Strict ontogenetic control vs. environmental plasticity: the stability of colour morphs in the Common Wall Lizard

Roberto Sacchi[1]

roberto.sacchi@unipv.it

[1]Università degli Studi di Pavia

Stefano Scali[2]

Museo di Storia Naturale di Milano

Marco A.L. Zuffi[3]

University of Pisa

Marco Mangiacotti[1]

[1]Università degli Studi di Pavia

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Abstract

Whether the complex colour polymorphisms of lizards are governed by rigid genetic control or by the type of phenotypic plasticity recently discovered in side-blotched lizard (*Uta stansburiana*) remains a critical question for understanding the maintenance of these systems. In this study, we utilized a four-year capture-recapture dataset to investigate if adult common wall lizards (*Podarcis muralis*) exhibit similar plastic transitions or follow a fixed developmental program. Using standardized digital photography and Residual Randomization Permutation Procedures (RRPP), we quantified Hue and Saturation variation across 167 adult individuals (216 transition events). Our results reveal an exceptional degree of categorical stability, with 97.7% of individuals retaining their discrete colour morph among captures; the rare transitions observed were exclusively unidirectional, involving initially white individuals acquiring pigmentation. While individual identity accounted for over 45% of the spectral variance, low repeatability coefficients (ICCs < 0.13) indicate high intra-morph chromatic uniformity. Phenotypic trajectory analysis demonstrated that as individuals grow, morphs significantly diverge in both colour intensity and direction. Notably, the yellow morph follows a unique, morph-specific maturation path, suggesting a canalized developmental program that enhances signal clarity with age. Unlike the reversible hormonal plasticity documented in *U. stansburiana*, the *P. muralis* system appears driven by a fixed ontogenetic commitment. These findings suggest that this polymorphism is maintained by a robust genetic architecture where adult maturation might serve to minimize cross-talk among socio-sexual signals, highlighting the analysis of adult ontogenetic trajectories as a vital practical tool for deciphering the evolution of complex biological polymorphisms.

Introduction

Colour polymorphism is defined as the presence of multiple, discrete colour variants, or morphs, within a single population, where the rarest morph is too common to be maintained solely by recurrent mutation [1,2]. This phenomenon is widely taxonomically distributed across diverse animal groups, ranging from arthropods to vertebrates, including fish, reptiles, and birds [3]. Colour polymorphic systems are highly significant in evolutionary biology as model systems for examining the processes that generate and maintain phenotypic variation, as colour morphs serve as easily observable markers for tracking changes in allele frequencies over time [4]. Further, colour polymorphism is important because it is often associated with alternative reproductive strategies and a suite of correlated behavioral, physiological, and life-history traits, such as varying testosterone levels, immune responses, and metabolic rates [5–7]. A prime example is the "rock-paper-scissors" mating system in the side-blotched lizard (*Uta stansburiana*), where orange (aggressive), blue (mate-guarding), and yellow (sneaker) morphs coexist through frequency-dependent selection, which favors rare phenotypes and maintains a dynamic equilibrium [5].

According to Gray and McKinnon [8], the maintenance of these systems relies on a balance between disruptive selection (acting within populations to favor extreme phenotypes) and divergent selection (acting among different environments), often mediated by visual heterogeneity and sensory bias [3]. These selective forces build up co-adapted gene complexes where colour is linked to strategy, often through chromosomal inversions or regulatory mechanisms like hormone systems [3]. However, this equilibrium can be destabilized by environmental shifts or the colonization of new areas, leading to the selective loss of morphs and eventual morph fixation [9]. This transition has been described as a driver of morphic speciation [10], where the loss of a morph triggers character release [3,9]. The process frees the genome from the evolutionary constraints of maintaining multiple phenotypes, allowing the remaining morphs to specialize rapidly toward new adaptive optima and accelerated phenotypic evolution [9]. Consequently, colour polymorphism serves as a key factor in speciation, effectively facilitates reproductive isolation and the eventual formation of new species in sympatry [11–13].

Genetic systems controlling colour polymorphism range from single regulatory switch genes to complex supergenes that maintain linked sets of adaptive traits by suppressing recombination [14–18]. In species such as the ruff (*Philomachus pugnax*) and the white-throated sparrow (*Zonotrichia albicollis*), morph determination is governed by supergenes originating from large chromosomal inversions, which allow for the stable inheritance of correlated suites of behavioral, physiological, and morphological traits [16,19]. Other models are controlled by a few autosomal loci of major effect, which act as master regulatory switches that trigger distinct biochemical pathways, such as pteridine and carotenoid synthesis, to determine discrete phenotypes, and likely exert pleiotropic effects on associated behavioral, physiological, and life-history traits [12,17]. In many butterflies, mimetic polymorphism is determined by single developmental switches, often transcription factor loci [14,15,20]. Furthermore, structural mutations like the insertion of transposable elements, as seen in the cortex gene of the peppered moth (*Biston betularia*), can act as molecular triggers for major phenotypic shifts [20]. These diverse genetic architectures ensure that distinct morphs are perceived as discrete signals by receivers, facilitating frequency-dependent selection and the maintenance of biodiversity within population.

