



The effect of coloration and temperature on sprint performance in male and female wall lizards

SUSANNE R. K. ZAJITSCHKEK^{1,2*}, FELIX ZAJITSCHKEK^{1,3}, DONALD B. MILES^{1,4} and JEAN CLOBERT¹

¹Station d'Ecologie Expérimentale du CNRS a Moulis, USR 2936, 09200 Moulis,, France

²Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

³Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

⁴Department of Biological Sciences, Ohio University, 131 Life Sciences Building, Athens, OH 45701, USA

Received 19 February 2012; revised 1 May 2012; accepted for publication 2 May 2012

Phenotypic coloration in animals is often expected to have a signalling function, but it may also evolve as a correlated trait that reflects life-history strategy, social strategy, or ecological divergence. Wall lizards (*Podarcis muralis*) exhibit substantial colour variation, with both males and females being red, white, yellow, or a mixture of these colours. However, the biological significance of these colour morphs remains unknown. Here we investigate the relationship between coloration and temperature-dependent locomotor performance in an attempt to identify the adaptive significance of colour variation in this species. We investigate the maximum sprint speed of males and females of each of these colour morphs across seven different temperatures, using general additive mixed models (GAMMs). We predicted that the different sexes and colour phenotypes would exhibit differences in sprint speed performance, potentially indicating a correlation between coloration and adaptation into different ecological niches. We found no difference in performance of the discrete colour morphs, but amongst individuals that exhibited red coloration, those with a greater percentage of red were slower than those with less red coloration. This suggests a trade-off between red coloration and high sprint performance in this species. Furthermore, larger animals performed better, independent of colour and sex. Finally, we found no relative or absolute difference between males and females in their sprint performance. Taken together, our results suggest that there is no sex-specific or colour morph-specific differentiation in the use of microhabitats in this species. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **107**, 573–582.

ADDITIONAL KEYWORDS: colour morph – GAMM – general additive mixed model – Lacertidae – locomotor performance – *Podarcis muralis* – polymorphism – sex differences – TPC, temperature performance curve.

INTRODUCTION

Understanding how variation in coloration relates to whole-organism performance is key to understanding the evolution and maintenance of discrete colour morphs, as well as continuous colour variation, within species. Coloration and performance are often both

intimately linked to fitness. Colour morphs are often associated with traits that reflect different life-history strategies and adaptations to different habitats (e.g. Ahnesjö & Forsman, 2006; Unsicker *et al.*, 2008). These different strategies are characterized by the nature of the trade-offs that optimize evolutionary fitness of colour morphs in different ways, and may contribute to the maintenance of observed polymorphisms, via balancing (negative frequency dependence; Gray & McKinnon, 2007; Zajitschek & Brooks, 2008) or disruptive selection (Zajitschek &

*Corresponding author.
E-mail: susanne.zajitschek@cosmoid.com

Brooks, 2008). Colour traits that vary continuously, on the other hand, are often linked to individual quality, and may therefore vary in response to an individual's environment or its capacity to secure resources that are needed for the expression of the coloration. A well-known example of this is seen for carotenoid-based yellow, orange, or red coloration, which is costly to express and often signals individual quality (e.g. in guppies, Kodric-Brown, 1989; Locatello *et al.*, 2006; Gouldian finches, Pryke *et al.*, 2007).

The selection pressures that lead to variation in colour traits can also lead to differences in whole-animal performance traits, which are also closely linked to evolutionary fitness (Husak & Fox, 2008; Irschick *et al.*, 2008). This can be the case in species displaying discrete polymorphisms (Sinervo *et al.*, 2000; Huyghe *et al.*, 2007), as well as in those with continuous colour traits (Vanhooydonck *et al.*, 2005; Meyers *et al.*, 2006).

Ectotherms provide excellent systems for testing how performance varies between individuals of different coloration. In ectotherms, performance is tightly linked to ambient temperature, and this relationship can have far-reaching effects on individual fitness (Huey & Kingsolver, 1989; Angilletta, Niewiarowski & Navas, 2002; Martin & Huey, 2008). Basic thermoregulatory behaviour in ectotherms is necessary for any kind of activity, which can be achieved by up- or down-regulating temperature (i.e. behavioural thermoregulation) during the time of activity (Stevenson, 1985). Moreover, optimal temperatures for performance traits such as sprint speed, endurance, and bite force are expected to link to phenotype (e.g. colour variation), particularly if an individual phenotype reflects adaptation to different ecological niches. Expressing this from an adaptive viewpoint, species or phenotype-specific thermal preferences are expected to match temperatures that serve to maximize fitness (Angilletta *et al.*, 2002). However, the co-adaptation between thermal optima and accomplished thermal preferences seems to have evolved only partially in a wide range of species, not representing the hypothetical perfect match (Bennett, 1980; Huey & Bennett, 1987; Angilletta *et al.*, 2006; Martin & Huey, 2008).

In addition to absolute peak performance values, we can also study how body temperature affects the location of the performance peak and the shape of the temperature performance curve. In general, locomotor performance tends to increase steadily from the critical minimum temperature towards higher temperatures, up to an optimal temperature at which performance is maximal, and beyond which performance drops more rapidly as the temperature approaches lethal levels (Chen, Xu & Ji, 2003; Pinch &

Claussen, 2003; Kingsolver, Ragland & Shlichta, 2004; Lailvaux & Irschick, 2007). Although the location of the peak of a curve gives information about the temperature at which the peak maximum sprint speed is reached, and about the absolute value of sprint speed at that temperature, the shape of the curve and its overall location can vary as well. A biologically meaningful classification of performance curve variation considers the variation in peak height along the vertical performance axis (e.g. slower–faster), the variation in the location of the performance curve along the horizontal temperature axis (hotter–colder), and the shape of the performance curve. Narrower curves may be indicative of specialist strategies, whereas wider curves may reflect a generalist strategy (Huey & Kingsolver, 1989; Izem & Kingsolver, 2005).

