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Article *in* The American Naturalist · May 2021

DOI: 10.1086/715186

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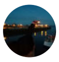

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VOL. 198, NO. 3 THE AMERICAN NATURALIST SEPTEMBER 2021

Climate Shapes the Geographic Distribution and Introgressive Spread of Color Ornamentation in Common Wall Lizards

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Submitted September 16, 2020; Accepted March 23, 2021; Electronically published June 25, 2021

Online enhancements: supplemental PDF. *Dryad data:* https://doi.org/10.5061/dryad.s1rn8pk7c.

ABSTRACT: Climate can exert an effect on the strength of sexual selection, but empirical evidence is limited. Here, we tested whether climate predicts the geographic distribution and introgressive spread of sexually selected male color ornamentation across 114 populations of the common wall lizard, *Podarcis muralis*. Coloration was highly structured across the landscape and did not reflect genetic differentiation. Instead, color ornamentation was consistently exaggerated in hot and dry environments, suggesting that climate-driven selection maintains geographic variation in spite of gene flow. Introgression of color ornamentation into a distantly related lineage appears to be ongoing and was particularly pronounced in warm climates with wet winters and dry summers. Combined, these results suggest that sexual ornamentation is consistently favored in climates that allow a prolonged reproductive season and high and reliable opportunities for lizard activity. This pattern corroborates theoretical predictions that such climatic conditions reduce the temporal clustering of receptive females and increase male-male competition, resulting in strong sexual selection. In summary, we provide compelling evidence for the importance of climate for the evolution of color ornamentation, and we demonstrate that geographic variation in the strength of sexual selection influences introgression of this phenotype.

Keywords: sexual selection, geographic variation, lizard, introgression, coloration.

Introduction

Biologists have known for centuries that species vary across their range, but unraveling the causes of this variation remains a major challenge. Geographic mosaics of phenotypic variation are the result of both historical processes as well as ongoing selection and gene flow. To identify the putative causes of selection, it is therefore necessary to have detailed knowledge about history, such as population divergence, range expansion, and secondary contact. Secondary contact zones in particular provide powerful frameworks to establish whether selection contributes to phenotypic variation across the landscape (Barton and Hewitt 1989; Orteu and Jiggins 2020). Occasionally, particular phenotypes and genes introgress from one lineage into another, a phenomenon that can occur when the trait confers an adaptive advantage in the recipient lineage (adaptive introgression; Hedrick 2013; Arnold 2016). Thus, concordance between environmental predictors of phenotypic variation within a lineage and environmental predictors of phenotypic introgression between lineages would provide particularly strong evidence that selection is operating and may help to identify its underlying causes (Uy and Stein 2007).

Climate is a strong candidate for geographic mosaics in selection, including selection on traits involved in competition for sexual partners. Climate commonly influences resource distribution, activity patterns, and reproductive cycles, all of which can modulate the strength of sexual selection (Emlen and Oring 1977; Shuster and Wade 2003; Cornwallis and Uller 2010; Macías-Ordóñez et al. 2013; Garcia-Roa et al. 2020). For example, a short activity season with synchronized breeding of females will tend to reduce the opportunity for males to mate multiply, resulting

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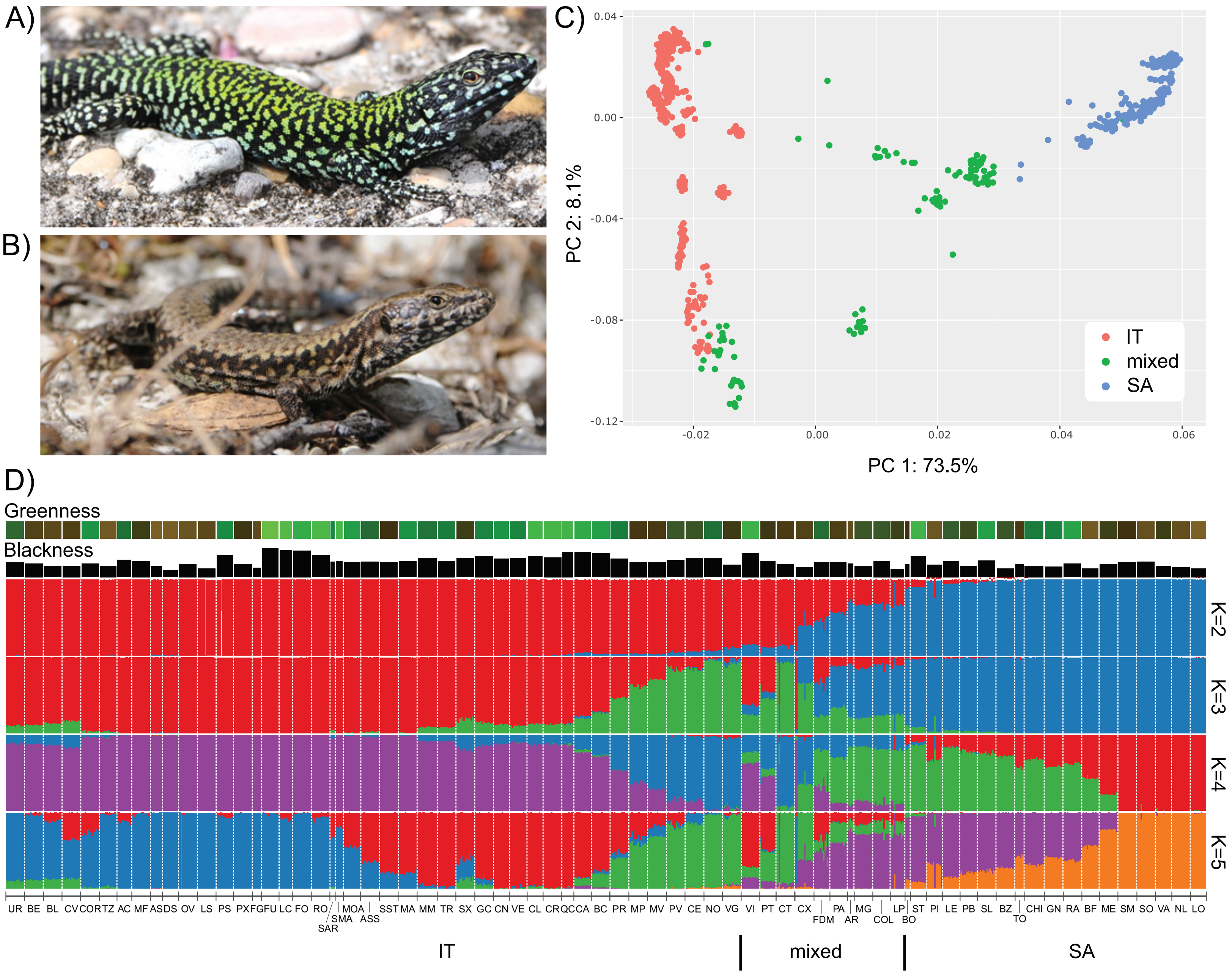
American Naturalist, volume 198, number 3, September 2021. Published by The University of Chicago Press for The American Society of Naturalists. https://doi.org/10.1086/715186


A)
B)
C)
PC 2: 8.1%
0.04
0.00
-0.04
-0.08
-0.12
-0.02
0.00
0.02
0.04
0.06
PC 1: 73.5%
● IT
● mixed
● SA
D)
Greenness
Blackness
K=2
K=3
K=4
K=5
UR BE BL CV CO RTZ AC MF AS DS OV LS PS PX FG FU LC FO RO SAR SMA MOA ASS SST MA MM TR SX GC CN VE CL CRQ CCA BC PR MP MV PV CE NO VG VI PT CT CX FDM PA AR MG COL LP BO ST PI LE PB SL BZ TO CHI GN RA BF ME SM SO VA NL LO
IT
mixed
SA

in weak sexual selection (Shuster and Wade 2003). As a result, sexual ornaments, such as the size or intensity of color patches, should be exaggerated in climates that allow intense competition for mates. Climate can also impose demands on the physiological and behavioral traits that sexual ornaments are supposed to signal (Evans and Gustafsson 2017; Garcia-Roa et al. 2019; Moore et al. 2019). Local adaptation to climate can therefore make coloration uninformative or prohibitively expensive.

