Mechanisms of regulation and maintenance of color polymorphism in the common wall lizard (*Podarcis muralis*)

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Color polymorphism is widespread in animals, but mechanisms underlying morph evolution and maintenance are not yet completely understood. Among Reptiles, color polymorphism is quite common, particularly in Saurians, where it has often a genetic basis and is frequently associated with the evolution of alternative behavioural strategies (competitive and/or reproductive) related to social context. Both sexes of the common wall lizard (*Podarcis muralis*) show three pure and three intermediate color morphs on ventral scales. In this study, I looked for associations between physiological traits, female reproductive strategies and colour morphs by analysing haematological profiles and primary reproductive efforts in *P. muralis* females.

1 Introduction

### 1.1 Evolutionary significance of color polymorphism

Identifying the processes maintaining both phenotypic and genetic variability in wild populations is a major challenge in conservation and evolutionary biology, since phenotypic variation is the raw material upon which natural and sexual selection work. A hot topic in this field research is to understand the mechanisms that generate and maintain intra-specific color variation, i.e. color polymorphism (hereafter CP, [1]), and its functions. Often, the functional significance of CP is linked to the mechanism that maintains it [2]. However, discrete color signals as those found in polymorphic species may be non-adaptive correlates of some physiological or fitness character of selective value, or alternatively, they may accomplish such a wide range of different functions, e.g., conveying information on social and reproductive status or competitive ability of its bearer [3] or supporting ecological functions such as anti-predatory, thermoregulatory or habitat's use strategies [4,5]. Therefore, it is difficult to get back to the mechanisms producing and maintaining CP. Assuming an adaptive function of CP [6], the costs of producing alternative colorations may be very similar [7], and therefore, rather than to signal individual quality, discrete color morphs may advertise different life history and behavioural strategies, for instance, alternative reproductive strategies [8].

In lizards, individuals often occur in discrete, stable, genetically based colour morphs, which coexist at different relative frequencies within the same population [9,10]. Since CP is often expressed only ventrally in many species, rather than accomplishing anti-predatory or thermoregulatory functions, it may be associated with alternative behavioural (territorial/reproductive) strategies in males as well as in females, which clearly underlie different physiological profiles related to different genotypes.

1.2 Color polymorphism in the common wall lizard (*Podarcis muralis*)

The common wall lizard (*Podarcis muralis*), a small lizard (snout-vent length, SVL, 45–75 mm) widespread in southern and central Europe, exhibits in both sexes three discrete color morphs differing in throat and belly coloration (i.e. red-, yellow- and white-throated, [11]); in addition, three intermediate

