapolations to the rest of the group (Garland and Adolph, 1994). Instead, further studies would be necessary in order to elucidate the ecological significance and plasticity of locomotor performance and habitat use in these lizards. First, understanding how maximal locomotor capacities are used in nature (e.g. for prey capture, predator avoidance, social encounters) is essential for deciphering their true evolutionary potential (Irschick et al., 2005a; Husak, 2006). Further, it would be relevant to investigate how morphological and locomotor traits are involved in shaping individual fitness (survival, mate acquisition, outcome of antagonistic encounters) in this group of lizards, and how these effects may vary across different environments.

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Appendices

Appendix S1: Descriptive statistics for the raw biometric characters in males and females of *Podarcis bocagei and P. g. lusitanicus*. Values are given as mean ± standard deviation (top) and range (bottom). See Material and Methods for variable abbreviations.

	Podarcis bocagei		*P. g. lusitanicus*	
	Males	Females	Males	Females
TRL	24.42 ± 0.68 17.51–32.03	28.58 ± 0.66 18.88–34.56	25.69 ± 0.48 22.17–32.11	26.37 ± 0.67 18.78–33.47
HW	7.4 ± 0.21 5.43–9.39	6.52 ± 0.07 5.21–7.7	7.09 ± 0.16 5.51–8.25	5.89 ± 0.08 4.91–6.75
HH	5.43 ± 0.13 3.9–6.56	4.63 ± 0.08 3.97–5.45	4.53 ± 0.08 3.71–5.55	3.98 ± 0.08 3.17–4.76
FLL	16.33 ± 0.27 11.27–20.17	15.07 ± 0.31 12.35–17.7	16.21 ± 0.16 13.97–18.72	13.24 ± 0.18 11.56–14.63
HLL	27.11 ± 0.71 18.68–32.8	24.28 ± 0.40 19.44–29.98	26.12 ± 0.44 22–30.42	21.45 ± 0.24 18.96–23.72
HL	17.59 ± 0.48 12.88–21.27	15.65 ± 0.20 12.69–17.9	17.99 ± 0.31 14.46–19.62	14.78 ± 0.18 12.23–16.75

Appendix S2: Descriptive statistics for the microhabitat use traits (in the point of observation) in males and females of *Podarcis bocagei* and *P. g. lusitanicus*. Values are given as mean ± standard deviation (top) and range (bottom). See Material and Methods for variable abbreviations.

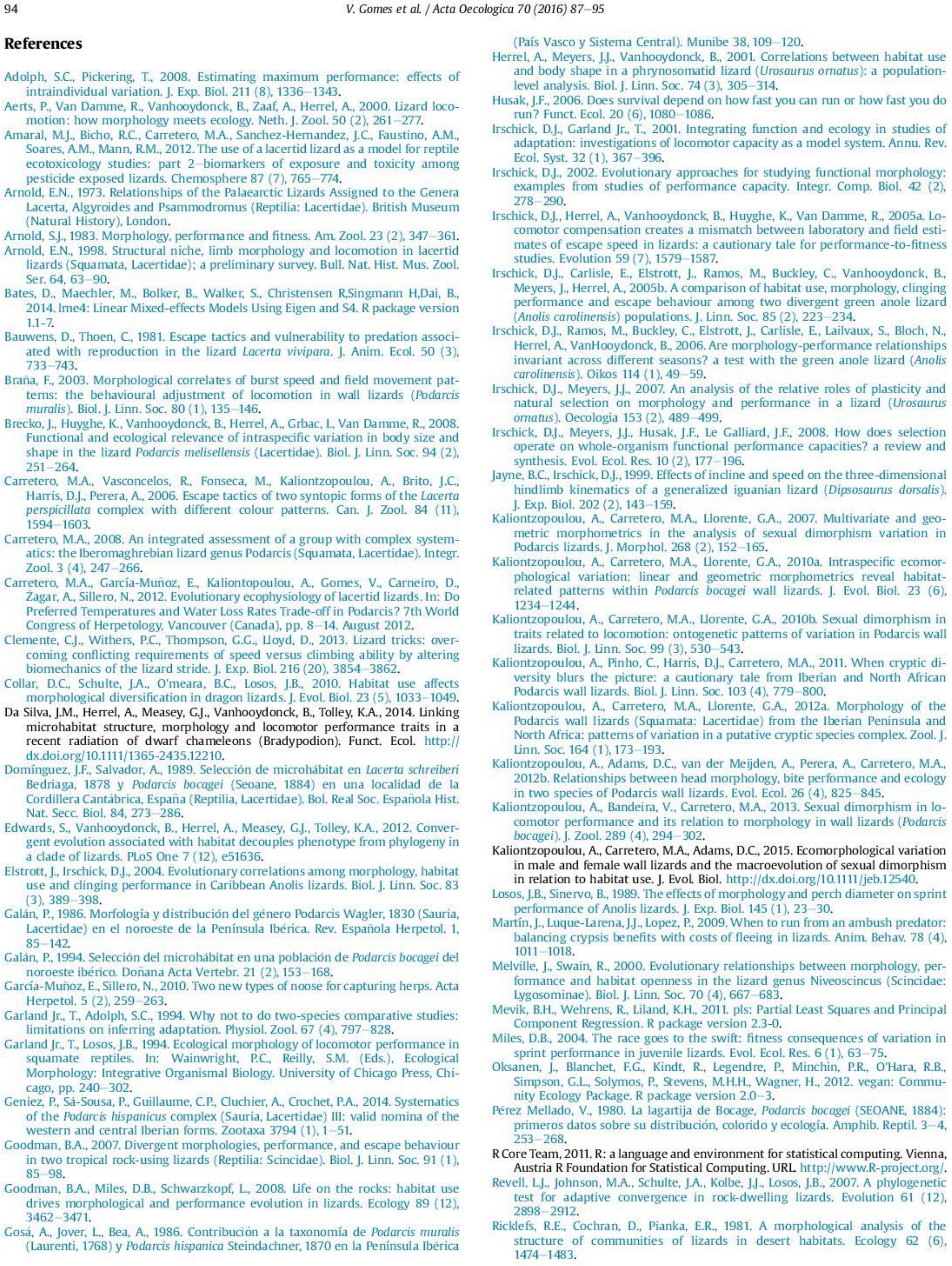
	Podarcis bocagei		*P. g. lusitanicus*	
	Males	Females	Males	Females
HGR	0.83 ± 0.05 0–1.4	0.8 ± 0.04 0–1.6	0.72 ± 0.06 0.1–1.2	0.71 ± 0.05 0–2
INC	22.72 ± 2.15 0–90	30.90 ± 2.84 0–90	34.75 ± 5.47 0–90	26.75 ± 3.58 0–90
ROCKD	0.41 ± 0.02 0.1–0.9	0.4 ± 0.02 0.1–0.9	0.63 ± 0.13 0.25–2.5	0.79 ± 0.11 0.2–2.5
SOIL %	4.25 ± 1.10 0–60	3.49 ± 0.9 0–30	3.50 ± 1.82 0–30	4.88 ± 2.16 0–70
VEG%	55.48 ± 1.40 20–90	53.55 ± 1.60 20–80	47.25 ± 2.98 20–70	49.63 ± 2.72 5–80
ROCK %	40.26 ± 1.32 0–80	42.83 ± 1.67 0–80	49.25 ± 3.21 30–80	45.00 ± 2.70 0–95

Appendix S3: Descriptive statistics for the locomotor performance in males and females of *Podarcis bocagei* and *P. g. lusitanicus*. Values are given as mean ± standard deviation (top) and range (bottom). See Material and Methods for variable abbreviations.

	Podarcis bocagei		*P. g. lusitanicus*	
	Males	Females	Males	Females
SPR.max	118.39 ± 10.02 37.09–211.74	99.83 ± 8.53 31.17–152.48	115.82 ± 8.53 81.33–195.13	110.32 ± 13.78 34.15–184.46
MAN.max	72.97 ± 7.26 33.6–110.54	51.06 ± 6.05 13.07–89.2	44.73 ± 6.58 12.74–107.38	60.77 ± 8.59 18.69–105.38
CLI60.max	93.77 ± 6.09 38.66–131.17	70.49 ± 10.07 12.22–123.27	34.2 ± 4.87 15.1–71.58	40.01 ± 7.56 16.04–98.72
CLI90.max	38.28 ± 6.3 8.04–87.65	33.72 ± 6.66 5.06–84.97	14.88 ± 2.86 3.43–37.54	14.78 ± 4.01 5.72–60.14
CLI60s.max	64.47 ± 8.54 21.96–119.97	52.44 ± 8.28 8.39–105.57	25.37 ± 4.82 6.01–63.47	28.3 ± 7.36 7.77–76.16

Appendix S4: Results of ANCOVA comparisons performed on multivariate set of maximal locomotor speeds (mSPEEDS) to examine the effect of species (SP), sex, their interaction (SP*SEX) and SIZE and Weight as covariate. Df: Degrees of freedom, F: F-statistic, p: corresponding p-value. Significant effects are marked in bold letter. See Material and Methods for variable abbreviations.

	Df	F	p
(Intercept)	1	1430.12	**3.01E-49**
SP	1	7.79	**2.24E-05**
SEX	1	0.51	0.77
SIZE	1	0.76	0.58
Weight	1	2.89	**0.02**
SP:SEX	1	1.18	0.33
Residuals	50		



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TOOLS AND TECHNIQUES

Instantaneous Versus Interval Speed Estimates of Maximum Locomotor Capacities for Whole-Organism Performance Studies

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Abstract Numerous studies have demonstrated the ecological and social relevance of different aspects of animal locomotor performance, as locomotion is frequently required to capture prey, escape from predators, find mates and defend territories. Typically, maximal performance is quantified using two alternative methods: across distance intervals or across time intervals, the later by extracting the speed observed between a specified number of video frames. Here, we analyze how this choice may influence statistical inference and the derived biological interpretations. Our results indicate that data obtained using 10-cm intervals are categorized, not normally distributed, exhibit lower individual repeatabilities and have four times more variance compared to the data obtained by quantifying speeds across short time intervals. These results have important methodological implications, as they suggest that the choice of method of speed quantification substantially influences the quality and bias of maximal performance descriptors. This may in turn have a profound influence on ecomorphological inference, as it affects our capacity of detecting variation in performance within and across groups, and associations between locomotor performance and other traits.