Climatic effects on sexual selection are likely to be particularly important for ectotherms, as their physiological functions and reproduction are inherently temperature dependent (Angilletta 2009; Sinervo et al. 2010). Indeed, temperature has been suggested to be a major driver of spatial and temporal variation in the intensity and form of sexual selection in ectotherms (Garcia-Roa et al. 2020). However, there is a conspicuous lack of empirical evidence that climate maintains variation in sexually selected characters (e.g., McLean et al. 2015; Moore et al. 2019; Rosenthal and Elias 2019; for discussion, see Cornwallis and Uller 2010; Macías-Ordóñez et al. 2013; McLean and Stuart-Fox 2014; Svensson 2019; Garcia-Roa et al. 2020).

Wall lizards (genus *Podarcis*) exhibit striking geographic variation in coloration, which has puzzled naturalists for well over a century (e.g., Eimer 1881; Cyrén 1924). Colors are typically expressed more in males than in females and are known to function as sexual displays in male-male contests (Pérez i de Lanuza et al. 2013). One of the most extreme cases of within-species variation occurs in common wall lizards (*Podarcis muralis*) on the Italian peninsula (fig. 1). Here, the species varies from the ancestral brown color to an intense green and shows a pronounced variation in black coloration on the throat, chest, and belly (see fig. 1 and "Results"). Within a population, green and black coloration are more highly expressed in males and are positively correlated with each other and with other male traits under intrasexual selection (e.g., body size and head size; While et al. 2015; Heathcote et al. 2016; MacGregor et al. 2017*a*). The expression of coloration itself does not appear strongly affected by the environment: phenotypic differences between individuals and populations remain consistent across seasons, in captivity, and in nonnative populations introduced to much cooler climates (e.g., Schulte et al. 2012; While et al. 2015; MacGregor et al. 2017*b*).

The green-and-black common wall lizard phenotype has been referred to as a distinct subspecies (*P. muralis nigriventris* Bonaparte, 1838) and evolved within what we here refer to as the Italian (IT) lineage of the species (distributed throughout central Italy; fig. 1; Yang et al. 2018). However, the species' extremes of the phenotype can be found even within this lineage (Yang et al. 2018; see "Results"). Furthermore, analyses of phenotypic and genomic data have shown that the green-and-black phenotype has introgressed into the distantly related Southern Alps (SA) lineage, despite limited overall gene flow (While et al. 2015; Yang et al. 2018, 2020). Introgression is supported by the close association between phenotypic and genome-wide clines (Yang et al. 2018). Phylogenetic reconstruction of introgressed sequences suggests that the green-and-black phenotype originated in the south of the IT lineage and subsequently spread northward to the contact zone between the IT and the SA lineages, and farther into the SA lineage (Yang et al. 2018, 2020). Studies of experimental populations demonstrate that the highly asymmetric genetic and phenotypic introgression is a result of green-and-black IT males being behaviorally dominant over the ancestral phenotype, making IT males more likely to hybridize with females of other lineages than vice versa (While et al. 2015; Heathcote et al. 2016; MacGregor et al. 2017*a*).

The geographic variation in common wall lizard coloration combined with secondary contact and introgression provide an outstanding opportunity to test for climate-mediated selection. To this end, we first quantify variation in male color ornamentation across the landscape. Second, we determine to what extent phenotypic variation within the IT lineage can be explained by genetic differentiation or climate. Third, to confirm a role of climate-mediated selection, we test whether the same climatic factors that explain variation within the IT lineage also can explain the introgressive spread of male color ornamentation into the SA lineage.

Methods

Study Area

Between 2012 and 2018, we collected morphological and coloration data on common wall lizards from 114 locations

Figure 1: *A*, Male *Podarcis muralis* from the Italian (IT) lineage with an exaggerated green-and-black phenotype. *B*, Male *P. muralis* from the IT lineage with the ancestral phenotype. *C*, Results from a principal components analysis of the individual single-nucleotide polymorphism data, illustrating the separation between the IT and the Southern Alps (SA) lineages and different degrees of admixture. *D*, Results from the admixture analysis for $K = 2$–5 illustrate the variation in greenness and blackness within each of the two main lineages (IT and SA; $K = 2$) and the poor concordance between lizard coloration and genetic structure within both lineages with increasing K (for full results for $K = 2$–10, see fig. S1). Colors of the admixture plot indicate genetic clusters. Color panels above the admixture plot represent the intensity of the average dorsal greenness (light green = more intense green dorsal color), and the height of the black bars represents the average proportion of black pigmentation on the chest (ranging from 0 to 1) for each population. Photo credit: Nathalie Feiner.

across central and northern Italy (table S1; fig. S1; tables S1–S7 and figs. S1–S14 are available online). The sampling covered a broad range of climates, represented by locations from the coast to those about 1,500 m above sea level. All populations were visited during the breeding season (from late March until early May) and individuals were captured opportunistically (using noosing) from suitable habitats. Typical habitats of wall lizards include stone walls or concrete structures delimiting gardens, roadsides and agricultural landscapes with walls, fences or other structures, fields with bushes or other features that provide structural complexity, or open forest. Each location includes a variety of habitats, but they are most commonly centered on a village or human-made structures (e.g., abandoned buildings, rural roads) and the areas surrounding these (e.g., olive plantations, forest). We refer to each of the sampling locations as a "population."

Phenotypic Data

On capture, lizards were measured, and traits were recorded, including snout-to-vent length (SVL) and total length (both ±1 mm) with a ruler and head width and head length (both ±0.01 mm) with a caliper. Body mass (±0.01 g) was recorded using a digital scale. All individuals were then photographed dorsally and ventrally with a Canon EOS 350D digital camera (Canon, Lake Success, NY) using an X-rite Colour-Checker chart as background. Intensity of the dorsal coloration (greenness) of each individual was scored in the field by two of the authors (G.M.W. and either T.U. or M.R.M.) using a scale from 1 to 10 (with 1 being completely brown individuals and 10 representing the most intense green color; While et al. 2015). This scoring is highly repeatable within and across observers and correlates well with spectrophotometry and with scoring from digital photographs (for data and details, see While et al. 2015; MacGregor et al. 2017*a*). The extent of black ventral coloration (blackness) was scored from digital photographs by quantifying the proportion of black to nonblack pixels on each lizard's chest (While et al. 2015), a variable that ranges from zero to one. We also collected tissue samples from the tail, or toes when the tail was lost, for later DNA extraction. Lizards were released back at their point of capture.

