Close encounters of the three morphs: does color affect aggression in a polymorphic lizard?

Stefano Scali[1], Marco Mangiacotti[2], Roberto Sacchi[2], Alan Jioele Coladonato[2], Mattia Falaschi[3], Luca Saviano[1], Marina Giulia Rampoldi[1], Matteo Crozi[2], Cesare Perotti[2], Francesco Zucca[2], Elisabetta Gozzo[1], Marco Alberto Luca Zuffi[5]

[1] Natural History Museum of Milan, Corso Venezia 55, I-20121, Milano, Italy

[2] Department of Earth and Environmental Sciences, University of Pavia, Via Taramelli 24, I-27100, Pavia, Italy

[3] Department of Environmental Science and Policy, University of Milan, Via Celoria 26, I-20133 Milano, Italy

[4] Natural History Museum, University of Pisa, Via Roma 79, I-56011 Calci (Pisa), Italy

Correspondence

Dr. Stefano Scali, Natural History Museum of Milan, Corso Venezia 55, I-20121, Milano, Italy

Email: stefano.scali@comune.milano.it

ACKNOWLEDGMENTS

We are grateful to all the students who helped us during the field work, in particular: Carlotta Pasquariello, Mara Battaiola, Cristian Matellini, Simone Buratti, Francesca Baccalini, Sara Pozzi, Thomas Zabbia, Pietro Rodigari, Stefano Pezzi, Ilenia Bresciani. We also thank Dr. Sara Matilde

Masseroli of the "Sovraintendenza Archeologica, Belle Arti e Paesaggio per le Province di Como, Lecco, Monza e Brianza, Pavia, Sondrio e Varese" for the technical support within the archeological site. The authors do not have any conflict of interest.

ABSTRACT

Color polymorphism is genetically controlled, and the process generating and maintaining morphs can affect speciation/extinction rates. Color badges are useful signals in intraspecific communication because they convey information about alternative strategies and can potentially decrease unnecessary conflicts among different color morphs. Competition and aggressive interactions among color morphs can contribute to polymorphism maintenance. This could lead to an uneven spatial distribution of morphs in a population because the local frequency of each morph establishes the intensity of the competition and the fitness of each male. We used a polymorphic lizard, *Podarcis muralis*, to assess if aggression varies among morphs under two contrasting hypotheses: a heteromorphic vs. a homomorphic aggression. We used laboratory mirror tests after lizard color manipulation, and we verified the consistency of results with an analysis of the spatial distribution of morphs in a wild population. Both the experiments confirmed that aggression is more common during homomorphic than heteromorphic contests. The adoption of alternative behavioral strategies that minimize risks and costs could facilitate the stable coexistence of the phenotypes and reduce competition. A bias in aggression would advantage rarer morph, which would suffer less harassment by common morphs obtaining a fitness advantage. This process would be negatively-frequency-dependent and would stabilize polymorphism, possibly contributing to sympatric speciation.

KEYWORDS

Color polymorphism, homomorphic aggression, mirror test, *Podarcis muralis*, lacertid lizard, morph spatial distribution, alternative strategies

INTRODUCTION

Color polymorphism occurs when two or more heritable color morphs "coexist in temporary or permanent balance within a single interbreeding population […] in such frequencies that the rarer cannot be due solely to mutation" (Huxley, 1955). Morphs are genetically controlled and can evolve by both natural and sexual selection, and the process generating and maintaining it can affect speciation rates and/or extinction rates, either positively or negatively (Gray & McKinnon, 2007; Hugall & Stuart-Fox, 2012). In several species of polymorphic lizards, morphs generally associate with alternative reproductive strategies, modulated by complex interactions among environmental pressures (e.g., social interactions and individual density), and therefore represent locally adapted optima (Roulin, 2004; Sacchi, Rubolini, et al., 2007; Sinervo & Lively, 1996; E. Svensson, Sinervo, & Comendant, 2001). When males of different morphs associate with opposite breeding strategies, the intensity of the aggressive behavior may vary among morphs. Color may predict the outcome of a dyadic encounter, irrespective of other asymmetries in size, residency, or prior experience (Hover, 1985; Sinervo & Lively, 1996; Thompson & Moore, 1991a, 1991b). For example, green males of the tree lizard (*Urosaurus ornatus*) are more likely to dominate orange ones despite their lower size (Hover, 1985); orange-throated males of side-blotched lizards (*Uta stansburiana*) are highly aggressive and dominant over the other morphs (Sinervo & Lively, 1996), as well as red males of the Australian painted dragon (*Ctenophorus pictus*) are more likely to win dyadic contests with yellow males (Healey, Uller, & Olsson, 2007). However, in other polymorphic lizard species, the role of color morph is often ineffective at predicting the outcome of male-male contests (Sacchi et al., 2009; Stuart-Fox & Johnston, 2005). For example, color morph does not predict the contest outcome or aggression levels for two species of dragon lizards, even if a marginal effect may

