UNIVERSITY OF CALIFORNIA, MERCED

Causes and Consequences of Color Polymorphism

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Quantitative and Systems Biology

by

Kinsey M. Brock

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2021

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2021

DEDICATION

This dissertation is dedicated to my parents, Cynthia and Douglas Brock, who loved and supported me as a child in their arms and as a scientist across the world in pursuit of our dream.

And to Jessica Blois, who kept the dream alive.

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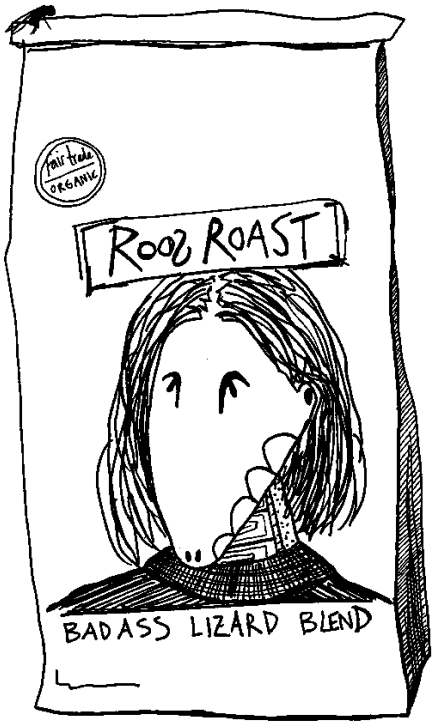

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Belasen, A., **Brock, K.M.**, Li, B., Chremou, D., Valakos, E., Pafilis, P., Sinervo, B., and Foufopoulos, J. 2016. Fine with heat, problems with water: microclimate alters water loss in a thermally adapted insular lizard. *Oikos*.

Mossman, A., **Brock, K.M**., ***Culhane, K.***, ***Miller, Z.***, Pafilis, P., Donihue, C.M. 2016. An extreme new record of *Natrix natrix* (Linnaeus, 1758) from a Mediterranean islet in Greece. *Herpetozoa*.

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CAUSES AND CONSEQUENCES OF COLOR POLYMORPHISM

Kinsey M. Brock
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One hundred and fifty years after Darwin and "On the Origin of Species," understanding how phenotypic variation is generated, maintained, and lost remains a central goal in evolutionary biology. Color polymorphic species are model systems for examining phenotypic variation in nature because discrete color variants are phenotypic markers of underlying genetic variation, or allele frequencies. Color polymorphism is the presence of multiple discrete, heritable color phenotypes within a single breeding population. Color morphs have evolved in thousands of species across the plant and animal kingdoms. Lizards have repeatedly evolved strikingly similar color polymorphisms in distantly related lineages, providing an opportunity to examine the effects of heritable phenotypic variation on micro- and macroevolutionary dynamics, intraspecific geographic variation, correlated traits, and associated evolutionary processes.

Herein, I take an interdisciplinary approach to understand the causes and consequences of color polymorphism at multiple biological scales, from molecules to macroevolution. In Chapter 1, I studied hundreds of individuals within a single population of lizards (Aegean wall lizard, *Podarcis erhardii*) to characterize a previously undescribed color polymorphism and identify color morph-correlated traits important for lizard fitness. I found that *P. erhardii* has three pure color morphs (orange, yellow, and white), and that male morphs display significant differences in body size traits, bite force, and chemical signal profiles extracted from their femoral pore exudate, whereas female morphs do not differ in head and body size dimensions or their maximum bite force capacity. I expand on these differences in traits in Chapter 2, where I conducted behavioral experiments on male *P. erhardii* color morphs from the same population to elucidate morph differences in behavior. Laboratory experiments revealed that male color morphs differ in their ability to access a limited space resource, and exhibit different levels of aggressive, bold, and signaling behaviors depending on the color morph identity of their competitor. In Chapter 3, I sampled lizards and measured environmental variation from 46 island populations across the range of *P. erhardii* to reconstruct the evolutionary history of color polymorphism and identify morph-environmental associations. I found that color polymorphism is likely the ancestral state of *P. erhardii*, and that morph variation is likely due to morph loss, which occurs at a much faster rate than evolutionary gains of color polymorphism. I also found that morph diversity seems to be lost in an ordered fashion, and that the rare orange morph is associated with cooler, wetter habitats, which are disappearing with climate change. Finally, in Chapter 4, I demonstrate that color polymorphism is a driver of diversification in the lizard family Lacertidae. Taken together, my dissertation suggests that the causes of color polymorphism is variation in the environment, and the consequences are variation within species that lead to the generation of new species.

Trait Differences Among Discrete Morphs of a Color Polymorphic Lizard, *Podarcis erhardii*

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ABSTRACT

Color polymorphism defies evolutionary expectations as striking phenotypic variation is maintained within a single species. Color and other traits mediate social interactions, and stable polymorphism within a population is hypothesized to be related to correlational selection of other phenotypic traits among color morphs. Here, we report on a previously unknown throat color polymorphism in the Aegean Wall Lizard (*Podarcis erhardii*) and examine morph-correlated differences in traits important to social behavior and communication: maximum bite force capacity and chemical signal profile. We find that both sexes of *P. erhardii* have three color morphs: orange, yellow, and white. Moreover, orange males are significantly larger and tend to bite harder than yellow and white males. Although the established color polymorphism only partially matches the observed intraspecific variation in chemical signal signatures, the chemical profile of the secretions of orange males is significantly divergent from that of white males. Our findings suggest that morph colors are related to differences in traits that are crucial for social interactions and competitive ability, illustrating the need to look beyond color when studying polymorphism evolution.

INTRODUCTION

Understanding processes that generate and maintain phenotypic variation is a fundamental goal in evolutionary biology. Color polymorphism, or the presence of multiple genetically determined color phenotypes that coexist within a breeding population, can be found in many species across the tree of life (Gray & McKinnon, 2007; Hugall & Stuart-Fox, 2012). Color polymorphic species offer a unique opportunity to study evolutionary processes underlying phenotypic variation (Ford, 1945; Huxley, 1955) such as natural and sexual selection (Kapan, 2001; Corl *et al.*, 2010a; Seehausen *et al.*, 1999), gene flow (Harley *et al.*, 2006), and genetic drift (Runemark *et al.*, 2010), because color morphs can be used as phenotypic proxies for genetic markers (reviewed in Roulin, 2004; Svensson, 2017). However, we still have a poor understanding of how color polymorphism evolves, and of the evolutionary processes underlying its maintenance (reviewed in Gray & McKinnon, 2007; McLean & Stuart-Fox, 2014).

Defining the number of color morphs and identifying other distinct characteristics among morphs are necessary first steps in understanding evolutionary mechanisms involved in color polymorphism maintenance. Color polymorphism may vary from just two color morphs, in the case of the spiny spider *Gasteracantha fornicata* where yellow and white morphs are tuned to the local environment and coloration of sympatric flowers (Kemp *et al.*, 2013; White *et al.*, 2017), to systems where many color morphs occupy a wide range of habitats with varying predation pressures, such as the 20 color types in the Central American strawberry poison frog, *Oophaga pumilio* (Rudh *et al.*, 2007; Hegna *et al.*, 2013). Color polymorphism may be limited to one sex (Andrés *et al.*, 2000; Van Gossum *et al.*, 2001; Kim *et al.*, 2019; Moon & Kamath, 2019) or morph types may vary within and between the sexes (Sinervo *et al.*, 2000; Martin *et al.*, 2013), suggesting that color variants are likely under some form of sexual selection (Wellenreuther *et al.*, 2014). In some species, color morphs indicate age or social rank (Thompson & Moore 1991; Martin *et al.* 2013). Further, social interactions among morphs can dictate morph diversity across populations (Pérez i de Lanuza *et al.*, 2017). Thus, the number of morphs and the maintenance of color polymorphism can be the result of natural selection, sexual selection, both natural and sexual selection, and sometimes perhaps even neutral processes (Gray & McKinnon, 2007; Rudh *et al.*, 2007; Runemark *et al.*, 2010; Pérez i de Lanuza *et al.*, 2018). But across taxa, one thing remains clear: the key to understanding color polymorphism lies in identifying the number of morphs and morph-correlated characteristics and the broader context in which these alternative phenotypes are operating and interacting with each other.

Color morphs often differ in multiple traits besides color (e.g. behavioral and physiological reproductive strategies [Sinervo & Lively, 1996; Sinervo *et al.*, 2000; Vercken & Clobert, 2008; Galeotti *et al.*, 2013], hormone levels and immune function [Huyghe *et al.*, 2009; Galeotti *et al.*, 2010], body size [Huyghe *et al.*, 2007], and other phenotypic characters [Lank *et al.*, 1995]). Distinct behavioral tactics and other traits associated with different color morphs are likely the result of multivariate correlational selection for particular trait combinations (Blows & Brooks, 2003; Blows *et al.*, 2003). Investigating morph-correlated traits is important for understanding how morphic variation is maintained, as the evolution of color polymorphism could be the result of selection on the color polymorphism itself, on a trait correlated with the color

polymorphism, or suites of traits possessed by different morphs (reviewed in Gray & McKinnon, 2006). Progress has been made on morph-correlated traits and their potential role in color morph maintenance in a few well-studied systems (Sinervo & Lively, 1996; Calsbeek *et al.*, 2010), but our understanding of how these traits evolve and their function in maintaining phenotypic diversity within species remains fragmented.

Lizards provide a good system to study the causes and consequences of color polymorphism because color morphs have evolved several times in squamates (e.g. Iguania: *Ctenophorus decresii*, McLean *et al.*, 2014; *Sceloporus grammicus*, Bastiaans *et al.*, 2013; *Uta stansburiana,* Sinervo & Lively 1996; *Urosaurus ornatus,* Thompson & Moore 1991; Lacertidae: *Iberolacerta monticola* López *et al.*, 2009; *Zootoca vivipara*, Vercken & Clobert, 2008; *Podarcis gaigeae*, Runemark *et al.*, 2010; *Podarcis muralis*, Pérez i de Lanuza *et al.*, 2019). Most importantly, all of these lizard species share a similar color polymorphism presented as distinct color badges on the throat, suggesting a similar evolutionary origin and function (Stuart-Fox & Ord, 2004). Color polymorphism is common among lacertid lizards, and the lacertid genus *Podarcis* is highly color polymorphic (Sacchi *et al.*, 2007; Huyghe *et al.*, 2007; Runemark *et al.*, 2010; Pérez i de Lanuza, Sillero & Carretero, 2018). Previous studies of color polymorphic *Podarcis* species have identified color morph differences in size and survival (Calsbeek *et al.*, 2010), ability to win staged contests (Huyghe *et al.*, 2012; Abalos *et al.*, 2016), absolute maximum bite force capacity and head muscle mass (Huyghe *et al.*, 2008), and circulating hormone levels associated with aggressive behaviors (Huyghe *et al.*, 2009). A lizard's body size and bite force capacity are critical functional traits that directly relate to its ability to acquire and defend resources (Huyghe *et al.*, 2005; Donihue *et al.*, 2015). In color polymorphic *Podarcis* species, male morphs differ in their maximum bite force capacity (Huyghe *et al.*, 2007; Pérez i de Lanuza *et al.*, 2014), largely due to morph differences in head size (Huyghe *et al.*, 2009). Color morphs may have different head morphologies due to different levels of circulating hormone levels, such as testosterone, associated with muscle development (Huyghe *et al.*, 2009b; Regnier & Herrera, 1993). Morphs may also differ in head size and bite force as a consequence of partitioning their dietary niche (Lattanzio & Miles, 2016), as differences in diet hardness can influence lizard bite force among species and even populations (Herrel *et al.*, 1999; Herrel *et al.*, 2004; Donihue *et al.*, 2016). In lizards, male size and bite force are important determinants in the outcome of agonistic and mating encounters and can indicate overall male quality (Tokarz, 1985; Lailvauz *et al.*, 2004; Huyghe *et al.*, 2005; Hardy & Briffa, 2013). Thus, color morph differences in bite force can play a significant role in an individual's social status.

In addition to visual signals such as color, chemical signals also play an important role in intra-specific communication and social organization in reptiles (Mason & Parker, 2010; Martín & López, 2014; Baeckens 2019). In lacertids, male lizards have distinct femoral glands that produce a lipid- and protein-rich exudate (López & Martín, 2002; López *et al.*, 2002; Mayerl *et al.*, 2015; Mangiacotti *et al.*, 2017). Both males and females strongly rely on chemical cues and use information from these secretions, particularly from the lipophilic fraction (Martín & López, 2015), to select mates (López *et al.*, 2002), judge competitive ability (Carazo *et al.*, 2007) and dominance status (López *et al.*, 2002). In some lacertid species, discrete male color morphs are also chemically polymorphic in

waxy secretions exuded from their femoral pores (Pellitteri-Rosa *et al.*, 2014; Mangiacotti *et al.*, 2019a). This suggests morph-correlated chemical signals may be used to indicate male quality and health status and be involved in mate choice. These discoveries suggest sexually selected traits such as size, bite force, and chemical communication are important for social interactions that may influence morph fitness (López *et al.*, 2009; Lopez-Darias *et al.*, 2015; Pérez i de Lanuza *et al.* 2017). Thus, we postulate these traits mediating social interactions are likely important to the evolutionary maintenance of color polymorphism in lizards.

The Aegean wall lizard (*Podarcis erhardii*) has been widely studied ecologically (Pafilis *et al.*, 2009; Brock *et al.*, 2015; Lymberakis *et al.*, 2018), but remains an understudied species when it comes to color polymorphism, though it displays variation in throat color within populations (Arnold & Burton, 1978). To date, there have been no studies on the variable throat colors displayed by *P. erhardii*. Here we undertake the first study examining color morphs in this species. We examined the relationships among color, bite force, and chemical signal profile in a large island population of lizards which exhibit variation in throat colors. Specifically, we investigated whether throat color can be reliably discriminated into three discrete color morphs, and if color morphs differ in two traits important to lizard social behavior: maximum bite force and chemical profiles from exudate secreted from male femoral pores. Our main questions and predictions are:

(1) Do the throat color patches on adult *P. erhardii* represent discrete color morphs?
Given previous work in other *Podarcis* species (e.g. Huyghe *et al.*, 2007; Calsbeek *et al.*, 2010; Andrade *et al.*, 2019), we expect that variation in *P. erhardii* throat color is discrete and can be discriminated into three morph types: orange, yellow, and white, and that both sexes contain the same three color morphs.

(2) Do color morphs differ in their maximum bite force capacity?
We postulate that color morphs will have different maximum bite force capacities and associated differences in morphological traits important to lizard bite force such as body size and head morphometrics.

(3) Do male color morphs differ in their chemical signal profile?
Given previous work on chemical signatures from other color polymorphic lacertids (López *et al.*, 2009; Pellitteri-Rosa *et al.*, 2014; Mangiacotti *et al.*, 2019a), we expect that *P. erhardii* morphs benefit from conveying information on their life strategies (or polymorphism) not only through color, but also through scent. Therefore, we predict that polymorphism in coloration matches polymorphism in chemical signal profile in *P. erhardii*.

MATERIALS AND METHODS

Study species.

The Aegean Wall Lizard (*Podarcis erhardii*) is a small to medium-sized ground-dwelling lacertid with an adult snout-vent length (SVL) of 45-78 mm and a tail approximately twice as long as the body (Gruber, 1987, Figure1A.). This species exhibits substantial variation in dorsal color, pattern, body size, morphology, and behavior across its range (Brock *et al.*, 2015; Donihue *et al.*, 2015; Marshall *et al.*, 2015). Similar to other color polymorphic *Podarcis* species (Andrade *et al.*, 2019), *Podarcis erhardii* is known to have variable throat coloration within populations (Arnold & Burton, 1978; Figure 1B.), and displays geographic variation in the frequency of throat color morphs across island and mainland populations (K.M. Brock unpublished data). This species of wall lizard is endemic to the southern Balkans and occurs on hundreds of Aegean islands (Valakos *et al.*, 2008; Speybroeck *et al.,* 2016). True to their vernacular name, they are typically encountered in habitats that are a mixture of dry stone walls surrounded by a mixture of low, spiny phrygana vegetation and grasses.

Study area.

We conducted our study in the terraced agricultural village of Moni (elev. 590 meters a.s.l., 37º04'54.1" N, 25º29'35.0" E) in the foothills below Profitis Ilias peak on Naxos island. Naxos is the largest island (440 km²) in the Cyclades island cluster (Aegean Sea, Greece) and harbors *P. erhardii* from low elevation sandy substrates with sparse vegetation to mid-elevation montane landscapes with diverse dwarf scrub communities. We chose Moni village because the site is largely representative of habitats on the island. The site is situated off a remote hiking path lined with dry stone walls that run through terraced agricultural plots. Vegetation at the site is a mixed matrix of grasses, sclerophyllous evergreen maquis, phrygana (*Euphorbia acanthothamnos*), and olive trees (*Olea europaea*).

Field methods.

Adult *P. erhardii* were captured from our study site in Moni during the month of May in 2017 and 2018. Lizards collected in 2017 include both adult females and males, while lizards in 2018 consist of only males used for exudate chemical analysis. To avoid sampling the same males from 2017 in 2018, we only used individuals with complete un-autotomized tails as all lizards from 2017 had 20 mm of tail tip taken for specimen collection. Upon capture, we sexed lizards; adult males were determined from their enlarged femoral pores and swollen tail base, and adult females were distinguishable by their smaller heads, longer bodies relative to head size, and absence of femoral pores. Lizards with a SVL less than 45 mm were deemed immature and inappropriate to include in color and morphometric comparisons, and immediately returned to their capture site. Animals were transported from the field to the laboratory in individual cloth lizard bags for subsequent measurement. All research was conducted in accordance with the University of California, Merced Institutional Animal Care and Use Committee (IACUC protocol AUP17-0002) and permits provided by the Greek Ministry for Environment and Energy (codes Ψ4Γ64653Π8-ΗΛ5 and Ω8Δ84653Π8-ΒΞΧ assigned to K.M. Brock).

Throat color measurements and analysis.

We collected lizard throat color data from 47 females (N = 8 orange, 21 yellow, and 18 white individuals) and 85 males (N = 31 orange, 29 yellow, and 25 white individuals) in May 2017 (2017 lizards were also used in bite force analyses). To quantify color, lizard throat color patches were first measured with an Ocean Optics Flame S-UV-VIS Fibre Optic Spectrometer 200-850nm (Ocean Optics Inc. Dunedin, FL, USA) and Xenon pulse light source connected to a probe with a fibre optic cable. Measurements were calibrated with a white WS-1-SL Labsphere Diffuse Reflectance Standard (Spectralon, Ocean Optics). Spectra were collected by placing the spectrometer illumination probe perpendicular to the surface of the throat 5 mm away from the skin (Pérez i de Lanuza & Font, 2015; Badiane *et al.*, 2017). Measurements were a circular point sample 3mm × 3mm, and we took six measurements of lizard throat color patches at landmark throat scale locations (Figure 1B) to avoid measurement bias and capture potential variation across the entire signal (Pérez i de Lanuza *et al.*, 2019).

Lizards were visually examined by K.M. Brock and assigned to one of three throat color categories, or morphs: orange, yellow, or white. Throat color signals in this species are comparatively simple and clear to discriminate by eye (Figure 1B) compared to other species that have mottling or fine throat color patterning (Teasdale *et al.*, 2013). For each lizard, we averaged the six throat spectra from the throat patch using the *aggspec* function in the R package 'pavo' to calculate one spectral measurement per lizard for analysis (Maia *et al.*, 2019). Averaged spectra were then smoothed to reduce noise in our reflectance curves (Teasdale *et al.*, 2013). To determine our smoothing parameter, we assessed reflectance curves against our raw, unsmoothed curves and set our smoothing span option to 0.2 nm to preserve shape and minimize noise. We extracted a suite of 23 colorimetric variables pertaining to hue, saturation, and brightness from our smoothed total reflectance data using the *rspec* function in the 'pavo' package in R (Montgomerie, 2006; Maia *et al.*, 2019). We selected 10 of the extracted colorimetric variables (mean brightness, intensity, UV chroma, yellow chroma, green chroma, blue chroma, red chroma, contrast, and hue; Supplemental 1) for their relevance to the trichromatic visual system of wall lizards (Martin *et al.*, 2015), robustness to smoothing correction (Maia *et al.*, 2019) and satisfaction of collinearity assumptions of K-means clustering and Linear Discriminant Function Analysis (LDFA) analyses. These 10 variables were used in all of our color analyses.

We first determined the optimal number of color morph categories in each sex we used an unsupervised K-means clustering analysis. We used the *clusGap* function in the R package 'cluster' (v.2.0.7-1, Maechler *et al.*, 2019), which calculates a goodness of clustering measure with a gap statistic (Tibshirani *et al.*, 2002). The gap statistic uses the output from the K-means clustering algorithm and compares the total within-cluster dispersion for different values of K with their expected values under a null reference distribution of the data (Tibshirani *et al.*, 2002). The estimated optimal number of clusters is where the gap statistic is maximized, or furthest away from a random uniform distribution of points. We *a priori* set the potential number of K-means to 6, given the number of color morphs in other *Podarcis* species (Huyghe *et al.*, 2007; Calsbeek *et al.*, 2010; Runemark *et al.*, 2010; Andrade *et al.*, 2019).

Finally, we used a Linear Discriminant Function Analysis to predict color morph groupings from our 10 colorimetric predictor variables and assure accuracy of our visual

assignments using the *lda* function from the 'MASS' package (v 7.3-50) in R (Venables & Ripley, 2002). LDFA determines group means of the 10 colorimetric predictor variables for each morph and computes, for each individual, the probability of belonging to different color morph categories. We then tested if color morphs significantly differed based on the same 10 colorimetric variables using a Wilk's Lambda test.

For both the K-means clustering analysis and LDFA, we analyzed females and males caught in 2017 separately to reliably identify morph types for each sex. All analyses and data visualization were performed in R (v. 1.1.456) (R Core Team, 2018).

Bite force measurements and analysis.

We collected bite force data from adult females and males in May 2017. These same lizards were used in the color analyses mentioned above. We measured lizard bite force with a purpose-built bite force meter consisting of two metal bite plates connected to a Kistler force transductor (type 9203; Kistler Inc., Switzerland) and pivot over a microcaliper fulcrum (full bite force meter specifications in Herrel *et al.*, 1999). Bite plates were placed toward the anterior of the lizards' mouth in straight alignment with the lizards' body to ensure consistent replication across individuals (Lappin & Jones, 2014). We recorded bite force of each individual in three repeated trials and used the hardest bite as our measure of maximum bite force (Anderson *et al.*, 2008; Donihue *et al.*, 2015). Lizard size is known to positively correlate with bite force capacity in this system (Donihue *et al.*, 2015), so we also measured several lizard head and body features that may explain variation in bite force among morphs. Morphometric data taken for each lizard include: SVL, head length (tip of snout to posterior of parietal scale), head width (at the widest point just posterior of ear opening), and head height (at posterior of parietal scale). Lizard morphometric data used in bite force analyses were taken with a Mitutoyo 500-171-30 Absolute Scale Digital Caliper.

Maximum bite force is known to differ substantially between the sexes across many taxa for social and ecological reasons (Herrel *et al.*, 1996; Herrel *et al.*, 2007; Sagonas *et al.*, 2014; Donihue, 2016). Keeping this pattern in mind, we analyzed the sexes separately (N = 45 females, N = 81 males). We assessed bite force data for normality and subsequently removed 4 outliers from the dataset that were well below the minimum first quartile, most likely due to a poor grip on the bite plate. Bite force and morphometric data were normally distributed and did not require transformation for analysis. We used ANOVAs and post hoc Tukey HSD tests to investigate univariate differences in bite force related head traits among morphs. To evaluate the influence of SVL and color morph on bite force, we ran Generalized Linear Models (GLMs, Dobson, 1990) with maximum bite force as the dependent variable and SVL and color morph as independent variables. GLMs were run using the *glm* function in the 'stats' package (v3.5.1) in R (R Core Team, 2018). All analyses and data visualization were carried out in R (v. 1.1.456) (R Core Team, 2018).

Collection of glandular secretions and chemical analysis.

In 2018, we re-visited our study population in Moni and collected glandular secretions and corresponding throat color measurements from 39 adult lizards (N = 11 orange males, N = 15 yellow males, and N = 13 white males). Since the femoral glands

of females are vestigial and non-active (Mayerl *et al.*, 2015), only males were sampled. Immediately after the lizards were captured, we collected femoral gland secretions by gently squeezing around the femoral pores. The secretions were subsequently transferred to glass vials with glass inserts sealed with Teflon-lined lids. Blank controls were also created to exclude any contaminants from the handling procedure or the environment and to examine potential impurities in the solvent or analytical procedure. All vials were, thereafter, stored at −20°C before chemical analysis.

The identification of each chemical compound and estimation of its relative abundance (as percentage) was assessed using gas-chromatography-mass spectrometry (GC-MS). Here, we used the same methodology and protocol as described in earlier studies (e.g. López *et al.*, 2009; López *et al.*, 2009; Baeckens *et al.*, 2018; Supplemental 2). Because we were interested in examining differences among different color morphs in the overall chemical profile, we determined the relative amount of each compound as the percent of the total ion current (TIC) as in García-Roa *et al.* (2018).

Prior to statistical analyses of chemical profiles, proportions were logit transformed by taking the natural logarithm of proportion / (1-proportion). A constant value (0.01) was added to eliminate zero values in the data set allowing logit transformation. This compositional analysis corrects for the non-independence of proportions (Aebischer *et al.*, 1993). To test for differences in the chemical profiles of lizards belonging to different color morphs, we performed a single factor permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001; McArdle & Anderson, 2001). To do so, we first calculated Euclidean distances between every pair of individual samples to produce a resemblance matrix that formed the basis of the PERMANOVA (set at 999 permutations). To assess among-morph differences in more detail, we investigated the chemical profiles further using a canonical analysis of principal coordinates (CAP, Anderson & Willis, 2003) and a principal component analysis (PCA). Next, we performed univariate analyses of variance (ANOVAs) to test for inter-morph differences in chemical profiles based on principal component scores of the first few principal axes. We also tested for body size-dependent variation in the chemical signal design of *P. erhardii* by regressing SVL against the scores of the principal components. Data were analyzed in R v3.6.1 (R Core Team, 2018) and the software PRIMER v6.1.13 (Clarke & Gorley, 2006) with the 'PERMANOVA' +v1.0.3 add-on package (Anderson *et al.*, 2008).

RESULTS

An unsupervised K-means cluster analyses of 10 colorimetric variables (Table 1) revealed that the optimal number of color clusters for both females and males is 3 (Table 2). For both females and males, the global optimal number of K-means was 3, with a maximum gap statistic at K-means = 3 (Table 2).

Linear discriminant function analyses of the same 10 colorimetric variables (Table 1) discriminated between the three color morph classes (Figure 2A & 2B) in *P. erhardii* with some overlap in the 95% confidence ellipses of orange and yellow morphs for both sexes (Figure 2C & 2D). Results from LDFA of females and males show that the three color morphs are discernable (Figure 2C & 2D), and color morphs significantly differ based on the 10 colorimetric variables (Wilks' Lambda $p < 0.001$). Morph

predictions from LDFAs for both females and males closely matched observed morph assignments (Table 3). For females, multivariate discrimination of color morph based on the 10 colorimetric variables was significant (Wilks' Lambda = 0.042, $F(2,44) = 13.533$, $p < 0.001$). For males, multivariate discrimination of color morph based on the same 10 colorimetric variables was also significant (Wilks' Lamdba = 0.029, $F(2,82) = 35.729$, $p < 0.001$).

Maximum bite force was strongly positively correlated with all head morphometrics (head length, head width, head height) and head morphometrics were also strongly positively correlated with body size in both sexes (Table 4), so we focus here on the relationship between bite force, color morph, and body size because body size drives head size. Maximum bite force in male lizards was strongly positively correlated with lizard body size (Linear regression maximum bite force ~ SVL, adj. $r^2 = 0.644$, $p = 0.002$, $N = 81$, $df = 79$, Figure 3A), and male color morphs had significantly different SVLs (ANOVA SVL ~ morph $F(2,78) = 11.6$, $p < 0.05$, Table 4, Figure 3A). A post hoc Tukey HSD test showed that orange males had significantly longer SVLs than white and yellow males, which did not differ from each other in SVL (Table 4). We then analyzed differences in bite force among male morphs that accounted for lizard size in a GLM that included morph and SVL as fixed effects, and found no significant effect of morph on maximum bite force capacity (GLM maximum bite force ~ morph + SVL, $F(3,77) = 13.41$, morph $p > 0.05$, SVL $p < 0.001$).

Female maximum bite force was also positively correlated with body size (Linear regression maximum bite force ~ SVL, adj. $r^2 = 0.704$, $p < 0.001$, $N = 46$, $df = 44$, Figure 3B). In contrast to males, female color morphs did not differ in body size (ANOVA SVL ~ morph $F(2,43) = 0.651$, $p = 0.527$, Figure 3B, Table 4), or head morphometrics (Table 4). In a GLM that included morph and SVL as fixed effects, we did not detect a significant effect of morph on maximum bite force for female color morphs of *P. erhardii* from the same population (GLM maximum bite force ~ morph + SVL, $F(3,42) = 14.72$, morph $p > 0.05$, SVL $p < 0.001$).

From the femoral gland secretions of male *P. erhardii* (N = 39) originating from the study population in Moni (Naxos, Greece), we could identify 81 different lipophilic compounds (Supplemental 3). Considering all individuals together, secretions were mainly a mixture of steroids (average ± SE % of TIC: 68.4 ± 1.21%), waxy esters (17.7 ± 0.82%) and tocopherol (5.6 ± 0.88%). Fatty acids (3.3 ± 0.40%), alcohols (1.8 ± 0.10%), and aldehydes (1.4 ± 0.11%) were present in intermediate concentrations. Amides (0.6 ± 0.13%), ketones (0.5 ± 0.06%), a terpenoid (0.3 ± 0.06%), and furanones (0.3 ± 0.03%) were the four chemical classes with the lowest average proportion. On average, the five most abundant chemicals were cholesterol (39.8 ± 1.37%), campesterol (7.9 ± 0.41%), α-tocopherol (5.6 ± 0.88%), the 1.2-ethanediyl ester of hexadecanoic acid (5.5 ± 0.47%), and β-sitosterol (4.2 ± 0.45%).