Common wall lizards develop their green-and-black coloration only when they reach maturity and therefore only adult males with an SVL >50 mm were included in the phenotypic analyses. We calculated the average blackness and greenness for each population, retaining only populations with data on at least five males (for sample sizes for each population, see table S1). The final phenotype data set consists of 1,801 male individuals from 114 populations (supplemental files 1 and 2, available in the Dryad Digital Repository [https://doi.org/10.5061/dryad.s1rn8pk7c; Ruiz Miñano et al. 2021]).

DNA Sequence Data

The data set for the phenotypic analyses contains a subset of 71 populations for which we also generated genetic data (for up to seven females and seven males per population) using double digest RAD sequencing (ddRAD) on an Illumina HiSeq 2500 platform (Novogene, Hong Kong). The protocol and downstream analyses are explained in detail elsewhere (Yang et al. 2018, 2020). In brief, we used Stacks version 2.2 to obtain single-nucleotide polymorphisms (SNPs; Catchen et al. 2013) and mapped clean reads to the reference genome of *Podarcis muralis* (PodMur_1.0; Andrade et al. 2019). One SNP was retained per RAD tag to exclude strongly linked loci in the data set, SNPs with depth <10 were removed as low-depth loci, and SNPs with depth >95th percentile were removed to avoid PCR duplicates or SNPs from high-complexity regions (Fan et al. 2016). The individuals with average depth of coverage for all SNPs <8 were also removed from the whole data set. Plink (Chang et al. 2015) was used to filter the SNPs with minimum minor allele frequency <0.05, deviation from Hardy-Weinberg equilibrium ($P < .05$), or with missing rate >0.1 in each population. Finally, we excluded individuals with genotyping rates <90%. After quality filtering, this data set consisted of 35,227 SNPs for 896 individuals from 71 populations (data are available in GenBank [accession no. PRJNA486080]).

Population Genetic Analyses

SNPs were used for two purposes. First, we revisited the population genetic structure across the study region (see Yang et al. 2018, 2020). We conducted a principal component analysis (PCA) based on genetic distance in Plink 1.9 (Chang et al. 2015). We then used Admixture 1.3.0 (Alexander et al. 2009) to estimate individual admixture by varying the coancestry clusters (K) from 2 to 10. Tenfold cross-validation was applied to evaluate the fit of the data to different number of clusters. Second, we tested whether, within the IT lineage, differences in male coloration between populations were associated with genetic differentiation. We used multiple matrix regression with randomization (MMRR; Wang 2013) with the Euclidian phenotypic distance for (standardized) greenness and blackness as the dependent matrix. The independent predictors were the genetic distance (pairwise population F_{ST}) and climatic distance (Euclidian distances between the two climatic principal components [PCs]). All distance matrixes were standardized (Wang 2013). These analyses were performed only within the IT lineage, representing individuals with the probability of being assigned to the IT lineage (Q-score) >0.9, as estimated by Admixture for $K = 2$, and only populations with at least five genotypes.

Climatic Variables

We extracted 19 bioclimatic variables from Worldclim 2.0 (Fick 2017) at a 30 arcsec spatial (1 km^2) resolution for all the sampling locations used in the phenotypic analyses. These bioclimatic variables contain mean, maximum, minimum, and seasonality data for both temperature and precipitation (see table S2) and thus capture information about climatic regimes that influence lizard activity, including its seasonality (e.g., Adolph and Porter 1993; Olsson et al. 2011*a*). We identified the major climatic axes of variation across these locations using PCA (ade4 package; Dray and Dufour 2007). The number of retained PCs for downstream analyses was calculated using the broken-stick method (Legendre and Legendre 2012).

The PCA supported the retention of PC1 and PC2, which explained 79.5% (55.5% and 24%, respectively) of the variation in the 19 climatic variables. The loadings are reported in table S2. Overall, PC1 represents a gradient from hot and dry climate (negative values) to cool and wet climate (positive values), and the geographic distribution of the scores clearly separates out lowland regions from mountains (see fig. 2*A*). PC2 captures seasonal variation in temperature and precipitation. Negative values of PC2 represent locations with mild, relatively wet winters and hot and dry summers. Positive values of PC2 represent locations with higher annual variation in temperature and where both winter and summer seasons experience substantial rainfall (table S2). This results in a pronounced shift in PC2 from the coast and farther inland (see fig. 2*B*). These PCs reflect well the Köpper-Geigen climate classification map of Italy (Beck et al. 2018; compare fig. 2*A*, 2*B* with fig. S2).

Spatial Analyses

Phenotypic introgression of greenness and blackness is accompanied by genetic exchange of a very limited part of the genome (Yang et al. 2018). As a result, even individuals and populations that are overwhelmingly (>99.999%) assigned to the SA lineage on the basis of the >35,000 unlinked SNPs we employ in this study can exhibit highly exaggerated green-and-black coloration (While et al. 2015; Yang et al. 2018, 2020). To test (i) whether climate explains the geographic variation in male coloration within the IT lineage and (ii) whether the same climatic conditions explain geographic variation in the introgression of the color phenotypes into the SA lineage, we therefore classified populations into three categories: Italian (IT; if $Q_{\mathrm{IT}} > 0.90$), Southern Alps (SA; if $Q_{\mathrm{IT}} < 0.10$), and hybrids ($0.10 \leq Q_{\mathrm{IT}} \leq 0.90$). Populations without genetic data were assigned to these categories on the basis of the inferred location of the hybrid zone (see "Results" and Yang et al. 2020). This resulted in a total of 58 populations assigned to the IT lineage, 37 populations assigned to the SA lineage, and 19 populations assigned to the hybrid zone.

For each of the three categories of populations, our aim was to establish the unique and shared contributions of climate (the focal predictor of selection on color ornamentation), a linear latitudinal and longitudinal trend (expected if the color ornaments originated and spread from the south; Yang et al. 2018), and more complex spatial structure across the landscape (arising from historical and contemporary limits to gene flow and selection).

To this end, we analyzed the spatial phenotypic structure using distance-based Moran eigenvector maps (dbMEMs). This method is explained by Legendre and Legendre (2012), and the information required to carry out the analyses in R (using the package adespatial; Dray et al. 2012) can be found in Borcard et al. (2018). Briefly, dbMEM variables are the eigenvectors of a spatial matrix calculated from the geographical coordinates of the sampling locations (Dray et al. 2006; Legendre and Legendre 2012). These eigenvectors represent orthogonal spatial descriptors of the variation in the focal traits (e.g., greenness and blackness) across sampling locations at different scales, from broad to fine scale (defined by Moran's I). The eigenvectors can therefore be used as spatial explanatory variables in multiple regression or redundancy analysis (RDA; which is an extension of regression models for multivariate dependent variables). The resulting canonical axes from the RDA represent the spatially structured variation in the focal traits, and these can be subject to further analyses, for example, to test whether the spatially structured variation can be explained by environmental factors (in this case, climate). Furthermore, it is possible to perform a variation partitioning analysis to quantify both unique and shared fractions of phenotypic variation explained by spatial and environmental (here climatic) predictors. Thus, this provides a comprehensive assessment of the geographic variation in phenotype and the association between phenotype and climate from fine to broad spatial scales.

