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RESEARCH ARTICLE

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Sexual color ornamentation, microhabitat choice, and thermal physiology in the common wall lizard (*Podarcis muralis*)

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Abstract

Common wall lizards (*Podarcis muralis*) in Italy show a striking variation in body coloration across the landscape, with highly exaggerated black and green colors in hot and dry climates and brown and white colors in cool and wet climates. Males are more intensely colored than females, and previous work has suggested that the maintenance of variation in coloration across the landscape reflects climatic effects on the strength of male–male competition, and through this sexual selection. However climatic effects on the intensity of male–male competition would need to be exceptionally strong to fully explain the geographic patterns of color variation. Thus, additional processes may contribute to the maintenance of color variation. Here we test the hypothesis that selection for green and black ornamentation in the context of male–male competition is opposed by selection against ornamentation because the genes involved in the regulation of coloration have pleiotropic effects on thermal physiology, such that ornamentation is selected against in cool climates. Field observations revealed no association between body coloration and microhabitat use or field active body temperatures. Consistent with these field data, lizards at the extreme ends of the phenotypic distribution for body coloration did not show any differences in critical minimum temperature, preferred body temperature, temperature-dependent metabolic rate, or evaporative water loss when tested in the laboratory. Combined, these results provide no evidence that genes that underlie sexual ornamentation are selected against in cool climate because of pleiotropic effects on thermal biology.

KEYWORDS

coloration, metabolism, microhabitat, pleiotropy, *Podarcis muralis*, sexual selection, thermal physiology

[Correction added on 21 August 2024, after first online publication: The third author's name has been corrected]

1 | INTRODUCTION

Animal coloration often varies across climatic and environmental gradients (Cole & Endler, 2015; Dale et al., 2015; Doutrelant et al., 2016; Fitzpatrick, 1994; Runemark et al., 2010; Stelbrink et al., 2019; Wallace, 1877). The extent of this variation can be the result of spatial variation in the functional significance of color. For example, animals can use color to reduce predation risk through camouflage or aposematism (reviewed in Ruxton et al., 2019), as an extravagant sexual signal to attract mates or ward off competitors (Candolin, 1999; Endler, 1983) or to provide thermoregulatory benefits (Clusella Trullas et al., 2007; Stuart-Fox et al., 2017). Geographic variation in predation pressure, intensity of competition for mates, or climate can, therefore, alter the strength and direction of selection on coloration across the landscape, contributing to adaptive divergence in coloration.

Selective pressures do not act in isolation. Different forms of selection can act on coloration in concert. For example, in alpine populations of toad-headed agamas (*Phrynocephalus putjatai*) dark coloration functions both in camouflage and thermoregulation (Sun et al., 2024). However, coloration can also reflect trade-offs between different forms of selection (Chen et al., 2013; Shultz & Burns, 2017). For example, in tiger moths, selection on dark coloration in cool climates, because it provides thermoregulatory benefits, is opposed by natural selection arising from increased conspicuousness to predators (Hegna et al., 2013; see also Kraemer et al., 2019; Moore et al., 2019). In butterflies, wing melanization is favored by natural selection at high altitudes but opposed by male preference for less melanized females across the elevational range (Ellers & Boggs, 2003). These trade-offs can also play out at a mechanistic level. For example, many of the key pigments that underpin variation in coloration (e.g., melanin, carotenoids, and pteridines) have pleiotropic effects on physiological and behavioral traits (Ducrest et al., 2008; Olson & Owens, 1998; Roulin & Ducrest, 2011; Roulin, 2016; San-Jose & Roulin, 2018; Svensson & Wong, 2011). For example, genes associated with melanocortin pathways, which regulate melanistic coloration, affect female sexual receptivity, aggressiveness, stress response, as well as the regulation of metabolic rate and body temperature (see Ducrest et al., 2008 for a review). Genes associated with carotenoid and pterin pigmentation have also been suggested to be involved in metabolic processes (Johnson & Hill, 2013; Longo, 2009; Powers & Hill, 2021), locomotor ability (Jiang et al., 2020) and reproduction (Surai, 2002). In environments where these genes reduce fitness through their pleiotropic effects on these traits (e.g., metabolic rate, reproduction, aggression) they have the potential to constrain positive selection on coloration and thus contribute significantly to patterns of color variation across the landscape (Ducrest et al., 2008).

Common wall lizards (*Podarcis muralis*) in Italy exhibit striking variation in body coloration. At one end of this continuum, individuals representing the ancestral phenotype for this species have brown dorsal coloration with limited black coloration on their ventral surface (here after referred to as the "ancestral phenotype"; Figure 1a). At the other end of this continuum, individuals exhibit phenotypes that are characterized by bright green dorsal coloration and extensive black coloration, in particular on the ventral surface (hereafter referred to as the "nigriventris phenotype" sensu Bohme, 1986; Figure 1b). Previous research has shown that the nigriventris phenotype has a relatively recent origin, having emerged around modern day Rome (Yang et al., 2018). Males that express the nigriventris phenotype are dominant over males that express the ancestral phenotype in experimental contact zones, resulting in large differences in courtship and reproductive success (i.e., Heathcote et al., 2016; MacGregor et al., 2017; Pérez i de Lanuza et al., 2013; While et al., 2015). This has facilitated the spread of the nigriventris phenotype across the landscape where it has replaced the ancestral phenotype first in its parental lineage and then via introgression into a distantly related wall lizard lineage (Yang et al., 2018, 2020). However, the spread of this phenotype both within and between lineages is not uniform, it is strongly mediated by climate. Specifically, the current distribution of the nigriventris phenotype is associated with a hot and dry climate with the ancestral phenotype maintained in cool and wet climates (Miñano et al., 2021). One explanation for this pattern is climatic effects on the strength of sexual selection. Specifically, warm and dry climatic conditions reduce the temporal clustering of receptive females and increase male–male competition, providing a climatic context for the individuals with the nigriventris phenotype to be selected (Miñano et al., 2021 see also García-Roa et al., 2020; Olsson, Schwartz, et al., 2011; Olsson, Wapstra, et al., 2011). However, the maintenance of such fine-scale patterns of color variation in the presence of high gene flow (e.g., Miñano et al., 2022; Yang et al., 2018), would require extraordinarily strong climatic effects on the intensity of male–male competition. Thus, additional processes may contribute to the maintenance of color variation within this species.

