

Distinguishing discrete polymorphism from continuous variation in throat colour of tree lizards, *Urosaurus ornatus*

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Within population colour variation in animals is ubiquitous and can range from discrete polymorphism to continuous variation. Distinguishing discrete polymorphism from continuous variation can be challenging, and this hinders our ability to test hypotheses about colour variation. We tested whether throat colour variation in the ornate tree lizard [*Urosaurus ornatus* (*U. ornatus*); Baird & Girard, 1852] is discrete or continuous using photographs of 617 individuals from seven study sites in the Chiricahua Mountains of Arizona, USA. Using principal component analyses on ten colour variables derived from photographs, we found throat colour to be highly repeatable for both males and females. Cluster analyses suggested that there are different groups of individuals based on their throat colours in males and in females, but distinguishing between the groups was difficult due to significant overlap in colour. Therefore, it appears that there is a significant amount of continuous variation in both male and female tree lizard throat colours. We suggest quantifying trait variation before assuming a colour trait is discrete. By using numerical descriptors of colour, more information is retained than by using discrete groups. Quantifying individual variation in colour is important for linking colour with other traits such as reproductive strategy, immune function, and size and for testing hypotheses about the evolution and maintenance of colour polymorphism.

ADDITIONAL KEYWORDS: alternative strategies – colour morphs – photograph – reptile.

INTRODUCTION

Within population colour variation is widespread in animals (Thompson & Moore, 1991; Hill *et al.*, 1994; Sinervo & Lively, 1996; Blouin-Demers *et al.*, 2013) and ranges from discrete polymorphism to continuous variation. Discrete colour morphs with correlated traits, such as behaviour, reproductive tactics, and body size, have been reported in a wide variety of taxa (Knapton & Falls, 1983; Sinervo & Lively, 1996; Knapp *et al.*, 2003; McKinnon & Pierotti, 2010). For example, the Gouldian finch (*Erythrura gouldiae*) exhibits two genetically determined head colour morphs (red and black) that differ in aggression and in immune response because the morphs differ in their endocrine responses and in their sensitivity to conspecific density (Pryke *et al.*, 2007). Other colour traits vary continuously between individuals of a population (Hill & Montgomerie, 1994; Salvador *et al.*, 1996; Weiss,

2006). For instance, female striped plateau lizards (*Sceloporus virgatus*) display significant variation in the size and intensity of their orange throat patch, and patch size and colour are correlated with clutch size and parasite load (Weiss, 2006). The cause and evolutionary maintenance of colour variation between individuals of a population have long been of interest to ecologists and evolutionary biologists (Sinervo, Bleay & Adamopoulou, 2001; Roulin, 2004; Gray & McKinnon, 2007).

Interindividual colour variation can be under genetic control, environmental control, or a combination of both (Gray & McKinnon, 2007; Hubbard *et al.*, 2010). Discrete colour polymorphism is the presence of at least two genetically determined colour morphs within a population (Ford, 1945). Multiple colour morphs are a source of genetic diversity in populations, and can have several ecological consequences, including decreased intraspecific competition (Smith & Skulason, 1996), increased abundance, and increased resilience to extinction (Forsman *et al.*, 2008). Continuous colour

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variation between individuals can be under genetic control. For example, plumage colour in tawny owls (*Strix aluco*) is highly heritable, ranges from grey to reddish brown, and has adaptive value (Brommer, Ahola & Karstinen, 2005). Continuous colour variation between individuals can also be under environmental control, in which case colour could indicate between-individual differences that are correlated with fitness (Wilson & Nussey, 2010). For example, male house finch (*Haemorrhous mexicanus*) plumage brightness signals nutritional status because brightness is a function of diet-derived carotenoids deposited in feathers (Hill & Montgomerie, 1994; Hill *et al.*, 1994). In several other species, colour patch size and intensity are correlated with body condition, clutch size, and immunocompetence (Weiss, 2006). Colour patches may thus provide honest signals of individual quality (McGraw & Ardia, 2003).