Following Legendre and Legendre (2012), we first tested for and, if significant, removed any overall linear relationship between phenotype and latitude/longitude by regressing the phenotypic data against population x-y coordinates and retaining the residuals. This ensures that the dbMEM model spatial structure cannot simply be captured by a linear association between phenotype and spatial coordinates. A linear trend can arise for several reasons, including spatially restricted gene flow, a cline in environmental conditions, or because the characters originated only recently and spread under (possibly environment-dependent) selection. We subsequently created a geographic distance matrix

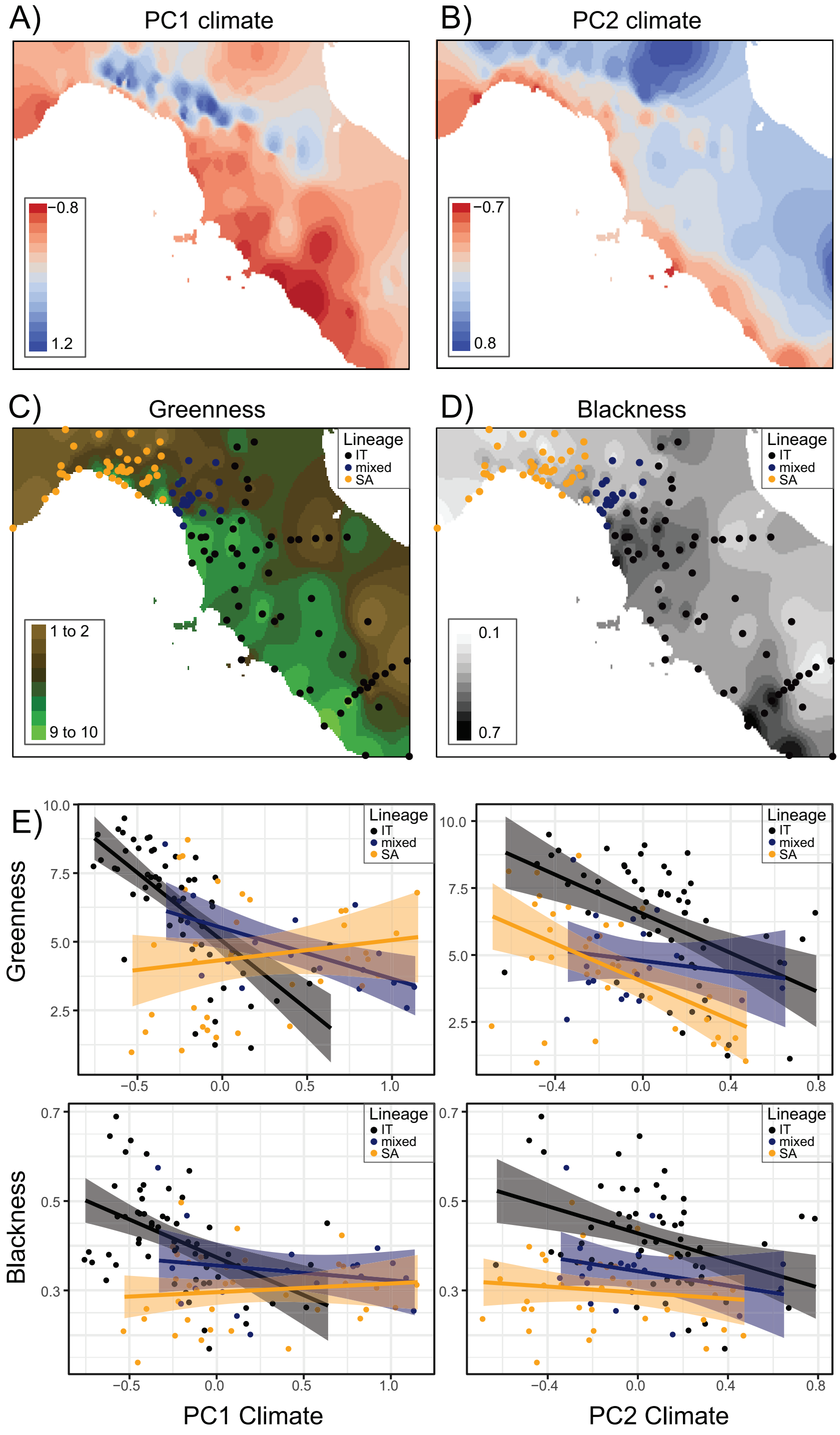


Figure 2: Summary of climatic and phenotypic variation in the study area. *A*, Climatic principal component 1 (PC1), showing a gradient from hot and dry (negative values, red) to cool and wet (positive values, blue). *B*, Climatic principal component 2 (PC2), showing a gradient from mild, wet winters and warm to hot, dry summers (negative values, red) to cool winters and warm summers with significant precipitation even during the driest month (positive values, blue). *C*, Geographic distribution of the average intensity of male green dorsal coloration. Light green represents more intense coloration. *D*, Geographic distribution of the average proportion of male black pigmentation on the chest (ranging from 0 to 1). Black dots in *C* and *D* represent populations of the Italian (IT) lineage, orange dots represent populations of the Southern Alps (SA) lineage, and blue dots represent the hybrid zone (mixed origin). Interpolation of coloration on the map uses inverse distance weights. *E*, Correlations between greenness and blackness and PC1 and PC2 for each of the lineages and the hybrid zone proper.

for all the locations where each location was linked within a minimum distance, using the longest edge in a minimum spanning tree (Fortin and Dale 2005). We first ran a global RDA on the population phenotypic data (average greenness and blackness) and used forward selection to identify significant dbMEMs (Blanchet et al. 2008). These significant dbMEM variables were then used as variables in the final RDA, resulting in one or more canonical axes that represented the spatially structured variation in greenness and blackness. To test whether any of this spatial variation in phenotype was explained by climate, the fitted population scores from the significant canonical axes were regressed on the two climatic PCs using a linear model. Statistical significance was assessed using ANOVA. Finally, we performed a variance partitioning analysis (Peres-Neto et al. 2006; Legendre and Legendre 2012). This method (implemented in the vegan package: Borcard et al. 2018; Oksanen et al. 2019) uses partial RDAs to quantify the unique and shared fractions of the phenotypic variation explained by the linear trend (x, y coordinates), the spatial structure (dbMEMs), and the two climatic PCs. Statistical significance and unique and shared contributions of climate, linear trend, and spatial structure (dbMEM) were determined by ANOVA using 1,000 permutations. These spatial analyses and variance partitioning analyses were subsequently repeated for the 43 IT populations for which we had genetic data, which allowed us to add genetic distance as an additional source of the observed phenotypic variation across the landscape. To this end, we calculated pairwise F_{ST} between populations and used a PCA on the dissimilarity matrix (Legendre and Andersson 1999) to obtain ordination axes. The first three axes (retained using the broken-stick method) were subsequently used as explanatory variables in partial RDAs and variance partitioning analyses as described above.