The colour polymorphism in side blotched lizard (*U. stansburiana*) was traditionally thought to be controlled by a single autosomal locus OBY, featuring three co-dominant alleles [21]. Recent genomic research has overturned this last model, revealing that the polymorphism is actually governed by a two-haplotype system coupled with phenotypic plasticity [12]. Authors identified the regulatory region of the sepiapterin reductase gene (SPR, the same as in *P. muralis*) as the molecular basis, which utilizes pleiotropy to couple visual signals, such as pteridine pigments, with behavioral traits through neurotransmitter synthesis. Rather than three alleles, the population possesses only orange (O) and blue (B) haplotypes, where individuals with the OO genotype express the orange morph, while those with at least one B haplotype (OB or BB) develop into either blue or yellow morphs depending on the context. Indeed, the yellow "sneaker" morph lacks a distinct genetic background, and emerges through phenotypic plasticity among B-background males that are initially unable to secure a territory. Specifically, genomic evidence shows that approximately 91% of yellow "sneaker" males share the same genetic background as blue morphs (possessing at least one B haplotype), meaning yellow is not a distinct genetic variant but a conditional strategy. Interestingly, this gene/plasticity hybrid system appears significantly more effective at maintaining the stable "rock-paper-scissors" dynamics against genetic drift than the traditional three-allele model [12].

The role of plasticity further supported by the observation that morphs can actually change their colour in response to social and environmental cues [22,23]. Specifically, B-haplotype individuals that are initially unable to secure a territory express yellow colouration, but can irreversibly transform into the blue territorial morph if a territory becomes available late in the season. Hormonal control is also involved in colour change in a way that the irreversible transformation of yellow sneakers into blue territorial morphs is driven by a significant increase in plasma testosterone, which simultaneously enhances physical performance traits like endurance and sprint speed [23].

The observation of ontogenetic colour changes within morphs, such as those documented in *U. stansburiana*, is scientifically significant as it highlights the potential influence of phenotypic plasticity on trait expression and behavioural strategies. Specifically, the temporal stability of a morph provides a diagnostic framework: when colouration remains constant throughout an individual's lifespan, the polymorphism is likely governed by rigid genetic control. In contrast, the occurrence of colour transitions implies that phenotypic plasticity, potentially driven by hormonal shifts or environmental cues, plays a pivotal role. Consequently, species exhibiting such plasticity offer a dynamic window into how organisms optimize their reproductive tactics in fluctuating social environments, distinct from the fixed strategies typical of genetically determined polymorphisms.

The common wall lizard (*Podarcis muralis*) species displays a prominent ventral colour polymorphism consisting of three primary "pure" morphs, white (W), yellow (Y), and orange (O), as well as "mixed" mosaic variants such as white-orange (WO) and yellow-orange (YO) [24]. Notably, while the most intense erythroic morph has been historically described as 'red' (e.g., [24]), here we adopt the term 'orange' to align with the nomenclature used for other species (such as *U. stansburiana*), and to reflect the genetic architecture described by Andrade et al. (2019). The genetic system in this species appears to be strictly genetic, where colouration responds to a two regulatory regions near the SPR gene (pteridine metabolism) and the BCO2 gene (carotenoid metabolism), each with two alleles, whose combination generate all the phenotypes [13,17]. Transitioning among these states occurs during development: all juveniles are born white, and as they grow toward sexual maturity some develop yellow pigmentation or orange scales, whereas other remain dully white [25]. Crucially, while younger lizards show these developmental shifts, the colour morphs appear to be ontogenetically stable once individuals reach sexual maturity, suggesting that adults do not undergo colour changes [26]. However, certain colour transitions between yellow and yellow/orange morphs (theoretically associated with genotypes oo and OO/Oo at the SPR locus, respectively) have been observed at low frequencies and interpreted primarily as ontogenetic changes [25]. These transitions, particularly trajectories moving toward orange, were attributed to developmental processes, but during a period (ten years ago) when the role of phenotypic plasticity in morph expression and reproductive strategies was not fully understood. While research suggests that morphs are ontogenetically stable once individuals reach sexual maturity, these conclusions based on relatively small capture-recapture datasets, and lacked extensive longitudinal evidence regarding specific adult transitions. Consequently, definitive data on the presence or absence of adult morph transitions in this species remain insufficient. In light of the recent work by Corl et al.[12] on *U. stansburiana*, this missing information becomes now crucial. Determining if such transitions occur in adult *P. muralis* is essential to understanding whether plasticity plays a similar role in regulating morph expression and behavioral strategies in the common wall lizard. Furthermore, such data could reveal if these transitions are linked to shifts between alternative behavioral strategies or, rather, to physiological triggers, such as changes in testosterone or gonadotropin levels, that affect the synthesis and accumulation of pigments in the skin. In this study, we utilize capture-recapture data collected over a four-years period to analyze colour changes in adult common wall lizards. Our objective is to better understand: i) whether and how morph transitions occur, and ii) how colouration within specific morphs shifts as individuals age.

Results

The transition matrix analysis performed on 216 recapture events confirms an exceptionally high degree of phenotypic stability in adult lizards (Table 1). Overall, 97.7% (211/216) of individuals retained their initial colour morph between captures, and only five individuals (one male and four females) changed colour with growing. When examining specific trajectories, these deviations were primarily associated with the acquisition of pigmentation by initially white individuals, or minor losses of pigment intensity. Individuals presenting the orange phenotype showed absolute stability (100% retention, n = 30).