subsist only during fights between unfamiliar opponents (Stuart-Fox & Johnston, 2005). Such discrepancies can be explained by the occurrence of multiple mixed signals, so that color badges could not be the only way lizards use to communicate their fighting abilities. In fact, many other signals such as posture, movements, or chemical secretions can mediate the opportunity of engaging in physical combats (e.g., Van Dyk & Evans, 2008; Martin & Lopez, 2015). Furthermore, prior antagonistic experience could override color importance in contests because a defined hierarchy can be established between individuals, avoiding unnecessary combats (Stuart-Fox & Johnston, 2005). One of the mechanisms that can help to maintain variation in a population is negative frequency-dependent selection resulting from some processes, such as predation (Olendorf et al., 2006; Qvarnström, Vallin, & Rudh, 2012), sexual selection (Sinervo & Lively, 1996) and intraspecific competition (Seehausen & Schluter, 2004), that guarantee an advantage to rare morphs. Morphs could reduce intraspecific competition by divergence in resource use (i.e. a character displacement). This process is acknowledged as one of the possible mechanisms for sympatric speciation (Qvarnström et al., 2012; Svensson, 2017) and makes the study of color polymorphism particularly attractive for evolutionary researchers. Aggression biases evolved to avoid unnecessary conflict among morphs, but in some cases, morphs adopt alternative strategies that are an outcome of male-male competition and maintain genetic variation and enhance the reproductive success of each morph under context-dependent control (Bleay, Comendant, & Sinervo, 2007; Hurtado-Gonzales & Uy, 2010). In this scenario, males of rare morphs should receive less aggression from other males because they do not share the same resources, and thereby they should experience a fitness advantage (Qvarnström et al., 2012; Seehausen & Schluter, 2004). The spatial scale at which behavioral interactions among morphs confer a fitness benefit is the composition of morph within a neighborhood, because the local frequency of each morph establishes the intensity of the competition in that neighborhood, and consequently the fitness of each male (Zamudio & Sinervo, 2003). When morphs associate with alternative breeding strategies, the fitness of a given male depends on the number of males that come in direct competition with him, and consequently on the

relative frequency of morphs in its neighborhood. For example, the three male morphs of the side-blotched lizard (*Uta stansburiana*) exhibit a combination of alternative breeding strategies that interact in a cyclical dominance hierarchy, where A > B, B > C, C > A (Sinervo & Lively, 1996). Orange males are aggressive and defend a territory, blue-males are aggressive but are mate-guarder, and yellow males are not aggressive and behave like sneakers (i.e. opportunistic individuals that do not own a defined territory or partner and mate with females occurring in other lizards' territories). Each morph has specific behavioral traits that allow it to outcompete only one of the other two. So, orange males outcompete yellow males but are defeated by blue males. There is experimental evidence that the frequency-distribution of morphs in a neighborhood of each male directly affects its fitness, in a way that orange males in a neighborhood of blue males achieve much less fitness than orange males in a neighborhood of yellow males (Zamudio & Sinervo, 2003). Therefore, the analysis of the spatial distribution of morph at the neighborhood scale can reveal information on the competitive interactions among the strategies behind morphs. In both cases (e.g., aggression bias or trade-off of alternative strategies), negative-frequency dependent selection from the evolved differential aggression could lead to rare-male advantage and maintain polymorphism,

The common wall lizard (*Podarcis muralis*) is a small (50–70 mm adult snout–vent length, SVL) diurnal lizard of central and south-eastern Europe (Sillero et al., 2014), whose males vigorously fight for territories, showing a marked territorial behavior (Edsman, 1990; Sacchi et al., 2009). Previous studies demonstrated that this species expresses a pigment-based ventral color polymorphism in both sexes with three discrete color morphs (white, yellow, and red, Sacchi et al. 2013; Sacchi, Scali, et al. 2007). Morphs are genetically determined (Andrade et al., 2019) and correlations between morphs and aggressiveness remain controversial (Abalos, Pérez i de Lanuza, Carazo, & Font, 2016; Coladonato et al., 2020; Sacchi et al., 2009). In a previous study using a resident-intruder design, Sacchi et al. (2009) showed that simple rules such as residency and body size differences determine the outcome of agonistic encounters but the authors did not find any

effect of color on male aggression or fighting success. By analyzing dyadic encounters in a neutral arena, Abalos et al. (2016) found that the black patches on ventral ornamentation emerged as a good predictor of contest outcome independently of morphs, even if red males lost fights against heteromorphic males more often than yellow or white males. However, this effect could be due to a correlation with the size of black patches. The plasmatic concentration of testosterone also did not differ among morphs (Sacchi et al., 2017). However, it varied seasonally, as yellow males maintained significantly higher T-levels over time and displayed a stronger subsequent decline. The hormone profile did not differ between red and white males (Sacchi et al., 2017). Accordingly to seasonal variation in hormone profile, Coladonato et al. (2020) were able to detect morph-specific differences in the seasonal pattern of variation in the aggressive behavior of yellow males compared to males of the white morphs, but not among white, yellow, and red males. Overall, no clear picture emerges from these studies, so a study able to prevent any confounding effect given by staged contest is needed to fully understand the role of visual badges in releasing aggressive behaviors and the strategies adopted by each morph.

A recent study highlighted the effectiveness of mirror experiments for measuring the intensity of the aggressive behavior in lizards, demonstrating that they do not show self-recognition and attack the mirror image as a true "rival" (Scali et al., 2019). Mirror tests have the great advantage of allowing the experimenters to control for the effect of asymmetries in size and residence/motivation, as well as color signal, since each individual can be acclimated in one cage until it becomes resident before facing an intruder having the same size and giving positive feedback during aggressive contests (Scherer, Buck, & Schuett, 2016). In this study, we used mirrors to assess if aggression varies among morph after removing the main determinants of contest outcome in this species (i.e. size and residency, Sacchi et al. 2009). In detail, we explored two contrasting hypotheses: i) a heteromorphic aggression hypothesis (three morphs hypothesis, hereinafter 3MH), where each morph compete with others to verify if some morphs are more aggressive than others or if some morphs are more aggressive towards a specific morph; ii) a homomorphic aggression hypothesis (hereinafter HH),

where each morph shows a higher aggression level when faced with an opponent displaying the same color (i.e. the same strategy). Even if the two hypotheses are not mutually exclusive, we chose to test both because the homomorphic one could be masked by the heteromorphic one in a three-level comparison. Furthermore, there is no evidence of a cyclic or intransitive system (sensu Permogorskiy, 2015 and Sinervo & Lively, 1996), nor a hierarchical one in the common wall lizard's polymorphism, so no a priori assumptions could be made about which morph dominates which one. In any case, our experiment could shed new light on the competitive interactions among morphs of this species. These hypotheses were tested in two ways: i) a laboratory experiment where we used mirrors to test aggression after color manipulation, and ii) an intensive field sampling where we assessed if the spatial distribution at neighborhood level of different morphs supports the results of the laboratory experiment.