For many species that display variation in colour, it is unclear whether the colour trait is discrete or continuous. This is a serious shortcoming because testing hypotheses about how other phenotypic traits are related to colour, and about how colour variation is maintained evolutionarily, requires knowing whether the trait is constrained to a finite number of unordered values (discrete) or whether it is continuous (Roulin, 2004; Cote *et al.*, 2008). In instances where colour is a true discrete polymorphism, an individual's genotype can be inferred from its phenotype (Sinervo, Svensson & Comendant, 2000; Sinervo *et al.*, 2001; Corl *et al.*, 2010). In instances where colour is a continuous trait and in instances where colour is under environmental control, however, an individual's genotype cannot be inferred from its phenotype (Cote *et al.*, 2008). Several mechanisms, such as frequency-dependent selection, assortative mating, or disassortative mating, can explain the evolutionary maintenance of discrete colour polymorphism (Gray & McKinnon, 2007). If individuals cannot be assigned reliably to a genotype based on their phenotype, then these alternate mechanisms cannot be tested without genetic data. The maintenance of continuous colour variation often depends on sexual selection or on variation in resources and the traits related to resource acquisition (Salvador *et al.*, 1996). Testing whether colour variation is discrete or continuous can inform whether individuals can be accurately assigned a genotype based on their phenotype, and can help identify possible mechanisms that maintain colour variation. A more complete understanding of the genes associated with colour polymorphism requires data from breeding experiments to measure heritability and genome wide associations.

There are methodological challenges associated with quantifying colour variation and with distinguishing discrete from continuous variation. There are cases

where colour polymorphism was deemed discrete and where phenotype was used to infer genotype (Vercken *et al.*, 2007; Vercken, Sinervo & Clobert, 2008) that were later deemed to represent continuous colour variation (Cote *et al.*, 2008). Colour is usually measured in one of three ways: qualitative scoring by researchers (Carpenter, 1995; Hews *et al.*, 1997; Corl *et al.*, 2010), spectrophotometry (Cogliati, Corkum & Doucet, 2010; Lattanzio, Metro & Miles, 2014), or analysis of photographs (Stevens *et al.*, 2007; Teasdale, Stevens & Stuart-Fox, 2013). Qualitative scoring is useful when there is a marked discrete polymorphism easily detected with the human visual range. However, this method is unreliable if there are subtle differences between colours or if there are signals outside the human visual range (for instance, flowers that reflect ultraviolet light; Chittka *et al.*, 1994). Spectrophotometry allows colours to be measured quantitatively and within the spectrum of wavelengths that the receivers perceive. One of the criticisms of using spectrophotometry, however, is that fine-scale transitions between patches of different colours could be missed (Vercken *et al.*, 2008); analyzing high-quality photographs can overcome this difficulty by examining differences in colour for each pixel. If the colour trait of interest reflects wavelengths only within the human visual range, then analyzing photographs proves to be an effective method for measuring colour variation.

Lizards are ideal to quantify colour variation because their mating and social systems often involve visual communication (Fleishman *et al.*, 1997), and many species display marked intra- and inter-population variation in colour (Hews *et al.*, 1997; Teasdale *et al.*, 2013). However, lizards may vary continuously in wavelength reflected, or they may occur in discrete morphs as a result of different alleles in a single gene or in a group of tightly linked genes (Sinervo & Lively, 1996). The ornate tree lizard, *U. ornatus*, displays a variety of throat colours that are used in visual signaling between competitors and between potential mates (Fig 1). Both males and females display throat colour variation, which has been assigned to discrete morphs (Zucker & Boecklen, 1990; Thompson & Moore, 1991; Lattanzio & Miles, 2014), but colour variation has not been quantified to test this assertion formally. Spectrophotometry indicated that throat colour patches of *U. ornatus* do not reflect significant amounts of light in the ultraviolet range (Lattanzio *et al.*, 2014). Our objectives were to develop a method for quantifying individual variation in lizard throat colour using photographs, to determine whether colour variation is repeatable, and to test whether tree lizard throat colour variation is discrete (as assumed by previous authors) or continuous.