Complementary Univariate Analyses

To complement the multivariate analyses, we ran univariate spatial analyses using Moran eigenvector-based spatial models. This is particularly useful to assess the patterns of introgression, as our measure of green dorsal color is effectively fixed for pure individuals from the SA lineage (i.e., they all exhibit the brown ancestral state), while the extent of black ventral spotting is variable. Thus, analyzing greenness and blackness separately may reveal patterns that are difficult to discern in a multivariate analysis. As for the multivariate analyses, we ran analyses separately for the IT lineage, the SA lineage, and the hybrid zone, using linear random effect regression models that eliminate residual spatial dependence using Moran eigenvectors (Murakami et al. 2017; Murakami and Griffith 2019; implemented using the spmoran package 0.2.0; Murakami 2020).

Briefly, for each trait (standardized mean greenness or blackness) and data set (IT, SA, or hybrids), we (1) tested for a linear relationship between the focal trait and latitude/longitude and, when the model was significant, detrended the data, (2) defined neighboring populations using the minimum spanning tree and select positive eigenvectors, and (3) ran a random effects model with the focal trait as dependent variable and climatic PC1 and PC2 as explanatory variables, while controlling for the spatial connectivity with the forward-selected Moran eigenvectors. In addition, this model provides information on the spatial scale of the observed pattern; a scaled Moran's I (i.e., the Moran.I/max (Moran.I) ratio) value close to 1 indicates large-scale spatial structure, while a value close to 0 indicates a local scale spatial structure. Finally, we ran an alternative model that allows the spatial scales of individual covariates of the model (i.e., climatic PC1 and PC2) to vary (Murakami et al. 2017). Spatially varying coefficients were included only if these improved the model fit (i.e., setting x_sel = TRUE), which was assessed using Akaike information criterion. This approach makes it possible to assess whether the climatic dependence varies across the landscape. Such variation would arise, for example, if introgression of green and black coloration is ongoing, since it would imply that climate fails to predict the color far away from the secondary contact zone. All the statistical analyses were performed in R version 3.6.2. Data have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.s1rn8pk7c; Ruiz Miñano et al. 2021).

Results

Population Genetic Structure

A PCA on the SNP data revealed that the first PC (variance explained = 73.5%) separated all individuals into two clusters that correspond to the SA and IT lineages, with several intermediate clusters of individuals in between (fig. 1*C*). The second PC (variance explained = 8.1%) reflected the divergence within the IT lineage from north to south. The Admixture analysis was consistent with PCA when the number of presumed ancestral clusters (K) was set to two (fig. 1*D*). Increased K supported the divergence between SA and IT, the hybrid zone, and the within-lineage differentiation (figs. 1*D*, S3). Out of the 71 populations with genetic data, 43 populations were assigned to the IT lineage ($Q_{IT} > 0.90$), 18 populations were assigned to the SA lineage ($Q_{IT} < 0.10$), and 10 populations classified as showing clear signs of hybridization (i.e., $0.10 \leq Q_{IT} \leq 0.90$; fig. 1*D*). The genetic substructure within the two lineages was strongly discordant with the spatial distribution of the green-and-black phenotype (fig. 1*D*). For example, there were two geographic regions with particularly exaggerated coloration within the IT lineage. One region is located near Rome (e.g., populations FU, LC, RO, FO, SMA) and another is near Pisa (e.g., CR, QC, CA, VI; fig. 2*C*, 2*D*), but these regions do not cluster together genetically (fig. 1*D*). Our population genetic analysis also confirms the extensive introgression of the green-and-black phenotype into the SA lineage (fig. 1*D*).

Discordance between genetic and phenotypic patterns of variation across the landscape was statistically supported by MMRR on the 43 populations belonging to the IT lineage. Genetic differentiation did not contribute significantly to the phenotypic distance between populations ($\beta_G = -0.17$, $P = .10$), while both climatic distance ($\beta_C = 0.32$, $P < .001$) and geographic distance ($\beta_{Geo} = 0.23$, $P = .001$) played significant roles. The lack of a relationship between genetic and phenotypic variation allowed us to proceed with spatial modeling of phenotypic variation within the IT lineage in analyses that included populations without genetic data.

Climate Explains Male Color Ornamentation within the IT Lineage

We first analyzed the data for the IT lineage, as this is where the green-and-black phenotype originated (Yang et al. 2018). The green-and-black phenotype showed a linear spatial trend within the IT lineage, with the most intense greenness and blackness in the southwest of the lineage's distribution ($F_{2,55} = 17.17$, $P > .001$; figs. 2*C*, 2*D*, S4). The remainder of the analyses therefore proceeded on the detrended data. Forward selection returned four statistically significant dbMEMs (figs. S5, S6), which describe the spatial variation in phenotype from broad to very fine scales (e.g., compare MEM2 and MEM8; fig. S6) The RDA model with these dbMEMs explained 32.5% of the total variance in green-and-black coloration ($F_{4,53} = 7.83$, $P < .001$) and returned one statistically significant canonical axis (RDA1: $F_{1,55} = 31.65$, $P < .001$; RDA2: $F_{1,55} = 0.87$, $P = .92$; fig. 3*A*).

Linear regression models using the first canonical axis scores as the response variable showed that the spatial variation in phenotype was significantly related to climate PC1 but not climate PC2 (table 1), with green-and-black phenotypes being more common in areas that are hot and dry (figs. 2, 3*A*). Partial RDAs and variance partitioning demonstrated that the unique fractions explained by climate, dbMEMs, and the linear trend were all statistically significant (table S3). Overall, climate explained 40.2% of the variation in green-and-black phenotype across the landscape (fig. 3*A*). Most of this variation (34.7%) was spatially structured and mostly accounted for by the linear trend (fig. 3*A*), which reflects latitudinal and longitudinal collinearity with climate (fig. 2). The remaining 5.5% of the variation explained uniquely by climate represents climate-phenotype associations on a finer scale than that captured by the global linear trend and dbMEMs. The spatial structure captured by the dbMEMs and the linear trend accounted for an additional 25.9% of the variation in the green-and-black phenotype, resulting in 36.6% of variation left unexplained by any of the three variables (fig. 3*A*).

Analyses of greenness and blackness separately corroborated that both colors are generally exaggerated in hot and dry climates, but the effects are substantially stronger for greenness than for blackness (all analyses on detrended data; table 2; fig. 2*E*). Greenness was also marginally affected by PC2 (table 2). Allowing for spatially varying coefficients suggested that the effect of PC1 was consistent across the distribution of the IT lineage, but PC2 showed some evidence for spatially varying coefficients (fig. S7; table S4). For blackness, the model suggested that the impact of PC1 also varied across the landscape (fig. S8; table S4).

To further confirm that genetic relatedness did not bias these results, we reran the dbMEM and variance partitioning analyses on the 43 populations for which we had genetic data, including genetic distance as an additional explanatory variable in partial RDAs (see the "Supplementary Results" section in the supplemental PDF, available online). The results confirmed that climate accounted for >40% of the phenotypic variation, of which about 5% was unique to climate (supplemental PDF, "Supplementary Results," table 1.3; fig. 1.5). Consistent with the MMRR analysis, genetic distance alone was a rather poor predictor of spatial variation in color ornamentation within the IT lineage (for full details, see "Supplementary Results" in the supplemental PDF).