METHODS

Laboratory methods

Thirty-six adult male lizards (12 for each pure morph) were captured by noosing during the reproductive period (April-June 2018) in five sites in the Milan province (northern Italy). Animals were carried to the Natural History Museum of Milan and housed in individual plexiglass boxes (40 × 40 × 30 cm) with a refuge (a hollow brick) positioned near one box's wall, water *ad libitum* and fed with three mealworm (*Tenebrio molitor*) larvae/day. A sheet of absorbent paper was used as substrate, so that the terrarium could keep the resident odor and the lizards could consider it as their territory. The vertical sides of the boxes were covered externally with white paper sheets, to avoid external stimuli and wall reflectance.

After three days, lizards were tested in the same terrarium where they were acclimated, removing water and food. The ventral color was manipulated painting throat and belly with water-based

tempera colors, randomly assigning all the color combinations with four replicas for each combination. This means that the twelve lizards from each morph were divided into three subsamples of four individuals, and each subsample was treated with one of the three colors (white, yellow, and red). Each individual was tested for four consecutive days, maintaining the same coloration used in the first treatment, to verify response consistency. A total of 144 trials were performed during the experiment (12 individuals × 3 morphs × 4 trials).

A heating lamp (ZooMed Repti Basking spot lamp, 150 W) was turned on for 15 minutes before each trial to achieve an optimal body temperature similar for all the individuals (Sannolo et al. 2014). A photographic set was then placed on the terrarium providing uniform led lightning and a webcam (Microsoft Life Cam HD-3000) was used to record the trial. The lizard was put under the refuge for one minute while the mirror (30 × 15 cm) was positioned at the opposite side of the terrarium. When the camera was turned on, the refuge was removed and the behaviors were recorded for 15 minutes. All the lizards were released, healthy, at the capture sites after the experiments.

The videos were analyzed using BORIS (Behavioral Observation Research Interactive Software), an open-source software (www.boris.unito.it (Friard & Gamba, 2016)). We counted the number of bites against the mirrored image to quantify the aggressive response to the stimulus. Since some lizards never bit the mirrored image, to prevent zero-inflation problems, the number of bites was transformed to the probability of bites during the trial (BP = no. of interactions with bites/no. of total interactions). We defined "interactions" any time the lizard entered the half of the terrarium bearing the mirror. Individuals who never interacted with the mirror in any trial were discarded from the following analyses. Some other displays were observed during the experiments (e.g., tongue-flicking, hand-shaking, tail-shaking, etc.), but we only considered those ones that could be interpreted as aggressive interactions towards the opponent and not those related to stress or explorative behavior (Coladonato et al., 2020; Sacchi et al., unpublished data). The lack of attacks

against the mirrored images by some individuals could be due to stress or fear, since individual responses can differ according to the provided stimulus (static models, regular mirrors, non-reversing mirrors or real opponents). Previous studies demonstrated that static models are not effective with common wall lizards, because movement is a key releaser for aggressive displaying (Scali et al., 2019). Non-reversing mirrors can be appropriate with some species, because they elicit a more pronounced aggressive response and less pronounced neurobiological fear response, characteristic of interactions with real conspecific opponents (Li et al., 2018). However, non-reversing mirrors, which provide a true reflection, are effective especially for species that perform head-tail displays, but this is not the case of common wall lizards, that face the opponents with head-head displays. Furthermore, we were interested in the role of throat and belly coloration, that can be seen only during the latter kind of display, so we chose to use regular mirrors in our experiments.

Field methods

Field sampling was performed in the archaeological site of Castelseprio (Province of Varese; UTM 32 T 489077E, 5063874 N; 338 m a.s.l.). The field site is not the same as the collection sites used for the lab experiment, but they are located within a 30 km range, within a species distribution continuum, so we are confident that no significant differences among populations occur as far as behavior is concerned. The site is on the top of a hill and is characterized by stone ruins in an open area with grass or bare soil surrounded by natural deciduous woods. The study area is 2380 m² wide, and ruins cover about 10% of the whole surface and their height ranges from 30 cm to 5 m. Common wall lizards are scattered on the ruins and along the wood borders of the whole study area. Field sampling was performed by ten researchers on 3rd April 2017, (09:00-18:00), during lizards' reproductive period, intensively searching across the whole area. We restricted the sampling to a single day in order to have a stationary picture of the distribution of lizards in the study area. We

captured a total of 255 lizards by noosing, including 206 adults lizards (SVL>56 mm, Sacchi et al., 2007). Lizards were marked with a non-toxic color to avoid pseudoreplication and released after recording their sex, morph, and position. To be sure that the position errors were small enough, compared to inter-lizards distances (e.g., less than 10 cm), lizards were located on a high-resolution orthoimage obtained by the use of a Remotely Piloted Aircraft System (Fig. 1; Supplementary material): the map had mean horizontal and vertical errors of 3.5 and 2.2 cm, respectively. Analyses were performed on 144 adult males, excluding females, juveniles, and three males (yellow-red) that cannot be unambiguously assigned to a single morph.