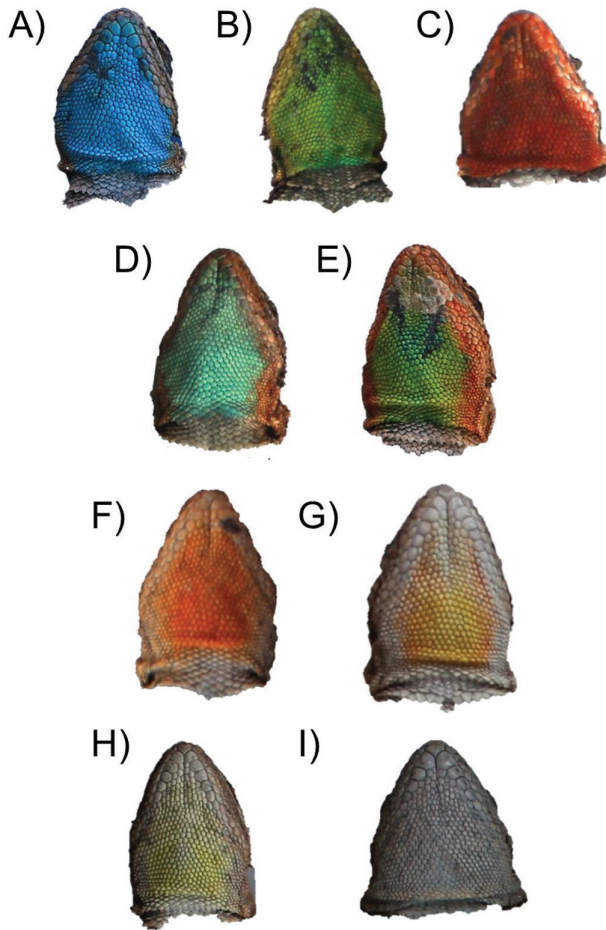


Figure 1. *Urosaurus ornatus* colour morphs from the Chiricahua Mountains of Arizona, USA categorized into (A) blue, (B) green, (C) orange, (D) orange-blue, and (E) orange-green for males, and (F) orange, (G) orange-yellow, (H) yellow, and (I) white for females.

METHODS

PHOTOGRAPHS

U. ornatus (317 males and 300 females) were captured by noose from seven study sites in the Chiricahua Mountains in southeastern Arizona, USA from May to August in 2014 and 2015. The study sites were in similar habitats along riparian corridors and were separated by a maximum of 16 km. All work was approved by the University of Ottawa Animal Care Committee (protocol BL-2300) and was conducted with scientific collecting permits from the Arizona Fish and Game Department (SP674341 and SP713940). We used a medical cauterizer to give lizards a unique mark (Ekner *et al.*, 2011), and we photographed their throat colour patch in the field with a light background at ambient temperature and lighting conditions (without

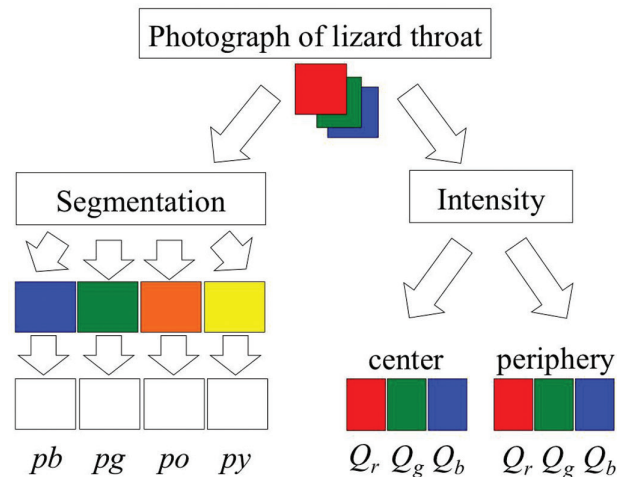


Figure 2. Schematic diagram of steps to extract the ten quantitative variables used to analyze photographs of *Urosaurus ornatus* throat colour from the Chiricahua Mountains of Arizona, USA.

a flash) with a Canon Rebel EOS T3 DSLR camera and a 18–55 mm lens. The lighting and temperature conditions represent the range in which lizards are active and engage in intraspecific signaling. Based on visual inspection in the field, male lizards were classified into discrete throat colour morphs: blue, green, orange, orange-blue, or orange-green (Fig. 1A–E). Morphs with two colours had an outer perimeter of orange with a central patch of either blue or green. Female lizards were classified as: orange, orange-yellow, white, or yellow. Orange-yellow females had an outer perimeter of orange with a central white patch (Fig. 1F–I).

LINEARIZING AND EQUALIZING COLOUR CHANNELS

To use photographs for the analysis of colour, a digital camera's three sensors (corresponding to red, green, and blue wavelengths) should respond linearly and equally to increases in light intensity (Stevens *et al.*, 2007). We tested this by photographing colour cards with grey standards of varying reflectance (X-Rite ColorChecker Passport) in the field under the same lighting conditions as the lizard photographs. We constructed linearization equations (Stevens *et al.*, 2007; Bergman & Beehner, 2008; White *et al.*, 2015) based on 102 measurements of grey colour cards ranging in reflectance from 3 to 90%:

$$Q_r = a_1 * (b_1^r) \quad (1)$$

$$Q_g = a_2 * (b_2^g) \quad (2)$$

$$Q_b = a_3 * (b_3^b) \quad (3)$$

Where Q is the known reflectance value from the grey colour standard, a and b are constants (for each camera sensor), and r , g , and b are the pixel scores from the camera's three sensors (red, green, and blue). Grey colour standards are designed to reflect all wavelengths equally. The RGB colour scores were extracted from photographs using the software ImageJ (Schneider, Rasband & Eliceiri, 2012). Using these data, we fit equations with the *nls* function in R (R Core Team, 2015) to estimate a and b for each colour sensor. We confirmed that linearized pixel scores (Q) of grey colour standards were equal in each sensor using paired t -tests. All photographs of lizards were then linearized and equalized using eqn. [1–3] before further analyses.