phenotypes (white-yellow, white-red and yellow- red) regularly occur in both sexes, and therefore a maximum of six morphs are recognizable in this species (pers. obs., [12]; Fig. 1). Juveniles show monomorphic white ventral color at hatching, and throat and belly colorations start to develop at the time of sexual maturity (i.e., at the second year of life). Thereafter, individuals maintain the same color through all their life [13].
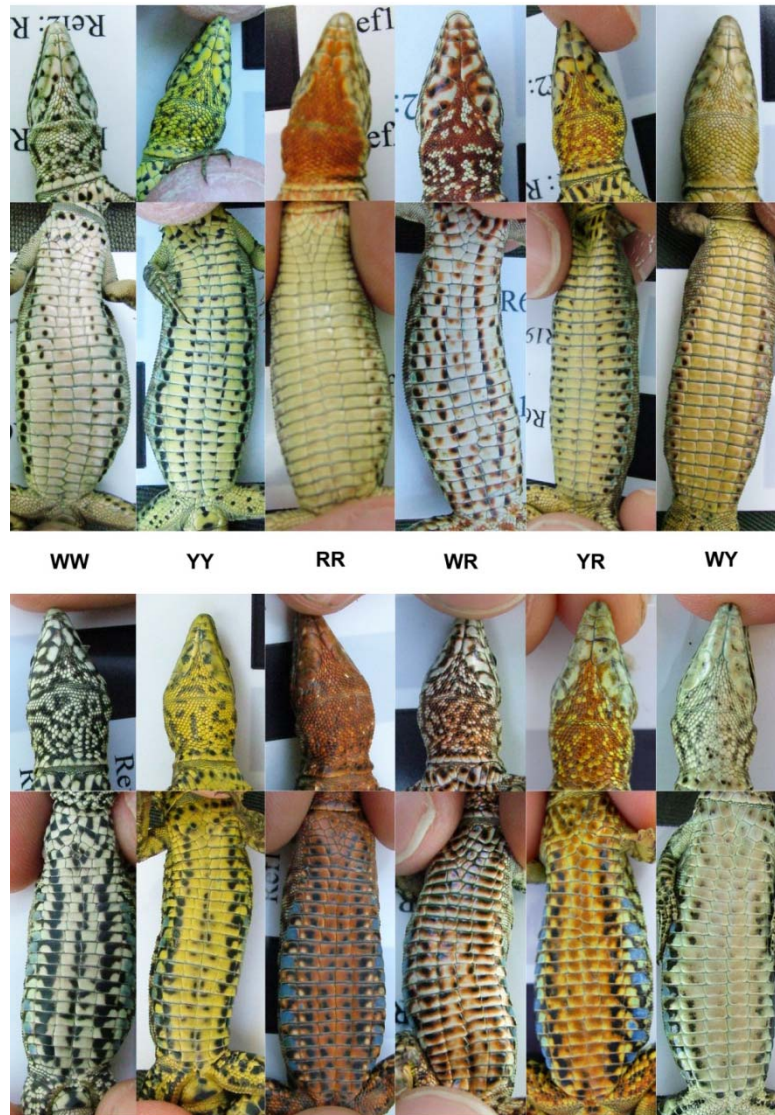
Fig. 1 Ventral CP in the common wall lizard (top row: females, bottom row: males). Discrete (WW: white, YY: yellow, RR: red) and intermediate morphs (WR: white-red, YR: yellow-red, WY: white-yellow).

To date, the adaptive function, of CP in this species is largely unknown, but as this color pattern is ventral, it is not expected to play any role in thermoregulation or in predation avoidance. Therefore, it is more likely to act as a social cue, signalling, e.g., different morphological, physiological and life-history

traits according to alternative behavioural strategies, upon which reciprocal mate selection could be based.

In this study, I looked first for the association between color morphs and alternative female breeding strategies under the hypothesis that CP may be (at least partially) genetically determined in the common wall lizard. Therefore, I looked for the occurrence of different life history traits of individuals, namely clutch and egg size, as occurs in highly genetically determined polymorphic species such as the side blotched lizard (*Uta stansburiana*) and the common lizard (*Lacerta vivipara*) [14,15]. Second, I aimed at identifying covariations between color morphs and physiological traits, determining the haematological profiles of color morphs in order to test for differences among morphs in body condition and blood parameters upon capture.

2 Material and methods

2.1 Sampling and Housing

In spring 2008, 2009, 2010 (February to March), I captured by noosing (i.e., using a slip knot of sewing thread attached to the end of a 2-m pole, [16]) sexually mature lizards (SVL >50 mm) in 11 sites in Pavia town and in the surroundings. Most of them were farms with cows and all have large availability of ancient brick walls with plenty of shelters. In each site, I captured males and virgin females, (i.e. females that had not mated yet in the current season as determined by the lack of male's scars on their belly, typically being present after copulation, [17]), in order to obtain mating and egg laying in captivity. Each individual was measured by a digital calliper (accuracy $\pm$ 0.1 mm) for snout-vent length (SVL), weighed (accuracy $\pm$ 0.1 g) and transferred to the Department of Animal Biology (Pavia) within 2 hours from capture. Overall, I housed 128 adult lizards per year for a total of 384 individuals (192 males and 192 females).

