The control of color change in the Pacific tree frog, *Hyla regilla*

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Abstract: A number of environmental variables have been identified as affecting anuran color, but rarely have the interactions between these variables been investigated. In attempt to elucidate the function of color change, we conducted a within-subject, full factorial experiment designed to determine the simple and interactive effects of background, temperature, and light intensity on the rate of color change in the Pacific tree frog (*Hyla regilla* Baird and Girard, 1852). Color was investigated holistically, as well as by decomposing it into its constituent parts (hue, chroma, and lightness), using digital photography. The rate of color change was faster on the green versus the brown background, at 10 versus 25 °C, and at low versus high light intensity. There was also a significant effect of the interaction between background color and temperature on the rate of color change. We found increased rates of hue, chroma, lightness, and color change with increasing initial hue, chroma, lightness, and color distances between the Pacific tree frog and its background, respectively. In addition, initial color distance covaried with changes in environmental variables. After controlling for initial color distance, and thus the effects of background matching, background color and temperature still showed a significant interaction for their effects on rate of color change. These results suggest that crypsis (i.e., background matching) is not the only function of physiological color change in *H. regilla*. Physiological color change may also be used to hydro- and (or) thermo-regulate.

Résumé : On sait que plusieurs variables de l'environnement affectent la coloration des anoures, mais on a rarement étudié les interactions entre ces variables. Afin de comprendre la fonction du changement de coloration, nous avons mené une expérience factorielle intra-groupe complète destinée à déterminer les effets simples et interactifs de l'arrière-plan, de la température et de l'intensité lumineuse sur le taux de changement de coloration chez la rainette du Pacifique (*Hyla regilla* Baird et Girard, 1852). La photographie digitale nous a permis d'étudier la coloration de manière holistique et aussi en la décomposant en ses parties constituantes (la teinte, la pureté et la brillance). Le taux de changement de coloration est plus rapide sur un arrière-plan vert que sur un brun, à une température de 10 °C plus qu'à 25 °C et davantage aux intensités lumineuses faibles qu'aux fortes. Il y a aussi une forte interaction entre la couleur de l'arrière-plan et la température sur le taux de changement de coloration. Les taux de changement de teinte, de pureté, de brillance et de coloration augmentent en fonction directe des différences respectivement de teinte, de pureté, de brillance et de coloration initiales entre la rainette et son arrière-plan. De plus, les différences initiales de coloration sont en corrélation avec les changements dans les variables de l'environnement. Une fois prise en compte la différence initiale de coloration, et ainsi les effets de l'harmonisation avec l'arrière-plan, la coloration de l'arrière-plan et la température montrent encore une interaction significative dans leurs effets sur le taux de changement de coloration. Ces résultats indiquent que le camouflage (i.e., l'harmonisation avec l'arrière-plan) n'est pas la seule fonction du changement physiologique de coloration chez *H. regilla*. Le changement physiologique de coloration pourrait aussi être impliqué dans l'hydrorégulation et (ou) la thermorégulation.

[Traduit par la Rédaction]

Introduction

Physiological color change (hereinafter referred to as color change) is a feature of many anurans whereby skin color changes rapidly because of a change in the relative state of pigment dispersion within pigment cells (Nery and Lauro Castrucci 1997). Although the high degree of variation across landscapes and between seasons in color morphologies for the Pacific tree frog (*Hyla regilla* Baird and Girard, 1852) has been suggested to be a result of biotic and abiotic selective pressures (Resnick and Jameson 1963; Jameson and Pequegnat 1971), the selective pressures and exact function of color change are still unclear. This is true not only for *H. regilla* but also for anurans in general. In

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anuran amphibians, color change may be used for avoiding predators (crypsis) in addition to water balance and (or) thermoregulation (Hoppe 1979; King and King 1991; King et al. 1994). It is likely that the most important aspect of color change is to increase cryptic value (Kats and Van Dragt 1986), but the relative importance of color change to hydro- and thermo-regulation is still unclear. This is due in part to the assumption that all frogs lose water as rapidly as a free water surface, which negates any potential effect of color on temperature (Tracy 1976; Stevenson 1985). Many anurans do lose water at the same rate as a free water surface, but a number of *Hyla* species have been shown to have comparatively low rates of water loss (Wygoda 1984, 1988). Thus, for these relatively impermeable species, color change may be important for thermoregulation in addition to background matching (Spotila et al. 1992). In general, there appears to be disagreement concerning what functions color change plays in anurans.