SEGMENTATION AND QUANTIFYING COLOUR VARIATION

To quantify individual colour variation, we measured the proportion of the throat of each lizard covered by each colour and the intensity of colour for two zones of the patch (Fig. 2). To obtain the proportion of different colours in lizard throats, we converted linearized photographs into segmented black-and-white photographs (Teasdale *et al.*, 2013) that represent the colours displayed by our *U. ornatus* populations. The distinct colours represented in our populations were: blue, green, orange, yellow, and white. To determine threshold limits for these colours, we used pixel scores from 25 to 35 measurements of each colour from linearized photographs of lizard throats. Instead of the raw linearized pixel scores from each sensor, we used the proportion of that sensor's value relative to the other sensors (Teasdale *et al.*, 2013) to standardize for lighting intensity [e.g., proportion blue: $pb = b/(r + g + b)$ for each pixel]. We created a classification tree using the R package *rpart* (Therneau *et al.*, 2015) to classify pixels into each colour using these proportions. The partitioning was highly accurate at separating pixel colours (classification error = 2.7%). The splits from this partitioning analysis were used to segment each lizard photo into four black-and-white photos that represented blue, green, orange, and yellow pixels. The proportion of white was not measured and was assumed to comprise the remainder of the throat not assigned to any of the other colours. In each segmented photo, all pixels were assigned 1 or 0. For example, in the blue segmented photo all blue pixels were 1, and all others were 0. Finally, the proportion of a lizard's throat composed of each colour was determined using the software ImageJ (Schneider *et al.*, 2012) by counting the number of pixels that were 1 for each segmented photograph and by dividing by the total number of pixels.

We also measured the colour intensity of throat patches in two zones of 0.25 cm² for every lizard. The mean pixel scores of each colour channel (Q_r , Q_b , and Q_g) on linearized photos were measured using ImageJ

at the center of the throat and at the posterior left side of the throat. These two zones captured variation in colour expression between individuals. Therefore, for each lizard photograph, we had ten variables to describe throat colour: four proportions to describe the relative size of each colour, three intensity scores from the center of the throat patch, and three intensity scores from the periphery of the throat patch.

PRINCIPAL COMPONENT ANALYSIS OF COLOUR VARIATION

To derive a composite measure of colour variation, we conducted a principal component analysis (PCA) on the variables describing lizard throat colour variation. For males, we used all ten variables. For females, we only used eight variables because no individuals displayed significant blue (maximum 6% of throat) or green (maximum 11% of throat). Variables were scaled to have a mean of 0 and an SD of one to account for uneven variance prior to conducting the PCA.

REPEATABILITY OF COLOUR MEASUREMENTS

To assess repeatability of colour measurements, we used a subset of photographs of lizards that were recaptured between 2 weeks and 1 year apart. We analyzed males ($n = 77$ lizards) and females ($n = 71$ lizards) separately because they display different colours. We assessed repeatability of the throat patch colour variables with intraclass correlation coefficients (ICC) that compared within individual variation to between individual variation (Lessells & Boag, 1987). Since we had ten colour variables for each photo, we calculated ICCs using the first two axes of the PCA. To test whether the assignment of individuals to morphs was repeatable, we also assigned individuals to morphs using photographs, unaware of their initial morph assignment in the field. We compared whether field and photograph morph assignments were consistent and whether individuals were assigned to the same morph in the field on their two capture occasions.

IS COLOUR EXPRESSION SIMILAR BETWEEN SITES?

We ensured lizard throat colour variation was similar at each of the seven study sites. We used ANOVAs with PC1 and PC2 as dependent variables and colour morph, site, and the interaction between colour morph and site as independent variables. If throat colour expression differed between sites, we predicted there would be a significant effect of site or an interaction between site and colour morph on PC1 or PC2. We conducted separate analyses for males and females because they differed in the range of colours expressed. For the analysis of males, we excluded orange individuals ($n = 5$),

green individuals ($n = 21$), and one study site ($n = 8$) because there was inadequate replication to estimate coefficients. For females, we excluded one study site ($n = 4$) because there was inadequate replication to estimate coefficients.

