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**Original Article**

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# Original Article

# Drivers of continuous colour variation in the Madeiran wall lizard (*Teira dugesii*)

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## ABSTRACT

Animal coloration is often shaped by a myriad of factors that lead to differences in colour through changes in the chromatophores. Depending on how this variation is partitioned, coloration is often categorized as continuous or polymorphic. However, the boundaries between these two categories are not always clear. Here, we investigated whether the ventral coloration of the Madeiran wall lizard (*Teira dugesii*) varies continuously or corresponds to discrete colour morphs, via by-eye colour classification and visual modelling. By combining these two approaches, we show that *T. dugesii* coloration varies continuously and that colour classifications based on anthropomorphic approaches alone are ill suited to describe animal coloration. We also tested the influence of size, body condition, and sex as possible factors that might explain differences in *T. dugesii* coloration. We found that body condition, and especially size and sex, explain a great proportion of the variability observed in this species. These differences point to an effect of ontogeny, which might play a major role in colour development owing to the longevity of this species. Moreover, the sexual dichromatism that this species shows is indicative of an effect of sexual selection on coloration, perhaps explained by differences in circulating hormones.

**Keywords:** colour; continuous variation; sexual dichromatism; *Teira dugesii*; visual modelling

## INTRODUCTION

Colour traits are extremely variable in animals. Given that they are involved in multiple functions, their occurrence and variation across taxa have been associated with a variety of evolutionary processes (Grether *et al.* 2004, Wellenreuther *et al.* 2014, Dunn *et al.* 2015). Coloration often differs between species, and even within species and populations, either continuously (e.g. Albertson *et al.* 2014, Demars *et al.* 2022) or as discrete colour phenotypes (i.e. colour polymorphism; Ford 1945, Gray and McKinnon 2007). Characterizing trait variation within species, in addition to the factors that promote such diversity, is essential to understanding the role of colour and, ultimately, phenotypic variability in biological diversification.

Colour polymorphisms in lizards have been highly influential in shaping the modern understanding of how selection operates to maintain variation in natural populations (e.g. Sinervo and Lively 1996, Roulin 2004). But although many examples of colour variation can be found across lizard species (Olsson *et al.* 2013, Stuart-Fox *et al.* 2020), it is not always so straightforward to define whether colour varies continuously or whether it corresponds to discrete colour morphs (Paterson and Blouin-Demers 2017). For example, ventral colour variation in *Zootoca vivipara* has been classified as polymorphic by some authors (Vercken *et al.* 2007, 2008), whereas other studies suggest that *Z. vivipara* coloration varies continuously (Cote *et al.* 2008). Even for species in which colour morphs are clearly described as categorically different, intramorph variability can be important. Such is the case in the common wall lizard (*Podarcis muralis*), a colour-polymorphic species in which five colour morphs have been described (white, yellow, and orange pure morphs and

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white–orange and yellow–orange mosaic morphs; e.g. Pérez i de Lanuza *et al.* 2013). In this species, epistatic interactions between the alleles of two genes (independently linked to deposition of orange pterins and yellow carotenoids) lead to intramorph variability in the orange colour morph (Andrade *et al.* 2019, Aguilar *et al.* 2022). Besides genetically determined variation, colour can also vary within populations throughout time, owing to a combination of ontogenetic and environmental effects (Carretero 2002, Bohórquez-Alonso and Molina-Borja 2014, Zhang *et al.* 2023).

Ultimately, coloration in reptiles and other vertebrates is dependent on the pigments and structures present in the chromatophores (Bagnara and Matsumoto 2007). For example, bright yellow, orange, and red colours are commonly associated with the xanthophores, where carotenoid and/or pterin pigments are deposited (Bagnara and Matsumoto 2007, Ligon and McCartney 2016, but see San-Jose *et al.* 2013), whereas brown-to-black colours are often linked to melanophores, where melanin is deposited (Bagnara and Matsumoto 2007, Ligon and McCartney 2016). Moreover, light-scattering purine platelets present in the iridophores are often associated with blue colorations (Bagnara *et al.* 2007). Yet, these colour-producing mechanisms are not mutually exclusive and frequently interact, strongly increasing colour diversity in reptiles (San-Jose *et al.* 2013, Shawkey and D'Alba 2017).

The Madeiran wall lizard, *Teira dugesii* (Milne-Edwards, 1829) is a lacertid lizard endemic to the Madeira archipelago (Portugal) that has been introduced in the Azores archipelago, mainland Portugal, and the Canary Islands (Silva-Rocha *et al.* 2016, Ferreira *et al.* 2023). Previous studies on this species highlight an exceptional variability in dorsal and ventral coloration (Crisp *et al.* 1979, Báez 1990). For ventral coloration in particular, individuals can show different combinations of white, yellow, orange, and blue colours (Báez 1990; see Fig. 1A). By classifying individuals into discrete colour classes for the dorsal and ventral coloration, Baez (1990) reported that the frequencies of these classes differed between males, females, and juveniles. However, these studies did not use objective techniques to analyse colour, hence these colour classifications might be affected by biases such as anthropomorphism by omission (Rivas and Burghardt 2002). Therefore, applying objective approaches will probably provide a better understanding of how colour varies in this species.

Our aim with this study was to characterize the stunning diversity in ventral coloration of *T. dugesii*, combining subjective ('by-eye' colour classes) and objective approaches (reflectance spectrophotometry and visual modelling) to understand whether coloration in *T. dugesii* corresponds to alternative colour morphs and to test the degree to which both approaches converge. We also assessed whether variation in colour is associated with traits such as sex, size, and body condition, with the aim of identifying selective forces that could explain colour variation in this species.

## MATERIALS AND METHODS

### Sampling

We randomly sampled 199 adult *Teira dugesii* individuals (83 females and 116 males) from eight populations across the island of Madeira from 16 to 23 March 2022 (Fig. 1B; see Supporting Information, Table S1). After capture, individuals were transported to the Estação de Biologia Marinha, in Funchal (Madeira, Portugal), where they were maintained prior to their release within the next 2–3 days. From this sample, we collected data on their snout–vent length (SVL; measured to the nearest .01 mm with digital callipers), weight (measured to the nearest .01 g with digital scales), and sex (based on sexual secondary characters and hemipenis eversion; Báez 1990). We also calculated a body condition index (BCI) as the residuals of the regression of the logarithm of weight on the logarithm of SVL, separately for each sex (e.g. Unglaub *et al.* 2018).