Sexes were held separately indoor under a natural light–dark cycle in individual transparent plastic jars (20 × 30 × 20 cm) provided with a newspaper sheet as substratum, a water tank and a shelter, and fed with 3 mealworms each day. Plastic jars were placed under an UV-B lamp (18 W) in order to provide the daily UV requirements for calcium and vitamin D fixation, and an incandescent lamp (25 W) for heating. UV lamps were switched on for 3 h a day (from 10.00 to 13.00), while incandescent lamps were set alight for 6 h a day (from 11.00 to 17.00). Males and females were then paired to obtain clutches. Females laid two clutches on average (range 1-4 in our experimental conditions), but in this study I used only the first clutches, since the energy females allocate to their first breeding attempt mainly derives from stored reserves, while later clutches depend on current energy intake [18] and thus, if breeding strategies actually exist, it is most likely to discover them by studying the first clutch.

At the end of the experiment (late June) all lizards were returned to their capture sites and all animals increased significantly their body mass between capture and release.

2.2 Egg Laying and Collection

Each jar (both outdoor and indoor) was supplied with a small plastic box (10 × 10 × 5 cm) filled by wet sand to allow egg laying. Boxes were carefully checked for eggs every morning, and laid eggs were gently removed, measured with a digital calliper (minor and major diameters to the nearest 0.01 mm) and weighed (Sartorius R200D balance, accuracy 0.01 mg). Overall, I collected 163 first clutches (2008: 42; 2009: 59; 2010: 62), because 29 females did not lay at all (2008: 22; 2009: 5; 2010: 2). Mean egg weight, length and width, as well as mean clutch mass were collected for 148 clutches out of 163, because 15 clutches were wholly formed by decayed (presumably unfertilized) eggs and were not measured.

2.3 Blood sampling and leukocyte counts

During 2008, blood samples were collected in the laboratory from the postorbital sinus [19] as smears at capture and release for all lizards. No lizards suffered long-term consequences from this procedure. Blood slides were prepared by placing a drop of blood directly onto a glass slide and smearing it with a second slide to produce a blood layer 1 cell thick. Leukocytes, red blood cells and haemoparasites were counted by randomly scanning blood smears at 630× magnification under oil immersion following standard routines [20]. In each scanned field (50 fields), red blood cells were counted and leukocytes classified as heterophils, eosinophils, neutrophils, basophils, lymphocytes and monocytes. The heterophil:lymphocyte ratio (H:L) was calculated as a sensitive measure of immune suppression and long-term stress; an increase in this ratio may reflect a reduction in the responsiveness of the immune system due to an increase of glucocorticoids (e.g., corticosterone in reptiles) plasma level following a stressful experience [21].

3 Results

3.1 Relationships between color morphs and alternative female breeding strategies

Females laid 4.33 ± 0.09 eggs on average in their first clutch (range: 1-8, $N = 163$ clutches), with a mean weight of 0.35 ± 0.01 g (range: 0.11 - 0.75 g) and a mean size of $11.7 \pm 0.06 \times 7.2 \pm 0.05$ mm (egg length range: 9.1 - 15.3 mm; egg width range: 4.6 - 10.1 mm). Larger and heavier females produced larger and heavier clutches, but clutch size was negatively correlated with egg length ($r = -0.43$, $p < 0.0001$), indicating that larger clutches consisted of smaller eggs, and *vice versa*; therefore, females traded-off between clutch and egg size.

