JOURNAL OF Evolutionary Biology

doi:10.1111/j.1420-9101.2010.01978.x

Multivariate phenotypes and the potential for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral colour morphs

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Keywords:

alternative strategies;
colour signals;
fitness optima;
polymorphism;
selection.

Abstract

A major goal in evolutionary biology is to determine how phenotypic variation arises and is maintained in natural populations. Recent studies examining the morphological, physiological and behavioural differences among discrete colour morphotypes (morphs) have revealed several mechanisms that maintain discrete variation within populations, including frequency-dependence, density-dependence and correlational selection. For example, trade-offs over resource allocation to morphological, physiological and behavioural traits can drive correlational selection for morph-specific phenotypic optima. Here, we describe a ventral colour polymorphism in the wall lizard (*Podarcis muralis*) and test the hypothesis that morphs differ along multivariate axes defined by trade-offs in morphological, physiological, and immunological traits. We show that ventral colour is a discrete trait and that morphs differ in body size, prevalence of infection by parasites and infection intensity. We also find that morphs differ along multivariate phenotypic axes and experience different multivariate selection pressures. Our results suggest that multivariate selection pressures may favour alternative optimal morph-specific phenotypes in *P. muralis*.

Introduction

A central challenge in evolutionary biology is determining how phenotypic variation is preserved in natural populations despite persistent selection (Bull, 1987; Houle, 1992; Rowe & Houle, 1996). Selection acts directly on phenotypic variation via individual interactions with the environment and with members of the same or different species (e.g. social selection; Sinervo *et al.*, 2001). The complexity of these interactions makes it difficult to isolate mechanisms that maintain phenotypic variation, and as such, much of our current understanding comes from studies investigating the underlying function of discrete phenotypic polymorphisms that are easily tractable in the wild (Brodie, 1992; Pryke & Griffith, 2006).

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Elaborate colour signals are common in many species of vertebrates (Seehausen & van Alphen, 1998; Roulin, 2004) and invertebrates (Sandoval & Nosil, 2005; Svensson *et al.*, 2005) and represent some of the most striking and well-documented examples of discrete phenotypic variation. Discrete colour signals can have a variety of functions. They may advertise social status (Hover, 1985), mate compatibility (Pryke & Griffith, 2009) or alternative life history or behavioural strategies (Lepetz *et al.*, 2009). Often, the functional significance of a colour polymorphism is linked to the very mechanisms that maintain it. For example, in morphs of the lek-breeding ruff (*Philomachus pugnax*), colour morphs exhibit alternative mating strategies that drive negative frequency–dependent selection. Negative frequency–dependence is believed to maintain multiple morphs within a population (Widemo, 1998).

More generally, alternative reproductive, anti-predator or resource-holding strategies among morphs drive the evolution of morph-specific optimal trait combinations (multiple fitness optima) that represent alternative

solutions to the problem of optimizing fitness in the face of tradeoffs (Whitlock *et al.*, 1995; Sinervo, 2000; Roulin, 2004). That is, when limited resources prevent individuals from investing maximally in all morphological, physiological and life history traits, individuals must allocate resources among traits to optimize reproduction and survival in light of trade-offs (Roulin, 2004). Correlational selection on colour signals together with morphological, physiological and life history traits may lead to alternative, morph-specific solutions to the problem of trade-offs, resulting in differences among morphs in traits like clutch size, immune function and behaviour (Sinervo & Svensson, 2002). Understanding the phenotypic syndromes associated with discrete polymorphism is the first step to understand the maintenance of multiple morphs within a population.

Lizards provide excellent systems to explore the evolution and maintenance of colour signals, as discrete colour polymorphisms have been described in multiple species, where two or more discrete colour morphs co-exist within a population (Sinervo & Lively, 1996; Vercken *et al.*, 2007). Alternative fitness optima among morphs may be driven by trade-offs that exist between the fitness benefits and energetic costs of allocating resources to traits that optimize reproduction, performance and defences against predators and parasites. For example, trade-offs may exist between the allocation of resources to performance (e.g. stamina) and parasite avoidance (e.g. immunocompetence). Physiological performance (e.g. stamina) may contribute to the ability to defend territories, acquire mates and escape predators in lizards (Garland *et al.*, 1990). Complex immune responses by hosts are costly (Demas *et al.*, 1996; Raberg *et al.*, 2002; Hanssen *et al.*, 2004), and thus immunocompetence may represent a potential trade-off with performance in resource allocation. Similar trade-offs have been shown to be important factors affecting the life histories of other lizard species with alternative morphs (Vercken *et al.*, 2010), and understanding differences among colour morphs in morphology, physiology and survival has proven central to identifying the ecological significance and maintenance of colour morphs.