Table 1
Transition matrix of ventral colour morphs between consecutive captures in adult *Podarcis muralis*. Rows represent the morph at the first capture (t_1), and columns represent the morph at the subsequent capture (t_2). The diagonal (bold) represents stable individuals.

t_1 White (ry)	**t_2 White (ry)**	**t_2 Orange (Ry)**	**t_2 Yellow (rY)**	**t_2 Yellow-Orange (RY)**
	45	**1**	**2**	**0**
t_1 Orange (Ry)	0	**30**	0	0
t_1 Yellow (rY)	1	0	**111**	0
t_1 Yellow-Orange (RY)	0	0	1	**25**

Similarly, yellow (oY) and yellow-orange (OY) morphs were highly stable (99.1% and 96.2%, respectively). The white (oy) morph acted as the primary source of variation, with three individuals (6.2%) developing pigmentation: one transitioned to orange (Oy) and two to yellow (oY). Only two cases of pigment "loss" were recorded: one yellow individual was reclassified as white (oY -> oy), and one yellow-orange individual lost the orange component, transitioning to yellow (OY -> oY). The RRPP model for the hue (Table 2) revealed a highly significant effect of individual identity, which accounted for approximately 45.6% of the total spectral variance ($F_{166,200}$ = 1.509, P < 0.001). However, these differences were insufficient to generate high repeatability of hue within individuals, as ICCs did not exceeded 0.082 (Table 2). In other words, the large sample size provides the model with enough power to detect differences among individuals (df_{num}= 166), yet these differences were too subtle to effectively distinguish individuals from one another basing on hue alone. In contrast, the morph effect within individuals was not significant ($F_{3,200}$ = 2.986, P = 0.202), indicating that the hue of colour morphs remained stable within individuals, and no systematic ontogenetic transitions between discrete morph categories occurred during the study period. Interestingly, we found a significant effect of replications ($F_{4,200}$ = 1.421, P = 0.010), which suggests a directional shift in colour expression, even though it accounted for only 1% of the total variance (Fig. 1). Finally, the interaction between morph and replicates was non-significant ($F_{9,200}$ = 0.954, P = 0.343), demonstrating that the observed temporal shifts in colouration (directional error) were consistent across all morphs and did not follow morph-specific trajectories.

Table 2
ANOVA statistics from the Residual Randomization Permutation Procedure (RRPP) assessing the effects of colour morphs on Hue and Saturation in *Podarcis muralis*. Morphological variation was analyzed as a single four-level factor (White, Orange, Yellow, and Yellow-Orange) and through binary contrasts (Orange/Non-orange and Yellow/Non-yellow), while accounting for individual identity and measurement replicability. Repeatability metrics, including the standard Intraclass Correlation Coefficient (ICC), the absolute agreement coefficient (ICCa), and the concordance coefficient (ICCc), are provided for each model to distinguish between random measurement error and systematic error (reps).

Source of variation	**Hue**					**Saturation**				
	Df	**SS**	**R²**	**F**	**P**	**Df**	**SS**	**R²**	**F**	**P**
White/Orange/Yellow/Yellow-orange										
Individuals	166	3.274	0.456	1.509	< 0.001	166	0.756	0.490	1.544	< 0.001
Morphs	3	0.117	0.016	2.986	0.202	3	0.014	0.009	1.608	0.075
Reps	4	0.074	0.010	1.421	0.010	4	0.025	0.016	2.130	0.015
Morph x Reps	9	0.112	0.016	0.954	0.343	9	0.034	0.022	1.295	0.089
Residual	200	2.618	0.364			200	0.590	0.382		
	ICC = 0.080; ICCa = 0.082; ICCc = 0.082					ICC = 0.093; ICCa = 0.098; ICCc = 0.099				
Orange/Not-orange										
Individuals	166	3.665	0.511	1.694	< 0.001	166	0.790	0.512	1.597	< 0.001
Orange	1	0.083	0.011	6.354	0.054	1	0.011	0.007	3.777	0.067
Reps	4	0.072	0.010	1.380	0.018	4	0.025	0.016	2.092	0.016
Orange x Reps	3	0.049	0.007	1.243	0.086	3	0.007	0.005	0.839	0.341
Residual	208	2.711	0.378			208	0.620	0.402		
	ICC = 0.114; ICCa = 0.115; ICCc = 0.116					ICC = 0.102; ICCa = 0.106; ICCc = 0.107				
Yellow/Not-yellow										
Individuals	166	3.886	0.541	1.736	< 0.001	166	0.863	0.560	1.735	< 0.001
Yellow	1	0.004	0.001	0.290	> 0.999	1	0.001	0.001	0.433	0.470
Reps	4	0.061	0.008	1.134	0.097	4	0.024	0.016	2.004	0.020
Yellow x Reps	3	0.034	0.005	0.841	0.657	3	0.014	0.009	1.518	0.155
Residual	208	2.805	0.391			208	0.624	0.404		
	ICC = 0.126; ICCa = 0.127; ICCc = 0.127					ICC = 0.125; ICCa = 0.130; ICCc = 0.130				

The RRPP model focusing on the hue of the orange pigmentation (Orange vs. Non-orange) confirmed that individual identity is a significant driver of spectral variation, accounting for 51.1% of the total variance ($F_{166,208}$= 1.694 P < 0.001). However, this individual effect was associated with relatively low repeatability, with ICCs values around 0.11 (Table 2), indicating that, as for the previous model, differences are too subtle to allow for a reliable discrimination of orange and not-orange individuals based on hue alone. In contrast, the effect of the orange grouping factor showed a much higher degree of consistency. The morph effect was marginal in terms of variance ($F_{1,208}$ = 6.354, P = 0.054), indicating that the hue of orange morphs remained relatively stable and repeatable within individuals, confirming that no systematic transitions between orange and non-orange status occurred during the study period. Temporal dynamics, captured by the reps term, remained significant ($F_{4,208}$ = 1.380, P = 0.018), reinforcing the hypothesis of a directional shift in colour expression. Importantly, the interaction between orange pigmentation and replicates was still not significant ($F_{3,208}$ = 1.243, P = 0.086), indicating that this intensification of colour occurs uniformly across both orange and non-orange individuals.

