



# Common Wall Lizard Females (*Podarcis muralis*) do not Actively Choose Males Based on their Colour Morph

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## Abstract

Identifying the processes that lead to the evolution and maintenance of links between colour morphs and behavioural strategies has implications for the evolution of reproductive isolation and sympatric speciation. Sexual selection may play a significant role in the evolution of colour pattern complexity in reptiles, particularly when there are fitness consequences associated with mating with males of different colour morphs. In this article, we explored if common wall lizard females (*Podarcis muralis*) actively select males according to their morph in a colour-assortative pattern using a multiple-choice experiment with both visual and chemical cues. We failed to identify female active mate choice, as females did not choose males based on male colouration or femoral pore secretions. Indeed, females equally entered the three preference compartments and spent nearly the same amount of time within them, irrespective of both colour and odour of males. Consequently, our results do not support the hypothesis that colour polymorphism in this species may be driven by colour-assortative mating promoted by females. However, we cannot exclude the possibility that females may choose males according to their colour following a flexible choice strategy, nor the possibility that females actively discriminate among males according to qualities that are not directly related to morph-specific strategies.

## Introduction

Identifying the proximal mechanisms leading to the evolution and maintenance of colour polymorphism has relevant consequences on the understanding of evolutionary processes, particularly the evolution of reproductive isolation and sympatric speciation (Huxley 1955; West-Eberhard 1986; Pryke & Griffith 2006; McKinnon & Pierotti 2010). Morphs are generally associated with the occurrence of alternative strategies, which involve complex combinations of behavioural, morphological, physiological and life history characteristics (Sinervo & Lively 1996; Svensson et al. 2001; Sacchi et al. 2007a, 2009). Therefore, colour morphs represent alternative, locally adapted optima (Calsbeek et al. 2010). Maintenance of alternative

strategies within a single species is generally regarded as the result of the equilibrium between opposite selective pressures: disruptive selection (i.e., correlational selection), which promotes the association between life history traits and colouration, and the stabilizing selection (e.g., frequency dependent selection), which preserve gene flow among morphs (Sinervo & Svensson 2002; Noble et al. 2013). Because the equilibrium between alternative strategies is not necessarily stable under changing conditions, some morphs can be favoured whereas other may decline; thus peculiar phenotypes can be fixed through allelic selection thereby promoting sympatric speciation (i.e., exclusive expression of only one alternative, West-Eberhard 1986). Morph fixation may be accompanied by 'character release' and fast divergence from

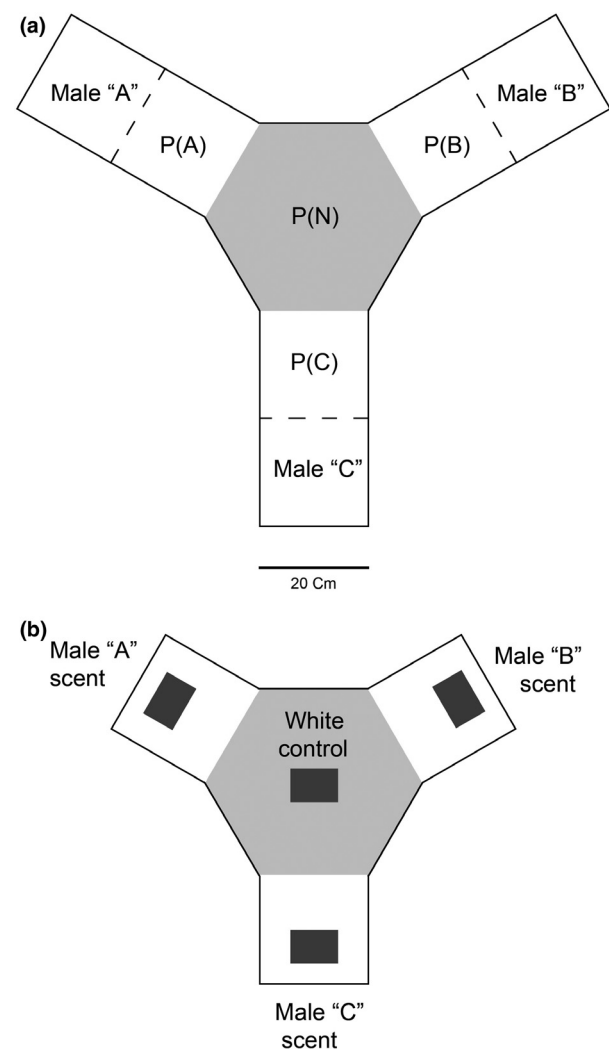
the ancestral condition, thereby promoting sympatric speciation ('morphic speciation', [West-Eberhard 1986](#); [Corl et al. 2010](#)).

