

Fixed Green and Brown Color Morphs and a Novel Color-Changing Morph of the Pacific Tree Frog *Hyla regilla*

Wendy H. Wente* and John B. Phillips†

Department of Biology, Indiana University, Bloomington, Indiana 47405

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ABSTRACT: Pacific tree frogs *Hyla regilla* are typically either green or brown in dorsal coloration. The frequency of green and brown individuals is known to fluctuate seasonally. Previous investigators have generally assumed that the green and brown body colors represent a “fixed” polymorphism and that seasonal changes in the proportion of the two body colors are a consequence of differential survival of the two color morphs. Here we report that, in addition to the “fixed” (i.e., non-color-changing) green and brown morphs of *H. regilla*, there are some individuals that can change hue between green and brown. The distribution of color-change ability in our study population is bimodal, suggesting that “color changers” are a distinct morph rather than one extreme of a continuous distribution of color-change ability. Our findings suggest that background brightness, not hue, triggers color change in the newly discovered morph and that this change requires days to weeks to occur. Such slow color change is not well suited for making short-term changes in color as a frog moves between differently colored substrates. Rather, seasonal changes in habitat characteristics and/or microhabitat use are likely to maintain color-change ability. Color polymorphism and color-change ability appear to represent alternative responses to divergent selection for crypsis in a heterogeneous, seasonally variable environment.

Keywords: color polymorphism, color change, spectral reflectance, principal component analysis, spectral segment classification.

* Corresponding author. Address for correspondence: United States Geological Survey Forest and Rangeland Ecosystem Science Center, 3200 Jefferson Way, Corvallis, Oregon 97331; e-mail: wendy_wente@usgs.gov.

† Present address: Biology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24060; e-mail: jphillip@vt.edu.

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When a selective pressure such as predation favors crypsis, color polymorphism (Tordoff 1980; Endler 1988) and individual color change (Waring 1963; Heinen 1994) have been suggested as alternative solutions to the problem of background color matching in an environment consisting of spectrally heterogeneous microhabitats. Polymorphisms with two or more genetically fixed patterns or colors have been described in a variety of organisms including, for example, insects (Agawa and Kawata 1995), snails (Cook 1986), fish (Barlow et al. 1990), snakes (King 1987; Brodie 1989), and frogs (Hoffman and Blouin 2000). A similarly diverse group of organisms has been found to exhibit reversible changes in coloration (brightness, hue, or saturation; Bagnara and Hadley 1973; Brakefield 1996; Hazel and West 1996; Montgomerie et al. 2001). In some organisms, including insects (Hazel et al. 1987), barnacles (Lively 1986b), and anurans (Pfennig 1992) among many others, plasticity is limited to a period of development and is nonreversible (polyphenism). Other species have a more flexible response and can switch between phenotypes (Portmann 1959; Nielsen 1980; Camargo et al. 1999; Hanlon et al. 1999). Nevertheless, polyphenism and reversible plasticity can be considered under the same umbrella of phenotypic plasticity.

Phenotypic plasticity and polymorphism typically have been presented as distinct alternatives expected to arise under different genetic conditions, and therefore, any single population is expected to evolve only one of the two strategies (Levins 1968; Marshall and Jain 1968). The contrasting strategies of fixed polymorphism and color change can be framed in the context of the specialist versus generalist responses to coarse- and fine-grained environments (Levins 1968). A fixed-color polymorphism can be thought of as a specialist strategy where individuals expressing a particular phenotype enjoy higher fitness when the environment consists of large definable patches or when individuals show a preference for patches in which they enjoy a fitness advantage. In the case of alternative color morphs, individuals that match the most prevalent background will have an advantage over nonmatching phenotypes. Color change is an example of phenotypic plasticity and repre-

sents a generalist strategy (van Tienderen 1997). In this case, when different types of patches are available, an individual can utilize more than one. Individuals have the ability to respond to a complex environment by expressing a phenotype that is more advantageous in a particular patch (microhabitat).