Finally, the analysis of yellow pigmentation (yellow vs. non-yellow) further emphasized the effect of individuals on spectral profiles. Individual identity accounted for 54.1% of the total variance of the hue, showing an even stronger statistical signal than in previous models ($F_{166,208}$ = 1.736, P < 0.001). Consistent with these findings, the repeatability for yellow hue was low, with the three ICCs around 0.127 (Table 2), suggesting that individual differences remain difficult to resolve despite the strong statistical effect. The presence of yellow pigment itself had no significant effect on the overall spectral variation ($F_{1,208}$ = 0.290, P > 0.999), suggesting that the yellow component also remain stable across replicates within individuals. Temporal effects and their interaction with yellow pigmentation (reps and ylw x reps) were both non-significant ($F_{4,208}$ = 1.134, P = 0.097 and $F_{3,208}$ = 0.8416.354, P = 0.657, respectively). This indicates that, unlike the orange colour, which showed a directional ontogenetic increase, the yellow chromatic component remains static over time.

The pattern of variation for the saturation component of colour morphs followed the same pattern as for the hue. The RRPP model for morphs confirmed the primary role of individual identity, which explained approximately 49.0% of the total variance ($F_{166,200}$= 1.544, P < 0.001). Despite this individual-driven variation, overall repeatability for saturation across morphs was low (Table 2). The morph effect was non-significant ($F_{3,200}$= 1.609, P = 0.075), further supporting the observation that variability in saturation levels within individuals does not depend on transitions between morphs. However, we observed a significant effect of replications ($F_{4,200}$= 2.130, P = 0.015), accounting for 1.6% of the variance. This indicates a consistent directional shift in colour intensity over time (Fig. 1). Crucially, the interaction between morph and replicates was non-significant ($F_{9,200}$= 1.295, P = 0.089), suggesting that the rate of saturation change is uniform across all morphs, rather than being driven by morph-specific developmental trajectories.

The RRPP model for saturation in relation to orange pigmentation (Orange vs. Non-orange) confirmed that individual identity remains the primary driver of variation, accounting for 51.2% of the total variance ($F_{166,208}$= 1.597, P < 0.001), but repeatability was still low (Table 2). The effect of the orange grouping factor was marginal but not significant ($F_{1,208}$= 3.777, P = 0.067), suggesting that transitions between orange and not-orange status do not explain the variability of saturation levels within individuals. Temporal dynamics (reps) showed a significant effect ($F_{4,208}$= 2.092, P = 0.016), indicating a directional shift in saturation as individuals age. The interaction (orange x reps) was non-significant ($F_{3,208}$ = 0.839, P = 0.341), confirming that this intensification of colour intensity occurs consistently across both groups, regardless of the presence of orange pigment.

When examining yellow pigmentation (Yellow vs. Non-yellow), individual identity explained an even greater portion of the variance, reaching 56.0% ($F_{166,208}$= 1.735, P < 0.001). This model yielded the highest repeatability coefficients for saturation among the three analyses (0.12, Table 2), but was again too low to effectively distinguish individuals from one another. Unlike the results for hue, the yellow factor had a clearly no significant impact on saturation levels ($F_{1,208}$= 0.433, P = 0.470), and the interaction between yellow and replications was also non-significant ($F_{3,208}$= 1.518, P = 0.155). Interestingly, the temporal effect (reps) remained significant in this model ($F_{4,208}$= 2.004, P = 0.020), suggesting that while yellow individuals do not differ from non-yellow ones in their overall intensity, all individuals undergo a shared ontogenetic increase in saturation over time.

The trajectory analysis provided further insights into the dynamics of colour change between the initial and final captures (Table 3). When considering the four discrete morphs (Fig. 1), we found significant differences in both the magnitude (size: $F_{3,159}$ = 6.590, P = 0.004) and the direction of CPTs (angle: $F_{3,159}$ = 1.660, P < 0.001) for Hue. While body size (SVL) consistently influenced the amount of chromatic shift ($F_{1,159}$ = 4.381, P = 0.048), the interaction between morph and SVL was non-significant, suggesting that the rate of change is generally similar across groups. However, the analysis of Saturation trajectories across the four morphs revealed an even stronger signal, with morph identity significantly affecting both the magnitude ($F_{3,159}$ = 20.52, P < 0.001) and the direction ($F_{3,159}$ = 1.607, P = 0.001) of the intensification process. Overall, these CPTs indicate that as individuals grow, they tend to progressively increase pigment loading, intensifying their colouration (Fig. 1). More importantly, the significant differences in trajectory angles demonstrate that this ontogenetic change is not uniform; instead, the chromatic profiles of the different morphs tend to diverge from one another over time, leading to a more pronounced phenotypic differentiation as the lizards reach maturity.