Here, we report on a discrete ventral colour polymorphism in the common wall lizard (*Podarcis muralis*). Populations of *P. muralis* from Southern France are polymorphic in ventral colour (orange, yellow, white and bicolour morphs that have combinations of ventral scales of two colours; Fig. 1). We tested the hypothesis that selection acts differentially among *P. muralis* colour morphs to create alternative, morph-specific phenotypic optima. We hypothesized that morphs differ in size, stamina, immune function, parasitemia and viability. We chose to study these traits because they are all important in either intraspecific interactions (i.e. mate acquisition or competition; (Robson & Miles, 2000), contribute to overall fitness (Svensson *et al.*, 2001) or have been identified by previous studies as involved in the maintenance of polymorphism (Sinervo & Lively, 1996). Multiple phenotypic optima among morphs are a potential indicator of alternative behaviour or reproductive strategies and may provide a mechanism to explain how multiple morphotypes can be maintained within populations of this species.

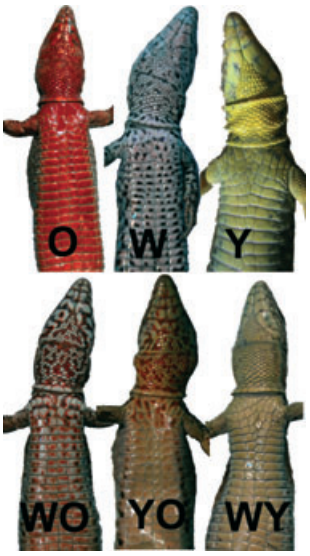

Fig. 1 *Podarcis muralis* ventral colour morphs. Upper row shows the three solid morphs (orange, white and yellow), and the lower row shows the three bicolour morphs consisting of alternating scales of two colours (orange-white, orange-yellow, and white-yellow).

Methods

Study system and design

Podarcis muralis is a small [snout-vent length (SVL) 48–67 mm] lacertid lizard common across central and southern Europe (Barbault & Mou, 1988). Males and females are commonly found in large aggregations on stone-walls, buildings and natural rock outcroppings, where males compete for access to females during the breeding season (Street, 1979). Females are polyandrous (Oppliger *et al.*, 2007), producing 1–3 clutches yearly during the April-July breeding season (Nembrini & Oppliger, 2003). From June 2007 to August 2008, we studied seven *P. muralis* populations (Moulis, Balague, Oust, Core, Aulus, Guzet, and Luzenac) in southern France along an elevation gradient from 430 to 1400 m. Lizards were captured by hand or using a silk noose attached to the end of a pole. Upon capture, each lizard was sexed; we recorded ventral colour, SVL (nearest mm), mass using a Pesola spring scale (to the nearest 0.1 g) and took a digital photograph to record ventral coloration. All individuals were brought into the laboratory, and running endurance (stamina) was estimated as

the time that lizards ran around a 1-m diameter racetrack while prompted by tapping with a soft paintbrush. Running lizards on a circular racetrack is a commonly used, repeatable technique for measuring stamina and comparable to measuring stamina on a treadmill (Garland, 1984; Clobert *et al.*, 2000). The end of each stamina trial was determined when the lizard could no longer right itself when placed on its back. All stamina trials were conducted on the same day as capture, and we standardized body temperature by placing individuals in an incubator at 32 °C for 5 min before trials. All lizards were returned to their initial point of capture within 24 h.

Colour morph stability

We measured between-year stability of ventral coloration for all six morphs by comparing the visual morph classification recorded during our survival study in 2007 with the visual morph classification recorded in 2008 ($N = 51$). If morph classifications differed between 2007 and 2008, we compared digital photographs of the individual between years to verify colour change. In addition, to estimate the repeatability of visual morph classifications, we presented 55 digital photographs of *P. muralis* ventral colours to nine untrained observers and one trained field assistant (independent scientist at field station that regularly aided in field work and was trained to identify morphs). Each participant recorded a morph category (orange, yellow, white, yellow-orange, white-orange or white-yellow) for each digital photograph. Repeatability of morph classification for each participant was recorded as the per cent of photographs correctly classified (i.e. correct if the participant identified the lizard as the same morph as recorded by B.C., who recorded morph scores for all individuals in both years).