The mixed model analysis confirmed that clutch size increased according to yellow color females relative to white and red ones ($\beta_{yellow} = 0.23 \pm 0.09$, $F_{1,225.9} = 6.79$, $p = 0.010$, Fig. 2), although significantly so only for putative homozygous yellow females, and by red color × SVL ($\beta_{interaction} = 0.07 \pm 0.03$, $F_{1,223.8} = 4.53$, $p = 0.034$, Fig. 3), larger red females (red, white-red and yellow-red) laying larger clutches compared to smaller red females (Fig. 3).
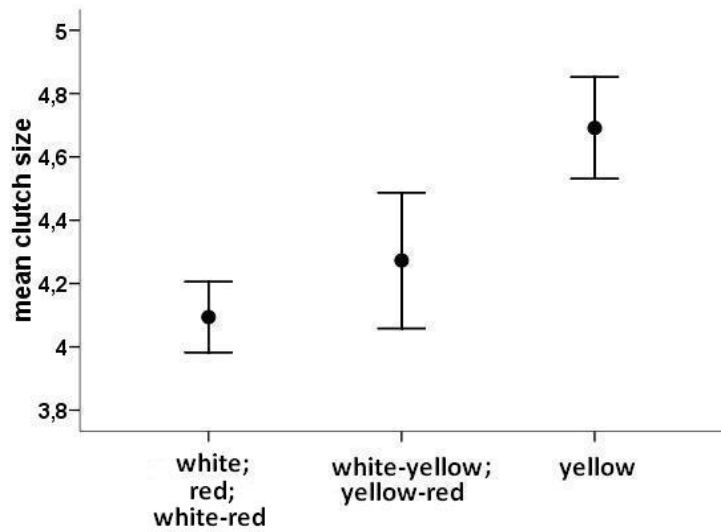
Fig. 2 Mean clutch size of wall lizard females in relation to female color. Bars represent mean s.e..
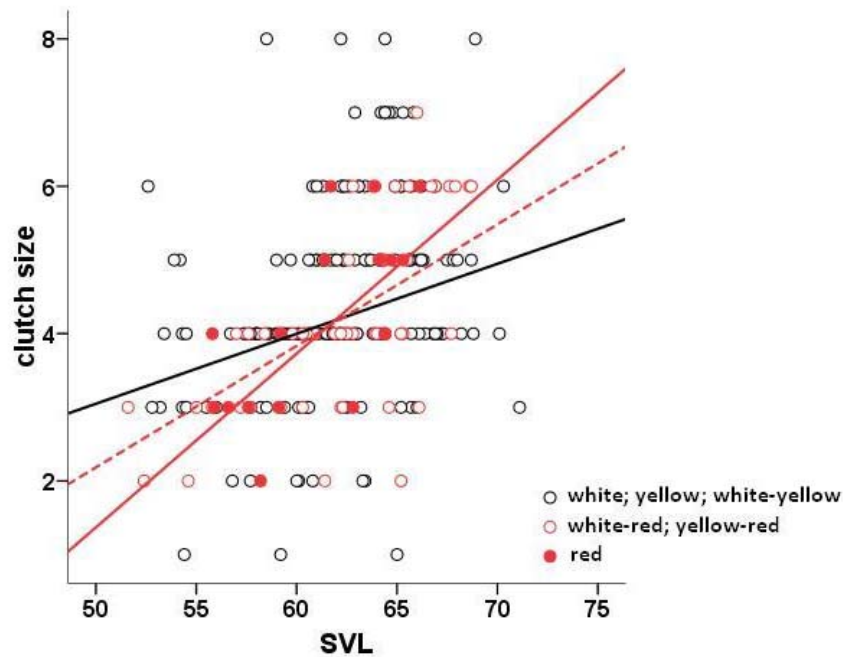

Fig. 3   Effect of morph $\times$ SVL on clutch sizes of color morph females.

Egg mean weight significantly decreased according to yellow females relative to white and red ones ($F_{1,181.9} = 5.11$, $p = 0.025$), yellow females laying lighter eggs. Concerning the egg length, the mixed model showed a pattern exactly opposite to that found for clutch size: in this case, yellow and red

females $\times$ SVL were associated with decreasing egg size (β_{yellow} = -0.22 $\pm$ 0.07, $F_{1,189.4}$ = 10.54, p = 0.001, $\beta_{\text{interaction}}$ = -0.05 $\pm$ 0.02, $F_{1,191.9}$ = 4.91, p = 0.028). Yellow and white females (yellow, white and white-yellow) laid smaller eggs independently of their size, whereas all red females laid smaller eggs when larger, and larger eggs when smaller, suggesting an age-modulated strategy of breeding investments.