One potential explanation for the observed variation in coloration across climatic gradients is that it not only relects the result of positive sexual selection for the nigriventris phenotype in warmer environments but also natural selection against the nigriventris phenotype in cooler environments. This would occur if the melanin and carotenoid pigments that are responsible for the differences in coloration between the ancestral and nigriventris phenotype (Feiner et al., 2024) were underpinned by pleiotropic genes, affecting traits that provide a fitness disadvantage in cool climates. Based on existing knowledge (see above), the most likely candidate traits are those associated with thermal preference and temperature-dependent performance. Here we tested if such differences in thermal preference and performance are intrinsically linked to ornamentation, and thus might contribute to geographic variation in coloration. To achieve this, we conducted laboratory assays to test if thermoregulatory and metabolic traits differed between individuals expressing the two-phenotypic extremes (ancestral vs. nigriventris). We combined this with field studies that explored the extent to which individuals with different coloration differed in their field active body temperature or segregated with respect to their microhabitat use (measured as the proportion of vegetation, buildings, bare ground, and climatic properties of their capture locations). If the geographic distribution of the nigriventris phenotype is partly explained by antagonistic pleiotropy caused by inherent associations between coloration and thermal physiology, we

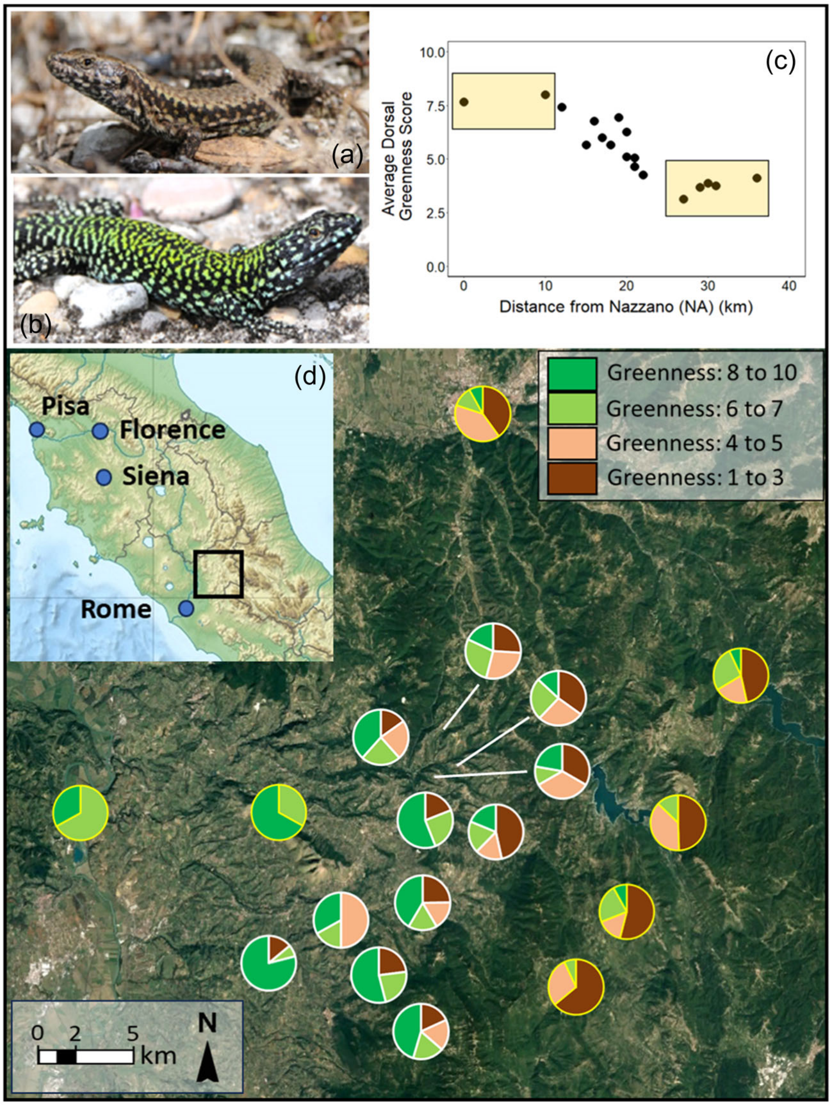

FIGURE 1 (a) and (b) A male *Podarcis muralis* with the ancestral brown-and-white phenotype and the exaggerated "nigriventris" green-and-black phenotype respectively. (c) and (d) the distribution of dorsal greenness across the 18 populations sampled from Nazzano in the west to Lago del Salto in the east. (c) The change in average greenness in each population as a function of distance from west to east. (d) Pie charts indicating the percentage of lizards scored in each of four broad categories of dorsal greenness (based on greenness scores spanning 1–10). Populations at the extreme ends of this distribution (highlighted in orange) were excluded from further analysis. Population Vicovaro, not shown, was excluded due to low sample size. The insert to (d) provides the geographical context for the study location within Italy.

can make the following predictions: (i) individuals with the nigriventris phenotype will have higher preferred body temperature, maintain a lower resting metabolic rate at upper test temperatures, and a higher critical thermal minimum (CT_{min}) than individuals with the ancestral phenotype in the laboratory. (ii) Individuals with the nigriventris phenotype will occupy warmer and drier microhabitats in the field, matching their thermal requirements and ameliorating the effects of possible trade-offs between thermal physiology and sexual selection. (iii) Individuals with the nigriventris phenotype will be active at higher temperatures in the field.