The binary contrast for orange pigmentation (Orange vs. Non-orange, Table 3) confirmed these trends, showing significant differences in the magnitude and direction of CPTs for both Hue (size: $F_{1,163}$ = 4.468, P = 0.044; angle: $F_{1,163}$ = 1.811, P < 0.001, Fig. 1) and Saturation (size: $F_{1,163--}$ = 41.11, P < 0.001; angle: $F_{1,163}$ = 1.999, P = 0.001, Fig. 1). Again, the accumulation of pigments (Size) was correlated with SVL (Saturation: $F_{1,163}$ = 4.853, P = 0.037), but no morph-specific ontogenetic rate was detected (Table 3). The most compelling results emerged from the yellow pigmentation analysis (Yellow vs. Non-yellow). The presence of yellow pigment significantly influenced the trajectory of colour change for both Hue and Saturation in terms of magnitude and direction (Table 3). Crucially, for the yellow morphs, we observed a significant interaction between the grouping factor and SVL for both the magnitude (size: $F_{1,163}$ = 4.147, P = 0.035) and the direction (angle: $F_{1,163}$ = 5.481, P = 0.042) of Hue change. A similar interaction was found for the direction of Saturation change ($F_{1,163}$ = 1.149, P = 0.011). This indicates that, unlike the other chromatic components, the ontogenetic development of yellow colouration follows a unique, morph-specific trajectory, where both the rate and the pattern of colour maturation diverge significantly, as individuals grow.

Table 3

Results of phenotypic trajectory analysis for Hue and saturation changes in throat colouration of *Podarcis muralis* morphs between initial and final captures. The analysis evaluates differences in trajectory magnitude (Size) and direction (Angle) across the four discrete morphs and their binary combinations (Orange/Non-orange, Yellow/Non-yellow). Body size (SVL) and its interaction with morph were included to test for ontogenetic effects on colour change.

Source of variation		**Size**					**Angle**				
		Df	**SS**	**R^2**	**F**	**P**	**Df**	**SS x 10^6**	**R^2**	**F**	**P**
Four morphs											
H	morph	3	0.024	0.108	6.590	0.004	3	21.48	0.030	1.660	< 0.001
	Svl	1	0.005	0.024	4.381	0.048	1	5.035	0.007	1.167	0.007
	Morph x svl	3	0.001	0.003	0.159	0.880	3	13.50	0.019	1.043	0.053
	Residuals	159	0.190	0.866			159	686.1	0.945		
S	morph	3	0.011	0.269	20.52	< 0.001	3	20.43	0.029	1.607	0.001
	Svl	1	0.001	0.031	7.048	0.017	1	4.171	0.006	0.984	0.609
	Morph x svl	3	0.001	0.007	0.510	0.615	3	13.32	0.019	1.047	0.057
	Residuals	159	0.028	0.694			159	673.9	0.947		
Orange/Not-orange (Hue)											
H	orange	1	0.004	0.026	4.468	0.044	1	11.71	0.011	1.811	< 0.001
	svl	1	0.004	0.024	4.064	0.046	1	7.514	0.007	1.162	0.007
	orange:svl	1	0.001	0.001	0.001	0.958	1	6.690	0.006	1.035	0.166
	Residuals	163	0.149	0.950			163	1053.6	0.976		
S	orange	1	0.005	0.196	41.11	< 0.001	1	4.970	0.012	1.999	< 0.001
	svl	1	0.001	0.023	4.853	0.037	1	2.386	0.006	0.960	0.685
	orange:svl	1	0.001	0.006	1.180	0.237	1	2.423	0.006	0.974	0.563
	Residuals	163	0.022	0.776			163	405.3	0.976		
Yellow/Not-yellow											
H	ylw	1	0.004	0.084	16.43	0.001	1	9.034	0.011	1.868	< 0.001
	svl	1	0.003	0.065	12.73	0.001	1	5.361	0.007	1.108	0.042

Source of variation		Size					Angle				
		Df	SS	R²	F	P	Df	SS x 10^6	R²	F	P
Four morphs											
	ylw:svl	1	0.001	0.021	4.147	0.035	1	5.481	0.007	1.133	0.021
	Residuals	163	0.039	0.830			163	788.3	0.975		
S	ylw	1	0.003	0.198	44.40	0.001	1	8.420	0.010	1.607	0.001
	svl	1	0.001	0.063	14.04	0.001	1	5.434	0.006	1.037	0.173
	ylw:svl	1	0.001	0.012	2.743	0.077	1	6.023	0.007	1.149	0.011
	Residuals	163	0.010	0.727			163	854.1	0.977		

Discussion

Our analysis reveals a complex picture of colour dynamics in adult common wall lizards, characterized by high categorical stability coupled with subtle, yet significant, ontogenetic shifts. The transition matrix analysis underscores an exceptional degree of phenotypic stasis, with over 97% of individuals retaining their discrete colour morph between captures. This stability is particularly absolute in orange phenotypes (none of them shifted to other morphs), while the rare transitions observed were almost exclusively limited to initially white individuals gradually acquiring pigments. Such results, corroborated by RRPP models, suggest that once a morph is established in the adult stage, it remains a permanent trait of the individual's phenotype. On the other hand, the high statistical significance of individual identity in RRPP models stems primarily from the clear chromatic divergence between discrete morphs (i.e., the stark differences between orange, yellow, and white). While the model effectively captures this broad inter-morph variance, it does not translate into high repeatability at the individual level (ICCs < 0.13). These low ICC values indicate that individuals within the same morph category share nearly identical spectral profiles; the subtle shifts between them are insufficient to allow for reliable discrimination, suggesting that intra-morph variation is characterized by a high degree of chromatic uniformity.