Ethical statement

This research was carried out in conformity with current European and Italian laws on animal use in scientific research (authorized by the "Ministero dell'Università e della Ricerca", MIUR, prot. 0002154/PNM, 2016, 2nd March, valid over 2016-2018) and Italian laws for the access to the Archeological Sites (authorized by the "Ministero dei Beni e delle Attività Culturali e del Turismo", prot. n. 1441/MIBACT-SAR-LOM, 2017, 17th March). All the experiments were performed in accordance with national guidelines for the care and use of animals in laboratories and all the animals captured for laboratory and field experiments were released healthy at the capture site.

Laboratory statistical analyses

Laboratory data were analyzed using a generalized linear mixed (GLMM) model with a binomial error distribution, using bite probability (BP) as the response variable. For the 3MH hypothesis, we used a model with morph (three-levels factor: W, Y, R), treatment (three-levels factor: W, Y, R), and their interaction as fixed factors, trial (entering as a continuous variable given the constant time between subsequent trials) as covariate controlling for a potential habituation effect (Peeke, 1984);

the lizard ID was used as a random factor on the intercept to account for repeated trials. For the HH hypothesis, we used a model with treatment (recoded into a two-level factor: homomorphic, heteromorphic) as the fixed factor, trial as the control covariate, and ID as the random intercept. While the 3MH model tests both for intransitivity or hierarchy and for homomorphic vs. heteromorphic hypotheses, the HH model only tests for homomorphic vs. heteromorphic aggression. In the latter case the statistical power is higher due to data pooling.
Predictor significance was evaluated by likelihood-ratio (LR) tests (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Analyses were performed under the R 3.45.2 statistical environment (R Development Core Team, 2018), using the package "glmmTMB" (Brooks et al., 2017).

Field statistical methods

The same hypotheses (3MH and HH) used for lab experiment were tested on field data. First, we mapped the distribution of all males (Fig. 1) and computed for each focal lizard the distance to: the nearest white (d_W), the nearest yellow (d_Y), the nearest red (d_R), the nearest homomorphic (d_{Ho}), and the nearest heteromorphic (d_{He}) conspecific. Secondly, we computed the differences in the minimum distance for each morph pair ($\Delta_{RW} = d_R - d_W$; $\Delta_{YW} = d_Y - d_W$; $\Delta_{RY} = d_R - d_Y$) associated to each focal lizard, and between the homomorphic and heteromorphic minimum distances ($\Delta_{HH} = d_{Ho} - d_{He}$): this way, we obtained a measure of the relative proximity of males of the three morphs, or of homomorphic and heteromorphic males to each focal lizard. In the end, we averaged these Δs according to the hypothesis to be tested: under 3MH, for each focal color morph we derived the three average, $\overline{\Delta_{RW}}$, $\overline{\Delta_{YW}}$, $\overline{\Delta_{RY}}$; under HH, the average $\overline{\Delta_{HH}}$. Departure of the average Δs from zero would indicate non-random distribution (repulsion or attraction among certain color morphs). However, since the number of individuals of the three morphs was not balanced (white = 81, yellow = 36, red = 27), differences in $\bar{\Delta}s$ may simply reflect the relative abundance of a given morph (i.e. a rarer morph can show a higher distance just by chance). This is true also for $\overline{\Delta_{HH}}$: even in a scenario

where morphs relative abundance was balanced (1/3 white, 1/3 yellow, 1/3 red), the probability of being closer to a heteromorphic would be higher just because of chance. To account for this spatial bias, we adopted a data permutation procedure, and built the expected average $\bar{\Delta}s$ null distribution given the observed point pattern and assuming color morph not to affect the spatial distribution of male lizards. We first permuted lizard color morph (no. of permutations = 999), and we re-computed all the distances (d) and distance differences (Δ). We thus obtained the null distributions of all the average $\bar{\Delta}$s for each focal color morph (3MH hypothesis), and the null distribution of $\overline{\Delta_{HH}}$ (HH hypothesis). We then assessed the probability of each observed $\bar{\Delta}$s being larger or smaller than the respective null distribution (depending on the observed value being larger or smaller than the mean null value). Even in this case, the statistical power of the 3MH model is lower than the HH model due to data pooling in the latter test.

RESULTS

Laboratory experiment

Out of the 36 lizards tested, six males never responded to the stimulus, i.e. they never moved after refuge removal or they never enter the half of the terrarium bearing the mirror. They were considered as failed trials and excluded from the analysis. From the remnant 30 focal lizards, we obtained 119 usable trials; one was excluded because the focal lizard did not enter the half cage with the mirror. So, 29 lizards had four replicas, and one had three.

The 3MH model for BP did not reveal any significant main effect (morph: LR, $\chi^2 = 0.359$, d.f. = 2; P = 0.836; treatment: LR, $\chi^2 = 1.539$, d.f. = 2; P = 0.463; morph × treatment: LR, $\chi^2 = 5.295$, d.f. = 4, P = 0.258; trial: $\chi^2 = 3.650$, d.f. = 2; P = 0.056). The effect of trial number was borderline significant, with a weak negative effect on BP ($\beta_{estimate} = -0.170$; $\beta_{SE} = 0.089$). On the opposite, the random effect of the individual identity was highly significant (LR, $\chi^2 = 75.018$, d.f. = 5; P < 0.001),

and accounted for 18.34% of the total BP variance, highlighting the occurrence of a strong variation among individual propensity to bite the mirrored image.

Considering the HH model, trial was confirmed almost significant (LR, $\chi^2 = 3.656$, d.f. = 1; P = 0.056) and with an identical coefficient estimation ($\beta_{estimate} = -0.170$; $\beta_{SE} = 0.089$) as 3MH model. The re-coded two-level treatment showed a significant effect, instead (LR, $\chi^2 = 5.584$, d.f. = 1, P = 0.0181), which highlighted a higher aggression level during homomorphic contests compared to heteromorphic ones (Fig. 2). Again, the individual identity was highly significant (LR, $\chi^2 = 43.367$, d.f. = 1; $P < 0.001$) and explained 19.80% of the total variance.

