

## Morph-specific immunity in male *Podarcis muralis*

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**Abstract.** Permanent colour polymorphism in lizards is maintained by complex interactions between environmental pressures and physiological traits (such as immune responsiveness) that differ among morphs. In this study we investigated whether T-cell mediated immune response vary among male colour morphs in the trimorphic (white, yellow and red) common wall lizard, *Podarcis muralis*. We found that yellow males showed a lower immune response compared to both red and white males, whose responses were similar. Thus, immune responsiveness is morph-specific in male common wall lizards, suggesting that this physiological trait could play an important role in maintaining colour polymorphism in this species. Moreover, immune responsiveness significantly increased with increasing male size, irrespective of colour morph, indicating that it could be regarded as a condition-dependent trait.

Intrasexual colour variability is widespread among lizard species, and has been interpreted as an adaptive compromise between conflicting selective pressures arising from social, antipredatory, thermoregulatory behaviours, and sexual selection (Cooper and Greenberg, 1992; Olsson and Madsen, 1998; Martin and Forsman, 1999; Lopez et al., 2004; Stuart-Fox et al., 2006). In many species of lizards, polychromatism is transient, and colours badges are either shown only during the breeding period (Rand, 1989, 1990), or change during ontogeny, as, for example, in *Psammodromus algirus*, where larger and older males show orange nuptial colouration and are dominant over smaller and younger males, which, albeit sexually mature, do not express colour signals (Martin and Forsman, 1999). By contrast, in other species, individuals occur in discrete colour morphs, which are permanent throughout their whole life, without seasonal changes, and coexist syntopically (Thompson and Moore, 1991b; Carpenter, 1995; Sinervo and Lively, 1996). In these species, polymorphism is maintained by

differences in fitness among morphs, which are modulated by complex interactions among environmental pressures (e.g. social interactions and individual density), genetic differences in the response to those pressures, and morph-specific variability in physiological parameters such as immunological condition (Sinervo and Lively, 1996; Sinervo et al., 2000; Svensson et al., 2001a, b; Comendant et al., 2003; Zamudio and Sinervo, 2003). For example, female side-blotched lizards (*Uta stansburiana*) surrounded by many territorial neighbours had suppressed immune function, and the variation in immunological condition has different effects on the fitness of the two heritable morphotypes of this species (Svensson et al., 2001a, b). These relationships cause a genetic correlation between female colour throat and immune responsiveness, thus promoting and maintaining throat colour polymorphism (Svensson et al., 2001a, b).

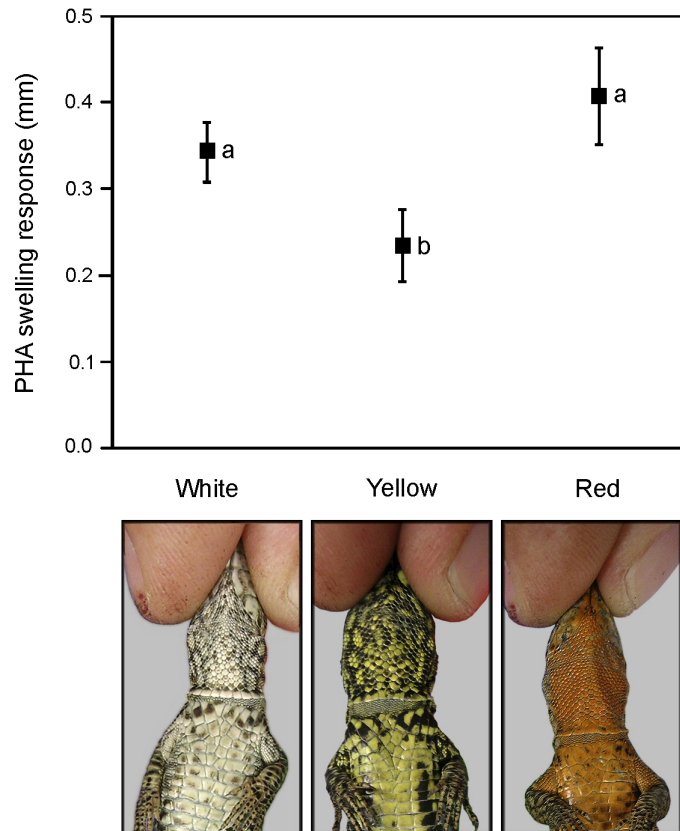
In this study, we investigate whether male immune responsiveness vary according to colour morphs of common wall lizards (Cheylan, 1988), as assessed by the delayed cutaneous hypersensitivity (DCH) test. Males of the common wall lizard (*Podarcis muralis*) show three discrete morphs differing according to throat and belly colouration (i.e. red, yellow and white, see also fig. 1), which develop after the second year of life (Cheylan, 1988). Such colour