To model and analyse thermal performance curves, we use additive models that are more flexible than globally fitted polynomials because they can model local changes in the mean response much better than the typically used lower order polynomials, without making assumptions about the shape of the curves. Despite a whole suite of other methods having been proposed for this task (Izem & Kingsolver, 2005; Angilletta, 2006), our approach, which has been widely used to model non-linear relationships in other research areas, including biomedical sciences, should add to the ability of researchers to model temperature performance curves and test for differences in their characteristics in a well-established, comparably easy-to-implement statistical framework.

Here we investigate the relationship between both coloration and sex with whole-organism performance (specifically, sprint speed) in the European wall lizard (*Podarcis muralis*). This species exhibits substantial variation in ventral coloration, with both discrete morphs (white, yellow, and red), as well as continuous variation in the form of discrete mixtures of these colours, such as red scales and yellow scales on the same animal (Sacchi *et al.*, 2007; Calsbeek, Haselquist & Clobert, 2010). Whereas the biochemical basis for red coloration is not known for wall lizards specifically, in reptiles more generally the expression of orange and red coloration is determined by the presence of both carotenoids and the pigment pterin (Steffen & McGraw, 2007). Traditionally, the coloration in *P. muralis* has been regarded as a discrete colour polymorphism (Calsbeek *et al.*, 2010; Galeotti *et al.*, 2010), as the classification into 'pure' colour morphs and 'mixed' colour morphs is easily achievable. The persistence of the colour over multiple seasons (Calsbeek *et al.*, 2010) suggests a genetic basis to the expression of this trait. However, the mixed colour morphs found in the Midi-Pyrénées region of southern France display immense, age-independent, variation in the relative composition

of their coloration. Here, a 'red–white' morph may exhibit a ventral coloration that is composed of 90% red pigments and 10% white, or along a continuous scale towards the other extreme, where a 'red–white' morph may be 90% white with only 10% red coloration. In addition, the frequencies of the colour morphs are extremely skewed, with most of the populations consisting of mainly white, red, and white–red individuals, with purely yellow individuals being extremely rare. Therefore, ecologically important information may be lost if this continuous spectrum in the mixed colour classes is entirely disregarded. This is particularly important, as to date we do not know whether ventral coloration in *P. muralis* is governed directly by genetic mechanisms or by condition dependence.

Little is known about the adaptive significance of colour variation in this species (but see Calsbeek *et al.*, 2010; Galeotti *et al.*, 2010), which occurs both in males and females, but may be sexually dimorphic in some populations (Martín *et al.*, 2008). However, there is some indication that red coloration in this species can have detrimental effects, as it has been found that red morphs have higher parasite loads and higher infection intensities, and lower immune function, endurance capacity, and survival, compared with white morphs (Calsbeek *et al.*, 2010). In another study, a complex interaction of body size and coloration was found, where larger red morphs were not as good at fending off parasites when under stress, compared with smaller red individuals (Galeotti *et al.*, 2010). Differing selection pressures might be driving optimal morph phenotypes, and colour morphs with different ventral coloration exhibit differently resolved trade-offs regarding performance and immunological traits (Calsbeek *et al.*, 2010). This was evident in the selection gradients for infection intensity, which showed positive trait correlations for white morphs, but negative trait correlations in red morphs; however, whether these diverging selection pressures translate into the partitioning of different thermal niches has not yet been investigated.

Here, we estimate temperature performance curves for the sprint performance of male and female wall lizards, examining the discrete colour morphs as well as the proportions of each coloration within the mixed colour morph classes, to test the following predictions.

1. Does coloration act as a signal of genetic quality or is it co-adapted to performance in this species – we expect individuals displaying different coloration to perform differently.
2. Is sprint speed performance under direct or indirect sexual selection in this species – we expect it to be higher in males than in females. This trait

could be mediated by differences in size, as in general larger lizards are better sprinters.

3. Is colour variation related to ecological strategies or differences in microhabitat use – we expect differences in the shape of temperature performance curves between colour morphs.

MATERIAL AND METHODS

STUDY SPECIES

The common wall lizard, *P. muralis*, is an abundant, small lacertid lizard species that occurs in central and southern Europe between about 40°N and 50°N. It is a ground-dwelling, active forager, and prefers environments that combine open surfaces with hiding spaces, which include man-made structures such as stone walls, old buildings, and graveyards.

Male and non-gravid female lizards were caught in March and April 2009 near Saint Giron (Ariège, Midi-Pyrénées, France). They were held in the laboratory with average room temperatures in the range of 20–25 °C with a 12-h light/12-h dark cycle. Individuals were kept in single terraria (35 × 18 × 22 cm) containing a mixed soil and gravel substrate as well as stones and halves of clay flower pots for spatial heterogeneity, and to provide hiding space and basking opportunities. Heat and light were provided using individual reflector lamps (Osram Concentra) and UV radiation (Sylvania Reptistar fluorescent lamps). Water was provided in small plastic Petri dishes and was refreshed daily. Lizards were fed either one cricket (*Acheta domesticus*) or one mealworm beetle larva (*Tenebrio molitor*) each per day. Animals were kept in the laboratory for 1 week to acclimatize before experiments started. After the experiments, animals were released back into their population of origin.