3.2 Covariations between color morphs and haematological parameters

The H:L ratio varied significantly according to sampling time$\times$sex$\times$morph$\times$SVL (Fig. 4). This variation involved both yellow and red females as well as yellow males: in particular, among yellow females, larger individuals significantly increased (i.e. they get worse) and smaller individuals decreased their H:L index (i.e. they get better) after captivity (Fig. 4). This trend was reversed in red females, where smaller individuals, which showed lower H:L values than larger individuals at capture, presented raised H:L index (i.e. they get worse) at release, while larger individuals maintained their high H:L values (Fig. 4). Finally, larger yellow males showed higher H:L values at release with respect to capture (i.e. they get worse after captivity). H:L values of white males and females and of red males did not show any size-related variation either at capture or release.
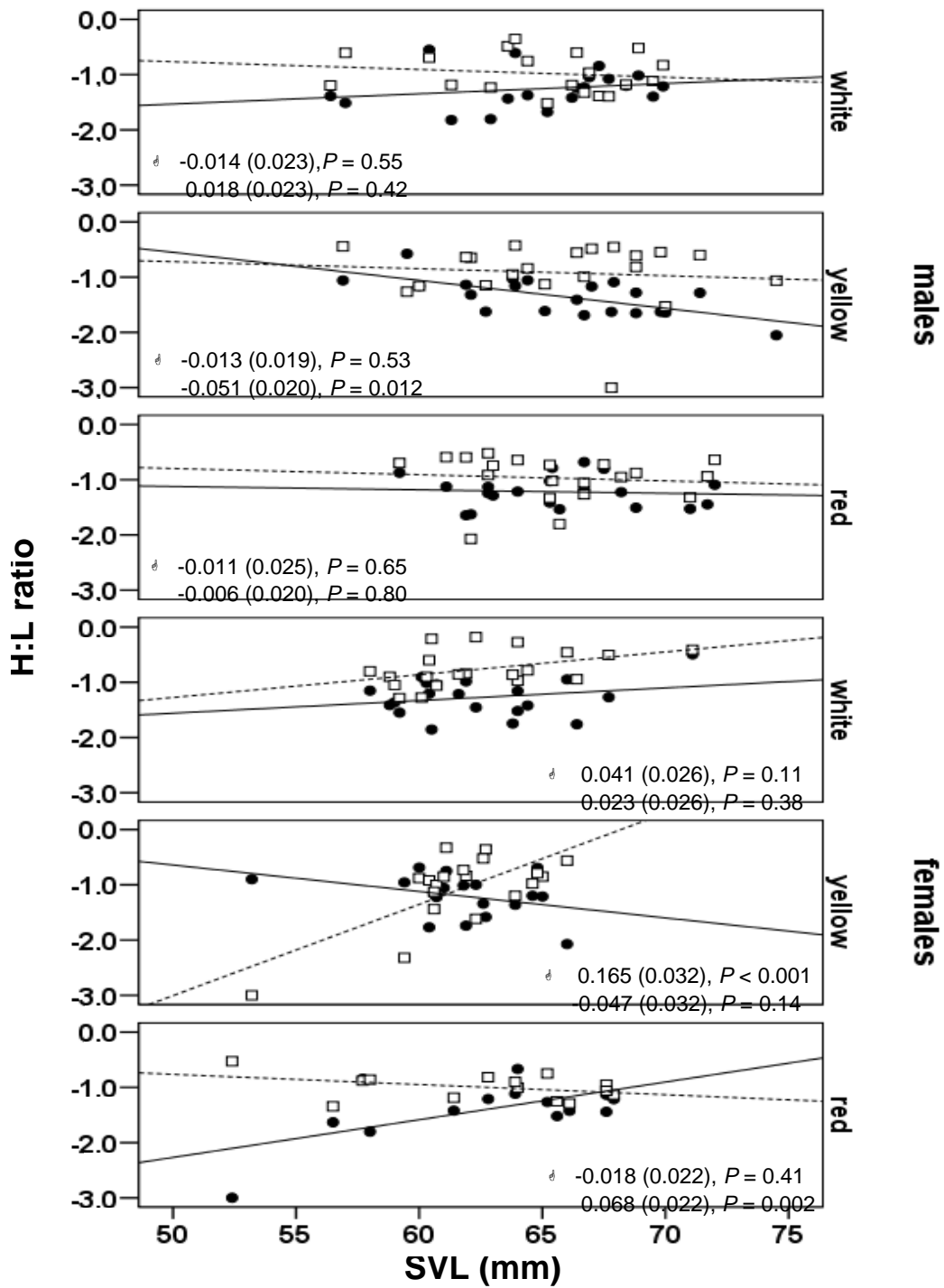
Fig. 4 Variation in H:L ratio according to time of sampling, sex, color morph and SVL of lizards (capture: black dots and solid line, release: open squares and broken line).