The PERMANOVA, based on the resemblance matrix comparing the three morphs, showed borderline significant differences in the chemical profile of the three color morphs (pseudo $F(2,38) = 1.587$, $p = 0.067$). Pairwise PERMANOVA tests indicated statistically significant differences in the chemical composition of the glandular secretions, specifically, between orange and white morphs ($t = 1.590$, $p = 0.021$). There were no significant differences between yellow and white morphs ($t = 1.125$, $p = 0.243$)

and orange and yellow morphs ($t = 0.991$, $p = 0.435$). The CAP analysis classified 51.28% of the chemical profiles into the correct population using leave-one-out cross-validation ($\delta = 0.97$, $p = 0.389$, $m = 34$ axes; Figure 4A).

The first four principal components jointly explained 64.0% of the variation, with the first axis (29.0%) being strongly affected by α-tocopherol (loading = 0.70), eicosyl 9-octadecenoate (0.27), the 1,2-ethanediyl ester of hexadecanoic acid (-0.25), octadecanoic acid (0.25), and tetradecyl 9-octadecenoate (-0.24). We used the scores of the first four principal components (PCs) to test for among-morph differences using four separate univariate analyses of variance (ANOVAs). These tests indicated that PC1 varied significantly among color morphs ($F(2,36) = 4.273$, $p = 0.0216$; Figure 4B). A post hoc LSD multi-comparison (with Bonferroni correction) showed a significant difference between white and orange color morphs (Table 5), with orange males having higher proportions of α-tocopherol, eicosyl 9-octadecenoate, and octadecanoic acid, but lower proportions of 1,2-ethanediyl ester of hexadecanoic acid and tetradecyl 9-octadecenoate. There were no significant among-morph differences in PC2, PC3, and PC4 (all $F \leq 0.669$; $p \geq 0.518$). Lastly, variation in the chemical composition of the glandular secretion of *P. erhardii* could not be explained by variation in body size as none of the PCs showed a significant link with SVL (all $F \leq 1.382$, $p \geq 0.175$).

DISCUSSION

Prior to this study, *P. erhardii* was known to have variable throat color (Arnold & Burton, 1978; Runemark *et al.*, 2010), but its status as a color polymorphic species was never recognized as the number and types of color morphs were unknown. Multiple species in the *Podarcis* genus are color polymorphic (Andrade *et al.*, 2019), and *P. erhardii* can now be counted among them. We used quantitative analyses of spectral reflectance data to reliably classify *P. erhardii* into three discrete color morphs: orange, yellow, and white. We have also established that both sexes have the same three color morphs, which is not always the case even among species of color polymorphic lizards (e.g.: *Ctenophorus decresii* [Rankin *et al.*, 2016], *Urosaurus ornatus* [Hews & Moore, 1995], *Uta stansburiana* [Sinervo & Lively, 1996; Sinervo *et al.*, 2000]). Determining the number and types of morphs within color polymorphic species is an essential first step toward understanding the evolution and maintenance color polymorphism , and identifying morph-associated traits relevant to fitness is the logical next step toward that goal.

In some color polymorphic species, morph color can indicate age or social rank (Thompson & Moore, 1991; Martin *et al.*, 2013), and thus individuals may have the ability to change morph over their lifetime (Carpenter, 1995). In a color polymorphic Phrynosomatid lizard, *Urosaurus ornatus*, females retain an orange throat color badge into adulthood while males tend to experience ontogenetic throat color change from orange to blue, suggesting some social function (Carpenter, 1995). In our study population, orange males tend to be larger than yellow and white males, and it is possible that male color morphs exhibit ontogenetic color change or experience different growth rates. A recent study on the genomic basis of throat color polymorphism in closely related *P. muralis* found that throat color is controlled by genetic differences at two small regulatory regions of the genome, is heritable, and shared by seven species across the

Podarcis clade (Andrade *et al.*, 2019). The evolutionary maintenance of color polymorphism across the *Podarcis* clade seems to be the result of retained ancient genetic variation and hybridization (Andrade *et al.*, 2019). We do not yet know if *P. erhardii* shares the same genomic architecture as other color polymorphic *Podarcis* species, or if temporary ontogenetic shifts can alter morph state (Andrade *et al.* 2019). Further research is needed that incorporates skeletochronological assessment of age and long-term monitoring of individual color *in situ* to fully understand the underlying mechanisms that control throat color in this species and the entire *Podarcis* clade.

A lizard's bite force is directly related to its ability to acquire and defend essential resources for survival and reproduction (Huyghe *et al.*, 2005), such as food (Verwaijen *et al.*, 2002; Huyghe *et al.*, 2007), territory (Husak *et al.*, 2006), and mates (Vitt & Cooper, 1985). We predicted that distinct color morphs would have different maximum bite force capacities. We found that body size varied substantially between male color morphs, with orange males exhibiting significantly larger body and head size than yellow and white males, while female morphs did not significantly differ in and size dimensions. When controlling for body size, we do not observe a difference in bite force between color morphs in either sex. The proximate driver of variation in bite force is body size, and orange males tend to be larger than yellow or white males and thus have relatively stronger bites. Our results are comparable to findings from closely related *P. melisellensis* (Huyghe *et al.*, 2009), where orange males have larger head dimensions and also bite significantly harder than yellow and white males. It is well-established that body size and bite force matters in determining fight outcomes in male lizards (Vitt & Cooper, 1985; Anderson & Vitt, 1990; Sacchi *et al.*, 2009). Further, male-biased sexual size dimorphism is also indicative of sexual selection for larger male size, either through intra-sexual selection via male-male competition over territory and mates or inter-sexual selection through female mate choice (Shine, 1989). Given that *P. erhardii* males have larger heads and body size than females, and male color morphs differ in their size and bite force capacity, it is plausible that male morphs use different strategies for survival and reproduction. Alternative color morph strategies specifically related to fitness are common, and likely play a role in balancing selection that maintain morphs through time, such as the different behavioral reproductive tactics employed by male morphs that generate differences in access to mates in *Uta stansburiana* (Sinervo & Lively, 1996). Interestingly, we did not find the same pattern in female color morphs, which exhibited no difference in body size dimensions or bite force capacity. Few studies on color morph differences in morphology, performance, behavior, and physiology include females (Sinervo *et al.*, 1996; Huyghe *et al.*, 2009; Bastiaans *et al.*, 2013), which combined with long-held assumptions about female reproductive behavior has hindered our understanding of underlying mechanisms of evolution (Kamath & Losos, 2017). An interesting and open question is whether female color morphs differ in their reproductive strategies and behaviors (Vercken & Clobert, 2008; Galeotti *et al.*, 2013; Ortega *et al.*, 2015), particularly if they have a preference for males based on color (Perez i de Lanuza *et al.*, 2013), morph-correlated traits, or both. Future study on *P. erhardii* color morph reproductive strategies and their effects on fitness are needed to provide insight into mechanisms that balance and maintain color polymorphism.

Our findings show that the chemical composition of the glandular secretions of *P. erhardii* males varies among color morphs, and that this variation is body size-independent. Although the established polymorphism in discrete colors only partly matched the observed intraspecific variation in chemical signal signatures (as the chemical profiles of yellow males could not be distinguished statistically from white or orange males), the chemical profile of the secretions of orange males did significantly differ from those of white males. While comparable findings on color polymorphism partially matching chemical polymorphism have been reported for two other lacertid species, *Iberolacerta monticola* (two color morphs; López *et al.*, 2009) and *Podarcis muralis* (three color morphs; Pellitteri-Rosa *et al.*, 2014; Mangiacotti *et al.*, 2019a), to our knowledge no such findings have been reported in any other color polymorphic vertebrate taxa.

One hypothesis on why animals would benefit from broadcasting their individual morph state (and potentially alternative strategies) in different ways is to enhance signal effectiveness (redundant signaling hypothesis; Johnstone, 1996; Partan & Marler, 1999; Partan, 2013). The use of different signal modalities, both visual and chemical, might increase the chance that the message is accurately perceived by the receiver. For instance, including scent marks in ones signaling repertoire could be beneficial as they work in darkness and can remain operative in the absence of signaler (Müller-Schwarze, 2006). Yet, our findings show an incomplete overlap in visual and chemical polymorphism. One potential reason for this is methodological. We took a multivariate approach in our analyses to statistically test for morph differences. However, it could also be that lizards may discriminate between color morphs solely based on the absence or presence of a single compound, on the absolute concentration of a specific chemical, or on a specific combination of molecules (Wyatt, 2014). Alternatively, the visual and chemical signals of *P. erhardii* may not be redundant, but may convey different information to the receiver (multiple signaling hypothesis, Hebets & Papaj, 2005). For instance, in another lacertid lizard, *Lacerta schreiberi*, large color patches on the body of adult males and the chemical composition of their femoral secretions provide different information on the individual's levels of carotenoids and vitamin E (Kopena *et al.*, 2014). As such, exploiting a bimodal signaling system allows animals to convey multiple messages at once. More elaborate behavioral research is necessary to find out (1) whether color morph-specific differences in chemical signatures are functional, in that lizards can discriminate based on scent alone, and (2) whether lizards can discriminate color morphs on the multicomponent profile of the femoral secretions or if discrimination is based solely on individual compounds and changes in their proportions. In addition, since recent findings suggest that also the proteinic fraction of femoral secretions can play a role in lizard intraspecific communication (Mangiacotti *et al.*, 2017; 2019a,b,c), future studies that combine proteinic and lipophilic assessments of chemical signals should be encouraged.

Our results show that orange males tend to have higher proportions of α-tocopherol, eicosyl 9-octadecenoate, and octadecanoic acid, but lower proportions of the 1,2-ethanediyl ester of hexadecanoic acid and tetradecyl 9-octadecenoate than the other two color morphs. Previous research has shown that in lacertids, α-tocopherol (= vitamin E) and octadecanoic acid are two important compounds for intraspecific communication,

with the relative proportions of lipids in the femoral gland secretions providing information on overall male quality (Martín & Lopez, 2014; Martín & Lopez, 2015). Several studies have found that α-tocopherol can be exploited as an honest sexual signal, with high proportions of the compound increasing the attractiveness of a male's scent to female conspecifics (Kopena *et al.*, 2011; García-Roa *et al.*, 2017). Our finding that orange males produce secretions with high proportions of α-tocopherol (which indicate 'high quality' in other lacertid males) are consistent with our finding that orange males are the largest morph with the hardest relative bite force in comparison to the other two morphs. Additionally, Martín *et al.* (2007, 2008) showed that octadecanoic acid can act as a chemical ornament in lacertids signaling individual health, with lizards in good health (indicated by a high T-cell-mediated immune response) having low proportion of octadecanoic acids in their secretions. In this study, we observe that orange *P. erhardii* males secrete high proportions of octadecanoic acid, which partially goes against our earlier argument on orange males being the morph in 'best condition'. Still, it might be that the low immune response and associated high proportion of secreted octadecanoic acid is the result of orange males having high levels of testosterone. This is not unlikely since the immunosuppressive effect of testosterone is well documented in lizards (e.g. Belliure *et al.*, 2004; Oppliger *et al.*, 2004), and so is the observation that color morphs experience dissimilar hormone levels (e.g. Sinervo *et al.*, 2000; Huyghe *et al.*, 2009; Sacchi *et al.*, 2017). Chemical signals from males may interact with other male morph-correlated traits and behaviors, as well as female preference to balance the relative fitness of color morphs through time. Endocrinological and immunological research is required to determine whether *P. erhardii* color morphs differ in hormone levels and immunocompetence, and whether these may cause among-morph differences in chemical signal profile and fitness.

CONCLUSION

We found that *P. erhardii* male color morphs tend to differ in two important lizard social traits: their maximum bite force capacities (driven by body size differences) and chemical signal signatures. These observations suggest that male differences in physical and chemical traits may play an important role in color polymorphism maintenance via social interactions. Further investigation into male morph competitive ability and female morph mate preference will shed light on how these traits relate to sexual signaling and morph persistence. Throat color polymorphism has been associated with sexual selection in lizards (Sinervo & Lively, 1996; Sinervo & Calsbeek, 2006). Competition between males over females often results in male-biased sexual size dimorphism and male attributes that signal status, fighting ability, and fitness (Enquist & Leimar, 1983; Andersson, 1994). It is currently unknown whether *P. erhardii* prefers the scent of certain morphs with certain qualities, or whether there is assortative mating based on color, size, scent preference, or behavior. Future work that integrates color morph behavior, reproductive strategies, and phenotypic traits is needed to determine the role of social traits in the maintenance of color polymorphism.

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Figures

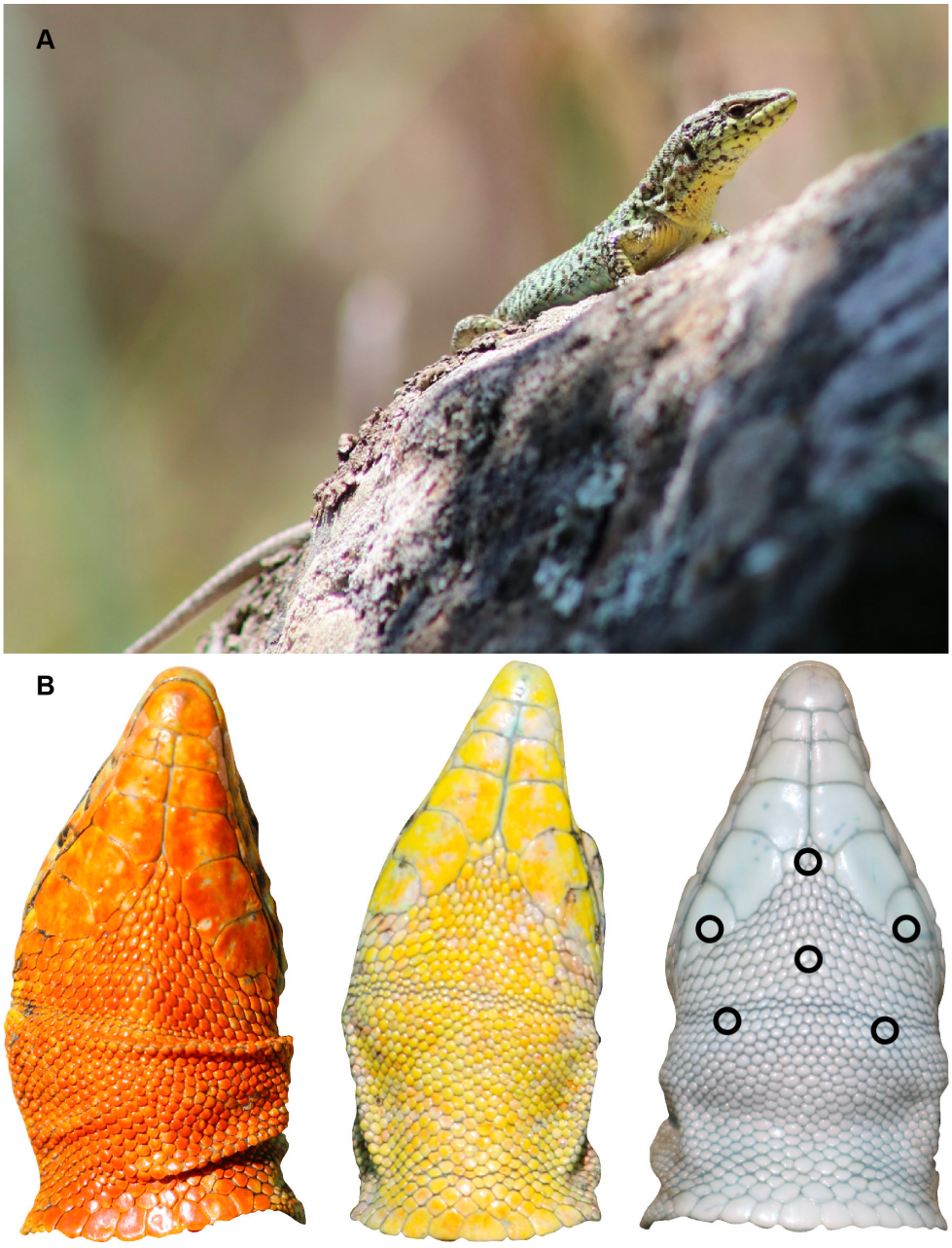

Figure 1. (A) An adult male yellow morph *P. erhardii* basking on a dry stone wall in Moni, Naxos, Greece. (B) Throat colors of adult male *P. erhardii* from our study population in Moni, Naxos, Greece. In *P. erhardii*, color polymorphism is restricted to the throat region and the rest of the venter is usually white. Black circles on the throat of the white color morph indicate the six landmark locations where we took spectrometer measurements.

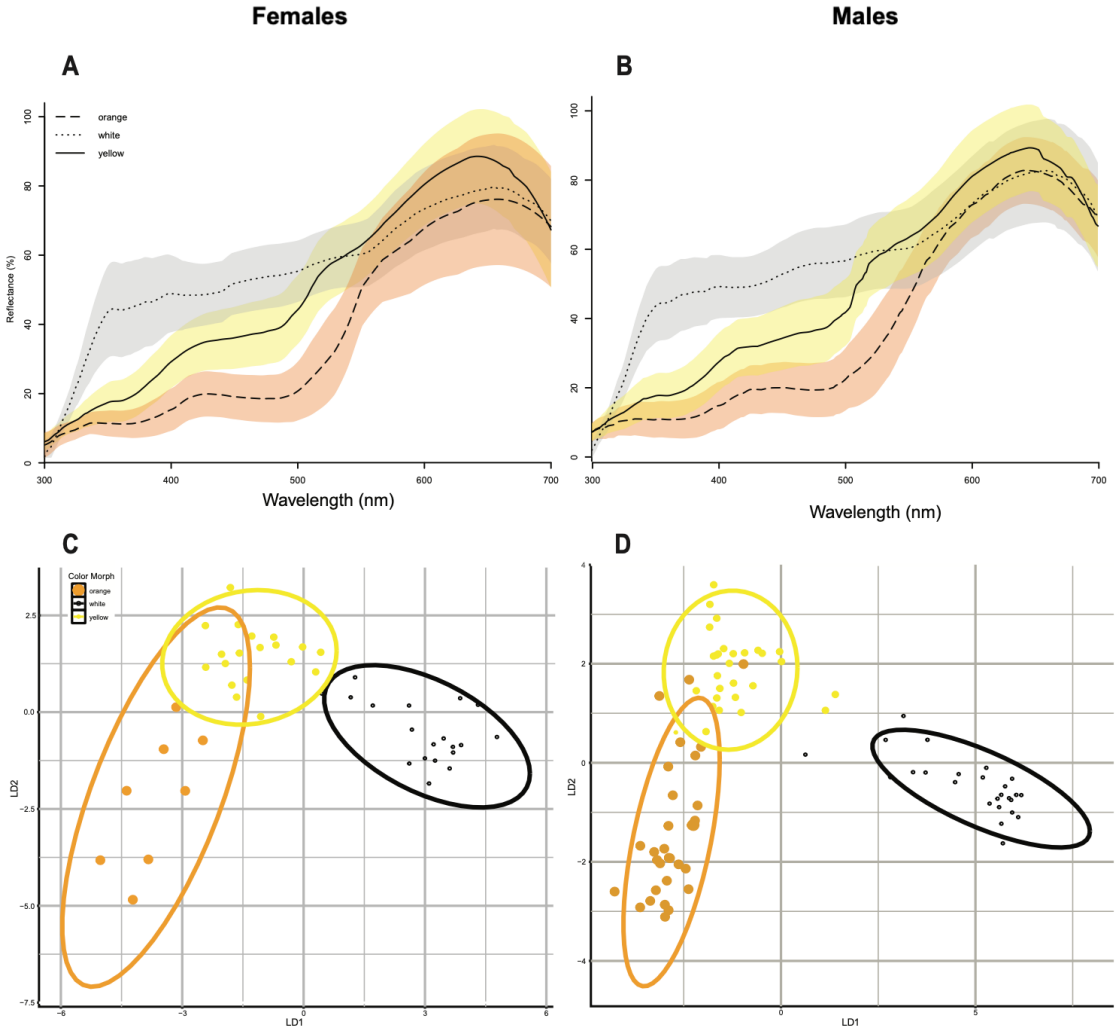

Figure 2. Color morphs in *Podarcis erhardii*. (A) Smoothed spectral reflectance curves of P. erhardii female color morphs. (B) Smoothed spectral reflectance curves of P. erhardii male color morphs. The average reflectance curves in (A) and (B) for orange morphs is represented by a long-dashed line, the average for yellow morphs is represented by a solid line, and the average for white morphs is a dotted line. (C) Linear discriminant function analysis of morphs from female color data. (D) Linear discriminant function analysis of morphs from male color data. For females and males, the first linear discriminant function (x-axis, LDA1) separates orange and yellow morphs from white morphs, with no overlap in the 95% confidence interval of white morphs. The second linear discriminant function (y-axis, LDA2) separates orange and yellow morphs, with some overlap in both females and males.

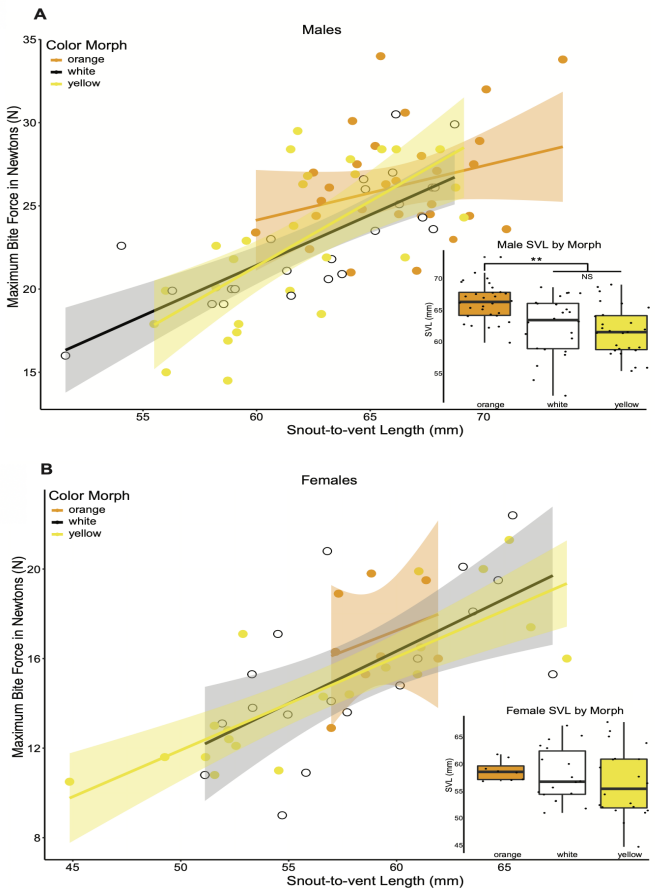

Figure 3. Relationship between maximum bite force and lizard body size (SVL) among color morphs. (A) Relationship between SVL and maximum bite force capacity in male color morphs. Bite force had a positive relationship with body size across all male color morphs. Within morphs, a significant positive correlation between maximum bite force and SVL was detected for yellow (Pearson corr = 0.663, R^2 adj = 0.417) and white (Pearson corr = 0.781, R^2 adj = 0.593, $p < 0.001$) morphs, while no significant correlation was detected for orange morphs (Pearson corr = 0.282, lm R^2 adj = 0.047, $p = 0.13$). Boxplot of male snout-to-vent length (SVL) by color morph. Orange males had significantly longer SVLs than white and yellow males (ANOVA SVL ~ morph $F(2,78) = 11.6$, $p < 0.001$, Tukey HSD orange-white $p = 0.001$, orange-yellow $p < 0.001$, denoted by a double asterisk). (B) Relationship between SVL and maximum bite force capacity in female color morphs. Bite force also increased with body size across all female color morphs. We detected a significant positive relationship between maximum bite force capacity and SVL for yellow (Pearson corr = 0.808, lm R^2 adj = 0.634, $p < 0.001$) and white (Pearson corr = 0.637, lm R^2 adj = 0.369, $p < 0.005$) morphs. The relationship between bite force capacity and SVL was positive but not statistically significant for orange females (Pearson corr = 0.298, lm R^2 adj = -0.063, $p = 0.473$), possibly due to small sample size (N = 8). Boxplot of female snout-vent length (SVL) by color morph. We did not detect a significant difference in SVL among female color morphs.

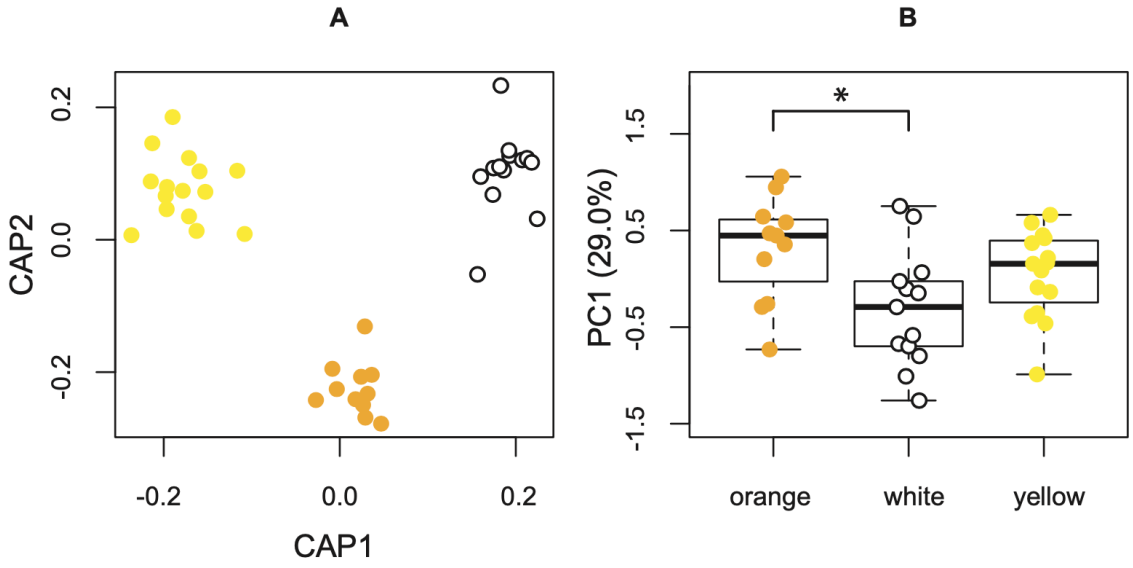

Figure 4. Chemical analyses of pore exudate. (A) A representation of the two first axes of the canonical analysis of principal coordinates (CAP) showing classification of the chemical profile of the three *P. erhardii* color morphs. (B) A boxplot showing inter-morph differences in PC1 scores. Asterisk annotates statistical significance.

Tables

Table 1. Results from linear discriminant function analyses testing for the presence of three discrete color morphs in *Podarcis erhardii*. Coefficients of linear discriminants are shown for each of the 10 colorimetric variables used in LDFA of females from 2017 (N = 47), males from 2017 (N = 85).

	2017 Females N = 47		2017 Males N = 85	
Linear Discriminant	Linear Discriminant 1	Linear Discriminant 2	Linear Discriminant 1	Linear Discriminant 2
Mean brightness	0.092	0.138	-0.213	0.088
Intensity	0.105	0.156	0.101	0.051
UV chroma	-1318.082	11529.59	-867.469	5969.393
Yellow chroma	133.069	-63.721	-12.332	-105.373
Blue chroma	-1299.786	11522.04	-846.666	5934.362
Green chroma	-1355.169	11550.36	-888.693	6035.815
Red chroma	-1395.448	11597.21	-887.764	6007.215
Contrast	-0.155	-0.246	0.049	-0.105
Spectral saturation	-6.105	-2.555	-9.871	0.476
Hue	0.129	0.041	-0.019	0.027

Table 2. Observed vs. predicted frequencies of color morph category assignments from linear discriminant function analyses. For 2017 females and males, our models including 10 colorimetric variables predicted all observed yellow individuals to belong to the yellow morph category. Observed orange individuals were never predicted to be white, and observed white individuals were never predicted to be orange. In a few instances, observed orange and white individuals were predicted to belong to the yellow morph category.

		Observed orange	Observed yellow	Observed white	Sum
2017 Females	Predicted orange	7	0	0	7
	Predicted yellow	1	20	1	22
	Predicted white	0	0	18	18
	Sum	8	20	19	47
2017 Males	Predicted orange	27	0	0	27
	Predicted yellow	4	29	1	34
	Predicted white	0	0	24	24
	Sum	31	29	25	85

Table 3. Gap statistic results from K-means cluster analysis. For both females and males, the optimal number of clusters in the colorimetric data was 3. Maximized gap statistic, where K-clusters are maximally distant from each other, is indicated in bold.

	2017 Females N = 47		2018 Males N = 85	
K-means	Gap statistic	Std. Error	Gap statistic	Std. Error
1	0.272	0.035	0.309	0.025
2	0.363	0.033	0.440	0.024
3	**0.385**	0.031	0.502	0.022
4	0.358	0.031	0.492	0.022
5	0.366	0.032	0.496	0.021
6	0.382	0.032	0.495	0.021

Table 4. ANOVA and Tukey HSD tests of SVL and head morphometric variables among color morphs. Females and males were analyzed separately. Bolded values with an asterisk denotes a significant difference in size between color morphs detected at alpha = 0.05.