COLOUR MORPH CLASSIFICATION, COLORATION ESTIMATION, AND MORPHOMETRICS

In *P. muralis*, the throat and belly of males and females are coloured red (r), white (w), and yellow (y), or are a discrete mixture of two of these colours (rw, ry, yw; see Fig. 1). We classified each individual lizard into one of these colour groups (from here on referred to as colour morphs) by visual inspection with the use of a reference colour map. In addition, we estimated the percentages of the respective colours (herein referred to as percentage colour) to allow for the analysis of coloration on a continuous scale, and thereby examined the influence of coloration on temperature-dependent performance on a fine scale (Table 1). To do this the coloured ventral scales of each lizard were assessed and the relative proportions of the colours present were estimated. To ensure the

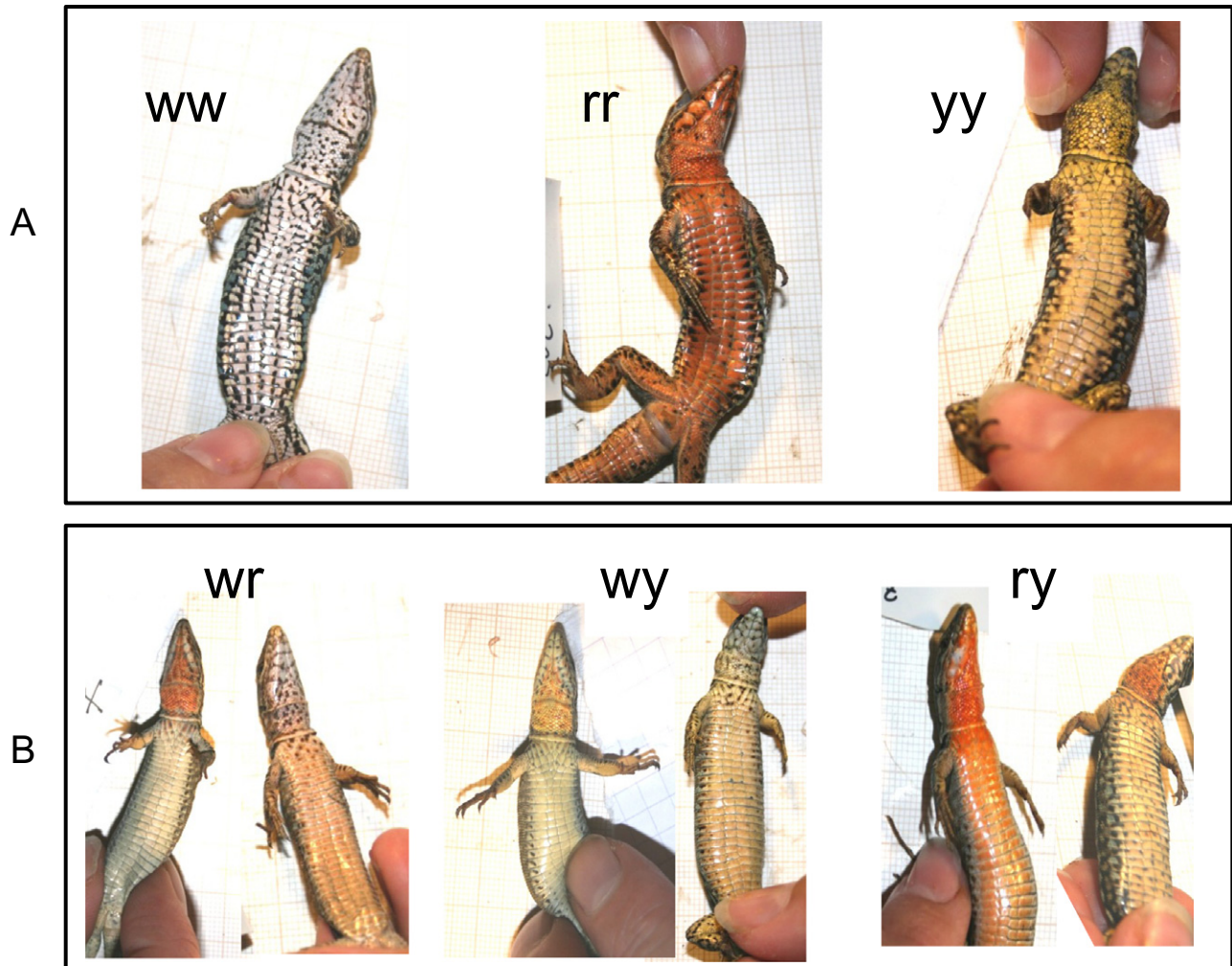


Figure 1. The different colour morphs of European wall lizards (*Podarcis muralis*): (A) pure colour morphs were white (ww), red (rr), or yellow(yy); (B) mixed colour morphs were white–red (wr), white–yellow (wy), or red–yellow (ry), with considerable variation in the relative proportions of the two colours present on each individual.

robustness of this measure, the first and second author independently classified and scored the colour percentages of each lizard and then compared scores. This measure was highly repeatable across experimenters, with scores never differing by greater than a 10% margin. In these cases the animal was inspected again and re-scored until a unanimous score was reached. We did not use spectrophotometric measurements of coloration in this study, as pure colour morphs in this species can be easily distinguished visually, and because visual classification of the morphs has been shown to be more reliable in this species than spectrophotometric analysis, when analysing mixed colour morphs (Calsbeek *et al.*, 2010). This is because mixed color morphs possess a mixture of discretely colored scales (e.g. red scales and white scales), rather than homogeneously coloured scales

of intermediate coloration (e.g. pink scales), and spectrophotometric measures are unable to capture colour variation with this spatial structure (Vercken, Sinervo & Clobert, 2008). The frequencies of the colour morphs found in the study area were highly skewed towards red, white, and red–white individuals, with yellow and yellow–white individuals being extremely rare (Table 1). This observation is typical for the study area (J. Clobert, unpubl. data), but in stark contrast to the colour frequencies of *P. muralis* populations studied elsewhere in Europe (e.g. Sacchi *et al.*, 2007).