### Colour data collection

To characterize the coloration of these animals, we measured the reflectance of their throat and belly using a FLAME-T-UV-VIS spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics Inc., Dunedin, FL, USA) calibrated with a white diffuse reflectance standard (Spectralon, Labsphere Inc., North Sutton, NH, US). All measures were conducted by placing the spectrometer probe 3 mm away from the skin, forming an angle of 90°. We recorded the average of 20 scans using an integration time of 30 ms and a boxcar width of 10. After inspection of the spectral measurements, we decided to focus on the belly spectra, where coloration was more variable (see Fig. 1) and the size of the scales was bigger, thus reducing the measurement error (Badiane *et al.* 2017). We also measured the reflectance of six representative stones randomly collected from three of the sampled populations (São Vicente, Lombo do Moleiro and Eira do Serrado) and obtained an averaged spectrum of all rocks (due to the similarity in their reflectance spectra) to be used as background for the visual modelling (see below). To complement the spectral measurements, we recorded digital images of the ventral side of the animals using a portable scanner (Canon Lide 400; Canon, Tokyo, Japan).

### Observer-based colour classification

To evaluate whether variation in ventral coloration was continuous or discrete, we started by testing for consistency between observers in *a priori*-defined categorical classification. This type of classification has been used for the study of colour in many species (e.g. Cain *et al.* 1960, Blanco and Bertellotti 2002, Reichard *et al.* 2009), including lizards (e.g. Carpenter 1995, Corl *et al.* 2010, Bastiaans *et al.* 2014). Based on a preliminary exploration of the digital pictures, we defined a total of six ventral colour classes: white, white–blue, yellow, yellow–blue, orange, and orange–blue. This classification is based on the fact that lizards can alternatively show white, yellow or orange ventral coloration, which may or may not be combined with blue. Similar classes are commonly defined in other polymorphic lizards (see Stuart-Fox *et al.* 2020). Individual pictures were independently scored by three observers (P. Aguilar, P. Andrade, and G.P.L.). When all observers assigned an individual to the same class, that class was used for further analyses. When observers disagreed on the classification, the individual colour class that was selected by two of the three observers was used. Only in a few cases (5 of 199 lizards) did each of the three observers choose a different colour class, and in these cases a random class out of those was assigned. Using this colour classification, we calculated the

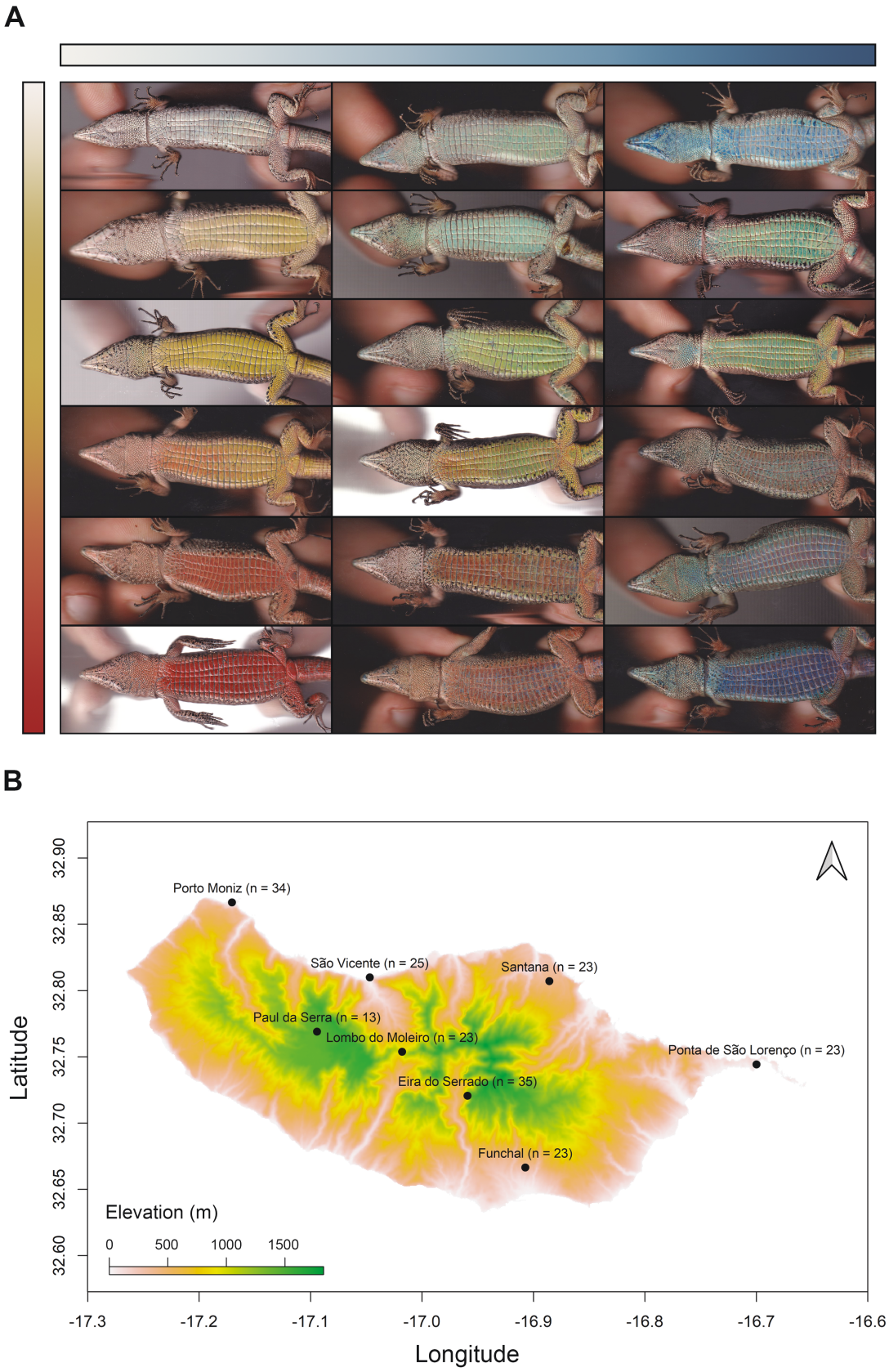


**Figure 1.** Ventral colour variation in the Madeiran wall lizard (*Teira dugesii*). A, representative ventral colour variability in this species, which can result from a combination of white, yellow, orange, and blue hues. B, sampling localities and number of individuals collected in each of them, within the island of Madeira.

proportion of the individuals that were unanimously assigned to the same colour class.