The Role of Predation in the Evolution of Alternative Strategies

Differential selection on color morphs by visual predators is a commonly cited explanation for the evolution of fixed-color polymorphisms (Stimson and Berman 1990; Hoffman and Blouin 2000), as well as individual color-change ability (Waring 1963; King et al. 1994). When the environment is spatially or temporally variable with respect to the availability of different color patches, color change and color polymorphism can be two strategies for achieving crypsis to avoid detection by predators. Individual behavior, in concert with color morphology, can enhance the effectiveness of crypsis if individuals have a behavioral preference for the microhabitat that most closely resembles their own morphology (Kats and VanDragt 1986; Morey 1990; Hazel and West 1996; Seehausen 1996). Evidence for a link between predator avoidance and background choice behavior has been obtained in laboratory studies, which demonstrated that tree frogs located on a mismatched color background are more vulnerable to both bird (Tordoff 1980) and snake (Morey 1990) predators.

Some authors have suggested that differential selection by predators on color morphs might be influenced by seasonal or geographic variation in the availability of matching background substrates (Pyburn 1961; Nevo 1973). Changes in color-morph frequencies across a species range have been shown to parallel changes in the availability of differently colored substrates (Nevo 1973). Color-morph frequencies have also been shown to track changes in available substrate colors and substrate patterns seasonally (Pyburn 1961; Jameson and Pequegnat 1971; Matthews 1971; Nevo 1973). These geographic and seasonal changes in morph frequency may reflect changes in the ability of visual predators to detect different morphs as habitat color changes seasonally.

In this article, we describe a species in which different individuals in the same population are either fixed in color (color polymorphism) or have an ability to change color (phenotype plasticity). Earlier investigators reported that the Pacific tree frog *Hyla regilla* exhibited a color polymorphism in which individuals are fixed in hue but able to change in brightness (Brattstrom and Warren 1955; Resnick and Jameson 1963). Resnick and Jameson (1963) suggested a simple two-gene autosomal inheritance system as the basis for the *H. regilla* color polymorphism. Other

authors have noted color change in this species, but the underlying genetic basis of the system remains unclear (Mackey 1958; Haugen 1992). We report here that the color polymorphism in *H. regilla* appears to include both non-color-changing (brown or green) and color-changing morphs within the same natural population. Our findings suggest that color change by some individuals is likely to play a role in the observed seasonal changes in the proportion of green and brown individuals in this species (Jameson and Pequegnat 1971; Schaub and Larsen 1978). Furthermore, we show that the time course of color change (requiring days to weeks rather than hours) and the environmental cues that trigger color change (background brightness, not hue) are consistent with a response to long-term seasonal variation in habitat characteristics rather than to differences in the substrates that an individual encounters during daily movements. The presence of both individuals exhibiting a fixed-color polymorphism and individuals that can change color within a single population provides a rare opportunity to compare the advantages and disadvantages of specialist and generalist strategies in a spatially and temporally heterogeneous environment.

Methods

Study Animals

Hyla regilla were collected on Vandenberg Air Force Base in Santa Barbara County, California, during the winter and spring from 1996 to 1999. Frogs were housed, in accordance with current animal care regulations at Indiana University, either singly in clear plastic containers or communally in terrariums containing plants and a soil and bark substrate. Animals were maintained on a 12L : 12D photoperiod throughout the year and fed crickets and houseflies two to three times each week.

Measurement of Frog Coloration

Reflectance spectra from the dorsal surface of each frog were measured with a spectroradiometer (Photo Research Spectrascan model 714) using the standard beam method (Endler 1990). Radiance data were collected every 4 nm (the spectral resolution of the instrument) from 384 nm to 700 nm. In an otherwise darkened experimental chamber, light was provided by means of a tungsten-halogen bulb housed in a reflective cone 6 cm in diameter located approximately 20 cm from the frog. Kodak LabLEADER Analytical White Reflectance Standard (BaSO₄) was used as a reference (~99% reflection across the range of wavelengths sampled). The spectroradiometer had an acceptance angle of 2° and was oriented perpendicular to the frog's skin surface (orientation was approximate since the

