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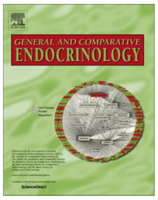

Research paper

Seasonal variations of plasma testosterone among colour-morph common wall lizards (*Podarcis muralis*)

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ABSTRACT

Sexual steroids influence reproductive behaviours and promote secondary sexual traits. In male lizards, increasing levels of testosterone (T) bolster conspicuous colouration, stimulate territoriality, and trigger antagonistic interactions among rivals. Moreover, in colour polymorphic species, reproductive strategy, aggressiveness and T levels can differ between morphs. Therefore, T level is considered as an important mechanism that regulates the expression of colour polymorphism and sexual behaviours of males. But in the polymorphic territorial wall lizard (*Podarcis muralis*), a lack of relationship between morphs and aggressiveness challenges the notion that T plays such a role. To examine this issue, we compared adult T levels among three colour morphs (white, yellow and red) through repeated sampling during the mating season. High T levels were observed at the onset of the mating season followed by a significant decrease, a pattern documented in other lizard species. Mean T levels did not differ among morphs. However, yellow males maintained significantly higher T levels over time and displayed a stronger subsequent decline. Overall, in this species, seasonal T patterns differ among morphs, not mean values. Previous studies revealed that T suppresses the immune response; suggesting that a strong initial investment promoted by high T levels may trade-off against immunity (maintenance). Further experimental investigations are required to clarify the relationship between T and reproductive effort in polymorphic species that exhibit complex temporal pattern of T levels.

1. Introduction

Proximal mechanisms that underlie the maintenance of colour polymorphism (CP) within animal populations are involved in reproductive isolation and speciation (Huxley, 1955; West-Eberhard, 1989; Corl et al., 2010). The persistence of different colour morph subpopulations within a given population is generally associated with the coexistence of alternative reproductive strategies, each one involving specific trade-offs among behavioural, morphological, physiological, and other life history characteristics (Sinervo and Lively, 1996; Svensson et al., 2001; Sacchi et al., 2007a, 2009). Different morphs may represent alternative locally adapted optima within a single species (review in Sinervo and Svensson, 2002). Because the equilibrium between alternative strategies is not necessarily stable under changing conditions, some morphs can be locally favoured or may colonize novel environments whereas other may decline; thus, peculiar phenotypes can be fixed through allelic selection thereby promoting sympatric speciation ('morphic speciation'; see West-Eberhard, 1989; Corl et al., 2010).

Many reptile species contain populations with marked colour polymorphism and thus offer suitable models to investigate these issues. In lizards for example, distinct colour morphs coexist within populations (Thompson and Moore, 1991a,b, 1992; Carpenter, 1995; Sinervo and Lively, 1996; Sinervo and Zamudio, 2001; Olsson et al., 2007; Sacchi et al., 2007b), and may have a genetic basis (Thompson et al., 1993; Sinervo et al., 2001; Sinervo and Zamudio, 2001). In many cases, this colour polymorphism is associated with alternative reproductive strategies (Sinervo and Lively, 1996; Zamudio and Sinervo, 2000) and is stabilised by both natural selection (e.g. negative frequency-dependent selection,

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Sinervo and Lively, 1996, disruptive selection, Bree and Rosenblum, 2006; genetic drift, Runemark et al., 2010) and sexual selection (Wellenreuther et al., 2014). Side-blotched lizards (*Uta stansburiana*) provide a well-known example of this phenomenon: three male colour morphs are respectively associated with aggressive, sneaky, and female-guarding mating strategies (Sinervo and Lively, 1996; Sinervo et al., 2000).