ARE THE COLOUR MORPHS DISCRETE?

We used linear discriminant function analyses (DFA) to determine whether the morphs identified in the field could be distinguished based on quantitative throat colour variables. Separate DFAs were constructed for males (ten colour variables) and females (eight colour variables) because the morphs assigned in the field differed by sex. In addition, we also performed support vector machine (SVM) classification using tuned parameters for cost and gamma (e1071 package; Meyer *et al.*, 2014) to verify whether individuals could be classified into colour groups. We again conducted separate analyses for males and for females. We computed correct classification rates for DFA and SVM as a measure of how well morphs identified in the field could be distinguished based on quantitative throat colour variables. Both SVM and DFA maximize the distances between group centroids based on a priori groupings, but identifying the various morphs a priori is subjective. Therefore, we also conducted cluster analyses on the numerical colour variables to identify morphs objectively a posteriori. We used the *Mclust* function (Fraley & Raftery, 2002) to create clusters using normal mixture models and the Bayesian Information Criterion (BIC) to determine the optimal number of clusters and their structure with the default models of variance and covariance (Fraley & Raftery, 2002). The model with the highest BIC was considered the most supported. We again conducted separate cluster analyses for males and females.

RESULTS

PRINCIPAL COMPONENT ANALYSIS OF COLOUR VARIATION

For males, the first two components of the PCA explained 74% of the variance in throat colour (Fig. 3A). The first component (PC1) had heavy variable loadings for the proportion of the throat that was blue, and for the pixel scores for the blue sensor (Q_b) in the center and at the periphery of the throat (Table 1). Therefore, males with more blue in their throats had higher PC1 scores. The second component (PC2) had high variable loadings for the proportion of the throat that was orange, pixel scores for the red sensor (Q_r) in the center and at the periphery of the throat, and negative loadings for the proportion of the throat that

was green and the pixel scores for the green sensor (Q_g). Therefore, males with low PC2 scores had more green throats, and males with high PC2 values had more orange throats (Fig. 3A).

For females, the first two components of the PCA explained 83% of the variance in throat colour. The variables with the heaviest loadings for PC1 were proportion of the throat that was orange and the pixel scores for the red sensor (Q_r) in the center and at the periphery of the throat (Table 1). The variables with the heaviest loadings for PC2 were proportion of the throat that was yellow and the pixel scores for the green sensor (Q_g) in the center and at the periphery of the throat (Table 1). Generally, females with higher PC1 scores had more orange throats and females with higher PC2 scores had more yellow throats (Fig. 3B).

REPEATABILITY OF COLOUR MEASUREMENTS

Repeatability (ICC) estimates were high for principal components describing colour variation in throat patches. For males, the estimates were 0.83 and 0.74 for PC1 and PC2, respectively. For females, ICC estimates were 0.65 and 0.76 for PC1 and PC2, respectively. Field and photograph morph assignment was consistent in 86% (356/413) of cases for males and 77% (298/388) of cases for females. For males, 89% (49/55) of the inconsistent cases involved distinguishing blue from orange-blue individuals. For females, 56% (55/90) of inconsistent cases involved distinguishing orange-yellow from orange or yellow. For individuals with at least two captures, 4% (3/77) of males and 31% (22/71) of females were classified as a different colour morph when recaptured. Therefore, colour measurements from photographs were repeatable between captures for individual lizards, but categorical morph assignment was not always consistent between field and photograph assessment, or between captures of the same individual in the field.

IS COLOUR EXPRESSION SIMILAR BETWEEN SITES?

In males, there was no significant effect of site ($F = 0.671$, d.f. = 5, 361, $P = 0.646$) and no interaction between site and colour morph ($F = 0.543$, d.f. = 10, 361, $P = 0.859$) on PC1. There was no effect of site ($F = 0.610$, d.f. = 5, 361, $P = 0.693$), but there was a significant interaction between colour morph and site on PC2 ($F = 5.768$, d.f. = 10, 361, $P < 0.001$). The interaction was driven by two individuals with orange-green throats at one site. When these two individuals were removed, the interaction was no longer significant ($F = 1.765$, d.f. = 10, 359, $P = 0.066$). In females, there was a significant effect of site ($F = 6.185$, d.f. = 5, 360, $P < 0.001$) and no significant interaction between colour morph and site