Spectrophotometry of ventral colour

To determine whether our visual morph classifications were compatible with discrete categories discernable by vertebrate photoreceptor systems, we conducted spectrophotometry analysis (Ocean Optics USB 2000; Ocean Optics Inc., Dunedin, FL, USA) of ventral lizard coloration on 150 individuals from Core, Aulus, Guzet and Oust. Using COLOR PROJECT 1 software (developed by Jean-Marc Rossi, Laboratiore d'Ecologie, Université Pierre et Marie Curie, Paris; Vercken *et al.*, 2007) we calculated hue, chroma, brightness and two measurements (long-medium (LM) and medium-short (MS)) from Endler's segment classification system (Endler, 1990) for wavelengths corresponding to the visual spectrum (400–800 nm). This system uses the basic properties of vertebrate photoreceptor systems to identify visual signals 'seen' by vertebrates based on spectrophotometry analysis of hue, chroma and brightness (Endler, 1990). LM, as seen by humans, measures the distinction between red and green, and MS is the distinction between yellow and blue. Together, LM and MS approximate the colour signal received by the vertebrate visual system in the range of the visual spectrum.

Infection rate and infection intensity

Podarcis muralis lizards in Southern France serve as hosts for blood-borne haemogregarine parasites. Haemogregarines (Phylum *Apicomplexa*) are intracellular blood parasites transmitted by blood-feeding mite vectors and are commonly found in lizard, snake, and frog hosts (Sorci, 1996). Haemogregarines cause anaemia, decreased stamina and decreased tail regeneration capabilities in lizards (Oppliger & Clobert, 1997). We collected a blood sample from each individual's post-orbital sinus and made a blood smear using standard protocols (Schall, 1986). Smears were fixed in methanol for 1 min and stained with giemsa prior to storage. Under 100× magnification, infection intensity was measured as the number of haemogregarine parasites/1000 red blood cells and infection rate was scored as 0 (no parasites found during a 4 min scan of blood smear) or 1 (haemogregarine found during 4 min scan of blood smear).

Immunocompetence

In August 2007, we measured immune function for a subset of individuals from three populations (Core, Aulus and Oust, $N = 85$) in the laboratory. We measured humoral immunocompetence as the ability of an individual's immune system to mount a response (proliferate antibodies) when challenged with a novel antigen (*Tetanus toxoid*). Challenging the immune system with a novel antigen is a common technique used to measure humoral immunocompetence in reptiles and birds and provides a standardized measure of the immune system's ability to produce a specific antibody response against a novel antigen (Svensson *et al.*, 1998, 2001; Hasselquist *et al.*, 2001, 2007). We vaccinated free-ranging individuals in our study populations with 50 μL of *T. toxoid* vaccine (Colorado Serum Company) and boosted with an additional 50 μL after 14 days. Seven days after boosting, we collected blood from the post-orbital sinus using 100-μL heparinized capillary tubes. Blood samples were kept on ice until centrifugation (<8 h) to extract plasma and stored at −40 °C until further analysis. Humoral immunocompetence was measured as the relative concentration (in milli optical densities min^{-1}; mOD min^{-1}) of antibodies in plasma produced in response to vaccination with *T. toxoid*, i.e. we measured the secondary antibody response to the specific antigen. This would mimic a situation where the animal is re-exposed to a certain parasite. We used a standard ELISA, where the secondary antibody was a rabbit-anti-lizard immunoglobulin antibody, to measure the response to *T. toxoid*

antigen in the blood plasma (see Svensson *et al.*, 1998, 2001 for complete methods).

During July 2007, we also measured humoral immunocompetence in one natural population (Luzenac). Upon initial capture, lizards were challenged with 50 μL of *Tetanus toxoid* and released. All individuals were permanently and uniquely marked for survival analyses (see Survival) and thus could be identified at subsequent recaptures. Lizards were recaptured a second time for boosting (mean = 6.6 days, SD = 2.2 days) and a third time (mean = 3.87 days after boosting, SD = 1.88 days) to collect a blood sample from the post-orbital sinus for the ELISA analysis (see Methods). Because the results of the immunization experiment differed significantly between the field and laboratory, we analysed all results from the Luzenac population separately from the remaining populations, in which a subset were measured for immune response in the laboratory (see Results).

Survival

We measured the proportion of individuals surviving from the 2007 to 2008 breeding seasons in population 'Luzenac', which was located on and around a stone church and cemetery enclosed by a stone wall. We chose this site for our viability study because it is surrounded on all four sides by unsuitable lizard habitat (busy highway/street on two sides and large agricultural field on two sides) existing effectively as an island of suitable lizard habitat. All individuals were permanently and uniquely marked with a combination of coloured elastomer dyes injected in the ventral forelimbs and hindlimbs (Nauwelaerts *et al.*, 2000). In May 2008, we recaptured all surviving individuals by conducting multiple daily walking censuses of the entire study site, capturing all lizards sighted. Most lizards were caught within 14 days, and censuses were continued for 60 days to maximize the probability that every lizard in the study population had been captured. Lizards marked in 2007 that were not recaptured in 2008 were considered to have died or permanently left the population. We also conducted exhaustive censuses of adjacent lizard populations within approximately 1 km of the study site to account for any potential dispersal off the study site. We did not find any marked lizards from our study population at another location.