Morphometric ANOVA	Post hoc comparisons	Male Difference	Male *P (adj)*	Female Difference	Female *P (adj)*
Male SVL F(2,78) = 11.6 Female SVL F(2,43) = 0.527	white-orange	-3.748	**0.001***	-0.805	0.932
	yellow-orange	-4.601	**< 0.001***	-2.268	0.568
	yellow-white	-0.853	0.709	-1.463	0.675
Male head length F(2,78) = 14.64 Female head length F(2,43) = 0.221	white-orange	-1.161	**< 0.001***	-0.158	0.892
	yellow-orange	-1.33	**< 0.001***	-0.523	0.285
	yellow-white	-0.169	0.821	-0.365	0.360
Male head width F(2,78) = 12.41 Female head width F(2,43) = 0.469	white-orange	-0.865	**< 0.001***	-0.062	0.957
	yellow-orange	-0.879	**< 0.001***	-0.184	0.669
	yellow-white	-0.014	0.997	-0.123	0.744
Male head height F(2,78) = 11.1 Female head height F(2,43) = 0.822	white-orange	-0.417	**0.043***	-0.287	0.431
	yellow-orange	-0.422	**0.032 ***	-0.245	0.531
	yellow-white	-0.005	0.999	-0.043	0.968

Table 5. A post hoc LSD multi-comparison (with Bonferroni correction) among male morphs for PC1. A significant difference detected at alpha = 0.05 is bolded with an asterisk.

Post hoc comparisons of 2018 male morph chemical profiles	Difference	*P (adj)*
orange - white	0.629	**0.019***
orange - yellow	0.265	0.649
white - yellow	-0.364	0.237

Morph Identity Predicts Social Behavior and Contest Outcomes in a Polymorphic Lizard (*Podarcis erhardii*)

ABSTRACT

Space is a limited resource that many animals need to perform basic functions such as feeding and reproducing. Competition over access to space can induce aggressive and signaling behaviors, which may result in differential access to crucial resources related to survival and fitness. The Aegean wall lizard, *Podarcis erhardii*, is a tri-color polymorphic lizard that eponymously inhabits, among other habitat types, dry stone walls where they access food, safely thermoregulate, shelter from predators, and display to other lizards. Adult male color morphs exhibit physical differences in size and chemical signaling profiles, but nothing is known about potential morph-specific differences in behavior. Many color polymorphic species have behavioral morphs, which may play a role in morph evolution and maintenance. Here, we conduct the first behavioral experiments on *P. erhardii* color morphs to identify morph competitive ability and characterize morph differences in behaviors involved in accessing and occupying a limited space resource. Experimental contests over limited heated space on a rock wall in a neutral laboratory arena revealed that male color morph identity, not size or individual identity, predicts inter-morph contest outcomes. We also found that male color morphs exhibit different levels of aggressive and display behaviors. Considering these results, we discuss the role of male color morph behavior in morph evolution, persistence, and loss.

KEYWORDS

Color polymorphism, Alternative morph tactics, agonistic behavior, visual signaling, chemical signaling, Aegean wall lizard, *Podarcis erhardii*

INTRODUCTION

Color polymorphic species are ideal systems for understanding how phenotypic variation evolves and is maintained within populations (Ford, 1945; Gray & McKinnon, 2007). Color polymorphism is the presence of two or more genetically determined color phenotypes within a single interbreeding population (Huxley, 1955), and has been identified in a wide range of taxa, from invertebrates to birds (Jamie & Meier, 2020). Intra-specific color morphs often, if not always, exhibit additional differences in traits besides color (McKinnon & Pierotti, 2010; Stuart-Fox *et al.*, 2020), such as morphology (Brock *et al.*, 2020), physiology (Huyghe *et al.*, 2009), and behavior (Sinervo & Lively, 1996). These multi-trait differences between color morphs can evolve via correlational selection, whereby genetic correlations of certain combinations of heritable traits are favored (Roulin, 2004). A mystery surrounding alternative morph phenotypes in natural populations is their long-term maintenance: how does color polymorphism persist in the face of natural selection and other evolutionary forces such as genetic drift, which tend to reduce genetic variation in populations (Roulin, 2004; Runemark *et al.*, 2010)? Although polymorphic species may differ in the number of color morphs and types of morph-correlated traits, a prevailing similarity across these systems seems to be that morph

diversity is maintained by some type of balancing selection for alternative multivariate morph phenotypes (reviewed in Gray & McKinnon, 2007 and Stuart-Fox *et al.*, 2020). Thus, identifying morph-correlated traits and the context in which they function is essential for understanding how color polymorphism is maintained within populations.

In many color polymorphic species, color morphs exhibit morph-specific behavioral strategies (Brodie, 1992; Sinervo & Lively, 1996; Sinervo *et al.*, 2001; Dijkstra *et al.*, 2008; Kupper *et al.*, 2016; Barcelo-Serra *et al.* 2020). Often, these morph-specific behavioral strategies are under correlational selection and are involved in predator avoidance or reproduction, and thus have fitness consequences (Brodie, 1992; Kupper *et al.*, 2016). Alternative morph phenotypes, whether tied to predator avoidance, foraging, mating, or aggression, must somehow be balanced such that no morph allele frequency dominates and becomes fixed in the population. The relative frequency of morphs can remain at a stabilized equilibrium, but in many color polymorphic systems morph frequencies oscillate through time (Gross, 1991; Sinervo & Lively, 1996; Olendorf *et al.*, 2006). Interactions between morphs can generate balancing or frequency-dependent selection that maintains the polymorphism within the population (Sinervo & Lively, 1996; Dijkstra *et al.*, 2008; Iversen *et al.*, 2019), though the nature of these interactions varies widely from species to species. Understanding how color morphs interact is an important step toward identifying alternative strategies and mechanisms that influence morph fitness, frequencies, and maintenance.

Access to resources such as quality habitat, food items, and mating opportunities is crucial for animal survival and fitness, and is limited by competition with conspecifics and avoidance of predators (Andersson, 1994). If morphs have different abilities to access resources that enhance fitness, those behaviors that confer an advantage, or disadvantage, can influence the frequency of morph alleles in color polymorphic populations (Sinervo & Lively, 1996). High levels of aggression and boldness are often associated with dominance and greater reproductive success (Ficken *et al.*, 1990; Kingston *et al.*, 2003). Color morphs in birds (Pryke, 2006; Horton *et al.*, 2012), lizards (Abalos *et al.*, 2016), and fish (McKaye & Barlow, 1976) distinctly vary in levels of aggression. Moreover, morph color, or some combination of morph traits, can signal information about competitive ability to the receiver. These signals could also be used differently depending on if the receiver belongs to the same or different color morph (Bruinjé *et al.*, 2019). In some species, morphs employ different aggressive behaviors in competitive situations based on color morph identity (Tinghitella *et al.*, 2018). In many instances male morphs exhibit aggression bias, and they tend to be more aggressive toward individuals of the same color (Dijkstra *et al.*, 2007; Horton *et al.*, 2012). Same-morph aggression bias could generate a frequency-dependent advantage for the morph at the lowest frequency in the population, since it would experience overall lower levels of aggression (Seehausen & Schluter, 2004). Nevertheless, negative interactions such as competition and territoriality are not the only interactions that may shape coexistence and evolution (Kamath & Wesner, 2020). Morphs may use color in combination with other signaling traits to identify situations in which it is advantageous to yield, cooperate, or share (Smith & Price, 1973; Sinervo & Lively, 1996). Studies of alternative morph strategies have centered around male aggression and reproduction, and far less is known about non-

aggressive morph interactions, signaling, and how these interactions vary by morph type within color polymorphic populations.

The Aegean wall lizard, *Podarcis erhardii* (Gruber, 1987), is a color polymorphic lacertid lizard with multiple discrete color morphs in both sexes. Females and males have throat badges that are orange, yellow, or white (Brock *et al.*, 2020). Female color morphs within a population do not differ in body size or head dimensions, but orange males have significantly larger body and head sizes than yellow and white male morphs (Brock *et al.*, 2020). Body size and mass have strong relationships with resource holding potential and the ability to persist in contests (Parker, 1974; Arnott & Elwood, 2009). Due to differences in head size dimensions, male *P. erhardii* color morphs also have different bite force capacities (Brock *et al.*, 2020), which is a known predictor of contest success in lizards (McLean & Stuart-Fox, 2015). The larger orange morph tends to bite harder than the yellow and white morphs, which do not differ in their maximum bite force capacities (Brock *et al.*, 2020). Further, male morphs in this species have significantly different proportions of chemical compounds in exudate secreted from their femoral pores (Brock *et al.*, 2020), which can be used for myriad signaling functions in lacertids (reviewed in Martín & Lopez, 2014), including territory demarcation (Aragón *et al.*, 2001), male rival assessment (López & Martín, 2002), and female choice (Gabirot *et al.*, 2013). Most *Podarcis* wall lizard species are color polymorphic (Brock *et al.*, 2021 [Chapter 4]), and male morphs in *Podarcis* species are known to display different levels of aggression, hormones, and chemical signal profiles (Huyghe *et al.*, 2009; Abalos *et al.*, 2016; Brock *et al.*, 2020). However, few studies have examined potential morph differences in their ability to access limited resources like high-quality habitat, and experiments that quantified multiple types of behaviors outside of pure aggression, such as visual and chemical signaling, are limited (but see McLean & Stuart-Fox, 2015 and Bruinjé *et al.*, 2019).

Here, we conducted the first study on color morph behavior in *P. erhardii*. Our aim was to identify morph-specific behavioral strategies that may play a role in the maintenance of diverse phenotypes within a population. To test this, we staged one-on-one encounters between adult males in a neutral arena with a heated rock wall resource to determine each morph's ability to maintain access to the rock wall and to observe the behaviors of each color morph during intra- and inter-morph contests. We quantified aggressive, bold, and signaling behaviors and duration of rock wall access in experimental contests to answer three main questions: 1) Does color morph identity predict an individual's ability to win one-on-one contests over a limited resource? 2) Do adult male *P. erhardii* color morphs perform behaviors at different frequencies? And do these frequencies change based on contestant morph identity? And 3) Do morphs exhibit significantly higher levels of aggression toward like-morphs?

We hypothesized that morph color would predict contest outcomes over a limited space resource. Specifically, we predicted that the orange morph would win more against white morphs and yellow morphs, probably due to their larger size in nature and potentially higher levels of testosterone. We also hypothesized that color morphs would exhibit behaviors at different frequencies during contests over a limited heated space, and that the frequency of these behaviors would vary depending on the opponent's morph identity. Finally, we hypothesized that the orange morph would perform more aggressive

behaviors toward other morphs, and that color morphs would engage in more aggressive behaviors with morphs of their own kind compared to morphs of other colors.

MATERIALS AND METHODS

Study species.

The Aegean wall lizard (*Podarcis erhardii*, Bedriaga, 1882) is a small lacertid species with an adult snout-vent-length between 45-80 mm and a tail twice as long as the body (Valakos *et al.*, 2008). *Podarcis erhardii* is endemic to the southern Balkans and has a distribution that stretches across southern Bulgaria, North Macedonia, Albania, and Greece, including hundreds of Aegean islands (Valakos *et al.*, 2008). This species occurs in a variety of habitats ranging from rocky desert islets, sandy arid shores, mixed low spiny vegetation and grasses, and montane forest regions up to 2000 m in elevation. As their vernacular name suggests, these lizards are typically found on dry stone walls where they can access food items, prominently display to conspecifics, and thermoregulate under cover. *Podarcis erhardii* is diurnal, and is most active from 0800 - 1200 and 1700 - 1900 during spring and summer. The breeding season typically lasts from April to June with the last hatchlings of the year emerging in August.

Our current knowledge of *P. erhardii*'s behavior is limited, and mostly consists of its physiology, thermoregulatory and anti-predator behavior (Li *et al.*, 2014; Brock *et al.*, 2015; Marshall *et al.*, 2016; Belasen *et al.*, 2017; Pafilis *et al.*, 2019). Relatively little is known about intra-specific interactions and behaviors in this species and virtually nothing is known about color morph behavior. However, throughout its range and in a variety of ecological contexts, *P. erhardii* is known to engage in intra-specific aggressive physical behavior such as biting that leaves scars, finger and tail consumption, and even cannibalism (Donihue *et al.* 2015; Madden & Brock, 2018). In small, isolated island populations with relatively low resource availability, adult lizards tend to exhibit more intra-specific scarring, digit loss, tail autotomy rates (Donihue *et al.*, 2015).

Sampling.

We conducted our study on Naxos, the largest Cycladic island located in the central Aegean Sea. Adult male lizards were captured from a single population near the agricultural village of Moni on Naxos island (37º04'54.1" N, 25º29'35.0" E) in May 2018. We chose this population for its abundance of lizards, presence of all three color morphs, and diverse habitat with mixed vegetation that also contains a plethora of dry stone walls. Lizards were captured with a thread lasso attached to the end of an extendable fishing pole. We sexed lizards immediately upon capture by examining their enlarged femoral pores, swollen tail base, and larger block-shaped heads indicative of males. We determined lizards were mature adult males if they had a snout-vent-length (SVL, or the length spanning the tip of the nose to the vent) larger than 45 mm (SVL range = 55.41–71.12 cm, N = 60). We captured 60 lizards, 20 of each pure color morph (orange, yellow, and white). Lizards were placed in individual cloth bags and transported to the laboratory on Naxos for further measurement, temporary housing, and experimentation.

Animal housing and husbandry.

Lizards were housed individually in plastic terraria (20 cm width by 40 cm length by 20 cm height) with sand substrate gathered from their home site in Moni. Each terrarium contained a water dish and two rocks used in classic Greek stone wall construction for thermoregulation and refuge. Each terrarium was situated under a 40 W incandescent lamp and received 12 hrs of light per day. Light and temperature cycles were set to mimic field conditions (12 hrs light from 07:00 - 19:00hrs, 15°C average at night, and 26°C average during the day). Lizards were provided full spectrum light (Zoo Med ReptiSun 10.0 UVB Compact Fluorescent Mini Reptile Lamp, 13 W) thrice per week for 2 hrs to prevent metabolic bone disease (Adkins *et al.*, 2003). We covered all walls adjacent to other terraria with opaque paper to shield lizards from viewing their neighbors and minimize stress. Lizards were fed a diet of mealworms (*Tenebrio molitor*) dusted with Zoo Med Repti Calcium once per day and given water *ad libitum*. Lizards were given one week to acclimate to laboratory conditions before experiments commenced, and stayed in the laboratory for a total of one month before being released back to their exact capture location in Moni.

Ethogram.

Prior to experimentation, we generated an ethogram to catalogue contest behaviors exhibited by adult male lizards from our study population in their natural habitat (Table 1). Over four days, we conducted direct observations in the field during peak hours of activity (0800 - 1700) from a distance with binoculars to limit effects of our presence on the focal subject's natural behavior. We narrowed the list of potential behaviors in our ethogram to discrete, countable actions that were repeatedly observed in nature and could be readily identifiable during experimental contests.

Experimental set-up, contest design, and behavioral quantification.

To quantify morph-specific behavior and competitive ability, we staged 90 30-minute contests between two lizards in a neutral arena where neither morph had a potential residency advantage. Each contestant competed against the two other morph types and its own morph type for a total of three contests per individual. We designed contests to minimize potential effects of body size and contest order on contest outcome. First, we size-matched contestants by measuring their snout-vent-length (SVL) and allowed up to 10% difference in body size between contestants (Baird, 2013). Once contestants were size-matched for their three contests, we randomized the order of contests so that the order of morph encounters was uncoupled from contest order.

Contests were performed in a neutral arena unfamiliar to both lizards in the laboratory under standardized conditions. The arena consisted of a 60 by 60 cm open top square with a floor and 20 cm high walls constructed with laminate-coated particle board. The arena contained a pile of rocks in the middle that functioned as a dry stone wall. Two heat lamps were fixed to opposite sides of the arena and pointed at a 45-degree angle directly at the rock pile resource, simulating a sunny basking spot on a dry stone wall. We recorded each contest using a digital video camera (JVC full HD Everio) for post-experimental analysis. All contests occurred between the hours of 0800 and 1700 in the laboratory under standardized lighting conditions. We used our ethogram to score the number of behaviors performed during each contest. The amount of time an individual

spent on the heated rock wall during the 30-minute contest was calculated post-experiment from the video data (performed by C. Ayton). We defined the "winner" of a contest as the individual who spent the most time on the heated rock wall. The entire arena, including the rocks, were sanitized with 80% ethanol between trials to remove scent marks.

Lizard head and body size can influence contest outcomes, where larger individuals usually enjoy an advantage (Baird, 2013; Huyghe *et al.*, 2005). We measured SVL, head width (at the widest point of head just posterior of ear opening), and head height (at posterior of parietal scale) using a Mitutoyo 500-171-30 Absolute Scale Digital Caliper. We also noted several aspects of body condition known to affect lizard locomotor ability and correlated with competitive ability, such as whether the lizard had previously autotomized its tail (McElory & Bergmann, 2013), and number of conspecific bite scars. We tested if any of these metrics influenced contest outcomes.

Statistical analyses.

To test if color morph identity predicts inter-morph contest outcome, we used two Binomial Generalized Linear Mixed Models (GLMMs) with a logit link function. Because we used the same individuals in multiple trials, we split our analysis across two GLMMs to avoid non-independence issues. First, to test the effect of an individual's own morph on its likelihood of winning a contest, we modeled contest outcome as a binary "win" or "lose" response variable with focal morph as a fixed effect and individual ID as a random effect, since the same individual was involved in two contests. Second, to test if opponent morph was a predictor of contest outcome, we ran a second Binomial GLMM with contest outcome as a response variable, opponent morph as a fixed effect and individual ID as a random effect. To ensure that body condition wasn't a predictor of contest outcome, we first ran a series of Binomial GLMs with different body condition metrics as a predictor variable. No metrics of body condition (individual SVL, % difference in contestant SVLs, head depth, head width, whether an individual had previously autotomized their tail, and number of conspecific bite scars) were significantly associated with contest outcome (see section Morph identity and contest outcomes, Results), so we excluded these variables from further analysis.

To test for color morph differences in ability to retain a limited resource during inter-morphs contests, we used two-tailed t-tests comparing the amount of time focal and opponent males spent on the heated rock wall during each trial. Time data for each morph were assessed for normality using Shapiro Wilk's tests and F-tests. Orange and white lizard time data had unequal variances (F-test: $F = 0.219$, $df = 19$, $p = 0.01$), so we used a Welch's t-test to compare the amount of time orange and white morphs spent on the rock wall during orange vs. white inter-morph contests.

To test for morph differences in the frequencies of certain behaviors by contest type, we used Chi-square goodness-of-fit tests. In this context, the Chi-square goodness-of-fit test is used to determine if a certain behavior occurs with equal frequency among morphs.

To determine if morphs exhibit higher levels of aggression toward like-morphs, we quantified an aggression score for each battle for all individuals. Following other studies in lizards (McLean & Stuart-Fox, 2015; Abalos *et al*., 2016), we used a point

system for different aggressive behaviors. The most aggressive behavior, biting, was given the highest point value, followed by lunging and chasing. Biting, lunging, and chasing were given point values of three, two, and one, respectively. Aggression scores were calculated by summing the total number of points per individual per battle. We then compared aggression scores for each focal morph by battle type using One-way ANOVAs followed by post-hoc Tukey HSD tests.

We used R (v.1.1.456) to conduct all statistical analyses. We used the lme4 package to construct GLMMs, and the stats package for all other analyses. We set a significance level $\alpha = 0.05$ *a priori* and used this cut-off for all analyses.

Ethical note.

All research was conducted in accordance with the University of California, Merced Institutional Animal Care and Use Committee (IACUC protocol AUP17-0002) and permits provided by the Greek Ministry for Environment and Energy (codes Ψ4Γ64653Π8-ΗΛ5 and Ω8Δ84653Π8-ΒΞΧ assigned to K.M. Brock). No animals were permanently or mortally wounded while in our care.

RESULTS

Morph identity and contest outcomes.

We could identify a winner (defined as the individual who occupied the heated rock wall the longest) in all 90 contests, 60 of which were inter-morph contests (Figure 1). Overall, the white morph won the most inter-morph contests (white morph win count = 16/20 v. orange, 11/20 v. yellow, Figure 1), followed by the yellow morph (yellow morph win count = 9/20 vs. white, 13/20 vs. orange, Figure 1). Counter to our predictions, the larger orange morph won the fewest inter-morph contests (orange morph win count = 4/20 vs. white, 7/20 vs. yellow).

No body condition variables were significantly associated with contest outcome (contest outcome ~ SVL: Coeff. = -0.025, df = 179, p = 0.522; contest outcome ~ % difference in contestant SVL: Coeff. = -0.012, df = 179, p = 0.997; contest outcome ~ individual head depth: Coeff. = 0.066, df = 179, p = 0.873; contest outcome ~ individual head width: Coeff. = 0.367, df = 179, p = 0.422; contest outcome ~ tail autotomy: Coeff. = -1.204, df = 179, p = 0.505; contest outcome ~ number of conspecific bite scars: Coeff. = -0.166, df = 179, p = 0.063).

Out of 60 lizards, nine lost all three contests. Of the nine lizards that lost all three contests, seven were orange, one was white, and one was yellow. Seven lizards won all three contests: two orange, three white, and two yellow. Ten lizards only won their two inter-morph contests: six white and four yellow morphs. Eight lizards only won the intra-morph contest: four orange, one white, and three yellow morphs.

Focal morph identity and opponent morph identity predicted inter-morph contest outcomes over a limited space resource in a neutral arena (Table 2). In the focal morph model of inter-morph contest outcome, white and yellow morphs were associated with winning (Coeff. white = 1.726, $p < 0.01$; Coeff. yellow = 1.188, $p < 0.05$, df resid = 116). Facing the white or yellow morph in inter-morph contests was significantly associated with losing (Coeff. white = -1.762, $p < 0.001$, Coeff. yellow = -1.213, $p < 0.05$, df resid = 116).

Color morphs varied in the amount of time they spent accessing the heated rock wall in inter-morph contests (Figure 2A). In orange vs. white contests, we found that white lizards occupied the rock wall space significantly longer than orange morphs (Welch's t-test: $t = -3.363$, $df = 29.224$, $p = 0.002$). In white vs. yellow and yellow vs. orange inter-morph contests, morphs did not significantly differ in the cumulative amount of time spent on the heated rock wall (t-test white vs. yellow: $t = 1.285$, $df = 38$, $p = 0.207$, t-test yellow vs. orange: $t = -0.853$, $df = 38$, $p = 0.399$).

Morph differences in behavior frequencies.

Aggression.

We detected significant differences in aggressive behaviors displayed by color morphs in inter-morph contests (Figure 3, Table 3). Overall, across all contest types, the white morph always performed more aggressive behaviors than the other morphs.

In inter-morph contests, white morphs (W) bit yellow (Y) and orange (O) lizards three times more than yellow or orange lizards bit white lizards (Table 3). Inter-morph contests where a white morph individual was present had more instances of biting than contests without white morphs, and the white morph always bit the contestant more times than the contestant morph bit it. In intra-morph contests, the most biting occurred in white vs. white contests, followed by yellow vs. yellow, and orange vs. orange.

In inter-morph contests, we only detected significant differences in the amount of chasing between the yellow and orange morph in yellow vs. orange contests. In intra-morph contests, the frequency of chasing did not differ significantly among morphs (Intra-morph chasing $x^2 = 3.3$, $df = 2$, $p = 0.185$).

In inter-morph contests, significantly more lunges were performed by the white morph when facing orange morphs in orange vs. white contests. The frequency of lunging significantly differed among morphs in intra-morph trials (Intra-morph lunging $x^2 = 15.4$, $df = 2$, $p < 0.001$). White morphs lunged at each other more frequently than orange or yellow morphs in intra-morph trials (Intra-morph contest lunge counts: W vs. W = 55, Y vs. Y = 37, O vs. O = 20, N = 10 intra-morph contests per morph). Overall, across all contest types, the white morph always performed more aggressive behaviors than the other morphs.

Boldness.

Color morphs differed in the amount of bold behaviors displayed during inter- and intra-morph contests (Figure 4, Table 4). In inter-morph contests, the orange morph approached the other morphs less frequently. Yellow morphs approached orange morphs significantly more frequently than expected. Similarly, white morphs approached orange contestants significantly more frequently than orange morphs approached white morphs. In white vs. yellow inter-morph contests, white morphs approached yellow morphs more frequently than yellow approached white, and the difference was significant (Table 4). In intra-morph trials, white morphs approached each other more frequently than the other morphs approached each other.

The frequency of patrolling behavior differed amongst morphs in inter-morph contests, but not intra-morph contests (Table 4). In inter-morph contests, the white morph patrolled the rock more frequently than the other two morphs. In orange vs. white

contests, white morphs patrolled the rock 62 more times than orange morphs over 20 trials (Figure 4, Table 4). In yellow vs. orange inter-morph contests, morphs patrolled the rock wall about equally.

Chemical signaling and sensing.

Color morphs differed in the amount of certain chemosensory behaviors performed during inter- and intra-morph contests (Figure 5, Table 5). In inter-morph contests, the orange morph gaped at the yellow significantly more than the yellow morph gaped at the orange morph. The frequency of gaping did not differ among morphs in the other inter-morph contest types involving white morphs (Table 5). The white morphs gaped at each other more than orange and yellows morphs did in intra-morph contests.

While yellow and orange did not differ in the amount of head wiping in yellow vs. orange inter-morph contests, white morphs performed head wiping more than twice as often compared to the orange and yellow morphs in inter-morph contests (Table 5). White morphs also performed head wiping much more frequently during intra-morph trials than the other morphs (Figure 5).

The yellow and white morphs wiped their femoral pores on the heated rock wall more often than orange morphs in inter-morph contests (Table 4). In white vs. yellow contests, white and yellow morphs laid down femoral pore scent marks about equally. In intra-morph contests, white morphs wiped their femoral pores on the heated rocks three times more often than orange morphs and 12 times more often than yellow morphs (Table 5).

Tongue flicking was not performed often compared to other chemosensory behaviors, and we only detected a significant difference in the frequency of this behavior in the orange vs. white inter-morph contest. Yellow morphs performed tongue flicking more often than the other two morphs in intra-morph contests.

Visual signaling.

We found differences in the amount of visual signaling behaviors performed by morphs in both inter- and intra-morph contests (Figure 6, Table 6). Hand waving occurred at significantly different frequencies in yellow vs. orange and orange vs. white inter-morph contests (Table 6), and the orange morph exhibited this behavior fewer times in both inter-morph contest types. White and yellow morphs hand waved at similar frequencies in white vs. yellow inter-morph contests (Table 6). In intra-morph contests, yellow morphs waved at each other much more often than the other morphs.

The frequency of side displays differed significantly in all inter-morph contest types (Table 6). Yellow morphs side displayed more often than orange morphs in yellow vs. orange contests, white morphs side displayed more often than orange morphs in orange vs. white contests, and white morphs displayed their side to yellow more often in white vs. yellow contests (Table 6). White morphs also performed this behavior much more often than the other morphs in intra-morph contests (Figure 6).

The frequency of tail displaying also differed in all inter-morph contest types (Table 6). In inter-morph contests where a white morph was present, the white morph tail displayed more often (Table 6). In yellow vs. orange inter-morph contests, the orange morph tail displayed more often.

Throat displays were by far the most frequent behavior we observed during behavioral experiments. In inter-morph trials, the frequency of throat displays did not differ in white vs. yellow contests (Table 6). However, in inter-morph contests with an orange morph, the orange morph performed this behavior less frequently than the yellow or white morphs. In intra-morph contests, the morphs performed throat displays at substantially different frequencies, and the white morphs did this most often (Figure 6).

Inter- vs. Intra-morph aggression scores.

On average, none of the color morphs exhibited significantly higher levels of aggression toward like-morphs (Figure 7). The only significant differences we detected were in orange morphs, which exhibited significantly higher aggression scores in contests with white morphs compared to orange or yellow morphs (ANOVA $F = 4.72$, $df = 2$, $p = 0.013$; Tukey HSD orange vs white – orange vs. orange diff. = 4.5, $p = 0.039$; Tukey HSD yellow vs. orange – orange vs white diff. = -5.0, $p = 0.019$). White morphs had higher aggression scores in all battle types, on average (Figure 7), but their aggression scores did not significantly differ among battle types (ANOVA $F = 0.73$, $df = 2$, $p = 0.486$). Yellow morphs did not differ in aggression scores by battle type (ANOVA $F = 0.538$, $df = 2$, $p = 0.587$).

DISCUSSION

Results from our behavioral experiments on Aegean wall lizard color morphs show that these morphs differ in their ability to win staged contests and in the frequency with which they display different behaviors. Although we anticipated that morph identity would predict contest outcomes over a limited heated space on a rock wall, the winners and losers were not who we expected. White and yellow morphs were associated with winning, whereas the orange morph, which tends to be bigger and bite harder (Brock *et al.*, 2020), was associated with losing inter-morph contests. We also found that contests with white morphs had more activity overall, and that white morphs tended to be more active than contestant morphs they faced. Counter to our expectations, the white morph exhibited the most aggressive behavior compared to the other morphs in all contest types and no morphs exhibited significantly higher levels of aggression toward like-morphs. We expected orange morphs to be bolder than other morphs in inter-morph trials, following other color polymorphic systems where red-orange morphs have elevated levels of circulating testosterone and also tend to have more endurance and be more active (Sinervo *et al.*, 2000), but we found that orange morphs were actually the least bold across all contest types. Chemical scent marking and throat visual signaling tended to be more frequent in inter-morph contests, as we expected. Our results demonstrate the potential for correlated traits to be involved in color polymorphism in this species.

Contests over space are pivotal for male reproductive success, as females are often attracted to good quality habitat (Olsson *et al.*, 2013), and often remain within a certain space regardless of individual male presence (Edsman 2001). Counter to our expectations based on body size and bite strength (Brock et al., 2020), the larger orange morph lost more contests for space against white and yellow morphs. We constrained lizard body differences to an upper end of 10% difference, and neither body nor head size was significantly associated with winning any type of contest, inter- and or intra-morph.