Keywords Locomotion · Methodology · Performance · Sprint capacity

Introduction

Numerous studies have investigated whole-organism performance to disentangle the complex relationships among phenotypic traits that culminate in differences in fitness (Ghalambor et al. 2003; Miles 2004; Mowles et al. 2010; Santana et al. 2010; Herrel et al. 2012; McGinley et al. 2013; Irschick and Higham 2016). Locomotor performance is a function with a high ecological and social relevance, as animals need to search for prey, escape from predators, find mates and defend territories (Domenici 2001; Vásquez et al. 2002; Blumstein et al. 2004; Husak 2006; Husak et al. 2006; Husaket al. 2008; Mowles et al. 2010; Pruitt 2010; Irschick and Higham 2016). For this reason, several ecomorphological, physiological, genetic and parasitological studies have investigated how locomotor capacities vary across species, environments, and in relation to other organismal traits (Schall et al. 1982; Angilletta et al. 2002; Ghalambor et al. 2003; Goodman and Johnson 2011; Zamora-Camacho et al. 2015; Irschick and Higham 2016). For instance, locomotor performance has been used to establish links between morphological variation and habitat use. Indeed, different morphological properties are known to enhance specific locomotor capacities, which in turn match the ecological requirements of specific habitats (Irschick and Garland 2001), an evolutionary association which is studied under the framework of the ecomorphological paradigm (Arnold 1983).

To examine the ecological and evolutionary significance of locomotor performance in small animals, laboratory experiments are usually performed to quantify maximal sprinting capacity (see e.g. Punzo 2003; Blumstein et al. 2004; Miles 2004; Husak et al. 2006; Husak 2006; Médoc and Beisel 2008; Tulli et al. 2012; Collins et al. 2013; McGinley et al. 2013; Da Silva et al. 2014;

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Zamora-Camacho et al. 2015; Gomes et al. 2016). More recently, other types of locomotor performance have also been studied, such as climbing ability at different angles, maneuverability, endurance and acceleration capacity, in the case of terrestrial locomotion (Van Damme et al. 1997; Vanhooydonck and Van Damme 2003; Goodman et al. 2008; Herrel and Bonneaud 2012; Prenter et al. 2012; Tulli et al. 2012; Kaliontzopoulou et al. 2013; Gomes et al. 2016); or swimming speed, acceleration and maneuverability, in the case of fish and other aquatic organisms (Drucker and Lauder 2000; Domenici 2001; Rivera et al. 2006). In all such studies, the objective was to estimate the maximal performance capacity of individuals. This is because—setting behavior aside (i.e. Irschick et al. 2005)—biomechanical and physiological predictions establish links between maximal functional capacities and morphological traits such as skeletal elements, muscle morphology and composition, external morphology, or biochemical and hormonal traits, to mention just a few (Irschick and Higham 2016). These are then expected to translate into variation in how animals interact with the surrounding environment (Arnold 1983) or with each other (Husak and Fox 2008). However, some caution is necessary when recording maximum performance, as methodological procedures may also influence the results obtained and alter the biological conclusions extracted. This has been shown to be the case for biting performance—the second most frequently studied performance trait (e.g. Dessem and Druzinsky 1992; Dumont and Herrel 2003; Erickson et al. 2004; Herrel et al. 1999; van der; Meij and Bout 2004). Here, both biting substrate and bite out-lever have been shown to considerably influence the accuracy of bite force measurements (Dumont and Herrel 2003). With respect to locomotor performance, Losos et al. (2002) already suggested that the number of locomotor trials, or the inclusion of data from animals performing sub-maximally, could influence biological inferences. However, the influence of the method used to quantify individual speed, which is one of the most basic components of the experimental design, has not been previously explored.

In the case of terrestrial locomotion, maximal sprinting performance is typically measured by chasing small animals along a racetrack of 1–4 m long (Losos et al. 2002). To quantify individual functional performance one of two alternative methods is most frequently used: (1) recording the maximum speed observed across regular distance intervals; (2) extracting the instantaneous speed observed between a specified number of frames of video-recorded runs, corresponding to time intervals (Losos et al. 2002). Although both approaches are used quite frequently in the literature, they have never been compared. However, the equivalence between methods cannot be taken for granted, but rather needs to be verified, as the two approaches are conceptually expected to exhibit substantial differences. In principle, velocities obtained across distance intervals are thought to be prone to a higher influence of behaviour, as well as of the mode of locomotion of the species (Arnold 1998; Braña 2003; Kaliontzopoulou et al. 2013). From a more technical perspective, spatial precision and temporal resolution are the two characteristics that can lead to differences in the results obtained by using these two methodologies.

Here, we explored how the method used to estimate maximum running speed can influence the statistical properties of the obtained data and, in consequence, biological interpretations. In principle, these two methods are so different that estimates obtained from distance intervals or time intervals should not be comparable. However, since both are widely used in the literature, their statistical properties should be assessed when making inference on maximum running speed with organisms moving at non-constant velocity within a given spatial interval, in order to enhance experimental inference of functional hypotheses. For this purpose, we used locomotor performance data recorded for the lizard *Podarcis bocagei* (Seoane 1884). Lizards have been extensively used as models for ecomorphological studies (Arnold 1998; Vanhooydonck and Van Damme 2003; Huyghe et al. 2007; Brecko et al. 2008; Kaliontzopoulou et al. 2013; Gomes et al. 2016) because they exhibit a wide variety of locomotor modes (e.g. climbers, sprinters, matrix-dwellers, etc.), they explore many different habitat types, and their morphology and performance are easy to quantify (Garland and Losos 1994). Nevertheless, the conclusions drawn here are relevant for any study aiming to quantify animal locomotor capacities, at least when working with small, fast-moving animals, where spatial precision and temporal resolution can be an issue (e.g. swimming performance in amphibian larvae or fish, running performance in small mammals etc.).

Materials and Methods

Podarcis bocagei is a lacertid lizard that occurs in the NW Iberian Peninsula and is found in a variety of habitats: sandy areas with rocks, sparse vegetation, and stone walls that delimit agricultural fields (see e.g. Kaliontzopoulou et al. 2010). Lizards for this study were captured in the coastal zone around the Metropolitan Area of Porto (Portugal). We collected a total of 156 adult individuals, including 76 females and 80 males, by noosing (García-Muñoz and Sillero 2010), in October of 2012. Lizards were placed in cloth bags, transported to the laboratory, and housed in individual terraria, where they were fed with live mealworms and provided with water ad libitum. Before performance experiments, animals were allowed to rest for 2 days to ensure acclimation. After the conclusion of laboratory trials, all individuals were released back to the site where they had been captured.

For all individuals, sprint speed (SPR) on a horizontal surface was measured by chasing animals along a 1 m-long and 15 cm-wide racetrack, on a cork substrate (Van Berkum et al. 1989; Braña 2003). Cork was used as a substrate because it provides very good traction (Van Damme et al. 1997). All animals were tested three times in order to estimate individual repeatability. This experimental procedure is typically used to ensure that maximal performance capacity is recorded (Losos et al. 2002; Adolph and Pickering 2008). Trials for each individual were randomized to ensure that all lizards were tested during different times of the day and to avoid sequential repeats. We allowed a rest of at least 2 h between trials to ensure physical recovery of the individuals. All experiments were carried out at a room temperature of about 31 °C, which is approximately the selected body temperature of this species (Amaral et al. 2012). Prior to and in between trials, lizards were placed for at least 1 h in a terrarium exposed to an infrared light of 150 W to thermoregulate and attain their preferred body temperature (Veríssimo and Carretero 2009). All running trials were filmed with a digital camera (Canon EOS 60D) at a filming speed of 50 frames per second (fps). It is worth noting that although 50 fps could be a low filming speed for some species, *P. bocagei* is a small lizard and it is not a long-distance runner (Braña 2003); as such, this filming speed is adequate for the purposes of this study. Each run was scored as "bad" or "good", and the "bad" races, in which the animals completely stopped or turned around during the race, were eliminated (sensu Van Berkum and Tsuji 1987; Tsuji et al. 1989). The position of the lizard across each run was digitized using MaxTRAQ 2D motion analysis software (Innovision Systems Inc. 2009). The maximum performance capacity of each individual in each run was then estimated using two alternative methods: (1) as the maximum speed observed across the whole trial for any interval of two sequential frames (hereafter referred to as 'instantaneous speed'); and (2) as the maximum speed across any 10-cm interval. For each of the two methods, the highest speed recorded across the three trials was taken as an estimate of each animal's maximum performing capacity. To investigate how different time intervals can influence the estimation of maximum speed, we also extracted frame-interval speeds throughout the entire gradient between 2 and 50 frames (the minimum and maximum number possible with our video records), where we resampled the data to extract the maximum speed observed over complementary sets of longer sequences (e.g. for five frames we calculated speeds for frame sets 1–5, 6–10 etc.).

Based on these estimates, we performed several tests to explore the statistical properties of maximum speed values and compare them between methods. First, to visualize the distribution of speed estimates, we produced a bivariate graph and visually inspected the relationship between the data produced by the two methods. We then investigated if the two datasets were normally distributed using a Shapiro–Wilks normality test. To quantify the repeatability of our data, we examined the correlation between the mean and maximum speed recorded across the three repetitions of each individual (Huey and Dunham 1987; Van Berkum et al. 1989). To investigate if the two methods provided different estimates of maximum speed values, we performed a repeated-measures ANOVA on maximum speed, with method as a fixed factor. Finally, we used Levene's test to compare the variance observed among individuals of the two datasets. Because we found differences in means across methods (see Results) that could influence variance estimates, we also visualized differences in the degree of variability across datasets using the coefficient of variation—i.e. the standard deviation divided by the mean (Sokal and Rohlf 1995, pp. 97–98). All data were logarithmically transformed prior to analyses. All analyses were implemented in R (version 3.2.0) using RStudio (0.99.447).

Results

Data of instantaneous estimates of maximum speed followed a normal distribution ($W = 0.991$, p value $= 0.459$), but those obtained from 10 cm-intervals deviated from normality with a bias towards low speed values ($W = 0.931$, p value $= 8.04 \times 10^{-7}$; Fig. 1). The time frame number corresponding to a 10 cm-interval was 17.69 (Fig. 2). Repeatability was higher in the speeds estimated using time-intervals than in the data obtained using 10 cm-intervals (Fig. 2a). The repeated-measures ANOVA showed that the two methods produced significantly different maximum speed estimates, where the 10 cm-interval approach provided lower values compared to the time-interval speeds estimated across two sequential frames ($F = 11.98$; p value <0.001; Fig. 3). This was the result of a gradual decrease of maximum speed estimates with increasing length of the time interval used to quantify speeds (Fig. 2b). Indeed, the speeds obtained using a time interval were lower than those from 10 cm-intervals when time-interval speeds were obtained using five or more frames (Fig. 2b). Speed estimates obtained using 10 cm intervals exhibited approximately three times more total variance ($F = 1.593$; p value $= 9.29 \times 10^{-7}$; Fig. 2c), and about double the coefficient of variation (Fig. 2d), as compared to instantaneous speeds.