Prior to temperature sprint speed trials, every animal was measured for size to the nearest 0.1 mm (snout–vent length, SVL) using digital callipers (Fisher Scientific), weighed to the nearest 0.001 g on an electronic balance (Denver Instrument, MXX-123),

Table 1. Sample size (*N*) for each morph category, and summary statistics for size and weight

Colour morphs	rr	rw	ww	yr	yw	yy	<i>N</i>	Overall mean \pm SD
<i>N</i> _{males}	6	20	20	2	0	1	49	
<i>N</i> _{females}	1	19	11	10	12	1	54	
<i>N</i> _{males + females}	7	39	31	12	12	2	103	
SVL _{males} (mm)	61.17 \pm 5.64	59.55 \pm 4.07	59.10 \pm 4.92	56.00 \pm 5.66	na (<i>N</i> = 0)	65 (<i>N</i> = 1)		59.53 \pm 4.66
SVL _{females} (mm)	58 (N = 1)	58.53 \pm 3.22	59.82 \pm 4.58	60.10 \pm 2.69	57.58 \pm 3.87	53 (<i>N</i> = 1)		58.76 \pm 3.65
Weight _{males} (g)	6.02 \pm 0.82	5.90 \pm 1.01	5.49 \pm 1.25	4.84 \pm 0.49	na (<i>N</i> = 0)	6.93 (<i>N</i> = 1)		5.73 \pm 1.10
Weight _{females} (g)	3.89 (<i>N</i> = 1)	4.32 \pm 0.43	4.66 \pm 0.76	4.28 \pm 0.62	4.45 \pm 0.28	3.92 (<i>N</i> = 1)		4.36 \pm 0.54

Values are given as means \pm standard deviations (SDs), if *n* > 1. Males were heavier than females, but there was no sex difference in SVL (Δ AIC_{weight} = 49.80; model including sex, AIC = 263.10; model excluding sex, AIC = 312.90; Δ AIC_{svl} = 1.10, model including sex, AIC = 589.95; model excluding sex, AIC = 588.85). When analysed separately in males and females, there was no substantial difference between colour morph class in body size (males, Δ AIC = 3.04; model including morph, AIC = 277.02; model excluding morph, AIC = 273.98; females, Δ AIC = 2.23; model including morph, AIC = 286.97; model excluding morph, AIC = 284.74), or weight (males, Δ AIC = 2.11; model including morph, AIC = 144.52; model excluding morph, AIC = 142.41; females, Δ AIC = 0.66; model including morph, AIC = 87.19; model excluding morph, AIC = 86.53). In order to accommodate the skewed frequencies of colour morphs found in the sampling region, and to resolve the effect of colour on a fine scale, the colour percentage classification resulted in 57 animals in the 'red' colour class (28 ♂, 29♀), 26 animals in the 'yellow' colour class (3 ♂, 23♀), and 82 animals in the 'white' colour class (40 ♂, 42♀).

and had its ventral side photographed with a digital camera (Canon, 400D) under standardized indoor ambient light conditions.

SPRINT SPEED MEASUREMENTS

We measured the sprint speed of male and non-gravid female lizards, in a randomly assigned order, at a fixed sequence of seven different body temperatures (18, 29, 37, 35, 25, 33, and 40 °C, in that order). Prior to each sprint speed trial lizards were held for 30 min at the trial temperature in a temperature chamber (Liebherr, ET 651-8) to allow them to acclimatize. In previous tests on other individuals from the same species, we found 30 min to be the period of time at which a lizard's body temperature equals the ambient temperature (S. Zajitschek, unpubl. data). Only one temperature was tested per day in order to allow the animals sufficient time to recover between trials.

To begin a trial, lizards were chased down a 2-m-long racetrack at maximum speed with the help of a soft brush. We used sandpaper as a substrate to provide adequate traction. Along the track, infrared photocell/receptor stations were fixed at 0.25-m intervals (Miles & Smith, 1987), and were connected to a portable computer. The time it took a lizard to pass an interval was recorded using custom-built software (TxTools 5.0.0). Every animal performed two runs at a given temperature. The single fastest velocity from the 16 data points attained per individual was selected as our estimate of maximum sprint speed (m s^{-1}) for each temperature. There is a possibility that our sampling protocol slightly underestimated the maximum sprint speed values for each individual. A recent simulation study (Adolph & Pickering, 2008) estimated the average downwards bias of maximum performance data taken from two trials to be 20%, compared with 11% from five trials. However, a consistent bias would not change our conclusions. In addition, the repeatability of maximum sprint speeds between the two test runs, calculated as the intraclass correlation coefficient (Sokal & Rohlf, 1994) between the maximum sprint speed of the first and second trial runs, across all experimental temperatures, was high (0.72; 95% confidence interval = 0.68–0.75; $F_{(720,721)} = 6.16$; $P < 0.0001$).

STATISTICAL ANALYSIS

As a result of the different sample sizes per colour morph in each sex, we tested for differences in size and weight by colour morph using sex-specific general linear models (GLMs), with the categorical variable 'colour morph' as a fixed effect. Models including 'colour morph' were compared with models without this variable, which had only the mean included as

a constant. For these and all further analyses, we excluded groups that had $n < 3$ (i.e. yy morphs in colour morph class analyses).

We used general additive mixed models (GAMMs) in the package MGCV 1.5–6, available within R (R Development Core Team, 2009), to estimate the shape of temperature performance curves (TPCs) and to test how factors and covariates influenced the shape and location of these curves. GAMMs are an ideal tool for such an analysis, as they are very flexible in modelling the shape of nonlinear relationships. Non-parametric smoothing functions are used on sections of the data, and these response curves are connected at their end points to generate an overall smooth curve (Wood, 2006). In addition to the non-parametric smoothing function, parametric fixed and random predictor terms can also be included, as in a conventional mixed-effects model. To visualize model predictions as response surfaces, we used the function `vis.gam` in package MGCV.