2 | METHODS

2.1 | Field microhabitat preference

Field data collection was conducted during the wall lizard breeding season, from March 28 to May 7, 2019, in 19 locations across the Rieti and Rome regions of central Italy (Table S1). Wall lizards within this region express the full range of coloration, from individuals exhibiting the ancestral (brown-and-white) phenotype to individuals exhibiting the nigriventris (green-and-black) phenotype (Figure 1; Figure S1). The high variation in coloration within a small geographic region (see below), high lizard abundance, and lack of population genetic structure (Miñano et al., 2022; Yang et al., 2018) is ideal for the purposes of this study.

We selected study locations within this region that had a wide diversity of possible microhabitats (e.g., towns, abandoned buildings, forest with and without rivers, vegetation, and other structures in fields and along country roads). We sampled lizards within each of these locations to address the aims outlined above. Field observations and sampling were performed during the hours of lizard activity (~9.00 to ~18.30) on days suitable for lizard activity. Lizards were captured (using noosing) opportunistically. Upon capture, we measured lizard body temperature (T_b) within 20 s of the lizard being captured using a cloacal thermometer (ET 959 Thermometer with a chromel-alumel [K type] thermocouple) (accuracy ± 0.5% of reading or ±1°C, whichever was greater). In total, we collected 316 individuals with exact point of capture (±5 m) recorded using a handheld Global Positioning System (GPS).

Using the GPS coordinates, we overlaid lizard locations onto a digitized map of the study region in Google Earth Pro and established the proportion of each lizard capture location represented by one of five microhabitat features: roads, buildings, vegetation, bare ground, major water sources. To achieve this, we established a buffer around each individual data point of 20 m diameter. This encompasses the average home range of a *P. muralis* (Abalos et al., 2020; Edsman, 1986) and was designed to capture the microhabitat features within a particular individual's home range. We then divided each lizard's home range into 5 × 5-m grid cells, resulting in 16 grid cells per individual, and assigned each grid cell to one of the five microhabitat features outlined above. This was achieved by creating a feature layer for each population and then assigning each grid cell to the most common feature layer that occurred within that grid. We could then use these data to calculate the percentage cover of the five feature classes within each lizard's home range. In addition to scoring microhabitat features ex situ, we also scored several aspects of the microhabitat in situ. This included surface temperature (T_{surf}) (using the thermometer and thermocouple combination described above) measured 5 mm from surface where the lizard was first observed, as well as air temperature (T_{air}) and humidity (using a thermometer with hygrometer [Velleman DEM 105]) measured at or above the point of capture of each lizard. We also estimated the percentage canopy cover and the presence or absence of a competing species, the Italian wall lizard *Podarcis siculus* (within 10 m of where the lizard was observed). *P. siculus* occurs in hotter and drier locations than *P. muralis* but the ranges of the two species overlap. *P. siculus* has been shown to be a dominant competitor in intraspecific interactions including those with other *Podarcis* species (Capula et al., 1993; Downes & Bauwens, 2002; Herrel et al., 2008; Nevo et al., 1972), which could impact the microhabitat use of *P. muralis*.

Following collection of microhabitat data, we measured morphology (snout-to-vent length [SVL], total length, head length, head width, body mass) and photographed the lizards dorsally and ventrally with a Canon EOS 350D digital camera (Canon 130 U.S.A., Inc.) using an X-rite Color-Checker chart as background. We focussed on two color traits that characterize differences between the ancestral and nigriventris phenotype; dorsal greenness and ventral blackness. Intensity of the dorsal coloration ("greenness") of was scored using a scale from 1 to 10 ("1" being completely brown individuals and "10" representing the most intense green color; see While et al., 2015). This scoring is highly repeatable within and across observers and correlates well with objective spectrophotometry and with scoring from digital photographs (for data and details, see MacGregor et al., 2017; While et al., 2015). 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) and thus ranged from zero to one. All individuals were then returned to the place where they were captured with the exception of 48 individuals who were returned to the Lund University for the assaying of thermal physiological traits (see below).

2.2 | Statistical analysis

We first explored broad spatial variation in coloration across our 19 study populations to identify locations that were suitable to test for associations between coloration and microclimate (e.g., locations that contained individuals exhibiting the full variation in color). An initial inspection of the data revealed that, as expected based on our previous work (e.g., Miñano et al., 2021), there was a strong cline in coloration associated with a west-east increase in altitude. This resulted in individuals exhibiting predominantly the nigriventris phenotype in the west and the ancestral phenotype in the east (Figure 1c,d). Because the aim of this study was to test for associations between coloration and microhabitat variables where individuals exhibit the full range of color phenotypes, where the climatic context is similar across populations, and where individuals are as genetically similar as possible, we focused our microhabitat study on populations in the middle of this cline. This resulted in the removal of seven populations (Nazzona and Fara Sabino in the west and Rieti, Lago del Salto, Paganico, Pozzaglia Sabino, and Orvinio in the east) from the data set (Table S1). An additional population (Vicovaro) was removed from the data set because of extremely low sample size ($n = 2$). The removal of these populations reduced our sample size from 316 lizards from 19 populations to 233 lizards from 11 populations.