Empirical and experimental results suggest that complex sets of morphological and behavioural traits can be controlled by a limited number of physiological mechanisms. Hormones, notably sexual steroids, can exert such pleiotropic effects. For instance, development, body shape, colouration, fertility and behaviours are all influenced by androgen steroids, notably testosterone levels (T-level) in vertebrates (Brantley et al., 1993; Ketterson and Nolan, 1992; Cox et al., 2004; Oliveira, 2004; Hau, 2007). Circulating androgen steroid levels play key roles in the development and maintenance of colour morphs and associated behavioural traits in various taxa; for instance in fish (Cardwell and Liley, 1991), birds (Pryke et al., 2007), and lizards (Moore et al., 1998; Olsson et al., 2007; reviewed in Oliveira et al., 2008). In male lizards, experimentally elevated T-level fosters a strong motivation to defend territories against rivals (i.e. increasing movements, activity, and aggressive display; Moore and Marler, 1987), and presumably increases mating success compared to control individuals (Fox, 1983; DeNardo and Sinervo, 1994). However, T is a double edge sword, as stated by the Immunocompetence Handicap Hypothesis (Folstad and Karter, 1992), a notion supported by experimental evidences collected in different vertebrates taxa (reviewed in Roberts et al., 2004 and Foo et al., 2016). For instance, differential T-levels that determine alternative colour morphs in Gouldian finch are involved in the trade-off between aggressiveness and immunity (Pryke et al., 2007). In reptiles, high T-level can decrease immune functions, favours parasite infections, stimulates risky behaviours, and thus degrades survival (Olsson et al., 2000; Klukowski and Nelson, 2001; Cox and John-Alder, 2007). Testosterone-treated male lizards are more aggressive and more conspicuous, but they also experience higher mortality (Marler and Moore, 1988). Overall, although trade-offs are not necessarily mediated by the HPG-axis, empirical and experimental studies on both avian and non-avian reptiles suggest that the relationship between morphs and reproductive strategies involves, at least partly, androgen steroids. Thus, assessing hormone levels provides a mean to examine evolutionary trade-offs involved in the occurrence of alternative morphs in natural populations (Huyghe et al., 2009).

Previous investigations of the relationships between colour morphs and T provided contrasted results in polymorphic lizards. In *U. stansburiana*, orange territorial males are characterized by elevated T-level, high endurance, strong activity, larger home range and a greater access to females than blue or yellow males (Sinervo et al., 2000). But in another species, *Podarcis melisellensis*, the three morphs did not differ in T-level or in the immunological parameters tested (Huyghe et al., 2009). In another genus, *Psammodromus algirus*, T-levels influence aggressiveness but not coloration or parasite load; this disconnection likely favours the expression of sneaking reproductive strategies in males (Salvador et al., 1997). Moreover, in *Urosaurus ornatus* aggressive and territorial orange-blue males have similar T-levels compared to less aggressive orange males; but T-level in non-territorial males was more subject to stress-induced decrease (Jennings et al., 2000). Overall, no clear picture emerges from these studies. Further investigations were therefore necessary to clarify the influence of T-level in the regulation of the expression of lizard colour morphs and of associated mating strategies in natural populations.

In the current study we examined different morphological and behavioural traits in relation with T-level in the highly polymorphic wall-lizard *Podarcis muralis*. In this small-sized lacertid species, males show marked territoriality and fight vigorously to prevent intrusions from rivals (Edsman, 1990; Sacchi et al., 2009). Ventral and throat coloration are polymorphic. Six distinct colour morphs have been identified, including three main phenotypes (i.e., white, yellow and red) and three intermediate phenotypes (white-red, yellow–red and white-yellow) (Cheylan, 1988; Sacchi et al., 2007a; Scali et al., 2013; Calsbeek et al., 2010). All these morphs can occur in both sexes and within the same population (Sacchi et al., 2007b). Importantly, each morph is characterized by different phenotypic traits: for example, body size (Sacchi et al., 2007b; Calsbeek et al., 2010), immuno-competence and stamina (Sacchi et al., 2007a; Martin et al., 2008; Calsbeek et al., 2010), homing behaviour (Scali et al., 2013), diet (Scali et al., 2016), and stress responses (Galeotti et al., 2010). Dominance rules for the access to territories based on body size and residence were observed (Edsman, 1990; Sacchi et al., 2009). Surprisingly, investigations failed to find difference of aggressive behaviour or fighting attitude among morphs (Sacchi et al., 2009). However, in this previous experiment only one facet of male interactions was analysed: the response of a resident male facing an unknown intruder. Thus, other more complex and/or subtle effects representative of the natural situations experienced by each morph (e.g. several males interacting in their natural habitat/territory) were not examined (Olsson et al., 2007). In addition, the central role of chemical communication was not taken into account while the secretions of femoral pores differ among morphs (Pellitteri-Rosa et al. 2014); possible differential chemical signals tuning aggressiveness among males were not considered (Martín and López, 2015). Overall, *P. muralis* exhibits a nebulous pattern of polymorphism where available hormonal information does not match well with behavioural and morphological traits (e.g., Oppliger et al., 2004), thereby masking the potential role of androgen steroids on sexual behaviours. Further investigations were required to elucidate the mechanisms that underpin this unexpected lack of relationship and to better understand the complex patterns exhibited by this species.