Despite this static categorical stability, the temporal effect included within replicates and trajectory analyses provide evidence of a directional maturation process. As individuals age and grow (SVL), they undergo a progressive pigment loading, leading to an intensification of saturation and shifts in Hue, favoring morph discrimination. Crucially, this ontogenetic intensification is not uniform across the population. While orange pigmentation appears to accumulate at a consistent rate across all individuals, the development of yellow colouration follows a unique, morph-specific trajectory. The significant interaction between the yellow grouping factor and SVL indicates that yellow morphs do not merely intensify their colour, but diverge in both the magnitude and direction of their chromatic maturation compared to non-yellow lizards. Consequently, while young adults of the four morph may present a certain degree of overlapping in chromatic profiles, ontogenetic growth acts as a driver of phenotypic divergence, sharpening the distinctions among morphs as they reach full maturity. This suggests that the colour morph in *P. muralis* is not a fixed state reached at maturity, but a developmental pathway that continues to specialize throughout the individual's life.

The high phenotypic stability and the lack of reversible transitions observed in our study have profound implications for the mechanisms maintaining colour polymorphism in *P. muralis*. Our results provide compelling evidence for high phenotypic canalization sharply contrasting with the hormonal plasticity model described in *U. stansburiana* [12]. In the latter species, colour morph expression seems linked to a "stay-or-go" strategy where the SPR locus controls the expression of the orange and blue morphs and social cues (through hormones) drive the transition from blue to yellow morph [12]. Our data show that the colour polymorphism in *P. muralis* is more likely governed by a robust genetic architecture involving two key loci: SPR (controlling the orange-to-white transition) and BCO2 (controlling the yellow-to-white transition). The absence of reversible shifts in our recapture data confirms that, unlike the plastic responses seen in *U. stanburiana*, the chromatic identity in *P. muralis* represents a fixed developmental commitment, likely evolved to provide stable and reliable social signals. In this scenario, *P. muralis* morphs appear to be strongly canalized from the late juvenile or early adult stage. The rare transitions we recorded (for the large majority from white to pigmented states) do not seem to represent reversible plasticity but rather a unidirectional ontogenetic completion of a pre-determined genetic program. This suggests that once the SPR or BCO2 pathways are activated during maturation, the individual’s chromatic identity is locked, supporting a model of genetic determination that is remarkably resistant to environmental fluctuation.

If morphs represent different evolutionary stable strategies, the intensification of colour through morph-specific trajectories may serve to minimize phenotypic overlap, reducing "cross-talk" between different socio-sexual signals. Indeed, morph classification by human observers in polymorphic lizards is frequently complicated by continuous phenotypic variation and inconsistent terminology between researchers often occurred, even in *P. muralis*. The identification of "mosaic" phenotypes in common wall lizards, such as white-orange and yellow-orange [24,25], is hindered because colour patches are often too small for consistent measurement, and subadults with isolated orange scales may be misclassified when they are actually in a transient ontogenetic phase. Further, the expression of the yellow morph is strictly linked to the accumulation of dietary carotenoids, specifically lutein and zeaxanthin, regulated by the BCO2 locus [17]. Because these pigments cannot be synthesized de novo and must be acquired through the diet [27], the intensity of the yellow colouration is inherently susceptible to environmental factors, such as the local availability of resources and specific climatic conditions [28], even making difficult to assess whether colour variation is discrete or continuous, as seen in *Zootoca vivipara* [29]. Similar ambiguity exists for *Urosaurus ornatus*, where the same male morph has been described alternatively as "yellow" or "green" by different authors [30,31], while others labelled orange-throated males with blue patches as "blue-green" or "green" depending on subjective criteria [32,33]. Similarly, in *Ctenophorus modestus*, mixed orange-grey and yellow-grey phenotypes are not formally recognized because the relative extent of these colours varies continuously, making them visually indistinguishable from pure morphs without objective statistical clustering [4]. Even in *U. stansburiana*, identification is challenged by "blue-yellow" phenotypes, especially since pigment boundaries can create spectral shifts toward green [12]. Based on the sources provided, the observed divergence in colour morphs in Common wall lizards likely reflects the outcome of disruptive selection acting to maximize signal clarity and minimize phenotypic overlap, ensuring that visual ornaments function as reliable signals of strategy despite significant environmental and developmental noise [34]. By maximizing signal clarity as individuals grow, the system may facilitate the action of frequency-dependent selection in maintaining morphs, where each morph occupies a distinct socio-ecological role that is "locked in" during early development. Consequently, the maintenance of this polymorphism likely relies on a balance of selective advantages that are fixed for the life of the individual, rather than on the ability of individuals to plastically adjust their phenotype to fluctuating conditions.

The behavioural and physiological patterns observed in *P. muralis* morphs closely align with the theoretical framework for "strategy signals" described by Tibbetts et al. [34]. In this system, colour morphs function as discrete, multimodal visual ornaments that effectively categorize individuals into non-overlapping behavioural types. As predicted for badges of strategy, these traits exhibit high stability and low condition-dependence once established at sexual maturity, ensuring the signal remains a reliable indicator of an individual's fixed socio-ecological role. The tight linkage between ventral colouration and a suite of co-adapted traits, including immune response, endocrine profiles, and reproductive tactics [6,35–38], confirms that these morphs operate as integrated phenotypic markers that minimize social cross-talk and optimize individual fitness within a competitive landscape.

In conclusion, our findings highlight a relevant divergence in evolutionary strategies: while *U. stansburiana* relies on social plasticity, *P. muralis* seems to exhibit a strictly canalized genetic program. This confirms that monitoring ontogenetic colour shifts in adult populations is not only a powerful tool for distinguishing between fixed and plastic traits, but also a practical framework for evaluating the mechanisms that maintain phenotypic diversity in nature.