Natural selection has been traditionally assumed to be involved in the maintenance of colour morphs, notably negative frequency dependent selection (reviewed in [Gray & McKinnon 2007](#)). However, sexual selection may also play a significant role in the maintenance of colour pattern complexity in animals, particularly when individuals of alternative morphs do not mate randomly ([Wellenreuther et al. 2014](#)). In a recent review on sexual selection in polymorphic species, [Wellenreuther et al. \(2014\)](#) showed that colour assortative mating is common across animals and discussed how it facilitates or constrains the maintenance of colour polymorphism.

Lizards offer a good model to investigate the evolution and maintenance of colour polymorphism, because polymorphic systems are quite common and some underlying mechanisms have been already hypothesized and tested ([Thompson & Moore 1991](#); [Thompson et al. 1993](#); [Sinervo & Lively 1996](#); [Zamudio & Sinervo 2000](#); [Sinervo et al. 2001](#); [Sinervo & Zamudio 2001](#); [Huyghe et al. 2007](#); [Sacchi et al. 2007b](#); [Galeotti et al. 2013](#); [Runemark et al. 2010](#)). In contrast with birds, lizards offer the advantage of living at high population density, being widespread and easy to be captured, manipulated as well as maintained in captivity over long period, without altering their normal behaviours. Notably, most studies have investigated the effect of natural selection on colour polymorphism, but only few have explored the contribution of sexual selection, specifically that of female preference, on its maintenance. Indeed, assortative mate choice may be relevant for a female when male morphs contribute differently to her fitness ([Healey et al. 2008](#); [Pérez i de Lanuza et al. 2012](#)). However, few studies have actually addressed female preference with respect to male morphs, and those that have revealed inconsistent results ([Alonzo & Sinervo 2001](#); [Hamilton & Sullivan 2005](#); [Healey et al. 2008](#); [Lattanzio et al. 2014](#)). For example, *Uta stansburiana* females did not mate assortatively according to colour morphs, but followed flexible mate choice rules according to population density ([Alonzo & Sinervo 2001](#)). By contrast, females of Australian painted dragon (*Ctenophorus pictus*) did not discriminate between single males of different head colour but preferentially associated with polymorphic as opposed to monomorphic male dyads, possibly to increase the likelihood of mating with different males ([Healey et al. 2008](#)). Furthermore, [Lattanzio et al. \(2014\)](#) recently found that *Urosaurus ornatus* females actually discriminate male

partners, but mate preference did not relate with the colour of male dewlap, but rather with the secretions of their femoral pores.

The common wall lizard (*Podarcis muralis*) is an European lacertid showing six distinct colour morphs, including three pure (i.e., white, yellow and red) and three intermediate phenotypes (white-red, yellow-red and white-yellow, see [Fig. 1](#) in [Sacchi et al. 2013](#) for a panoramic of male and female morphs). Morphs occur in both sexes and within the same population,



**Fig. 1:** Drawing of the arenas used for female preference tests with visual (a) and chemical (b) stimulations. In (a), dashed lines denote the opaque walls, while the areas P(A), P(B) and P(C) are the areas in which females were considered to express preference towards male A, male B or male C. The area P(N) denotes the neutral area, in which females were considered to have not expressed a preference. In (b), dark grey boxes in white compartments denote the paper sheets with the secretions from the femoral pores of males, while the box in the grey hexagonal compartment represents the clean paper used as white control.

but their relative frequencies in Northern Italy are highly variable among populations (Sacchi et al. 2007a,b). Yellow-throated females are r-strategists, whereas white-throated females are K-strategists while red females have been supposed to follow a mixed strategy (Galeotti et al. 2013). Additionally, the breeding performance of female morphs may be not independent of that of males, and yellow females are most efficient when experimentally mated with males of their own colour (Galeotti et al. 2013). These findings suggest that assortative mating between colour morphs should occur (Galeotti et al. 2013), and field observations of morph mating behaviour seem to support this conclusion (Pérez i de Lanuza et al. 2012). Recently, we found that femoral pore secretions of males match colour morphs, giving the females the opportunity to choose their partners accordingly (Pellitteri-Rosa et al. 2014).