frog's body did not provide a flat surface). The angle of incidence of the light source was 45° to the surface being measured, to reduce specular reflectance. We checked sample placement, focus, and specular reflectance through the instrument's viewing port before each sample measurement. To measure skin color, an individual frog was restrained by hand and held with its head oriented at right angles to the overhead light source, which was suspended from a stand opposite the spectroradiometer. If the frog moved during the procedure, the measurement was discarded and repeated. A radiance measurement was collected from the dorsal surface of the animal, followed immediately by a measurement of radiance from the white standard placed in the same location and orientation as the frog's skin surface. Patterned portions (striping or spotting) of the dorsal skin surface were avoided when taking the radiance measurement so that only a measurement of the underlying dorsal skin color was collected. By comparing each dorsal radiance spectrum with the radiance measure of the standard (BaSO_4) collected immediately afterward, we reduced any variation in measurements that might have resulted from voltage fluctuations to the light source. Frog skin reflectance was calculated by dividing the skin sample radiance by the white standard radiance at each wavelength interval. Spectral reflectances were also collected from a number of background substrates using the same methods.

Color Change

Terrarium/Clear Box Experiment. Initially, color change by some frogs was noticed when frogs were switched from terrariums containing plants and dark brown soil to clear plastic containers that lacked a dark substrate. Some frogs that had appeared brown in color to the observer after being housed in the terrarium exhibited green body coloration after spending several weeks in the clear container. In contrast, some frogs, including both brown and green individuals, maintained the same dorsal body coloration under both housing conditions. To quantify the ability of some frogs to change color (specifically hue), we collected spectral reflectances from the dorsal surface of 67 individuals after they were housed in the terrarium for at least 3 wk and from the same individuals after exposure to the clear box treatment for at least 3 wk. These measurements were used to develop a method of classifying fixed green, fixed brown, and color-changing morphs based on a ratio of middle-wavelength to long-wavelength reflectance (see "Results").

Causes of Color Change: Hue versus Brightness. To determine whether background color (specifically hue) drives color change in *H. regilla*, 16 frogs that were observed to

change color after being switched from the terrarium to clear boxes were housed individually in identical clear plastic containers surrounded on the four sides and the bottom by brown (brown paperboard similar in reflectance to brown frogs), green (dried green leaves similar in reflectance to green frogs), black, or white surfaces. A subset of five frogs experienced all four backgrounds; four frogs experienced green, black, and white backgrounds; four frogs experienced only brown and white backgrounds; and three frogs experienced only green and white backgrounds. The tops of the containers were clear, admitting light from a diffuse incandescent light source, that is, four GE Wattmiser 120-W flood lamps mounted in the ceiling 2.4 m above the test chambers behind a 1.3×1.3 -m square of white Plexiglas that served as a diffuser. The experiment was carried out in an environmental chamber to reduce variation in temperature ($21^\circ \pm 2.0^\circ\text{C}$) over the course of the test period. Humidity in the test room generally ranged between 45% and 60%. Measurements of spectral reflectance of the dorsal surface of each frog were collected after a 4-wk period of exposure to each of the four backgrounds (i.e., green, brown, white, or black). Hue angle was calculated for each frog from the reflectance curves following the segment classification method (Endler 1990). Background hue was calculated using the same method as frog hue. We completed regression analyses to assess the effects of background brightness and hue on frog hue angle.

Comparison of Color-Morph Groups. To compare the three color-morph groups, we employed two methods: spectral segment classification (Endler 1990) and principal component analysis (Cuthill et al. 1999; Grill and Rush 2000; see app. A in the online edition of the *American Naturalist*). Spectral segment classification takes into account the three variables of color: hue, chroma (or saturation), and brightness (Endler 1990). Hue and chroma are responsible for determining the shape of any given reflectance curve. Brightness estimates the overall level of reflectance but not the shape of the reflectance curve.

To apply spectral segment classification to reflectance data, the reflectance spectrum of the frog's skin was divided into four equal segments (384–460 nm, 464–540 nm, 544–620 nm, 624–700 nm). By comparing nonadjacent segments of the spectrum, segment classification enabled us to construct a simple color space and calculate a numerical value for hue based on attributes of a generalized model of a vertebrate visual system with two color opponency channels, LM (which compares signals from the long-wavelength region of the spectrum with the shorter middle-wavelength region) and MS (which compares the longer middle-wavelength region of the spectrum with the short-wavelength region [Endler 1990]). In human perception, LM represents the comparison made be-