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**Figure 1.** PHA swelling response of the three colour morphs of male common wall lizards. Bars represent means  $\pm$  1 SE and different letters denote significant differences between morphs ( $P < 0.05$ ).

morphs are permanent, and individuals maintain the same colour throughout their life, without seasonal changes (Cheylan, 1988; R. Sacchi and S. Scali, unpubl. data). Morph relative frequency is highly variable among populations, the white morph being generally more frequent, although populations where red and yellow morphs prevail are not uncommon (Cheylan, 1988; R. Sacchi and S. Scali, unpubl. data).

We aimed at detecting an association between colour polymorphism and intensity of the immune response, which would be a prerequisite for the comprehension of the physiological mechanisms maintaining colour polymorphism in this species.

We captured adult male lizards (snout-vent length, SVL,  $>50$  mm, Barbault and Mou, 1988) at 4 sites in the surroundings of Pavia (Northern Italy,  $45^{\circ}11'N-9^{\circ}9'E$ ), between 29 April and 2 June 2005. At all sites, the white

morph prevailed over the others, accounting for 40-62% of adult males. Body length (SVL) of all individuals was measured to the nearest 0.5 mm and each male classified as white, yellow or red according to throat and belly colour (fig. 1). Overall, 40 males (white:  $N = 23$ , yellow:  $N = 8$ , red:  $N = 9$ ) were used in this study. Animals were kept in cotton bags, taken to the laboratory and individually housed in 15-l plastic terraria for the duration of the experiment (one day, see below). The day following the experiment all males were released at the site of capture. The DCH test is based on a subcutaneous injection of phytohaemagglutinin (PHA), a mitogen which causes local swelling and oedema, driven by mitogenesis and infiltration of T-lymphocytes into the injected tissue (Goto et al., 1978). T-cell-mediated immune response was assessed as follows: thickness of the right foot was measured (at a mid point between the third and fourth finger) using a pressure sensitive micrometer (cod. SM112, Alpa S.p.A., Milan, Italy, accuracy of 0.01 mm), then it was injected with 0.03 ml of a solution containing 50 mg of PHA (Sigma, L-8754) in 10 ml phosphate buffered saline (PBS) (Oppliger et al., 2004). Since this test had been widely used in birds, but only in a few recent studies of reptiles (Svensson et al., 2001; Belliure et al., 2004; Oppliger et al., 2004; Berger et al.,

2005), we performed a preliminary analysis of the response on a separate set of 15 individuals to determine the peak swelling time. On the day following capture, we injected one foot with the PHA solution and measured the thickness immediately before injection and 6, 12, 18, and 24 h after injection. To control for swelling due to the injection in itself, in this test the other foot was injected with the same amount of PBS. The peak swelling occurred 6 h after PHA injection (data not shown). The response to PHA injection of the 40 experimental males was expressed by the change in thickness of the PHA-inoculated foot (thickness 6 h after inoculation minus thickness before inoculation; see Oppliger et al., 2004).

To analyse the association between male colour and the response to PHA injection, we adopted a mixed model analysis of variance, where PHA swelling response was included as the dependent variable, throat colour as a 3-level factor, while SVL was included as a covariate to control for the effect of male size. The site of collection was included as random factor to control for among-sites variation in immune-response. Significance level of the random effect was tested using the likelihood-ratio test (LRT, one-tailed) comparing the models with and without the random factor (see Littell et al., 1996). All statistical analyses were performed using the SAS system (v. 9.0). Means and parameter estimates are reported together with their associated standard error (SE).