Testosterone exerts immunosuppressive effects in the wall lizard (Oppliger et al., 2004), and this immune response is morph-specific in males: yellow males show a lower immune response compared to both red and white males (Sacchi et al., 2007a). To further assess the possible roles of testosterone, we compared the T-level of adult males from different morphs monitored repeatedly during the breeding season. Indeed, T-level fluctuates markedly during the breeding season in reptiles, and this dynamic component should be taken into account (Naulleau et al., 1987; Aldridge et al., 1990; Rostal et al., 1994). We thus compared T-level variations during the breeding season in different adult male colour morphs through repeated sampling. We notably expected that yellow males would exhibit higher global T-levels compared to the other morphs because they display a lower immune response. This expectation was based on the assumed trade-off between competitive demands. Given the complexity of the colour polymorphism in this species, we did not further speculate about the relationships between colour morphs and reproductive strategies. We initially tested if there were differences in adult T levels between morphs, and then examined if this sexual steroid is involved in associated phenotypic and behavioural traits (e.g. homing behaviour).

2. Materials and methods

2.1. Individual collection and marking

Adult common wall lizards (snout-vent length, SVL > 54 mm, Sacchi et al., 2012) were collected by noosing during April-July 2006, between 0900 and 1400 h, in four farms in the surroundings of Pavia (Northern Italy; 45°11′31″ N, 9°9′11″E). Lizards were assigned to three groups according to their morph: white, yellow (including pure-yellow and white-yellow individuals), red (including pure-red and white-red individuals). This classification was adopted since the intensity of morph colouration shows both ontogenetic and geographic variation, especially for white-yellow and white-red individuals (Sacchi et al., 2013). Yellow-red individuals ($n = 12$) could not be classified unambiguously, and thus were excluded. Each individual was measured (to the nearest 0.1 mm using a calliper) for snout vent length (SVL), photographed for individual identification as described by Sacchi et al. (2010), and then released at the exact point of capture.

At each farm, we performed three successive capture periods to encompass the mating season (period 1: 4th April to 4th May; period 2: 11th May to 8th June; period 3: 12th June to 3rd July). Overall, we processed 463 males, but only 76 individuals were captured at least twice and were included into the analyses that focused on temporal variations (see Table S1 for details).

2.2. Blood sampling and testosterone assays

Immediately after capture, we collected a sample of blood from each individual (75–100 μl), drawn from the retro-orbital plexus with a heparinized glass capillary tube (MacLean et al., 1973). Blood samples were stored in a cooled box, and brought into the laboratory of the University of Pavia. Within three hours from collection, tubes were centrifuged at 10000 rpm for 5 min and the plasma was stored frozen at -25 °C until assays.