tween the red and green portions of the spectrum, while MS is the comparison between yellow and blue. Each spectral reflectance curve was reduced to a single data point within the segment classification space (color space). The abscissa (MS) represented the difference between the long-middle (544–620 nm) and short- (384–460 nm) wavelength portions of the reflectance spectrum. The ordinate (LM) represented the difference between the long- (624–700 nm) and short-middle (464–540 nm) wavelength portions of the reflectance spectrum. In spectral segment color space, chroma (C) was represented as the distance from the origin to the (MS, LM) spectral coordinate. Hue (H) was calculated as the arc cosine (LM/C) and corresponded to the value of an angle clockwise from the LM axis to the spectral coordinate (Endler 1990). Since each of the segment comparisons was divided by the total reflectance of the reflectance spectrum, overall reflectance did not affect the color space coordinates. As a result, two spectra with the same hue and chroma but different brightness (total reflectance) would fall on the same point in the color space.

Segment classification enabled us to quantify the ability of certain frogs to change hue while others remained relatively fixed in hue. Spectral segment classification, however, relies on a simple model of vertebrate visual processing based on inputs from three receptor types (L, M, and S), with signals compared in a two-channel opponency system (LM and MS) similar to that found in many, but not all, vertebrates (Endler 1990). Because of its utilization of broad segments of the spectrum and its reliance on a two-channel opponency model, spectral segment classification will not necessarily be a good model for the visual system possessed by *H. regilla* and/or some of its predators. Thus, although spectral segment classification is a valuable method for visualizing the trends in the spectral data, for our largest data set from the black/white substrate experiment, we employed principal component analysis as an additional method that avoids the assumptions inherent in the spectral segment method (see app. A).

Color Change and Characterizing the Proportion of Color-Changing and Nonchanging Frogs: The Black/White Experiments. To confirm the effects of substrate brightness on color change in *H. regilla* and to determine the proportion of fixed brown frogs, fixed green frogs, and color changers, a larger number of frogs were used in a series of black/white substrate experiments. Frogs were housed individually in identical clear plastic containers surrounded by either black or white surfaces in the same environmental chamber described earlier. Measurements of spectral reflectance were collected from each frog every week for a period of 4 wk. At the end of the first 4 wk, frogs exposed to the black substrate were switched to the white substrate

for an additional 4 wk (and vice versa). Measurements of spectral reflectance were again collected once each week. Finally, the experiment was concluded after a third 4-wk period on the original substrate color. The black/white substrate experiment was repeated with three groups of frogs resulting in a total of $n = 155$.

Time Course of Color Change. To more accurately assess the time course of hue change in color-changing frogs, we collected dorsal reflectance measurements at more frequent intervals from two small groups of frogs on black and white backgrounds. Eight known changers that had been housed most recently in clear containers so that they were green in color were placed on the black background, and spectral reflectance was measured after 3, 7, 14, and 21 d of exposure. A separate sample of nine known changers that had been most recently housed in terrariums (and were brown in color) were placed on the white background, and spectral reflectance was measured at the same time points. We used regression to test for a relationship between frog hue angle and time spent on the black or white background.

Results

Color-Morph Classification: Terrarium/Clear Box Experiment

On the basis of our initial observations of the color change by some individuals during the terrarium/clear box experiment, we devised a simple classification scheme for distinguishing green, brown, and color-changer morphs. In figure 1, 67 frogs housed in both the terrariums and clear boxes exhibited bimodal distributions of hue angles (calculated using the spectral segment classification method). Both distributions exhibit the same local minimum at a hue angle of 55°–60°. Since frogs with hue angles greater than 60° generally exhibit spectral reflectances resembling those of fresh green leaves and frogs with hue angles less than 55° generally resemble the reflectance of dead (brown) leaves, this local minimum provides a convenient criterion for distinguishing “green” versus “brown” frogs. Color changers were defined as individuals that exhibited a change in hue angle that spanned the local minimum at 55°–60°. Although convenient, this method of classifying the color morphs depends on having a complete reflectance spectrum for each individual and on the particular spectral segment classification method that we have chosen. Therefore, this discrimination method is likely to be of limited utility to other investigators who lack a spectroradiometer comparable to the one used in this study and/or use a different method of quantifying frog color. To facilitate future com-