Focussing on individuals from the remaining 11 populations, we first generated estimates of microhabitat. To achieve this, we subjected four microhabitat features (percentage of vegetation, roads, bare ground, buildings) as well as canopy cover to a principal component analysis using the princomp function in r (R Core Team, 2023). Percentage water was excluded from the principal component analysis because it occurred at so few locations (<3%). We centered log-ratio transformed our percentage variables before analysis to account for the compositional nature of this data (Aitchison, 1986). The first principal component (PC1) captured the major axis of microhabitat variation, explaining 32% of the variation, and was characterized by strong positive loadings for canopy cover, vegetation, and bare ground and strong negative loading for roads and buildings (Table S2; Figure S2). The second principal (PC2) component explained an additional 30% of the variation in habitat features each and was characterized by the separation between bare ground (negative) and vegetation and canopy (positive) (Table S2; Figure S2). The third principal component (PC3) explained 24% of the variation and separated out roads (negative) and buildings (positive) (Table S2; Figure S2). The remaining two principal components explained 14% and 0% of the variation in microhabitat respectively (Table S2; Figure S2). Because PC 1, 2, and 3 represented the major axes of variation in microhabitat we retained only these for subsequent analysis.

We then used a series of general linear models to explore whether there was evidence that individuals with green and/or black coloration (characteristic of individuals with the nigriventris phenotype) occupied different microhabitats to those with brown and/or white coloration (characteristic of individuals with the ancestral phenotype). The global model for these analyses included our first three habitat principal components outlined above, the presence of *P. siculus* (y/n)) as well as two microclimate variables (air temperature, humidity at capture) as explanatory variables. We did not include location as a random effect in these models because (a) the locations are not suitably differentiated to be classed as true populations (see Miñano et al., 2022; Yang et al., 2018) and (b) including location as a random effect resulted in poor model fit. We ran this global model separately for each sex, because of the sexual dimorphism in our target phenotypic traits (dorsal greenness, ventral blackness; See Table S3 and Figure S3 for tests of sexual dimorphism).

Due to uncertainty in model structure, we took a model-averaging approach (Burnham & Anderson, 2004). For each model run, the explanatory variables produced a set of candidate models that were then compared using the AICc (the Akaike information criterion for small sample sizes) using the MuMIN package (Barton & Barton, 2015), with the lowest AICc value indicating the best model fit. A subset of models was generated by calculating the difference between the AICc value of the best-fitting model and all other models using a cut-off of two AICc as the criterion for inclusion in the subset. The relative importance of each variable (sum of weights) was then calculated from this model subset (Burnham & Anderson, 2004). All continuous variables were scaled (to a mean of zero and a standard deviation of 1) before being included as predictors in the models. For each model, we used the DHARMa package (Hartig, 2017) to check for deviations from model assumptions. Female blackness was log-transformed before analysis.

We repeated the above model averaging approach to test for an association between coloration and field active body temperature. Body temperature at capture was included as a dependent variable with dorsal greenness and ventral blackness as predictor variables. We also included the three habitat principal components, air temperature, humidity, and an individual's mass as covariates. While dorsal greenness and ventral blackness are correlated with one another in both males ($r = 0.35$, $p < 0.001$) and females ($r = 0.50$, $p < 0.001$), these correlations were considered sufficiently modest in this data set to allow inclusion in the same model.

2.3 | Quantification of thermal physiological traits in the laboratory

We tested for differences between color phenotypes in several thermal physiological traits including CT_{min}, preferred body temperature (T_{bpref}), resting metabolic rate, and evaporative water loss: all collected in the laboratory. For all laboratory traits, we captured individuals at the extreme ends of the distribution of coloration from the same populations as the field data outlined above (e.g., where individuals express the full range of coloration; hereafter referred to as Sabino) ($n = 48$). Within this population, we caught individuals that corresponded to a color score between 1 and 3 (representing individuals exhibiting the ancestral phenotype; $n = 25$) on the standard scale used in previous studies (While et al., 2015) and individuals that corresponded to a color score between 8 and 10 (representing individuals exhibiting the nigriventris phenotype; $n = 23$). In addition, we captured individuals in two locations where all individuals exhibited either the ancestral (Fagge; $n = 22$) or nigriventris (Santa Marinella; $n = 23$) phenotype. These "pure" and "mixed" color populations were separated by <100 km (straight line flight distance), along the pronounced phenotypic and climatic cline from the coast to 1000 masl (Miñano et al., 2021) (see Figure S1).

In the laboratory, a single male was housed with either one or two females from the same population in a single terrarium (590 mm × 390 mm × 415 mm). Each terrarium contained a fine layer of sand, a shelter and a basking spot with a heat lamp (from 9:00 a.m. to 4:00 p.m., 60 W). This created a thermal gradient in the terraria from approximately room temperature (max 24°C) to ~40°C. From May until August, before the lizards were tested (see below), the room lights were set to a 12 h light, 12 h dark light regime and the temperature of the room varied from 20°C (8:00 p.m. to 7:00 a.m.) to 24°C (7:00 a.m. to 8:00 p.m.). From the end of August to November (i.e., until hibernation), the room was set to 14 h light, 10 h dark light regime, and the temperature in this case varied from 15°C (6:00–8:00 p.m.) and 22°C (8:00 a.m. to 6:00 p.m.). Lizards were fed meal worms and crickets every other day and sprayed with water daily. Water was provided ad libitum.

All individuals were tested for four thermal physiological traits: CT_{min}, preferred body surface temperature (T_{bpref}), resting metabolic rate, and evaporative water loss. These traits are commonly used for assessing thermal adaptation (Angilletta, 2009; Huey & Stevenson, 1979). Full descriptions of our methodology for each of these traits is contained within the Supporting Information and we only supply brief details here. CT_{min} was quantified using the righting reflex test (Spellerberg, 1972). This test consists of placing lizards on their back at decreasing temperatures (to a minimum testing temperature of 2°C). The natural reflex is for the lizards to right themselves immediately. The temperature at which lizards are no longer able to right themselves represents an important physiological threshold. Individuals ($n = 31$) who could still right themselves at the lowest temperature tested (2°C) were given a CT_{min} of 1°C. T_{bpref} was quantified from thermal images taken of individuals housed in a box with a thermal gradient of between 20°C and 50°C. Resting metabolic rate was measured as consumed oxygen ($\dot{V}O_2$) and produced carbon dioxide ($\dot{V}CO_2$) using a FC-10 O_2 and a CA-10 CO_2 analyzer (both from Sable Systems, Las Vegas NV, USA) at 21°C, 27°C and 33°C. Produced carbon dioxide ($\dot{V}CO_2$) was used as our final measure of metabolic rate. Evaporative water loss was calculated as H_2O vapor production ($\dot{V}H_20$) during the metabolic rate measurement.