In this article, we performed a multi-choice experiment in order to assess if female common wall lizards choose males according to colour morph (i.e., if they are male-morph specialist). Hamilton & Sullivan (2005) rightly caution against staging mate choice experiments on single male traits, as females may base mate choice decisions on multiple traits. Therefore, we allowed females to choose males basing on both visual and chemical signals and comparing their patterns of response under these two different communication channels. If females actively seek partners and mate in a colour-assortative way, our specific prediction is that a female faced with the choice of three males with different colouration should approach more frequently and spend more time with that of her own morph. Given that femoral pore secretions are correlated with male morph (Pellitteri-Rosa et al. 2014), we also predict that females presented with the scents of three males differing in colour should be attracted to the scent of their own colour morph.

## Methods

### Subjects and Housing Conditions

We captured sexually mature lizards (SVL > 50 mm, Sacchi et al. 2012) by noosing in five sites in the surrounding of Pavia (Lombardy, Northern Italy), between February and March 2014. To ensure that females were receptive, we captured not mated females as soon as they appeared in the field. Common wall lizard females do not store sperm over the winter, so they mate at the beginning of the season (Pellitteri-Rosa et al. 2012), and their status (mated vs. not mated) can easily be assessed in the field by

visually checking the presence of mating scars (Bauwens & Verheyen 1985; Bajer et al. 2010; Galeotti et al. 2010). Males were collected in sites well apart (> 5 km) from that in which we captured females. Since the intensity of morph colouration shows both ontogenetic and geographic variation, especially for white-yellow and white-red individuals (Sacchi et al. 2013), we only considered four female morphs, i.e. white, pure yellow, pure red and yellow-red. Each individual was measured using a digital caliper (accuracy 0.1 mm) for SVL, weighed (accuracy 0.1 g), and transferred to the laboratory within 2 h from capture. Lizards were housed individually in plastic cages (20 × 30 × 20 cm) provided with a paper sheet as substratum, a small tile as a basking site and a single brick as shelter. We provided mealworms as food and water *ad libitum*. All lizards were maintained and fed for at least 1 wk before trials were started, and released at their capture sites at the end of the experiment. Overall, we housed 32 females (eight individuals for each morph) and 42 males (14 individuals for each morph but yellow/red, which were not considered as stimulus). Before release, we again weighed a sub-sample of individuals, and found that all of them maintained their original body mass (change in grams of the average mass, paired-sample *t*-test; females: +0.16 g,  $t_{22} = 1.372$ ,  $p = 0.18$ ; males: -0.01 g,  $t_{23} = -0.192$ ,  $p = 0.85$ ).

### Experimental Design

We formed 14 groups of three males ('triads' hereafter), by randomly choosing one male of each colour morph, matched for size, but randomly assorted concerning both lateral colour patches and blue spots. As body mass and SVL were strongly related in all morphs (females:  $r_p = 0.72$ ; males:  $r_p = 0.94$ ), and body mass could vary according to the amount of food eaten before capture, we used SVL as a measure of body size. Mean SVL of males was  $63.6 \pm 0.9$  mm (range 51.4–75.5 mm), and mean size difference between males within a given triad was 3.3% of SVL (range: 0–10.2%). Mean SVL of females was  $61.7 \pm 0.7$  mm (range 52.2–70.1 mm), and did not differ among morphs ( $F_{3,28} = 1.052$ ,  $p = 0.38$ ). Each of the 32 females was randomly assigned to one of the 14 triads, so triads were used on average in 2.4 trials (range 1–4). We therefore tested female preference in two experiments: in the first one the female responded to a simultaneous visual presentation of the three males (hereafter 'visual experiment'), while in the second one the female was tested with the scents of the same three males used in the visual trial