Field experiment

No difference was observed in the mean distance between individuals when morph pairs were compared (Fig. 3), i.e.: mean distance from red morph is similar to that from white ($\Delta_{RW} = 7.53$ m; P = 0.272) and yellow morph ($\Delta_{RY} = 1.63$ m; P = 0.246); the mean distance from a yellow morph is equal to the distance from a white morph ($\Delta_{YW} = 5.90$ m; P = 0.378). The mean distance between homomorphic and heteromorphic individuals resulted instead significantly higher than null mean distances ($\Delta_{HH} = 4.53$ m; P = 0.043; Fig. 3).

DISCUSSION

Our experiment demonstrated that the common wall lizard shows a morph-specific aggressiveness. Males of this species are often aggressive towards conspecifics and this was demonstrated by the three morphs lab experiment, where almost all the individuals performed at least one aggression towards their mirrored image. The comparison of the number of aggressive interactions between pairs of morphs, did not reveal any prevalence in attacking a specific morph. However, when data

were pooled and analyzed under the homomorphic vs. heteromorphic hypothesis, a significant aggression was observed towards individuals belonging to their same morph.

Morph-specific aggression has been studied in the past years to understand mechanisms underlying polymorphism maintenance with contrasting results. Many studies were conducted on fish, mainly on cichlids, famous for their spectacular intraspecific diversity. In some cases, males compete more heavily with other males of the same color, as in our case study (Dijkstra, Hemelrijk, Seehausen, & Groothuis, 2008; Dijkstra et al., 2010; Dijkstra, Seehausen, Pierotti, & Groothuis, 2007; Seehausen & Schluter, 2004; van Doorn, Dieckmann, & Weissing, 2004). In these cases, rare male phenotypes would receive less aggression than common male phenotypes, and this could generate frequency-dependent selection, as rare morphs are more likely to gain higher dominance status as a result of reduced harassment from competing males (Dijkstra et al., 2010). If males bias their aggression towards similar rivals that share the same resources, rare morphs should receive less aggression from other males and thereby experience an advantage in establishing breeding territories (Dijkstra et al., 2007; Qvarnström et al., 2012; Seehausen & Schluter, 2004). Although this mechanism does not contribute to the emergence of reproductive barriers, it can pave the way for sympatric speciation (Dijkstra et al., 2008).

A previous study demonstrated that common wall lizards can discriminate colors and can, consequently, recognize individuals belonging to their own morph (Pérez i de Lanuza, Abalos, Bartolomé, & Font, 2018). This ability presumably contributes to different results in staged contests, with red males having the lower fighting ability (Abalos et al., 2016), and to assortative mating, with homomorphic male-female pairs more common than the heteromorphic pairs (Pérez i de Lanuza, Font, & Carazo, 2012). Assortative mating could be advantageous also in terms of fitness, since non-random mating produces clutches with different characteristics, in accordance with the r, K, and mixed strategies demonstrated for the common wall lizard (Galeotti et al., 2013). Under these assumptions, throat and belly coloration can be considered as a visual badge that

common wall lizards use in intraspecific communication. Even if color polymorphism of common wall lizards occurs both in males and females, sexual selection seems to be driven mainly by male choice, whereas the importance of female choice is still debated (Sacchi et al., 2015, 2018). If males preferentially choose homomorphic females, then homomorphic males should be considered as the direct competitors for this resource, so the higher aggression level observed in our experiment can be explained as a need to defend the territories from intruders competing for the same potential mates (Seehausen & Schluter, 2004). This conclusion is supported by the lack of differences in aggression in the 3MH model. Similar results were obtained for the tawny crevice dragon (*Ctenophorus decresii*), an agamid with four discrete morphs (Teasdale, Stevens, & Stuart-Fox, 2013), that showed a higher aggressive behavior during homomorphic contests when models were presented to free individuals (Yewers, Pryke, & Stuart-Fox, 2016). The color perception of the opponent seems fundamental in triggering aggression also in other species, such as the ornate tree lizard (*Urosaurus ornatus*), as demonstrated by experiments where colors were manipulated (Hover, 1985).

Our field experiment supported these results, as the mean distance among individuals resulted significantly different when homomorphic and heteromorphic males were compared, in accordance with the HH model. In contrast, no difference was observed in the 3MH model, highlighting no variation in the mean distance between pairs belonging to the three morphs. This result suggests a non-random distribution of males in the study area, in a sort of "repulsive" effect of homomorphic pairs. A similar result was observed in haplochromine cichlids of Lake Victoria, where breeding territories of individuals of different colors are closer than those of individuals of the same color (Dijkstra, Seehausen, Gricar, Maan, & Groothuis, 2006; Seehausen & Schluter, 2004). The uneven spatial distribution of male common wall lizards could be reinforced by the individual recognition given by scent marks, that contribute to give information about neighbors. In fact, in lacertids, femoral secretions convey information about neighbor characteristics, such as size, weight, and

familiarity (Carazo, Font, & Desfilis, 2008). Proteins, in particular, give information about the identity and morph in *P. muralis* (Mangiacotti, Fumagalli, et al., 2019; Mangiacotti, Gaggiani, et al., 2019). Lacertid lizards are also able to remember the spatial location of scent marks (Carazo et al., 2008), so males could build a spatial map of neighboring rivals. In this way, they can decide which neighbors could exert a major threat to their territories and address aggression against their direct competitors, minimizing both the energetic costs of territory defense and the risks of suffering injuries or predation, according to the paradigm of the "dear-enemy effect" (Carazo et al., 2008; Tumulty, 2018; Whiting, 1999; Ydenberg, 1988).