Next, we trimmed the spectral data to match the lacertid sensitivity visual range (300–700 nm; e.g. Loew *et al.* 2002). We also smoothed the spectra via local regression smoothing using a value of .2, which proved to reduce the noise while preserving the shape of the spectra (see Maia *et al.* 2019). To summarize the spectral differences between individuals, we calculated three complementary variables: (i) luminance (total reflectance); (ii) chroma; and (iii) hue (peak location) (Maia *et al.* 2019). Spectral analyses were performed in R v.4.0.3 (R Core Team 2022) using the package 'pavo' v.2.7.1 (Maia *et al.* 2019). Using these data, we tested whether these colour classes differed spectrally (i.e. luminance, chroma, and hue) through Kruskal–Wallis tests, when significant we applied *post hoc* comparisons between groups using Dunn's test and corrected for multiple testing (Benjamini and Hochberg 2000; false discovery rate of .05).

### Visual model-based clustering

To test for unbiased (observer-independent) clustering, we carried out a classification of the lizards following a model-based clustering approach. For this, we fitted a psychophysical colour vision model (Vorobyev and Osorio 1998), through which we can determine whether differences between two spectra can be discriminated by the receiver under a given illuminant spectrum. Such differences are commonly estimated as just noticeable differences (JNDs). Following Siddiqi *et al.* (2004), values greater than three JNDs indicate that colours can be discriminated by the receiver, and values between one and three JND indicate that they are barely discriminable. To fit the visual model, we used cone absorbances and abundances from the lacertid lizard *P. muralis* (UVWS, 367 nm; SWS, 456 nm; MWS, 497 nm; and LWS, 562 nm; in proportions of 1:1:1:4; Martin *et al.* 2014), the closest *T. dugesii* relative for which these data are available. Although the visual ecology of these species is likely to show slight differences, available evidence suggests that the retina of different diurnal lizard species shows a rather similar set of cone pigments (Fleishman 2024, including lacertids; Pérez i de Lanuza and Font 2014).

Using these data, we estimated the cone sensitivities through the *sensmodel* function implemented in pavo v.2.7.1 (Maia *et al.* 2019), which relies on templates from Govardovskii *et al.* (2000) and Hart and Vorobyev (2005). We used an irradiance spectrum corresponding to daylight conditions 'D65' and set the value of the Weber fraction to .05 (e.g. Delhey *et al.* 2015, Pérez i de Lanuza *et al.* 2018). Then, following the methodology proposed by Delhey *et al.* (2015), we quantified differences in chromatic variation between individuals. We first calculated the *x*, *y*, and *z* chromatic coordinates from the chromaticity diagram, which result from the conversion of the quantum catches of each cone (Delhey *et al.* 2015). These coordinates indicate the different stimulation of the cones, where variation in the *zz*-axis indicates the stimulation of the long (L) cones relative to medium (M), short (S), and very short (VS) cones, whereas variation in the *yy*-axis corresponds to the stimulation of M cones relative to the S and VS cones, and finally, variation in the *xx*-axis indicates stimulation of the S cones relative to the VS cones (Delhey *et al.* 2015). We did this by using the 'jnd2xyz' function from pavo v.2.7.1, which implements the framework from Pike (2012), through which *xyz* coordinates are calculated based on perceptual units (JNDs). Given that the resulting *xyz* coordinates were highly correlated, we summarized the information through a principal components analysis on the covariate matrix to preserve the perceptual distances (Delhey *et al.* 2015). We then tried to identify potential groups within our sample using a model-based clustering approach, using the first two principal components (PCs) resulting from the previous analysis as input. This was done using the mclust R package v.6.0.0 (Scrucca *et al.* 2016), where several combinations that differed in the number of clusters (from 1 to 10) and the models used (which differed in their covariance parametrization) were compared. Then, we selected the most adequate combination of model and number of clusters using the Bayesian information criterion (BIC). Finally, we also compared how the by-eye colour classes corresponded to the clusters identified by the two best models.

### Colour volume overlap

To further evaluate the colour variability of the Madeiran lizard, we used the visual model described above. We compared the chromatic volume overlap of the colour classes defined for *T. dugesii* with the volume overlaps between colour morphs of *P. muralis* (e.g. Pérez i de Lanuza *et al.* 2013, 2018). Here, we would expect a relatively greater colour volume overlap for *T. dugesii* if the ventral coloration of this species was continuous. We restricted the analysis of the volumes of *T. dugesii* to white, yellow, and orange colour classes, because *P. muralis* does not show ventral blue coloration. To standardize the comparison, we randomly selected the same number of *P. muralis* spectra per colour class as those of *T. dugesii* (28 white, 41 yellow, and 57 orange lizards) from a previous study (Aguilar *et al.* 2022). Volumes and their overlap (i.e. overlapped volume divided by the sum of the two volumes compared) were calculated using the *voloverlap* function from the package pavo v.2.7.1 (Maia *et al.* 2019).

### Colour discrimination of by-eye colour classes

To test whether these human-defined colour classes agreed with the colour discrimination capabilities of lizards, we used the lizard model defined above. We used a natural background (i.e. stones) as reference for the contrasts between colour classes and tested whether comparisons between colour patches belonging to different by-eye classes were discriminable for lizards. We did this by performing distance-based permutational analyses of variance (PERMANOVAs) on the chromatic and achromatic distances with 999 permutations, with the by-eye colour class as an explanatory variable (Maia and White 2018), using the *pairwise.adonis* function (Arbizu 2017). We also generated 95% confidence intervals using the *bootcoldist* function from pavo v.2.7.1 (Maia *et al.* 2019) with 1000 replicates.