Climate Explains Introgression of Male Color Ornamentation

Next, we tested whether climate also explains the introgression of the green-and-black phenotype. To this end, we first analyzed the distribution of greenness and blackness for populations that were genetically assigned to the Southern Alps lineage ($Q_{SA} > 0.90$). In this lineage, the green coloration follows closely genetic introgression (Yang et al. 2018), and introgressed populations also exhibit more extensive black coloration (While et al. 2015; Yang et al. 2018).

The introgression of the green-and-black phenotype into the SA lineage showed a strong linear trend ($F_{2,34} = 12.08$, $P < .001$; fig. S9), with a decline in color ornamentation toward the west and north (fig. 2*C*, 2*D*). The dbMEM analyses for the SA lineage therefore proceeded on the detrended data. Forward selection returned four statistically significant dbMEMs (figs. S10, S11), which describe the spatial variation in phenotype from broad to very fine scales (e.g., compare MEM2 and MEM10; fig. S10) The overall

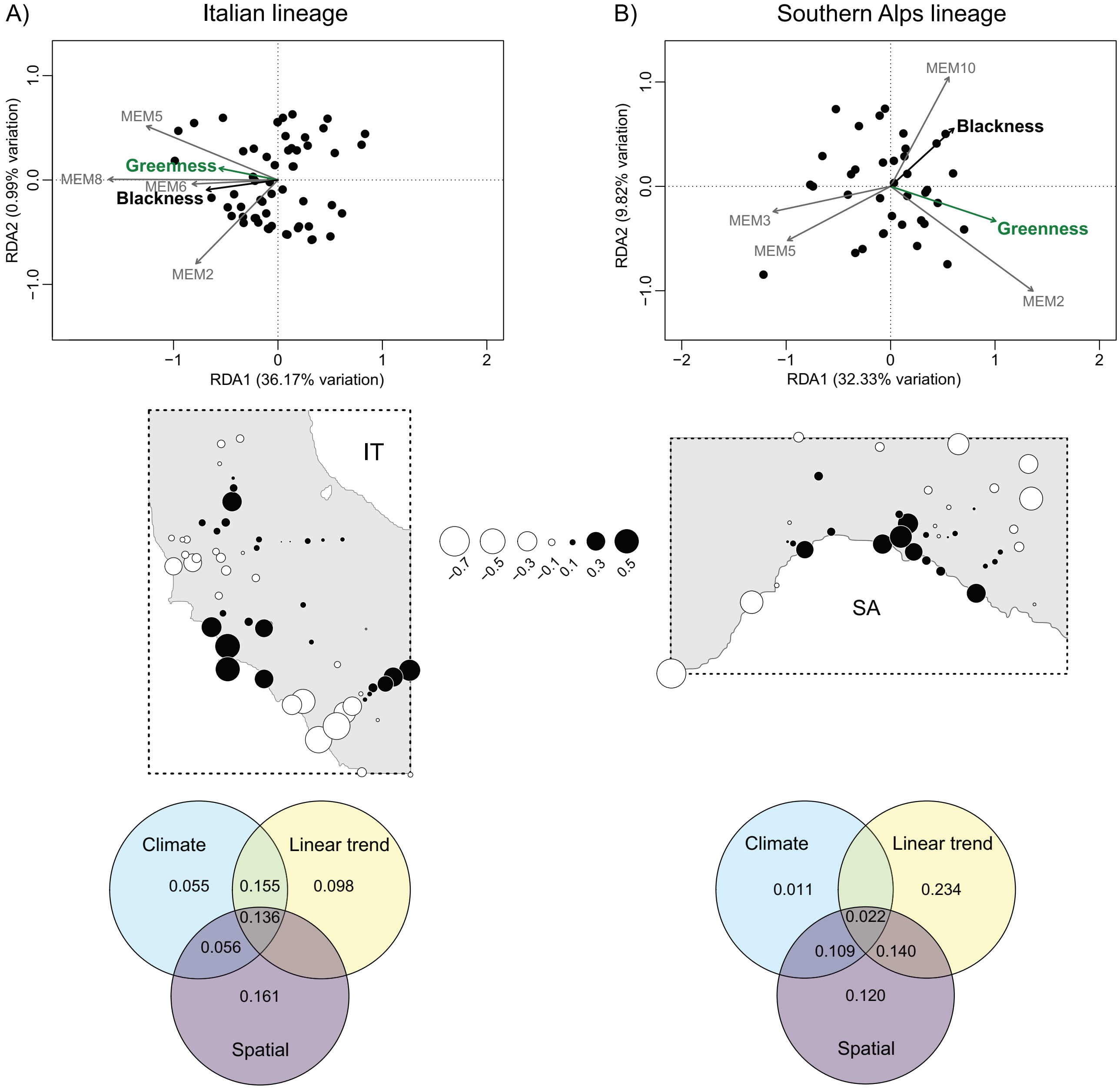


Figure 3: Results from the distance-based Moran eigenvector maps (dbMEM) analyses and variance partition of the coloration data for the Italian (IT) lineage (*A*) and the Southern Alps (SA) lineage (*B*). The top row shows redundancy analysis (RDA) triplots with two canonical axes (RDA1 and RDA2). Black circles represent populations, gray vectors represent the dbMEM predictors, and green and black vectors represent the response variables greenness and blackness, respectively. The middle row shows fitted scores of the first canonical axis of the RDA (i.e., RDA1 from top panel). Each dot represents a population; the size and color of the dots indicate the fitted score of RDA1, with white dots representing negative scores and black dots representing positive scores. For interpretation on its relationship to greenness and blackness, compare with the plots in the top row (e.g., for the IT lineage in *A*, populations represented by large white dots on the map are those with the most intense green-and-black phenotype). The bottom row shows variance partitioning of the coloration data with unique and shared fractions of explained variation. Empty fractions are estimated to have zero variance.

model explained 34.9% of the total variation in green-and-black coloration ($F_{4,32} = 5.83$, $P < .001$) and returned one statistically significant canonical axis (RDA1: $F_{1,34} = 19.00$, $P < .001$; RDA2: $F_{1,34} = 5.77$, $P = .12$; fig. 3*B*).

Regressing the fitted scores of the first canonical axis revealed that the spatially structured variation in green-and-black phenotype is primarily related to PC2 (table 3; fig. 2*E*), with the green-and-black phenotype being more

Table 1: Linear regression of the fitted scores for the first canonical axis (redundancy analysis [RDA] 1) on the two climatic principal components (PCs) in the Italian lineage

Coefficients	Estimate ± SE	t	P
Intercept	.066 ± .040	1.63	.11
PC1	.271 ± .100	2.70	.009
PC2	−.008 ± .108	−.07	.95

Note: For the overall model, $F_{2,55} = 4.11$, $P = .02$, adjusted $R^2 = 0.10$, and residual error = 0.219. Negative scores for RDA1 represent populations with more exaggerated colors.

common in environments characterized by consistently warm climates with wet winters and dry summers (i.e., coastal regions; figs. 2, 3*A*). Partial RDAs and variance partitioning demonstrated that climate explained 13.2% of the phenotypic variation, the large majority (12.1%) of which was structured by the dbMEM and linear trend (fig. 3*B*). The unique fraction (1.1%) explained by climate was not statistically significant (table S5). Spatial structure and the linear trend accounted for an additional 49.4% of the phenotypic variation, leaving 37.9% of the variation unexplained (fig. 3*B*).