Statistical analyses

Antibody titre scores were log transformed, and infection intensity (# parasites/1000 red blood cells) was log + 1 transformed prior to analysis. Spectral scores (LM and MS) were square-root transformed (after adding 0.02 to make all values nonnegative) prior to statistical analysis to obtain approximately normal distributions (Gotelli & Ellison, 2004). Individuals weighing <3.0 g were considered juveniles and were excluded from all analyses. We tested whether individuals were consistently categorized as the same morph between years using a chi-square test. To determine whether morphs had significantly different LM (distinction of wavelengths between red and green) and MS (distinction between red and blue) segment classification scores, we performed separate least-squares analyses of variance (ANOVA) with morph as the independent variable and LM and MS as dependent variables. We detected no significant effect of population on LM (ANOVA $F_{4,140} = 0.31$, $P = 0.87$) and MS (ANOVA $F_{4,140} = 0.71$, $P = 0.59$) scores, and thus population was not included as a covariate in this analysis. To reduce the dimensionality of LM and MS to one variable describing ventral colour, we performed a principal components analysis of LM and MS. We compared PC1 among morphs using an ANOVA, with morph as the independent variable and PC1 as the dependent variable, and applied a Tukey's HSD test to compare mean PC1 scores among morphs.

We tested for differences among morphs in SVL, stamina, humoral immune function, infection rate and infection intensity, by sex, population and morph (including two-way interaction terms) using ANOVA for continuous dependent variables and nominal logistic regression for nominal variables.

We tested for differences in survival (0 or 1) among morphs using a nominal logistic regression. We measured viability selection on stamina and infection intensity using a full factorial nominal logistic regression of survival on standardized trait values (mean = 0, standard deviation = 1), including morph and the interaction terms and including SVL as a covariate (Lande & Arnold, 1983; Phillips & Arnold, 1989). We excluded humoral immune function from the selection analysis because of the small number of individuals for which immune function was successfully measured in the field. In addition, white-yellow morphs were excluded from our selection analyses, owing to their low frequency in the wild ($N = 3$), to increase our statistical power when comparing selection among morphs. However, results of a model with white-yellow morphs included did not differ qualitatively from the results presented in the following text. To explore how selection was acting within each morph type, we conducted the above-mentioned selection analysis by morph and replaced survival (nominal variable) with relative fitness (continuous variable) to calculate selection gradients (Lande & Arnold, 1983). We report the results for the purpose of pattern interpretation only, as sample sizes by morph are not large enough to calculate accurate selection gradients.

To determine whether morphs differ in multivariate phenotypic space for trait combinations that are under differential selection by morph, we conducted a principal components analysis on the combined populations (Luzenac excluded) of the trait combinations found to be under differential selection by morph (stamina and

infection intensity) in the above-mentioned selection analyses, including SVL as a covariate. All statistical analyses were performed in JMP v 6.02.

Results

Classification and stability of colour morphs

Ventral colour was stable between years (χ^2, d.f. = 25, $P < 0.001$). Between-year comparisons of digital photographs showed that for two of the 51 individuals (~4%), there was a change in ventral colour (for one male from white to white-orange and for another male from yellow-orange to orange). In the repeatability of visual morph classification trial, untrained observers correctly classified (i.e. classified as the same morph as classified by B.C.) colour morph for 84% of digital photographs, and the trained observer correctly classified colour morph for 93% of the photographs.

Spectrophotometric analysis of ventral colour showed differences among morphs in colour classification variables LM (ANOVA $F_{5,144} = 41.15$, $P < 0.0001$) and MS (ANOVA, $F_{5,144} = 48.50$ $P < 0.0001$). Principal components analysis of LM and MS provided a first principal component that explained 93% of variance with large positive loadings on both LM (0.707) and MS (0.707). Morphs differed significantly in PC1 (ANOVA $F_{5,144} = 49.87$, $P < 0.0001$) where white, yellow and orange morphs all remained significantly different from each other in *post hoc* comparisons (Tukey-Cramer HSD, $q = 2.89$, $\alpha = 0.05$), and mean PC1 scores for bicolour morphs were intermediate between their respective solid colour classes (e.g. mean PC1 score for white-orange morphs was intermediate between the mean score for white and the mean score for orange; Fig. 2).