We only tested the effect of a resident male towards an intruder male without analyzing the response of an intruder vs. a resident lizard. In this case, other factors could intervene, such as motivation to defend an unknown territory. This hypothesis could be tested by inserting the focal lizards in unknown boxes, different from those used for acclimation. Indeed, a previous study with staged contests demonstrated that the motivation of resident individuals could be a driving force to the contest outcome (Sacchi et al., 2009), so the aggressive response could be lowered by this condition and the role of color morph underestimated.

In conclusion, male aggression in the common wall lizard seems to be morph-dependent. The adoption of alternative behavioral strategies that minimize risks and costs of unwanted conflicts could facilitate the stable coexistence of the phenotypes (Dijkstra et al., 2006; Yewers et al., 2016). A bias in aggression to like-colored males would benefit the rarer morph, which would suffer less harassment by common morphs and obtain a fitness advantage. This process would be negative-frequency-dependent and would stabilize polymorphism in the populations (Dijkstra et al., 2008; Dijkstra et al., 2006; Seehausen & Schluter, 2004).

REFERENCES

Abalos, J., Pérez i de Lanuza, G., Carazo, P., & Font, E. (2016). The role of male coloration in the outcome of staged contests in the European common wall lizard (*Podarcis muralis*). *Behaviour, 153*, 607–631. doi:10.1163/1568539X-00003366

Andrade, P., Pinho, C., Pérez i de Lanuza, G., Afonso, S., Brejcha, J., Rubin, C. J., Carneiro, M. (2019). Regulatory changes in pterin and carotenoid genes underlie balanced color polymorphisms in the wall lizard. *Proceedings of the National Academy of Sciences of the USA, 116*, 5633–5642. doi:https://doi.org/10.1073/pnas.1820320116

Bleay, C., Comendant, T., & Sinervo, B. (2007). An experimental test of frequency-dependent selection on male mating strategy in the field. *Proc. R. Soc. B, 274*, 2019-2025. doi:10.1098/rspb.2007.0361

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Bolker, B. M. (2017). "glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling.". *The R Journal, 9*(2), 378-400.

Carazo, P., Font, E., & Desfilis, E. (2008). Beyond 'nasty neighbours' and 'dear enemies'? Individual recognition by scent marks in a lizard (*Podarcis hispanica*). *Animal Behaviour, 76*, 1953–1963. doi:10.1016/j.anbehav.2008.08.018

Coladonato, A. J., Mangiacotti, M., Scali, S., Zuffi, M. A. L., Pasquariello, C., Matellini, C., Sacchi, R. (2019). *Morph-specific pattern of aggression throughout the season in Podarcis muralis.* Paper presented at the Program and Abstracts of the XX European Congress of Herpetology (S.E.H.), Milan (Italy).

Coladonato A. J., Mangiacotti, M., Scali, S., Zuffi, M. A. L., Pasquariello, C., Matellini, C., Sacchi, R. (2020). Morph-specific seasonal variation of aggressive behaviour in a polymorphic lizard species. *PeerJ, 8*, e10268. doi: 10.7717/peerj.10268

Dijkstra, P. D., Hemelrijk, C. K., Seehausen, O., & Groothuis, T. G. G. (2008). Color polymorphism and intrasexual competition in assemblages of cichlid fish. *Behavioral Ecology, 20*(1), 138–144. doi:10.1093/beheco/arn125

Dijkstra, P. D., Lindström, J., Metcalfe, N. B., Hemelrijk, C. K., Brendel, M., Seehausen, O., & Groothuis, T. G. G. (2010). Frequency-dependent social dominance in a color polymorphic cichlid fish. *Evolution, 64*, 2797–2807. doi:https://doi.org/10.1111/j.1558-5646.2010.01046.x

Dijkstra, P. D., Seehausen, O., Gricar, B. L. A., Maan, M. E., & Groothuis, T. G. G. (2006). Can male-male competition stabilize speciation? A test in Lake Victoria haplochromine cichlid fish. *Behavioral Ecology and Sociobiology, 59*(5), 704-713. doi:10.1007/s00265-005-0100-1

Dijkstra, P. D., Seehausen, O., Pierotti, M. E. R., & Groothuis, T. G. G. (2007). Male–male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex. *Journal of Evolutionary Biology, 20*, 496–502. doi:10.1111/j.1420-9101.2006.01266.x

Edsman, L. (1990). *Territoriality and competition in wall lizards.* (PhD Thesis), University of Stockholm, Sweden,

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution, 7*(11), 1325–1330. doi:10.1111/2041-210X.12584

Galeotti, P., Sacchi, R., Pellitteri-Rosa, D., Bellati, A., Cocca, W., Gentilli, A., Fasola, M. (2013). Colour polymorphism and alternative breeding strategies: effects of parent's colour morph on fitness traits in the common wall lizard. *Evolutionary Biology, 40*, 385–394. doi:https://doi.org/10.1007/s11692-012-9222-3

Gray, S. M., & McKinnon, J. S. (2007). Linking color polymorphism maintenance and speciation. *Trends in Ecology & Evolution, 22*(2), 71–79. doi:10.1016/j.tree.2006.10.005

Healey, M., Uller, T., & Olsson, M. (2007). Seeing red: morph-specific contest success and survival rates in a colour-polymorphic agamid lizard. *Animal Behaviour, 74*, 337–341. doi:10.1016/j.anbehav.2006.09.017

Hover, E. L. (1985). Differences in aggressive behavior between two throat color morphs in a lizard, *Urosaurus ornatus*. *Copeia, 1985*(4), 933–940.