Using GAMMs to estimate TPCs allowed us to test for differences in TPCs along the maximum sprint speed axis (vertical shift), the temperature axis (horizontal shift), or for TPC shape differences. If a GAMM that includes separate smoothing functions of temperature for each group of interest (e.g. one for each sex) is supported most, this is evidence for a vertical or a horizontal shift in TPC, or both. The results can then be further described by comparing visualized response surfaces for the separate groups. If a model with a general temperature smoothing function and the group variable, fitted as a fixed effect, is the most supported model, this is evidence for a difference in maximum sprint speed (on the vertical axis) across all temperatures tested between the groups tested, and for a similar shape and horizontal location of the TPC of different groups. We built GAMMs using a Gaussian error distribution with an identity link function, and included an autoregressive moving average ARMA (1,1) correlation structure to account for temporal autocorrelation between speed measurements in each individual over all temperatures. The ARMA (1,1) structure explained the most variation arising from correlated within-individual measurements, out of other possible error correlation structures [`corARMA (0,1)`, `corARMA (1,0)`, `corAR (1)`, `corCAR (1)`, `corLin`, `corGaus`, `corExp`].

First, we tested for sex differences in TPC characteristics, independent of the colour of individuals. Then we proceeded to estimate the effect of ventral coloration on the shape and location of the TPC. To test for the effect of categorical colour morphs, we compared a model that included separate temperature smoothing functions for each colour morph against a model with a general temperature smoothing function and colour morph as a fixed effect.

The effect of percentage colour on maximum sprint speed was tested by performing a GAMM that included a general smoothing function for temperature. The percentage colour variable of one colour was included either as a third-degree polynomial in the fixed effects part of the model or as an additional smoothing function.

To test the importance of model terms, we always first defined a global model that included all meaningful model terms and then compared all possible candidate models that differed in model terms retained from the initial model. The null model, which is the least complex model tested, was either a model that only included SVL as a covariate or an intercept-only model, which indicates no effects of any explanatory variable tested. For model selection we used Akaike's information criterion (AIC), and a difference in AIC (Δ AIC) of ≥ 2 between two models was taken as evidence that the data provided better support for the model with the smaller AIC (Burnham & Anderson, 2002). Δ AIC is always reported as the AIC of the poorer model minus the AIC of the better model, i.e. it refers to the comparison of two candidate models, as explained in the text, and not to the difference in AIC between a candidate model and the overall best model. We confirmed the global goodness-of-fit of the best models using residual and predicted versus actual data plots (Bolker, 2008).

RESULTS

In all analyses we included SVL as a covariate and therefore tested effects on the body size-corrected maximum sprint speed (see Table 1 for details on morphometric measurements). Weight was not included, as it was highly correlated with SVL and its inclusion as a covariate did not improve the fit of the basic model with a general smoothing function of temperature (Δ AIC = 0.46).

THE ROLE OF COLORATION: MORPH DIFFERENCES IN TEMPERATURE-DEPENDENT SPRINT SPEED

To test for differences between all discrete colour morphs in the TPC of maximum sprint speed we first pooled the data from males and females, as we found no overall sex effect (Δ AIC = 20.15; model including sex, AIC = 2446.56; model excluding sex, AIC = 2426.41). We found no effect of colour morph (model without colour, AIC = 2365.93), either when modelled with colour morph-specific temperature smoothing functions (Δ AIC = 32.01, AIC = 2397.94) or when modelled with colour as a fixed effect (Δ AIC = 1.71; 2367.64).

Similar to the results using discrete colour morphs, the effect of percentage colour on TPC was not sex

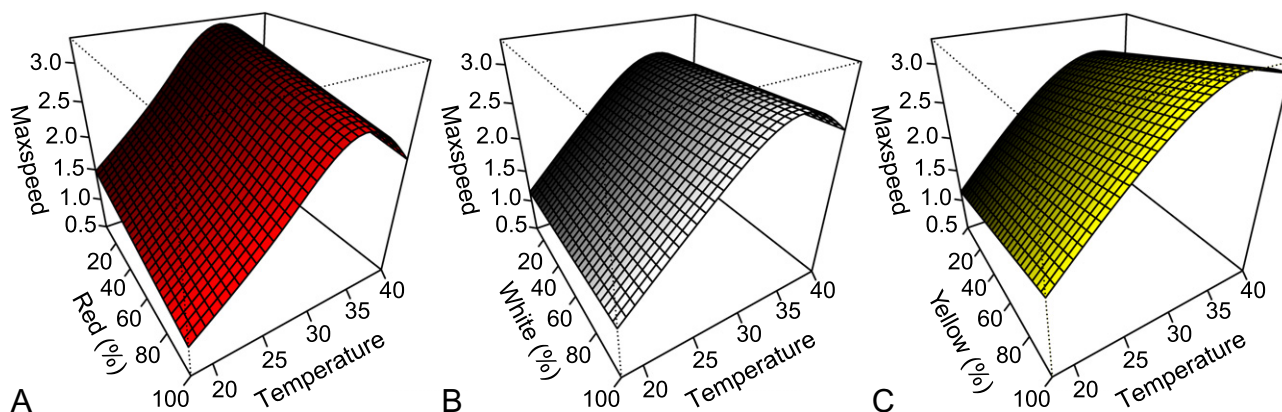


Figure 2. Temperature performance curve (TPC) surfaces showing the effect of percentage colour on maximum sprint speed (m s^{-1} , labelled 'Maxspeed'), across the temperature range ($^{\circ}\text{C}$). Please note that only the effect of percentage red had explanatory power for changes in sprint speed. Specifically, we found that animals that had higher percentages of red coloration displayed slower maximum sprint speeds (A). White coloration did not affect maximum sprint speed (B). Despite the apparent effect of yellow coloration on sprint speed (C), individuals bearing more yellow coloration were statistically not proven to be faster than those with less yellow colour. The differences in the curvature across temperatures between the different colours were also not statistically substantiated.