### Effect of population, size, sex, and body condition on coloration

To understand whether coloration is influenced by sex, size, or body condition, we carried out two sets of analyses. First, we tested whether the proportion of each by-eye colour class differed between sexes using a $\chi^2$ test and carried out a *post hoc* test on the residuals of the $\chi^2$ test. We also ran a $\chi^2$ test to check whether

the distribution of by-eye colour classes differed between populations. Then, we tested for differences between by-eye colour classes in SVL and body condition through Kruskal–Wallis tests and fitted *post hoc* Dunn's tests when the results were significant. *Post hoc* tests were corrected for multiple testing (Benjamini and Hochberg 2000; false discovery rate of .05).

For the second approach, we first tested whether populations showed differences in their overall luminance, chroma, and hue using the Kruskal–Wallis test. Then, to understand the effect of sex, size, and body condition on the spectral variables, we controlled for differences between populations. We did this by fitting linear mixed models with luminance, chroma, or hue as the dependent variable, and we included SVL, sex, the interaction between SVL and sex, and BCI as fixed factors and population as a random term. When the interaction was not significant, we removed it from the model. We tested for model assumptions, namely normality, homogeneity of variance, overdispersion, and autocorrelation of the residuals for all models. For models that showed slight departures from normality, we applied the Box–Cox transformation (Box and Cox 1964), and in models showing non-homogeneous variance, we modified the weight of the observations based on the variance of the residuals (e.g. Rosopa *et al.* 2013). Linear mixed models were performed and assessed using the R packages 'lme4' (Bates *et al.* 2009), 'car' (Fox *et al.* 2012), and 'performance' (Lüdecke *et al.* 2021).

Finally, using the same visual model detailed above, we checked whether overall differences in colour between males and females were discriminable for lizards by fitting distance-based PERMANOVAs on the chromatic and achromatic distances with 999 permutations, with sex as an explanatory variable (Maia and White 2018), and calculated the 95% confidence intervals using the *bootcoldist* function from PAVO v.2.7.1 (Maia *et al.* 2019) with 1000 replicates.

## RESULTS

### Observer-based colour classification

We identified a large degree of variation in ventral coloration in *T. dugesii*, with all different pigmentation types being present in several populations (Supporting Information, Fig. S1). For the colour classes defined by eye, 61.3% of the individuals ($N = 122$) were assigned to the same colour class unanimously, whereas for 36.2% of the individuals ($N = 72$) two of the three observers agreed on the same colour class. Finally, for a small proportion of the individuals (2.5%, $N = 5$), each observer selected a different colour class. Using the classification based on the digital pictures, we found differences between the six by-eye colour classes in luminance ($\chi^2 = 95.462$, d.f. = 5, $P < .001$), chroma ($\chi^2 = 99.36$, d.f. = 5, $P < .001$), and hue ($\chi^2 = 74.018$, d.f. = 5, $P < .001$) (Fig. 2). Moreover, differences between by-eye colour classes were particularly associated with the long-wavelength region of the spectra (see Fig. 2). In addition, individuals showing blue coloration had an overall lower reflectance, and their hue was shifted towards lower wavelengths. However, not all of the pairwise comparisons between the by-eye colour classes differed in the set of spectral variables tested (see Supporting Information, Table S2).

### Visual model-based clustering

After obtaining the *xyz* visual space coordinates of each spectrum from the visual models, we summarized this information with a principal components analysis and retained the first two PCs, which explained most of the chromatic variance. PC1 explained 79.14% of the variance (SD = 5.8, range = 38.8), had a moderate positive loading for *x* (.618), a strong negative loading for *z* (−.785), and a weak loading for *y* (.035). PC2 explained 20.09% of the variance (SD = 2.9, range = 16.2) and had a strong positive loading for *x* (.772), a moderate positive loading for *z* (.615), and a weak loading for *y* (.157). To illustrate the information contained in each of the PCs, we plotted the normalized reflectance spectra of each of the quartiles for each PC (Fig. 3). Then, we investigated whether clusters identified by the model-based approach (based on PC1 and PC2) corresponded to the by-eye defined classes. The best model (VEV, ellipsoidal, equal shape; BIC = −2234.649) identified two clusters, whereas the second (EEV, ellipsoidal, equal volume and shape; BIC = −2238.421; BIC difference = −3.772) and third (EVV, ellipsoidal, equal volume; BIC = −2239.009; BIC difference = −4.360) best models identified three clusters. Overall, the distribution of the by-eye colour classes did not show a clear correspondence to the two and three clusters associated with the two best Gaussian finite mixture models (Table 1).

### Colour volume overlap

When comparing the chromatic volume overlap of orange, yellow, and white colour classes of *T. dugesii* with those of *P. muralis*, we observed that *T. dugesii* showed a greater overlap in the chromatic space (Supporting Information, Fig. S2). Specifically, for *T. dugesii* there was 1.37% of overlap between orange and yellow classes, whereas the volume overlap between yellow and white was 6%. For *P. muralis*, orange and yellow colour morphs showed .001% of overlap, and yellow and white colour morphs showed an overlap of .3%. Orange and white chromatic volumes did not overlap in either of the species.

### Colour discrimination of by-eye colour classes

Estimates of colour discrimination showed great variability depending on the colour classes that were compared, but despite such variation, for most of the comparisons, both chromatic and achromatic distances were higher than the one JND threshold (98.4% and 81% of the comparisons, respectively; Fig. 4; for the contrasts between the different by-eye colour classes, see Supporting Information, Fig. S3). This was particularly true for the chromatic contrasts, where 85.5% of the comparisons were above the three JNDs threshold, compared to the 47.9% for the achromatic contrasts. However, differences between certain colour classes (i.e. orange vs. orange–blue, yellow vs. white–blue, and yellow–blue vs. white) were often not discriminable chromatically or achromatically (Table 2; Supporting Information, Fig. S3).