Corroborating the RDA, univariate regression models with climatic predictors and dbMEMs explained 60% of the variation in greenness, and greenness was significantly related to climatic PC2 but not PC1 (table 4; fig. 2). Allowing spatially variable coefficients revealed that the impact of PC2 on greenness was strongest at intermediate distances from the zone of secondary contact (fig. S12; table S6). This reflects that populations that are on the coast, but far away from the hybrid zone proper, show no evidence of genetic or phenotypic introgression (see figs. 1*D*, 2*C*). Relative to greenness, blackness was less structured across the landscape, and neither of the climatic predictors reached statistical significance (table S6).

While the relatively low number of hybrid populations limited statistical inference for the hybrid zone proper (i.e., $0.10 \leq Q_{IT} \geq 0.90$), the data suggested very similar climate dependences as for the IT and SA lineage. The green-and-black phenotype showed a linear trend ($F_{2,16} = 3.64$, $P = .033$), with decreasing intensity toward the north (figs. 2*C*, 2*D*, S13), but there was no evidence of additional spatial structure (i.e., the RDA on the detrended data was not significant; $F_{6,12} = 0.95$, $P = .52$). We therefore tested for an effect of climatic PC1 and PC2 by running a multiple regression directly on the (standardized) greenness and blackness data. This showed a statistically significant effect of PC1 and a strong trend for PC2 (Pillai's approximate F-test; PC1: $F_{2,15} = 7.91$, $P = .004$; PC2: $F_{2,15} = 3.31$, $P = .064$; see fig. 2*E*). A partial RDA with climate PCs and linear trend revealed that climate accounted for 29.8% of the total variation in the green-and-black phenotype (fig. S14). Most of the explained variation (18.4%) was shared between the linear trend and climate, and neither of the two components explained a significant fraction on their own (table S7). Given the lack of more complex spatial structure, we did not conduct further univariate analyses using dbMEMs for the hybrid populations.

Discussion

The green-and-black color ornamentation in common wall lizards shows a pronounced variation throughout central and northern Italy, including on a small geographic scale. This variation is evident both within the IT lineage, where the green-and-black phenotype originated (Yang et al. 2018), as well as across a zone of secondary contact with extensive introgression of the color ornaments. Within lineages, geographic variation in coloration does not reflect genetic differentiation, with steep clines of black and green coloration across populations with extensive gene flow (e.g., eastward from Rome toward the Apennine mountains).

The striking geographic variation in coloration is difficult to explain unless it is maintained by strong spatial variation in selection (color expression shows limited direct environmental effects; see the introduction). While many unmeasured environmental factors will vary across the landscape, our analyses are consistent with climate being a major

Table 2: Spatial random effects regression models on standardized greenness and standardized blackness scores for the Italian lineage

	Greenness			Blackness		
Coefficients	Estimate ± SE	t	P	Estimate ± SE	t	P
Intercept	−.320 ± .093	−3.45	.001	−.190 ± .119	−1.59	.12
PC1	−1.118 ± .268	−4.17	.001	−.751 ± .344	−2.18	.03
PC2	.591 ± .308	1.92	.06	.091 ± .389	.23	.82

Note: The two climatic principal components (PCs) were entered as explanatory variables. For the greenness model, the standard error of the estimated spatial process = 0.47, residual standard error = 0.43, scaled Moran's coefficient = 0.16, and adjusted $R^2 = 0.61$. For the blackness model, the standard error of the estimated spatial process = 0.57, residual standard error = 0.56, scaled Moran's coefficient = 0.22, and adjusted $R^2 = 0.52$.

Table 3: Linear regression of the fitted scores for the first canonical axis (redundancy analysis [RDA] 1) on the two climatic principal components (PCs) in the Southern Alps lineage

Coefficients	Estimate ± SE	*t*	*P*
Intercept	−.073 ± .041	−1.76	.08
PC1	.130 ± .075	1.73	.09
PC2	−.377 ± .111	−3.40	.002

Note: For the overall model, $F_{2,34} = 6.34$, $P = .005$, adjusted $R^2 = 0.23$, and residual error = 0.212. Positive scores for RDA1 represent populations with more exaggerated colors.

source of this selection. First, within the IT lineage, green-and-black coloration was strongly associated with hot and dry climate. Second, a consistently warm climate with dry summers appears to promote introgression of color ornamentation into the distantly related SA lineage. That the best climatic predictor differed between the IT lineage and for phenotypic introgression into the SA lineage reflects the climatic variation across the sampling region and that introgression is ongoing. In fact, the results are remarkably concordant, as they establish that male color ornamentation—and in particular greenness—is most exaggerated in dry and hot Mediterranean summer climates (compare fig. 2*A*–2*D* with the climate types in fig. S1). This climate allows a long annual activity period, with consistent and high daily opportunities for activity during the main activity and reproductive season (i.e., spring and summer). In contrast, lizards express the ancestral phenotype at high elevation, which is generally characterized by hibernation during winter, a shorter activity season, and cool and unreliable thermal conditions during spring and summer.

More pronounced sexual ornamentation in environments with a prolonged reproductive season and reliable conditions for lizard activity is consistent with theoretical models of sexual selection (reviewed in Shuster and Wade 2003; Garcia-Roa et al. 2020). Sexual selection in wall lizards is predominantly a result of intense competition between males, who defend territories and females and occasionally venture into the territories of other males in search of mating opportunities (Edsman 1990; Heathcote et al. 2016; MacGregor et al. 2017*a*; Abalos et al. 2020). An extended and poorly synchronized breeding season can result in a sustained presence of receptive females, which allows dominant males to acquire a disproportionate mating success and results in stronger sexual selection compared with a shorter and more synchronous breeding season (Emlen and Oring 1977; Shuster and Wade 2003). This phenomenon is exemplified by a long-term study of sand lizards (*Lacerta agilis*) at the species' climatic extreme in Sweden. Warmer years prolonged the mating period, allowing males to mate with more females and resulting in more intense competition for fertilizations (Olsson et al. 2011*a*, 2011*b*). Female wall lizards do not choose males based on their coloration or morphology (e.g., Heathcote et al. 2016; MacGregor et al. 2017*a*; Abalos et al. 2020), and it therefore appears unlikely that differences in female choosiness contribute to the geographic variation in male coloration. As population genetic differentiation explained very little of the geographic variation in coloration, phenotypic divergence in male ornamentation, per se, likely has a weak effect on the evolution of reproductive isolation (Servedio and Boughman 2017; but see Yang et al. 2020 for how ornamentation can mediate the extent of introgression). More importantly in this context is that female wall lizards in cool climates lay fewer clutches and invest more in the first clutch (MacGregor et al. 2017*b*) and that breeding may be initiated more rapidly following hibernation (Bonnet et al. 1998). As a result, receptive females will tend to be clustered in time (at least within a given microhabitat), limiting the number of females a male can gain access to (Emlen and Oring 1977; Shuster and Wade 2003). Further field studies of the distributions of males and receptive females and direct estimates of reproductive success (from paternity; Olsson et al. 2019) could substantiate this explanation for the geographic mosaic of color ornamentation in common wall lizards and other species (for discussion, see Shuster and Wade 2003). We suggest that climatic effects on male activity and the distributions of receptive females is widespread in ectotherms and contribute