This was still a somewhat surprising result given that white and yellow morphs were smaller than orange morphs in most of the one-on-one contests we ran. Many examples from contest experiments in squamates suggest that larger body size is associated with winning (West-Eberhard, 1983; Andersson, 1994). In nature, lizards are not size-matched, as they were in our staged contests, and so similar observations should be collected from color morph interactions in wild populations to fully understand morph fitness consequences of contest outcomes over mates and quality habitat. It is possible that orange morphs may benefit from their size advantage in a natural setting where smaller white and yellow morphs might not challenge larger orange morphs. Nevertheless, our results do match a recent set of morph behavioral experiments conducted in *P. erhardii*'s close relative, *Podarcis muralis*, which has the same three color morphs (Abalos *et al.*, 2016). Contest experiments in both *P. muralis* (Abalos *et al.*, 2016) and *P. erhardii* males have similarly found that orange morphs have a propensity to lose inter-morph contests.

Correlational selection is multivariate selection that promotes non-random trait combinations (reviewed in McKinnon & Pierotti, 2010). Male *P. erhardii* color morphs are associated with morphological and chemical signaling traits (Brock *et al.*, 2020), which likely arise from correlational selection. In this study, we found that morphs also differ in their behavior, and that morph identity matters for winning contests and the types of interactions that morphs engage in with each other. We found that the larger orange morph exhibits lower levels of aggression and is the least active in an experimental setting. The white morph has the widest range of body sizes but overall tends to be smaller (Brock et al., 2020), and initiates interactions and displays elevated levels of scent marking and aggressive behaviors. The yellow morph seems to be an intermediate between orange and white in morphology, chemical signal design (Brock et al., 2020), and behavior. When selection acts on behavior, the fitness of an individual depends on the behavior of other individuals (Sinervo *et al.*, 2001). We did not find a rock-paper-scissors pattern of contest wins among morphs, where one morph consistently wins against another morph and is beaten by the other. Nor did we find that morphs were more aggressive toward like-morphs than unlike-morphs, which both seem to be involved in male fitness outcomes and maintaining color morph diversity in other color polymorphic species (Sinervo & Lively, 1996; Dijkstra *et al.*, 2007; Horton *et al.*, 2012). Correlational selection may produce morph variation in phenotype, but the correlated traits we have identified in *P. erhardii* color morphs here and in previous work do not alone explain how morph diversity is maintained; instead, these traits are likely involved with additional factors like female choice and morph-environment interactions.

The Moni source population of lizards has had higher frequencies of white morphs during opportunistic sampling trips in the breeding seasons from 2017-2019 (K.M. Brock, unpublished data), followed by yellow morphs, and orange morphs have consistently been the least common. In fact, white morphs are the most common in 10 different populations across the island of Naxos, the island where we performed our study. Geographic variation in morph frequencies is common, if not the norm (reviewed in McLean & Stuart-Fox, 2014). In some populations on Naxos, we failed to find any orange morphs. These orange-less populations tended to be hot and dry, with sparse vegetation and far from a water source. In other color polymorphic species, it is not

uncommon for environment-associated morphs to evolve (spiny orb weaver spiders, Kemp *et al.*, 2013; cichlids, Tinghitella *et al.*, 2018). In *P. muralis*, a close relative in the same genus as *P. erhardii*, there seems to be a partial divergence in color morph microhabitat use. *Podarcis muralis* orange morphs tend to prefer humid environments that are close to water (Pérez i de Lanuza & Carretero, 2018). Further, a distribution-wide study of the Side-blotched lizard (*Uta stansburiana*) found that the yellow morph, which is the least aggressive, is the first morph in that species to be lost from populations (Corl *et al.*, 2010). More *in-situ* behavioral observations and research into morph microhabitat use and morph diversity across many populations will provide further insights into morph-correlated traits and morph maintenance within and between populations.

Color morphs must maintain a minimum viable number of alleles in a population, or else those morphs will die out. So what maintains the orange morph in natural populations? One possibility is that contest outcomes are different in natural settings. Given the repertoire of behaviors we observed in nature and in the experimental setting, this seems unlikely but remains to be tested. Alternatively, assuming our experimental results are consistent with contests over quality habitat in nature, orange morphs may have some other unmeasured advantage over white and yellow morphs that enhances their fitness. Orange males tend to be larger than yellow and white males, and females show no significant size differences among morphs (Brock *et al.*, 2020). We performed our size measurements in May, which is the peak breeding season in this species (Valakos *et al.*, 2008). It is possible that orange males experience faster growth rates than the other male morphs, and reach sexual maturity earlier in the season, thus giving earlier access to mating opportunities. Color may be another possible unmeasured advantage the orange morph may hold, as it could be an honest signal of health quality and used in mate choice by females. Pigment-based animal coloration that ranges from red to yellow has been demonstrated to be an honest signal of individual quality and a predictor of fitness in birds (Pryke *et al.*, 2010), fish (Grether, 2000), and lizards (Fitze *et al.*, 2018). Additionally, chemical signals play an important role in lizard communication and can be used by females and males to judge competitive ability and dominance status (López & Martín, 2002). Orange and white *P. erhardii* males have significantly different throat colors and chemical signals in the waxy cuticle excreted from their femoral pores (Brock *et al.*, 2020). Of all the behaviors we measured, orange morphs performed throat displays more than any other behavior. Orange males could signal their quality in visual and chemical signals to females, who may choose orange males preferentially over white and yellow males, thus sustaining orange in the population. Additionally, if orange morphs can obtain enough matings via female preference, then orange morphs may not need to expend energy fighting over quality habitat to attract females and persist at lower frequencies in the population. *Podarcis muralis* color morphs mate assortatively but also engage in considerable heteromorphic matings (Pérez i De Lanuza *et al.*, 2012), suggesting that mate choice patterns may be involved in polymorphism maintenance. Mate choice experiments and behavioral studies that incorporate female morphs are needed to better understand color polymorphism maintenance in *P. erhardii*.

Color polymorphic species have enhanced our understanding of evolution, and the maintenance of genetic diversity within species (Svensson, 2017). Here, we showed that male color morphs in *P. erhardii* differ in their ability to win staged contests over space

and in the amount of aggressive, boldness, and signaling behaviors in a neutral arena. Although male color morphs differ in head and body size, chemical signal design (Brock *et al.*, 2020, and frequency of different behaviors, it is still unclear whether these morph-correlated traits are involved in mating and fitness outcomes in nature. The cumulative effects of natural and sexual selection likely shape morph genotypes and phenotypes (McKinnon & Pierotti, 2010), morph relative fitness (Sinervo & Lively, 1996; Sinervo & Zamudio, 2001), and morph diversity maintenance in and across populations (Corl *et al.*, 2010). To understand what promotes color morph diversity and coexistence, it will be helpful to know what environmental and ecological factors are associated with morph loss and morph frequencies in populations through time. We also advocate for more research on morph-correlated traits in both males and females that explore morph physiology, mating behaviors, and mating preferences to determine the nature of morph-specific strategies and the persistence of alternative phenotypes in this species.

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Figures

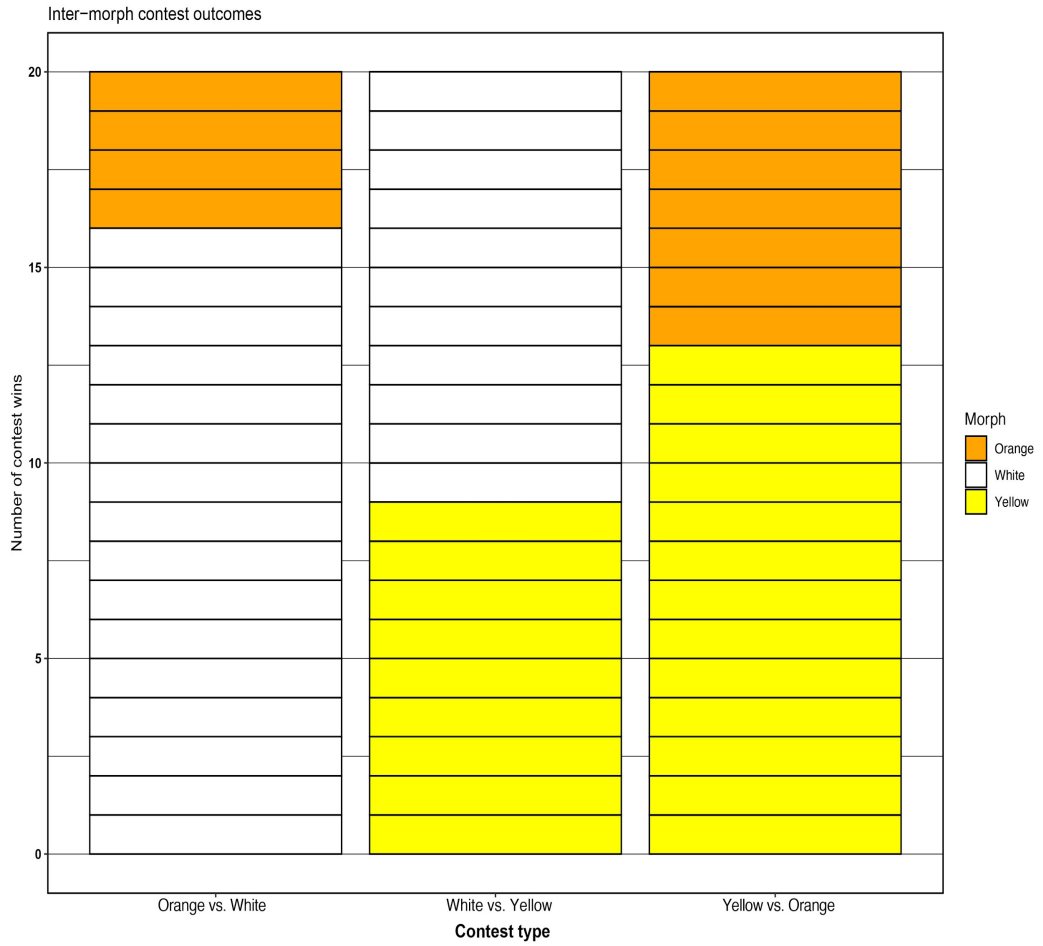

Figure 1. Number of inter-morph contest wins for each color morph by contest type. White morphs won the most inter-morph contests overall, and won more contests against both the orange and yellow morph. The orange morph won the fewest inter-morph contests overall, and did not win more contests than either the white or yellow morph.

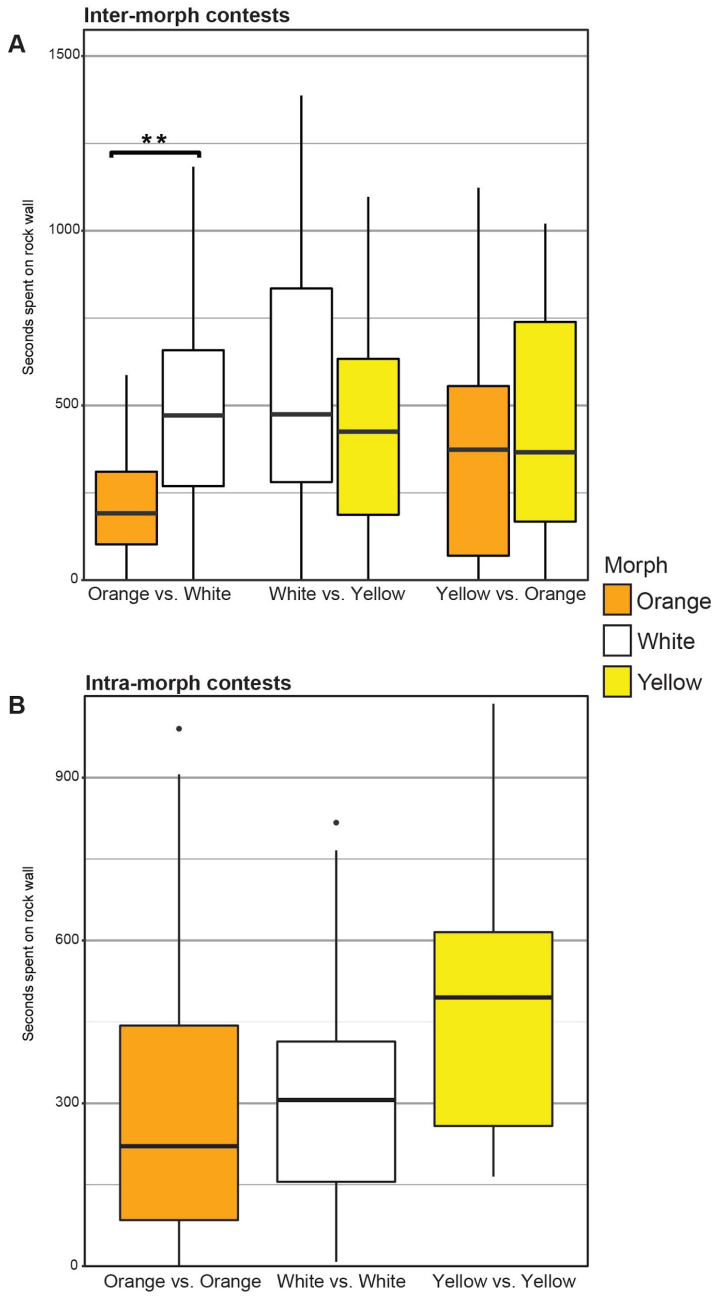

Figure 2. A. Amount of time morphs spent on the heated rock wall during inter-morph contests by contest type. Orange morphs spent significantly less time on the rock wall than white morphs in orange vs. white contests (Welch's t-test $p < 0.01$, 2A. denoted with an asterisk). 2B. Average amount of time morphs spent on the heated rock wall during intra-morph contests by contest type. While yellow morphs spent more time on the rock overall in intra-morph contests, we detected no significant differences in the amount of time different morphs spent on the rock wall when in contest with an individual of the same morph color.

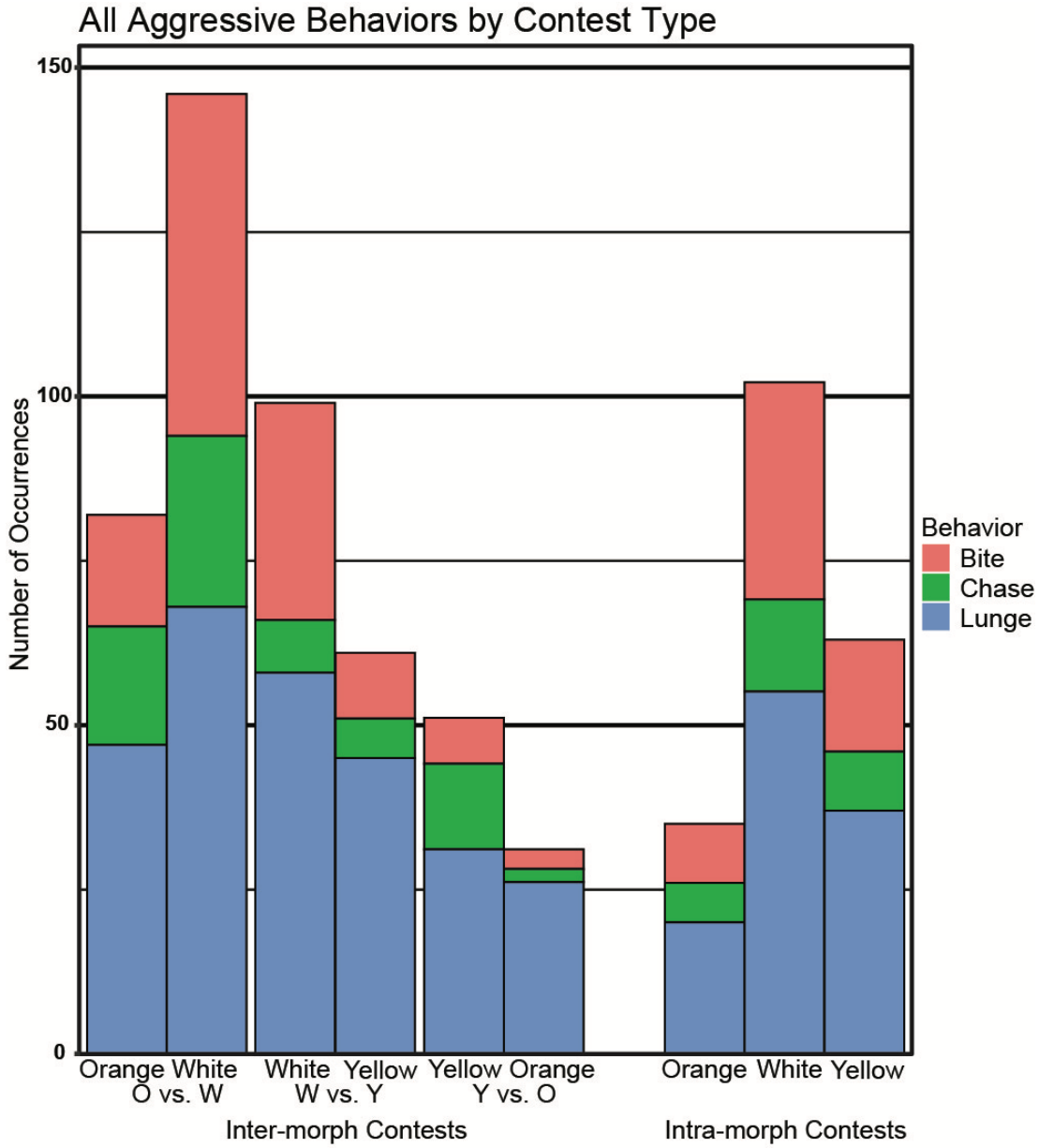

Figure 3. Cumulative numbers of behaviors by morph and contest type. Contests with white morphs had more aggressive behaviors. Orange morphs performed fewer aggressive behaviors across contest types. Yellow morphs performed fewer aggressive behaviors than white morphs and more aggressive behaviors than orange morphs and seem to express intermediate levels of aggression.

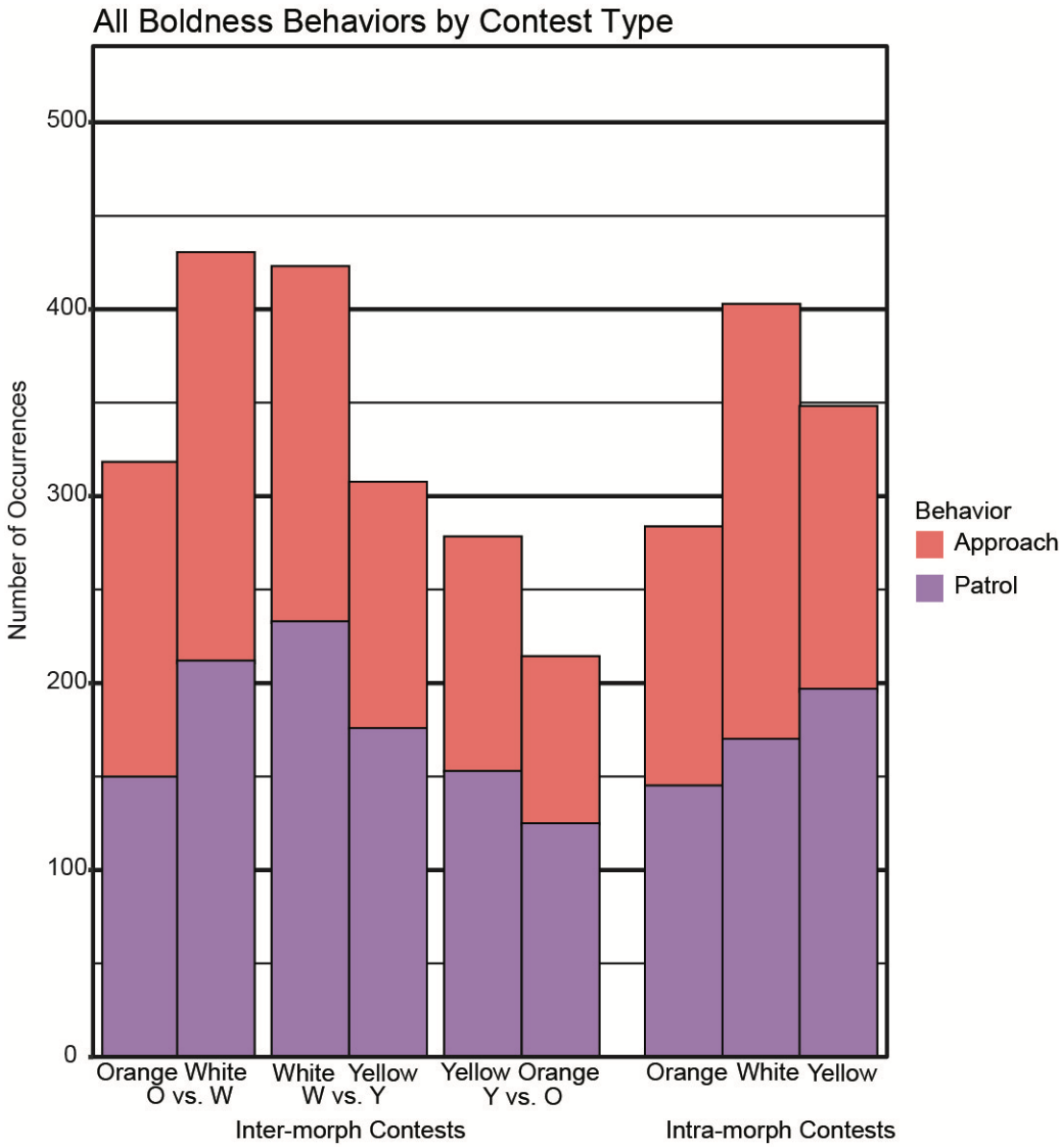

Figure 4. Cumulative number of bold behaviors performed by morphs by contest type. White morphs were bold more often than the other morphs.

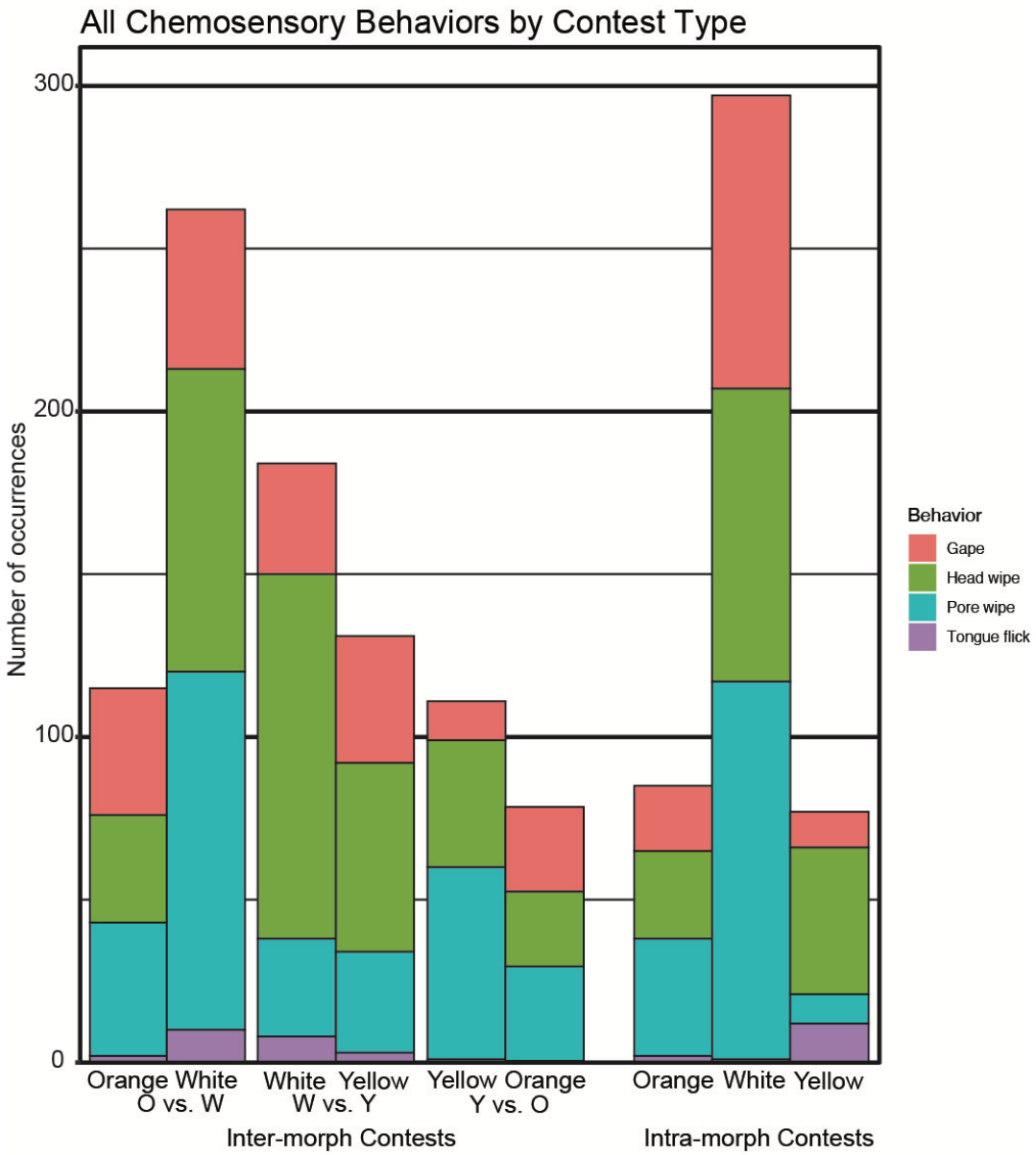

Figure 5. Cumulative number of chemosensory behaviors performed by morphs by contest type. Orange and yellow morphs performed scent marking behaviors (head and pore wiping) more often in inter-morph contests than intra-morph contests.

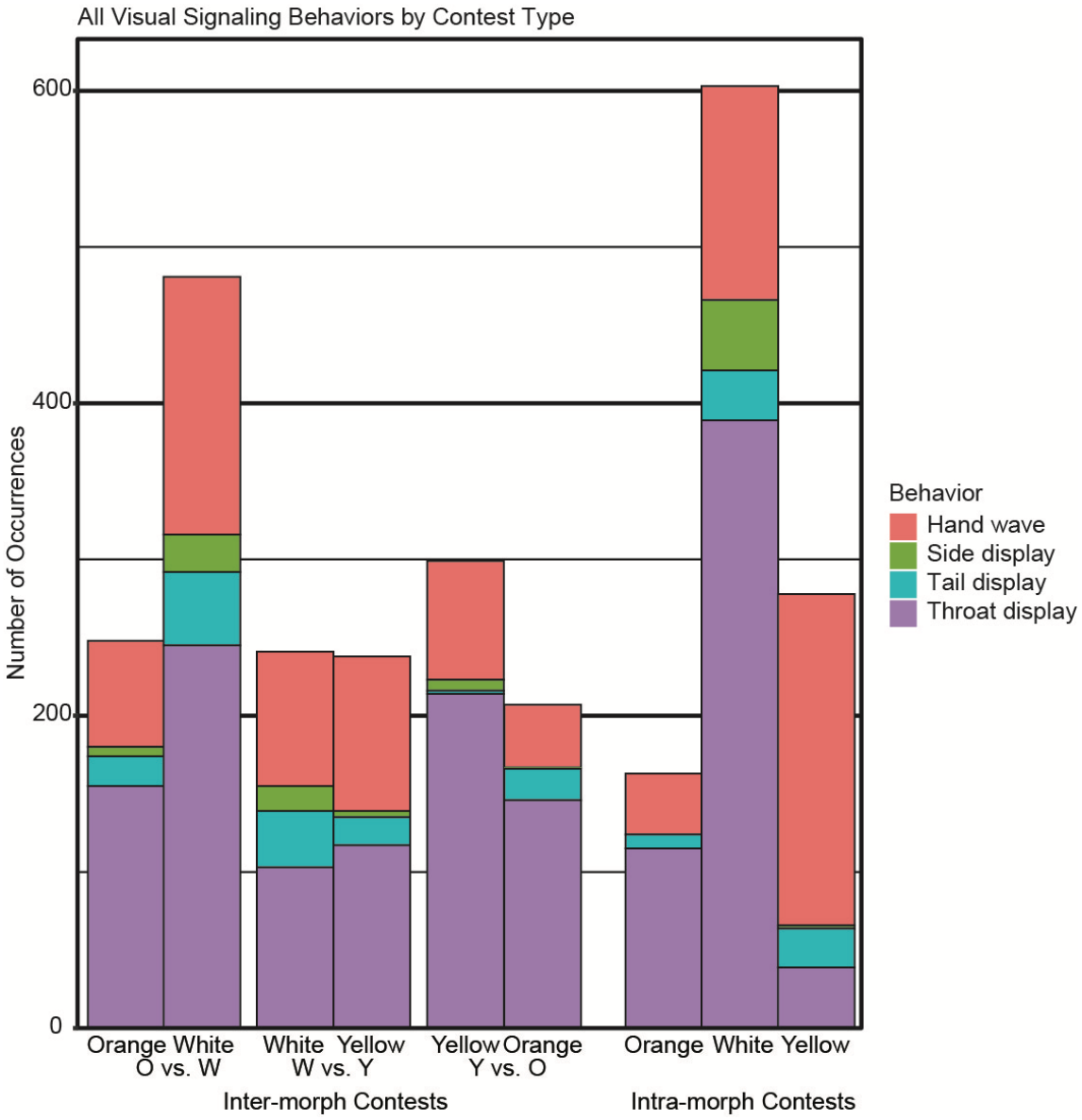

Figure 6. Orange and yellow morphs did not perform throat signaling behavior significantly more frequently than white morphs in inter-morph contests. Visual signals were performed frequently in white intra-morph trials.