Testosterone assays were performed in the Centre d'Etudes Biologiques of Chizé using a highly sensitive Radio-Immuno-Assay (RIA) method on 50 μl of plasma after di-ethyl-ether extraction (extraction efficiency 93 ± 10%, mean ± SD). The cross reactivity of the specific antibody (Sigma Laboratory) with other steroids was low (cross reactivity for B/B0 on antibodies anti-testosterone: 5α-dihydrotestosterone: 17.8%; 5β-androstene-3β, 17β–diol: 1.4%; 5 α-androstene-3β, 17β-diol: 1.2%; androstenedione: 1.4%; epitestosterone: 0.7%; progesterone: 0.07%). The relatively high level of cross-reactivity with DHT was not problematical because T is the main androgen in squamate reptiles (Crews et al., 1978; Courty and Dufaure, 1979). Most of the variations can be attributed to varying levels of T; further DHT and T variations exhibit parallel trends (Courty and Dufaure, 1979). The sensitivity of the assay was 7.8 pg by tube. Intra- and inter-assay variations were low, respectively 6% and 12% (Bonnet and Naulleau, 1996; Sereau et al., 2010).

2.3. Statistical analyses

Because individuals were captured and sampled more than once, possible effects of pseudo-replication were considered. First, we used MCMC-glmm with lizard-ID as a random factor, sites and morphs as the factors and T (log-transformed) as the dependent variable. For some analyses, notably when body size, site, period and morphs were all included, the models did not converge and the results were not reliable. Therefore we used a MANOVA for repeated measures with successive T values as the dependent variable. However, several individuals were not sampled at each of the three mating periods; therefore we performed the tests with a reduced sample size ($n = 20$ lizards) and then with the full sample size ($n = 76$ lizards) by using mean values calculated for each period and each morph as surrogates for missing cases. This method, although routinely used, introduces a bias (as any surrogate technique), and also enables controlling for pseudo-replication of individuals on the p values. To evaluate this last issue, we performed permutation tests by shuffling individuals among and within farms and performed the MANOVA using the same variables. Both approaches (repeated MANOVA with surrogates versus permutations) led to slightly different results.

Table 1
Morphological measures (mean ± SE) of male morphs from the four sampling sites. SVL stands for snout vent length.

Variable	Morph	Site1	Site2	Site3	Site4
SVL (mm)	White	64.8 ± 1.1	66.7 ± 1.1	67.9 ± 0.5	65.6 ± 1.9
	Yellow	67.4 ± 0.9	64.2 ± 2.0	67.2 ± 0.7	67.2 ± 1.6
	Red	63.3 ± 3.4	–	67.9 ± 1.5	67.6 ± 0.0
Body Mass (g)	White	6.8 ± 0.4	7.2 ± 0.4	8.2 ± 0.3	7.0 ± 0.5
	Yellow	8.2 ± 0.0	6.4 ± 0.8	7.8 ± 0.2	8.0 ± 0.4
	Red	6.5 ± 1.1	–	7.8 ± 0.4	7.7 ± 0.0

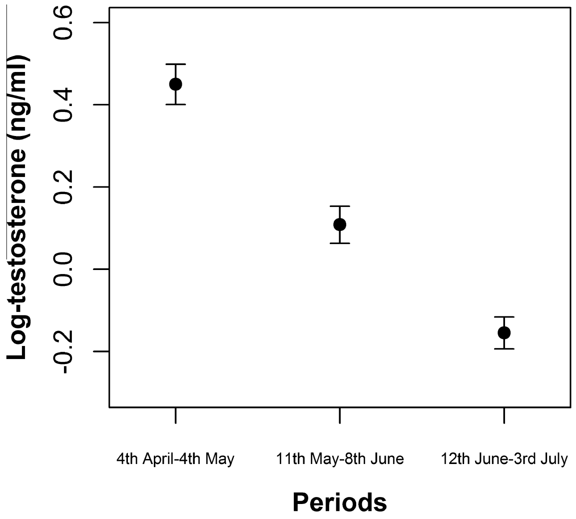

Fig. 1. Variation of testosterone plasma levels (log transformed) in males of the Common Wall lizard males over the three sampling periods. Points and bars represent means ± 1SE respectively ($n = 76$ in each period).

For simplicity we present only the results from the repeated MANOVA but the results from the permutation tests are provided in a Supplementary file.

3. Results

3.1. Comparing body size among colour morphs and sites

We found no morphological differences among morphs and sites (Table 1), although lizards from site 3 were slightly heavier than those from other sites (two-way factor ANOVAs with log-T recorded in the first capture as the dependent variable, site, morph and their interaction as predictors; statistics are reported in Table S2 in the Supplementary materials).