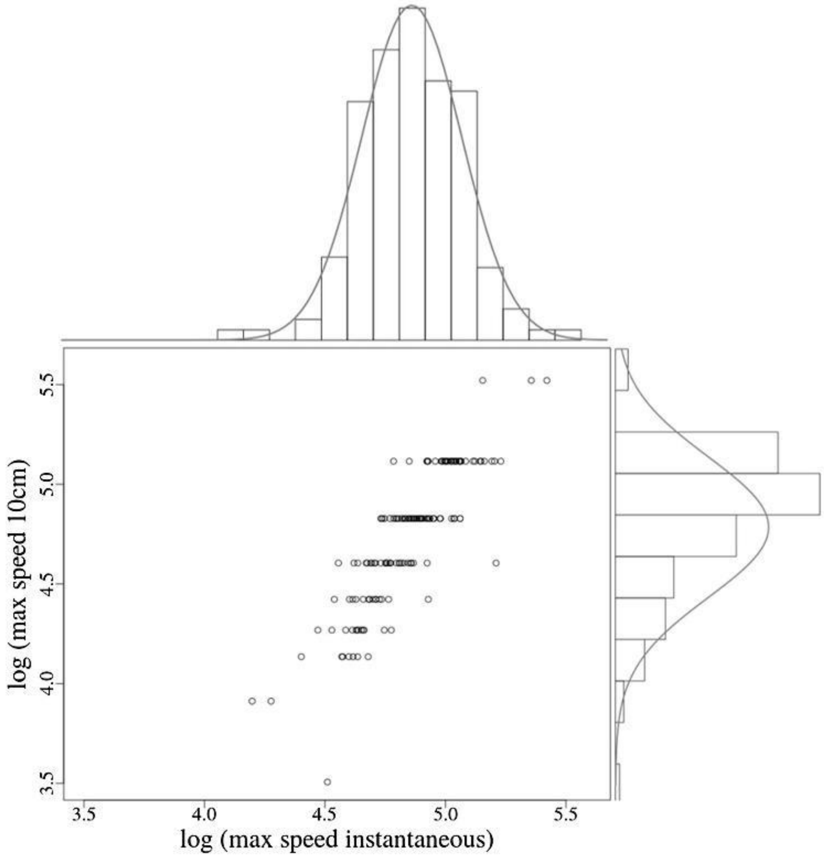

Fig. 1 Relationship between maximum speed from data of 10 cm-interval and instantaneous speed, with the representation of the histograms with the normal curve represented

Discussion

Our comparison of the data obtained using two frequently implemented methodologies for quantifying locomotor performance in animals revealed substantial differences in the estimates of maximum speed, which exhibit different statistical properties. Previous observations had suggested that these two methodologies may differ in resolution and accuracy, where instantaneous speeds were expected to provide better estimates than those calculated across distance intervals (Kaliontzopoulou et al. 2013). Indeed, the comprehensive set of comparisons conducted here revealed that the data obtained across 10 cm-intervals deviated from normality (Fig. 1) and exhibited lower means (Figs. 2b, 3), lower individual repeatabilities (Fig. 2a), and higher variances among individuals (Fig. 2c) when compared to instantaneous speed estimates. These results suggest that the approach used for extracting estimates of maximal performance considerably influences the quality of the data obtained. As a result, our capacity for detecting differences in performance across groups of interest or for identifying associations between functional and other (e.g. anatomical, morphological, physiological, behavioural) traits may become compromised. Such methodological effects may entangle biological inference, and addressing them is of relevance for enhancing studies of functional performance.

Our results showed that data from 10 cm-intervals did not follow the normal distribution and exhibited a higher variance as compared to instantaneous speed data. These statistical properties are most likely a result of behavioural modulation of movement patterns. Indeed, *Podarcis* lizards are not long-distance runners but instead perform short bursts of running alternated with frequent pauses (Braña 2003). These pauses within distance intervals can lead to an underestimation of the maximum running potential of each individual, as shown by the lower mean velocity estimates obtained when using distance interval estimates. Even individuals that are highly motivated when performing locomotor experiments will exhibit this running behaviour. Furthermore, burst-pause running patterns will vary randomly among individuals, as well as across trials of the same individual and across intervals within trials (Avery et al. 1987).

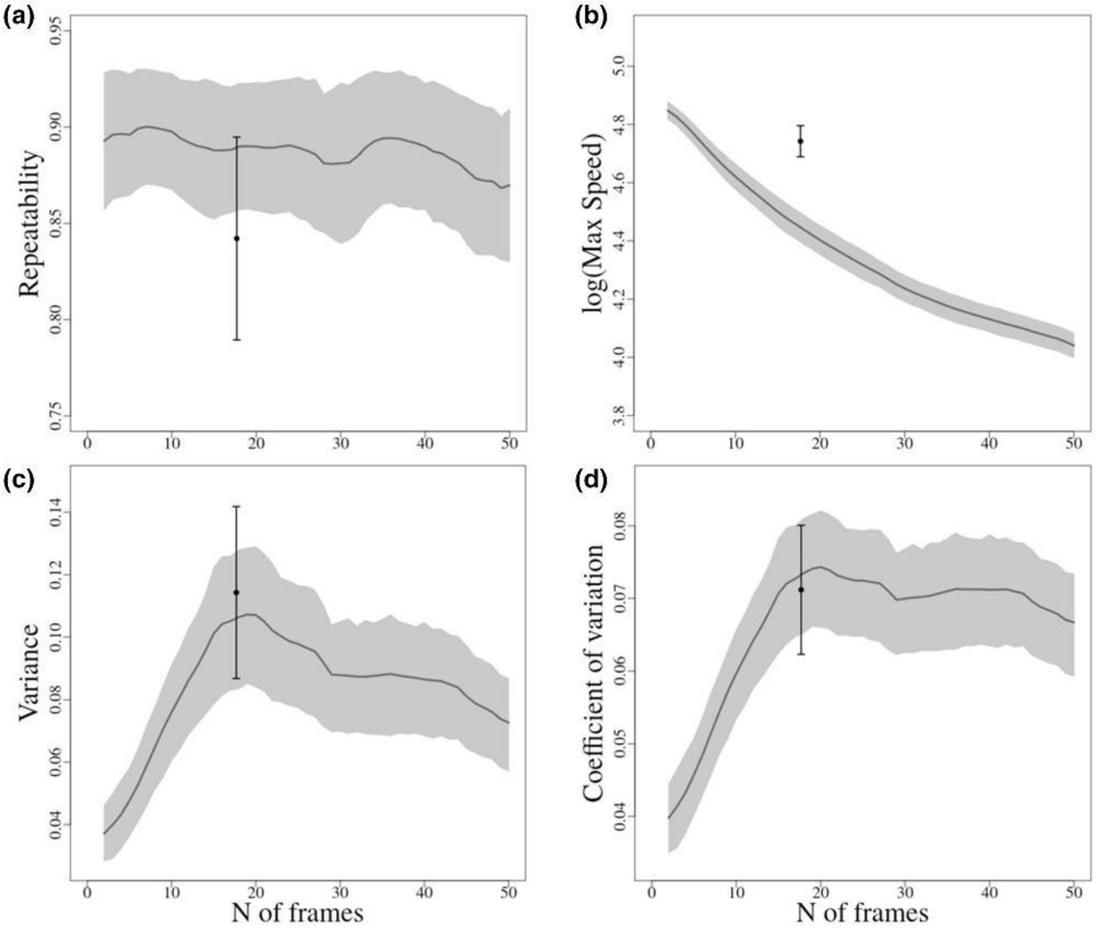

Fig. 2 Mean value for **a** repeatability—correlation between mean and maximum speed by individual, **b** maximum speed, **c** variance of maximum speed and **d** coefficient of variation of maximum speed. The *x-axis* represents the examined gradient of number of frames used to define time intervals for extracting speed estimates. The value of each descriptor estimated using 10 cm-interval data is placed at the mean corresponding number of frames (*black*). Confidence intervals around the mean value of each descriptor (*shaded grey area*) were calculated using bootstrapping with 1000 random samples

As a result, speed data obtained across intervals exhibit higher variance among individuals and lower individual repeatability compared with time-interval speed data. Note that this effect is already quite marked when considering sprint speed on a flat surface, but it is expected to have an even stronger effect when considering other types of locomotion, such as climbing or manoeuvring. Performance trials conducted on tilted surfaces or including obstacles are generally more challenging and, as a result, animals tend to stop more frequently than on a flat surface (pers. obs.), a fact that increases this behavioural effect of burst-pause movement on speed estimates. Similarly, we expect the effect of locomotor pattern to be much more prominent when investigating different species, possibly engaging in different running strategies. In such cases, this behavioural effect may vary more extensively than across individuals of the same species, as in the example dataset used here.

In addition, as highlighted by Losos et al. 2002, it is also important to always keep in mind the effect of motivation. To motivate an animal to run during laboratory trials, a predator attack is simulated where the animals escape from the researcher. Therefore, maximal functional performance measures obtained in the laboratory have been associated with escape performance in the field (e.g. Martín and López 1999; Diego-Rasilla 2003). When escaping from a predator, lizards evaluate the costs and benefits of fleeing,

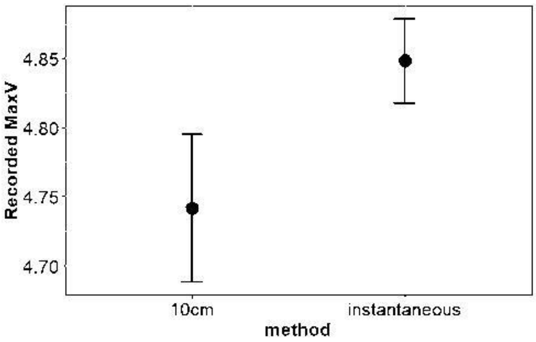

Fig. 3 Least-squares means for each method—maximum 10 cm-interval speed and maximum instantaneous speed. *Vertical bars* denote 95% confidence intervals

only moving when the predator represents a serious threat (Carretero et al. 2006; Cooper and Peréz-Mellado 2004; Martín and López 1999). Many lizards adopt different tactics when escaping from predators—e.g. they may not allow the predator to come very close (increasing approach distance), or they may stay close to their refuge (decreasing the distance fled). The last case is the most common in *Podarcis* wall lizards, where fleeing distance is usually quite short (Cooper et al. 2009; Diego-Rasilla 2003, pers. obs. of the authors). Comparing laboratory experiments and field tests is important to understand how behaviour may mould performance (Husak and Fox 2006; Irschick et al. 2005; Losos and Irschick 1996). When we try to link maximal performance measured in the laboratory with observations of behaviour in the field, the method used to estimate maximal performance becomes important. In this context, different methods may be more adequate depending on the ecological or social task of interest. In the case of escape or foraging behaviour, during which the animals normally run longer distances, interval estimates could be used. However, if faster tasks, as antagonistic behaviour, are being investigated, instantaneous estimates should be preferred to accurately capture individual variation at a short temporal scale.