Hugall, A. F., & Stuart-Fox, D. (2012). Accelerated speciation in colour-polymorphic birds. *Nature, 485*, 631-634. doi:10.1038/nature11050

Hurtado-Gonzales, J. L., & Uy, J. A. C. (2010). Intrasexual competition facilitates the evolution of alternative mating strategies in a colour polymorphic fish. *BMC Evolutionary Biology, 10*, 391. doi:http://www.biomedcentral.com/1471-2148/10/391

Huxley, J. (1955). Morphism and evolution. *Heredity, 9*(1), 1-52.

Li, C. Y., Hofmann, H. A., Harris, M. L., & Earley, R. L. (2018). Real or fake? Natural and artificial social stimuli elicit divergent behavioural and neural responses in mangrove rivulus, *Kryptolebias marmoratus*. Proceedings of the Royal Society B: Biological Sciences, *285*, 20181610. doi: http://dx.doi.org/10.1098/rspb.2018.1610

Mangiacotti, M., Fumagalli, L., Cagnone, M., Viglio, S., Bardoni, A. M., Scali, S., & Sacchi, R. (2019). Morph-specific protein patterns in the femoral gland secretions of a colour polymorphic lizard. *Scientific Reports, 9*(1), 8412. doi:https://doi.org/10.1038/s41598-019-44889-7

Mangiacotti, M., Gaggiani, S., Coladonato, A. J., Scali, S., Zuffi, M. A. L., & Sacchi, R. (2019). First experimental evidence that proteins from femoral glands convey identity-related information in a lizard. *Acta Ethologica, 22*, 57–65. doi:10.1007/s10211-018-00307-1

Martin, J., & López, P. (2015). Condition-dependent chemosignals in reproductive behavior of lizards. *Hormones and Behavior, 68*, 14-24. doi: http://dx.doi.org/10.1016/j.yhbeh.2014.06.009

Menegoni, N., Giordan, D., C., P., & Tannant, D. D. (2019). Detection and geometric characterization of rock mass discontinuities using a 3D high-resolution digital outcrop model generated from RPAS imagery–Ormea rock slope, Italy. *Engineering Geology* (252), 145–163. doi:10.1016/j.enggeo.2019.02.028

Olendorf, R., Rodd, F. H., Punzalan, D., Houde, A. E., Hurt, C., Reznick, D. N., & Hughes, K. A. (2006). Frequency-dependent survival in natural guppy populations. *Nature, 441*(1), 633–636. doi:10.1038/nature04646

Peeke, H. V. S. (1984). Habituation and the Maintenance of Territorial Boundaries. In H. V. S. Peeke & L. Petrinovich (Eds.), *Habituation, Sensitization, and Behavior* (pp. 393–421). Orlando, Florida: Academic Press, Inc.

Pérez i de Lanuza, G., Abalos, J., Bartolomé, A., & Font, E. (2018). Through the eye of a lizard: hue discrimination in a lizard with ventral polymorphic coloration. *Journal of Experimental Biology, 221*, jeb169565. doi:10.1242/jeb.169565

Pérez i de Lanuza, G., Font, E., & Carazo, P. (2012). Color-assortative mating in a color-polymorphic lacertid lizard. *Behavioral Ecology, 24*, 273–279. doi:https://doi.org/10.1093/beheco/ars164

Permogorskiy, M. S. (2015). Competitive intransitivity among species in a biotic community. *Biology Bulletin Reviews, 5* (3), 213–219. doi: https://doi.org/10.1134/S2079086415030068

Qvarnström, A., Vallin, N., & Rudh, A. (2012). The role of male contest competition over mates in speciation. *Current Zoology, 58*(3), 493−509. doi:10.1093/czoolo/58.3.493

R Development Core Team. (2018). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Vienna. Retrieved from http://www.Rproject.org

Roulin, A. (2004). The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews, 79*, 815–848. doi:10.1017/S1464793104006487

Sacchi, R., Coladonato, A. J., Ghitti, M., Mangiacotti, M., Scali, S., Bovo, M., & Zuffi, M. A. L. (2018). Morph-specific assortative mating in common wall lizard females. *Current Zoology, 64*(4), 449-453. doi:10.1093/cz/zox055

Sacchi, R., Ghitti, M., Scali, S., Mangiacotti, M., Zuffi, M. A. L., Sannolo, M., Pellitteri-Rosa, D. (2015). Common Wall Lizard Females (*Podarcis muralis*) do not Actively Choose Males Based on their Colour Morph. *Ethology, 121*, 1145–1153. doi:10.1111/eth.12431

Sacchi, R., Pellitteri-Rosa, D., Bellati, A., Di Paoli, A., Ghitti, M., Scali, S., Fasola, M. (2013). Colour variation in the polymorphic common wall lizard (*Podarcis muralis*): An analysis using the RGB colour system. *Zoologischer Anzeiger, 252*(4), 431–439. doi:10.1016/j.jcz.2013.03.001

Sacchi, R., Pupin, F., Gentilli, A., Rubolini, D., Scali, S., Fasola, M., & Galeotti, P. (2009). Male-male combats in a polymorphic lizard: residency and size, but not color, affect fighting rules and contest outcome. *Aggressive Behavior, 35*, 274–283. doi:10.1002/ab.20305

Sacchi, R., Rubolini, D., Gentilli, A., Pupin, F., Razzetti, E., Scali, S., Fasola, M. (2007). Morph-specific immunity in male *Podarcis muralis*. *Amphibia-Reptilia, 28*, 408–412. doi:10.1163/156853807781374700

Sacchi, R., Scali, S., Mangiacotti, M., Sannolo, M., Zuffi, M. A. L., Pupin, F., Bonnet, X. (2017). Seasonal variations of plasma testosterone among colour-morph common wall lizards (*Podarcis muralis*). *General and comparative endocrinology, 240*, 114–120. doi:https://doi.org/10.1016/j.ygcen.2016.09.012