Although many abiotic factors may affect skin coloration, temperature, light intensity, and background have received the most attention as ecological determinants of color change (see King et al. 1994). Previous studies have looked at only one or two of these variables (e.g., Nielsen and Dyck 1978; King et al. 1994). Hence, many of the possible interactions among these three factors are still unknown. In a natural system, frogs are exposed to all variables simultaneously, so it is important to study their interactions to determine how these ecological factors work together to influence frog coloration. This knowledge will improve our understanding of the specific roles that color change may play in the ecology of anurans.

Another relatively unexplored area is the rate of color change. When a frog moves, it is more likely to be detected by predators than when it is stationary (Morey 1990), and stationary frogs that more closely match their background are less likely to be eaten by visual predators such as garter snakes and predatory birds (Tordoff 1980; Morey 1990). Therefore, rapid color change is most important during the few minutes after a frog becomes stationary following a change in microhabitat. This should be especially true when the contrast is high between the frog's color and that of its new habitat. Previous experiments on color change have typically examined only a frog's final color state. This approach does not allow us to understand the information-rich intermediate stages, which may be crucial to a frog's survival. For example, Kats and Van Dragt (1986) observed the rate of color (lightness) change to be proportional to the difference in color between *Hyla crucifer* (Wied-Neuwied, 1838) and its background. Hence, the rate of color change seems to be an ecologically significant yet largely unstudied component of color change.

Crypsis is defined as the matching between the color and pattern of an organism and a random sample of the background that it is viewed against, as perceived by another organism (Endler 1978). Thus, crypsis in terms of color is due to the combined effects of the constituent parts of color. These constituents are lightness (i.e., a measure of how bright a color is), hue (which tells one if the color is yellow or green, etc., and quantified as the color's angle on the 360° color circle), and chroma (i.e., a measure of how much gray is in the color). Similarly, changes in hue, chroma, and lightness may affect the temperature and (or) the water regime of an anuran owing to changes in absorbance of solar energy. Hence, it is logical to assume that all three components of anuran color should change simultaneously. However, most existing studies only examine whole color responses without looking into the constituent color components (e.g., Edgren 1954; Iga and Bagnara 1975) or they break color into its components without looking at whole color change (e.g., Nielsen 1979; Kats and Van Dragt 1986; King et al. 1994). Therefore, it is important to study the simple and interactive responses of color components in the context of whole-animal color change for a clear understanding of the ecological significance of color change.

Traditionally, human observation (e.g., Edgren 1954; Iga and Bagnara 1975; Kats and Van Dragt 1986) and reflectance spectrophotometry (e.g., Nielsen 1980; King and King 1991; King et al. 1994; Wente and Phillips 2003) have been the two methods employed in color change studies. Digital imagery overcomes the subjectivity of human observation and the invasiveness of reflectance spectrophotometry (Nielsen 1978*a*, 1978*b*; Zuk and Decruyenaere 1994) and is a reliable method (e.g., Villafuerte and Negro 1998; Gerald et al. 2001; Garcia and Sih 2003; Garcia et al. 2003). Yet, digital cameras capture light only in the human visible spectrum, and potential predators of *H. regilla* (e.g., birds and reptiles) may be sensitive to ultraviolet light beyond the human visible spectrum (Sillman et al. 1997; Honkavaara et al. 2002). Since perceived color is a result of the specific visual system of the perceiving organism (Endler 1978), this limitation of digital imagery may be problematic. Wente and Phillips (2003) have compared the spectral reflectance of brown and green *H. regilla* and found that these tree frogs show the same reflectance of ultraviolet light. Because *H. regilla* does not change color in the ultraviolet part of the spectrum, our study, although limited to the human visual spectrum, should produce robust color change patterns.

In this study, we hope to provide insight into whether color change is used only for background matching or if it has other functions such as temperature and (or) water regulation. To answer this question, we manipulated important abiotic variables simultaneously, examined the rate of color change, and investigated the constituent parts of color in addition to whole color via digital imaging techniques. Specifically, we investigated the individual and interactive effects of temperature, light intensity, and background on the rate of hue, lightness, chroma, and whole color change in *H. regilla*. We hope this approach will bring new insights and greater clarity to the function of physiological color change.

Methods

We used 20 adult *H. regilla* collected from Englehorn Pond in Ellensburg, Washington, in the fall of 1999. These tree frogs were selected to maximize the range of color morphologies (for tree frog descriptions see Table 1). Since there is no evidence that age or size affects the color change ability in adults (Straub 2001; A. Buchan, personal communication), age and size were not controlled. All tree frogs were housed communally in a 54-L aquarium (60 cm × 30 cm × 30 cm) maintained inside a Percival Scientific envi-

Table 1. Qualitative descriptions lightness, hue, and chroma values for the color of all experiment Pacific tree frogs (*Hyla regilla*).