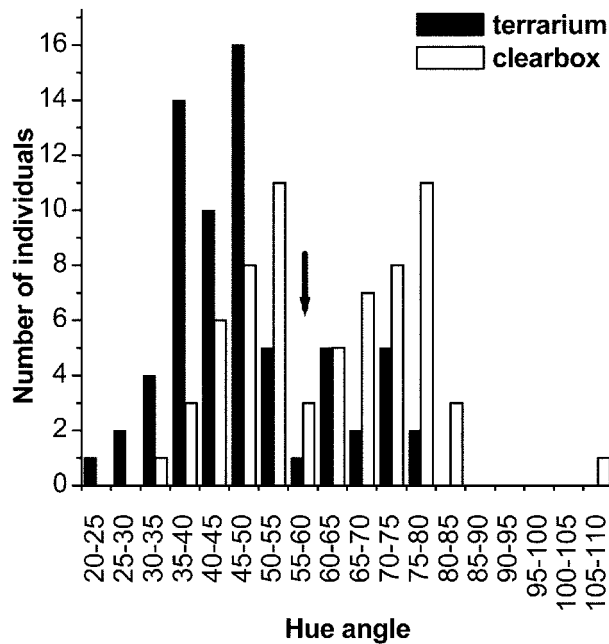


Figure 1: Hue angle distributions recorded for all frogs ($n = 67$) following the terrarium (black bars) and clear box (open bars) treatments. The local minimum in both hue angle distributions between 55° and 60° is indicated with an arrow.

comparisons of morph frequency data collected from different populations of *Hyla regilla* and by different investigators, we developed a simpler classification scheme based on the ratio of only two wavelengths, one in the midwavelength region of the spectrum, where the reflectance of green frogs is maximal, and a second at the long-wavelength end of the spectrum, where the reflectance of brown frogs is maximal. We chose 540 nm since it represented the midwavelength peak in the average reflectance measurements of *H. regilla*. We chose 650 nm (actually 652, which was the closest wavelength value obtainable with our spectroradiometer) to represent the longer wavelength portion of the spectrum above the crossover point in green and brown reflectance spectra (roughly 600 nm; see fig. 2A) because this fell within the range of typical vertebrate visual sensitivity. For *H. regilla*, a hue angle value of between 55° and 60° (where the hue angle minima occurred in fig. 1) corresponds to a 540/650 nm ratio of 0.9. All frogs with a 540/650 nm ratio of greater than 0.9 had hue angles above 60° (i.e., “green”), and a majority of frogs (91%) with a ratio below 0.9 had hue angles below 55° (i.e., “brown”). The ratio of 0.9 provided a convenient criterion to distinguish the three color morphs. Frogs exhibiting a range of 540/650 nm reflectance ratios spanning the criterion value of 0.9 (i.e., able to shift between green and brown hues) were designated as “color changers.” Indi-

viduals whose ratio remained above 0.9 throughout the experiment were designated as “nonchanging green,” and those whose ratio remained below 0.9 as “nonchanging brown.”