Sacchi, R., Scali, S., Pupin, F., Gentilli, A., Galeotti, P., & Fasola, M. (2007). Microgeographic variation of colour morph frequency and biometry of common wall lizards. *Journal of Zoology, 273*, 389–396. doi:10.1111/j.1469-7998.2007.00342.x

Scali, S., Sacchi, R., Falaschi, M., Coladonato, A. J., Pozzi, S., Zuffi, M. A. L., & Mangiacotti, M. (2019). Mirrored images but not silicone models trigger aggressive responses in male Common wall lizards. *Acta Herpetologica, 14*(1), 35–41. doi:10.13128/Acta_Herpetol-24651

Scherer, U., Buck, M., & Schuett, W. (2016). Lateralisation in agonistic encounters: do mirror tests reflect aggressive behaviour? A study on a West African cichlid. *Journal of Fish Biology, 89*(3), 1866–1872. doi:10.1111/jfb.13069

Seehausen, O., & Schluter, D. (2004). Male–male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proceeding of the Royal Society of London B, 271*, 1345–1353. doi:https://doi.org/10.1098/rspb.2004.2737

Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P. A., Vences, M. (2014). Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia, 35*, 1–31. doi:10.1163/15685381-00002935

Sinervo, B., & Lively, C. M. (1996). The rock-paper-scissors game and the evolution of alternative mating strategies. *Nature, 380*, 240–243. doi:https://doi.org/10.1038/380240a0

Stuart-Fox, D. M., & Johnston, G. R. (2005). Experience overrides colour in lizard contests. *Behaviour, 142*, 329-350. doi:10.1163/1568539053778265

Svensson, E., Sinervo, B., & Comendant, T. (2001). Condition, genotype-by-environment interaction, and correlational selection in lizard life-history morphs. *Evolution, 55*(10), 2053–2069. doi:https://doi.org/10.1111/j.0014-3820.2001.tb01321.x

Svensson, E. I. (2017). Back to basics: using colour polymorphisms to study evolutionary processes. *Molecular Ecology, 26*, 2204–2211. doi:10.1111/mec.14025

Teasdale, L. C., Stevens, M., & Stuart-Fox, D. (2013). Discrete colour polymorphism in the tawny dragon lizard (*Ctenophorus decresii*) and differences in signal conspicuousness among morphs. *Journal of Evolutionary Biology, 26*(5), 1035–1046. doi:https://doi.org/10.1111/jeb.12115

Thompson, C. W., & Moore, M. C. (1991a). Syntopic occurrence of multiple dewlap color morphs in male tree lizards, *Urosaurus ornatus*. *Copeia, 1991*(2), 495–503.

Thompson, C. W., & Moore, M. C. (1991b). Throat colour reliably signals status in male tree lizards, *Urosaurus ornatus*. *Animal Behaviour, 42*, 745–753. doi:https://doi.org/10.1016/S0003-3472(05)80120-4

Tumulty, J. P. (2018). Dear Enemy Effect. In J. Vonk & T. K. Shackelford (Eds.), *Encyclopedia of Animal Cognition and Behavior* (pp. 1–4). Cham, Switzerland: Springer International Publishing.

van Doorn, G. S., Dieckmann, U., & Weissing, F. J. (2004). Sympatric Speciation by Sexual Selection: A Critical Reevaluation. *The American Naturalist, 163*(5), 709–725. doi:10.1086/383619

Van Dyk, D. A., & Evans, C. S. (2008). Opponent assessment in lizards: examining the effect of aggressive and submissive signals. *Behavioral Ecology, 19*, 895–901. doi: 10.1093/beheco/arn052

Whiting, M. J. (1999). When to be neighbourly: differential agonistic responses in the lizard *Platysaurus broadleyi*. *Behavioral Ecology and Sociobiology, 46*, 210–214. doi:10.1007/s002650050611

Ydenberg, R. C. (1988). Neighbours, strangers, and the asymmetric war of attrition. *Animal Behaviour, 36*, 343–347. doi:https://doi.org/10.1016/S0003-3472(88)80004-6

Yewers, M. S., Pryke, S., & Stuart-Fox, D. (2016). Behavioural differences across contexts may indicate morph-specific strategies in the lizard *Ctenophorus decresii*. *Animal Behaviour, 111*, 329–339. doi:http://dx.doi.org/10.1016/j.anbehav.2015.10.029

Zamudio, K. R., & Sinervo, B. (2003). Ecological and social contexts for the evolution of alternative mating strategies. In S. F. Fox, J. K. McCoy, & T. A. Baird (Eds.), *Lizard social behavior* (pp. 83–106). Baltimore and London: The Johns Hopkins University Press.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). GLM and GAM for count data. In A. F. Zuur, E. N. Ieno, N. J. Walker, A. A. Saveliev, & G. M. Smith (Eds.),

Mixed Effects Models and Extensions in Ecology with R (pp. 209–243). New York, NY: Springer-Verlag.

FIGURE LEGENDS

FIGURE 1 Position of the 144 adult males considered in the analyses. Orthoimage was obtained by the use of a Remotely Piloted Aircraft System, as described in the supplementary material (Menegoni, Giordan, C., & Tannant, 2019).

FIGURE 2 Effect of treatment and morph on the biting probability (BP), according to the heteromorphic aggression model (3MH, left panel), and on the homomorphic aggression model (HH, right panel). For 3MH model, all morph × treatment combinations were shown. Grey points = observed values; horizontal thick lines = predicted BP values; color or grey shaded area = 95% confidence interval of predictions.

FIGURE 3 Observed distance differences (black lines) compared to the distribution of the simulated distance differences (color or grey shaded areas). The left panel shows all morph combinations under the 3MH hypothesis, while the right panel shows the average differences between homomorphic and heteromorphic individuals (HH hypothesis).