Tree frog No.	Lightness	Hue	Chroma	Description
1	122.12	–72.02	45.76	Green with brown patches
2	121.28	–82.57	43.24	Brown with brown–red tinge
3	92.26	–68.78	30.73	Brown with green sides
4	147.19	–78.65	39.87	Brown with gray sides
5	115.44	–69.96	40.82	Brown with green sides
6	97.64	–68.28	28.98	Gray–brown
7	116.66	–81.02	34.27	Red back with gold sides
8	157.06	–68.61	45.84	Brown with green sides
9	103.1	–67.71	31.35	Gray with green fringes
10	82.73	–78.61	30.85	Brownish gray
11	153.14	–77.39	41.31	Brown with green undertones
12	126.81	–71.25	35.24	Brown
13	85.22	–71.68	29.91	Brown with gray sides
14	121.31	–75.57	39.84	Grayish green with copper tinges
15	130.78	–77.73	42.78	Gold with green sides
16	124.31	–67.06	39.41	Brown with green sides
17	120.05	–59.72	40.07	Green
18	138.35	–76.74	36.45	Brown
19	118.1	–77.97	37.60	Brown
20	125.89	–57.57	46.20	Green with brown patches

Note: Different treatment combinations will give different color values, so only data from the green background, high light intensity, 10 °C treatment are reported. As such, these values are not meant to represent absolute characteristics but rather to give a general idea of the difference between the tree frogs.

ronmental chamber (Percival Scientific, Inc., Perry, Iowa). Photoperiod was set to a 12 h light : 12 h dark cycle and temperature was held constant at 17.5 ± 0.5 °C. Each tree frog was provided water ad libitum, fed one or two crickets per week, and toe-clipped prior to the study for positive identification of individuals. During the course of our study, no significant mass loss was noted among the tree frogs, so we assume that the rate at which they were fed was appropriate to minimize potential effects of hunger on color change. All individuals were returned alive to Englehorn Pond in the spring of 2000. Animals were cared for following the guidelines of the Canadian Council on Animal Care and Central Washington University Animal Use and Care protocol No. 91-R-0023. Animals were collected under Washington Department of Fish and Wildlife permit No. 99-272 issued to D. Darda.

Table 2. Lightness, hue, and chroma values for the two experimental backgrounds and two common habitats (i.e., brown and green cattails) of the *H. regilla* study population.

	Lightness	Hue	Chroma
Background			
Brown	134.97±0.63	40.48±0.47	16.98±0.06
Green	197.83±0.53	–46.97±0.12	76.74±0.21
Habitat			
Brown cattails	172.5	45.9	185.2
Green cattails	140.84	–53.02	29.83

Note: Data reported are from pictures taken with tree frog No. 1 in high light intensity and at 10 °C and are means ± SE. We control for light intensity, temperature, and individual tree frog not because they actually change the background color but rather because these factors can create variation in the measured values of the background. As such, these values are not meant to represent absolute characteristics but rather to give a general idea of the difference between the two backgrounds.

Experimental design

To examine the effects of light intensity, temperature, and background on the rate of color change in adult *H. regilla*, we used a full factorial within-subject design. (Note that the within-subject design controls for variation owing to different color morphs and sexes of individual tree frogs.) Light intensity, provided by regular (i.e., not full spectrum) fluorescent and incandescent bulbs, was either 15 or 30 μmol photons·m^{-2}·s^{-1}, temperature was 10 or 25 °C, and background colors were either bright green or dark brown (see Table 2). We used 20 individuals in a full factorial combination of variables to give 160 trials, each run in random order.

Light intensity was manipulated by changing the number of lighted 35-W fluorescent bulbs (Royal Philips Electronics, Amsterdam, the Netherlands) and light intensity was measured with a LI-190 quantum sensor (LI-COR, Inc., Lincoln, Nebraska). Natural habitats of *H. regilla* are composed of enumerable colors that vary in hue, chroma, and lightness. As such, we did not attempt to match all color variables of the experimental backgrounds to any specific natural colors. Instead, we chose experimental background colors that qualitatively matched natural habitat colors. Table 2 gives color descriptions for the experimental backgrounds and two habitats commonly occupied by *H. regilla* (habitat data from Straub 2001). Since our backgrounds varied in hue, chroma, and lightness, we simply refer to this treatment as background. Although we refer to the two levels of this treatment as green and brown, this does not imply that the difference in hue between the two backgrounds is

the most important color variable in eliciting a color change response.