All assays were performed after the reproductive season, between August and November 2019. Each assay was completed over a maximum of 19 days with the order of animals trialed randomized with respect to phenotype and source location. Before each test, lizards were fasted for 24 h, but water was kept ad libitum. Lizards were weighed before and after each test. Individuals were measured with a minimum of 3 weeks between each test

(CT_{min}, $T_{bpref,}$ resting metabolic rate), to ensure lizards were fully recovered between measurements.

2.4 | Statistical analysis

For this part of the study, we specifically targeted individuals exhibiting the two phenotypic extremes (nigriventris vs. ancestral phenotype). As a result, these individuals exhibited extreme values for dorsal green coloration (e.g., greenness scores from 1 to 3 for lizards exhibiting the ancestral phenotype and 8–10 for individuals exhibiting the nigriventris phenotype). Furthermore, because of the tight correlation between dorsal greenness and ventral blackness observed across these two phenotypic extremes the lizards selected also differed in their ventral blackness (ancestral phenotype = 16 ± 0.02% ventral blackness *vs.* nigriventris phenotype = 43 ± 0.03% ventral blackness; $F_{1,84} = 62.47$, $p < 0.001$). This allowed us to include a single, categorical, coloration predictor in our models that captured this variation (hereafter referred to as color morph).

To analyze differences in thermal physiology between the two color morphs we ran a series of general linear mixed models (LMMs) using the lme4 package (Bates et al., 2015) in R. Each model included a thermal physiological trait as the dependent variable with color morph (nigriventris vs. ancestral) as well as body mass and region (Sabino, Santa Marinella, Fagge) as predictor variables. For resting metabolic rate and water vapor production, we included the testing temperature (21°C, 27°C, and 33°C) and the interaction between testing temperature and color morph as additional predictor variables. We included different random effects depending on the trait of interest. This included testing box for Tb_{pref} and the experimental box in which individuals were placed and lizard ID for metabolic rate. CT_{min} was analyzed using a cox-proportional hazards model with the coxph function in R (Therneau, 2023). The response variable for these models took the form of a survival curve where "mortality" was considered as the point when an individual could no longer right itself. Color morph was included as a fixed factor along with body mass and region.

All continuous variables were scaled (to a mean of zero and a standard deviation of 1) before being included as predictors in the models. For each model, we used the DHARMa package (Hartig, 2017) to check for deviations from model assumptions. Resting metabolic rate (VCO_2) and water vapor production (VH_2O) were log-transformed before analysis. As above, body mass was scaled before being included as a predictor in models.

3 | RESULTS

3.1 | Field microhabitat preference

There was effectively no evidence that individuals with different body coloration representative of the ancestral and nigriventris phenotypes (e.g., Dorsal Greeness and Ventral Blackness) segregated at small spatial scales with respect to any microhabitat feature (Figure 2; Tables S4–S6). For five of the six sex/trait combinations, the null model was included in the set of top-performing models (Tables S4 and S5). Across all trait and sex combinations, only the presence of *Podarcis siculus* was included in all top models (Tables S4 and S5) and exhibited a consistent direction of effect (Table S6). Specifically, the presence of *Podarcis siculus* was associated with small body size, brown dorsum, and white ventral for both sexes (Figure 2). This effect was statistically significant for male body size and female ventral blackness (Table S6; Figure 1). Humidity exhibited a statistically significant association with female body mass, with heavier females found in more humid areas (Tables S5 and S6; Figure 1). No other predictors were identified as significant of any of the three phenotypic traits for either males or females (Table S6).

The body temperature of lizards caught in the field ranged from 14°C to 38°C with an average field active temperature of 29.27 ± 0.20°C. Air temperature at capture was the only significant predictor of an individual's field active body temperature (Tables S7 and S8, Figure 3) with air temperature included in the best-performing model for both males and females (Table S7). Individuals had a higher field active temperature when caught at higher air temperatures. Dorsal greenness was included in the top-performing models for both males and females but was not identified as a significant predictor of field active body temperature in either sex (the direction of this effect was opposite for the two sexes; Figure 2). Ventral blackness was not included in the top-performing model for either males or females. Finally, there was limited evidence that field active body temperatures differed between individuals occurring in different microhabitats (Tables S7 and S8; Figure 3). The exception to this was that females with higher values for PC 1 (lower canopy and vegetation cover) had higher field active body temperatures than individuals with lower values for PC 1.

3.2 | Quantification of thermal physiological traits in the laboratory

Individuals exhibiting the nigriventris phenotype and those exhibiting the ancestral phenotype did not differ from one another in their CT_{min} (ancestral phenotype: 3.13 ± 0.31°C, nigriventris phenotype: 3.34 ± 0.27°C). This was true for both males ($\chi^2 = 0.01$, $p = 0.99$) and females ($\chi^2 = 0.21$, $p = 0.65$) (see Table S9 for full model outputs including hazard ratios). There was also no difference in CT_{min} between individuals sampled from the three different regions (males: $\chi^2 = 1.61$, $p = 0.45$; females $\chi^2 = 1.96$, $p = 0.37$). The distribution of phenotypes amongst the 31 individuals who righted themselves at the lowest temperature tested and were therefore assigned a CT_{min} of 1°C (the next lowest temperature) was slightly biased towards individuals with the ancestral phenotype ($n = 19/45$) compared to nigriventris phenotype ($n = 12/48$). However, this bias was not statistically significant ($\chi^2 = 2.37$, df = 1, $p = 0.12$). The preferred body temperature (T_{bpref}) of lizards in our sample was 34.42 ± 0.12°C. There was no difference between individuals with the nigriventris