The SVL of males was on average  $63.9 \pm 3.3$  mm and did not vary among morphs (mixed model analysis of variance:  $F_{2,37} = 1.59$ ,  $P = 0.22$ , white males:  $63.8 \pm 3.2$  mm; yellow males:  $66.1 \pm 3.0$  mm; red males:  $62.2 \pm 3.5$  mm), nor among collection sites (LRT,  $\chi^2 = 0$ ,  $df = 1$ ,  $P = 0.5$ ).

The intensity of the T-cell mediated immune response varied according to male throat colour ( $F = 3.48$ ,  $P = 0.042$ ) (fig. 1), but not to collection site (LRT,  $\chi^2 = 1.79$ ,  $df = 1$ ,  $P = 0.15$ ). Scheffe's *post-hoc* tests showed that yellow males had a lower immune response compared to both white and red males (yellow-white:  $P = 0.045$ ; yellow-red:  $P = 0.014$ , fig. 1), while the PHA swelling response did not differ between red and white males ( $P = 0.26$ , fig. 1). The immune response increased according to male SVL ( $F_{1,33} = 4.98$ ,  $P = 0.033$ , estimate:  $0.011 \pm 0.005$ ). The interaction between colour morph and SVL was not significant ( $F_{2,32} = 2.01$ ,  $P = 0.15$ ), implying that the positive relationship between SVL and immunity did not differ among morphs.

Our study suggests that male common wall lizards belonging to different morphs show a difference in cell-mediated immunity. This finding is of interest for understanding the evolutionary processes controlling and maintaining colour polymorphism in this species. Although simple genetic mechanisms may control the expression of lizard morphotypes (e.g., Sinervo and Zamudio, 2001), colour could also be under strong natural and sexual selection, that may favour particular combinations of life-history traits in the different morphotypes (Sinervo et al., 2000). In this scenario, colour polymorphism of male common wall lizards could be maintained by morph-specific interactions between environmental/social pressures and genetically determined variations in physiological traits. Therefore, the existence of morph-specific variability in immune responsiveness of male common wall lizards suggests that this important life-history trait might play an important role in maintaining polymorphism in this species. In other words, a reduced immune responsiveness might be the cost yellow males pay to be more efficient in other life history traits that confer to them a higher fitness in specific environmental/social conditions compared to males of the other morphs.

Several mechanisms could mediate the association between colour morphs and immunity. There is evidence that density-dependent social interactions from neighbours have different effects on immune responsiveness of lizards, the sign of which may vary in different morphs (Sinervo et al., 2000; Svensson et al., 2001a, b; Comendant et al., 2003; Zamudio and Sinervo, 2003). Indeed, genetic differences in sensitivity to density fluctuation have been suggested to play an important role in maintaining genetic polymorphisms for immunological traits (Lochmiller and Dabbert, 1993; Lochmiller, 1996). Thus, different genetic fixed levels of sensitivity to crowding among morphotypes might explain the reduced immune responsiveness of yellow males. The association between morphs and immunity could be medi-

ated by differential effects of circulating levels of steroid hormones in the three morphotypes and their concomitant immunosuppressive effects (Lochmiller, 1996; Folstad and Karter, 1992). In this context, crowding or increased aggressive interactions resulting from high individual density might result in different levels of circulating corticosterone and testosterone in the yellow males compared to other males, causing the different levels of immune suppression (Belluire et al., 2004; Oppliger et al., 2004; Berger et al., 2005).

Alternatively, lower immune responsiveness of yellow males might be the result of a differential colour-based susceptibility of individuals to environmental stressors, such as temperature, predators or parasite infestations.

A further important finding emerging from this study is that the intensity of T-cell mediated immune response increased with increasing male SVL, independently of male morph. Body size is correlated with age in lizards and it is the male trait that more frequently predicts dominance as well as the outcome of male-male combats (Stamps and Krishnan, 1994a, b, 1998; Carpenter, 1995; Martin and Forsman, 1999; Haenel et al., 2003). Therefore, the link between size/age and immune response may reflect the ability of the larger and dominant males to invest more resources in immune system functioning than smaller subordinate males, perhaps as a consequence of preferential access to resources.

In conclusion, results of this study show that immune system functioning varies according to colour morph in male common wall lizards, and this association could contribute to maintain colour polymorphism in this species. In addition, male body size positively predicted the intensity of T-cell mediated immune response irrespective of colour morph, thereby indicating that immune system functioning can be a condition-dependent trait in lizards.

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