3.2. Seasonal changes of T

Plasma T levels decreased markedly over time; displaying a threefold decline from the first period to the end of the mating season: 3.14 ng/ml, 1.73 ng/ml, and 0.93 ng/ml (Linear mixed model with log-T as the dependent variable, lizard-ID as a random factor, and the three periods as the factor, reduced sample size: Likelihood $\chi^2_2 = 60.146$, $P < 0.001$; using surrogates: $\chi^2_2 = 117.92$, $P < 0.001$, Fig. 1). Therefore, for the following analyses we took into account this strong time effect.

3.3. Changes in T in the different colour morphs

The most consistent results (Table 2 and Table S3) considering the set of possible analyses were the interaction of periods with colour morphs and sites (Fig. 2). All colour morphs presented similar T at the beginning of the mating season. The yellow lizards maintained higher T levels from the first to the second period, then exhibited an abrupt drop and displayed the lowest level at the end of the mating season (Fig. 2). The period × site interaction revealed that the patterns of T plasma level over time were not similar among sites, as two out four groups maintained high T levels from the first to the second period (Fig. S1 in supplementary materials). However, this difference was due to the prevalence of yellow males in those two sites with respect to the others, in which the white was the most frequent morph (see Table S1 for details). Overall, colour morph was the major factor in the differential dynamic of T-levels among groups.

4. Discussion

Testosterone levels of adult *P. muralis* varied markedly among individuals (ranging from 0.11 to 23.5 ng/ml) and over time (decreasing during the mating season from 3.14 ng/ml in April to 0.93 ng/ml in early July). These results are in line with previous lizard studies (Smith and John-Alder, 1999; Edwards and Jones, 2001; Calisi and Hews, 2007; Husak et al., 2007). For example, strong inter-individual variations (0.04–77.8 ng/ml) and high mean plasma concentration of testosterone (36.1 ng/ml) were observed in male *Sceloporus pyrocephalus* during the breeding season (Calisi and Hews, 2007); high values were also recorded during the breeding season in *Anolis carolinensis* (ranging from 9.31 to 13.78 ng/ml, depending on body mass) and dropped to low levels in the post-breeding season (1.78–2.23 ng/ml; Husak et al., 2007). In *Tiliqua nigrolutea* Edwards and Jones (2001) found that plasma T levels peaked in spring (October, 10.9 ng/ml) after the onset of agonistic male-male interactions and at completion of spermiogenesis, then decreased to 8.8 ng/ml during the mating period (November), to reach 5.3 ng/ml in summer (December) when males become reproductively quiescent. In lizards, seasonal changes of testosterone levels correlate with seasonal variations of attributes of male to male competition: aggressiveness (Greenberg and Crews, 1983; Yang and Wilczynski, 2002), territoriality (Moore, 1987, 1988; Knapp et al., 2003; Watt et al., 2003), and bite force (Huyghe et al., 2009). For example, male *A. carolinensis* begin to display territorial and aggressive behaviours in spring, in coincidence with an increase of testis size and raising blood T concentration, suggesting that sexual behaviours are testosterone-dependent (Crews, 1975). Overall, high T levels promote the acquisition of high-quality territories and thus influence reproductive success in male lizards (Fox, 1983; Tokarz, 1995; Olsson et al., 2007).

Common wall lizards establish a territory that they patrol and actively defend against intruders (Edsman, 1990; Sacchi et al., 2010). In Northern Italy, most male to male combats are observed between early spring and mid-June (Edsman, 1990), although interactions can occur all over the year. We found that testosterone plasma levels peaked in April, decreased during the following months, and thus matched the seasonal pattern of male territoriality. This correlation supports the hypothesis that sexual activity, territoriality and sexual interactions among males might be testosterone dependent in *P. muralis*, as observed in other reptiles (Naulleau et al., 1987; Bonnet and Naulleau, 1996; Nelson, 2005; King and Bowden, 2013; Goldey and van Anders, 2015; Baird et al., 2001; Aragón et al., 2004). The lack of difference of aggressive behaviours among morphs in *P. muralis* (Sacchi et al., 2009) parallels the lack of difference of mean T levels we observed in this study. However, the study on aggressiveness only focused on absolute differences among morphs, and did not account for seasonal variations, masking possible temporal specificities. Our unpublished results suggest that modulations of aggressive behaviour intensity over the season actually occur. This chronological factor should be investigated with experiments involving repeated trials within individual, both early and later in the breeding season; and also considering short term (daily) fluctuations (Olsson et al., 2007).