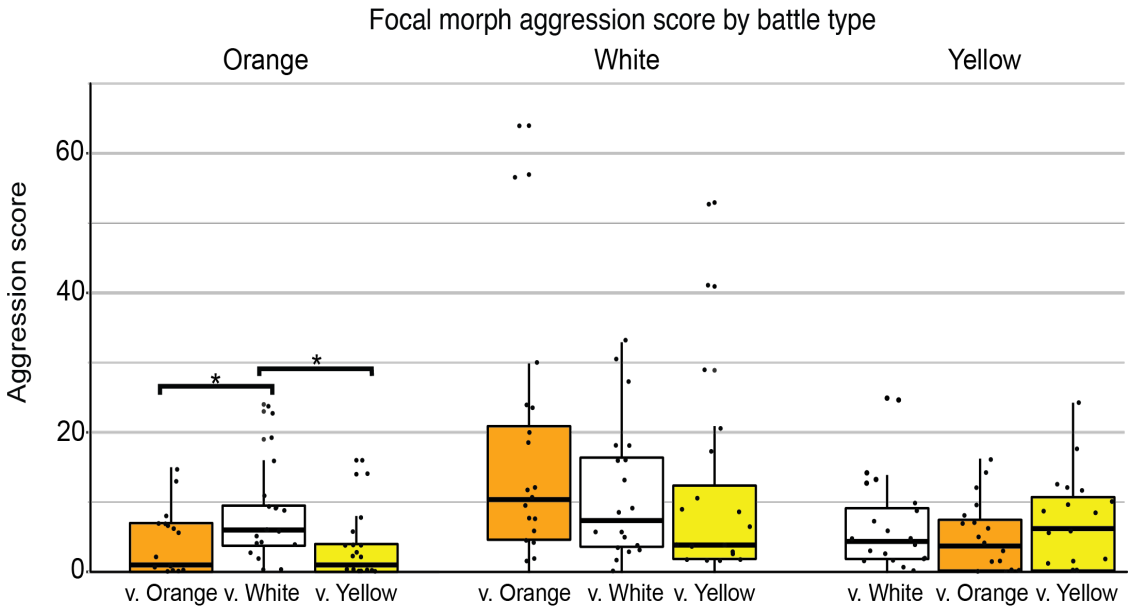

Figure 7. Color morph aggression scores by battle type. Color morphs did not exhibit significantly higher levels of aggression toward like-morphs. Orange morphs did exhibit significantly higher levels of aggression toward white morphs compared to orange morphs and yellow morphs (denoted with asterisks).

Tables

Table 1. Ethogram of contest behaviors exhibited by adult male *P. erhardii*.

Behavior	Description	Category
Bite	Bites opponent	Aggression
Chase	Quickly follows opponent	
Lunge	Hits opponent with head with a fast, forward motion	
Approach	Walks toward opponent	Boldness
Patrol	Walks the perimeter of the top of the rock wall	
Gape	Opens mouth toward opponent	Chemical signaling
Head wipe	Wipes head on substrate	
Pore wipe	Wipes femoral pores on substrate	
Tongue flick	Flicks tongue out of mouth	
Hand wave	Lifts hand in a circular, waving motion	Visual signaling
Side display	Orients body perpendicular to opponent and dorsolaterally flattens to display side to opponent	
Tail display	Wriggles tail at opponent	
Throat display	Extends throat upward in direction of opponent	

Table 2. Relationships between contest outcome and morph identity in inter-morph contests (N = 60 contests). Both models included individual lizard identification (ID) as a random effect. Significant results are denoted with an asterisk.

Focal Morph Model	**Estimate Fixed effect**	**Std. Error Fixed effect**	**z value Fixed effect**	**p-value Fixed effect**	**Variance (Std. Dev.) Random effect = ID**
Intercept	-0.984	0.376	-2.614	0.009*	0.064 (± 0.254)
White	1.726	0.534	3.233	0.001*	
Yellow	1.188	0.503	2.361	0.018*	
Opponent Morph Model	**Estimate Fixed effect**	**Std. Error Fixed effect**	**z value Fixed effect**	**p-value Fixed effect**	**Variance (Std. Dev.) Random effect = ID**
Intercept	1	0.381	2.636	0.008*	0.274 (± 0.524)
White	-1.762	0.529	-3.334	< 0.001*	
Yellow	-1.213	0.506	-2.396	0.017*	

Table 3. Chi-square goodness-of-fit tests on frequency of morph aggressive behaviors in inter- and intra-morph contests. Significant deviations from equal frequencies are bolded with an asterisk.

Contest Type	Behavior	Focal Morph	Observed	Expected	Difference (Obs-Exp)	Chi-square and p-values
Inter-morph contests	**BITING** Focal morph bit contestant morph of different color	Y vs. O	Y = 7 O = 3	5	Y = 2 O = -2	1.6 p = 0.206
		O vs. W	O = 17 W = 52	34.5	O = -17.5 W = 17.5	17.8 **p < 0.001***
		W vs. Y	W = 33 Y = 10	21.5	W = 11.5 Y = -11.5	12.3 **p < 0.001***
	CHASING Focal morph chased contestant morph of different color	Y vs. O	Y = 13 O = 2	7.5	Y = 5.5 O = -5.5	8.1 **p = 0.004***
		O vs. W	O = 18 W = 26	22	O = -4 W = 4	1.5 p = 0.228
		W vs. Y	W = 8 Y = 6	7	W = 1 Y = -1	0.286 p = 0.593
	LUNGING Focal morph lunged at contestant morph of different color	Y vs. O	Y = 31 O = 26	28.5	Y = 2.5 O = -2.5	0.44 p = 0.508
		O vs. W	O = 47 W = 68	57.5	W = -10.5 O = 10.5	3.8 **p = 0.05***
		W vs. Y	W = 58 Y = 45	51.5	Y = -6.5 W = 6.5	1.6 p = 0.200
Intra-morph contests	**BITING** Focal morph bit morph of same color	O vs. W	9	19.666	-10.666	15.2 df = 2 **p < 0.001***
		Y vs. Y	17		-2.666	
		W vs. W	33		13.334	
	CHASING Focal morph chased morph of its own same color	O vs. O	6	9.666	-3.666	3.3 df = 2 p =0.185
		Y vs. Y	9		-0.666	
		W vs. W	14		4.334	
	LUNGING Focal morph chased morph of same color	O vs. O	20	37.333	-17.333	15.4 df = 2 **p < 0.001***
		Y vs. Y	37		-0.333	
		W vs. W	55		17.667	

Table 4. Chi-square goodness-of-fit tests on frequency of morph boldness behaviors in inter- and intra-morph contests. Significant deviations from equal frequencies among morphs are bolded with an asterisk.

Contest Type	Behavior	Focal Morph	Observed	Expected	Difference (Obs-Exp)	Chi-square and p-values
Inter-morph contests	**APPROACH** Focal morph walked toward contestant morph of different color	Y vs. O	Y = 126 O = 90	108	Y = 18 O = -18	6 **p = 0.014***
		O vs. W	O = 169 W = 220	194.5	O = -25.5 W = 25.5	6.7 **p = 0.009***
		W vs. Y	W = 191 Y = 132	161.5	W = 29.5 Y = -29.5	10.8 **p = 0.001***
	PATROL Focal morph walked perimeter of rock wall excluding morph of different color	Y vs. O	Y = 153 O = 125	139	Y = 14 O = -14	2.8 p = 0.093
		O vs. W	O = 150 W = 212	181	W = -31 O = 31	10.6 **p = 0.001***
		W vs. Y	W = 233 Y = 176	204.5	W = 28.5 Y = -28.5	7.9 **p = 0.004***
Intra-morph contests	**APPROACH** Focal morph walked toward morph of same color	O vs. O	139	175.333	-36.333	30 df = 2 **p < 0.001***
		Y vs. Y	153		-22.333	
		W vs. W	234		58.667	
	PATROL Focal morph walked perimeter of rock wall excluding morph of same color	O vs. O	145	170.666	-25.666	7.9 df = 2 p = 0.019
		Y vs. Y	197		26.334	
		W vs. W	170		-0.666	

Table 5. Chi-square goodness-of-fit tests on frequency of morph chemosensory behaviors in inter- and intra-morph contests. Significant deviations from equal frequencies among morphs are bolded with an asterisk.

Contest Type	Behavior	Morphs	Observed	Expected	Difference (Obs-Exp)	Chi-square and p-values
Inter-morph contests	**GAPE** Focal morph gaped at contestant morph of different color	Y vs. O	Y = 12 O = 26	19	Y = -7 O = 7	5.2 **p = 0.023***
		O vs. W	O = 39 W = 49	44	O = -5 W = 5	1.1 p = 0.286
		W vs. Y	W = 34 Y = 39	36.5	W = -2.5 Y = 2.5	0.34 p = 0.558
	HEAD WIPE Focal morph wiped head on surface during contest with a morph of a different color	Y vs. O	Y = 38 O = 24	31	Y = 7 O = -7	3.2 p = 0.075
		O vs. W	O = 33 W = 93	63	O = -30 W = 30	28.6 **p < 0.001***
		W vs. Y	W = 112 Y = 59	85.5	W = 26.5 Y = -265	16.4 **p < 0.001***
	PORE WIPE Focal morph wiped femoral pores on surface during contest with a morph of a different color	Y vs. O	Y = 59 O = 29	44	Y = 15 O = -15	10.2 **p = 0.001***
		O vs. W	O = 41 W = 110	75.5	O = -34.5 W = 34.5	31.5 **p < 0.001***
		W vs. Y	W = 30 Y = 31	30.5	W = -0.5 Y = 0.5	0.02 p = 0.898
	TONGUE FLICK Focal morph flicked tongue out during contest with a morph of a different color	Y vs. O	Y = 1 O = 0	0.5	Y = 0.5 O = -0.5	1 p = 0.317
		O vs. W	O = 2 W = 10	6	O = -4 W = 4	5.3 **p = 0.021***
		W vs. Y	W = 8 Y = 3	5.5	W = 2.5 Y = -2.5	2.3 p = 0.123
Intra-morph contests	**GAPE** Focal morph gaped at contestant morph of same color	O vs. O	20	40	-20	91.1 df = 2 **p < 0.001***
		Y vs. Y	11		-29	
		W vs. W	89		49	
	HEAD WIPE Focal morph wiped head on	O vs. O	27	54	-27	39 df = 2
		Y vs. Y	45		-9	

	surface during contest with morph of the same color	W vs. W	90		36	**p < 0.001***
	PORE WIPE Focal morph wiped femoral powers on surface during contest with morph of the same color	O vs. O	36	53.666	-17.666	115.4 df = 2 **p < 0.001***
		Y vs. Y	9		-44.666	
		W vs. W	116		62.334	
	TONGUE FLICK Focal morph flicked tongue out during contest with morph of the same color	O vs. O	2	5	-3	14.8 df = 2 **p < 0.001***
		Y vs. Y	12		7	
		W vs. W	1		-4	

Table 6. Chi-square goodness-of-fit tests of frequencies of visual signaling behaviors performed by color morphs in inter- and intra-morph contests. Significant deviations from equal frequencies among morphs are bolded with an asterisk.

Contest Type	Behavior	Morphs	Observed	Expected	Difference (Obs-Exp)	Chi-square and p-values
Inter-morph contests	**HAND WAVE** Focal morph waved at contestant morph of different color	Y vs. O	Y = 76 O = 39	57.5	Y = 18.5 O = -18.5	11.9 **p < 0.001***
		O vs. W	O = 69 W = 165	117	O = -48 W = 48	39.4 **p < 0.001***
		W vs. Y	W = 86 Y = 99	92.5	W = -6.5 Y = 6.5	0.914 p = 0.339
	SIDE DISPLAY Focal morph displayed side to contestant morph of different color	Y vs. O	Y = 7 O = 1	4	Y = 3 O = -3	4.5 **p = 0.034***
		O vs. W	O = 6 W = 24	15	O = -9 W = 9	10.8 **p = 0.001***
		W vs. Y	W = 16 Y = 4	10	W = 6 Y = -6	7.2 **p = 0.007***
	TAIL DISPLAY Focal morph displayed tail to contestant morph of different color	Y vs. O	Y = 2 O = 20	11	Y = -9 O = 9	14.7 **p < 0.001***
		O vs. W	O = 19 W = 47	33	O = -14 W = 14	11.9 **p < 0.001***
		W vs. Y	W = 36 Y = 18	27	W = 9 Y = -9	6 **p = 0.014***
	THROAT DISPLAY Focal morph displayed throat to contestant morph of different color	Y vs. O	Y = 212 O = 145	178.5	Y = 27.5 O = -27.5	12.6 **p < 0.001***
		O vs. W	O = 151 W = 246	198.5	O = -47.5 W = 47.5	22.7 **p < 0.001***
		W vs. Y	W = 103 Y = 119	111	W = -8 Y = 8	1.2 p = 0.283
Intra-morph contests	**HAND WAVE** Focal morph waved at contestant morph of same color	O vs. O	40	125	-85	115.6 df = 2 **p < 0.001***
		Y vs. Y	210		85	
		W vs. W	125		0	
	SIDE DISPLAY Focal morph displayed its side	O vs. O	0	15.666	-15.666	82.5 df = 2
		Y vs. Y	2		-13.666	

	to contestant morph of same color	W vs. W	45		29.334	**p < 0.001***
	TAIL DISPLAY Focal morph displayed its tail to contestant morph of same color	O vs. O	9	22	-13	12.6 df = 2 **p = 0.002***
		Y vs. Y	25		3	
		W vs. W	32		10	
	THROAT DISPLAY Focal morph displayed its throat to contestant morph of same color	O vs. O	118	176.333	-58.333	370.4 df = 2 **p < 0.001***
		Y vs. Y	32		-144.333	
		W vs. W	379		202.667	

The Odyssey:
Evolution of Color Polymorphism Across the Aegean Archipelago

ABSTRACT

Color polymorphism is an extreme type of intraspecific variation that can drive speciation. Color polymorphic species, in which multiple color phenotypes coexist within a population, often vary geographically in the number and types of morphs present in populations, which could be an important step toward speciation. We reconstructed the evolutionary history of color polymorphism among 46 distinct populations covering the species' geographic distribution of Aegean wall lizards (*Podarcis erhardii*), a color polymorphic Greek island endemic species. Color polymorphism (orange, white, or yellow morphs) is likely the ancestral state of this species, and variation in morph diversity across the landscape is most likely the result of morph loss that happens at a much faster rate in populations than evolutionary gains of color polymorphism. We found repeated population-level loss of the same morph type and similar morph frequencies across distinct populations that experience low levels of gene flow. We also found morph-environment associations, and that the orange morph, which was repeatedly the first morph absent from populations, is associated with cooler, wetter habitats.

INTRODUCTION

Intra-specific color morphs represent extreme phenotypic variation that could be the starting material for speciation (West-Eberhard, 1986; Gray & McKinnon, 2007; Corl *et al.*, 2010; Hugall & Stuart-Fox, 2012; Brock *et al.*, 2021[Chapter 4]). In color polymorphic species, morphs are genetically-determined, usually differ in other traits besides color (McKinnon & Pierotti, 2010), and coexist within breeding populations (Huxley, 1955). However, the frequency and presence of different color morphs varies among populations in most color polymorphic species (reviewed in McLean & Stuart-Fox, 2014). A key aim in evolutionary biology is to understand drivers of phenotypic variation and consequences of its loss, and intraspecific color morphs that vary geographically provide an excellent system to investigate phenotypic variation at multiple spatial and evolutionary scales (Svensson, 2017).

Color polymorphic species show great variation in color morph frequencies and color morph diversity across their geographic range in insects (Takahashi *et al.*, 2011), lizards (Corl *et al.*, 2010; McLean *et al.*, 2015), and birds (Roulin, 2004). High levels of gene flow between populations help maintain balanced morph frequencies and morph diversity in populations (Gray & McKinnon, 2007), thereby decreasing the chance for

morph loss, morph divergence, and speciation (Merilaita, 2001). When gene flow is low between color polymorphic populations, variation in morph frequencies and diversity may reflect local selection pressures (Nosil, 2012; McLean *et al.*, 2015).

Patterns of geographic variation in genetically-based phenotypes, such as color morphs, may be driven by selection (Gray & McKinnon, 2007; Svensson, 2017). If morph fitness is dependent on some aspect of the environment, and gene flow is maintained between populations of different selective environments, we would expect to see morph frequencies gradually change across the landscape according to that variable (Takahashi *et al.*, 2011). If gene flow is limited between populations across the landscape, or polymorphic species experience steep gradients in environmental variables, then local selection pressures may result in unbalanced morph frequencies or local morph extinction. If a population loses a morph, this can lead to rapid phenotypic divergence between remaining morphs and can be a precursor to speciation (West-Eberhard, 1986; Corl *et al.*, 2010). Nevertheless, few studies have explored color morph frequencies and diversity across many populations to identify ecological, environmental, and evolutionary drivers of morph evolution. Thus, the relative contributions of evolutionary history, ecology, and the environment in shaping morph diversity and frequencies across the landscape remain unclear.

We documented color morph diversity and inferred evolutionary relationships for 44 distinct island populations and two mainland populations of the Aegean wall lizard (*Podarcis erhardii*), a color polymorphic lacertid lizard widely distributed across the Aegean islands to understand temporal and spatial patterns of morph variation. The islands *P. erhardii* inhabits were once one landmass that subsequently fragmented into hundreds of ecologically diverse islands since the last glacial maximum (Foufopoulos & Ives, 1999). Islands vary in age (Brock *et al.*, 2015), or time of fragmentation from another landmass, based on a combination of geomorphology, sea floor depth, and tectonic activity, allowing us to assess the rate of morph evolution across distinct island populations (Poulos, 2009). Island sizes span orders of magnitude and exhibit a range of lizard predator regimes and habitat types to test for morph environmental associations (Figure 1). Specifically, we investigated the following questions: (1) What is the evolutionary history of color polymorphism among island populations of *P. erhardii*? (2) How many times has color polymorphism been gained and lost across distinct island populations? (3) Are morphs associated with certain aspects of the environment?

METHODS

Lizard sampling.

Field work was conducted over three field seasons (2017-2019) during the lizard breeding season from May-July. Lizards and associated environmental and ecological data were collected from 46 unique sites spanning 44 islands and two mainland

populations across the geographic distribution of *P. erhardii* (Figure 2). Field sites were chosen by reconnaissance satellite imagery to identify island landscape features and selecting sites that best represent available habitat of that island. Multiple populations were sampled at larger islands, whereas small uninhabited islets likely represent one population. Lizards were collected opportunistically at each site during their diurnal activity periods (0800-1300 and 1600-1800). We obtained a minimum sample size of 30 lizards for most populations. We captured lizards opportunistically using extendable fishing poles with a small thread lasso attached to the end. Lizards were kept in individual, breathable cloth bags between capture and measurement.

For every individual we sampled, A 20mm section of tail tissue was snipped from the posterior of the tail and preserved in RNAlater for preservation and subsequent DNA extraction.

Field work and lizard sampling procedures were carried out with permission from the Greek Ministry of Environment (codes □ΨΓ64653Π8-Η□Λ5, □□Ω8Δ84653□Π-Β□ΞΧ, and 6ΥΛΥ4653Π8-ΠΞΓ issued to K.M. Brock) and were in compliance with the University of California, Merced Institutional Animal Care and Use Committee (IACUC protocol AUP17-0002).

Color morph scoring.

We scored morph color following a three-step procedure. First, K.M. Brock scored morphs in the field upon capture. Morphs could belong to one of six categories: orange, orange-white, white, white-yellow, yellow, or yellow-orange. Second, another color morph score was given independently by a field assistant. Visual scores of morph color agreed 1,293 out of 1,311 sampled individuals. Finally, we used the average reflectance spectral measurement extracted from six spectrophotometer readings of the throat patch, to make final morph scores for disputed scores made by eye (N = 18). To ensure accuracy of population-level morph diversity measurements, we re-visited as many populations throughout the study as possible (Table 1). To ensure a 95% chance of sampling a color allele if it is present at 5% in the population, we obtained a minimum sample size of 30 lizards for most populations (Corl *et al.*, 2010). Sample sizes for each population are given in Table 1.

Ecological and environmental measurements.

We took both individual- and site-level ecological and environmental measurements. Individual-level measurements taken in the field included a GPS coordinate taken from the point we first sighted the lizard and substrate temperature from point of first sight. Further, for each individual we conducted a survey of the following in a $3m^2$ quadrant surrounding the point we first sighted the lizard: number of refugia, nearest refuge type, perch height, nearest vegetation type, light level (scored from 1-3

with 1 being full sun and 3 being full shade). Site-level measurements conducted in the field included predator surveys (see Brock *et al.*, 2015), intra-specific lizard density surveys (see Donihue *et al.*, 2015). We also noted the presence or absence of dry stone walls, humans, goats, or water sources.

We extracted several environmental variables *post hoc* from individual lizard GPS coordinates. Individual-level environmental measurements from GPS coordinates include: elevation, latitude, longitude, normalized vegetation index (NDVI), and normalized moisture index (NDMI). NDVI and NDMI were calculated in LandSat explorer (www.livingatlas2.arcgis.com) using the change detection tool. We specified vegetation and moisture as the rendering layers and used the calendar year the individual was sampled as our start and end dates for measurement.

Site-level variables calculated *post hoc* include island area, island age, and sitewide percent vegetation cover. We gathered island area data for most islands using published literature and government sources (Foufopoulos & Ives, 1999; Poulos *et al.*, 2009). For some of the smaller uninhabited islands, we calculated island area using Google earth using the measure distance and area tool, tracing the outline of the island, and generating an area calculation. We used island age estimates from published sources (Brock *et al.*, 2015) where possible. To determine the time of separation for islands without published age data, we used bathymetry data derived from navigation charts and sonar measurements collected by J. Foufopoulos in the field, as well as geomorphological reconstructions of past sea-level change from the literature (Pirazzoli, 1991; Hurston *et al.*, 2009, Poulos *et al.*, 2009). Sitewide percent evergreen vegetation cover was calculated using Google earth satellite imagery from the date of sampling (Brock *et al.*, 2015). Island ecological and environmental characteristics are detailed in Table 1.

Molecular data generation.

A total of 337 lizards from 30 study populations were included in phylogenetic analyses (Table 1). We cut up 3 cm of tail tip and placed pieces in 125 microliters of proteinase K overnight for digestion. Total genomic DNA was extracted from tail tissue preserved in RNAlater using a Qiagen Dneasy Blood and Tissue Kit following the manufacturer's protocol. To isolate and amplify the mitochondrial cytochrome b gene, we used selective primers in polymerase chain reaction (PCR). The mitochondrial gene cytochrome b and some flanking regions were amplified with the primers IguaCytob_F2 (5'-CCACCGTTGTTATTCAACTAC-3') and IguaCytob_R2 (5'-GGTTTACAAGACCAATGCTTT-3') to give a 1174 base pair (bp) fragment. Amplification conditions for cytochrome b involved an initial denaturation step of 2 minutes at 94º C, 35 cycles of 10 seconds at 95º C, 20 seconds at 50º C, and 90 seconds at 72º C, and a final extension of the last step. Reamplification was conducted in 1 microliter of amplification mix under the aforementioned PCR conditions but with an

annealing temperature of 55º C. PCR products were run on a 1.5% agarose gel to ensure target fragment length (1174 bp) and quality. Sequencing was carried out by the University of California, Davis sequencing facility.

Data for this project will be publicly available on Dryad upon publication.

Sequence data and phylogenetic inference.

We aligned cytochrome b sequence data in AliView (v.1.25) using MUSCLE (v.3.8.425) with default parameters. We then assessed the gene sequence alignment for the most appropriate model of evolution for tree inference using the Akaike Information Criterion in jModelTest (v.2.1.10) using default parameters (Darribe *et al.*, 2012).

An intra-specific population-level phylogeny of *P. erhardii* was inferred in a Bayesian framework in BEAST 2 (v.2.5.1) using the StarBEAST plug-in (Heled & Drummond, 2010). In BEAST 2, we used the General Time Reversible site model of evolution with a gamma distribution and invariant sites (GTR+Γ+I) from jModelTest with all frequencies set to empirical and no additional parameters estimated. We specified an uncorrelated relaxed molecular clock with a log-normal distribution, which assumes each branch has an independent rate (Drummond *et al.*, 2006). We set the species tree population size function to linear with a constant root and population mean of 1.0. We set the species tree prior to a Yule Model (pure-birth) with a log-normal distribution on the species birth rate. Prior distribution of the cytochrome b gene clock was set to exponential, and the population mean prior was set to log-normal. We used a Metropolis-Coupled Markov Chain Monte Carlo (MCMC) method for Bayesian phylogenetic sampling. We specified the MCMC chain length to run for 1,000,000 steps and sampled every 1,000 steps. We generated a maximum clade credibility tree in TreeAnnotator and discarded the first 20% of sampled trees as burn-in.

Estimating the evolutionary history of color polymorphism in P. erhardii populations.

To estimate the evolutionary history of color polymorphism in *P. erhardii*, we performed ancestral state reconstruction using the inferred cytochrome b phylogeny and color polymorphism trait data we collected. Ancestral state reconstructions were carried out in R using a maximum likelihood approach with the ‘ace’ function in the ‘ape’ package (Paradis & Schliep, 2018). We also estimated ancestral states and the associated uncertainty of our discretely valued color polymorphism trait at each node in the tree using a continuous-time Markov chain model, or Mk model in the ‘phytools’ package (Revell, 2012). We fit a single-rate model with equal transition rates and an all-rates-different model that allows backward and forward transition rates between states to have different values and compared model log-likelihood scores using a likelihood ratio test with α set to 0.05 *a priori* to select the best fit model. We summarized results from our

final all-rates-different Mk model by mapping the empirical Bayesian posterior probability pie charts for each node (Figure 3).

Simulating morph frequencies to understand probability of observed morph diversity.

Islands are known for founder effects and genetic drift, which are dramatic events that usually result in greatly reduced genetic diversity by chance. In our case, it is possible that our observed morph loss and diversity across islands could be the result of random genetic drift from ancient sea-level rise. If this were the case, we would expect sampled simulated (uncorrelated) data to match our observed data. If our observed morph loss data do not match sampled simulated data, then we have some evidence that morph presence across islands is not due to random events.

To identify the probability of our observed morph diversity results, we conducted a simulation study. We observed 34 populations with one or more missing morphs (Figure 2). We observed 32 populations without orange morphs, 22 populations without yellow morphs, and 20 populations without both orange and yellow morphs (Figure 2). The overall observed morph frequencies from our entire dataset (N = 1,311 lizards) were orange: 0.05, yellow: 0.12, white: 0.83. We created a pool of morphs with these frequencies, and simulated our sampling numbers for all 46 sites 100 times with replacement to see how many times out of 100 we see similar orange, yellow, and orange yellow morph loss.

Morph environmental associations.

We tested for morph differences in several ecological and environmental variables important to lizard life history and fitness including the following: elevation (m), substrate temperature (ºC), field body temperature (ºC), moisture index (NDMI), vegetation index (NDVI), and the percentage of open ground within a 3 m^2 plot. Lizards are ectothermic, and variables such as elevation and temperature are important for basic physiological functions and thermoregulation. The Aegean islands experience a typical Mediterranean climate, with hot, dry summers, and cool, wet winters. Most Aegean islands we sampled do not have water sources, and so moisture from vegetation is likely important to lizard survival. Vegetation can provide critical refuge from the intense midday sun as well as predators. We tested for morph differences in these variables using One-way ANOVAs and Tukey HSD tests.

All analyses were performed in RStudio (v 1.3.1093).

RESULTS

The evolutionary history of color polymorphism in island populations of Podarcis erhardii.

We identified 12 tri-color polymorphic populations, 13 di-morphic populations, and 21 monomorphic populations (Figure 2). However, we could only use a subset of these observations in ancestral reconstruction to match the 30 populations we sequenced (Table 1). Because we used a binary metric of color polymorphism for ancestral state reconstruction, color polymorphic populations include any population with more than one color morph allele (N = 17 color polymorphic populations, 13 monomorphic populations, Figure 3).

A likelihood ratio test supported the more heavily parameterized all-rates-different transition probability matrix where all possible transitions between states receive distinct parameters (ARD log-likelihood = -8.9335, ER log-likelihood = -19.1055, likelihood ratio test $p < 0.05$, df =1).

Ancestral state reconstruction using the all-rates-different transition matrix revealed that the most recent common ancestor of all *P. erhardii* populations was most likely color polymorphic (Scaled likelihood at the root, color polymorphic = 89%, monomorphic = 11%, Figure 3). The proportion of total evolutionary time spent in the color polymorphic state was 66%, and just 33% in the monomorphic state. Results from sampling 1,000 character histories conditioned on the all-rates-different transition matrix detected nine total character changes in color polymorphism. We estimated eight transitions from polymorphism to monomorphism and potentially one transition from monomorphism to polymorphism (see Schoinoussa in Figure 2). Estimates of evolutionary transition rates from poly- to monomorphism (Rate index estimate = 0.0323) were higher than transitions from mono- to polymorphism (Rate index estimate = 0.0002), suggesting that color polymorphism is more easily lost than gained at the population-level.

Simulated vs. observed morph frequencies and diversity

When we compared simulated morph loss values to our observed morph loss data, we found that the simulated instances of orange, yellow, and orange and yellow morph loss were much lower than observed morph loss, suggesting that observed morph loss is likely not completely due to random chance (Simulations: loss of orange from populations = 29%, loss of yellow from population = 9%, loss or orange and yellow = 6%). Our observed rates of morph loss were: orange = 69%, yellow = 48%, orange and yellow = 41%.

Morph environmental associations.

Color morphs were found at significantly different elevations. We found morph differences in the average elevation at which they were observed (ANOVA $F = 74.95$, df = 5, $p < 0.001$) across our entire dataset. A *post hoc* Tukey HSD test revealed that morphs with orange alleles (orange, orange-white, yellow-orange) were present at

significantly higher elevation than morphs without orange alleles (Tukey HSD $p < 0.05$). When we subsetted the data to only include populations with all three morphs, we again found the same pattern where morphs with orange alleles (orange, orange-white, yellow-orange), on average, were found at higher elevation than morphs without orange alleles (ANOVA $F = 26.66$, $df = 5$, $p < 0.001$, Tukey HSD $p < 0.05$).