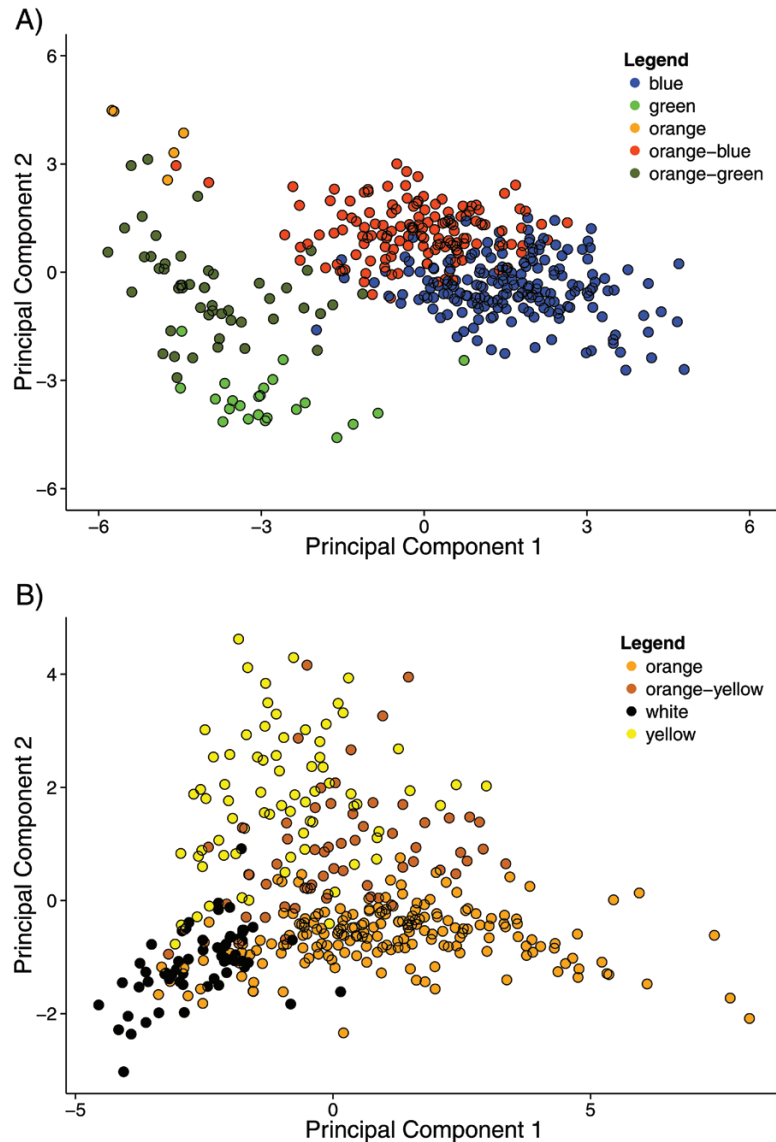


Figure 3. Biplot of principal component analyses on throat colour variation in *Urosaurus ornatus* from the Chiricahua Mountains of Arizona, USA based on photographs of (A) males ($n = 413$ captures of 317 individuals), and (B) females ($n = 388$ captures of 300 individuals) and coded by their assignment to throat colour morphs in the field.

on PC1 ($F = 1.374$, d.f. = 15, 360, $P = 0.157$). The site effect arose because one site had lower PC1 scores than the other sites, indicating there may be different frequencies of throat colour morphs between sites. The non-significant interaction indicates that throat colour morphs had similar quantitative colour expression between sites. There was no significant effect of site ($F = 1.540$, d.f. = 5, 360, $P = 0.177$) and no significant interaction between throat colour morph and site on PC2 ($F = 1.374$, d.f. = 15, 360, $P = 0.157$). Overall, therefore, we believe that throat patch colours were similar at each site for males and for females, and that our conclusions drawn from

pooling individuals from different sites are thus robust to variation between these sites.

ARE THE COLOUR MORPHS DISCRETE?

The DFA for males correctly classified 88% (363/413) of lizards to their colour morph designation in the field. The SVM classification (using a cost of 0.5 and a gamma of 0.125) for males correctly classified 91% (375/413) of lizards to their colour morph designation in the field. The morph with the highest misclassification rate was orange-green (16% of cases misclassified with DFA). For the male cluster analysis, the best

Table 1. Variable loadings for the first two components from principal component analyses on variables describing throat colour patches for male ($n = 413$ captures of 317 individuals) and female ($n = 388$ captures of 300 individuals) *Urosaurus ornatus* from the Chiricahua Mountains of Arizona, USA.