### Effect of population, size, sex, and body condition on coloration

Together with the differences in the proportion of the by-eye colour classes between populations ($\chi^2 = 62.718$, d.f. = 35, $P = .003$; Supporting Information, Fig. S1), we

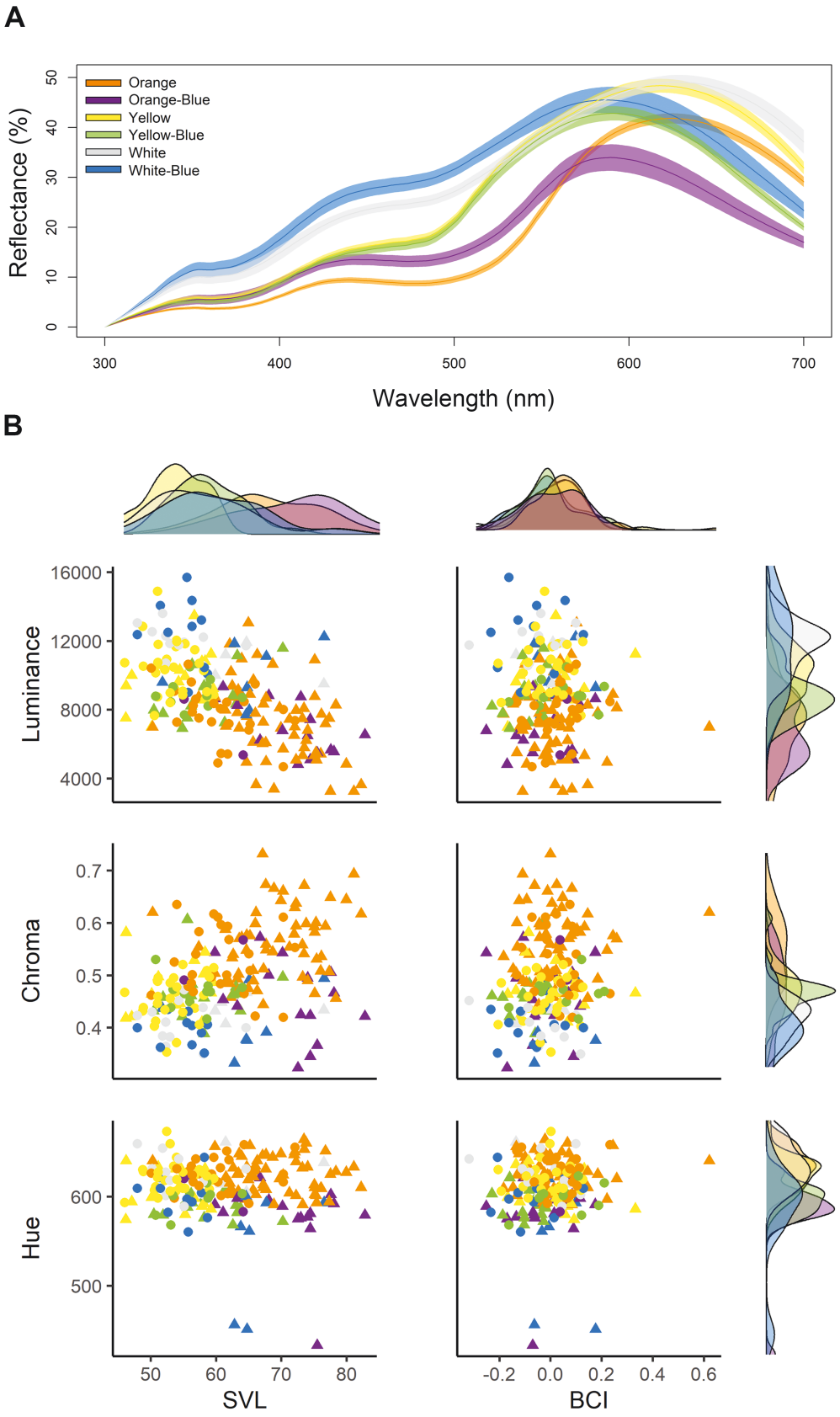


**Figure 2.** Spectral data of *Teira dugesii* ventral coloration, based on the groups defined by eye. A, reflectance spectra divided by colour class (the continuous lines indicate the average, and the shaded areas correspond to the SEM). B, relationship between snout–vent length (SVL) or body condition index (BCI) and the spectral variables (luminance, chroma, and hue); individuals are grouped by colour class. Males are represented as triangles and females as circles.