(hereafter 'scent experiment'). The trial arenas (one for each experiment) were modified after LeBas & Marshall (2001) and Bajer et al. (2010), which were designed for testing spatial association between a female and a male. This association is an important pre-requisite for assortative female 'mate choice'. Our arenas were composed of a central hexagonal box (20 cm side) connected to three 20 × 20 cm compartments oriented at 120° one each other (Fig. 1). The central hexagonal compartment was regarded as neutral area for the female, while the three annexed compartments were defined as the preference ones (Fig. 1). Their floors were raised by 2 mm with respect to the central hexagonal compartment to prevent passive access by female due to random walking (i.e., females could remain within the neutral area by moving along the steps). In the visual experiment, the three preference compartments were separated by a transparent plexiglass divider from the male compartments (20 × 20 cm, Fig. 1a), while in the scent experiment the bottom of the preference compartments was completely opaque (Fig. 1b), like all the walls of the arena. In the scent experiment, the absorbent paper sheets used as substratum in the housing cages of males were used to obtain the scents and secretions from the femoral glands of lizards (Lopez et al. 2002), and were fixed to the floor of the three preference compartments. A clean paper was additionally fixed in the centre of the hexagonal neutral compartment as white control. Paper sheets were removed from the male cages and placed in the arena immediately before each experiment began. Trials were conducted indoor, from 17 March to 22 April between 08.00 and 16.00. Equal lighting was maintained throughout this arena with the aid of a suspended 75 W lamp. In order to control for body temperature, we placed each lizard in a thermally controlled terrarium (20 × 30 × 20 cm, 32°C) for 10 min, so when the trial started body temperatures (measured using a hand-held IR thermometer, Lafayette TRP-39IR, at a distance of 5 cm) were on average  $32.8 \pm 0.3^\circ\text{C}$  (range: 26.0–38.5°C) in females, and  $32.7 \pm 0.3^\circ\text{C}$  (range: 23.5–38.0°C) in males. The starting body temperature did not significantly differ among female morphs ( $F_{3,60} = 1.081$ ,  $p = 0.36$ ), either among male morphs ( $F_{2,93} = 0.374$ ,  $p = 0.69$ ). Then we gently moved individuals from pre-heating cages into the arena: males were assigned randomly among preference compartments, while females were placed in the centre of the hexagonal compartment within a opaque small box 5 min before the trial was started. The box was then removed and female behaviour was observed and video recorded with a SONY

Super Night Vision Camera (M020-s53-001) connected by a 20 m isolated wire to a laptop PC located in a room adjacent that housing the arena. Females were observed continually for 1 h and did not show any sign of stress during the trials. The arenas were carefully washed with detergent between subsequent trials in order to remove any chemical stimuli left by lizards from the previous trial. At the end of each trial body temperatures of both males and females were recorded in order to control for the effects of heat dispersion on female activity. Ending temperatures were on average  $25.6 \pm 0.3^\circ\text{C}$  (range: 20.8–33.0°C) in females, and  $24.5 \pm 0.3^\circ\text{C}$  (range: 20.0–32.0°C) in males, and did not differ among female morphs ( $F_{3,60} = 1.457$ ,  $p = 0.24$ ) either among male morphs ( $F_{2,93} = 0.015$ ,  $p = 0.98$ ). If a female had not approached any of the preference compartments at least once, the trial was repeated with a new triad of males. Over all, we obtained 64 successful recordings, two for each female.

#### Female Preference Variables

Video recordings of female behaviour were analysed to measure (1) the total time spent in each preference compartment, (2) the number of accesses to each preference compartment, (3) the maximum duration of a single access for each preference compartment and (4) the latency to explore the arena (i.e., the time until the first movement). We considered a lizard entering a preference compartment when both the forehands were on its floor. The total time and maximum duration were both interpreted as a measure of attraction to the male, and consequently an evaluation of the female preference. The latency and number of accesses to the compartments were regarded as a measure of the propensity of the female to explore the arena, and consequently they represent indexes of female activity (or the intensity of stimulation) rather than of female mate preference.

#### Statistical Analyses

As three measures for every preference variable but latency were simultaneously collected (one for each male morph), we used a multivariate analysis of variance (MANOVA) to compare the total time, the maximum duration and the access number among female morphs. In this analysis, the matrix of the three measures was the dependent variable, and three separate analyses for each preference variable were performed. The predictors were the female morph, the female body size (SVL), the female starting and ending body

temperatures (the Pearson correlation coefficient between these temperatures being  $r_p = 0.23$ ), the experiment (i.e., visual vs. scent). Additionally, we added the female morph  $\times$  experiment interaction in order to account for a different response of morphs to visual and chemical cues, and the female morph  $\times$  female body size interaction to control for a differential effect of age among morphs. The size of males was not included in the analysis as preliminary tests have shown that none of the female preference variable was related with it (statistics not shown). Multivariate homogeneity of variance was achieved in all cases, so raw data were used in the analyses. In order to control for the effects of the replication of both male triads over the females and females over the experiments (each female was indeed tested once for visual and once for chemical cues), we performed a linear mixed model (LMM) for each of the three measures within every female preference variable. In these models, the same fixed effects as in MANOVAs were used, and the female identity nested within the male triad entered as the random component. The significant effects of the random effects were assessed using likelihood-ratio  $\chi^2$  tests.