Color acclimation

To measure color change during a trial, each tree frog was enclosed in a 60 mm × 15 mm plastic petri dish. Because the height of the dish was slightly taller than the tree frog, the dorsal surface of the tree frog remained nearly perpendicular to the camera. During acclimation, each tree frog was placed on a black background in a Percival Scientific environmental chamber at the experimental temperature and light intensity for that trial. The acclimation period was approximately 2 h. This was sufficient for the tree frog to complete its darkening on the black background and become settled within the dish. The acclimation period was used to minimize effects of handling and temperature shock and to maximize the amount of darkening before a trial.

Image capture and data analysis

At the end of the acclimation period, the tree frog was quickly moved onto the specified background (green or brown). Our apparatus, containing a Kodak DC260 digital camera (768 × 512 pixels; Eastman Kodak, Rochester, New York) (Fig. 1), was immediately placed over the dish containing the tree frog and the first picture was manually taken with the chamber door open, a process that took approximately 1 min to complete. The camera then automatically captured pictures at 1-min intervals for 2 h thereafter.

The digital images were loaded into Adobe PhotoShop version 5.0 (Adobe Systems Inc. 1998). We analyzed all pictures acquired from minutes 2–11 and then the 10 pictures from minutes 15–60 at 5-min intervals. Preliminary data (unpublished) showed that color change was mostly complete within 1 h, so our analysis did not include pictures taken after the first hour. Blurry or distorted images resulting from animal movement were discarded.

Histograms were produced from the color of the tree frog's dorsal surface (minus limbs and eye surface) for lightness and position of the tree frog's color on the *a* and *b* color axes using the CIE 1976 *L*a*b** color space model. This color space is composed of three orthogonal axes. It can be visualized as the lightness axis (*L*) standing vertically and the *a* and *b* axes lying in the same plane as each other. The lightness value simply gives lightness, but the *a* and *b* axes give the color coordinates for the measured color on the two-dimensional color circle. Using these coordinates, it is then possible to describe the color's hue (*H*) and chroma (*C*), which are calculated via the following formulae (CIE 1986):

$$H = \tan^{-1}(b/a)$$

$$C = \sqrt{(a^2 + b^2)}$$

(Note that the output for three *L*a*b** axes is from 0 to 255. Without a negative axis, all hue and chroma values will be altered and final results skewed. We avoided this problem by subtracting 127.5 from all *a* and *b* values. This was not necessary for lightness values because they are measured in one dimension.) To compare each tree frog with its background, color was also quantified for an adjacent area of the background. Since the color of the background does not change, it acts as a control for slight variations in exposure and picture quality. In the *L*a*b** color model, the three axes are orthogonal, so we calculated an Euclidean distance (ED) between the tree frog's color (f) and its background color (c). The following ED equation was used because it allowed us to track color change in a three-dimensional color space (CIE 1986):

$$\mathrm{ED} = \sqrt{((a_\mathrm{f} - a_\mathrm{c})^2 + (b_\mathrm{f} - b_\mathrm{c})^2 + (L_\mathrm{f} - L_\mathrm{c})^2)}$$

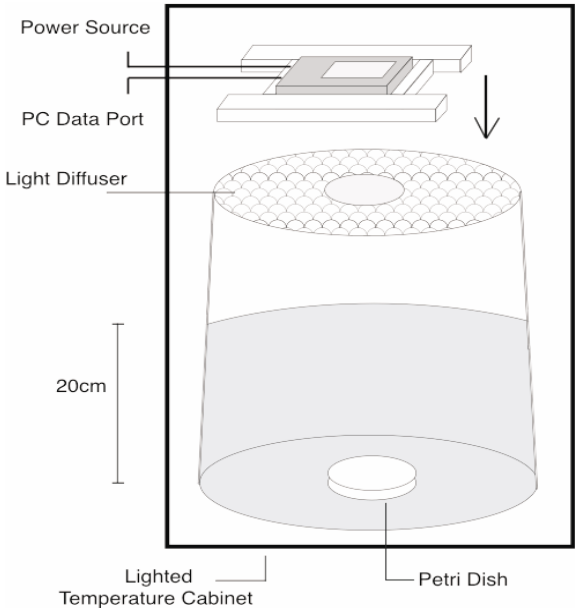
The smaller the ED, the better a tree frog matches the color of the background. (Note that lightness is orthogonal to hue and chroma, but hue and chroma are not orthogonal to each other. Thus, an ED cannot be calculated using these measures.) Differences between the tree frog and its background were also calculated for hue, chroma, and lightness.