Indeed, another related feature influencing the quality of maximal speed estimates is scale and its relationship with running mode. An inherent problem of speed analysis during animal locomotion is precisely the connection between temporal and spatial scale. Indeed, while trying to estimate maximum speed, one may choose to sample running trials using distance intervals, as traditionally done, or time intervals (in the form of number of frames). In either case, data collected at different temporal or spatial scales will not be comparable, as the resolution used for data recording will influence the estimated speeds (and their variance and repeatability, i.e. Fig. 2). The effect of scale on locomotor performance estimates is nicely illustrated by considering acceleration, instead of speed, and its variation. Acceleration is frequently examined in performance studies, as it is a critical feature of locomotion in many animals, particularly during escape from predators (Domenici 2001; Miles 2004; Vanhooydonck et al. 2006). However, it is also more sensitive to scale effects, as it occurs at a finer temporal scale and it therefore exhibits a higher dependence on the temporal resolution of the quantification approach. This inflation of variance estimates for speed measures artificially increases the overlap between the distributions of compared groups (e.g. sexes, habitat types, species). As such, the detectability of small differences among groups, or of the association of locomotion descriptors with other traits, will be more difficult when using a lower resolution.

Distance-interval estimates of maximal velocity have been used in several studies, where it was possible to detect differences between groups or correlate morphological traits with performance (Bauwens et al. 1995; Goodman et al. 2008; Gomes et al. 2016; Huyghe et al. 2007; Kaliontzopoulou et al. 2013; Vanhooydonck and Van Damme 2001; Vanhooydonck et al. 2006; Žagar et al. 2017). Most of these studies examined locomotor variation at the interspecific, and sometimes at the family, level (see Table 1). In these cases, the magnitude of variation is larger and identifying differences among groups and associating them to other traits as morphology is easier than it is within species (Kaliontzopoulou et al. 2010a; Losos and Miles 1994). However, when interested in intraspecific functional variation, the magnitude of variation across individuals is much smaller, and a method with more resolution and accuracy will provide a higher capacity for identifying significant variation among groups in locomotor performance, or covariation with other traits. For instance, Kaliontzopoulou et al. (2013) found an association between morphological traits and performance at the intraspecific level using instantaneous speed data, but not considering 10 cm-interval estimates of the same data. To obtain a more global view of how the difference between methods could affect biological inferences, we reviewed locomotor speed data from available intraspecific and interspecific studies. We focused mainly on studies examining species of the family Lacertidae, which are—evolutionarily speaking—more associated to our dataset (Table 1). Based on the maximal velocities reported in each study, we calculate the coefficient of variation observed across the groups of interest compared by the authors (i.e. sexes, ages, or populations, for intraspecific studies; and species, for interspecific studies), to contrast these values with the variation between methods retrieved in this study. The coefficients of variation reported in intraspecific studies are quite smaller than those observed when considering variation at the genus or family level (Table 1). Putting the results obtained

Table 1 Study system, compared groups, range, mean and coefficient of variation of maximum velocities, and method used to quantify locomotor capacity in different studies

Study system	Compared groups	Range (cm s^{-1})	Mean (cm s^{-1})	CV	Method	References
Podarcis melisellensis	Colour morphs	197.89–202.4	200.56	0.02	Interval	Huyghe et al. (2007)
Podarcis melisellensis	Age	142.69–181.8	163.56	0.24	Interval	Brecko et al. (2008)
Podarcis bocagei	Sexes	138.97–157.88	148.43	0.13	Interval	Kaliontzopoulou et al. (2013)
Podarcis bocagei	Sexes	139.56–161.21	150.39	0.14	Instantaneous	Kaliontzopoulou et al. (2013)
Podarcis bocagei	Sexes	99.83–118.39	109.11	0.17	Interval	Gomes et al. (2016)
Podarcis guadarramae	Sexes	110.32–115.82	113.07	0.05	Interval	Gomes et al. (2016)
Podarcis spp.	Species (2)	99.83–118.39	111.09	0.17	Interval	Gomes et al. (2016)
Podarcis spp.	Species (2)	108.88–202.68	155.78	0.6	Interval	Van Damme et al. (1997)
Podarcis muralis	Sexes	163.72–171.24	167.48	0.05	Instantaneous	Žagar et al. (2017)
Iberolacerta horvathi	Sexes	222.94–226.44	224.69	0.02	Instantaneous	Žagar et al. (2017)
Podarcis muralis, Iberolacerta horvathi	Species (2)	163.72–226.44	196.09	0.32	Instantaneous	Žagar et al. (2017)
Podarcis sicula	Sexes	173–175.6	174.3	0.01	Interval	Vervust et al. (2007)
Podarcis sicula	Sexes	182.8–184.9	183.85	0.01	Interval	Vervust et al. (2007)
Podarcis sicula	Islands	173–184.9	179.8	0.07	Interval	Vervust et al. (2007)
Gallotia simonyi	Age	171–253.4	212.2	0.39	Interval	Cejudo and Márquez (2001)
Gallotia stehlini	Age	236–330.2	283.1	0.33	Interval	Cejudo and Márquez (2001)
Gallotia spp.	Species (2)	171–330.2	247.65	0.64	Interval	Cejudo and Márquez (2001)
Psammodromus algirus	Sexes	129.27–155.79	142.53	0.19	Interval	Zamora-Camacho et al. (2015)
Psammodromus algirus	Sexes	222.89–225.58	224.24	0.01	Interval	Iraeta et al. (2011)
Psammodromus algirus	Sexes	224.17–231	227.59	0.03	Interval	Iraeta et al. (2011)
Psammodromus algirus	Habitats	222.89–231	225.91	0.04	Interval	Iraeta et al. (2011)
Lacertidae	Species (13)	109–334	207.46	1.09	Interval	Vanhooydonck and Van Damme (2001)
Lacertidae	Species (13)	90–313	188.02	1.19	Interval	Bauwens et al. (1995)
Lacertidae	Species (22)	87–298	213.96	0.99	Interval	Verwaijen and Van Damme (2008)
Methods	This study	120.8–129.85	125.33	0.07	Between both	This study

For interspecific studies, the number of species examined is reported between brackets

here in the context of those studies, the coefficient of variation obtained when comparing the two methods for data acquisition is similar to the values reported in intraspecific studies. This suggests that using lower-resolution estimates of maximal performance should not be problematic when examining highly differentiated groups (i.e. different species or genera), but it also reinforces the importance of using more accurate methods, as instantaneous speed estimates, when working at the intraspecific level.

Another effect related to spatial scale is that of the body size. Most studies investigating locomotor performance use photocells placed at regular intervals, the length of which is usually defined taking the size of the animal into account (e.g. Van Damme et al. 1997; Brecko et al. 2008; Goodman et al. 2008). This has consequences for the methodological definition of the quantified measures, as larger animals need to take fewer strides than smaller ones to cover the same distance. Furthermore, variations across studies in the selection of interval length exist even within specific groups, potentially hindering comparisons across them. For instance, different published studies investigating locomotion in *Podarcis* lizards have used different intervals to obtain maximum speed (Van Damme et al. 1997—25 cm; Braña 2003—20 cm; Kaliontzopoulou et al. 2013—10 cm). Our results advise against comparisons across these studies, as the time or length interval used to record running performance considerably affects the mean and variance of maximum speed estimates. Most importantly, this has severe consequences for the posterior use of data recorded over different intervals for meta-analyses, which would be of interest e.g. for conducting comparative studies across different species (Kramer and McLaughlin 2001; Iriarte-Díaz 2002; Van Damme et al. 2008).

In conclusion, the methodology used to obtain maximal performance capability estimates substantially modifies the statistical properties of such estimates. This may have an influence on ecomorphological inference, as it affects our capacity for accurately describing variation in performance and associations between locomotor performance and other traits (e.g. morphology, ecology, fitness). For instance, the higher variance that characterizes interval speed estimates may entangle statistical inference by increasing the overlap between different groups (e.g. populations or species inhabiting different habitats), making the detection of eventual differences more difficult. This is the same effect as that caused by the inclusion of individuals performing submaximally, which has been previously demonstrated to hinder the detection of interspecific differences in locomotor performance (Losos et al. 2002). Such effects may be more pervasive in locomotor performance studies and could have a stronger effect on ecomorphological inference than previously thought. Taking our results into account, the use of instantaneous speed estimates should be preferred over distance-interval estimates when possible. Given the increasing accessibility to high resolution, high-speed filming equipment, and also to computer programs that allow the automatic detection of animal position, obtaining instantaneous maximum speed estimates is becoming increasingly easier, with evident advantages for researchers interested in understanding the evolution of animal locomotion.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL PAPER

Run for your life, but bite for your rights? How interactions between natural and sexual selection shape functional morphology across habitats

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Abstract
A central issue in evolutionary biology is how morphology, performance, and habitat use coevolve. If morphological variation is tightly associated with habitat use, then differences in morphology should affect fitness through their effect on performance within specific habitats. In this study, we investigate how evolutionary forces mold morphological traits and performance differently given the surrounding environment, at the intraspecific level. For this purpose, we selected populations of the lizard *Podarcis bocagei* from two different habitat types, agricultural walls and dunes, which we expected to reflect saxicolous vs ground-dwelling habits. In the laboratory, we recorded morphological traits as well as performance traits by measuring sprint speed, climbing capacity, maneuverability, and bite force. Our results revealed fast-evolving ecomorphological variation among populations of *P. bocagei*, where a direct association existed between head morphology and bite performance. However, we could not establish links between limb morphology and locomotor performance at the individual level. Lizards from walls were better climbers than those from dunes, suggesting a very fast evolutionary response. Interestingly, a significant interaction between habitat and sex was detected in climbing performance. In addition, lizards from dunes bit harder than those from walls, although sexual differentiation was definitely the main factor driving variation in head functional morphology. Taking into account all the results, we found a complex interaction between natural and sexual selection on whole-organism performance, which are, in some cases, reflected in morphological variation.

Keywords Morphological traits · Habitat use · Performance · Natural selection · Sexual selection

Introduction

A central theme in evolutionary biology is to understand how phenotypic variation evolves and how the phenotype is related with the environment. Morphological traits are a substantial part of the phenotype: they are the structural components with which organisms interact with their environment. As such, they are involved in very different and crucial ecological and social functions, and they are subject to strong selective influences. Selection can be described as the relationship between variation in phenotypic traits and variation in fitness across individuals (Kingsolver and Huey 2003). Differences in an organism's functional morphology should result in differences in performance that directly affect fitness in a given environment (Arnold 1983; Emerson and Arnold 1989; Irschick 2002), yielding links between morphology, performance, and fitness that have been studied under the framework of the ecomorphological paradigm (Arnold 1983).