Variable	Males		Females	
	PC1	PC2	PC1	PC2
Proportion blue	0.39	0.13	–	–
Proportion green	–0.30	–0.37	–	–
Proportion orange	–0.30	0.36	0.85	–
Proportion yellow	–0.32	–0.34	–	0.70
Center Q_r	–0.36	–0.14	0.42	–
Center Q_g	–0.14	–0.19	–0.30	0.49
Center Q_b	0.38	0.22	–0.38	–0.28
Periphery Q_r	–0.34	0.39	0.41	0.18
Periphery Q_g	0.20	–0.55	–0.24	0.23
Periphery Q_b	0.36	–0.21	–0.38	–0.32

Table 2. Cluster assignments of *Urosaurus ornatus* from the Chiricahua Mountains of Arizona, USA based on the best normal mixture models of throat colour variation for males (three clusters; $n = 413$ captures of 317 individuals) and females (four clusters; $n = 388$ captures of 300 individuals) compared to their assignment to throat colour morphs in the field.

Throat colour		Cluster			
		1	2	3	4
Males	Blue	125	0	64	–
	Green	0	22	1	–
	Orange	0	5	0	–
	Orange-blue	83	2	61	–
	Orange-green	0	45	5	–
Females	Orange	145	27	3	17
	Orange-yellow	6	9	1	47
	White	0	57	0	3
	Yellow	2	4	1	70

model had three clusters with varying volume, shape, and orientation (BIC = 5653.29). This model had considerably more support than the best model with two (BIC = 5314.19) or four clusters (BIC = 5551.21). The three identified clusters in the best mixture model for males corresponded to individuals with (1) mostly blue throats, (2) blue and orange throats, and (3) orange, orange-green, and green throats (Table 2).

The DFA for females correctly classified 79% (310/388) of lizards to their colour morph designation in the field. The SVM classification (using a cost of 1 and a gamma of 0.125) for females correctly classified

82% (318/388) of lizards to their colour morph designation in the field. For the female cluster analysis, the best model had four clusters with varying volume, shape, and orientation (BIC = 4403.73). This model had more support than the best model with two (BIC = 4014.55) or three clusters (BIC = 4269.73). The identified clusters corresponded to individuals with throats that were (1) very orange, (2) pale orange or white, (3) orange and yellow, and (4) yellow (Table 2).

DISCUSSION

Analyzing tree lizard throat colour with photographs and PCA was very effective at quantifying inter-individual variation. Colour was highly repeatable (ICC > 0.74 for males, > 0.65 for females) between captures of the same individual. These estimates of colour repeatability were comparable to those of other species, including an agamid lizard (*Ctenophorus ornatus*; 0.65–0.91) and Green Lizards (*Lacerta viridis*; 0.89) using spectrophotometry (LeBas & Marshall, 2000; Vaclav, Prokop & Fekiac, 2007). The high repeatability of throat colour measurements, including captures more than a year apart, suggests that colour traits are fixed within individual *U. ornatus*. Using a numerical description of colour is objective and captures more variation between individuals than just morph assignment. Capturing more subtle variation between individuals is important for examining genetic and environmental influences on colour and for testing hypotheses about how colour is related to other phenotypic traits.

The DFA, SVM, and cluster analysis suggested there are discrete male morphs, but distinguishing them is difficult due to significant overlap in colour between morphs. The DFA (88% accurate) and SVM (91% accurate) were relatively accurate at classifying individuals into pre-determined morphs, and the cluster analyses suggested there was more than one morph (three clusters). However, the three morphs identified in the cluster analysis of males did not correspond to pre-determined morphs because one cluster included most green, orange, and orange-green individuals. These three morphs may not have been distinguished because of the limited number of individuals in each morph. Cluster analyses are less likely to detect morphs as group size decreases (de Craen *et al.*, 2006). There were only five individuals in the orange morph, and these individuals ended up in a cluster with the other less common morphs (green and orange-green). Correctly identifying rare morphs with cluster analyses will always be problematic.

Female colour displayed more continuous variation between individuals than male colour (Fig. 3B). The cluster analysis for females identified four morphs

that broadly corresponded to the pre-determined morphs of orange, orange-yellow, white, and yellow. However, identifying the boundaries of female morphs in parameter space was difficult and resulted in a higher rate of misclassification in the DFA and SVM classification for females. In the DFA, there was a 21% misclassification rate overall, but a 52% misclassification rate of orange-yellow females that lie in the center of the parameter space (Fig. 3B). In the SVM, there was an 18% misclassification rate overall, which demonstrates that continuous variation between morphs is real and not a consequence of the type of classification analysis used to distinguish between morphs. The relatively high number of females that were classified into different morphs when they were recaptured (28%) also suggests that female colour displays significant continuous variation, and that distinguishing between supposedly discrete morphs in the field may be unreliable.