specific ($\Delta\text{AIC} = 0.97$; model including sex, $\text{AIC} = 2426.56$; model excluding sex, $\text{AIC} = 2425.59$). When sexes were analysed together, the percentage red had a substantial effect on TPC ($\Delta\text{AIC} = 4.76$, model including red, $\text{AIC} = 1392.77$; model without red, $\text{AIC} = 1397.53$), with individuals that exhibit more red having on average a lower maximum sprint speed (0.006 m s^{-1} per 1% colour change; Fig. 2a), whereas the percentage white as well as the percentage yellow did not have an effect on maximum sprint speed ($\Delta\text{AIC}_{\text{white}} = 1.87$, model including white, $\text{AIC} = 1903.97$; model without white, $\text{AIC} = 1902.10$; $\Delta\text{AIC}_{\text{yellow}} = 1.02$; model including yellow, $\text{AIC} = 660.01$; model without yellow, $\text{AIC} = 658.99$; Fig. 2b, c).

COLOUR-INDEPENDENT SEX DIFFERENCES IN SPRINT SPEED ACROSS TEMPERATURES

Male and female TPCs for maximum sprint speed, modelled using sex-specific smoothing functions, did not differ ($\Delta\text{AIC} = 20.15$; model including sex, $\text{AIC} = 2446.56$; model excluding sex, $\text{AIC} = 2426.41$). Furthermore, the exclusion of SVL from the sex-independent model resulted in a poorer model fit ($\Delta\text{AIC} = 2.50$; $\text{AIC} = 2428.91$), showing a substantial effect of SVL. In Figure 3 we therefore present a single temperature- and size-dependent response surface, including data from all individuals.

MAXIMUM SPRINT SPEED AND OPTIMAL SPRINT TEMPERATURES

The highest value of maximum sprint speed of 2.96 m s^{-1} was estimated at a temperature of $34.50 \text{ }^{\circ}\text{C}$ ($= T_{\text{opt}}$). This estimate is derived from the model that

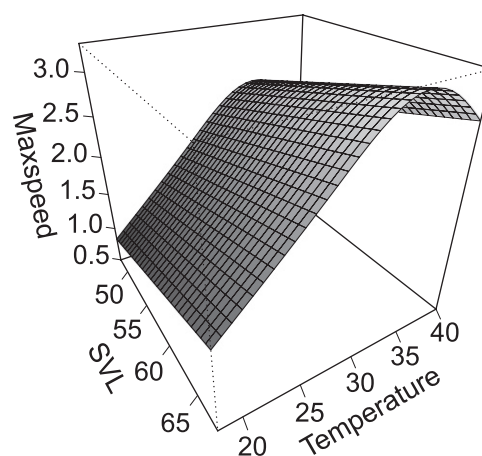


Figure 3. Response surface for maximum sprint speed (m s^{-1}), dependent on snout-vent length (SVL in mm), with data from both sexes combined.

fitted a general smooth function for maximum sprint speed values of all experimental animals. Split between the sexes, the optimal temperature for males was $T_{\text{opt}} = 33.05 \text{ }^{\circ}\text{C}$, with an estimated breadth of temperature range B_{95} (temperature range at which lizards can run at least at 95% of the highest value of maximum sprint speed; see Hertz, Huey & Nevo, 1983) between $29.23 \text{ }^{\circ}\text{C}$ and $37.85 \text{ }^{\circ}\text{C}$. In females, T_{opt} was estimated at $34.80 \text{ }^{\circ}\text{C}$, with a corresponding B_{95} ranging from $32.17 \text{ }^{\circ}\text{C}$ to $37.12 \text{ }^{\circ}\text{C}$.

DISCUSSION

We predicted that red coloration may influence performance in this species, as effects of red morphs

compared with different coloured animals have been observed in previous studies (Calsbeek *et al.*, 2010; Galeotti *et al.*, 2010). However, we did not find any effect of coloration examining the discrete colour morphs, but found that animals bearing relatively more red coloration performed worse compared with individuals with less red coloration, when investigating the relative importance of the proportion of any given colour. Second, we did not find the expected sex differences in sprint speed, as males were not faster than females, but the height of the TPC across all temperatures was positively dependent on body size (SVL), independent of sex. Third, there was no effect of sex either on the shape or location of the body size-corrected TPC, and therefore no sex effect on the optimum temperature at which maximum sprint speed peaked, nor on peak maximum sprint speed. Therefore, we found no evidence of ecological differentiation (according to Huey & Kingsolver, 1989; Izem & Kingsolver, 2005) between the morphs or the sexes.

In general, the TPC followed a distribution that is typical for ectothermic animals (see for example Huey & Kingsolver, 1989; Wilson, 2001; Angilletta *et al.*, 2002; Pinch & Claussen, 2003; Kingsolver *et al.*, 2004; Zhang & Ji, 2004; Lailvaux & Irschick, 2007; Ben-Ezra, Bulte & Blouin-Demers, 2008). Peak performances were reached at a T_{opt} of 34.5 °C, which also closely matches the selected temperature of 33.8 °C in the field for the species (Brana, 1991), and is very similar to the T_{opt} for sprint speed under laboratory conditions (35 °C; Bauwens *et al.*, 1995).

The finding that individuals with relatively more red coloration were slower than those with less red colour suggests a trade-off between sprint performance and the intensity of red coloration in *P. muralis*. This contradicts the hypothesis that red coloration could potentially reflect quality, and therefore indicate a superior performance ability in this species, and may instead indicate that the red coloration comes from the pigment pterin, which in contrast to carotenoid-based colour is endogenously produced (Steffen & McGraw, 2007), and is not necessarily a reliable signal for quality.