We plotted the hue, lightness, chroma, and color distances against time and fitted a logarithmic trend line to the data to determine their respective rates of change. This trend line took the following form:

$$Y = a\ln(x) + (b)$$

whereby *a* denotes the "logarithmic slope", *x* is the time in minutes since the start of the trial, and *b* is the *y* intercept. A logarithmic line was used because it visually fit the data best and previous studies have shown that the rate of tree frog color change decreases through time (Kats and Van Dragt 1986). The logarithmic slope was used as the rate of change and the *y* intercept was used as the initial Euclidean distance (IED).

Fig. 1. Internal view of the apparatus used to manipulate background color and capture images. The Pacific tree frog (*Hyla regilla*) was contained in the petri dish at the bottom and the colored background surrounded the tree frog horizontally and 20 cm vertically. Images were captured through a hole in the diffuser from the top of the apparatus and loaded into Adobe PhotoShop version 5.0. The data port is connected to the camera, which is placed on top of the diffuser during a trial, as depicted by the downward facing arrow.



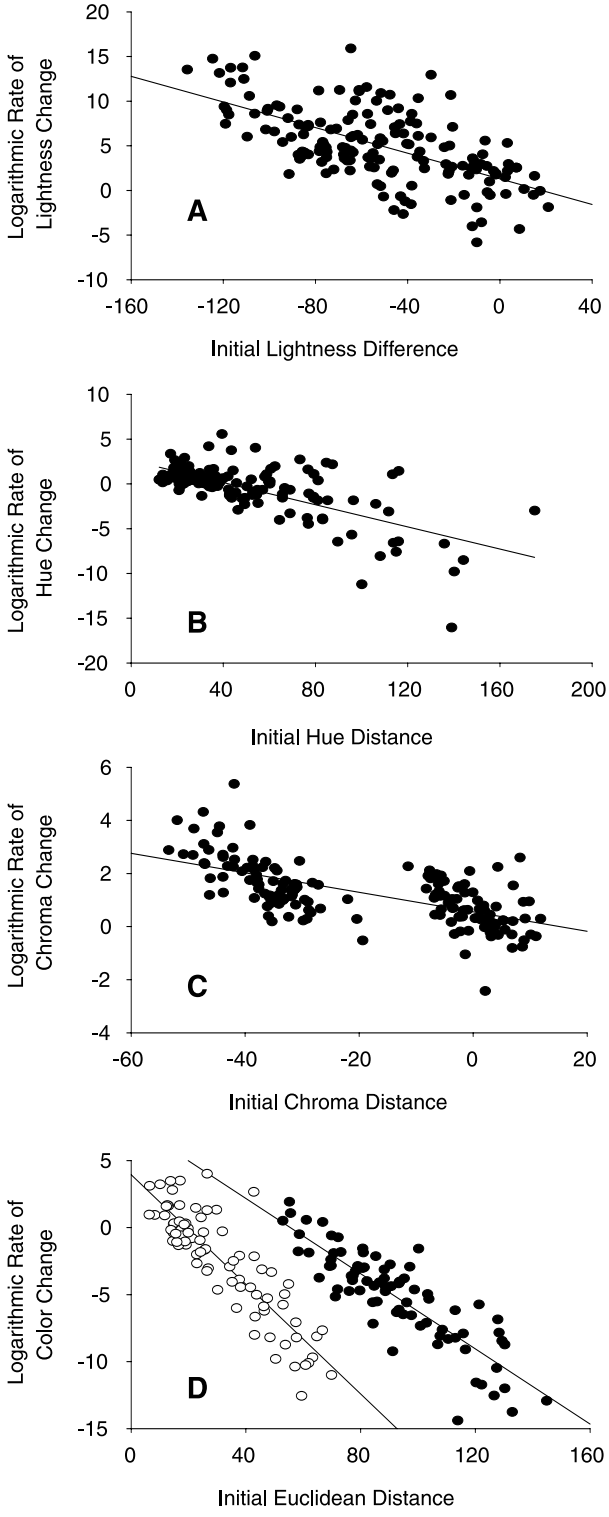


Fig. 2. Regression analysis showing the pattern of increasing rate of change with increasing initial distance for lightness (A), hue (B), chroma (C), and color (D) in *H. regilla*. Two trend lines are fitted to the color data to elucidate the effect of background (○, brown; ●, green) on the rate of color change seen in the ANOVA with initial Euclidean distance as an additional independent variable. Note that as rate and initial distance values move away from zero, the rate of change increases and the degree of matching between the tree frog and its background decreases. Each point represents one trial. Also, note that the rate equation is reported only for color change and given as rate × $(\ln(x + 1) - \ln(x))$ × points of Euclidean distance/time where x is the number of minutes since the start of the trial and time is in minutes, and the units of change are specific to each regression so that the chroma figure is in units of chroma distance, etc.