Selection pressures act on individuals, maximizing their chances for survival and reproduction, which is why the approach described by Arnold (1983) was initially put forward at the intraspecific level, and in particular highlighting

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the relevance of "*the analysis of adaptation within populations of conspecifics*" (Arnold 1983: 348). However, similar approaches have been used extensively to study macroevolutionary associations between morphology, performance, and ecology across species. For instance, *Anolis* lizards are one of the most remarkable examples of an adaptive radiation where sympatric species have repeatedly evolved divergent morphologies, performance, and behaviors as means of exploiting different microhabitats and enhancing niche segregation (Losos and Sinervo 1989). This and other textbook ecomorphological examples have attracted attention in part due to the remarkable morphological diversity they encompass, which makes patterns of variation easier to detect. Indeed, because the magnitude of variation is big, it is generally easier to identify differences among groups and associate them to hypothesized explanatory factors at higher taxonomic levels than within species (Losos and Miles 1994; Kaliontzopoulou et al. 2010a). However, when examining species that encompass a wide evolutionary array, in many occasions, the detected phenotypic patterns and associations across traits can be the result of adaptation or exaptation (sensu Gould and Vrba 1982), evolutionary signal (Blomberg and Garland 2002), or phenotypic plasticity (Losos et al. 2000) potentially making the interpretation of the results difficult. Hence, studies at the intraspecific level are relevant for detecting microevolutionary mechanisms and linking them to adaptive pressures that lead to phenotypic differentiation (Irschick et al. 2005a).

To optimize whole-organism performance, natural and sexual selection mold morphological traits that are involved in ecological functions (e.g., feeding, escape from predators, habitat and refuge use, competition with heterospecifics) and social interactions (e.g., antagonistic behavior, territory and mate acquisition, mating), respectively. Natural selection may influence performance and morphology to enhance the survivorship of the individuals when performing different tasks (Husak and Fox 2006). For instance, when escaping from predators, a fast locomotor performance will be the best strategy to avoid predation, but the probability of survival can be further augmented through morphological adaptations that facilitate the use of specific refuges (Goodman 2009). On the other side, sexual selection may influence performance and morphology to provide individuals with an advantage in mating through intrasexual competition and mate choice (Husak and Fox 2008). For instance, increased locomotor and bite performance enhance the capacity of males for defending territories and increase their mating possibilities (Husak et al. 2006; Husak and Fox 2008). These processes occur simultaneously, and they can be parallel, when both natural and sexual selection act on performance and/or morphological traits in the same way to enhance fitness, or opposite, if ecological and social functions impose conflicting performance demands (Husak et al. 2006; Husak and Fox 2008). The balance between natural and sexual selection is complex, but it can be generally inferred when observing variation in the degree of sexual dimorphism depending on the ecological context (Butler et al. 2000; Butler and Losos 2002; Husak and Fox 2008; Kaliontzopoulou et al. 2010a, 2012).

Lizards provide excellent models for studies of functional morphology because of their wide range of morphologies and performance skills, and of their presence in a variety of habitats (Arnold 1998; Garland and Losos 1994; Irschick and Garland, 2001; Irschick 2002). Further, they are usually sexually dimorphic in body size and shape, where males are frequently larger, with larger heads and longer limbs, whereas females tend to have longer inter-limb length (Kaliontzopoulou et al. 2012, 2013; Gomes et al. 2016; Irschick and Higham 2016). These differences probably result from sexual and fecundity selection, and they occur in morphological traits with a high functional relevance (e.g., Kaliontzopoulou et al. 2010b, 2012, 2013). This provides the opportunity of combining morphological, functional, and ecological data to investigate how natural selection—e.g., with respect to habitat variation—and sexual selection are integrated to shape functional and morphological diversity, and to decipher the evolutionary meaning of morphological variation. In the Mediterranean Basin, wall lizards (*Podarcis* spp.) provide a particularly interesting system for investigating phenotypic variation, with remarkable levels of intraspecific morphological variation and cryptic diversity. Several studies in this group have investigated variation in morphology and performance, and their relationship, both in relation to habitat and focusing on sexual dimorphism (Van Damme et al. 1997; Brecko et al. 2008; Kaliontzopoulou et al. 2012, 2013; Gomes et al. 2016). From an ecomorphological perspective, habitat use has been shown to be an important determinant of macroevolutionary phenotypic differentiation in head shape but not in body size or limb length in wall lizards, although patterns differed between the sexes (Kaliontzopoulou et al. 2015). At the intraspecific level, both limb and head morphology are known to differ across different habitat types of *P. bocagei*, but again the degree of differentiation between sexes varies between habitats, suggesting an interaction between natural and sexual selection (Kaliontzopoulou et al. 2010a). Interestingly, some of the morphological patterns retrieved in that study aligned with, while others contradicted, predictions of the ecomorphological paradigm, raising questions about the functional, and therefore evolutionary, significance of the observed variation.

Indeed, morphological differentiation does not always translate into functional variation, and the evidence available is frequently contradictory. In the context of sexual dimorphism, differences in morphology between males and females of *P. melisellensis* translate into different bite force capacities, but not different sprint speeds (Brecko et al. 2008). By contrast, two Iberian *Podarcis* wall lizards have been reported to differ in head morphology but this differentiation did not translate into bite force capacity, suggesting that ecological variation between species was only reflected on morphology but not on functional capacities (Kaliontzopoulou et al. 2012). In the case of locomotor performance, some studies have provided evidence of an association between morphological traits and locomotor performance (Kaliontzopoulou et al. 2013), but others have failed to detect this association (Van Damme et al. 1997; Vanhooydonck et al. 2000; Gomes et al. 2016). Thereby, it is not clear whether morphological evolutionary responses to habitat and sexual dimorphism are mediated by functional performance and, if so, which traits are involved and which is the exact link between morphology and function (Irschick et al. 2008).

In this study, we investigate how natural and sexual selection may contribute in shaping morphological traits and performance differently given the surrounding environment, at the intraspecific level. For this purpose, we selected populations of *P. bocagei* from two contrasting habitat types, representing saxicolous and ground-dwelling ecological habits. We tested whether individuals from different habitats differ in locomotor and biting performance, as we would expect under predictions of the ecomorphological paradigm. Further, we investigated to what extent and how individual variation in morphology translates into variation in functional performance, as predicted by biomechanical rules and as suggested by studies in other lizard groups. Based on previous observations on the morphology and ecology of this species, and considering ecomorphological patterns in other lizards, we hypothesize that individuals from dunes, which are generally ground-dwelling, have little chance to climb, live in more open habitats, and have higher and more rounded heads and longer limbs, will exhibit stronger bites and will be better sprinters. On the other hand, we expect individuals from agricultural walls, which have been generally considered as saxicolous, frequently using vertical surfaces and have flattened head and shorter limbs, to perform better when climbing and possibly exhibit reduced biting performance, as a result of physical constraints on head height. In addition, considering the well-known existence of male-biased sexual dimorphism in these lizards, we expect that males will exhibit stronger biting and locomotor performance. Finally, given that the ground-dwelling populations studied here are known to be morphologically more dimorphic than saxicolous ones (Kaliontzopoulou et al. 2010a), we were interested in investigating if this variation also translates into differences in the degree of sexual dimorphism in whole-organism performance between habitats. The mechanical constraints imposed by a saxicolous life translated in a less pronounced sexual dimorphism than in the animals living in open environments (Kaliontzopoulou et al. 2010a), and may lead to differences in functional capacities.

Material and methods

Study organism

Podarcis bocagei is a lacertid lizard endemic to the NW Iberian Peninsula, and it can be found in a variety of habitats: sandy areas with or without rocks, sparse vegetation, and stone walls that delimit agricultural fields (see e.g., Kaliontzopoulou et al. 2010a). Lizards for this study were captured in four localities: two dune areas (Madalena and Mindelo) and two sites with agricultural stone walls (Gião and São Mamede do Coronado). We collected a total of 156 adult individuals, including 76 females and 80 males (approximately 20 males and 20 females from each population), by noosing (García-Muñoz and Sillero 2010) in October 2012. Lizards were placed in cloth bags, transported to the laboratory, and housed in individual terraria, where they were fed with live mealworms and provided with water ad libitum. Before experiments, animals were allowed to rest for two days to ensure acclimation. After the conclusion of laboratory experiments, all individuals were released back to the site where they had been captured. We performed all experiments in autumn, that is, in the advanced post-reproductive season (Carretero et al. 2006), to exclude any potential effects of pregnancy on female performance (Bauwens and Thoen 1981) or due to low body condition immediately after reproduction on both sexes (Galán 1996).

Quantified parameters

In all individuals captured, we measured the following linear biometric traits: snout-vent length (SVL), trunk length (TRL), head length (HL), head width (HW), head height (HH), mouth opening (MO), forelimb length (FLL), and hind limb length (HLL), using electronic calipers (precision ± 0.01 mm; see Kaliontzopoulou et al. 2007 for a detailed description of variables). All morphological traits were ln-transformed for further analyses.

All experiments to quantify functional performance and examine microhabitat selection were carried out at a room temperature of about 31 °C, which is approximately the selected body temperature of the species (Amaral et al. 2012). Prior to and in between trials, lizards were placed for at least 1 h in a terrarium exposed to an infrared lamp of 150 W, allowing them to thermoregulate and attain their preferred body temperatures (Veríssimo and Carretero 2009).

For all individuals, three types of locomotor performance were measured (Kaliontzopoulou et al. 2013): sprint speed (SPR), climbing capacity (CLI), and maneuverability (MAN). Sprint speed on a horizontal surface was measured by chasing animals along a 1-m-long and 15-cm-wide racetrack, with a cork substrate (Van Berkum et al. 1989; Braña 2003). Climbing capacity was quantified by chasing animals up a similar racetrack, tilted to an angle of 60°. Cork was used as a substrate because it provides very good traction (Van Damme et al. 1997). To quantify maneuverability, a 0.5-m-long and 15-cm-wide pinboard was placed on the racetrack. This pinboard was made of 8-mm diameter pins placed at equal distances of 35 mm (Vanhooydonck et al. 2000). We allowed a rest of at least 2 h between trials to ensure physical recovery of the individuals. All trials were filmed with a digital camera (Canon EOS 60D) at a filming speed of 50 frames per second. Locomotor speed in the three types of racetracks was measured on different days, and the order in which animals were subjected to the tests was randomized. Each individual was tested three times in each type of racetrack to ensure that maximal locomotor capacity was recorded. Each run was scored as "bad" or "good," and the "bad" races, in which the animals turned around during the race, were eliminated (sensu Van Berkum and Tsuji 1987; Tsuji et al. 1989). The position of the lizard across each run was digitized using MaxTRAQ 2D motion analysis software (Innovision Systems Inc. 2009). The highest instantaneous speed (Gomes et al. 2017) recorded across the three trials was taken as an estimate of each animal's maximum performing capacity in each of the three types of racetrack and log-transformed for further analyses.