The latency was analysed using a linear mixed model, after the variable was log transformed to achieve normality, using the same predictors as in MANOVAs, and the same random structure as in previous LMM. Statistical analyses were performed using R ver. 3.1.0 (R Core Team 2014), and otherwise stated, reported values represent means and standard errors.

## Results

The latency did not significantly vary among female morphs, and was not affected by body temperatures

and female body size (Table 1). Interestingly, we found a significant difference between experiments (Table 1), as females started exploring the arena significantly earlier with olfactory than visual stimulation (Fig. 2). The random effect was also significant ( $LR\chi^2 = 16.5$ , d.f. = 1,  $p < 0.001$ ), but it mainly depended on the female identity, rather than on the male triad (intraclass correlation coefficient: females within triad: 0.70, triad: 0.01).

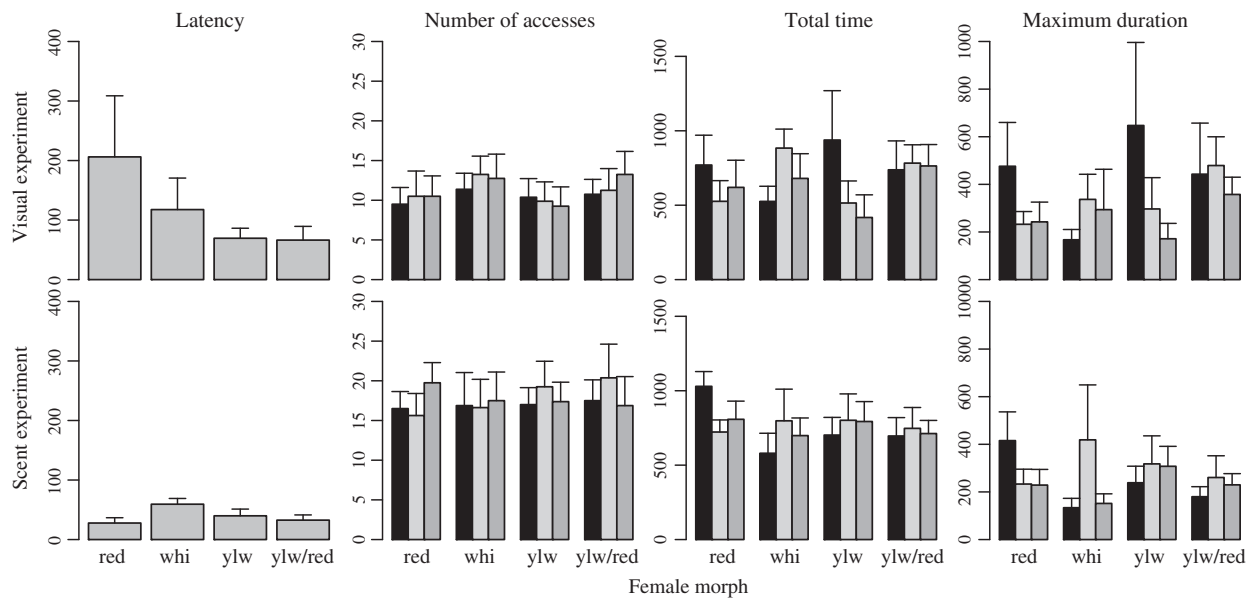
The MANOVA performed on the number of accesses to the preference compartments detected a significant effect for both the experiment (Pillai's trace = 0.241,  $p = 0.0038$ ) and the ending temperature (Pillai's trace = 0.246,  $p = 0.0033$ ), while no significant effects were found for all other predictors (Table 1). These results suggested that, irrespective of their morph, females entered the preference compartments more frequently in scent than in visual trials (Fig. 2; see Supplementary materials, table S1 for beta values), and were less active with faster cooling (see Supplementary materials, Table S1 for beta values). No significant effects were also detected for both total time and maximum duration within preference compartments (Table 1, Fig. 2). All the effects of the random factors assessed through linear mixed models were never significant ( $LR\chi^2 < 2.753$ , d.f. = 2,  $p > 0.10$ , see Supplementary materials, Table S2 for details).