Substrate temperatures differed amongst color morphs (ANOVA $F = 2.44$, $df = 5$, $p = 0.03$). A *post hoc* Tukey HSD test revealed that the pure orange morph had the lowest average substrate temperature, and was significantly lower than the white-yellow morph (Tukey HSD diff. = 5.59, $p = 0.05$). When we subsetted the data to only include populations with all three morph alleles present, we again found that the orange morph had the lowest average substrate temperature, however the difference was not significant (ANOVA $F = 0.76$, $df = 5$, $p = 0.581$). When we subset the entire dataset to analyze males and females separately, we found that female morphs from those populations did not differ significantly in substrate temperature (ANOVA $F = 1.49$, $df = 5$, $p = 0.19$) but male morphs did (ANOVA $F = 2.44$, $df = 5$, $p = 0.03$). A *post hoc* Tukey HSD revealed that pure orange male morphs from tri-morphic populations were found on significantly cooler substrates than male white-yellow morphs (Tukey HSD diff. = 7.51, $p = 0.02$).

Color morphs differed in their field body temperature. When we analyzed all lizards together, we found that color morphs differed significantly in body temperature (ANOVA $F = 3.38$, $df = 5$, $p = 0.005$). A *post hoc* Tukey HSD test revealed that pure orange and orange-white morphs had significantly lower body temperatures than pure yellow morphs (Tukey HSD yellow--orange diff. = 2.49, $p = 0.04$; Tukey HSD yellow--orange-white diff. = 2.05, $p = 0.03$), and morph with orange alleles had the lowest body temperatures. When we subset the data to only include populations with all three morph alleles present, we found that morphs significantly differed in their field body temperature (ANOVA $F = 3.74$, $df = 5$, $p = 0.003$), and a *post hoc* Tukey HSD test revealed that orange morphs had significantly lower body temperatures than the white and white-yellow morphs (Tukey HSD diff. white--orange = 3.59, $p = 0.003$; Tukey HSD diff. white-yellow--orange = 3.7, $p = 0.02$). When we subset the data further to only include females from tri-allelic sites, we found no difference in color morph body temperatures (ANOVA $F = 1.8$, $df = 5$, $p = 0.112$). When we analyzed males from tri-allelic populations, we found morph differences in body temperature (ANOVA $F = 5.5$, $df = 5$, $p < 0.001$) and orange morphs had significantly lower body temperatures than yellow, white-yellow, and white male morphs (Tukey HSD $p < 0.05$).

Color morphs exhibited differences in average normalized difference moisture index (NDMI), or the amount of moisture in the areas from where we sampled them. When we analyzed all lizards together, we found that color morphs differed significantly in NDMI (ANOVA $F = 17$, $df = 5$, $p < 0.01$). A *post hoc* Tukey HSD test revealed that all morphs with a white allele were found from drier spaces than the yellow morph (Tukey

HSD yellow--orange-white diff. = 0.05, p = 0.001; Tukey HSD yellow--white diff. = 0.07, p < 0.001, Tukey HSD yellow--white-yellow diff. = 0.08, p < 0.001). When we subset the dataset to only include females, we found that they significantly differed in average NDMI (ANOVA F = 9.84, df = 5, p < 0.001). A *post hoc* Tukey HSD test revealed that yellow females were found in more moist areas than white-yellow females (Tukey HSD yellow--white-yellow diff. = 0.09, p < 0.001). When we subset the data to only include males, we found that male morphs significantly differed in average NDMI (ANOVA F = 9.17, df = 5, p < 0.001). A *post hoc* Tukey HSD test revealed that orange and yellow males were found in areas that had higher average NDMI than white-yellow males (Tukey HSD p < 0.05).

Color morphs exhibited differences in average normalized vegetation index (NDVI), or the amount of green vegetation in the areas from where we sampled them. When we analyzed all lizards together, we found that morphs significantly differed in NDVI (ANOVA F = 26.33, df = 5, p < 0.001). A *post hoc* Tukey HSD test revealed that all morphs with an orange allele were found in areas with greater vegetation greenness, or NDVI (Tukey HSD p < 0.05). When we subset the data to only include females, we found that female morphs differed significantly in NDVI in areas from where we sampled them (ANOVA F = 16.87, df = 5, p < 0.001). A *post hoc* Tukey HSD revealed that female morphs with orange alleles and the yellow females were sampled from areas with significantly greater vegetation greenness than the white morph (Tukey HSD p < 0.05). When we subset the data to only include males, we found the exact same pattern as females (Tukey HSD p < 0.05).

DISCUSSION

By combining evolutionary history and contemporary ecological and environmental data, we determined that the common ancestor of *P. erhardii* populations was color polymorphic, and that color polymorphism probably evolved once in this species. We found that color polymorphism is lost in populations much more quickly than it is gained, following early morphic speciation theory (West-Eberhard, 1986). Although we found several interesting environmental differences between color morphs, we were unable to explain morph diversity with the variables we measured.

Much research to date has focused on intra-population morph divergence and sympatric speciation, yet the geographical context of morph variation among populations has gone under-explored. The few range-wide studies of morph variation in mainland species with contiguous distributions have found that morph frequencies are structured according to evolutionary history (Corl *et al.*, 2010) or associated with an environmental gradient (Gosden *et al.*, 2011; Takahashi *et al.*, 2011; McLean *et al.*, 2015), but rarely are these considered together.

Alleles with strong fitness effects will reach fixation more rapidly than those with low fitness effects (Fisher, 1930). We found repeated loss of the orange morph from many distinct island populations. Morph diversity, in general, seems to follow an ordered pattern, where the orange morph disappears first, followed by yellow, and white usually persists. We only detected three exceptions from this ordered pattern: two islands where orange and white persist (Folegandros and Agios Nikolaos) and one island where only yellow lizards persist (Serifos). We visited Folegandros only once, and sampled in late June. During our sampling trip to Folegandros, temperatures were extremely hot and we found it difficult to catch lizards. Thus, it is possible that we missed yellow individuals on Folegandros. However, we have visited Agios Nikolaos regularly for several years and have consistently only observed orange, white, and orange-white individuals. Serifos is the only island that seems to be fixed for one morph that is not white. The yellow lizards of Serifos are interesting case, as Serifos is an older island, one that separated from Cycladia quite early on in the break up of the proto-Cycladic block (Foufopoulos & Ives, 1999; Poulos, 2009). The neighboring satellite islet to Serifos, Vous, is one of the only islands where a morph other than the white morph is the most common.

Ecological and environmental variation is known to have strong effects on color morph evolution, maintenance, and frequencies within populations (reviewed in Gray & McKinnon, 2007). Orange morphs seemed to disappear at low elevation sites. Orange morphs also seem to prefer cooler substrates and environments with greater moisture. Orange morphs also tended to have lower field body temperatures. It is possible that the repeated loss of the orange morph is related to harsher dry environments with sparse vegetation, little moisture, and not quite enough shade to maintain a lower body temperature.

The next steps in this research will be to expand our phylogeny to include all 46 populations, and test for ecological and environmental drivers of morph loss.

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Figures

Figure 1. Habitat diversity across *Podarcis erhardii* sampling sites. 1A-H are nine different island sites where we sampled lizards. Large islands 1A-C, medium-sized islands 1D-F, and small islands 1G-I span an environmental and ecological gradient from dry and bare to more moist and green. All photos were taken in late May-June during the lizard breeding season.

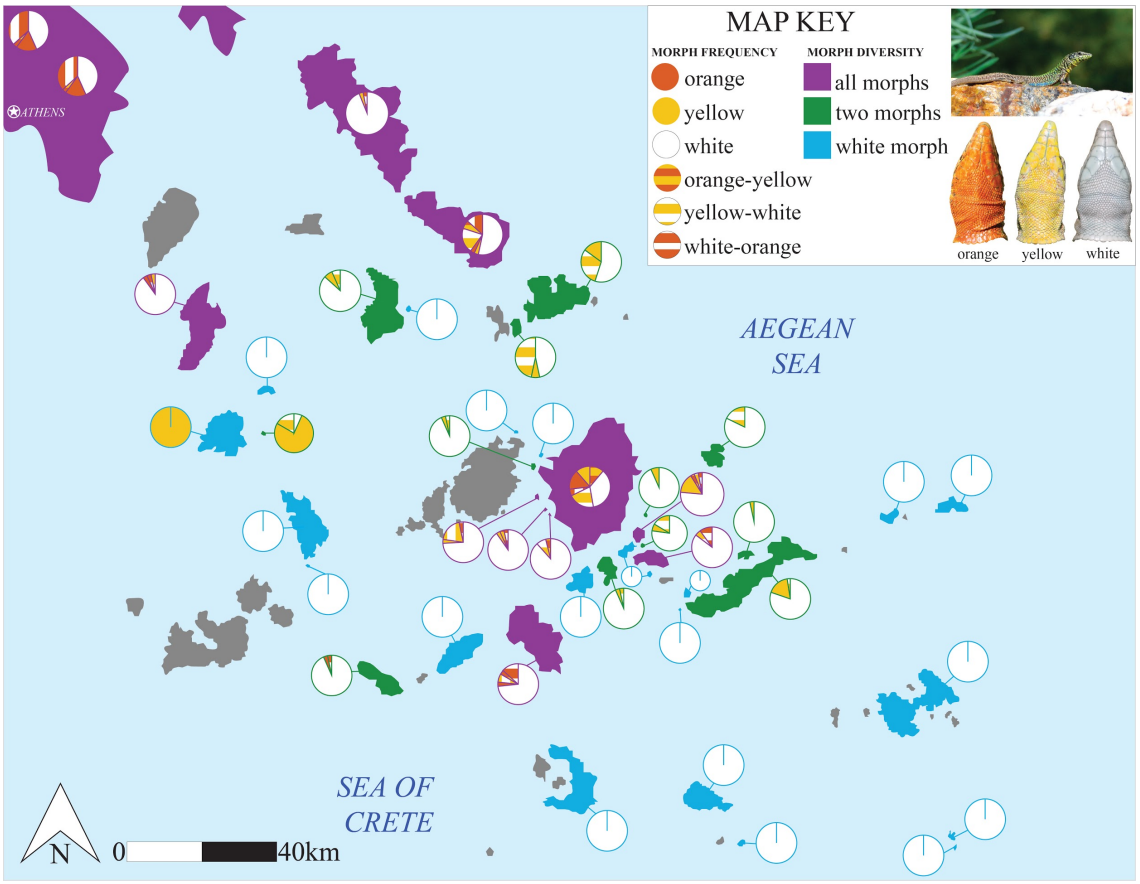

Figure 2. Map of color morph diversity and frequencies across the geographic distribution of *Podarcis erhardii*. Islands and the mainland are color coded by morph allelic diversity, ranging from 1-3. Morph frequencies are given for each site in pie chart form, and detail pure morphs and mosaic morph frequencies. Islands in grey were not sampled due to boat travel constraints or because *P. erhardii* do not inhabit that particular island.

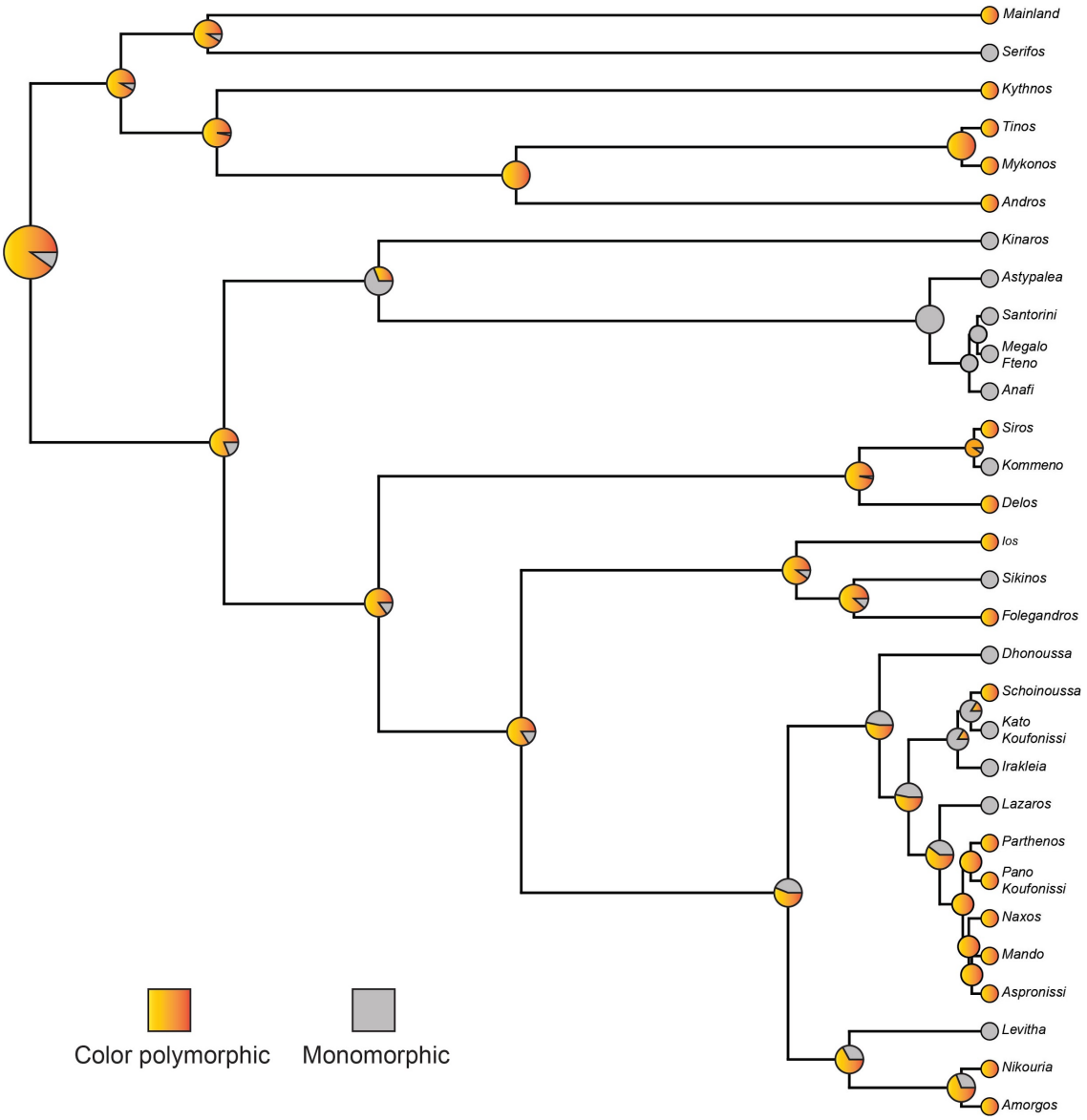

Figure 3. Evolutionary relationships and history of color polymorphism among 30 populations of *Podarcis erhardii*. Tips of the tree are labeled by island. Circles at the tips of the tree indicate the observed binary state of color polymorphic or monomorphic. Empirical Bayesian posterior probability pie charts at the nodes indicate inferred ancestral state probabilities.

Tables

Table 1. Island character data. Islands are listed from smallest to largest. The number of lizards sampled at that location is given under island name. Morph diversity indicates how many color morph alleles we found at that island (ranging from 1-3). Island age is the inferred period of time since that landmass last split from another landmass. Predator regime indicates the number of predator types at that site and ranges from zero to six, indicating no predator presence to maximum predator category diversity, respectively (see Brock *et al.*, 2015). Average Normalized Difference Moisture Index (NDMI) and average Normalized Difference Vegetation Index (NDVI) were calculated at the site-level from individual NDMI and NDVI data. Asterisks indicate the two mainland sites. A † symbol next to island names indicates sites we were able to revisit in a later year to confirm morph presence and frequencies.

Island	Morph diversity	Island area (km^2)	Island age (years)	Predator regime	Lizard density (/100m)	Site vegetation cover (%)	Average NDMI/NDVI
Mikri Vigla (25)	3	0.002	6100	0	6	0.4	0.02 / 0.16
Parthenos † (31)	3	0.004	5650	1	21	0.45	-0.05 / 0.12
Kommeno (18)	1	0.007	300	1	13	0.86	-0.03 / 0.13
Aspronissi † (31)	3	0.009	6100	1	12	0.8	0.04 / 0.12
Apollo's Gate † (11)	1	0.01	100	2	3	0.62	0.04 / 0.22
Kisiri (5)	1	0.012	5750	3	3	0.18	-0.03 / 0.17
Lazaros (31)	1	0.014	9100	0	19	0.78	-0.04 / 0.15
Mando † (30)	2	0.025	4	3	6	0.51	-0.001 / 0.16
Megalo Fteno (35)	1	0.059	9550	0	15	0.5	-0.06 / 0.19

Astakidopoulo (1)	1	0.085	8300	0	10	0.39	-0.05 / 0.18
Gaidouronissi (30)	1	0.133	7100	1	7	0.13	-0.03 / 0.26
Vous (30)	2	0.14	11700	0	3	0.9	0.09 / 0.42
Kopria (30)	2	0.14	11700	0	10	0.09	-0.04 / 0.19
Glaronissi (31)	2	0.16	5650	1	3	0.03	-0.05 / 0.26
Gramvoussa (30)	1	0.759	6700	3	3	0.51	0.03 / 0.21
Kitriani (10)	1	0.837	8100	1	2	0.41	0.04 / 0.32
Agios Nikolaos (34)	2	0.89	11900	2	10	0.4	0.06 / 0.3
Astakida (5)	1	0.98	36000000	0	10	0.3	-0.07 / 0.14
Serifopoula (30)	1	1.75	200000	1	12	0.19	-0.07 / 0.17
Nikouria † (30)	2	2.75	5700	3	6	0.9	0.09 / 0.35
Delos (15)	2	3.43	9500	5	2	0.31	-0.11 / 0.2
Kato Koufonissi (26)	1	3.89	5000	3	2	0.4	-0.04 / 0.23
Kinaros (30)	1	4.58	450000	2	6	0.91	0.01 / 0.04
Schoinoussa † (30)	2	8.83	9550	5	1	0.3	-0.03 / 0.17
Levitha (31)	1	9.12	450000	2	10	0.95	0.03 / 0.24

Dhonoussa † (17)	2	13.65	12800	4	2	0.48	-0.008 / 0.22
Keros (41)	3	15.05	9150	4	5	0.38	0.005 / 0.34
Irakleia † (32)	1	18.08	9800	6	3	0.44	-0.05 / 0.23
Folegandros (30)	2	32.38	11650	5	2	0.33	-0.04 / 0.2
Pano Koufonissi † (30)	3	37.5	9000	5	3	0.4	-0.006 / 0.2
Sikinos (30)	1	41.68	14100	6	1	0.22	-0.01 / 0.13
Anafi (30)	1	49	36000000	3	4	0.57	-0.01 / 0.23
Sifnos (30)	1	73.94	200000	5	4	0.45	0.08 / 0.36
Serifos † (45)	1	75.21	450000	4	9	0.75	0.14 / 0.35
Santorini † (33)	1	76.19	200000	4	3	0.18	-0.03 / 0.2
Astypalea † (25)	1	96.9	36000000	3	1	0.28	-0.02 / 0.18
Kythnos (30)	3	99.43	450000	4	5	0.82	0.18 / 0.41
Siros (30)	2	101	12800	5	9	0.82	0.06 / 0.32
Mykonos † (30)	2	105	11800	5	2	0.36	-0.01 / 0.17
Ios † (30)	3	109	11750	6	5	0.67	0.01 / 0.17

Amorgos † (41)	2	123	200000	5	9	0.8	0.003 / 0.25
Tinos (30)	3	197	5800	5	4	0.84	0.02 / 0.18
Andros (33)	3			6	5		0.16 / 0.41
Naxos † (98)	3			6	8		0.18 / 0.48
Parnitha** (30)	3			6	7		0.01 / 0.33
Mt. Olympus** (30)	3			6	6		0.02 / 0.42

Color Polymorphism is a Driver of Diversification in the Lizard Family Lacertidae

ABSTRACT

Color polymorphism – two or more heritable color phenotypes maintained within a single breeding population – is an extreme type of intra-specific diversity widespread across the tree of life. Color polymorphism is hypothesized to be an engine for speciation, where morph loss or divergence between distinct color morphs within a species results in the rapid evolution of new lineages, and thus, color polymorphic lineages are expected to display elevated diversification rates. Multiple species in the lizard family Lacertidae are color polymorphic, making them an ideal group to investigate the evolutionary history of this trait and its influence on macroevolution. Here, we produce a comprehensive species-level phylogeny of the lizard family Lacertidae to reconstruct the evolutionary history of color polymorphism and test if color polymorphism has been a driver of diversification. Accounting for phylogenetic uncertainty, we estimate an ancient origin of color polymorphism (111 MYA) within the Lacertini tribe (subfamily Lacertinae). Color polymorphism most likely evolved few times in the Lacertidae and has been lost at a much faster rate than gained. Evolutionary transitions to color polymorphism are associated with shifts in increased net diversification rate in this family of lizards. Taken together, our empirical results support long-standing theoretical expectations that color polymorphism is a driver of diversification.

KEYWORDS

Color polymorphism, state-dependent speciation extinction models, Lacertidae, trait-dependent diversification

INTRODUCTION

Understanding how diversity is generated and maintained within and across species is a fundamental goal of evolutionary biology. Color polymorphism, or the presence of two or more distinct genetically determined color phenotypes within a breeding population (Huxley 1955; Gray & McKinnon 2007), exemplifies extraordinary intra-specific phenotypic diversity. Despite its usefulness in developing population genetic theory (Ford 1945; Huxley 1955; Svensson 2017), relatively little is known about the evolutionary origins and macroevolutionary consequences of color polymorphism (Gray & McKinnon 2007; Jamie & Meier 2020). A longstanding hypothesis is that the presence of different genetically-based color morphs within populations may promote speciation (West-Eberhard 1986; Jonsson 2001; Gray & McKinnon 2007; Corl *et al.* 2010a,b; Hugall & Stuart-Fox 2012; McLean *et al.* 2014). In this scenario, a group of distinct morphs is established within a population, phenotypic variation among morphs gradually accumulates, and over time isolated populations with different morphs diverge, facilitating speciation (West-Eberhard 1986). Studies of color polymorphisms spanning fish (Jonsson 2001; Seehausen & Schluter 2004; Allender *et al.* 2003), birds (Hugall & Stuart-Fox 2012), and reptiles (Corl *et al.* 2012) all suggest the evolution of multiple

variable morphs as an impetus for speciation. However, after more than 50 years of research, few comparative studies exist that test this claim (but see Hugall & Stuart-Fox 2012).

The origins and maintenance of color polymorphism have long been subjects of fascination in evolutionary biology (Huxley 1955; West-Eberhard 1986; Roulin 2004; Stuart-Fox *et al.* 2020). Understanding the evolution of stable color polymorphisms has been difficult because the processes involved seem to operate across multiple biological scales – ranging from within and among population processes within species (reviewed in Gray & McKinnon 2007) to convergence and divergence in color polymorphisms among species (reviewed in Stuart-Fox *et al.* 2020). Theoreticians have argued that evolutionary branching events (e.g.: population divergence, speciation) provide a useful framework for generating predictions and testing alternative hypotheses regarding the likelihood that phenotypic polymorphisms evolve, persist, or diverge (West-Eberhard 1986; Leimar 2005). Leimar (2005) posited that multiple genetically determined phenotypes, or morphs, are less likely to persist within one lineage at an evolutionary branching event because a polymorphic system is inherently less stable than a monomorphic one. Indeed, studies of color polymorphic species have consistently found that some form of balancing selection is required for multiple discrete phenotypes to persist over evolutionary timescales (Sinervo & Lively 1996; Perez i de Lanuza *et al.* 2017). Further, empirical studies have found that geographic variation in the number and types of morphs among populations is usually explained by morph loss from populations, with little evidence for gaining morphs back (Corl *et al.* 2010b; Sacchi *et al.* 2007). In a phylogenetic context, we would thus expect novel gains of color polymorphism to evolve at a slower rate than monomorphism and that color polymorphism is lost at a faster rate than monomorphism across a phylogeny of species or populations (Corl *et al.* 2010b; Hugall & Stuart-Fox 2012). Given this, on a phylogenetic tree we would expect ancestral state reconstructions of the evolutionary history of color polymorphism to produce a pattern of ancestral polymorphic lineages diversifying and giving rise to monomorphic lineages.

A phylogenetic comparative approach can also be leveraged to test the hypothesis that color polymorphism is a driver of diversification (Morlon 2014; Morlon *et al.* 2020). First proposed by Huxley (1955), many theoretical (West-Eberhard 1986; Forsman *et al.* 2008) and empirical studies (Boughman 2001; Corl *et al.* 2010b) in groups across the tree of life have followed that also suggest multiple color morphs within species should lead to faster formation of new species (reviewed in Gray & McKinnon 2007). Forsman *et al.* (2008) present a series of ecological and evolutionary scenarios where color polymorphic species should have greater evolutionary potential for speciation compared to monomorphic species. According to Forsman *et al.* (2008) the evolution of multiple color forms within a population may increase evolutionary potential for speciation through increased utilization of diverse habitats and resources, and greater ability to successfully colonize and expand their range relative to monomorphic populations. Coupled with divergent selection, either natural, sexual, or their combination, alternative morph phenotypes spread across variable geographic contexts may increase the chance for unbalanced morph frequencies, rapid phenotypic divergence, and speciation (reviewed in McLean & Stuart-Fox 2014). Most well-studied color polymorphic species, from moths to lizards, exhibit population differences in the number and frequency of morphs (Grant

et al. 1998; Corl *et al.* 2010b; Stuart-Fox *et al.* 2020). Consensus from field studies of natural populations suggest that the disruption of balancing selection for multiple color morphs usually results in morph loss, and divergence in remaining morphs may progress quickly toward speciation (Corl *et al.* 2010b; Galeotti *et al.* 2003). If color polymorphic species are engines of speciation, then net diversification rates (speciation - extinction) should be greater within color polymorphic lineages compared to monomorphic lineages. To date, the claim that color polymorphism promotes diversification has largely gone untested at the macroevolutionary level. A single study by Hugall & Stuart-Fox (2012) found that in three of five families of non-passerine birds investigated, speciation rates were higher in color polymorphic lineages than monomorphic lineages. More comparative studies of speciose groups that have multiple independent origins of color polymorphism are needed to evaluate hypotheses of color polymorphism maintenance and its role in diversification.

Lizards from at least seven different families have repeatedly and independently evolved a similar color polymorphism on the head, throat, and/or ventral region (Stuart-Fox *et al.* 2020), which makes this trait ideal for comparative analysis. The lizard family Lacertidae (Oppel 1811) is an excellent group for a comparative study on the evolution of color polymorphism as the group is relatively speciose and multiple species spanning several genera are known to be color polymorphic (Vercken & Clobert 2008; Huyghe *et al.* 2009a,b; Runemark *et al.* 2010; Brock *et al.* 2020). Biologists have identified a similar throat color polymorphism in several genera across the family Lacertidae (Fig. 1), including *Iberolacerta monticola* (López *et al.* 2009), *Podarcis* species (*P. erhardii*, Brock *et al.* 2020; *P. gaigeae,* Runemark *et al.* 2010; *P. melisellensis*, Huyghe *et al.* 2009a,b; *P. muralis*, Pérez i de Lanuza *et al.* 2019), and *Zootoca vivipara* (Vercken & Clobert 2008). The Lacertidae is the most speciose family of squamates in the Western Palearctic comprising around 320 species distributed across Africa, Asia, and Europe (Arnold *et al.* 2007). While lacertids share a relatively similar body plan, they occur in a variety of habitats from xeric desert lands to montane forests, range widely in their geographic distributions, and exhibit diverse ecologies (Edwards *et al.* 2012; Garcia-Porta *et al.* 2019). The family currently comprises two major taxonomic sub-groups, the Gallotiinae and the Lacertinae. Lacertinae is further divided into two tribes, the Eremiadini and the Lacertini. There is likely some undescribed taxonomic diversity in both the Eremiadini and Lacertini tribes. This undescribed diversity is due to lack of molecular data and under-sampling in the Eremiadini and under-reported species-level diversity in the Lacertini which contains many geographically isolated sub-species and species with extraordinarily large geographic distributions, some on the order of several continents (Herczeg *et al.* 2003). These factors, combined with short internal branches and gene tree discordance, have made family-wide species-level phylogenetic inference for the Lacertidae difficult (Fu 2000; Arnold *et al.* 2007; Pyron *et al.* 2013; Baeckens *et al.* 2015; Garcia-Porta *et al.* 2019). However, the opportunity to leverage new data (Garcia-Porta *et al.* 2019) and analytical approaches mean the Lacertidae is now an ideal group for a comparative study on the evolution of color polymorphism.

In this study, we infer the evolutionary relationships among species in the family Lacertidae to reconstruct the evolutionary history of color polymorphism and test whether the evolution of this trait is associated with diversification rate shifts. We build a

comprehensive species-level phylogeny of the Lacertidae, including 262 species from all 42 described genera. We apply a multispecies coalescent approach that accounts for potential individual gene histories among species (in contrast to approaches used in previous studies, Fu 2000; Arnold *et al.* 2007; Pavlicev & Mayer 2009). Using this species-level tree, a distribution of possible trees from the posterior, a recently inferred time tree from Garcia-Porta *et al.* (2019), and trait simulations, we conduct the first family-wide investigation of color polymorphism. We identify previously undescribed color polymorphic lacertids, and assess long-standing hypotheses concerning the evolutionary origins and consequences of this trait. Specifically, we address the following questions: (1) What is the evolutionary history of color polymorphism in the Lacertidae? (2) Do color polymorphic lineages have elevated rates of diversification compared to non-color polymorphic lineages?