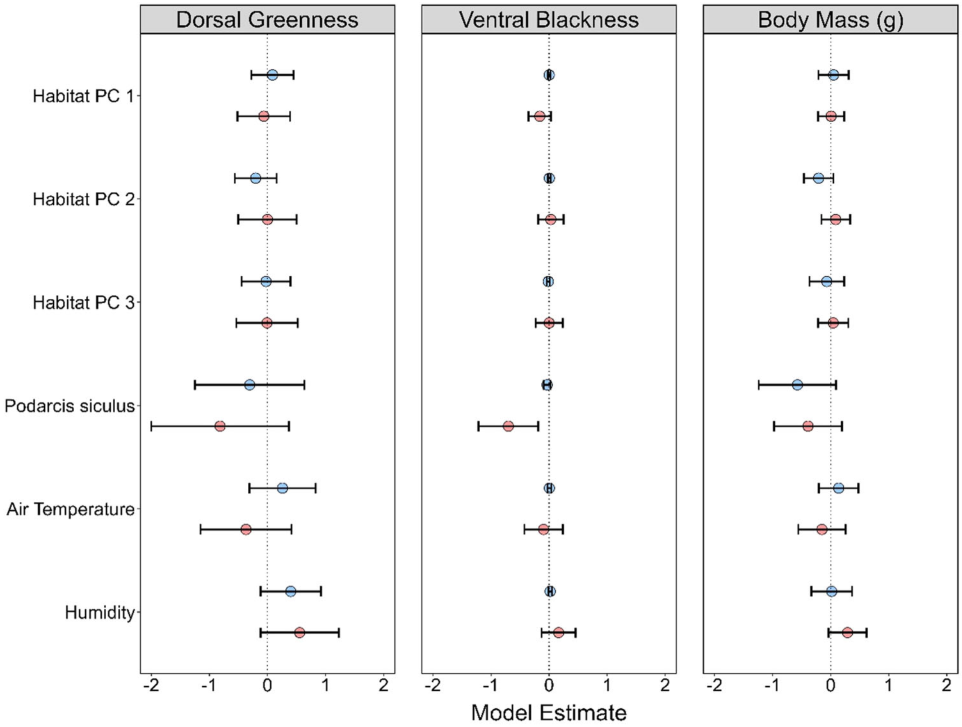

FIGURE 2 Model-averaged parameter estimates and their confidence intervals for each predictor variable plotted against the three key phenotypic traits; dorsal greenness, ventral blackness, and body mass. Blue dots indicate estimates from male models. Red dots indicate estimates from female models. These estimates are based on conditional model averages.

phenotype and individuals with the ancestral phenotype in their preferred body temperature (Table 1; Table S10) nor was there any evidence that lizards originating from different regions had different preferred body temperatures (Tables 1 and S10).

Resting metabolic rate ($\dot{V}CO_2$), measured as mL CO_2/min, differed significantly between temperatures tested, with individuals exhibiting higher metabolic rates at higher temperatures (Table 1; Table S11). There was no difference in resting metabolic rate between indiviudals exhibiting the nigriventris phenotype and those exhibiting the ancestral phenotype (Table 1; Table S11). Heavier males had a marginally higher resting metabolic rate than lighter males (Table S11). In females, individuals from the high-altitude population (Fagge) had marginally higher resting metabolic rate than individuals from either of the two lower altitude populations (Sabino and Santa Marinella) (Table 1; Table S11; Figure S8). Finally, individuals exhibiting the nigriventris phenotype did not differ from individuals exhibiting the ancestral phenotype in their water vapor production (Table 1; Table S12).

4 | DISCUSSION

The nigriventris phenotype of the common wall lizards, which is characterized by green dorsal coloration and black ventral coloration, is most exaggerated in hot and dry climates. Previous work has suggested that this is due to climate-modifying activity patterns and hence the strength of intra-sexual selection (Minano et al., 2021). However, the melanin and carotenoid pigments that are responsible for the differences in coloration between the ancestral and nigriventris phenotype (Feiner et al., 2024) can also have pleiotropic effects which could cause indirect selection on coloration (Ducrest et al., 2008; Olson & Owens, 1998; Roulin & Ducrest, 2011; Roulin, 2016; San-Jose & Roulin, 2018; Svensson & Wong, 2011) and thereby modulate the association between climate and coloration at a landscape scale. Our results do not provide any support for this hypothesis. First, individuals exhibiting the nigriventris phenotype did not differ from individuals with the ancestral phenotype in their critical minimum temperature, preferred body temperature, resting metabolic rate, or evaporative water loss. Second, there was no evidence for a correlation between the expression of the nigriventris phenotype and active body temperature in the field, nor was there any evidence that microhabitat use differed between individuals exhibiting color traits associated with the nigriventris and ancestral phenotypes. Thus, we conclude that it is unlikely that indirect selection on coloration—via selection on thermal tolerance, preference, or temperature-dependent metabolism—plays an important role in determining the geographic distribution of the nigriventris phenotype.