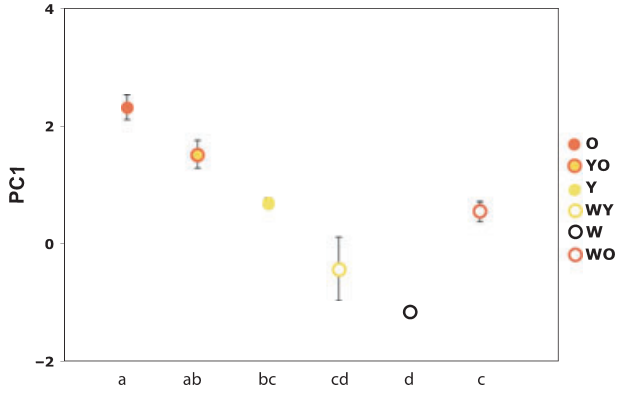

Fig. 2 Morph mean values for PC1, describing variance in ventral colour variables LM and MS for orange (O), yellow-orange (YO), yellow (Y), white-yellow (WY), white (W) morphs and white-orange (WO). Error bars represent ±one standard error. Morphs not connected by the same lowercase letter were significantly different in *post hoc* comparisons (Tukey's HSD, $\alpha = 0.05$).

Morphology and physiology of colour morphs

To determine whether morphs differed in morphological and physiological phenotypes, we compared SVL, stamina, humoral immune function, parasite prevalence and infection intensity of lizards by morph in the six combined populations (Moulis, Balague, Core, Oust, Aulus and Guzet; Fig. 3). There was a significant effect

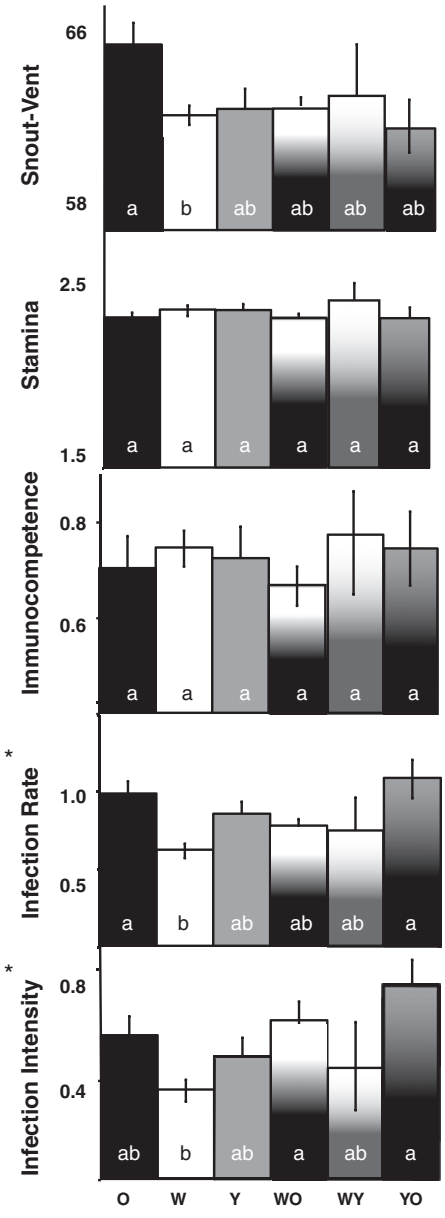

Fig. 3 Least squares mean snout-vent-length, stamina, immune function, infection rate and infection intensity of ventral colour morphs. Error bars represent ±one standard error. * Indicates that morphs mean trait values were significantly different ($P < 0.05$). Morphs not connected by a similar lowercase letter are significantly different in *post hoc* comparisons (Tukey's HSD, $\alpha = 0.05$).

of population on SVL (ANOVA $F_{5,317} = 21.58$, $P < 0.001$), stamina (ANOVA $F_{4,192} = 0.51$, $P = 0.0003$), infection rate (χ^2, d.f. = 5, $P = 0.003$) and infection intensity (ANOVA $F_{5,153} = 8.48$, $P < 0.0001$), and the following analyses include population designation as a fixed effect. In addition, infection rate and infection intensity were both significantly correlated with SVL (χ^2, d.f. = 5, $P = 0.0004$ and ANOVA $F_{1,163} = 9.01$, $P = 0.003$, respectively), and we included SVL as a covariate when comparing these traits among morphs. When considering variance in humoral immune function, neither the number of days to boosting, nor the number of days from boosting until bleeding affected immune response (ANOVA $F_{1,38} = 0.06$, $P = 0.80$, and ANOVA $F_{1,38} = 0.65$, $P = 0.42$). Mean deviation between repeated immune function measures was 6% of the mean value. Results of the immune challenge depended on whether tests were conducted in the laboratory or field (ANOVA $F_{1,129} = 6.09$, $P = 0.015$), and we analysed these two datasets separately (see Methods). However, there was no significant population effect in the laboratory challenge (ANOVA $F_{2,82} = 1.29$, $P = 0.28$). Because there was no effect of sex and no morph by sex interactions for any trait, we pooled the sexes in all analyses.