When negative results are obtained, a good practice would be to perform a power analysis for the statistical tests to rule out the possibility of false negatives. In our case the probability to detect a significant effect ranged from 0.90 at  $p = 0.05$  to 0.75 at  $p = 0.01$ . Therefore, we were confident that the lack of morph discrimination would actually be due to lack of female preference rather than to lack of statistical power of MANOVAs.

**Table 1:** Statistics of the analyses used to compare the responses of female colourmorphs in visual and scent experiments

Predictors	Response variables											
	Latency			Number of accesses			Total time			Maximum time		
	F	df	p	Pillai	df	P	Pillai	df	p	Pillai	df	p
Female morph	0.447	3, 31.2	0.72	0.092	3	0.65	0.132	3	0.65	0.123	3	0.69
Experiment	19.973	1, 30.7	<0.001	0.241	1	0.0038	0.052	1	0.46	0.072	1	0.30
Body size	0.136	1, 30.0	0.71	0.078	1	0.26	0.024	1	0.76	0.044	1	0.54
Start temperature	0.687	1, 36.2	0.69	0.146	1	0.054	0.049	1	0.48	0.012	1	0.90
End temperature	0.357	1, 39.2	0.55	0.246	1	0.0033	0.025	1	0.75	0.120	1	0.10
Morph $\times$ Experiment	2.622	3, 27.5	0.070	0.174	3	0.42	0.128	3	0.67	0.126	3	0.68
Morph $\times$ Body size	0.216	3, 27.8	0.88	0.121	3	0.71	0.226	3	0.21	0.162	3	0.48

Latency was analysed using a linear mixed model (denominator df are estimated using the Satterthwaite approximation), whereas all other variables were analysed through MANOVAs.



**Fig. 2:** Comparison of the responses of female morphs when facing with male colour morphs in visual (upper panels) and chemical (lower panels) experiments. Sample size is eight females for each morph and bars represent standard errors. Colours in bars represent male morphs; black: white male, light-gray: yellow male, and dark-gray: red male.

## Discussion

The hypothesis that colour-assortative mating would occur in the polymorphic common wall lizards was based on the observations that homomorphic male-female pairs in the wild are significantly more common than heteromorphic pairs (Pérez i de Lanuza et al. 2012), and chemical femoral secretions of males differ among morphs, thus having the potential to be used by different male morphs to signal their behavioural strategies to females (Pellitteri-Rosa et al. 2014). Here, we experimentally tested this prediction, and particularly whether females spatially associated with males of the same/different colour morph. Our experiments failed to identify active female mate choice based on male visual or chemical cues alone. Indeed, females of the three preference compartments equally spent nearly the same amount of time within them, irrespective of both colour and odour of males. These results do not support the hypothesis that assortative mating based on male colour morphs promoted by active female preferences might be involved in the maintenance of colour polymorphism in this species.

Previous experimental studies on other polymorphic species have failed to demonstrate assortative female preference based on male colour morphs (Alonzo & Sinervo 2001; Healey et al. 2008), with the only exception being the tree lizard (Lattanzio et al. 2014). In this species polymorphic males adopt different territorial strategies, and females choose males

accordingly: yellow females exhibit preference to avoid ultra-dominant males, whereas orange females prefer dominant ones. However, the female morphs of tree lizards responded to visual and chemical signals differently, and were capable of distinguishing among the male morphs using chemical signals alone (Lattanzio et al. 2014). Actually, our experiments showed that females were more interested by scents rather than colour of males, thus revealing that they probably assessed information conveyed by gland secretions. Apparently, females did not use this information for discriminating among male morphs.

Three main considerations might follow these experimental conclusions in the light of possible interactions between female choice and colour polymorphism. Firstly, our experimental design was strictly settled to explore colour-assortative mating, and consequently was not fit to effectively show different patterns of association. All females were in fact housed in the same conditions in order to remove any confounding effect due to lizard manipulation. Consequently, the experimental settings were not suitable to detect a flexible female choice, and this possibility remains an open question (Alonzo & Sinervo 2001; Alonzo & Sinervo 2001; Healey et al. 2008).