Throat colour in male *U. ornatus* is related to several other phenotypic traits. For example, colour influences dominance (Carpenter, 1995), trophic niche (Lattanzio, Miles & Lattanzio, 2016), and home range size (Moore, Hewst & Knapp, 1998) in other populations. However, those studies assumed throat colour to be discrete and have not considered the possibility of continuous variation in colour. Our data indicate significant continuous variation in several aspects of male throat colour, including colour intensity and the proportion of the throat patch of different colours. Therefore, we suggest that predictions about how colour relates to other traits would best be tested with numerical descriptors rather than with discrete categories. For instance, because of the known behavioural differences between individuals with different colours, individuals with different colours likely occupy different habitats. We predict that males with more blue (higher PC1) or green (lower PC2) and less orange should secure home ranges in better habitats because they are more dominant (Carpenter, 1995).

There is little known about how female tree lizard throat colour relates to other phenotypic traits. Some populations of tree lizards are monomorphic for female throat colour (Zucker & Boecklen, 1990), but at all of our study sites females varied from white to yellow and orange. In a related species [*Uta stansburiana* (*U. stansburiana*)], female throat colour is related to alternative reproductive strategies where morphs invest in offspring differently and their success fluctuates with density (Sinervo *et al.*, 2000, 2001). Recently, behavioural trials have shown that orange and yellow throated female tree lizards respond differently to courting males (Lattanzio *et al.*, 2014). However, in previous studies authors again assumed that colour was discrete and scored the trait with a small number of discrete values. The continuous variation between

white, orange, and yellow we observed in the present study suggests that female *U. ornatus* throat colour cannot be reliably assigned to one of a few discrete morphs.

Quantifying variation in colour is difficult because it may represent multiple phenotypic traits. The reflection of different wavelengths is caused by different cellular mechanisms, so it is possible that different genes or condition-dependent factors influence them. For example, blue is typically a structural colour caused by the scattering of light in iridiophores (Bagnara, Fernandez & Fujii, 2007), while orange and yellow are usually from carotenoid or pteridine pigments in xanthophores (Evans & Sheldon, 2014), and green is caused by a combination of cells that scatter light and contain yellow pigment (Bagnara *et al.*, 2007). Typically, carotenoid pigments are acquired through the diet and are thus influenced by the environment (Hill *et al.*, 1994), while pteridine pigments can be synthesized and expression is thus not influenced by diet (Hurst, 1980). Although the cellular basis of throat colour has not been examined in *Urosaurus*, both pteridines and carotenoids have been found in the throat colour patches of *U. stansburiana*, a closely related species (Haisten *et al.*, 2015). In addition, colour may vary continuously in one aspect, such as the proportion of blue on the throat, but vary discretely in another aspect, such as the presence of green. Considering the various mechanisms underlying colour expression, we expect continuous variation in colour to be common. Therefore, we suspect that quantifying different aspects of coloration as we did here will represent reality better than classification in a few discrete colour morphs, and will thus allow better tests of the various hypotheses that have been proposed as explanations of the presence and of the evolutionary maintenance of colour polymorphisms.

There are several promising lines of research to uncover the underlying causes of colour expression in *U. ornatus* and other lizards. First, genetic data from individual lizards could be used to test whether there is a link with colour expression, ideally using nuclear markers. Second, experimental crosses of different colour phenotypes, and in which the parental and offspring throat colours are measured quantitatively, could be used to determine the heritability of the colour traits. Finally, histological sections of throat colour patches could be used to determine which aspects of colour are structural and which aspects are derived from the environment.

We used photographs to demonstrate that both male and female *U. ornatus* exhibit significant interindividual variation in throat colour that is not easily categorized into discrete morphs. Our cluster analyses suggest there could be discrete throat colour morphs in this species, but that their parameter boundaries are not easily

distinguished due to significant within morph variation in throat colour. Heritability estimates and genetic mapping would provide additional evidence of whether throat colour in tree lizards represents a discrete polymorphism or a continuous trait. Using PCA to summarize colour expression can be used to test how colour is related to other traits without having to collapse interindividual variation into a few discrete morphs. Considering the suite of factors that influence colour expression, it seems unlikely that a single gene (or a few tightly linked genes) is the only influence on colour expression. It appears more likely that there is significant continuous variation between individuals caused by some combination of genetic and environmental factors. We encourage researchers to quantify formally interindividual variation in colour expression before assuming that it represents a discrete trait.

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