Variation in body size (SVL) was correlated with ventral colour morph, but this pattern was not statistically significant (ANOVA $F_{5,317} = 2.00$, $P = 0.08$). However, *post hoc* pair-wise comparisons revealed that orange morphs were significantly larger than white morphs (Tukey's HSD, $\alpha = 0.05$). We found no significant differences among morphs in stamina (ANOVA $F_{5,192} = 1.03$, $P = 0.40$; $N = 202$) or humoral immune response (ANOVA $F_{5,77} = 0.47$, $P = 0.79$).

The overall infection rate (±SE) by haemogregarine parasites was 81% (±0.03, $N = 181$). Colour morphs differed in infection rate (χ^2, d.f. = 5, $P < 0.0004$), and *post hoc* analysis (Tukey's HSD) revealed that this pattern was driven by the fact that orange and yellow-orange morphs had significantly higher prevalence of haemogregarines compared to white morphs. Morphs also had significantly different mean infection intensities (# parasites/1000 blood cells; ANOVA $F_{5,163} = 5.44$, $P = 0.0001$). *Post hoc* comparison showed yellow-orange and white-orange morphs had significantly higher infection intensities than white morphs. All significant differences described earlier remained significant after correcting α for multiple comparisons.

Survival

Colour morphs differed significantly in their probability of survival to our annual census (o<w<wo<y<yo<wy; χ^2, d.f. = 5, $P = 0.007$; $N = 124$). We detected no effect of sex, nor was there any significant morph by sex interaction. Our selection analysis revealed multiple differences in the form of selection acting among morphs (Table 1). Directional selection on stamina varied significantly by morph (χ^2, d.f. = 4, $P = 0.02$; $N = 117$). Quadratic selection on infection intensity (χ^2, d.f. = 4, $P = 0.003$; $N = 120$) and correlational selection on stamina and infection intensity (χ^2, d.f. = 4, $P = 0.043$; $N = 117$) were also significantly different as a function of morph. Selection gradients describing the form and intensity of selection were consistently different either in sign (±) or magnitude among morphs. For example, the selection gradient for correlated selection on stamina and infection intensity was negative in orange morphs ($\gamma = -1.04 \pm 2.08$) and positive in white ($\gamma = 0.42 \pm 0.32$; see Appendix 1 for full results).

Table 1 Results of the nominal logistic regression of survival on phenotypic traits with morph included as an interaction term. Directional selection on stamina, quadratic selection on infection intensity and correlational selection on stamina and infection intensity differed by morph.

Source	d.f.	χ^2	*P*-value
Snout-vent length	1	0.96739508	0.3253
Morph	4	4.03990362	0.4006
Stamina	1	9.76801e−7	0.9992
Stamina^2	1	1.16458e−6	0.9991
Infection intensity	1	0	1.0000
Infection intensity^2	1	1.90919e−5	0.9965
Infection intensity × stamina	1	4.05033e−6	0.9984
Stamina × morph	4	11.7999433	0.0189*
Stamina^2 × morph	4	8.73090804	0.0682
Infection intensity × morph	4	8.93365989	0.0628
Infection intensity^2 × morph	4	16.1765956	0.0028*
Infection intensity × stamina × morph	4	15.6841264	0.0035*

Finally, we compared morphs relative to major phenotypic axes for traits found to be under differential selection by morph mentioned earlier. The analysis revealed three principal components that explained 43%, 35% and 22% of observed variance, respectively (Table 2). Comparison of morphs along each principal component showed that morphs varied significantly with respect to PC1, a phenotypic axis contrasting values of SVL and infection intensity with stamina (ANOVA $F_{5,77} = 3.23$, $P = 0.01$). *Post hoc* comparisons show that orange individuals had a significantly higher mean value of PC1 than white individuals. Morphs also differed significantly along PC2, a phenotypic axis comprising positive values for SVL and stamina (ANOVA $F_{5,77} = 2.34$,

Table 2 Factor loadings on principal components of snout-vent length (SVL), stamina and infection intensity.