A second possible explanation is that females are not interested in the specific strategies related to morphs, but rather on the individual quality of males. Actually, lateral colour patches and blue spots (which do not differ among morphs) have been shown to be

condition-dependent expression, potentially functioning as complex multicomponent signals of male quality (Pérez i de Lanuza et al. 2014). By contrast, ventral polymorphic colouration apparently did not share this function (Pérez i de Lanuza et al. 2014). Thus, if females have been selected for actively searching for best partners despite their morphs, our experiments would not be able to detect such a kind of mate preference. This is because females would not be expected to spend time differentially among the preference compartments of the arenas.

The last alternative explanation is that female common wall lizards do not actively choose sexual partner before the copula at all, neither according to their morph, nor to their quality. Lizard females have been often pointed as 'passive lovers' (Noble & Bradley 1993), and despite several attempts, very few studies have convincingly demonstrated that females in lizards actively discriminate among males (Tokarz 1995; Olsson & Madsen 1998; Olsson 2001; Olsson et al. 2003). Thus, female common wall lizards could actually behave as passive lovers, accepting copulation attempts from every approaching male. The possibility that common wall lizard females might perform a cryptic post copulatory choice would still be open (Olsson & Madsen 1998; Pellitteri-Rosa et al. 2012), but pre-copulatory male–female interactions would be completely controlled by males.

An unexpected result of this study is that females were more interested in chemical cues rather than in male colouration, as they were active earlier and explored more frequently the preference compartments when presented with male scents. These findings might indicate that females not only do not actively promote mating from males, but rather they might look on them cautiously, probably in order to avoid male harassment, which has been already associated with increased female mortality and risk of injuries during mating in other species (Le Galliard et al. 2005; Healey et al. 2008). Indeed, common wall lizard males are highly aggressive in defending territories, and during courtship males try to stop females biting their flanks. Accordingly, the preference for low male bite force by female *P. melisellensis* (Huyghe et al. 2012) and the preference for the scent of older over young males (Lopez et al. 2003) have been both interpreted as a selection against male harassment. Colour polymorphism is not a signal of status or fighting ability in intrasexual conflicts (Sacchi et al. 2009), so females cannot prevent courtship attempts by highly aggressive males solely by looking for partners on the basis of their morphs. Higher activity in scent experiments (without any choice for a specific male)

may consequently follow a stressful condition following the perception of three males in their proximity without the possibility to see them. Our results might therefore reflect the avoidance of physical nuisance by males, rather than a real interest for them.

An alternative explanation of the higher mobility of females in scent experiments relies on the opposite roles played by chemical and visual cues in foraging and territorial strategy in lacertid lizards. Contrary with all iguanians, which are ambush predators relying mostly on visual cues, lacertids are active foragers, typically using chemosensory cues to locate prey and interact on conspecifics (Pianka & Vitt 2006). Several researchers have shown that femoral secretions may provide reliable information on several aspects of male quality, such as age (Lopez et al. 2003), health status, parasitic load and immune response (Lopez et al. 2006; Martin & Lopez 2006). In *P. muralis*, the effects of parasite load and immunocompetence on the relative proportions of lipophilic chemical compounds in femoral gland secretions have been demonstrated (Martin et al. 2008), as well as differences in some compounds (especially tocopherols and furanones) among morphs (Pellitteri-Rosa et al. 2014). Triads in our experiment were matched in size, and were randomly assorted concerning both lateral colour patches and blue spots, which are the only coloured traits with condition dependent expression, thus potentially conveying information about male quality (Pérez i de Lanuza et al. 2014).

In conclusion, our experiments with female common wall lizards revealed that there was no active choice for any male morph by females within their receptive period, thus not supporting the hypothesis that colour-assortative mating could maintain colour polymorphism in this species.

### Ethical Notes

Captures and lizard housing were carried out in accordance with European habitat directive (92/43/CEE) and Italian law for the use of animals in scientific research (Aut. Prot. 0011511/PNM).

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### Supporting Information

Additional supporting information may be found in the online version of this article:

**Table S1.** Effect size (mean  $\pm$  standard error) for the MANOVA performed on the number of accesses to the preference compartments by females.

**Table S2.** Results of the likelihood-ratio  $\chi^2$  tests used to evaluate the random effect in LMMs performed to control for the replication of females within male triads.