Table 4: Spatial random effects regression models on standardized greenness scores and standardized blackness scores for the Southern Alps lineage

	Greenness			Blackness		
Coefficients	Estimate ± SE	*t*	*P*	Estimate ± SE	*t*	*P*
Intercept	−.107 ± .093	−1.15	.25	−.001 ± .151	−.06	.95
PC1	−.119 ± .187	−.63	.53	−.125 ± .303	−.41	.68
PC2	−.987 ± .350	−2.82	.008	−.246 ± .552	−.45	.65

Note: The two climatic principal components (PCs) were entered as explanatory variables. For the greenness model, the standard error of the estimated spatial, process = 0.47, residual standard error = 0.42, scaled Moran's coefficient = 0.27, and adjusted $R^2 = 0.61$. For the blackness model, the standard error of the estimated spatial process = 0.71, residual standard error = 0.70, scaled Moran's coefficient = 0.16, and adjusted $R^2 = 0.13$.

to geographic mosaics in the strength of sexual selection in both vertebrates and invertebrates (Gosden and Svensson 2008; Snell-Rood and Badyaev 2008; Punzalan et al. 2010; Miller and Svensson 2014).

While climatic effects on the strength of sexual selection is a plausible explanation for the geographic variation in male ornamentation in common wall lizards, there are at least four alternative and complementary reasons why selection may maintain population differentiation in ornamentation across climatic regimes. First, green and black colors may be less effective as signals in cool and wet climates, for example, because of variation in light conditions and background matching (Endler 1992; McLean et al. 2014; Pérez i de Lanuza and Font 2016). Although such differences can be subtle, and cannot be ruled out, the explanation appears unlikely given that the typical habitats (e.g., dry stone walls, open forest) are similar across climatic regimes and for the two lineages. However, human activities have changed the Italian landscape over millennia, and some of the phenotypic variation observed today may reflect regional differences in habitats or other environmental factors that were pronounced in the past. Second, the green-and-black phenotype could be associated with significant fitness costs and benefits beyond communication to conspecifics (or arise as a side effect of communication). For example, variation in predation risk can vary with climate (Vucic-Pestic et al. 2011; Dell et al. 2014) and therefore impose a fitness cost on bright animals irrespective of sex (Martin and Lopez 2001; Marshall et al. 2015). We cannot exclude this possibility but have no reason to expect such large and geographically variable differences in predation risk for conspicuous animals. Third, colors could have biological functions other than communication, such as thermoregulation. Dark body coloration can be favored in cool climates if it helps the animals heat up faster and maintain higher equilibrium temperatures (Clusella Trullas et al. 2007). This prediction is opposite to the distribution of ventral blackness in common wall lizards, since it was most pronounced in hot and dry climates. However, it is notable that blackness showed a weaker and less consistent association with climate than dorsal greenness. Thus, although greenness and blackness are correlated at both individual and population levels (including during introgression), selection on blackness may be relatively weaker or more variable across climatic regimes. Fourth, the green-and-black phenotype could have an underlying genetic basis that affects other (i.e., noncoloration) traits that make lizards poorly adapted to cool climate. Indeed, there is extensive evidence from mammals and birds that melanization could be inherently pleiotropic, in particular with respect to physiology (San-Jose and Roulin 2018). The same applies to carotenoids and pterins (e.g., Svensson and Wong 2011), which may be involved in the green dorsal coloration. To evaluate this hypothesis, it will be necessary to establish how coloration is genetically, developmentally, and functionally integrated with other traits.

Our study identifies climate as a very important factor in the evolution of sexual coloration. However, there are undoubtedly other traits (e.g., food availability, habitat quality) that vary across the landscape and in turn mediate a range of factors (e.g., variation in population density, reproductive output, sex ratios) known to promote or relax sexual selection. Furthermore, selection is of course not the sole reason that animal coloration varies across the landscape. The poor concordance between genetic and phenotypic differentiation suggests that historical or contemporary restrictions on gene flow have little effect on the geographic distribution of male color ornamentation. However, several lines of evidence suggest that the spread of the phenotype is ongoing and hence is not at an equilibrium. Recent genetic analyses have revealed that the green-and-black phenotype in common wall lizards is rather recent and that it may have originated in the southwest of the current distribution of the IT lineage (Yang et al. 2018, 2020), which is where the characters are most exaggerated. Thus, the limited expression of the characters at the northern limit of the IT lineage, on the Po plain, is consistent with an ongoing spread of coloration from the south. Whether or not that is the case, an ongoing introgressive spread is strongly supported by the very strong decline in the introgression of green dorsal coloration with increasing distance from the hybrid zone. In fact, the analyses suggest that the green-and-black phenotype is absent from regions where it should be under positive selection (in particular along the coast farther west). We therefore conclude that this exaggerated body coloration is likely to continue its introgression where the climate allows high lizard activity and long reproductive seasons, further contributing to the mosaic distribution of coloration in this species.

Acknowledgments

We are grateful to the many field assistants and to Nathalie Feiner for discussion and help with the preparation of figures. The associate editor and two anonymous reviewers provided helpful comments on the manuscript. The Ministry of Education, University and Research (MIUR) provided all the authorizations for the study (2012–2013: Aut. Prot. PNM-0009344; 2014–2015: Aut. Prot. PNM-0011379; 2016–2018: Aut. Prot. PNM-0002154). This work was funded by the Royal Society of London, the National Geographic Society, the British Ecological Society, the Swedish Research Council, and a Wallenberg Academy Fellowship from the Knut and Alice Wallenberg Foundations (all to T.U.).

Statement of Authorship

M.R.M., G.M.W., and T.U. conceived and designed the study; M.R.M., G.M.W., W.Y., R.S., M.Z., S.S., D.S., and T.U. contributed to field data collection; M.R.M. and W.Y. performed the molecular laboratory work; and M.R.M. and T.U. analyzed the data with input from G.M.W., W.Y., and C.P.B. T.U. wrote the manuscript, and M.R.M., G.M.W., C.P.B., and T.U. reviewed and edited the manuscript.

Data and Code Availability

Data are available in the Dryad Digital Repository (doi: https://doi.org/10.5061/dryad.s1rn8pk7c; Ruiz Miñano et al. 2021) and in GenBank (accession no. PRJNA486080).

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Associate Editor: Daniel R. Matute
Editor: Erol Akçay

"The trumpet honeysuckle (*Lonicera sempervirens*) in the proper season, furnishes an important part of the food of *T[rochilus] colubris*." From "Ornithophilous Pollination" by Joseph L. Hancock (*The American Naturalist*, 1894, 28:679–683).