Factor loadings	PC1	PC2	PC3
SVL	0.502	0.686	−0.527
Stamina	−0.465	0.728	0.504
Infection intensity	0.729	−0.008	0.684
Per cent	43.29	34.69	22.02
Cum per cent	43.29	77.98	100.00

$P = 0.05$) and along PC3, which contrasts stamina and infection intensity with SVL (ANOVA $F_{5,77} = 5.30$, $P = 0.03$).

Discussion

We have provided evidence to support the hypothesis that alternative trait combinations in colour morphs of the common wall lizard represent alternative solutions to the problem of fitness optimization. Colour morphs differed in body size, parasite prevalance, infection intensity and differed slightly in running stamina and immune function. In addition, alternative combinations of these traits were favoured by natural selection in the different morphs. Our study illustrates the utility of studying discrete polymorphisms to understand the maintenance of variation within populations.

Colour morph classification and stability

Ventral colour in our study of common wall lizards was stable between years and differed (based on LM and MS scores) among the six colour morphs. Spectral analyses showed that the three pure morphs (orange, white and yellow) were readily distinguishable from one another by ventral colour, however not all bicolour morphs were distinguishable from morphs with which they share a colour (Fig. 2). This was likely because of constraints imposed by spectrophotometry methods. Because bicolour morphs have discrete scales of two colours, reflectance spectra showed either multiple peaks or large continuous peaks between the two colours. Thus, spectrophotometry analysis was unable to capture the discrete spatial structure of bicolour ventral scales (see also Vercken *et al.*, 2008). Our analysis identified two males that changed ventral colour (from white to white-orange and yellow-orange to solid orange) between years suggesting that rare colour changes may occur, and thus colour variation may be in part age or condition-dependent. However, individuals within other lizard species with genetically determined ventral colour polymorphism also exhibit rare colour changes (Sinervo *et al.*, 2000). Moreover, previous studies of *P. muralis* have also shown that ventral colour is stable throughout the lifetime of an individual (Cheylan, 1988; Sacchi *et al.*, 2007). Overall, the high repeatability of visual morph classification, significant differences in morph ventral coloration, and stability of ventral colour between years suggests that colour variation in *P. muralis* is discrete and may be mainly genetically determined.

Morphology, physiology and survival of colour morhs

We identified multiple differences among traits of *P. muralis* morphs that are commonly associated with alternative behavioural strategies, such as success in male–male competition (large body size; Fairbairn, 2007) and mate acquisition (increased stamina; (Klukowski *et al.*, 1998). We found that morphs differ in size (SVL), rate of infection with haemogregarine parasites, intensity of parasite infection (# parasites per 1000 red blood cells) and survival. Most observed differences among morphs in morphology and physiology were driven by orange or yellow-orange morphs having significantly different trait values compared to white individuals (Fig. 3). Orange morphs were larger and had higher parasite prevalence, higher infection intensities and tended to have lower humoral immune function, stamina and survival compared to white morphs. Yellow morphs had intermediate mean trait values compared to orange and white morphs. Our inability to discriminate between the mean trait values of all morphs in *post hoc* comparisons may be because of the fact that yellow, white-yellow and yellow-orange morphs were rare in all populations. We also found significant differences among morphs in multivariate phenotypic space. The major axis of phenotypic variation in *P. muralis* (PC1, Fig. 4) distinguished individuals of large body size, high infection intensity and low stamina (as in orange individuals) with individuals that have small body size, low infection intensity and high stamina (white individuals). Morphs differed significantly along this major axis of variation, with orange individuals having large PC1 scores and white individuals having small PC1 scores.

In addition to phenotypic differences, we found evidence for multiple different types of natural selection acting on morphs. Selection on stamina, infection intensity and on the two traits in combination all differed significantly by morph (Table 1). These results suggest that differences in multivariate phenotypes among morphs may be the result of morph-specific alternative phenotypic optima for these traits. Selection gradients estimated for these traits by morph showed consistent