MATERIALS & METHODS

Phylogenetic Inference of the Lacertidae.

GenBank data.

We used publicly available sequence data from GenBank to build our tree (Clark *et al.* 2016). We first pulled all Lacertidae single gene sequences from GenBank and identified five genetic markers for which there were at least 100 species with data for that marker. This filtering resulted in three mitochondrial genes (12s: N = 178 species; 16s: N = 189 species; cytochrome b: 239 species) and two nuclear genes (mos proto-onco gene: N = 142 species; recombination activating gene (RAG) 1: N = 117 species). Genes selected from the mitochondrial genome have been used extensively for phylogenetic reconstruction of Lacertidae in the past (Fu 2000; Edwards *et al.* 2012; Pyron *et al.* 2013; Baeckens *et al.* 2015; Baeckens *et al.* 2017), and the RAG-1 nuclear gene has been shown to evolve at a greater rate than other commonly used nuclear markers, which may allow for greater confidence both deep within the tree and at the tips (Portik *et al.* 2012; Edwards *et al.* 2012). To ensure taxonomic validity, sequences were georeferenced to verify they fell within species distributions as described on the regularly updated lacertid database, AG Lacertiden (www.lacerta.de, maintained by The Arbeitsgemeinschaft Lacertiden within the Deutsche Gesellschaft für Herpetologie und Terrarienkunde). Overall, we retrieved gene sequences for 262 lacertid species – approximately 82% of described lacertid diversity according to the AG Lacertiden database, covering all currently described genera (Arnold *et al.* 2007). Details of genetic data used per species and GenBank accession numbers for each genetic marker are provided in Supplementary Table 1. Sequences were aligned separately in AliView (v.1.25) using MUSCLE (v.3.8.425) with default parameters. Gene sequence alignments were then assessed for appropriate models of molecular evolution for tree inference using the Akaike Information Criterion in jModelTest (v.2.1.10) using default parameters (Darriba *et al.* 2012).

Species tree inference and divergence dating estimation.

We employed a multi-locus coalescent approach for species tree inference as individual gene histories can vary within closely related species (Maddison 1997;

McCormack *et al.* 2009). To do this with our five locus alignments, we used the full Bayesian method of species tree estimation in BEAST2 using the *BEAST template (v.2.5.1) (Heled & Drummond 2010; Bouckaert *et al.* 2014). We unlinked all site and molecular clock models, and linked the gene tree models for mtDNA alignments only. We used the GTR+Γ+I site model for each gene according to model comparison results from jModelTest with all frequencies set to empirical and no additional parameters estimated for computational efficiency. For all genes, we specified an uncorrelated relaxed molecular clock with a log-normal distribution that assumes each branch has its own independent rate (Drummond *et al.* 2006). The species tree population size function was set to linear with constant root and population mean set to 1.0. We set the species tree prior to a Yule Model (pure-birth) with a log-normal distribution on the species birth-rate. Prior distributions for all gene clock-rate priors were set to exponential, and the population mean prior was set to log-normal. To time-calibrate the phylogeny, we used the 'Sampled Ancestors' package in BEAUti2 to generate monophyletic taxon set hyper-priors according to fossil information from the literature (Hipsley *et al.* 2009). Time-calibrated outgroup nodes following Hipsley *et al.* (2009) include: (1) *Sphenodon punctatus – Cnemidophorus tigris*, 228.0 Mya (Sues & Olsen 1990), (2) *Cnemidophorus tigris – Rhineura floridana*, 113.0 Mya (Nydam & Cifelli 2002), and (3) *Rhineura floridana – Gallotia galloti*, 64.2 Mya (Sullivan 1985). All fossil hyper-priors were offset by the above dates, given a log-normal distribution with a mean of 1.0 and standard deviation of 1.25, and constrained to be monophyletic. We ran two independent species tree analyses with the same data and XML configuration for 1 billion MCMC generations and stored every 30,000th sampled tree. Posterior distributions of trees for the two independent BEAST2 runs were combined in BEAST2's logCombiner (v.2.5.1) with the first 20% of trees discarded as burn-in and the rest of the trees resampled at a lower frequency for a total posterior sample of 12,000 trees. A final maximum clade credibility tree, the tree from the reduced posterior sample that had the maximum sum of posterior probabilities on its n - 2 internal nodes, was generated in BEAST2's TreeAnnotator for use in comparative analyses.

The Evolutionary History of Color Polymorphism and Diversification in the Lacertidae.

Color polymorphism data.

We scored the presence or absence of color polymorphism for all described extant lacertid species (N = 320), including those not represented in our phylogeny. Examination of all extant taxa, including species lacking available genetic data and not represented in our phylogeny, was necessary to account for trait estimation proportions downstream in our state-dependent speciation and extinction (SSE) models. We scored color polymorphism from several georeferenced sources to ensure taxonomic validity, including online databases with photographs (www.inaturalist.org, www.lacerta.de), scientific literature (Huyghe *et al.* 2009a,b; López *et al.* 2009; Runemark *et al.* 2010; Brock *et al.* 2020 in press), and field guides (Valakos *et al.* 2008; Speybroeck *et al.* 2016). We *a priori* restricted our investigation to color polymorphism defined as species with multiple coexisting color morphs in the same geographic location. Further, we

focused on color polymorphism in the same trait on the same region of the body, as this is likely to share both a similar underlying genetic mechanism and subject to similar selective pressures across species (Andrade *et al.* 2019). In lacertid lizards, color polymorphism is expressed as colorful badges on the throat and colors vary from species to species (Runemark *et al.* 2010; Brock *et al.* 2020). Species were coded as color polymorphic if we could adequately identify they met all of the following criteria: 1) variation in color located on the throat, 2) variation in throat color is not result of ontogenetic color change and is present in adults, 3) individuals from the same location exhibit at least two different throat colors, and 4) variation in throat color is not strictly sexually dimorphic, males and females both exhibit at least two different color types. We were able to collect color polymorphism data for all described lacertids and thus had no missing trait data for both our MCC species tree and the Garcia-Porta *et al.* (2019) tree. Altogether, we identified 43 color polymorphic species spanning 10 genera.

Ancestral state reconstruction of color polymorphism in the Lacertidae.

To understand the evolutionary history of color polymorphism in lacertids, we used ancestral state reconstructions jointly estimated with character transitions and diversification rates in the HiSSE package with the 'MarginRecon' function (Beaulieu & O'Meara 2016). We present ancestral state reconstructions for both our MCC species tree and a recently published lacertid tree inferred in a maximum likelihood (ML) framework by Garcia-Porta *et al.* (2019) that differed somewhat in taxon representation and topology to test if our results were robust to phylogenetic uncertainty (Figure 2).

State-dependent diversification models.

To test our hypothesis that evolutionary transitions to color polymorphism are associated with elevated diversification rates, we used state-dependent speciation and extinction (SSE) models (Maddison *et al.* 2007). An advantage of SSE models is joint estimation of trait transitions and diversification rates (Maddison *et al.* 2007; Beaulieu & O'Meara 2016). The original binary state-dependent speciation and extinction (BiSSE) model calculates the probability that a group of extant species evolved as observed at the tips given a phylogenetic tree and a binary character under a simple model of evolution with six parameters (Maddison *et al.* 2007). The parameterization of a basic BiSSE model specifies two speciation rates (a rate for when a lineage is in state 0, and a rate for when a lineage is in state 1), two extinction rates (for lineages in state 0 and state 1), and two rates of character state transition (from state 0 to state 1 and *vice versa*). The hidden-state speciation and extinction (HiSSE) model framework is an extension of BiSSE that specifies additional parameters to account for diversification rate heterogeneity that is not associated with the observed trait (Beaulieu & O'Meara 2016). These "hidden states" represent unmeasured characters that could affect diversification rate estimates for the measured observed character (Beaulieu & O'Meara 2016). Thus, including hidden states allows us to estimate the effect of color polymorphism while controlling for other unmeasured correlated traits on diversification rate. The SSE model framework is statistically advantageous because BiSSE models are nested within HiSSE models, and maximum likelihood inference can be used to estimate a suite of alternative models and their parameters for subsequent hypothesis tests (see below). Biologically, SSE models

are desirable for our study because we are interested in both the evolutionary history of color polymorphism (historic transitions to and from color polymorphism) and if this character, or something unmeasured, is associated with increased speciation and extinction or not at all.

We performed BiSSE and HiSSE model tests on both our maximum clade credibility (MCC) species tree and the Garcia-Porta *et al.* maximum likelihood (ML) time tree in R with the 'HiSSE' package (Beaulieu & O'Meara 2016). We constructed a suite of character-dependent (BiSSE and HiSSE), character-independent models (CID-2 and CID-4), and null models that vary in the number of distinct transition rates, extinction parameters, and hidden states to test alternative hypotheses related to the evolution of color polymorphism and diversification rates in the family Lacertidae (Table 1). Briefly, the CID-2 and CID-4 models are BiSSE and HiSSE character-independent models, respectively. The CID-2 and CID-4 models contain the same number of distinct turnover and extinction fraction parameters that can vary across the tree as their analogous BiSSE and HiSSE models, but CID-2 and CID-4 explicitly specify that diversification is not linked to the observed character state (Beaulieu & O'Meara 2016). The BiSSE and HiSSE null models also contain the same number of transition rates as the BiSSE and HiSSE models, but the null models specify a constant rate of diversification across the tree (number of distinct turnover rates = 1). For all 11 SSE models run on our time-calibrated MCC species tree, we used the same estimated proportion of extant species (82% of all non-color polymorphic and 100% of color polymorphic extant species are represented in our tree) and did not constrain the root character state. For the Garcia-Porta *et al.* (2019) ML tree, we specified a slightly different estimated proportion of extant species due to differences in taxonomic representation (73% of all non-color polymorphic and 93% of color polymorphic extant species represented in the tree), otherwise, all other SSE model specifications are identical. Full SSE model parameterizations are given in Supplementary Material. Nested SSE models were compared using AICc scores, ΔAIC scores, and Akaike weights (Table 1) (Burnham & Anderson 2002).

Trait simulations and SSE model adequacy.

The main disadvantage of SSE models is the potential issue of choosing between model A and model B when model C is true (Caetano *et al.* 2018). To address this potential issue, we conducted a simulation study (similar to Portik *et al.* 2019) to identify the rate at which a character-dependent model of diversification (BiSSE or HiSSE) is falsely chosen as the correct model out of a subset of six different SSE models with uncorrelated simulated color polymorphism trait data on our empirical phylogeny. To simulate color polymorphism character data on our species tree and the Garcia-Porta *et al.* ML tree, we used the 'phytools' (v.0.6-60) 'sim.history' function (Revell 2012). For trait data simulation, we used the parameter estimations from the transition matrix from best fit HiSSE model run on the empirical data. We extracted the root state probabilities from our fitted models and used them to specify simulation root states. We then simulated character data on the empirical trees 10,000 times and randomly selected 1,000 simulations where at least 10% of tips were color polymorphic for SSE model adequacy investigation. We then ran a subset of six SSE models from our empirical study on our

1,000 simulations and extracted AIC scores for model comparison and adequacy evaluation (Fig. 3). Given that the simulated trait data are uncorrelated, we expect character-independent (CID-2 and CID-4) or null models (BiSSE null and HiSSE null) to have the lowest AIC scores most of the time on both trees.

Phylogenetic uncertainty and SSE model comparison.

A phylogenetic tree represents one hypothesis of evolutionary relatedness. Biological conclusions drawn from phylogenetic comparative methods are influenced by uncertainty in the timing and topology of those relationships, with the potential for misleading conclusions based on mis-estimating the true diversification history (Louca & Pennell 2020). To understand the robustness of our conclusions based on phylogenetic comparative analyses carried out on our MCC species tree we performed a sensitivity analysis using 1,000 randomly selected trees from the posterior distribution of 12,000 trees from our Bayesian tree inference. Here, we ran the same subset of six SSE models from trait simulation analyses with the same parameterizations (Table 1) on 1,000 possible phylogenies with our observed color polymorphism trait data. We extracted AIC scores for SSE models run on each tree for model comparison (Fig. 3). Finally, we extracted model parameter estimates from a subset of phylogenies from the posterior sample of 1,000 trees to understand how uncertainty in phylogeny affects net diversification and extinction fraction estimates (N = 50 phylogenies, Table 2). A similar analysis was not possible for the Garcia-Porta *et al.* (2019) ML tree because no posterior is generated from ML tree inference.

All data and code for this project are available on Dryad.

RESULTS

Phylogeny of the Lacertidae.

Phylogenetic inference from the combined two independent MCMC runs with 20% burn-in converged well (ESS values for posterior, likelihood, species coalescent, and all five gene trees > 200). We estimated a most recent common ancestor (MRCA) of all lacertids occurred 98.3 – 148.9 MYA (95% HPD = 98.299 – 148.988, average age = 121.285 MYA). Evolutionary relationships presented in our maximum clade credibility tree (Fig. 2a), recovered from our multi-locus full Bayesian species tree inference had some disagreements with other family-wide phylogenies (Hipsley *et al.* 2009; Garcia-Porta *et al.* 2019). The subfamily Gallotiinae, comprising *Gallotia* and *Psammodromus* genera, grouped together monophyletically consistent with previous studies (Arnold *et al.* 2007; Garcia-Porta *et al.* 2019). However, our inference placed Gallotiinae nested within Lacertinae with low node support (posterior probability = 0.76), which follows results from Fu (2000), but is in contrast to the hypothesis that the Gallotiinae and Lacertinae are two separate monophyletic subfamilies that comprise the Lacertidae (Arnold *et al.* 2007; Garcia-Porta *et al.* 2019). The two tribes within the subfamily Lacertinae (as most recently reviewed with 620bp of mtDNA and 64 morphological characters from 59 nominal species by Arnold *et al.* 2007), the Lacertini and Eremiadini, were not reciprocally monophyletic. The Lacertini tribe (Oppel 1811; Arnold *et al.* 2007) that usually comprise 18 genera from Europe, northwest Africa, and southwest and east Asia

largely grouped together, with 15 of 18 genera forming a monophyletic clade containing *Algyroides*, *Anatololacerta*, *Apathya*, *Archaeolacerta*, *Dalmatolacerta*, *Dinarolacerta*, *Hellenolacerta*, *Iberolacerta*, *Lacerta*, *Parvilacerta*, *Phoenicolacerta*, *Podarcis*, *Teira*, *Timon*, and *Zootoca*. The *Darevskia* and *Iranolacerta* genera that belong to the Lacertini tribe grouped monophyletically sister to the aforementioned Lacertini, but also include the *Eremias*, which are traditionally placed in the Eremiadini tribe. The last Lacertini genus, the *Takydromus*, which contains 24 species that have a far east distribution spanning eastern China, Japan, and southeast Asia grouped monophyletically but separate from the other Lacertini. The Eremiadini tribe diverge from other lacertids deep in our phylogeny, with the speciose *Acanthodactylus* and *Meroles* genera forming a monophyletic clade, the Afrotropical genera *Pedioplanis*, *Nucras*, *Poromera*, *Latastia*, *Pseuderemias*, *Heliobolus*, *Meroles*, *Ichnotropis*, *Vhembelacerta*, and *Australolacerta* forming a monophyletic clade. The remaining Eremiadini genera from Equatorial Africa, *Adolfus*, *Gastropholis*, *Holaspis*, *Congolacerta*, *Atlantolacerta*, and *Omanosaura* form a separate clade similar to Arnold *et al.* (2007). Most genera formed monophyletic groups with the exception of *Algyroides*, *Eremias*, and *Ophisops*.

The evolutionary history of color polymorphism in the Lacertidae.

We identified 43 color polymorphic extant lacertid species spanning 10 out of 42 currently described genera. The 10 genera containing color polymorphic species are all within the sub-family Lacertinae: *Algyroides* (2 of 4 spp.), *Anatololacerta* (5 of 5 spp.), *Apathya* (1 of 2 spp.), *Darevskia* (8 of 30 spp.) *Dinarolacerta* (1 of 2 spp.), *Hellenolacerta* (1 of 1 sp.), *Iberolacerta* (3 of 8 spp.), *Phoenolacerta* (2 of 4 spp.), *Podarcis* (19 of 23 spp.), and Zootoca (1 of 1 sp.). All 43 color polymorphic lacertid species are represented in the MCC species tree (Fig. 2a) and comparative analyses, and 40 color polymorphic species are contained in the ML tree (Fig 2b). We present ancestral state reconstructions estimated with HiSSE for both our MCC species and the ML tree proposed by Garcia-Porta *et al.* (2019) (Fig. 2a and b).

Ancestral state reconstructions on both the MCC and ML trees estimated that the most recent common ancestor of all lacertids was most likely not color polymorphic (Fig. 2a and 2b). Results from ancestral state reconstructions also suggest that color polymorphism is an ancient trait within lacertids, and is likely to have evolved by the most recent common ancestor of the Lacertini around 111 MYA (Fig. 2a and 2b, denoted with an asterisk).

State-dependent diversification in the Lacertidae.

Results from SSE analyses on the MCC species tree support a character state-dependent diversification model with hidden states, or HiSSE model (Akaike weight = 0.913, Fig. 2, Table 1). For the best fit model, turnover parameter estimates were asymmetrical between color polymorphic and non-color polymorphic lineages (MCC parameter estimates, Table 2). Estimated net diversification rates were higher in observed color polymorphic lineages (HiSSE model, Table 2). Parameter estimates for character transitions from color polymorphism to monomorphism were much higher than transitions from monomorphism to color polymorphism, providing further evidence that color polymorphism is more easily lost than gained (HiSSE model transition rate CP-1A

to NotCP-0A = 0.015, NotCP-0A to CP-1A = 2.061e-09, CP-1B to NotCP-0B = 0.247, NotCP-0B to CP-1B = 0.007). The other state-dependent diversification models had lower AIC values and greater Akaike model weights than trait-independent and null models of diversification (Table 1). Parameter estimates from trait-dependent diversification SSE models also detected higher rates of net diversification at evolutionary transitions to color polymorphism (Table 2). We estimated much higher character transition rates from color polymorphism to monomorphism (MCC HiSSE transition rates from poly- to monomorphism = 0.015, mono- to polymorphism = 2.06e-09; ML HiSSE transition rates from poly- to monomorphism = 0.057, mono- to polymorphism = 2.06e-09). All null and character-independent SSE models received very little support relative to state-dependent diversification SSE models, accounting for less than 0.1% of the Akaike model weight (Table 1). Complete details of all SSE model net diversification and extinction fraction parameter estimates are given in Table 2. Overall, for diversification analyses on the MCC species tree the HiSSE model accounted for more than 91% of the Akaike model weight, and model-averaged net diversification rate estimates extracted from tree tips (Figure 2D) support our hypothesis that evolutionary transitions to color polymorphism are associated with elevated diversification rates.

Model selection results from the same set of eleven SSE analyses performed on the Garcia-Porta *et al.* (2019) ML time tree are also given in Table 1. Again, we find greatest support for a HiSSE model with hidden states (Akaike weight = 0.999) and that trait-dependent diversification models have lower AIC values and greater Akaike model weights than trait-independent and null models. Estimated transition rates from color polymorphism to monomorphism were much higher than evolutionary transitions from monomorphism to color polymorphism (HiSSE model transition rate CP-1A to NotCP-0A = 35.464, NotCP-0A to CP-1A = 2.061e-09, CP-1B to NotCP-0B = 16.231, NotCP-0B to CP-1B = 2.066e-09). Model-averaged net diversification rate estimates extracted from the ML tree tips (Figure 2C) also suggest that color polymorphic lineages experience elevated diversification rates.

Trait simulations on empirical MCC and ML phylogenies and SSE model adequacy.

When we compared AIC values of six SSE models run on 1,000 trait datasets simulated with no correlation to diversification rates on the empirical MCC and ML Lacertidae phylogenies, we found that trait-dependent diversification models were chosen as the best fit model less than 2% of the time. For uncorrelated trait simulations performed on the MCC species tree, the BiSSE null model was selected as the best fit model most often (ΔAIC score = 0, 58.3% of simulations for BiSSE null model), followed by the HiSSE null model (ΔAIC score = 0, 36.8% of simulations). Character-independent models, CID-2 and CID-4, were rarely the best fit models on simulated trait data and also had low Akaike model weights compared to other models (3.4% of simulations CID-2 ΔAIC score = 0, 1.1% of simulations CID-4 ΔAIC score = 0, Fig. 3a). Character-dependent diversification models, BiSSE and HiSSE, were rarely the best fit models on simulated uncorrelated color trait data (ΔAIC score = 0, 0.1% of simulations for BiSSE model, 1.1% for HiSSE model, Fig. 3a).

For 1,000 uncorrelated trait simulations performed on the Garcia-Porta *et al.* (2019) ML tree, we produce similar results. Here, the HiSSE null model was chosen as the best fit

model most often (ΔAIC score = 0, 99% of simulations) (Fig. 3b). A trait-dependent diversification model was identified as the best fit model only 7 times (0.7% of simulations, HiSSE ΔAIC score = 0).
Ultimately, we recover a Type I error rate (trait-dependent diversification when there should be none) less than 1% of the time when we run a subset of six SSE models from our observed data on uncorrelated simulated trait data.

SSE model selection and parameter estimation from posterior distribution of trees.

SSE model selection using the empirical color polymorphism data performed on a posterior distribution of 1,000 trees identified trait-dependent diversification models, HiSSE and BiSSE, as the best fit models 99.4% of the time (Fig. 3c), which largely supports a color polymorphism-dependent diversification scenario in the Lacertidae. The HiSSE and BiSSE models also had greater Akaike model weights than null or character-independent diversification models (Fig. 3c). The HiSSE model was identified as the best fit model most of the time (ΔAIC score = 0, 60.6% of sampled trees). The BiSSE model, trait-dependent diversification with no hidden states was selected as the best fit model second-most often (ΔAIC score = 0, 38.8% of sampled trees). Of the 6 times the HiSSE null model was selected as the best fit model, it achieved a ΔAIC score > 1 only once. Out of 1,000 potential evolutionary histories of the Lacertidae, the BiSSE null model and character-independent models (CID-2 and CID-4) of diversification were never selected as best fit models.

SSE model net diversification parameter estimates extracted from a subset of 50 phylogenetic trees overlapped with the estimates obtained from the maximum clade credibility tree (Table 2).

DISCUSSION

Color polymorphism research has been dominated by the hypothesis that multiple genetically-based phenotypes can be a precursor to speciation, but with few comparative studies there has been a limited ability to test this hypothesis. We generated a comprehensive family-wide multi-locus species tree of the Lacertidae to elucidate the evolutionary history and macroevolutionary consequences of color polymorphism. Phylogenetic ancestral state reconstructions of the family from two different tree inferences of the relationships in the Lacertidae suggests the most recent common ancestor of all lacertids was most likely not color polymorphic, and there were probably multiple independent evolutionary transitions to color polymorphism throughout the family tree. We found that the evolution of color polymorphism from monomorphism happens at a much slower rate than evolutionary transitions from color polymorphism to monomorphism. This macroevolutionary-level finding follows empirical results from species-specific case studies that color polymorphism is more easily lost than gained from populations (Corl *et al.* 2010b; Runemark *et al.* 2010). Finally, we explored macroevolutionary dynamics within the Lacertidae using two summary phylogenies and a posterior distribution of possible trees to test the theory that color polymorphism is a driver of diversification. Amongst several alternative hypotheses that simultaneously consider evolutionary history, unsampled hidden states, and trait transitions, we found

multiple lines of support for our hypothesis that color polymorphic lineages diversify at a higher rate than monomorphic lineages.

The Lacertidae: Evolutionary history and color polymorphism.

Phylogenies are essential for addressing macroevolutionary hypotheses that use interspecific data, and a long-contested phylogeny has limited our understanding of the evolutionary history and macroevolutionary dynamics of the Lacertidae (Fu 2000). Molecular investigations of the entire family Lacertidae at the species level are rare, and topological relationships in the family tree remain controversial (Fu 1998; Harris *et al.* 1998; Fu 2000; Arnold *et al.* 2007; Mayer & Pavlicev 2007; Garcia-Porta *et al.* 2019). Phylogenetic uncertainty in the Lacertidae likely stems from a combination of early and recent bursts of diversification (Fu 2000; Garcia-Porta *et al.* 2019). Family-level phylogenies of lacertids usually recover low support deep within the tree and at nodes connecting short branches near the tips (Fu 2000; Arnold *et al.* 2007; Garcia-Porta *et al.* 2019). We find a similar pattern in our data, with an early period of rapid diversification deep within the tree and low support or non-traditional placement of short branch taxa. Fossils and genetic data from Europe help resolve relationships among morphologically convergent European lacertids, but limited genetic data and poor fossil records in other areas where lacertids currently occur, such as the Middle East, Africa, and Asia, hinder our ability to generate phylogenies with strong support deep in the tree and between subgroups in these lineages (Hipsley *et al.* 2009). In particular, Eurasian lacertids and Lacertini species with expansive geographic distributions tend to have uncertain placement in phylogenetic reconstructions (Fu 2000; Garcia-Porta *et al.* 2019). Twenty years later, and with far more molecular markers, we echo Fu's (2000) sentiment that to resolve nodes with low support in the lacertid family tree, future investigations should focus on interrogating species-level evolutionary relationships between a few widely distributed and contested genera that are probably not monophyletic (e.g.: the highly polyphyletic former Lacerta genus; Arnold *et al.* 2007).

Accommodating phylogenetic uncertainty is essential to evolutionary studies. Indeed, the one true evolutionary history escapes us due to missing data from past processes such as extinction and an incomplete fossil record, and from limitations in the present from missing data, taxonomic uncertainty, and the continuous and ephemeral nature of speciation (Huelsenbeck *et al.* 2000; Rosenblum *et al.* 2012; Louca & Pennell 2020). We account for phylogenetic disagreement and uncertainty with trait simulation studies, test suites of alternative hypotheses on 1,000 sampled trees from our Bayesian posterior, and run parallel comparative methods analyses on our own lacertid tree inference and that of others (Garcia-Porta *et al.* 2019). Through these methods, we find multiple lines of evidence all in agreement with regard to the evolutionary history of color polymorphism in the Lacertidae. Whether the Gallotinae truly belong sister to all Lacertinae (Arnold *et al.* 2007; Garcia-Porta 2019), or somewhere nested within (Fu 2000), the phylogenetic structure of the evolution of color polymorphism across extant lacertids places the first instances of color polymorphism deep within the tree, though most likely in the tribe Lacertini and not at the common ancestor of all lacertids (Fig. 2a and 2b).

The Lacertidae exhibit a high degree of color polymorphism, spanning 10 genera and comprising 43 species that share a similar throat color polymorphism. Phylogenetic and ancestral state reconstruction analyses co-estimated with diversification rates revealed that the ancestor of all lacertids was probably not color polymorphic, and that color polymorphism has been gained few times and lost many times throughout the evolutionary history of the Lacertidae. That the ancestor of all lacertids was most likely not color polymorphic is not surprising, given that the closest relatives of lacertids used as outgroup taxa are themselves not color polymorphic. Color polymorphism, however, appears to have evolved relatively early in the history of lacertids, during or shortly after an initial early period of diversification in the family. Color polymorphism seems to be a trait restricted to the Lacertini tribe, and most likely evolved in the group. Our results that recover an ancient origin of color polymorphism in the group also underline recent findings from a study of the highly polymorphic *Podarcis* group, which found patterns of molecular evolution at the color polymorphism pigmentation loci that indicate the alleles are of ancient origin (Andrade *et al.* 2019). We estimate that color polymorphism evolved fewer times than it has been lost throughout the evolutionary history of the Lacertidae, and that evolutionary transition rates from monomorphism to color polymorphism are much lower than transitions from color polymorphism to monomorphism, by up to seven times. This result is not surprising given that empirical studies of color polymorphic taxa at the species level report that morph loss in populations represents lost genetic variation that cannot likely be regained (Corl *et al.* 2010b). Our estimates indicate the rate of loss far exceeds the rate of gain of color polymorphism, which aligns with our expectations. The tendency for color polymorphism to be lost faster than it evolves follows from theory on morphic speciation (West-Eberhard 1986; Gray & McKinnon 2007). If color polymorphic species have highly variable or large geographic ranges where gene flow between populations is infrequent, populations that experience morph loss may diverge quickly genetically and phenotypically (Corl *et al.* 2010a,b), setting the stage for speciation. This scenario would generate a phylogenetic pattern where color polymorphic lineages give rise to daughter lineages that are monomorphic, which we see in our ancestral state reconstruction in the *Apathya*, *Darevskia*, *Dinarolacerta*, *Iberolacerta*, and *Phoenicolacerta* generic groups (Fig. 2).

An unexpected pattern emerges in the speciose *Podarcis* clade, where 19 out of 23 extant species are color polymorphic. If color morph loss or divergence progresses to speciation, we would expect to see polymorphic lineages give rise to monomorphic descendant lineages (Jamie & Meier 2020). Further, the only other comparative study on color polymorphism and diversification we are aware of found that color polymorphic lineages tend to be younger than monomorphic lineages (Hugall & Stuart-Fox 2012), which also aligns with early theoretical models of morphic speciation driven by morph loss and fixation (West-Eberhard 1986). However, the *Podarcis* group is not particularly young compared to other groups in the Lacertidae, nor does it exhibit many short branches like the color polymorphic *Darevskia* clade. So, what might explain persistent polymorphism in *Podarcis*? A recent genomic study that identified genes controlling color differences amongst morphs in *Podarcis muralis* also found some evidence for inter-specific color allele sharing with other *Podarcis* species (Andrade *et al.* 2019). To retain color polymorphism after speciation, the alleles for different morphs must be

present, either ancestrally or arising again through a novel mutation. Ancestral genetic variation may persist past speciation via introgression or standing ancestral variation (Andrade *et al.* 2019; Jamie & Meier 2020). If speciation rates are high, and genetic barriers between species are porous, there may be opportunities for introgressed morph alleles and morph persistence beyond complete speciation if a color polymorphic lineage comes into secondary contact with a monomorphic lineage (Jamie & Meier 2020). Taken together, these genomic and phylogenetic investigations of the *Podarcis* clade raise interesting implications for the role of hybridization and introgression in the evolution and long-term maintenance of color polymorphism and its relationship to speciation rates (Jamie & Meier 2020).