Bite force was measured using an isometric Kistler force transducer (type 9203, Kistler Inc., Winterthur, Switzerland) mounted on a vertical holder and connected to a Kistler charge amplifier (type 5058A, Kistler Inc., Winterthur, Switzerland). Bite force measurements were obtained by provoking the lizard to bite a pair of thin metal plates connected to the force transducer (see Herrel et al. 2001a for a detailed description). The tip of the metal plates where the lizard bit was delimited with a marker to ensure all lizards bit at an equal distance from the revolving arms and thus standardize the point of force exertion. Each lizard was tested five times to ensure that the maximal individual bite force per individual was registered. The maximum bite force measure per individual was retained and log-transformed for further analyses.

Statistical analyses

To represent total head size (HS), we used the scores of the first principal component calculated using HL, HW, HH, and MO. For this purpose, principal components of the covariance matrix of head dimensions were calculated using the function prcomp of stats package (R Development Core Team 2016). The four head dimensions had similarly high loadings of the same sign (MO, 0.52; HH, 0.50; HW, 0.49; and HL, 0.49) on the first principal component, which explained 91% of total variance, and was therefore taken to represent HS. To investigate if there were differences between habitats and sexes in size, we used an ANOVA comparison. Next, ANCOVA models were run using each measured morphological trait as the response variable, and habitat (HAB), population nested within habitat (POP), sex, and interaction terms (HAB×SEX and POP×SEX) as predictors, and SVL as a covariate. To examine whether habitats and sexes differed in locomotor performance, we performed ANOVA comparisons on each type of locomotor speed and bite force performance separately, with the same design as for morphology. We also performed ANCOVA comparisons on each type of locomotor speed and bite force performance separately with the same design and considering SVL—in the case of locomotor performance—and HS—in the case of bite force—as covariates. Throughout, we always used permutation-based ANOVA procedures using 1000 randomizations of Euclidean distance matrices as implemented in the adonis function of the vegan R-package (Oksanen et al. 2012).

To investigate the multivariate association between morphology and performance, we used two-block partial least-squares regression (PLS) as implemented in the plsr function of the pls R-package (Mevik et al. 2011). To investigate the association between morphology and locomotor performance (three variables: SPR, CLI, MAN), we only used head size and limb and trunk variables, as these traits are known to determine locomotor performance in these and other lizard species (Kaliontzopoulou et al. 2010a, 2013; Vanhooydonck and Van Damme, 2001 Cameron et al. 2013; Gomes et al. 2016). We first performed the PLS with raw variables, and then we repeated the analysis after correcting all locomotor performance and morphological variables for size effects through a regression on SVL. In the case of the association between morphology and biting performance, we only have one performance variable; however, we used two-block partial least-squares regression to be concordant with the previous analysis of locomotor performance. We only used head dimensions (HL, HW, HH, and MO), which are the main morphological determinants of bite force (e.g., Herrel et al. 2001a; Kaliontzopoulou et al. 2012). Similar to the approach for locomotor performance, we first performed the PLS with raw variables and then we re-run the analysis after size-correcting both bite performance and morphological variables through a regression on HS.

All statistical analyses were performed using R v. 3.3.1 (R Development Core Team 2016).

Results

ANOVA comparisons indicated that individuals from different habitats differed in size ($F = 8.135$, $p = 0.007$), where animals from dunes were larger in body size. ANCOVA comparisons using SVL as the covariate only revealed significant differences between habitats in relative head height, where individuals from dune environments had relatively higher heads than those from walls. ANCOVA also confirmed significant differences between the sexes in all morphological traits, where males had longer limbs and larger heads, but shorter trunks than females (Table 1).

ANOVA comparisons on locomotor performance showed that individuals from walls exhibited higher climbing capacities (Table 2, Fig. 1). Sex did not have a significant effect on locomotor performance. We also detected significant differences between population in sprint capacity. Furthermore, a significant interaction between habitat and sex was identified, where individuals of both sexes from walls exhibited similar locomotor performance, whereas in dunes, males were better climbers then females. ANCOVA comparisons using SVL as the covariate revealed a significant effect of body size on locomotor performance, but differences between habitats and the interaction between habitat and sex remained significant for climbing after accounting for variation in body size (Table 3, Fig. 1). ANOVA comparisons also showed that lizards from dunes bit harder than those from walls. Bite force was also significantly different between the sexes, where males attained a higher maximal bite force than did females (Table 2, Fig. 2). Finally, ANCOVA comparisons using HS as the covariate revealed a significant effect of head size on bite performance, and differences between habitats were still significant after accounting for variation in head size, and also differences between the sexes remained, at least in dunes (Table 3, Fig. 2).

Table 2 Results of ANOVA comparisons performed on each type of locomotor speed and bite force separately to examine the effect of habitat (HAB), population nested within habitat (POP), sex, and their interaction in Bocage's wall lizard—*P. bocagei*

		SPR	MAN	CLI	Bite
HAB	*F*	0.689	1.773	9.783	6.49
	p	0.419	0.187	**0.003**	**0.018**
SEX	*F*	1.117	1.035	3.068	81.123
	p	0.304	0.291	0.07	**0.001**
POP	*F*	10.771	2.184	2.689	0.927
	p	**0.001**	0.127	0.071	0.381
HAB×SEX	*F*	2.562	1.131	4.485	0.113
	p	0.132	0.252	**0.03**	0.716
POP×SEX	*F*	1.451	0.216	1.987	0.791
	p	0.26	0.839	0.139	0.476

Significant effects are marked in bold. See "Material and methods" for variable abbreviations

F, F-statistic; *p*, corresponding *p* value

Two-block partial least-squares regression revealed a significant association between trunk and limb morphology and locomotor performance ($r = 0.305$ and $p = 0.001$), where higher speeds are associated with longer trunks and limbs and smaller head size (Fig. 3). However, after size-correcting all variables, the significant association between morphological traits and locomotor performance

Table 1 Results of ANCOVA comparisons performed on each linear measurement separately with habitat (HAB), population nested within habitat (POP), and sex as predictors, and SVL as a covariate in Bocage's wall lizard—*P. bocagei*

		HL	HW	HH	MO	FLL	HLL
SVL	*F*	536.69	954.88	447.83	1130.57	508.69	758.89
	p	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
HAB	*F*	0.43	0.61	7.94	0.2	0.31	0.05
	p	0.503	0.432	**0.008**	0.643	0.551	0.826
SEX	*F*	209.35	349.7	112.12	462.35	193.41	412.58
	p	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
SVL×HAB	*F*	0.3	0.42	0.01	4.66	0.36	0.42
	p	0.6	0.518	0.907	0.034	0.532	0.528
SVL×SEX	*F*	6.75	23.13	12.83	24.54	11.73	22.54
	p	**0.014**	**0.001**	**0.001**	**0.001**	**0.002**	**0.001**
HAB×SEX	*F*	2.3	0.001	0.71	0.44	0.01	1.73
	p	0.137	0.979	0.386	0.482	0.922	0.169
SVL×POP	*F*	1.28	6.02	11.23	0.63	0.7	0.79
	p	0.273	**0.007**	**0.001**	0.544	0.487	0.458
SVL×HAB×SEX	*F*	1.77	0.12	0.23	0.47	0.01	0.02
	p	0.197	0.733	0.656	0.505	0.919	0.894
SVL×POP×SEX	*F*	4.38	2.92	1.77	0.53	0.8	3.34
	p	**0.013**	0.058	0.128	0.6	0.471	**0.038**

Significant effects are marked in bold. See "Material and methods" for variable abbreviations

F, F-statistic; *p*, corresponding *p* value

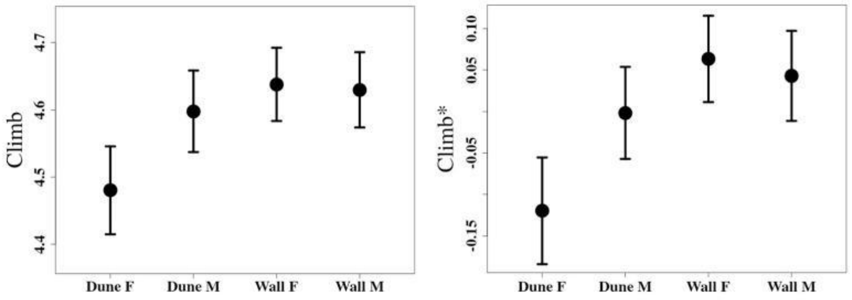

Fig. 1 Variation across groups of Bocage's wall lizard—*P. bocagei*—in climbing speed (left), and climbing speed corrected for SVL (right). Points represent means, and vertical bars denote 95% confidence intervals. Dune F, females from dunes; Dune M, males from dunes; Wall F, females from walls; and Wall M, males from walls

was lost ($r = 0.135$ and $p = 0.565$). Two-block partial least-squares regression also revealed a significant association between head morphology and bite performance ($r = 0.968$ and $p = 0.001$), where higher bite forces are associated with larger head dimensions (Fig. 4). This association remained significant after taking head size effects into account ($r = 0.365$ and $p = 0.001$), where relatively higher bite forces were associated with relatively wider and flatter heads, and with relatively longer jaws (Fig. 4).

Table 3 Results of ANCOVA comparisons performed on each type of locomotor speed and bite force performance separately with habitat (HAB), population nested within habitat (POP), and sex as predictors, and SVL (in the case of locomotion) or HS (in the case of bite) as a covariate (represented by X) in Bocage's wall lizard—*P. bocagei*

		SRP	MAN	CLI	Bite
X	*F*	14.911	2.308	8.908	2344.48
	p	**0.001**	0.124	**0.003**	**0.001**
HAB	*F*	3.182	2.969	16.134	3.867
	p	0.069	0.082	**0.001**	**0.048**
SEX	*F*	0.637	0.784	2.361	10.279
	p	0.414	0.368	0.118	**0.003**
X×HAB	*F*	1.939	0.328	0.25	0.572
	p	0.166	0.549	0.625	0.451
X×SEX	*F*	1.159	0.024	1.518	0.358
	p	0.259	0.882	0.23	0.548
HAB×SEX	*F*	2.828	1.343	5.085	0.686
	p	0.089	0.255	**0.024**	0.415
X×POP	*F*	9.409	1.814	2.348	1.085
	p	**0.001**	0.148	0.105	0.332
X×HAB×SEX	*F*	2.761	1.532	0.416	0.852
	p	0.123	0.234	0.508	0.37
X×POP×SEX	*F*	1.215	0.434	1.941	0.025
	p	0.305	0.606	0.132	0.974

Significant effects are marked in bold. See "Material and methods" for variable abbreviations

F, F-statistic; *p*, corresponding *p* value

Discussion

Our investigation of how functional performance mediates the evolutionary response of morphology to habitat revealed fast-evolving ecomorphological variation among populations of *Podarcis bocagei*, where a direct association existed between head morphology and bite performance. However, we could not establish links between limb morphology and locomotor performance at the individual level after correcting for size effects. Nevertheless, significant differences in climbing capacity existed between habitats, where individuals from walls were better climbers than those from dunes, suggesting a very fast evolutionary response of functional performance to habitat use, identifiable at the intraspecific level. Interestingly, a significant interaction between habitat and sex was detected in climbing performance. The same was the case for biting performance, although sexual differentiation was definitely the main factor driving variation in head functional morphology. Taking into account all the results, we found a complex interaction between natural and sexual selection on whole-organism performance, which are, in some cases, reflected on morphological variation. This complex interaction between different types of selective mechanisms results in context-dependent combined variation of morphological and performance traits, which sometimes confirms the ecomorphological paradigm and other times contradicts it.