Methods

Data collection

Data came from a fieldwork conducted from March to September between 2009 and 2012 on a farm in the surrounding of Pavia (Northern Italy). Lizards were captured using noosing by teams of four-six trained observers, measured for snout–vent length (SVL), sexed, and photographed ventrally to determine colour morph and allow individual photo-identification [39]. Colour morphs were coded using a pair of dichotomous variables corresponding to the SPR/BCO2 genetic system controlling colour expression: the presence or absence of orange pigmentation (org: O/o) and the presence or absence of yellow pigmentation (ylw: Y/y). The combination of these two variables defined four discrete morphs (Fig. 2): white (oy), yellow (oY), orange (Oy), and yellow–orange (OY). All individuals were released at the site of capture immediately after processing. A total of 761 individuals were recorded over the study period, including 387 females and 374 males, with an average of 190 captures per year (range: 93 in 2011 to 243 in 2009). Morph frequencies in the overall capture dataset were 183 white (84 females, 99 males), 380 yellow (214 females, 166 males), 117 orange (48 females, 69 males), and 81 yellow–orange (41 females, 40 males) individuals. For each lizard, the throat was photographed adjacent to a GretagMacBeth using a Nikon D50 camera with a resolution of 12 megapixels, equipped with a Nikkor 60 mm AF-S Micro lens, at a fixed distance of 18 cm. To standardize lighting conditions, photographs were taken in a 44 × 44 cm lightbox illuminated with two daylight 22 W circular neon tubes (Reporter 55100 Studio-kit).

From this sample, we selected 383 images corresponding to 167 adult lizards (82 males and 85 females) that were recaptured at least once over a minimum period of one month. The mean recapture frequency was 2.3 ± 0.6 times (range: 2–5), with a mean time interval between first and last captures of 266 ± 346 days (range: 33–1539 days). Regarding morphs, among the 82 males, 15 lizards were orange (Oy), 43 yellow (oY), 9 yellow-orange (OY), and 15 white (oy). Among the 85 females, eight displayed orange colouration, 51 yellow, 10 yellow-orange, and 16 white. Images were processed following Sacchi et al. (2013): we measured the colour of throat by selecting the areas of all scales showing the colouration (i.e. black spots were excluded) using the 'magic wand' tool (approximately 22000 pixels), and recording the RGB levels using the histogram palette. Finally, the RGB colour values were rearranged in the Hue, Saturation and Brightness (HSB) system.

Statistical analyses

To assess the ontogenetic stability of colour morphs in adult lizards, we adopted a two-step approach analyzing both discrete morph categories and continuous chromatic variables (HSB). First, we evaluated the stability of discrete morph assignments across recaptures. We constructed a transition matrix to calculate the probability of an individual retaining its initial morph or transitioning to a different one. We tested the hypothesis that morphs are fixed in adults by calculating the percentage of agreement between the morph assigned at two consecutive captures. The sample available for the analysis included 216 transitions.

To evaluate the stability of colouration beyond discrete morph categories, we analyzed the quantitative properties of the throat colourations using the HSB colour space. Rather than reducing colour information to summary statistics (e.g., mean or median), we retained the full chromatic complexity of each individual by extracting 512 values representing the frequency distribution of pixel values for Hue (H, Fig. 2) and Saturation (S). Brightness (B) was excluded from the analysis to minimize the confounding effects of lighting conditions and exposure. To assess the temporal consistency of these colour profiles and quantify morph repeatability within individuals, we employed a linear model evaluated via Residual Randomization Permutation Procedures (RRPP), a robust framework for testing high-dimensional data through residual permutation [40]. This method is particularly suited for complex datasets as it allows for accurate hypothesis testing without the strict distributional assumptions of traditional parametric statistics. Specifically, we used within subject replication implemented in the lm.rrpp.ws function in the "RRPP" package [40] in R to model the total colour variance into three components: individual identity (subject), morph, and measurement replicates within individual (reps). This hierarchical approach allowed us to specifically test for measurement error among morphs and directional shifts over time. In this model, the morph effect accounts for transition among colour morphs within individuals, allowing us to test for ontogenetic transitions. Three models for each analysis were run according to the way we coded morph: as a four-level factor including all possible colour combinations (white, orange, yellow, yellow-orange), or as a two-level factor, once for the orange component (org) and once for the yellow one (ylw). In all cases, the reps term represents the measurement error (ME) but also encompasses broad-scale temporal effects on colouration. Crucially, a significant effect of reps can indicate a directional increase in colour expression, such as the accumulation of pigments leading to a more saturated and extensive colouration over the body as the individual ages. Furthermore, the morph × reps interaction was included to quantify systematic error within morphs, testing whether colour shifts follow a morph-specific trajectory over the study period. Repeatability was further quantified using the Intraclass Correlation Coefficient (ICC) derived from the RRPP model components. Following the framework proposed by Collyer and Adams [41], we computed three distinct ICC metrics to partition measurement reliability: the standard ICC, which reflects the overall consistency of measurements; the adjusted ICC (ICCa), which accounts for absolute agreement by incorporating the variance attributed to systematic error (i.e., the *reps* effect); and the corrected ICC (ICCc), which isolates the measurement concordances by excluding the variance associated with directional temporal shifts. This multi-index approach allowed us to distinguish between random noise and systematic bias in our colour quantification, given that when ICC values diverge, systematic ME can be implicated. The analysis was conducted separately for Hue and Saturation vectors, using 999 permutations to generate significance levels and without reducing dimensionality via Principal Component Analysis to preserve the raw distributional data.