The most relevant result of our study was the difference in seasonal hormone profiles among morphs, but not in the mean absolute concentrations. Indeed, we found that at the beginning of the season, in April, every morph exhibited high testosterone plasma values, without any statistical difference among morphs. Similarly, averaging the T-mean values of each morph across the whole mating season did not permit to discriminate the morphs ($P = 0.78$), likely because strong inter-individual differences and changes over time generated strong variance with overlapping ranges among groups. However, examination of changes over time precisely revealed a strong colour morph effect. Indeed, in May hereafter the onset of the mating season, yellow individuals maintained highest hormone concentrations compared to other morphs, but they exhibited the lowest values in June-July. Red and white morphs exhibited similar profiles with a continuous progressive decrease. Thus, yellow males experienced high circulating testosterone over much longer time than the other morphs.

Testosterone may provide some benefit fitness to yellow males by bolstering the development of sexual ornamentation, increasing aggressiveness and courting behaviours, in addition to its obligate role in sperm production (Zeller, 1971; Stokkan, 1979; Wingfield et al., 1990; Ketterson and Nolan, 1992; Bonnet et al., 2016). However, the maintenance over time of high T-levels can also impose both metabolic and immunological costs. First, by promoting sexual characters and activity, testosterone increases metabolic rate, and thus may cause a degradation of body condition and/or survival rate (Marler and Moore, 1988; Abelenda et al., 1992; Marler et al., 1995; Buchanan et al., 2001). Second, high T-levels temper immune-competence and thus increase vulnerability to parasites (Zuk, 1990, 1996; Folstad and Karter, 1992; Wedekind and Folstad, 1994).

Table 2
Effect of morph, capture periods and morphology on plasma T levels from male Common Wall lizards as assessed by MANOVA for repeated measures in reduced ($n = 20$) and surrogate ($n = 76$) samples. See methods for detailed description of samples and their analyses.

Source	Reduced sample			Surrogate sample		
	df	*Pillai's trace*	*P*	df	*Pillai's trace*	*P*
Intercept	1	0.3473	0.027	1	0.0038	0.61
Morph	2	0.3393	0.083	2	0.0346	0.30
SVL	1	0.5218	0.004	1	0.0014	0.76
Site	3	0.2161	0.386	3	0.0025	0.98
Body mass	1	0.4891	0.005	1	0.0262	0.18
Session	1	0.2992	0.142	1	0.0051	0.84
Morph × Period	2	0.9314	0.004	2	0.2491	0.001
SVL × Period	1	0.2656	0.183	1	0.0100	0.71
Site × Period	3	1.0366	0.004	3	0.3064	<0.001
Body mass × Period	1	0.0070	0.962	1	0.0043	0.86

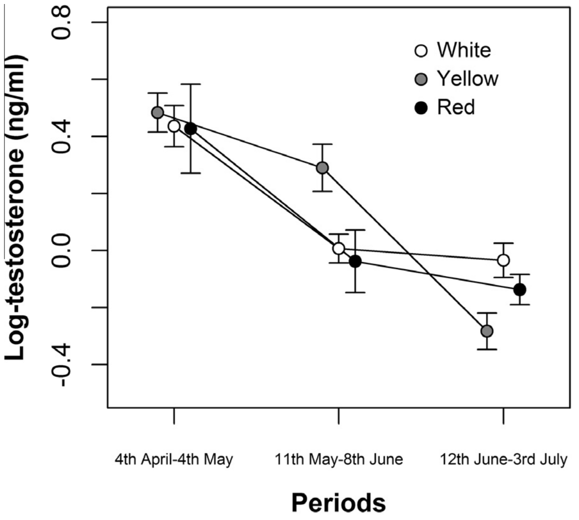

Fig. 2. Variation of testosterone plasma levels (log transformed) among colour morphs of Common Wall lizards over the three sampling periods. Points and bars represent means ± 1SE respectively (sample sizes: white males, *n* = 35; yellow males, *n* = 29; red males, *n* = 12).