Form-function associations

Indeed, under the ecomorphological paradigm, an association is predicted between morphology and performance as a response to different selective forces imposed by the environment, i.e., habitat structure in this study. However, here, we observe a complex pattern: we could detect an association between head morphology and bite force, but not between limb morphology and locomotor performance after correcting for size effects. Both pairs of functional morphology sets—head-biting and limbs-locomotion—are commonly associated as a response to environmental factors in lizard species (Losos and Sinervo 1989; Sinervo and Losos 1991; Melville and Swain 2000; Herrel et al. 2001b, Irschick et al. 2005b,

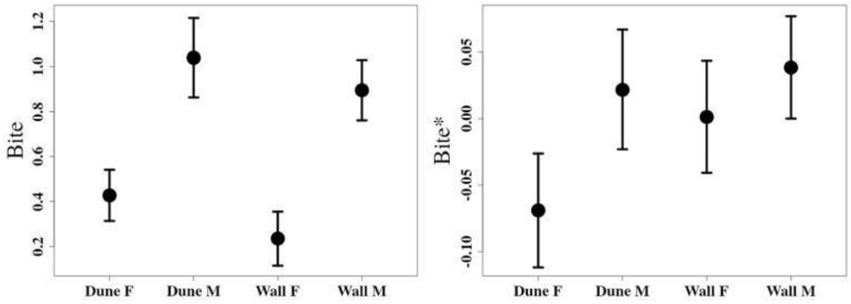

Fig. 2 Variation across groups of Bocage's wall lizard—*P. bocagei*—in bite force (left) and bite force corrected for HS (right). Points represent means, and vertical bars denote 95% confidence intervals. Dune F, females from dunes; Dune M, males from dunes; Wall F, females from walls; Wall M, males from walls

Kaliontzopoulou et al. 2013), but cases where this association was not found are also known (Vanhooydonck et al. 2000; Goodman et al. 2008), including several examples in *Podarcis* lizards (Van Damme et al. 1997; Vanhooydonck et al. 2000; Brecko et al. 2008; Kaliontzopoulou et al. 2012; Gomes et al. 2016). This lack of the typical functional morphological pattern in the locomotor system could be explained by factors other than limb length regulating locomotor performance, such as physiology, behavior, or biochemistry, or even by other morphological and locomotor traits not examined in this study being more relevant (Van Damme et al. 1997; Jayne and Irschick 1999; Braña, 2003; Revell et al. 2007; Vervust et al. 2007).

In the case of bite force, our results suggest an association with head morphology, which is largely driven by size effects and sexual size dimorphism (Fig. 4, left). However, after taking size effects into account, we see that head height (negative correlation) and head width and mouth opening (positive correlation) are the traits that may constrain bite force (Fig. 4, right). Following biomechanical rules and a common pattern in lizards and other organisms (Herrel et al. 2001a, b, 2005; Thomas et al. 2015), two-block partial least-squares regression suggests that higher bite forces are associated with relatively wider heads (Fig. 4). However, we also observed a negative correlation between head height and bite force, where individuals with relatively flatter heads had higher bite forces than those with relatively higher heads, a pattern that disagrees with predictions of biomechanical models of biting in lizards. It has been demonstrating that high bite forces are associated with wider and taller heads in several organisms (e.g., Herrel et al. 2001a, b, 2005; Thomas et al. 2015), indicating that external head measures are not good predictors of

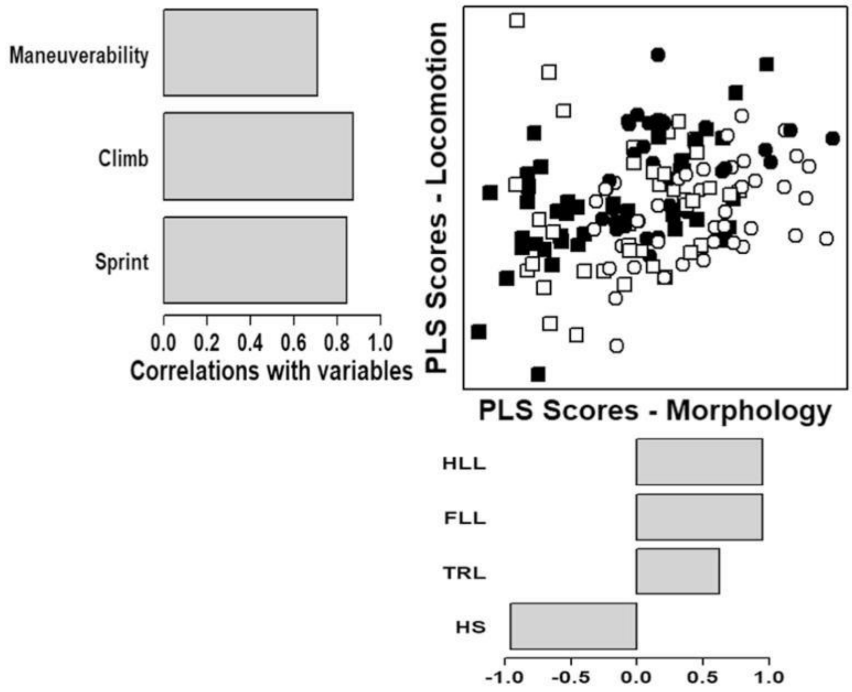

Fig. 3 Scatter-plot of individual Bocage's wall lizard—*P. bocagei*—scores of dune individuals (black squares, males; black circles, females) and wall individuals (white squares, males; white circles, females) obtained from partial least-squares (PLS) analysis between morphology (trunk length, TRL; forelimb length, FLL and hind limb length, HLL; and head size, HS) and locomotor performance (sprint, climb, maneuverability)—variables not corrected by size. Bar-plots next to morphology axis represent the correlations observed between that axis and locomotor performance

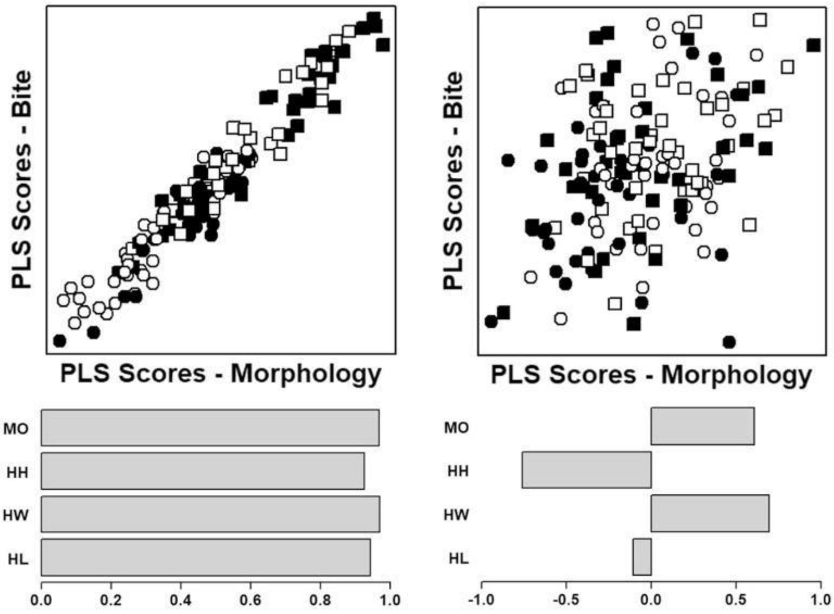

Fig. 4 Scatter-plot of individual Bocage's wall lizard—*P. bocagei*—scores of dune individuals (black squares, males; black circles, females) and wall individuals (white squares, males; white circles, females) obtained from partial least-squares (PLS) analysis between head morphology (head length, HL; head width, HW; head height, HH; mouth opening, MO) and bite force—left, variables not corrected by head size; right, variables corrected by head size. Bar-plots next to morphology axis represent the correlations observed between that axis and bite force

the pattern observed and other factors as internal muscle composition should be studied. Our results are in accordance with the known pattern of sexual dimorphism in *Podarcis bocagei* and other lizards, where males are larger in total body size, with relatively larger heads and more robust head shapes (Kaliontzopoulou et al. 2008), a pattern also known to translate into higher bite forces (Herrel et al. 2001a, b; Kaliontzopoulou et al. 2012). Higher bite performance in males is known to be associated with male dominance (Herrel et al. 1999; Husak et al. 2006; Huyghe et al. 2009), an advantage in antagonistic behavior between males for territory defense and/or mate acquisition and/or an advantage during copulation (Lappin and Husak 2005). The morphology-performance axis aligns with sexual differentiation (Fig. 4), pointing to sexual selection as a major force driving the joint phenotypic differentiation of head morphology and function; however, natural selection related to habitat type also seems to play a role in shaping whole-organism performance.

Functional morphological responses to habitat variation

The investigation of performance across habitats revealed that lizards from dunes bite harder than those from walls (Table 2, Fig. 2), as is expected given the larger body size observed in dune populations, and provided that animals with larger heads bite harder (Herrel et al. 2001a, b). Interestingly, however, when body size effects were taken into account, we found that animals from walls bite harder relative to their head size than those from dunes (Table 3, Fig. 2). This result indicates that the small head size observed in walls does not have repercussions on bite performance. Thereby, the potential physical constraints imposed by saxicolous habits—i.e., smaller heads being favorable in rocky environments to facilitate the use of small holes and rock crevices for refuge (Vitt et al. 1997; Revell et al. 2007)—do not influence bite force performance. A potential explanation for this pattern is different osteology and/or different jaw muscle insertion, orientation, or even length across habitats (Herrel et al. 1996, 1998, 2001b; Lappin et al. 2006; Huyghe et al. 2009). Indeed, this is a very feasible explanation, which merits further attention in future studies, as the feeding apparatus is known to evolve very fast in wall lizards, exhibiting remarkable short-term responses to selection pressures (Herrel et al. 2008). In fact, this observation adds to the evidence suggesting that this flexibility in the relationship between head morphology and function to maintain biting performance is common in *Podarcis*, both within and across species (Herrel et al. 1996; Kaliontzopoulou et al. 2012). As such, a type of many-to-one mapping of morphology on function seems like a plausible mechanism for balancing the requirements posed by natural (habitat use) and sexual selection (Alfaro et al. 2005).