It is important to note that while the thermal traits targeted here are commonly used for assessing thermal adaptation in ectotherms (Angilletta, 2009; Huey & Stevenson, 1979), they are not the only traits that may correlate with color pigmentation. Therefore, we

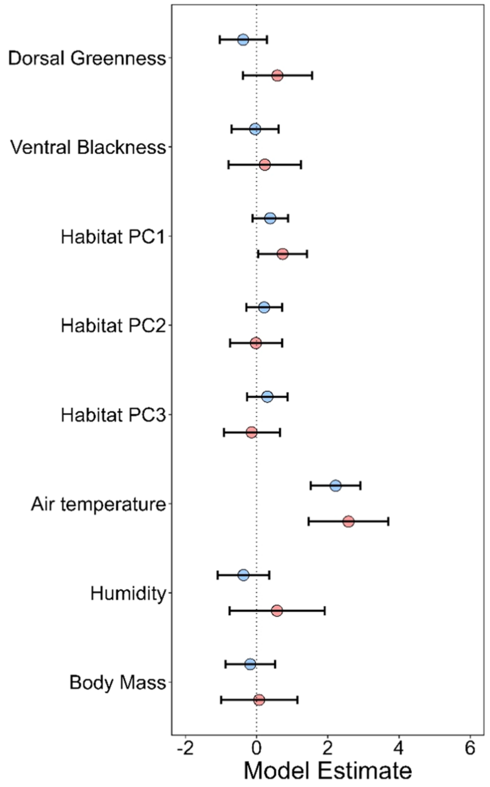

FIGURE 3 Model-averaged parameter estimates and their confidence intervals for each predictor variable plotted against field active body temperature. Blue dots indicate estimates from male models. Red dots indicate estimates from female models. These estimates are based on conditional model averages.

cannot exclude the possibility that other traits related to thermal physiology or thermoregulatory behavior may play a role. For example, coloration, in particular melanism, can affect heating rate, which is important for ectotherms that inhabit cool climates (Forsman, 1995; Walton & Bennett, 1993; White et al., 2002). While the nigriventris phenotype (which is characterized by increase melanism on the ventral surface) is strongly associated with hot, rather than cool, climate, such effects could still mediate the strength of the overall association between climate and coloration. Additionally, coloration may be mechanistically linked to traits that are not related to thermal physiology per se but whose effects on fitness are themselves temperature dependent—for example, stress tolerance, aggression, or sexual receptivity (Ducrest et al., 2008). If this is the case, color ornamentation may still be selected against in cool climates because of, for example, physiological costs associated with aggression. Therefore, while our results do not support an intrinsic link between coloration and thermal biology, additional work is still required to completely rule out the possibility that pleiotropic effects of genes involved in coloration do not contribute to weak or negative net selection on coloration in cool climates.

Our field study helped to evaluate the utility of laboratory assays in assessing the importance of thermal physiological trait variation in explaining geographic variation in coloration. Wall lizards typically inhabit a diversity of microhabitats, such as stone buildings, gardens, meadows, and forests. These microhabitats often differ in temperature and humidity (e.g., Pérez i Lanuza & Carretero, 2018), creating a mosaic of microclimates that individuals can exploit. Previous studies of vertebrates have shown that such habitat choice can allow individuals to occupy thermal conditions that match their thermal physiology (Adolph, 1990; Baudier et al., 2015; Edelaar et al., 2008; Moen & Wiens, 2017; Rozen-Rechels et al., 2019; Scheffers et al., 2013). Lizards can also adjust their activity patterns or basking behavior to further match their thermal environment (Bauwens et al., 1996; Caldwell et al., 2017; Grant, 1990; Kearney & Porter, 2004; Moss et al., 2023). Variation in microhabitat use and behavior can potentially mitigate the effects of thermal variability on organisms and may highlight differences in thermal adaptation that may not be readily apparent in our laboratory assessments. While the conflicting demands on microclimate use make it difficult to draw robust inference from observational data, we found no evidence that lizards with different coloration preferred any of the microhabitat variables measured. While we did not test for more subtle variation in microhabitat use (e.g., perch location) or variation in basking behavior, the general lack of any association between coloration and microhabitat supports our laboratory data on thermal physiological traits, further suggesting that color ornamentation and thermal biology are at best weakly integrated and contribute little to explaining the variation in coloration across climatic regimes (Minaño et al., 2021).

The only microhabitat characteristic that exhibited any consistency in its association with phenotypic variation was the presence of *P. siculus*. *P. siculus* is more exploratory, aggressive, and consume more food when in direct competition with other *Podarcis* species (Damas-Moreira et al., 2019, 2020). In extreme cases, this can result in the exclusion of other species from particular habitats (e.g., Capula et al., 1993; Downes & Bauwens, 2002; Herrel et al., 2008, Nevo et al., 1972). Such effects, if manifested in the context of *P. muralis*, may distort associations between phenotype and microhabitat. While the results were relatively weak overall, one interpretation is that individuals exhibiting the nigriventris phenotype (e.g., large green-and-black individuals) are less likely to co-occur with *P. siculus* because they are perceived as a greater threat to *P. siculus* individuals, compared to individuals exhibiting the ancestral phenotype that therefore are tolerated. Such patterns of competitive exclusion are common within species (e.g., Edsman, 1990; MacGregor et al., 2017). How interspecific competition contributes to selection on color ornamentation and influences the distribution of the two species on local and regional scales, deserves further study.

It is noteworthy that the laboratory measures of critical minimum temperature, preferred body temperature, and evaporative water loss did not reveal any differences between lizards from different altitudes (from 40 masl in coastal Santa Marinella to 1000 masl in inland Fagge). While our study was not designed to test for local adaptation per se, this finding is surprising, given that lizards from these regions experience quite drastic differences in climate. Indeed, we have previously shown signatures of genetic differentiation associated with climatic regimes across this geographic region, including candidate

TABLE 1 Post hoc comparison of mean estimates for color morph (ancestral vs. nigriventus), region (Fagge [high]; Sabino [intermediate]; Santa Marinella [low]), and incubation treatment (21°C, 27°C, and 33°C) factors.