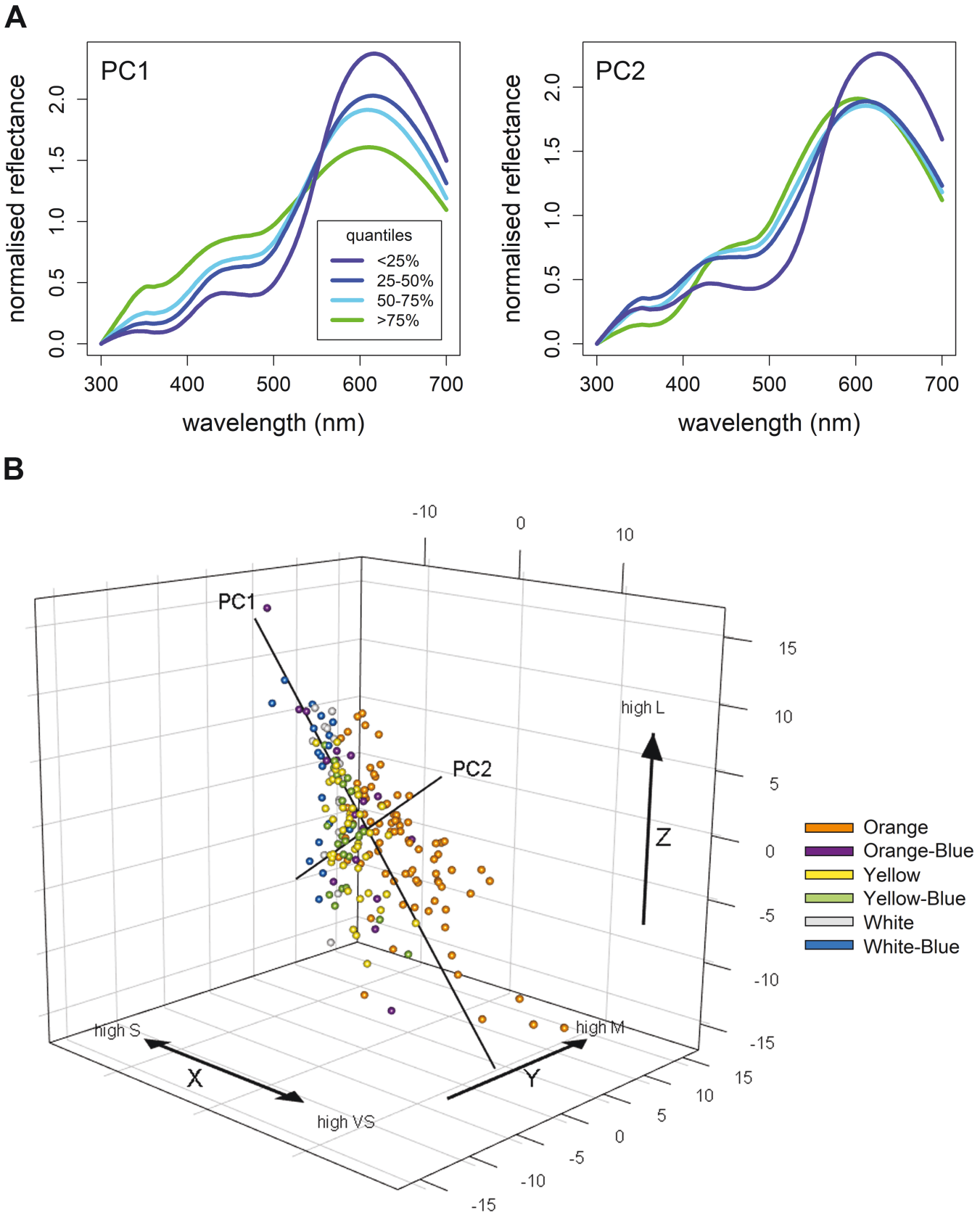


**Figure 3.** A, variability in the reflectance spectra based on the interquartiles of PC1 and PC2 calculated on the *xyz* coordinates. B, ventral chromatic variation of *Teira dugesii* individuals plotted on the visual space along with the axis of variation of PC1 and PC2. Colours correspond to the by-eye colour classes.

**Table 1.** Correspondence between the colour classes defined by eye and the clusters identified by the best and second-best models using a model-based approach on the first two principal components obtained from the *xyz* coordinates, associated with the degree of stimulation of the different cones.

| By-eye class | Best model | | Second-best model | | | Total |
|---|---|---|---|---|---|---|
| | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 3 | |
| Orange | 49 | 34 | 49 | 30 | 4 | 83 |
| Orange–blue | 10 | 7 | 10 | 7 | 0 | 17 |
| Yellow | 18 | 23 | 25 | 12 | 4 | 41 |
| Yellow–blue | 14 | 10 | 14 | 9 | 1 | 24 |
| White | 9 | 7 | 11 | 5 | 0 | 16 |
| White–blue | 11 | 7 | 9 | 8 | 1 | 18 |

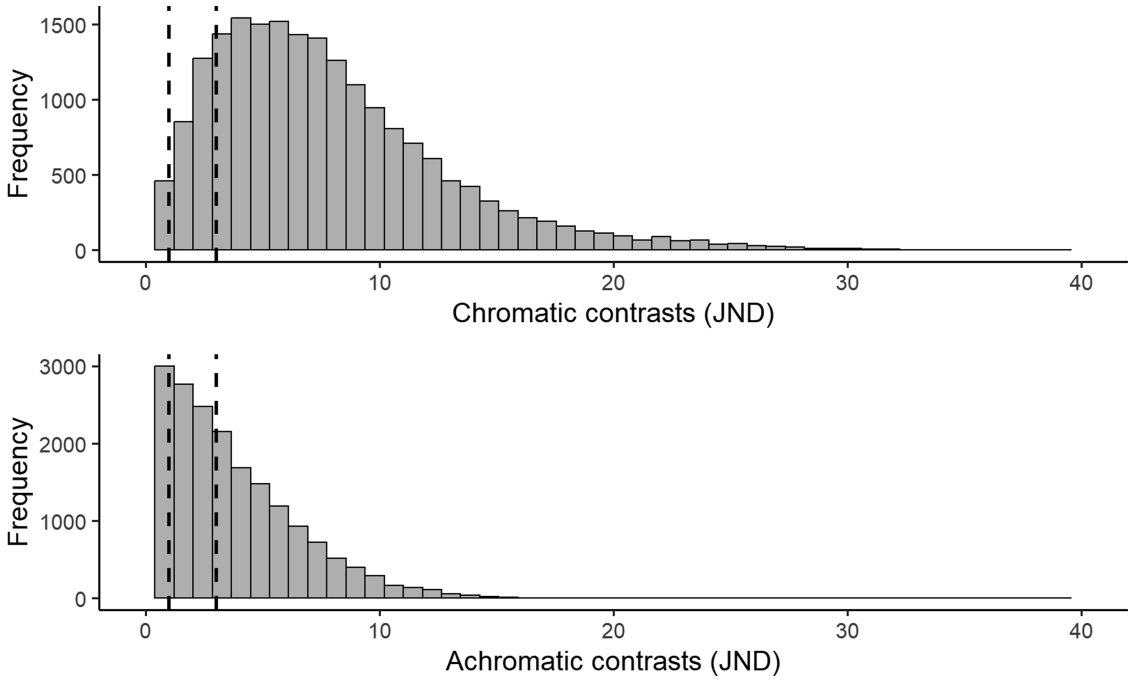


**Figure 4.** Chromatic and achromatic contrasts between the by-eye colour classes, estimated as perceptual units [just noticeable differences (JNDs)]. Vertical dashed bars indicate the visual thresholds corresponding to values of one and three JNDs.