Color polymorphism is associated with rapid diversification rates.

Theory suggests that dramatic intra-specific phenotypic diversity and the underlying processes that maintain it may promote rapid speciation in color polymorphic lineages (West-Eberhard 1986; Gray & McKinnon 2007; Forsman *et al.* 2008). We find evidence for this in the Lacertidae, where diversification rates are substantially faster in lineages in the color polymorphic state. Across many possible phylogenetic reconstructions of the Lacertidae, we consistently estimate that net diversification is almost double the rate in color polymorphic lineages than monomorphic lineages. Indeed, lacertids also exhibit faster character transition rates from color polymorphism to monomorphism, which is consistent with theory that suggests speciation occurs by morph loss and fixation of remaining morphs (West-Eberhard 1986). Trait simulations and state-speciation extinction models run on many possible trees, including a ML time tree inferred with a large phylogenomic dataset (Garcia-Porta *et al.* 2019), suggest that it is unlikely that the shape of the Lacertidae phylogeny produces false estimates of trait-dependent diversification. These findings are also supported by empirical studies that show repeated loss, fixation, and rapid divergence of morph types among populations of color polymorphic species (Corl *et al.* 2010b).

Animal color and pattern are important traits involved in processes such as mate choice, species recognition, and sexual selection, which can all play a role in accelerating speciation (Houde & Endler 1990; Roulin 2004). Across many color polymorphic species, morph color is often involved in intra-specific visual signaling to communicate myriad messages in a variety of social and environmental contexts (Gray & McKinnon 2007). In social contexts, morph color can indicate reproductive strategy (Sinervo & Lively 1996) and morph color is often a factor in mate choice (Pryke & Griffith 2007). In birds, reptiles, and fish, the prevailing environment and lighting conditions affect the efficacy and transmission of signals displayed by different color morphs (Gray & McKinnon 2007), and color morphs may segregate microhabitat to optimize signal transmission (Endler 1984). Thus, if sexual and/or natural selection pressures shift away from balancing color polymorphism toward favoring the phenotype of one or several morphs over another, divergent or directional selection could result in morph loss from a population. Because color polymorphic species inherently possess extreme variation, there exists increased opportunity for selection or drift to operate against any one of several distinct color morph phenotypes, which may explain elevated diversification rates in color polymorphic lineages. Theoretical expectations and empirical studies of

populations show that morph loss and fixation can result in rapid divergence (West-Eberhard 1986; Corl et al 2010a,b), but the microevolutionary processes operating within and between populations that disrupt balanced color polymorphisms and generate divergence remain less understood and generalizable (Chelini *et al.*, 2021 in review). Further study is needed to quantify the relative roles of natural selection, sexual selection, and drift in color polymorphism maintenance and speciation.

Ultimately, we show the color polymorphic condition in lacertids is associated with elevated diversification rates. Species have many traits, and it is unlikely that a single trait is the only factor that accounts for increased or decreased diversification rates. Indeed, the best fit model was trait-dependent diversification that included additional unobserved "hidden states" that are correlated with color polymorphism. The inclusion of hidden states in the HiSSE framework ameliorates confounding effects of unmeasured correlated traits on diversification by allowing for greater rate heterogeneity (Caetano *et al.* 2018; Patton *et al.* 2020), and thus alternative SSE models in our study measure the effect of color polymorphism while controlling for other correlated traits on diversification rates in the Lacertidae. Color polymorphism is usually accompanied by alternative morph-specific ecological, morphological, physiological, and behavioral syndromes, or correlated traits (Lattanzio & Miles 2016; Huyghe *et al.* 2009a,b; Sinervo & Lively 1996; Sinervo & Svensson 2002). Here, color and other heritable traits are likely subject to multivariate selection, wherein correlational selection builds up genetic correlations through linkage disequilibrium at loci underlying the traits (Sinervo & Svensson 2002). Correlation between color morphs and traits related to fitness, such as reproductive strategy (Sinervo & Lively 1996; Galeotti *et al.* 2013), or reproductive hormone levels (Huyghe *et al.* 2009b), or body size (Brock *et al.* 2020 in press), can produce color morphs with alternative adaptations that occupy different adaptive peaks (West-Eberhard 1986). When a color polymorphic lineage with multiple balanced adaptive peaks faces strong or novel selective forces, one or more of the peaks may shift, and a morph or morphs must cross "valleys" of selection to persist else they are lost. Another interesting possibility to explore in future studies is the relationship of habitat diversity to color polymorphism and diversification dynamics. Conceptual models suggest that habitat heterogeneity may promote color polymorphism, and divergent selection between populations could drive morph divergence and speciation (Forsman *et al.* 2008) and have also been shown to be related to sexual selection in lizards (Östman & Stuart-Fox, 2011). Thus, the nature of color polymorphism and correlated traits could increase the potential for color polymorphism to contribute to divergence amongst morphs and speciation.

Color polymorphism: linking micro-evolutionary process and macroevolutionary pattern.

Color polymorphism is widespread throughout the tree of life, but our understanding of the mechanisms underlying morph evolution and the processes that influence the shape of the tree remain fragmented. The evolutionary mechanisms that maintain alternative color phenotypes within a population are also likely involved in morph loss, divergence, and speciation (Gray & McKinnon 2007). These population-level processes, particularly the effects of natural and sexual selection and their relative

roles within and between populations, are of utmost interest (Jamie & Meier 2020). Color polymorphism is often studied at the level of a single species (Huyghe *et al.* 2009a,b; Corl *et al.* 2010a,b; Runemark *et al.* 2010), but macroevolutionary perspectives will provide deeper insights into the origin, duration of maintenance, and inter-specific persistence of color polymorphism (Gray & McKinnon 2007; Jamie & Meier 2020). Very few empirical studies link population-level evolutionary processes and the evolution of color polymorphism at the macroevolutionary scale (Hugall & Stuart-Fox 2012; Willink *et al.* 2019). Studies that investigate trait variation within species and use biologically meaningful versions of those inputs at the between-species level with the use of comparative methods are needed (Gray & McKinnon 2007). Such investigations will illuminate the connection between evolutionary process and macroevolutionary pattern. Color polymorphisms offer ideal model systems to study how micro-evolutionary dynamics shape macroevolutionary patterns of diversification. Our results show that the Lacertidae, in particular, offer a promising avenue for interrogating the relative contributions of different forms of selection on alternative phenotypes, and how population-level color morph dynamics scale up to influence macroevolutionary patterns.

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Figures

Figure 1. Throat color polymorphism in *Podarcis erhardii*, a Mediterranean lacertid species. In lacertid lizards, species spanning several genera exhibit a similar color polymorphism. Across all lacertid species in our study, color polymorphism is expressed at adulthood as colorful badges ventrally on the throat or belly region.

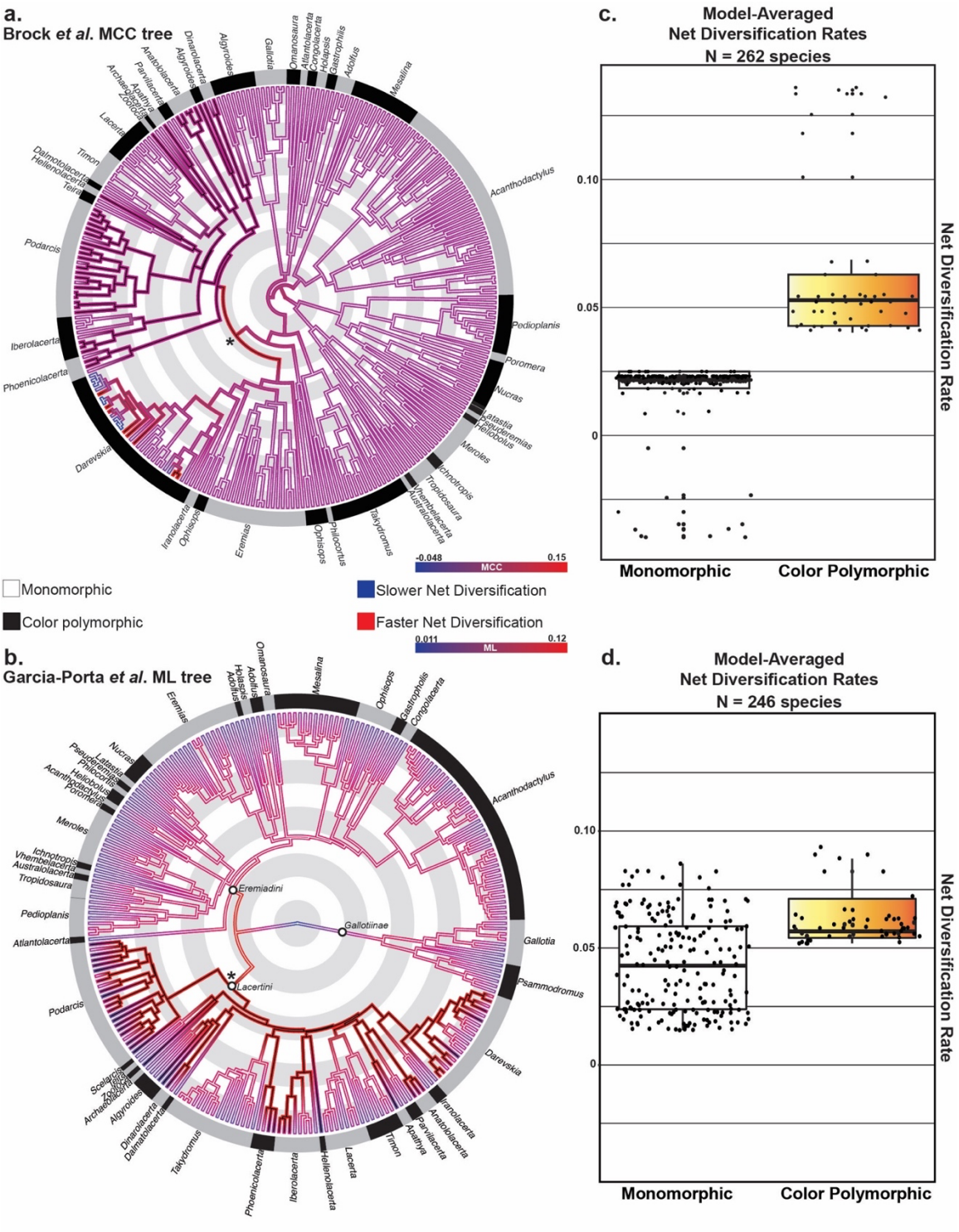


Figure 2. Evolutionary relationships of 262 species from the family Lacertidae. a) Phylogeny is the maximum clade credibility (MCC) tree calculated from the posterior of a full-Bayesian species tree inference from three mitochondrial and two nuclear loci. b) Phylogeny is the ML time tree inferred by Garcia-Porta *et al.*, (2019). In both trees,

genera are labelled and separated by black and gray bars. Observed color polymorphism character states reconstructed from the best-fit HiSSE models for each tree are in black and white. for extant taxa are given in circles at the tips (blue indicates observed species is color polymorphic, yellow indicates observed species is monomorphic). Diversification rates estimated from HiSSE models on both trees are in blue-red color gradient, where blue represents slowest estimated rates and red the fastest. On each tree, an asterisk denotes the oldest common ancestor with 99% probability of being color polymorphic. c) Model-averaged net diversification rates extracted from the tips of the Brock *et al.* MCC tree (a). Branches of the phylogeny are painted with net diversification rates from the best fit HiSSE model, and indicate the estimated tempo of net diversification, ranging from slowest rate. c) Model-averaged net diversification rates extracted from the tips of the Garcia-Porta *et al.*, (2019) (b). Model-averaged results are from all 11 SSE models.

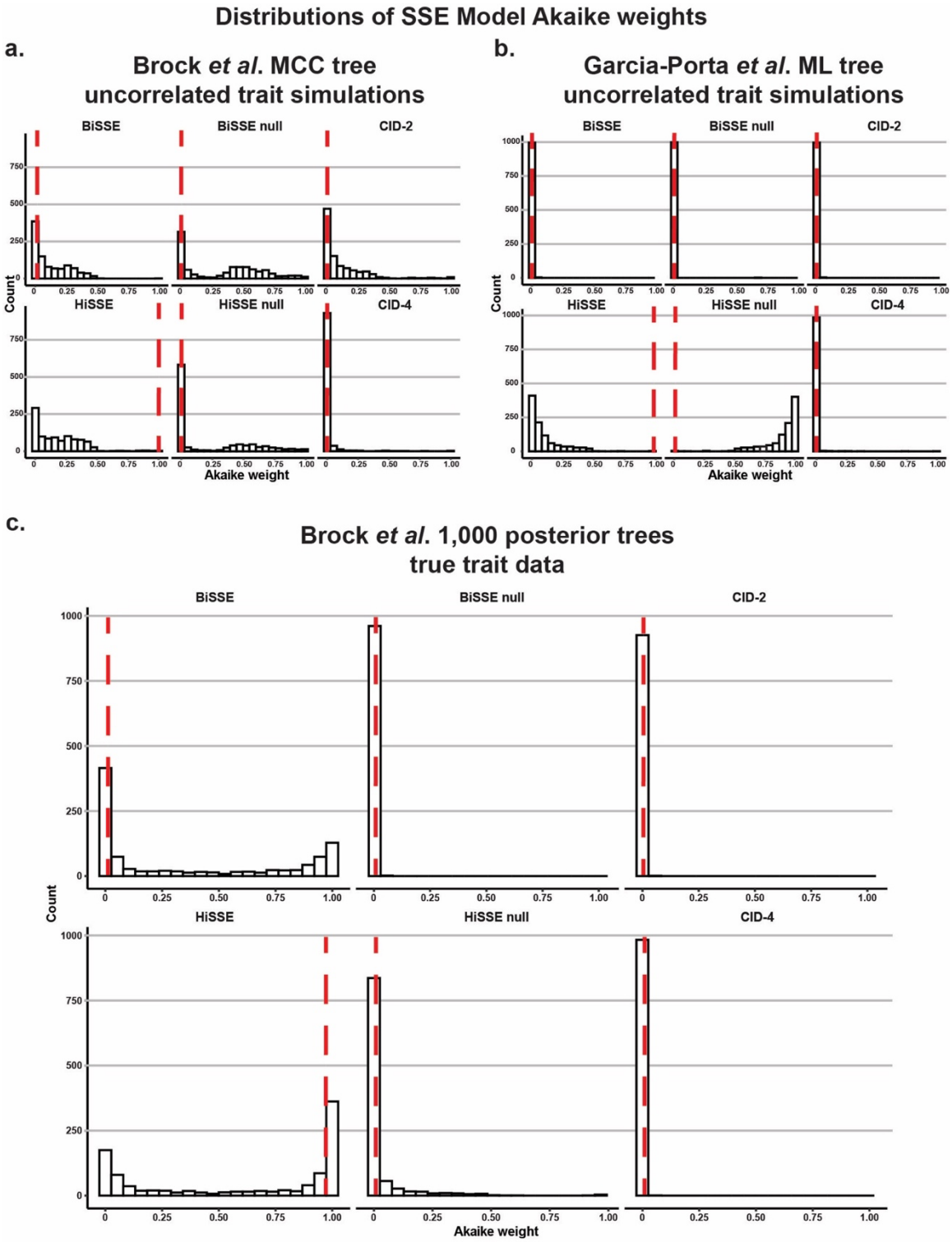


Figure 3. Distributions of Akaike model weights for six state-speciation extinction (SSE) models run on 1,000 simulated color polymorphism trait datasets (uncorrelated trait data run on true trees) and a posterior distribution of possible trees (real data run on 1,000 posterior trees). For a) and b), all simulations and models were performed with empirical

phylogenies. The horizontal axis (Akaike weight) refers to the relative probability of that SSE model compared to the other five competing SSE models run on the same uncorrelated simulated color polymorphism trait data. The dotted vertical lines indicate model weights from our empirical phylogeny and observed color polymorphism trait data for that same SSE model. Low model weight indicates relatively low support for the that hypothesis, and high model weight indicates greater support. c) Distributions of Akaike model weights for six SSE models run on 1,000 phylogenetic trees randomly selected from posterior distribution of possible trees and our empirical color polymorphism trait dataset. A higher model weight indicates greater relative support for that state-speciation extinction hypothesis.

Tables

Table 1. Summary of color polymorphism SSE hypotheses, model parameterization, and associated model fits from diversification analyses performed on the Brock *et al.* maximum clade credibility (MCC) species tree, averaged results for 1,000 trees from the posterior of Brock *et al.* species tree inference, and the Garcia-Porta *et al.* (2019) maximum likelihood tree. For the Brock *et al.* and Garcia-Porta *et al.* phylogenies, models are listed in best-fit order according to AIC value. Average AIC and ΔAIC score results from the six models run on the posterior are given in parentheses under the corresponding model run on the Brock *et al.* MCC tree. A trait-dependent diversification HiSSE model best explained the data for both phylogenies and the posterior.

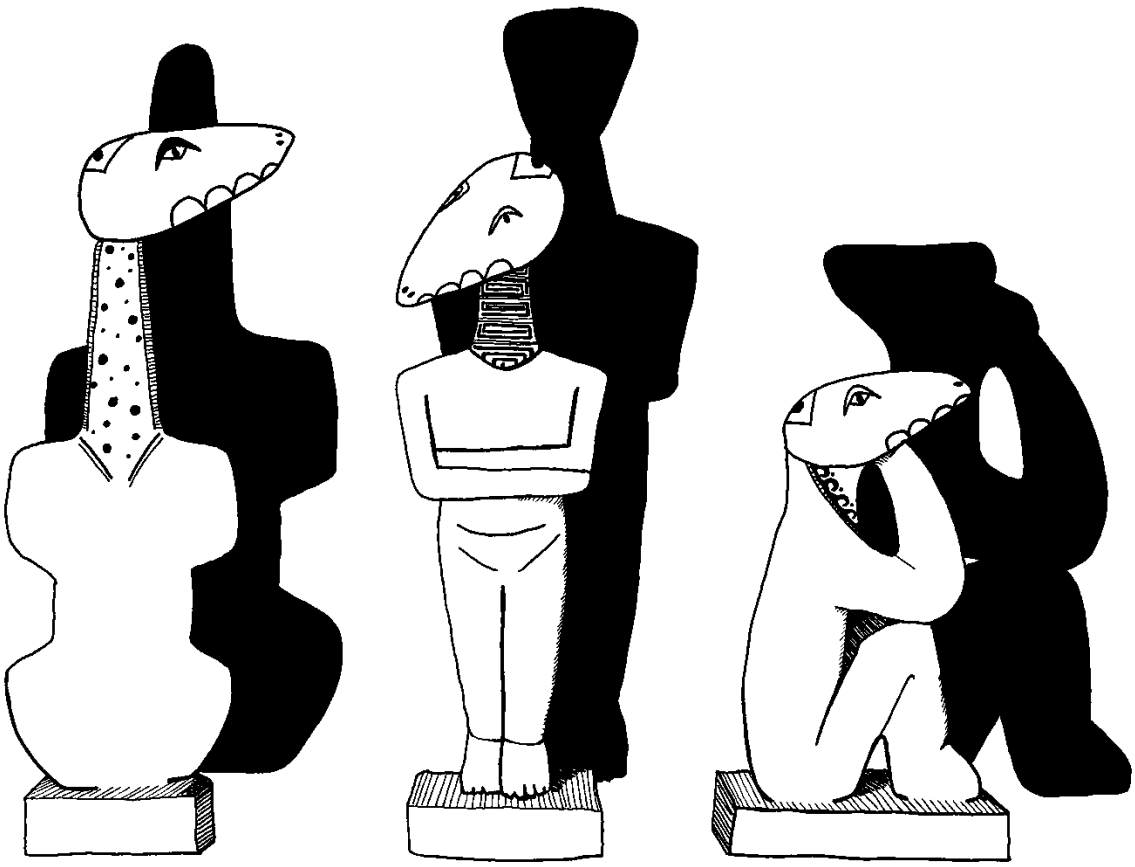
Model	AIC	Δ AIC	Akaike weights	Trait Dependent Diversification	N Distinct Turnover Rates	N Distinct Extinction Fractions	N Distinct Transition Rates
BROCK *et al.* MAXIMUM CLADE CREDIBILITY TREE AND 1,000 TREES FROM POSTERIOR							
HiSSE	2478.605 (2500±58.1)	0 (0)	0.913	yes	4	4	8
HiSSE No 1B	2483.477	4.872	0.079	yes	3	3	4
HiSSE No 0B	2489.169	10.564	0.001	yes	3	3	4
BiSSE 1 extinct frac	2491.563	12.958	0.0005	yes	2	1	2
BiSSE 2 extinct frac	2493.563 (2504±57.3)	14.958 (3)	< 0.0001	yes	2	2	2
BiSSE null	2511.294 (2519±57.5)	32.689 (20)	< 0.0001	no	1	1	2
CID-4 3 trans rate	2515.252	36.647	< 0.0001	no	4	4	3
CID-2 3 trans rate	2517.294	38.689	< 0.0001	no	2	2	3
HiSSE null	2528.909 (2512±57.5)	50.304 (12)	< 0.0001	no	2	2	1
CID-2	2541.242 (2539±59.1)	62.637 (46)	< 0.0001	no	2	2	1
CID-4	2544.542 (2538.1±59)	65.937 (45)	< 0.0001	no	4	4	1
GARCIA-PORTA *et al.* MAXIMUM LIKELIHOOD TREE							
HiSSE	2049.914	0	0.999	yes	4	4	8
HiSSE No 1B	2064.018	14.104	< 0.0001	yes	3	3	4
HiSSE No 0B	2066.914	17	< 0.0001	yes	3	3	4
BiSSE 1 extinct frac	2076.788	26.866	< 0.0001	yes	2	1	2
BiSSE 2 extinct frac	2078.788	28.874	< 0.0001	yes	2	2	2
BiSSE null	2081.417	31.503	< 0.0001	no	1	1	2
CID-4 3 trans rate	2081.879	31.965	< 0.0001	no	4	4	3
CID-2 3 trans rate	2083.417	33.503	< 0.0001	no	2	2	3

CID-4	2100.142	50.228	< 0.0001	no	4	4	1
CID-2	2114.789	64.875	< 0.0001	no	2	2	1
HiSSE null	2116.559	66.645	< 0.0001	no	2	2	1

Table 2. SSE model parameter estimates from comparative analyses on the Brock *et al.* maximum clade credibility (MCC) species tree from Bayesian inference, a posterior sub-sample of 100 species trees from Bayesian inference by Brock *et al.*, and the maximum likelihood (ML) tree from Garcia-Porta *et al.* (2019). Net diversification parameter estimates from the MCC species tree (Fig. 2a) are given for each SSE model under Spp. MCC. SSE model parameter estimates from all 11 models run on 50 phylogenies extracted from the posterior are given under Posterior where the first value is the average and the value in parentheses is the standard deviation of the estimate. SSE model parameter estimates from the Garcia-Porta *et al.* (2019) tree (Fig. 2b) are given under ML.

Model	Net Turnover (1A)	Net Turnover (0A)	Net Turnover (1B)	Net Turnover (0B)	Extinction Fraction (1A)	Extinction Fraction (0A)	Extinction Fraction (1B)	Extinction Fraction (0B)
HiSSE	**MCC** 0.052 **Posterior** 0.078 (0.025) **ML** 0.011	**MCC** 0.025 **Posterior** 0.032 (0.0.347) **ML** 2.06e-09	**MCC** 0.146 **Posterior** 0.265 (0.321) **ML** 0.093	**MCC** 0.019 **Posterior** 0.120 (0.139) **ML** 0.011	**MCC** 2.06e-09 **Posterior** 0.469 (1.09) **ML** 3.000	**MCC** 2.06e-09 **Posterior** 0.066 (0.277) **ML** 2.08e-09	**MCC** 2.06e-09 **Posterior** 0.388 (0.455) **ML** 2.06e-09	**MCC** 1.177 **Posterior** 0.192 (0.555) **ML** 2.06e-09
HiSSE No 1B	**MCC** 0.080 **Posterior** 0.078 (0.031) **ML** 0.045	**MCC** 0.020 **Posterior** 0.022 (0.022) **ML** 0.010	N/A	**MCC** 0.025 **Posterior** 0.035 (0.042) **ML** 0.012	**MCC** 0.433 **Posterior** 0.414 (0.050) **ML** 2.06e-09	**MCC** 0.065 **Posterior** 0.055 (0.032) **ML** 2.06e-09	N/A	**MCC** 2.06e-09 **Posterior** 2.06e-09 (0.003) **ML** 0.007
HiSSE No 0B	**MCC** 0.063 **Posterior** 0.106 (0.072) **ML** 0.127	**MCC** 0.026 **Posterior** 0.026 (0.003) **ML** 0.053	**MCC** 0.042 **Posterior** 0.038 (0.013) **ML** 2.06e-09	N/A	**MCC** 2.06e-09 **Posterior** 3.79e-03 (0.015) **ML** 2.06e-09	**MCC** 2.06e-09 **Posterior** 6.03e-04 (0.004) **ML** 2.06e-09	**MCC** 2.06e-02 **Posterior** 6.75e-02 (0.421) **ML** 3.000	N/A
BiSSE 1 extinct fraction	**MCC** 0.050 **Posterior** 0.050 (0.006) **ML** 0.067	**MCC** 0.026 **Posterior** 0.026 (0.003) **ML** 0.054	N/A	N/A	**MCC** 1.374e-08 **Posterior** 1.00e-08 (0.002) **ML** 2.06e-09	**MCC** 1.374e-08 **Posterior** 1.00e-08 (0.002) **ML** 2.06e-09	N/A	N/A
BiSSE 2 extinct fractions	**MCC** 0.050 **Posterior** 0.053 (0.002) **ML** 0.067	**MCC** 0.026 **Posterior** 0.027 (0.006) **ML** 0.054	N/A	N/A	**MCC** 2.06e-09 **Posterior** .007 (0.019) **ML** 2.06e-09	**MCC** 2.35e-09 **Posterior** 2.51e-09 (1.70e-09) **ML** 2.06e-09	N/A	N/A
BiSSE null	**MCC** 0.031 **Posterior** 0.032 (0.003) **ML** 0.057	**MCC** 0.031 **Posterior** 0.032 (0.003) **ML** 0.057	N/A	N/A	**MCC** 2.061e-02 **Posterior** 1.23e-08 (1.50e-08) **ML** 2.06e-09	**MCC** 2.061e-02 **Posterior** 1.23e08 (1.50e-08) **ML** 2.06e-09	N/A	N/A
HiSSE null	**MCC** 0.183 **Posterior** 0.671 (0.305) **ML** 2.06e-09	**MCC** 0.10283 **Posterior** 0.671 (0.305) **ML** 2.06e-09	**MCC** 0.028183 **Posterior** 0.647 (0.159) **ML** 0.060	**MCC** 0.028 **Posterior** 0.647 (0.159) **ML** 0.060	**MCC** 0.673 **Posterior** 0.343 (0.411) **ML** 3.01e-09	**MCC** 0.673 **Posterior** 0.343 (0.411) **ML** 3.01e-09	**MCC** 2.064e-09 **Posterior** 0.420 (0.426) **ML** 2.06e-09	**MCC** 2.064e-09 **Posterior** 0.420 (0.426) **ML** 2.06e-09
CID-2	**MCC** 0.014 **Posterior**	**MCC** 0.014 **Posterior**	**MCC** 0.032 **Posterior**	**MCC** 0.032 **Posterior**	**MCC** 2.06e-09 **Posterior**	**MCC** 2.06e-09 **Posterior**	**MCC** 2.06e-09 **Posterior**	**MCC** 2.06e-09 **Posterior**

	0.073	0.073	0.183	0.183	0.119	0.119	0.134	0.134
	(0.029)	(0.029)	(0.651)	(0.651)	(0.306)	(0.306)	(0.323)	(0.323)
	ML	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**
	2.06e-09	2.06e-09	0.061	0.061	3.17e-09	3.17e-09	2.06e-09	2.06e-09
	MCC	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**
	0.031	0.031	0.031	0.031	2.061e-09	2.061e-09	2.061e-09	2.061e-09
CID-2	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**
3 trans rates	0.074	0.074	0.186	0.186	0.121	0.121	0.137	0.137
	(0.155)	(0.155)	(0.658)	(0.658)	(0.309)	(0.309)	(0.326)	(0.326)
	ML	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**
	9.26e-04	9.26e-04	0.083	0.083	2.06e-09	2.06e-09	2.06e-09	2.06e-09
	MCC	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**
	0.031	0.031	0.009	0.009	2.061e-09	2.061e-09	2.061e-09	2.061e-09
CID-4	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**
	0.024	0.024	0.004	0.004	2.08e-09	2.08e-09	2.08e-09	2.08e-09
	(0.031)	(0.031)	(0.014)	(0.014)	(0.017)	(9.29e-11)	(9.29e-11)	(9.29e-11)
	ML	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**
	0.008	0.008	0.002	0.002	2.06e-09	2.06e-09	2.06e-09	2.06e-09
	MCC	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**	**MCC**
	0.020	0.020	0.042	0.042	2.061e-09	2.061e-09	2.061e-09	2.061e-09
CID-4	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**	**Posterior**
3 trans rates	0.021	0.021	0.125	0.125	2.06e-09	2.06e-09	2.06e-09	2.06e-09
	(0.006)	(0.006)	(0.259)	(0.259)	(0.005)	(0.005)	(0.005)	(0.005)
	ML	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**	**ML**
	0.099	0.099	0.003	0.003	2.19e-09	2.19e-09	2.06e-09	2.06e-09



To be continued…