It is important to note that to study aspects of color change, such as the effects of different color morphs, measurements of absolute color would be necessary, which is in contrast with our relative color measurements. To achieve absolute color change, it would be necessary to calibrate each picture to multiple color standards with known reflectance properties and to account for the absorbance/reflectance of any media the picture is taken through (i.e., the petri-dish cover).

Statistical procedures

To demonstrate the effects of background, temperature, and light intensity on the rate of color change, we first performed a repeated measures ANOVA on the rate of color change through PROC MIXED in SAS version 8 (SAS Institute Inc. 2000). We tested the compound symmetry, autoregressive (order 1), and unstructured covariance structures to determine which would be the most appropriate to give us the most accurate model. The compound symmetry covariance structure gave values for Akaike's Information Criterion (–410.8) and Schwarz's Bayesian Criterion (–411.8) that were closer to zero than did the other two covariance structures. Thus, compound symmetry covariance was used in our analysis, which also produced the most conservative F statistics (Littell et al. 1996). This analysis was also performed with IED as the dependent variable, for which compound symmetry produced values for Akaike's Information Criterion (–637.6) and Schwarz's Bayesian Criterion (–638.6) that were closer to zero than did the other two covariance structures.

We found the rate of color change to be correlated with IED, so we also included IED into our ANOVA model. Since the background, light intensity, and temperature treatments affected the IED (see Results), a formal ANCOVA could not be used (Littell et al. 1996). Thus, we included the IED in the model to look at how the environmental variables affected the rate of color change while controlling for the influence of IED. Again, we used compound symmetry in our analysis because it produced values for Akaike's Information Criterion (–322.7) and Schwarz's Bayesian Criterion (–323.7) that were closer to zero than did the other two covariance structures.

Results

Linear regression showed that the rate of hue, chroma, lightness, and color change were dependent on the initial color distance for hue (R = 0.691, v = 150, P < 0.0005), chroma (R = 0.644, v = 150, P < 0.0005), lightness (R = 0.621, v = 150, P < 0.0005), and ED (R = 0.708, v = 150, P < 0.0005), respectively (Fig. 2). Since each tree frog rep-

Table 3. Partial ANOVA output based on color data for *H. regilla*.

	Rate of colour change/initial Euclidean distance			
Environmental variable	Mean	SE	$F_{[1,19]}$	*P*
Background				
Green (G)	–5.13/92.1	0.394/2.41	31.60/821.9	<0.0001/<0.0001
Brown (B)	–2.57/31.9	0.448/1.92		
Temperature				
10 °C	–5.51/72.7	0.439/4.11	53.71/104.81	<0.0001/<0.0001
25 °C	–2.18/51.2	0.367/3.55		
Light intensity				
High	–3.21/59.2	0.406/3.86	7.93/6.75	0.011/0.018
Low	–4.49/64.7	0.472/4.19		
Background × temperature				
G10	–6.25/104	0.572/3.28	5.59/1.21	0.029/0.285
G25	–4.00/80.2	0.488/2.35		
B10	–4.77/41.5	0.652/2.78		
B25	–0.37/22.3	0.374/1.62		

Note: Values reported for the rate of color change and the initial Euclidean distance. The rate of color changes is given as rate × (ln(x + 1) – ln(*x*)) × points of Euclidean distance/time where *x* is the number of minutes since the start of the trial and time is in minutes. See Fig. 3 for elucidation of interaction. Nonsignificant variables and interactions are not reported. Note that when the initial Euclidean distance increases, the rate of color change also increases (i.e., becomes more negative).

Fig. 3. Interaction between background (○, brown; ●, green) and temperature on the rate of color change (solid lines) and initial Euclidean distance (broken lines) in *H. regilla*. The rate of color changes is given as rate × (ln(*x* + 1) – ln(*x*)) × points of Euclidean distance/time where *x* is the number of minutes since the start of the trial and time is in minutes. Note that the interaction on rate is significant, while the interaction on the initial Euclidean distance is not. SE bars are shown except for the mean rate of color change on the green background at 10 °C, which was removed for clarity because it obscured other SE bars. See Table 2 for statistics.