Immunosuppressive effects of testosterone have been experimentally documented in the Common Wall lizard through hormone implants: males with increased T-levels showed decreased T-cell mediated response and increased mean metabolic rate (Oppliger et al., 2004). However, colour morphs were not accounted in that experiment. Using subcutaneous injection of phytohaemagglutinin (PHA), we showed that cell-mediated immunity is morph-specific in the Common Wall lizard, yellow males having a lower immune response compared to both white and red males (Sacchi et al., 2007a). All above findings support the existence of a trade-off between testosterone plasma level and immune function, in accordance with the immune-competence handicap hypothesis (Folstad and Karter, 1992). This trade-off may underlie specific optima in terms of colour morph and physiology, conferring to each morph a higher fitness in specific environmental/social conditions compared to males of the other morphs. In white and red males, low T-profiles may offer health benefit at the expense of reducing mating success through a lower investment to defeat rivals and attract females.

Recently, we obtained experimental evidence supporting these notions. Challenging homing ability we showed that yellow lizards of both sexes achieved a higher return success than white and red ones; particularly when individuals were released at greater distances (Scali et al., 2013). Yet, homing ability is associated with boldness because shy individuals tend to remain sheltered whereas high T-levels promote boldness (Raynaud and Schradin, 2014). In addition, high T-levels trigger dispersal in polygynous species and increase home range (Raynaud et al., 2012; Marjamäki et al., 2013; Onyango et al., 2013). T-levels have been shown also to promote secretion production of femoral pores increasing male appeal to females (Beackens et al., 2016), thus yellow males could invest more energy in marking territories and attracting females. Overall, high-T profiles exhibited by males correlate with a 'high reproductive investment syndrome' where individuals display typical traits of intensive and demanding sexual behaviours: increasing movements, boldness, exploratory abilities, and perhaps aggressiveness. Sustaining intensive reproductive effort driven by high T levels can be achieved by individuals in high body condition; gradual variations between body condition and reproductive investment are expected in this first group (Aubret et al., 2002; King and Bowden, 2013). Alternatively (not exclusively), in polymorphic species discrete differences in terms of reproductive investments and associated costs are expected among morphs: each morph displaying a strategy mediated by a specific tuning of the trade-off among traits (Knapp et al., 2003). The wall lizard may belong to the second category.

The occurrence of physiological optima differing among colour morphs might play an important role in promoting and maintaining polymorphism in Common Wall lizards. However, further investigations are required, both in the field and in captivity, including other key hormones (e.g. corticosterone; Meylan et al., 2002; Knapp et al., 2003) and behavioural observations to finely measure reproductive investments versus reproductive status and associated costs (e.g. changes in body condition, survival). Variations of aggressiveness should be monitored over time to examine if seasonal patterns differ among morphs. Interestingly, in polymorphic fish a role-dissociation between the two main androgens was observed: 11-ketotestosterone being involved in coloration whereas testosterone influences sexual behaviours suggesting that the profile of androgens should be compared among morphs in other vertebrates (Cardwell and Liley, 1991).

Common Wall lizard offers interesting opportunities to address these issues. Indeed reproduction has been intensively studied in this species (Strijbosch et al., 1980; Barbault and Mou, 1988; Bejakovic et al., 1996; Ji and Braña, 2000; Sacchi et al., 2012; Le Hénanff et al., 2013) whereas the existence of three main colour morphs with intermediate morphs provides an exceptional option to test straightforward hypotheses. For instance, intermediate morphs should express intermediate degrees of the 'high reproductive investment syndrome'.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ygcen.2016.09.012.

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