The different ventral colorations explained only a small degree of variation in performance, which was independent of the shape of the TPCs. No difference in maximum performance (independent of temperature) has been found among colour morphs in neighbouring populations of wall lizards (endurance; Calsbeek *et al.*, 2010), nor has a difference been found in a related species from Croatia (endurance and sprint speed; Huyghe *et al.*, 2007).

Nevertheless, colour may act as a signal of a certain strategy regarding the trade-off between performance and another fitness correlate: for example, immune function. In alternative colour morphs, a trade-off like

this might not be as important, or might be resolved differently. Indeed, a recent study on this species found that the different morphs may experience different selection pressures. In particular, in the white and red male morphs selection analyses revealed reverse effects of coloration on endurance and infection intensity (Calsbeek *et al.*, 2010), suggesting that these morphs may follow disparate life-history strategies, and resolve trade-offs differently. In addition, complex sex- and morph-dependent physiological stress responses were found in another study on *P. muralis*, with some evidence that larger red morphs were not as good in fending off parasites when under stress, compared with smaller red individuals (Galeotti *et al.*, 2010). No such relationship was found for white or yellow individuals (Galeotti *et al.*, 2010). The negative effect of red coloration may be correlative, and in fact be caused by lower immune function. However, the significance of the red coloration requires additional studies investigating additional performance traits, hormonal analyses, and fitness and survival data in this species. In addition, behavioural observations on aggressiveness and sexual preferences may help to shed light on the role that natural and sexual selection play for the maintenance of the ventral coloration in these lizards.

Independent of coloration, our study shows a positive effect of body size on sprint performance across temperatures: larger animals run faster. This result was not found to be sex dependent, as males and females did not differ substantially in body size (SVL). Limb morphology has also been found to not differ between the sexes in this species (S. Zajitschek, unpubl. data). This finding contrasts a study in a sister species, *Podarcis melisellensis*, where variation in size could not explain the variation in maximum sprint speed, despite clear sexual dimorphism (Brecko *et al.*, 2008). In many other lizard species, however, performance differences between the sexes can be explained by differences in size (Huyghe *et al.*, 2007; Lailvaux & Irschick, 2007).

In addition, we had expected to find differences in the shape of the performance curves between the sexes, and between individuals of different coloration. The absence of a significant effect of the interaction of sex, temperature, and colour may indicate that neither the sexes nor the different colour morphs have specialized into different ecological niches. For example, a narrow shape of the performance curve might have indicated a specialization (Huey & Kingsolver, 1989; Izem & Kingsolver, 2005) towards a narrow temperature spectrum, and potentially very strong preferences for basking spots accommodating these needs. A wide performance curve would have indicated a generalist approach, with individuals that may be able to deal with a wide range of tempera-

tures without major sprint speed drawbacks. From our findings, however, it seems that males and females do not differ substantially in the use of thermal microhabitats, despite the fact that the optimal temperature for maximum sprint performance was almost 2 °C higher for females than for males. To further investigate this non-trivial difference between the sexes, studies on basking behaviour and observational data on thermal habitat use would be valuable.

In conclusion, the weak association between coloration and sprint performance across the measured temperature range in this species indicates that the different phenotypes might not be largely caused by thermal differentiation, and do not necessarily constitute adaptations to selection arising from alternative biophysical microhabitats. The major causes for the maintenance of the ventral coloration in wall lizards remain to be resolved, but potential alternative explanations may include sexual selection pressures or the fact that the observed polymorphism may indeed be selectively neutral.

ACKNOWLEDGEMENTS

We thank Gerlinde Zajitschek for invaluable help during the experiment. Megan Head, Simon Lailvaux, Duncan Irschick, Michael Angilletta, Fred Janzen and three anonymous reviewers provided helpful comments on the article. Funding was provided by the ANR sixième extinction MOBIGEN and the European programme Biodiversa TENLAMAS. The work was performed in accordance with the French Animal Ethics charter.