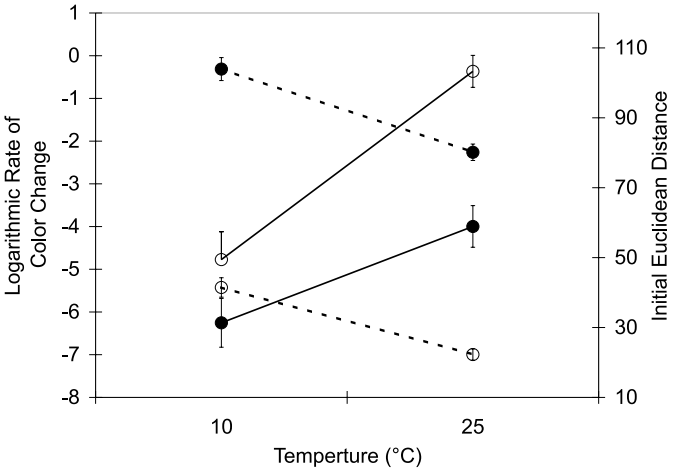
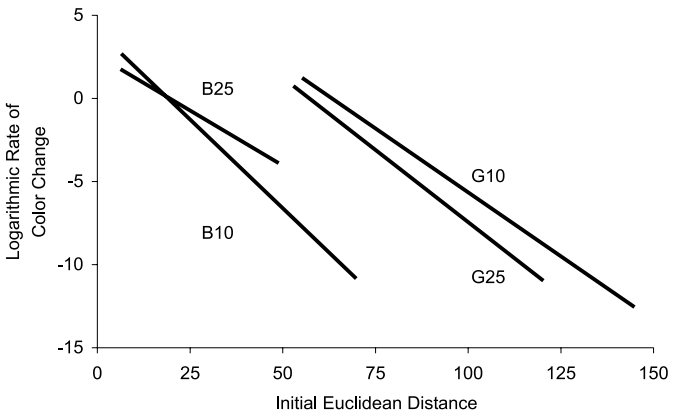


Fig. 4. Scatterplot (data points removed to expose trend lines) showing the interaction between background and temperature on the rate of color change in *H. regilla*. From trend line displacement, it is possible to determine the direction of significant effects shown by the ANOVA with initial Euclidean distance as an additional independent variable. G and B refer to green and brown backgrounds and 10 and 25 refer to those temperatures in degrees Celsius. See text for statistics. The rate of color changes is given as rate × (ln(*x* + 1) – ln(*x*)) × points of Euclidean distance/time where *x* is the number of minutes since the start of the trial and time is in minutes.



resents eight data points within these regressions, the assumption of independence was violated and formal regression analysis could not be performed. Thus, these statistics are reported only to suggest the potential that the rate of color change is driven by IED.

The repeated measures ANOVA also suggests a link between IED and the rate of color change. We found that the tree frogs changed color slower on the brown than on the green background and that they matched the brown background better than the green background. Similarly, high light intensity resulted in slower rates of color change than did low light intensity, and the tree frogs matched their background better at high light intensity as compared with low light intensity. Furthermore, they changed color slower at 25 °C than at 10 °C, and they matched their background better at 25 °C than at 10 °C (Table 3).

There was also an interaction for the effects of background and temperature on the rate of color change: on both

backgrounds, the rate of color change increased between 10 and 25 °C, but there was a greater increase on the brown background than on the green background (Fig. 3, Table 3). No other interactions between environmental variables were significant for the rate of color change, and no interactions were significant for IED.

The analysis that included IED as an independent variable revealed that the IED was correlated with the rate of color change ($F_{[1,132]} = 305$, $P < 0.0001$) and that the rate of color change was higher on the brown background than on the green background ($F_{[1,19]} = 143$, $P < 0.0001$) (Fig. 2D). There was also a significant interaction between background and temperature ($F_{[1,19]} = 29.46$, $P < 0.0001$). This interaction is such that the tree frogs were changing color faster at 10 °C when on the brown background but faster at 25 °C when on the green background (Fig. 4). No other interactions or main effects were significant for the rate of color change.