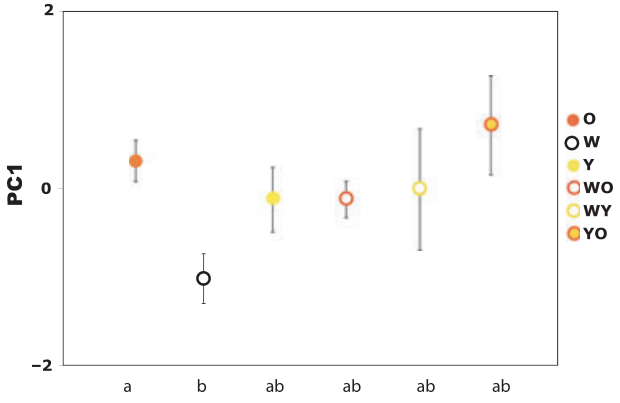

Fig. 4 Morphs varied significantly along PC1, which contrasts values of snout-vent length and parasite intensity with stamina (ANOVA $F_{5,57} = 3.24$, $P = 0.01$). Error bars represent ±one standard error. Morphs not connected by the same lowercase letter are significantly different in *post hoc* comparisons (Tukey's HSD, $\alpha = 0.05$).

differences in sign or magnitude. For example, selection gradients for directional selection on infection intensity and for correlated selection on infection intensity and stamina were opposite in sign in orange and white morphs. Selection favoured orange individuals with low infection intensity and high stamina, and white individuals with high infection intensity and high stamina. Thus, selection favoured negative trait correlations in orange individuals (i.e. high infection intensity and low stamina or low infection intensity and high stamina) and positive trait correlations in white individuals. This suggests that morphs may experience different trade-offs between values of stamina and infection intensity that optimize their survival.

The observed differences in selection among morphs are reflected in differences in morph multivariate phenotypes, where orange individuals have large values for PC1 (the negative correlation of stamina with SVL and parasitemia) and white individuals have small PC1 scores. Based on previous studies, it is likely that ventral colour in *P. muralis* is a signal of behavioural or life history strategy (Sinervo *et al.*, 2007), and results in alternative phenotypic optima among morphs. For example, large body size in orange individuals may provide an advantage when competing for preferred territories and could increase reproductive success in orange males. However, large body size is also correlated with high rates of infection with parasites and lower survival. Thus, smaller white males may invest more in immune function and longevity and obtain the same net lifetime reproductive success as orange males. We suggest that a fruitful next step would be to investigate the potential behavioural differences among morphs that may be correlated with the morphological and physiological differences illustrated here.

Acknowledgments

We thank Amandine Gasc for invaluable help in the field, Erik Svensson for access to the rabbit-anti-lizard antibody, J.J. Schall for assistance with field parasitemia methods, and N. Gotelli, R. Calsbeek and two anonymous reviewers for comments on an earlier draft of this manuscript. This research was supported by the CNRS, the Swedish Research Council (VR), the Swedish Research Council for Environment, Agricultural Science and Spatial Planning (Formas), the Gaige Fund Award from the American Society of Ichthyologists and Herpotologists, the Explorers Club Exploration Fund and a travel grant from the Society for Integrative and Comparative Biology.

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Appendix 1

Selection gradients (β) for stamina and infection intensity differed among ventral color morphs of lizards. Selection gradients differed in sign (±) or magnitude among morphs, however sample sizes for some individual morph classes (e.g. YO) were not large enough to accurately measure selection gradients.

Morph	Parameter	β	Standard error	P
Orange	Stamina	0.24	0.52	0.67
Orange	Stamina^2	0.28	0.39	0.50
Orange	Infection intensity	−0.02	0.59	0.97
Orange	Infection intensity^2	0.02	0.48	0.98
Orange	Stamina × Infection Intensity	0.26	0.98	0.80
White	Stamina	0.16	0.22	0.45
White	Stamina^2	−0.08	0.08	0.36
White	Infection intensity	0.11	0.19	0.55
White	Infection intensity^2	0.11	0.14	0.45
White	Stamina × infection intensity	0.20	0.16	0.21
White-Orange	Stamina	−1.05	0.79	0.22
White-Orange	Stamina^2	0.13	0.73	0.87
White-Orange	Infection intensity	−0.10	0.65	0.88
White-Orange	Infection intensity^2	−0.72	0.70	0.32
White-Orange	Stamina × infection intensity	−0.73	1.07	0.51

Appendix 1 (Continued).

Yellow	Stamina	2.81	1.29	0.12
Yellow	Stamina^2	1.47	1.26	0.33
Yellow	Infection intensity	−0.38	0.86	0.69
Yellow	Infection intensity^2	−0.51	1.30	0.72
Yellow	Stamina × infection intensity	4.82	2.25	0.12
Yellow-Orange	Stamina	−1.70	–	–
Yellow-Orange	Stamina^2	−14.79	–	–
Yellow-Orange	Infection intensity	0.29	–	–
Yellow-Orange	Infection intensity^2	−0.69	–	–
Yellow-Orange	Stamina × infection intensity	7.22	–	–

Received 12 February 2010; accepted 16 February 2010