REFERENCES

- Adolph SC, Pickering T. 2008.** Estimating maximum performance: effects of intraindividual variation. *Journal of Experimental Biology* **211**: 1336–1343.
- Ahnesjo J, Forsman A. 2006.** Differential habitat selection by pygmy grasshopper color morphs; interactive effects of temperature and predator avoidance. *Evolutionary Ecology* **20**: 235–257.
- Angilletta MJ. 2006.** Estimating and comparing thermal performance curves. *Journal of Thermal Biology* **31**: 541–545.
- Angilletta MJ, Bennett AF, Guderley H, Navas CA, Seebacher F, Wilson RS. 2006.** Coadaptation: a unifying principle in evolutionary thermal biology. *Physiological and Biochemical Zoology* **79**: 282–294.
- Angilletta MJ, Niewiarowski PH, Navas CA. 2002.** The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**: 249–268.
- Bauwens D, Garland T, Castilla AM, Vandamme R. 1995.** Evolution of sprint speed in lacertid lizards – morphological, physiological and behavioral covariation. *Evolution* **49**: 848–863.
- Ben-Ezra E, Bulte G, Blouin-Demers G. 2008.** Are locomotor performances coadapted to preferred basking temperature in the Northern Map Turtle (*Graptemys geographica*)? *Journal of Herpetology* **42**: 322–331.
- Bennett AF. 1980.** The thermal-dependence of lizard behavior. *Animal Behaviour* **28**: 752–762.
- Bolker B. 2008.** *Ecological models and data in R*. Princeton, NJ: Princeton University Press.
- Brana F. 1991.** Summer activity patterns and thermoregulation in the wall lizard, *Podarcis muralis*. *Herpetological Journal* **1**: 544–549.
- Brecko J, Huyghe K, Vanhooydonck B, Herrel A, Grbac I, Van Damme R. 2008.** Functional and ecological relevance of intraspecific variation in body size and shape in the lizard *Podarcis melisellensis* (Lacertidae). *Biological Journal of the Linnean Society* **94**: 251–264.
- Burnham KP, Anderson DR. 2002.** *Model selection and multimodel inference*, 2nd edn. New York, NY: Springer.
- Calsbeek B, Hasselquist D, Clobert J. 2010.** Multivariate phenotypes and the potential for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral colour morphs. *Journal of Evolutionary Biology* **23**: 1138–1147.
- Chen XJ, Xu XF, Ji X. 2003.** Influence of body temperature on food assimilation and locomotor performance in white-striped grass lizards, *Takydromus wolteri* (Lacertidae). *Journal of Thermal Biology* **28**: 385–391.
- Galeotti P, Pellitteri-Rosa D, Sacchi R, Gentili A, Pupin F, Rubolini D, Fasola M. 2010.** Sex-, morph- and size-specific susceptibility to stress measured by haematological variables in captive common wall lizard *Podarcis muralis*. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **157**: 354–363.
- Gray SM, McKinnon JS. 2007.** Linking color polymorphism maintenance and speciation. *Trends in Ecology & Evolution* **22**: 71–79.
- Hertz PE, Huey RB, Nevo E. 1983.** Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. *Evolution* **37**: 1075–1084.
- Huey RB, Bennett AF. 1987.** Phylogenetic studies of coadaptation – preferred temperatures versus optimal performance temperatures of lizards. *Evolution* **41**: 1098–1115.
- Huey RB, Kingsolver JG. 1989.** Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution* **4**: 131–135.
- Husak JF, Fox SF. 2008.** Sexual selection on locomotor performance. *Evolutionary Ecology Research* **10**: 213–228.
- Huyghe K, Vanhooydonck B, Herrev A, Tadic Z, Van Damme R. 2007.** Morphology, performance, behavior and ecology of three color morphs in males of the lizard *Podarcis melisellensis*. *Integrative and Comparative Biology* **47**: 211–220.
- Irschick DJ, Meyers JJ, Husak JF, Le Galliard JF. 2008.** How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evolutionary Ecology Research* **10**: 177–196.
- Izem R, Kingsolver JG. 2005.** Variation in continuous

- reaction norms: quantifying directions of biological interest. *American Naturalist* **166**: 277–289.
- Kingsolver JG, Ragland GJ, Shlichta JG. 2004.** Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* **58**: 1521–1529.
- Kodric-Brown A. 1989.** Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology and Sociobiology* **25**: 393–401.
- Lailvaux SP, Irschick DJ. 2007.** Effects of temperature and sex on jump performance and biomechanics in the lizard *Anolis carolinensis*. *Functional Ecology* **21**: 534–543.
- Locatello L, Rasotto MB, Evans J, Pilastro A. 2006.** Colourful male guppies produce faster and more viable sperm. *Journal of Evolutionary Biology* **19**: 1595–1602.
- Martin J, Amo L, López P. 2008.** Parasites and health affect multiple sexual signals in male common wall lizards, *Podarcis muralis*. *Naturwissenschaften* **95**: 293–300.
- Martin TL, Huey RB. 2008.** Why ‘Suboptimal’ is optimal: Jensen’s inequality and ectotherm thermal preferences. *American Naturalist* **171**: E102–E118.
- Meyers JJ, Irschick DJ, Vanhooydonck B, Herrel A. 2006.** Divergent roles for multiple sexual signals in a polygynous lizard. *Functional Ecology* **20**: 709–716.
- Miles DB, Smith RG. 1987.** A computerized racetrack for measuring sprint speed in cursorial animals. *Functional Ecology* **1**: 281–286.
- Pinch FC, Claussen DL. 2003.** Effects of temperature and slope on the sprint speed and stamina of the Eastern Fence Lizard, *Sceloporus undulatus*. *Journal of Herpetology* **37**: 671–679.
- Pryke SR, Astheimer LB, Buttemer WA, Griffith SC. 2007.** Frequency-dependent physiological trade-offs between competing colour morphs. *Biology Letters* **3**: 494–497.
- R Development Core Team. 2009.** *R: a Language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Sacchi R, Scali S, Pupin F, Gentilli A, Galeotti P, Fasola M. 2007.** Microgeographic variation of colour morph frequency and biometry of common wall lizards. *Journal of Zoology* **273**: 389–396.
- Sinervo B, Miles DB, Frankino WA, Klukowski M, DeNardo DF. 2000.** Testosterone, endurance, and darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior* **38**: 222–233.
- Sokal RR, Rohlf FJ. 1994.** *Biometry*. New York: Freeman.
- Steffen JE, McGraw KJ. 2007.** Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* **146**: 42–46.
- Stevenson RD. 1985.** The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *American Naturalist* **126**: 362–386.
- Unsicker SB, Kohler G, Linz J, Stein C, Weisser WW. 2008.** Colour morph related performance in the meadow grasshopper *Chorthippus parallelus* (Orthoptera, Acrididae). *Ecological Entomology* **33**: 631–637.
- Vanhooydonck B, Herrel AY, Damme RV, Irschick DJ. 2005.** Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? *Functional Ecology* **19**: 38–42.
- Vercken E, Sinervo B, Clobert J. 2008.** Colour variation in female common lizards: why we should speak of morphs, a reply to Cote *et al.* *Journal of Evolutionary Biology* **21**: 1160–1164.
- Wilson RS. 2001.** Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. *Journal of Experimental Biology* **204**: 4227–4236.
- Wood SN. 2006.** *Generalized additive models: an introduction with R*. Boca Raton, FL: Chapman and Hall/CRC..
- Zajitschek SRK, Brooks RC. 2008.** Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). *American Naturalist* **172**: 843–854.
- Zhang YP, Ji XA. 2004.** The thermal dependence of food assimilation and locomotor performance in southern grass lizards, *Takydromus sexlineatus* (Lacertidae). *Journal of Thermal Biology* **29**: 45–53.