Discussion

When looking at the effects of each environmental variable alone, we found the rate of color change to be slower on the brown background, at high light intensity, and at 25 °C relative to the green background, at low light intensity, and at 10 °C, respectively (Table 3). Similarly, when examining each variable in isolation, IED was smaller on the brown background, at high light intensity, and at 25 °C (Table 3). From these patterns, it is apparent that with a smaller IED, there is a corresponding decrease in the rate of color change or, conversely, an increase in the rate of color change with larger IEDs (Table 3). This relationship is shown in Fig. 2 and is true not only for whole color but also for hue, chroma, and lightness. Since all three color components change in response to their respective initial distances, it can be concluded that *H. regilla* has control over and can change its hue, chroma, and lightness during time periods on the order of minutes. The fact that all three color variables can be changed rapidly suggests that all three are important in physiological color change (Fig. 2).

From an ecological viewpoint, when a tree frog moves from one color environment to a different color environment, it will change color. One factor that will affect the tree frog's rate of color change is the IED between the tree frog and its new environment. If the previous and current color environments are sharply different, the tree frog will change color quickly in response to the large IED. One interpretation for this change is crypsis, which is suggested as a major function of color change (Jameson and Pequegnat 1971). Since *H. regilla* can change their hue, chroma, and lightness on the order of minutes in our study, we support the idea that physiological color change has evolved as a mechanism to allow rapid background matching as a tree frog moves from one location to another. This does not conflict with the recent findings that long-term (i.e., morphological; Nery and Lauro Castrucci 1997) hue change is not a mechanism used by this species to match substrates because tree frogs move between habitats more quickly than they can morphologically change from green to brown or from brown to green (Wente and Phillips 2003). Difference in time scale also explains that short-term physiological color change is elicited by a combination of hue, chroma, and lightness stimuli in our study, whereas lightness appears to be the only color variable that stimulates long-term morphological color change in *H. regilla* (Wente and Phillips 2003). The disparity may be due to different ecological roles of physiological and morphological color change.

In addition to the main effects of temperature, light intensity, and background on the rate of color change and IED, there was a significant interaction between background and temperature on the rate of color change. However, this same interaction was not significant for IED (Fig. 3). Since the rate of color change is driven by the IED in a linear fashion (see Fig. 2D), we would expect analogous patterns in the rate data that we see in the IED data. This should be realized so that an increase in IED is complemented by an increase in the rate of color change. This prediction is not supported because the lines fitting the IED data are parallel, whereas the lines fitting the rate data are not parallel (Table 3, Fig. 3). This suggests that, in addition to IED, there is some other factor that is also partially responsible for the rate of color change. In other words, these tree frogs use color change to cope with more than just low cryptic values.

Results from the ANOVA with IED as an independent variable further suggest the possibility of an additional function of color change. Using IED in the model allows us to compare color change rates without the influence of IED. Thus, observations can be made on color change patterns with the effect of crypsis removed. In this analysis, the interaction between background and temperature remains significant. We see that on the brown background, the tree frogs were changing color faster at 10 °C than at 25 °C, but on the green background, change was faster at 25 °C than at 10 °C (Fig. 4). At this point, it is difficult to know what is responsible for these specific patterns and what functions color change serves in *H. regilla*. Although, in this analysis, the effects of background matching are factored out, yet the tree frogs still change their color in response to environmental stimuli. This again suggests that color change has functions other than increasing cryptic value.

Color change could also be used for thermoregulation, since changes in color will affect the amount of absorbed solar energy (e.g., Hoppe 1979). Some researchers have rejected the possibility of thermoregulation because anuran skin is highly permeable to water. The argument is that, although tree frogs show variation in their solar absorbance, water loss increases with increased solar absorbance. This increase in water loss will maintain a tree frog's temperature at a constant level (Tracy 1976). This view, however, has been challenged by the discovery that some tree frogs can change the permeability of their skin by secreting a "waterproof" coating (Wygoda 1988; Lillywhite et al. 1997). If a "waterproof" tree frog increases its solar absorbance via color change, the extra energy will not dissipate. This is because the waterproof coating prevents the evaporative cooling effect seen in water-permeable species. Hence, a waterproof tree frog may be able to regulate its temperature by changing its solar absorbance via changing its body color. Alternatively, in relatively water-permeable species, water loss will increase with an increase in solar absorbance (Tracy 1976), so color change may help regulate the water balance in these species.

In conclusion, our study shows that there are complex interactions among ecological factors that determine color change patterns in *H. regilla*. We have shown that hue, chroma, lightness, and whole color respond to their initial color distances. We have also shown that factors in addition to background matching can drive the color change patterns in *H. regilla*. Further studies are needed to fully elucidate what these factors are, to understand their mechanistic basis, and to determine the multiple roles that color change plays in anurans in general.

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