A two-steps phenotypic trajectory analysis [42] was used to compare ontogenetic patterns in colour among morphs over the study period. This technique has the advantage of taking into account the dynamic aspect of the path that a specific trait follows during individual growth [42,43]. Here, the colour phenotypic trajectory (CPT) is represented by the vector that joins the starting and ending points (e.g., first and last captures) in the colour space. CPT is characterised by its length and its direction; consequently, two trajectories may differ because of their lengths (size), the angle between their directions (angle) or both [44]. Differences in vector size and angle among morphs were assessed using RRPP models with 999 random permutations of data as implemented in the function lm.rrpp of the “geomorph” package. The dependent variable was the distance matrix among individuals for sizes and angles of CPTs (in two separate models), whereas morphs, SVL, and their interaction were the independent terms. In these models the SVL term corresponds to the effect of reps in the repeatability models, therefore explicitly capturing the colour variance due to the ontogenetic change. Analyses were performed using R ver. 4.5.1 [45], and otherwise indicated values correspond to means and standard errors.

Declarations

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Data availability Statement: The colour data (Hue and Saturation) and individual covariates have been deposited in Zenodo under the accession link xxxxxxx

Author contributions: Roberto Sacchi (conceptualization, methodology, formal analysis, investigation, data curation, writing original draft), Stefano Scali (conceptualization, methodology, formal analysis, data curation, writing review and editing), Marco Mangiacotti (conceptualization, methodology, formal analysis, investigation, writing review and editing), Marco A.L. Zuffi (methodology, formal analysis, data curation, writing review and editing). All authors reviewed and approved the final version of the submitted manuscript.

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Figures

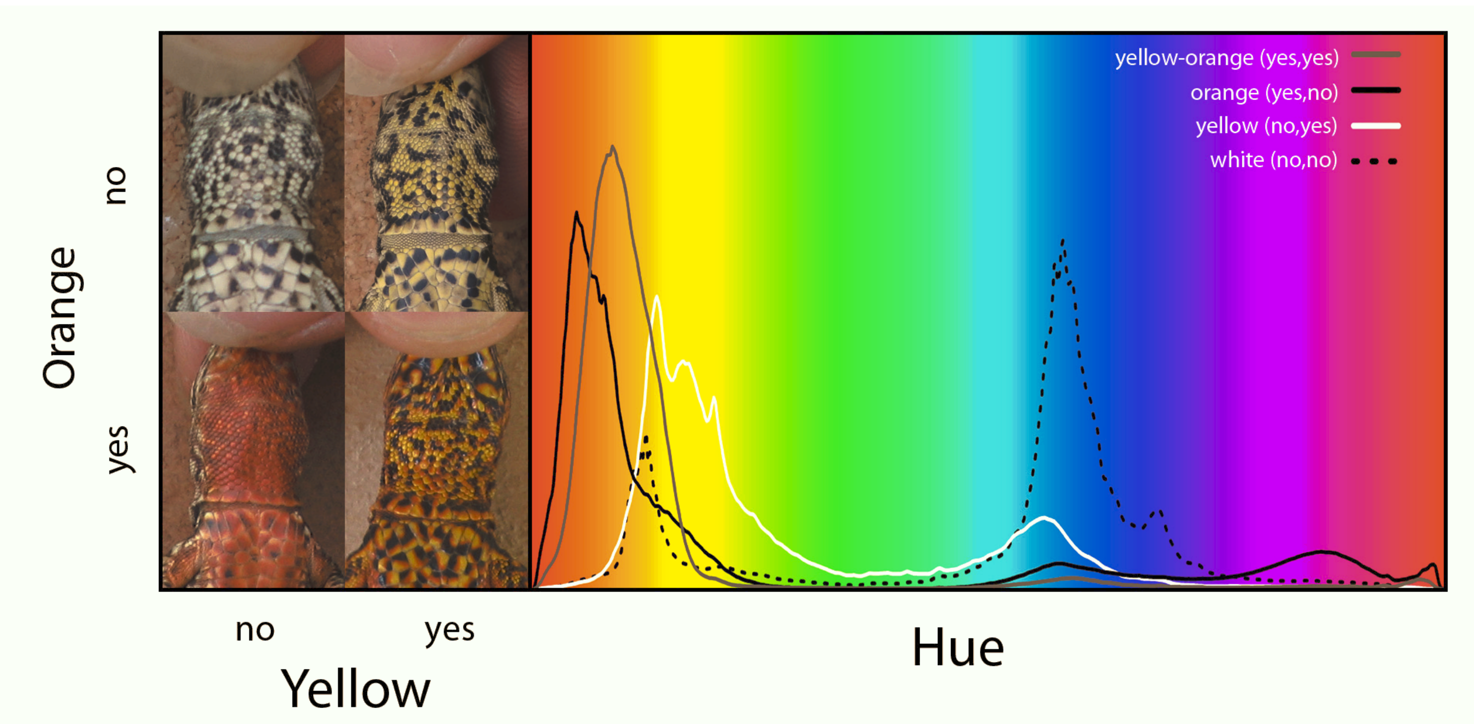

Figure 2

The four colour morphs of the common wall lizard (*Podarcis muralis*) as defined by the two binary variables (Orange/Non-orange and Yellow/Non-yellow). From the upper left, clockwise: White, Yellow, Orange, and Yellow-orange. The right panel illustrates the mean frequency distributions of hue values for each colour morph.