found that populations also differed in their overall luminance ($\chi^2 = 25.829$, d.f. = 7, $P < .001$), chroma ($\chi^2 = 36.838$, d.f. = 7, $P < .001$) and hue ($\chi^2 = 27.202$, d.f. = 5, $P < .001$) (Supporting Information, Fig. S4). After controlling for differences between populations, we observed that colour variability in *T. dugesii* was explained by the combination of sex, SVL, and BCI (Fig. 2B). The proportion of by-eye colour classes differed between the sexes ($\chi^2 = 26.034$, d.f. = 5, $P < .001$). These differences were significant for orange ($P = .012$; 50.9% in males and 28.9% in females), orange–blue ($P = .035$; 12.9% in males and 2.4% in females), and yellow ($P = .012$; 12.9% in males and 31.3% in females), but not for yellow–blue ($P = 1$), white ($P = .066$), or white–blue ($P = .508$). Overall, by-eye colour classes also showed differences in SVL ($\chi^2 = 95.385$, d.f. = 5, $P < .001$; see Supporting Information, Table S3), but not in their BCI ($\chi^2 = 8.293$, d.f. = 5, $P = .144$). Interestingly, orange and orange–blue individuals tended to be bigger. We also observed that none of the comparisons between classes that differed only in the presence or absence of blue (i.e. orange vs. orange–blue, yellow vs. yellow–blue, and white vs. white–blue) were significant (Supporting Information, Table S3). For the tests on the spectral variables, we found that differences in luminance were explained by the interaction between SVL and sex ($\chi^2 = 6.750$, d.f. = 1, $P < .001$), but not by BCI ($\chi^2 = 1.994$, d.f. = 1, $P = .158$). Specifically, bigger animals and males tended to show lower luminance (i.e. darker coloration), whereas females and smaller individuals usually attained higher luminance values (i.e. brighter colours). We also found an effect of SVL ($\chi^2 = 13.576$, d.f. = 1, $P < .001$) and BCI ($\chi^2 = 7.145$, d.f. = 1, $P < .001$) on the chroma, whereby animals that were bigger and had higher body condition showed more saturated (pure) colorations. But neither the interaction between SVL and sex ($\chi^2 = 1.666$, d.f. = 1, $P = .197$) nor sex itself had a significant effect on the chroma ($\chi^2 = 1.585$, d.f. = 1, $P = .208$). For the hue, there was a significant effect of sex ($\chi^2 = 4.416$, d.f. = 1, $P = .036$), whereby

**Table 2.** Distance-based PERMANOVAs on the chromatic and achromatic contrasts between colour classes (O, orange; OB, orange–blue; W, white; WB, white–blue; Y, yellow; YB, yellow–blue). Pseudo *F*-statistics, effect size estimates ($R^2$) and *P*-values indicate whether colour classes can be discriminated by the visual model of *Teira dugesii*. Significant *P*-values ($P < .05$) after Bonferroni's correction are highlighted in bold.

| Comparison | Chromatic contrasts | | | | Achromatic contrasts | | | |
|---|---|---|---|---|---|---|---|---|
| | *F* | $R^2$ | *P*-value | Adjusted *P*-value | *F* | $R^2$ | *P*-value | Adjusted *P*-value |
| O vs. OB | 4.093 | .04 | .026 | .390 | .112 | .001 | .885 | 1 |
| O vs. Y | 14.851 | .13 | .001 | **.015** | 22.646 | .186 | .001 | **.015** |
| O vs. YB | 8.807 | .067 | .001 | **.015** | 42.866 | .26 | .001 | **.015** |
| O vs. W | 6.72 | .06 | .003 | **.045** | 21.017 | .167 | .001 | **.015** |
| O vs. WB | 11.192 | .103 | .001 | **.015** | 23.338 | .194 | .001 | **.015** |
| OB vs. Y | 1.655 | .048 | .193 | 1 | 15.533 | .32 | .001 | **.015** |
| OB vs. YB | 3.707 | .062 | .009 | .135 | 25.585 | .314 | .001 | **.015** |
| OB vs. W | 2.602 | .063 | .051 | .765 | 14.093 | .265 | .001 | **.015** |
| OB vs. WB | 1.17 | .036 | .306 | 1 | 18.999 | .38 | .001 | **.015** |
| Y vs. YB | 15.049 | .209 | .001 | **.015** | 2.436 | .041 | .109 | 1 |
| Y vs. W | 11.002 | .216 | .001 | **.015** | 6.247 | .135 | .005 | .075 |
| Y vs. WB | .562 | .017 | .564 | 1 | .98 | .03 | .340 | 1 |
| YB vs. W | .12 | .002 | .946 | 1 | 2.484 | .038 | .088 | 1 |
| YB vs. WB | 9.479 | .147 | .001 | **.015** | 1.169 | .021 | .283 | 1 |
| W vs. WB | 6.908 | .154 | .003 | **.045** | 5.822 | .133 | .008 | .12 |

males showed lower hues in comparison to females, but there was no effect of the interaction between SVL and sex ($\chi^2 = .001$, d.f. = 1, $P = .972$), SVL ($\chi^2 = .199$, d.f. = 1, $P = .656$), or BCI ($\chi^2 = .519$, d.f. = 1, $P = .471$). These results were supported by the tests on the chromatic and achromatic contrasts, which indicated that differences in coloration between sexes were also significant (chromatic: $F = 7.539$, $R^2 = .037$, $P = .001$, adjusted $P = .001$; achromatic: $F = 13.924$, $R^2 = .066$, $P = .001$, adjusted $P = .001$). These differences were equal to 2.585 JNDs (confidence interval = 1.241–4.083) for the chromatic contrasts and 4.076 JNDs (confidence interval = 2.523–5.705) for the achromatic contrasts. In line with these results, we observed that two individuals recaptured after 2 years and 9 months showed a darker and more saturated coloration on the second capture (see Supporting Information, Fig. S5).

## DISCUSSION

Despite multiple historical and ongoing efforts to describe how animal coloration varies, we still lack a clear understanding of the underlying mechanisms that promote such variability in many species. This is particularly true for species in which the study of colour has been tackled using only subjective approaches based on human vision, often resulting in inadequate descriptions of phenotypic variation. Consequently, these classifications might not only be a poor proxy of the discrimination capabilities of conspecifics and predators but might also hinder the analysis of relevant biological aspects of coloration, such as the pigments and/or the structures involved in the production of colour. Here, by measuring the ventral reflectance of *T. dugesii* across the island of Madeira, we provide a comprehensive description, based on quantitative and perceptual-based evidence, of how ventral coloration varies in this species, and we identify some of the factors that contribute to such differences.