Response	Sex	Color morph	Estimate (95% CI)	Region	Estimate (95% CI)	Temp	Estimate (95% CI)
Preferred body temperature	Males	Nigriventris	34.7 (33.8–35.2)	Fagge (H)	34.3 (33.5–35.1)		
		Ancestral	34.5 (34.0–35.5)	Sabino (M)	34.5 (33.9–35.1)		
				Santa Marinella (L)	35.0 (34.1–35.9)		
	Females	Nigriventris	34.7 (33.7–35.7)	Fagge (H)	34.1 (33.0–35.1)		
		Ancestral	34.1 (33.2–35.1)	Sabino (M)	34.2 (33.2–35.1)		
				Santa Marinella (L)	35.0 (33.9–36.1)		
Metabolic rate (VCO_2)	Males	Nigriventris	0.012 (0.010–0.015)	Fagge (H)	0.012 (0.009–0.015)	21°C	**0.006 (0.005–0.007)a**
		Ancestral	0.010 (0.008–0.012)	Sabino (M)	0.011 (0.009–0.012)	27°C	**0.011 (0.010–0.013)b**
				Santa Marinella (L)	0.011 (0.008–0.015)	33°C	**0.020 (0.017–0.022)c**
	Females	Nigriventris	0.009 (0.008–0.010)	Fagge (H)	**0.012 (0.010–0.015)a**	21°C	**0.005 (0.005–0.006)a**
		Ancestral	0.010 (0.009–0.012)	Sabino (M)	**0.009 (0.008–0.010)b**	27°C	**0.010 (0.009–0.011)b**
				Santa Marinella (L)	**0.008 (0.007–0.010)b**	33°C	**0.016 (0.015–0.018)c**
Evaporative water loss (VH_2O)	Males	Nigriventris	164.02 (122.73–217.02)	Fagge (H)	172.43 (127.74–232.76)	21°C	**127.74 (109.94–148.41)a**
		Ancestral	194.41 (151.41–249.63)	Sabino (M)	196.37 (164.02–235.09)	27°C	**165.67 (142.59–192.48)b**
				Santa Marinella (L)	167.35 (115.58–244.69)	33°C	**207.42 (232.76–314.19)c**
	Females	Nigriventris	160.77 (138.38–186.79)	Fagge (H)	151.41 (123.96–184.93)	21°C	**113.29 (103.54–122.73)a**
		Ancestral	162.39 (139.77–186.79)	Sabino (M)	169.02 (149.90–188.67)	27°C	**157.59 (144.02–172.43)b**
				Santa Marinella (L)	164.02 (134.29–198.34)	33°C	**235.10 (214.86–254.68)c**

Note: Bolded estimates indicated estimates for which there were significant differences. Letters indicate significant differences among groups (a > b > c). Estimates for variables that were log-transformed before analysis (e.g., metabolic rate, evaporative water loss) have been back-transformed.
Abbreviation: CI, confidence interval.

genes that have previously been shown to be involved in both thermal physiology and coloration (Miñano et al., 2022). There is also direct evidence for local adaptation in terms of embryonic development. Specifically, embryos from high-altitude regions use less energy to complete development, that is, they develop faster without a concomitant increase in metabolic rate, compared with those from the low-altitude region (Pettersen, Ruuskanen, et al., 2023). Despite this, it was only resting metabolic rate in (female) lizards that differed between the high-altitude and low or intermediate sites, independent of coloration. While the effect size was relatively small (Figure S8), the higher resting metabolic rate in lizards from high altitudes could facilitate activity at cold temperatures and is in the direction predicted if there would be local adaptation (see also Plasman et al., 2020; Seebacher, 2005; White et al., 2012). In contrast, there were no differences in preferred body temperature or critical temperature minimum between regions. The latter is particularly noteworthy, as CT_{min} has been suggested to evolve rapidly, often being the first aspect of thermoregulation to become locally adapted following exposure to cool conditions (see Bodensteiner et al., 2021 for a review). For example, in *Lampropholis* lizards, critical thermal minimum is associated with increases in elevational gradients (Anderson et al., 2023). There are two caveats, however. First, CT_{min} has been shown to be highly dependent on acclimation times (e.g., Pintor et al., 2016), which can make it difficult to detect differences between groups of animals kept for several months in the laboratory. Second, a third of our individuals could still right themselves at the lowest temperature tested (2°C) which limited our ability to quantify the true CT_{min} of a subset of our population which may have restricted our ability to tease apart relatively subtle differences between regions (and/or phenotypes). Evidence for intraspecific variation in preferred body temperature is more mixed with some studies showing strong geographic clines in thermal preference (Rozen-Rechels et al., 2019) while others suggest thermal preferences are more rigid (Chabaud et al., 2022; Pettersen, Feiner, et al., 2023).

In summary, our results suggest limited, if any, differences in thermal physiological traits between common wall lizards with different coloration. This result holds for both males and females. We conclude that antagonistic pleiotropy between coloration and thermal biology produces, at best, a modest impact on the distribution of the green and black coloration on local and regional spatial scales. Therefore, the prevailing evidence supports climatic influences on sexual selection (Miñano et al., 2021) as the primary explanation for the remarkable diversity in wall lizard body coloration across the Italian landscape.

AUTHOR CONTRIBUTIONS

Maravillas Ruiz Miñano, Tobias Uller, and Geoffrey M. While designed and coordinated the study. Maravillas Ruiz Miñano, Tobias Uller, Amanda K. Pettersen, Luisa J. Fitzpatrick, and Geoffrey M. While performed fieldwork and collected samples. Maravillas Ruiz Miñano and Andreas Nord performed laboratory work. Maravillas Ruiz Miñano, Tobias Uller, and Geoffrey M. While analyzed the data. Maravillas Ruiz Miñano, Tobias Uller, Andreas Nord, and Geoffrey M. While interpreted the results. Maravillas Ruiz Miñano, Tobias Uller, and Geoffrey M. While wrote the manuscript with comments from, and final version approved by, all authors.

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DATA AVAILABILITY STATEMENT

The data and code used in this study are available on Zenodo (https://doi.org/10.5281/zenodo.12896800; Miñano et al. 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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