4 Conclusions

This study revealed an association between female color morph and breeding strategies, which may be compatible with the existence of a genetic control system of color morphs in common wall lizards. The alternative to genetic control should be a form of plastic morph determination, but theory suggests that plasticity can eliminate morphs [22]. Indeed, I found that the putative presence of the yellow females associated with larger clutches of smaller and lighter eggs, whereas pure white females combined with smaller clutches of larger and heavier eggs. Interestingly, pure red females resulted in a decrease of clutch size and an increase of egg size depending on body size (SVL), and therefore on the age of females. Actually, smaller/younger red females laid smaller clutches of larger eggs, while larger/older red females did the opposite.

Thus, concerning breeding strategy, yellow females seemingly adopted a pure r-strategy (many little eggs), and white females a pure K-strategy (few big eggs) independently of size/age. By contrast, red morph females were adopting a mixed, conditional K/r-strategy of reproductive allocation, behaving as K-strategists when young and as r-strategists when old. Therefore, populations of wall lizards appeared to be composed of at least two alternative pure-strategists (pure white and yellow females) and by mixed-strategists (pure and intermediate red females). The presence of a morph-related mixed ESS in breeding trade-offs further suggests that different color phenotypes may reflect different genotypes in this species.

For that concerning haematological parameters, I found that morphs of common wall lizards differed at capture depending on sex and size (which reflects age in reptiles, [23]), and in their haematological responsiveness to altered life-conditions related to captivity (Table 1). This suggests that different morphs show alternative fitness/physiological optima. Indeed, different haematological profiles and responsiveness to altered life-conditions may provide a mean to endure various kinds of stressors eventually resulting in differential fitness advantages for alternative morphs and age-classes.

Table 1　　Summary of variation in haematological parameters of common wall lizard morphs at capture and release.

Morph	Capture	Release
White	more heterophils in larger individuals	more eosinophils in larger females
Yellow	lower H:L values in larger males	more heterophils in larger individuals higher H:L values in larger females
Red	more heterophils in larger individuals more eosinophils in larger females higher H:L values in larger females	higher parasite loads in larger individuals

Response to long-term stressful conditions, such as those represented by captivity, differed among common wall lizard color morphs, implying a sex-, size- (age-) and morph-specific sensitivity to stress in this species, and hence a different physiological profile of the colour morphs. Differential color-based susceptibility of individuals to environmental stressors, such as temperature, predators, diseases or parasite infections may clearly depend on the genetic background in polymorphic species. This fits well with the hypothesis that different color morphs signal different genotypes, with different life-history and behavioural strategies as occurs in other lizard species [9,14].

What is the relevance of this study for current ideas about evolution and maintenance of CP? If female and male common wall lizards respond differently to chronic stress according to color morph and age, as these results suggested, then it is likely that response to an acute stress may vary in a mostly similar way, and this may have profound implications for the maintenance of CP in this species, since indirect selection may act differentially on physiological attributes (e.g., metabolic rate, immune response, resistance to stressors) that vary among color morphs.

Acknowledgements

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