Colour classes defined by eye differed in luminance, chroma, and hue. However, and despite these differences, the proportion of individuals assigned to either class differed substantially between the three experienced lizard observers who scored the ventral pictures. This highlights the difficulty of classifying highly variable species, such as *T. dugesii*, into discrete classes (i.e. colour morphs), something that was already noted by Báez (1990), which serves as a word of caution for descriptions of putative polymorphisms based solely on by-eye colour classifications. This outcome was to be expected if coloration in *T. dugesii* was continuous, as observed when plotting the individuals included in this study in the visual space, where the by-eye colour classes overlapped widely. Supporting this, the observed overlap in the chromatic space was greater for *T. dugesii* when compared with the colour morphs of *P. muralis*. On the same line, Gaussian finite mixture modelling on the spectral data suggested clustering patterns that were not consistent with the by-eye classification. Although some degree of discordance would be expected even if coloration corresponds to different colour morphs (Davison *et al.* 2019), these results are more congruent with a highly continuous ventral colour variation in *T. dugesii*.

We also investigated whether the colour classes we defined could be discriminated by conspecifics and therefore serve as a proxy of other life-history traits that might be correlated with colour (e.g. alternative life-history strategies). These analyses had mixed results, because not all colour classes could be discriminated. For example, the following contrasts were not discriminable for the lizard visual model: orange vs. orange–blue, yellow vs. white–blue, and yellow–blue vs. white. Yet, the fact that some colours classes are hardly discriminable does not preclude that coloration might convey information to other individuals. Again, these results warn against the classification of individual coloration based on human vision alone. Instead, this approach should always be complemented with spectral data and/or full-spectrum camera photography (e.g. Davison *et*

*al.* 2019, Nokelainen *et al.* 2022). This is of special importance when coloration varies in a way that might not be perceived by humans but can still be detected by other species. For example, Nokelainen *et al.* (2022) found that visual models tuned to represent conspecifics and predators could distinguish the two genotypes responsible for the white coloration (i.e. *WW* and *Wy*) in the hindwings of a polymorphic moth. On the contrary, human observers could only differentiate the two white genotypes from the *yy* genotype, associated with the yellow coloration in this species (Nokelainen *et al.* 2022). This is especially common when coloration shows a great degree of variability in the near-ultraviolet region of the spectrum, where differences in reflectance would not be evident for humans (e.g. Li *et al.* 2008, Font *et al.* 2009, Ficarrotta *et al.* 2022).

Our results also indicate that the coloration of *T. dugesii* differed between populations. However, owing to the low sample size of some of the populations, this finding should be confirmed in future studies. Moreover, unlike other lacertids, inter-individual differences in the expression of ventral colour variation in *T. dugesii* are less likely to be tightly linked to genetic variation, because we found significant differences in coloration associated with sex, size, and body condition. Together, these results suggest an important role for ontogenetic, historical, and/or environmental effects on colour expression. Bigger animals tended to be darker (i.e. lower luminance) and show more saturated (i.e. purer) colorations. Moreover, the fact that orange and blue colours occur more frequently in large individuals is supported by observations by Baez (1990), who also detected differences in the proportion of each of the colour classes he described between juveniles and adults of *T. dugesii*. These results point to the progressive accumulation of pigments after a certain size and/or age. This phenomenon has been observed in other lizard species, such as *Liolaemus fitzingerii*, in which larger and older individuals present more melanized ventral patches (Escudero *et al.* 2016). If this was the case, differences between white, yellow, and orange colours of *T. dugessi* could be related to the accumulation of carotenoids and/or pterins in the skin (e.g. Andrade *et al.* 2019, Stuart-Fox *et al.* 2020). Changes in coloration could also be linked to size- and/or age-related differences in the nanostructure of the iridophore layer (e.g. the development of guanine cells; Zhang *et al.* 2023), as blue coloration seemed to be more frequent in bigger individuals. This variation might also be explained by the differential deposition throughout development of some pigment, such as melanin (Bagnara *et al.* 2007, Shawkey and D'Alba 2017, Zhang *et al.* 2023). In fact, these mechanisms are not mutually exclusive, because the complex coloration of *T. dugesii* might originate from the interaction between different chromatophore layers. Given the lifespan records for *T. dugesii*, which suggest that they can live for at least 41 years in captivity (Magry and Heitmans 2021) and 16 years in the wild (Jesus 2012), the effect of ontogeny on the expression of colour is likely to be very important. Individuals with higher body condition also showed a more saturated coloration, which might indicate that coloration could act as a social signal (Peters *et al.* 2008, Megía-Palma *et al.* 2022). Yet, whether this correlation arises owing to differences in pigment allocation or structural coloration is unclear, since both colour-producing mechanisms can convey information about body condition in lacertids (Megía-Palma *et al.* 2016, 2022). Finally, sex had an effect on luminance and hue, whereby females tended to show higher luminance and lower hue. Overall, these differences were enough to be discriminated by lizards, possibly pointing to an effect of sexual selection in the evolution of *T. dugesii* coloration. This variability could be mediated by hormonal differences (Lindsay *et al.* 2016, Fresnillo *et al.* 2019). For example, Rankin and Stuart-Fox (2015) demonstrated that females of *Ctenophorus decresii* closely resembled male coloration after testosterone supplementation.

## CONCLUSION

Collectively, our results indicate that ventral coloration of *Teira dugesii* varies continuously and that such variability is affected by differences in size, sex, and body condition. Moreover, ventral coloration in *T. dugesii* might also vary seasonally (in den Bosch 1991, 1992, Sleijpen 1996). Future studies should aim to characterize the scale and timing of colour change. Likewise, dissecting the genetic and cellular basis of coloration would help to elucidate the mechanisms that produce such extraordinary colour variability. Overall, the Madeiran wall lizard provides an excellent model for the understanding of animal coloration and, in particular, intraspecific continuous variation.

## SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

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## CONFLICT OF INTEREST

None declared.

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## DATA AVAILABILITY

Data supporting the results have been deposited in Figshare (DOI: https://doi.org/10.6084/m9.figshare.27095854.v1).

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