By contrast to this maintenance of biting functionality despite contrasting ecological demands across habitats, locomotor performance capacities seem to align more to expectations (Table 2, Table 3, Fig. 1), we found that animals from walls were faster climbers than those from dunes. Here, natural selection seems to act upon these animals increasing their ability to climb. In other words, lizards from agricultural walls adapt to their natural environment by increasing their climbing speed, which enhances their probability of survival, while escaping from predators in perpendicular surfaces. Several other studies have provided evidence of a clear and strong association between habitat use and performance capacities across species (e.g., *Anolis*: Losos 1990a, b; Irschick and Losos 1999; Elstrott and Irschick 2004, subfamily Lygosominae: Melville and Swain 2000; Goodman et al. 2008, or other systems: Vanhooydonck and Van Damme 2003; Vanhooydonck et al. 2005; Tulli et al. 2011). The results obtained here support the idea that this kind of association can evolve quite fast, being detectable at the microevolutionary level, i.e., among a set of geographically nearby, genetically quite uniform populations of the same species (Pinho et al. 2011). As such, the pattern of differentiation in climbing performance described here represents a very fast functional response to habitat type, occurring in a time frame of about 10,000 years (Pinho et al. 2011). Such a fast, fine-scale response could be facilitated by proximate mechanisms like phenotypic plasticity or differential growth in different habitats, as is known to occur in the locomotor apparatus of other lizard groups (Losos et al. 2000; Kolbe and Losos 2005). Given the high flexibility of ontogenetic trajectories observed across *Podarcis* wall lizards (e.g., Kaliontzopoulou et al. 2010b; Piras et al. 2011), this seems like a reasonable hypothesis, which would need to be further explored experimentally in future studies.

Despite this positive, short-frame association between habitat use and locomotor performance, we also found some unexpected results. Specifically, taking into account several interspecific studies that showed an association between morphology, performance, and habitat use to understand better how natural selection shape whole-organism (e.g., Losos 1990a, b; Irschick and Losos 1999: Melville and Swain 2000; Vanhooydonck and Van Damme 2003; Elstrott and Irschick 2004; Vanhooydonck et al. 2005; Goodman et al. 2008; Tulli et al. 2011), we had predicted that lizards from dunes would be better sprinters than those from walls. However, a differentiation between the two types of habitats in sprint speed was not identifiable in our data. This reduced functional differentiation between habitat types in locomotor performance, which is the “classical” trait expected to vary due to its direct connection to structural habitat, could be a matter of scale, as at the sampled populations are geographically very close, allowing extensive gene flow between them, and they are known to share the most important part of their evolutionary background, being genetically quite uniform (Pinho et al. 2011).

Alternatively, individuals from walls may adapt to moving on inclined surfaces by increasing their climbing capacity, but at the same time maintain their ability to sprint in horizontal surfaces, which is still beneficial given the generally ground-dwelling habits of this species. Indeed, horizontal sprinting is associated with escape from predators (Husak and Fox 2006; Miles 2004) and it should be still under selection in environments where agriculture walls are predominant. In fact, lizards in such environments also escape from predators by sprinting horizontally on top of the walls or by jumping to the ground and sprinting to hide within the adjacent vegetation cover (personal observation). Indeed, *P. bocagei* is quite generalistic in its ecological habits (i.e., sensu Kaliontzopoulou et al. 2015) and maintain a high degree of flexibility in terms of habitat preference. Despite the marked contrast of habitat structure in the localities of origin of the studied populations, individuals did not exhibit differences in the main aspects of microhabitat choice under experimental conditions (Supp. Inf.). This observation suggests that, although morphology and functional performance vary across populations, a global flexibility is also maintained, reinforcing the possibility of an important role for phenotypic plasticity in mediating the observed patterns. Particularly in view of the lack of an association between locomotor performance and morphology observed here, it seems that climbing performance can be enhanced in saxicolous populations through mechanisms (e.g., physiology: Braña, 2003; behavior: Van Damme et al. 1997) which do not influence the capacity of the lizards to also maintain their sprinting performance. In fact, this lack of a trade-off between sprinting and climbing seems to be a recurrent pattern, which occurs at several evolutionary levels in lacertids, including populations of the same species (i.e., results presented here), lineages of the same species complex (Van Damme et al. 1997), and deeply differentiated species at the family level (Vanhooydonck and Van Damme 2001).

Interactions between natural and sexual selection

We have seen how sexual and natural selection shape functional and morphological diversity separately. However, the balance between both selective forces is complex and in this study result in differences in the degree of functional sexual dimorphism between habitats (Table 1, Table 2, and Table 3). Our results on climbing performance showed that individuals from dune habitats were more dimorphic than those inhabiting agriculture walls (Fig. 1). This is in accordance with the variation in the degree of morphological sexual dimorphism between different habitats previously reported for *P. bocagei* (Kaliontzopoulou et al. 2010a). While the same morphological pattern was not detected here, most probably due to the lower number of populations and individuals examined, we did identify differences between habitats in the degree of sexual differentiation in climbing capacity. The fact that individuals of the two sexes living in walls had similar climbing performance could be explained by the necessity of both sexes to escape from predators in inclined surfaces. By contrast, individuals from dunes (ground-dwelling) have less of a selective pressure in this direction, and exhibit a more pronounced difference between sexes in their climbing capacities, possibly associated to behavioral differences related to their social roles. Here, males, which normally have larger home ranges to patrol (Stamps 1983; Perry and Garland 2002; Diego-Rasilla and Perez-Mellado 2003), and move more extensively in search of mating opportunities, may benefit more by enhanced climbing capacities, while females seem more limited in this respect (Fig. 1). Nevertheless, such a hypothesis should be further investigated in the future.

Put together, the results obtained here highlight the potential for remarkable flexibility in morphology-function associations, and their potential for responding to environmental variation in very short evolutionary time intervals. This means that establishing whether morphological variation is associated with habitat use through influences on whole-organism performance, as dictated by the ecomorphological paradigm, is not necessarily straightforward, even using data on individuals at the intraspecific level. Interestingly, both the head-bite and limbs-locomotion functional systems seem to be quite flexible in wall lizards, but in different directions. In one case—bite performance—the same whole-organism performance capacities can be maintained despite varying external morphological properties. In the second case—locomotor performance—different performance capacities are achieved by individuals with similar morphological properties. This suggests that these lizards exhibit a particularly high complexity of the morphology-performance association, through the interference of other regulatory mechanisms such as muscle orientation, physiology, behavior, etc. Such complexity may uncouple performance and morphology and provide an explanation for success of this species in the ecosystems of NW Iberia, and also explain their potential for exhibiting extreme levels of intraspecific morphological variability. In this sense, morphology does not seem to be canalized through its integration with functional performance (sensu Klingenberg 2014), and as functionality can be maintained through other properties, external morphology is relatively free to vary. Further studies investigating the ecological significance of performance are required to fully understand how, and at which hierarchical level, these organisms' phenotypic traits respond to environmental variation. For this, investigating how lizards implement their maximal performance to perform different ecological and social tasks is important to understand their true evolutionary potential (Irschick et al. 2005c; Husak 2006).

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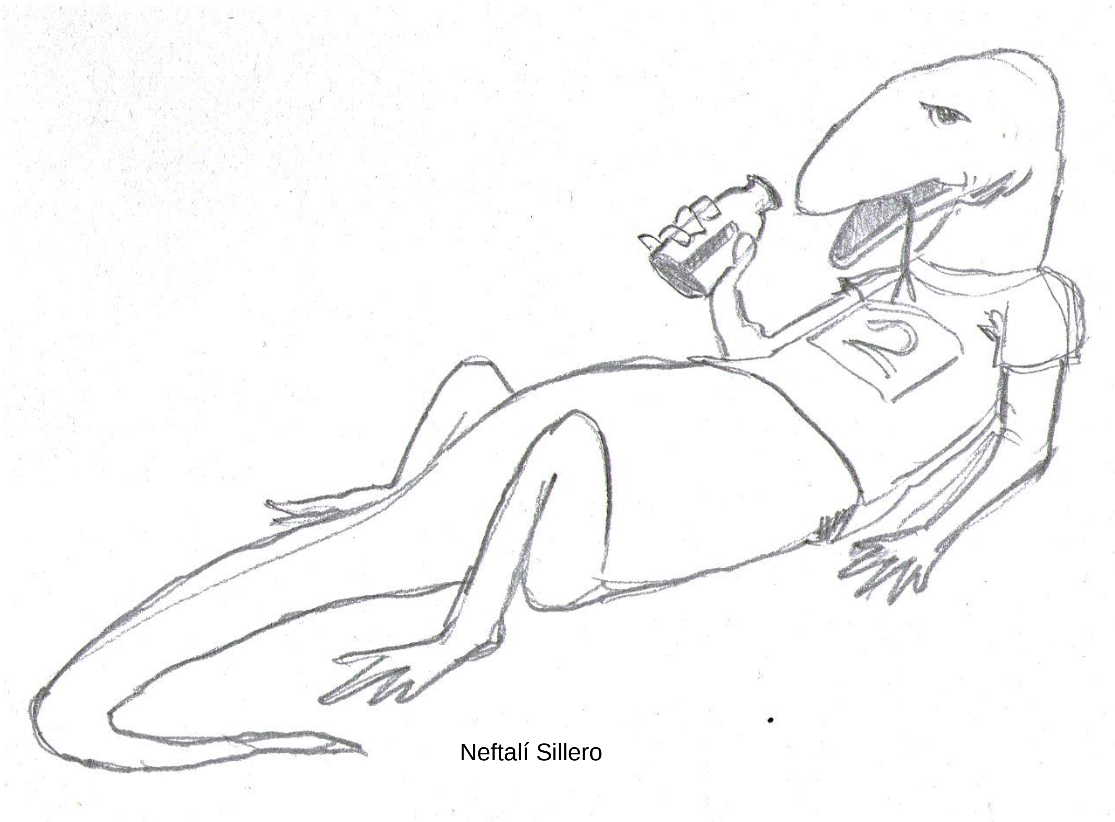

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