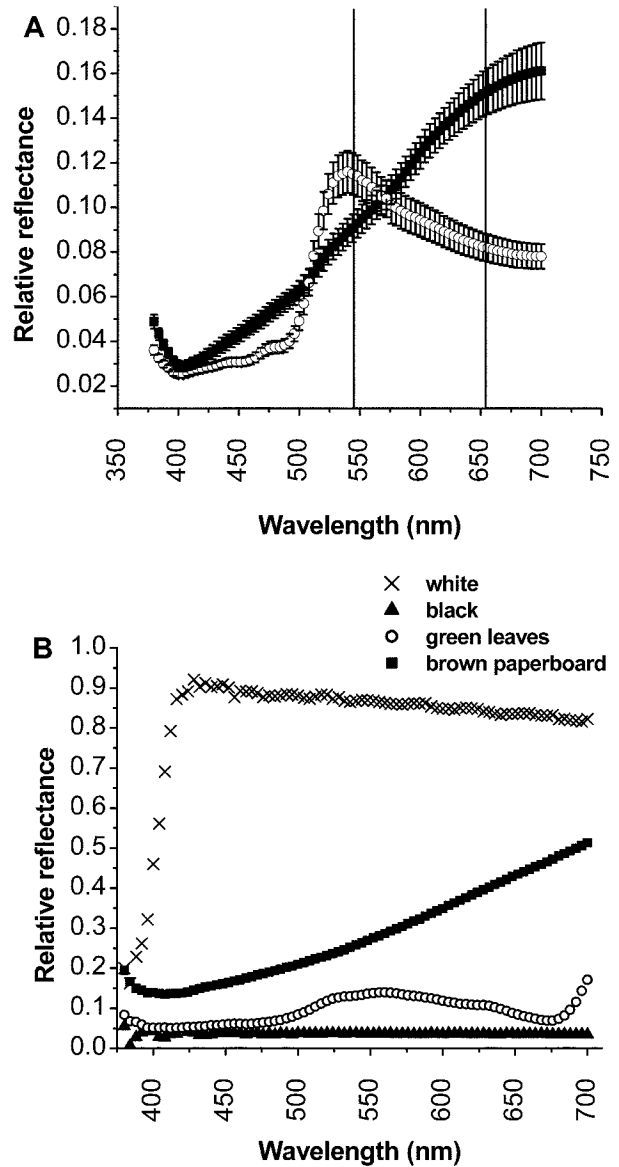


Figure 2: Frog and background substrate reflectance. A, Reflectance spectra of 12 nonchanging brown (filled squares) and 12 nonchanging green (open circles) *Hyla regilla*. The two spectral reflectance points (540 nm, 652 nm) used to calculate the ratio defining green and brown frogs are marked with vertical lines. Error bars indicate SEM. B, Reflectance spectra of the four backgrounds used in the experiments, including white (X), black (filled triangles), brown paperboard (filled squares), and dried green leaves (open circles).

Causes of Color Change: Hue versus Brightness, the Green/Brown Substrate Treatments

We tested the hypothesis that frogs with an ability to change color would respond to green and brown backgrounds by altering hue to match the background. A total of 16 frogs that had changed dorsal color when switched between light (clear box or white) and dark (terrarium or black) substrates (i.e., color changers) were exposed to a series of 4-wk treatments, including a white, black, green, and brown background (fig. 2B). Not all frogs experienced all the backgrounds (see “Methods”). We compared the total reflectance (brightness) of each background to the hue angles attained by the frogs at the end of the 4-wk treatment period (fig. 3A). There was a significant positive relationship between frog hue angle and background brightness ($r^2 = 0.69$, $F = 113.7$, $df = 1, 51$, $P < .0005$). There was no relationship between frog hue angle and background hue angle (fig. 3B; $r^2 = 0.016$, $F = 0.848$, $df = 1, 51$, $P = \text{NS}$). The hue values calculated for the black and white backgrounds are misleading because these backgrounds are achromatic. Because of this, we also compared the hue angles measured for frogs following the green and brown treatments and found no difference between the two groups (Watson’s $U_{0.05, 12, 9}^2 = 0.0723$, $P = \text{NS}$). Finally, a multiple regression of frog hue on both background brightness and background hue indicated that only background brightness, and not hue, was a significant predictor of frog hue ($r^2 = 0.698$, $F = 57.68$, $df = 2, 50$, $P < .0005$; $\beta_{\text{background hue}} = -0.086$, $t = -1.103$, $P = \text{NS}$; $\beta_{\text{background brightness}} = 0.826$, $t = 10.614$, $P < .0005$).

Characterizing the Proportions of “Color Changer,” “Nonchanging Green,” and “Nonchanging Brown” Morphs in a Natural Population of H. regilla: The Black/White Experiments

A combined sample of 155 *H. regilla* were subjected to the alternating 4-wk exposures to black and white substrates to characterize the magnitude and time course of the effects of substrate brightness on color change and to obtain an estimate of the proportions of the three color morphs in our study population. From this sample, 105 frogs were classified as nonchanging brown, 34 were classified as color changers, and 16 were classified as nonchanging green, using the 540/650 nm ratio method.

Ability to change hue angle in response to the black and white substrates was quantified for frogs who completed the 4-wk black/white experiment by plotting the change in hue angle between the minimum and maximum value recorded for each individual (fig. 4). While some ability to alter hue was recorded for all three groups, changers (defined by the 540/650 ratio) exhibited a significantly

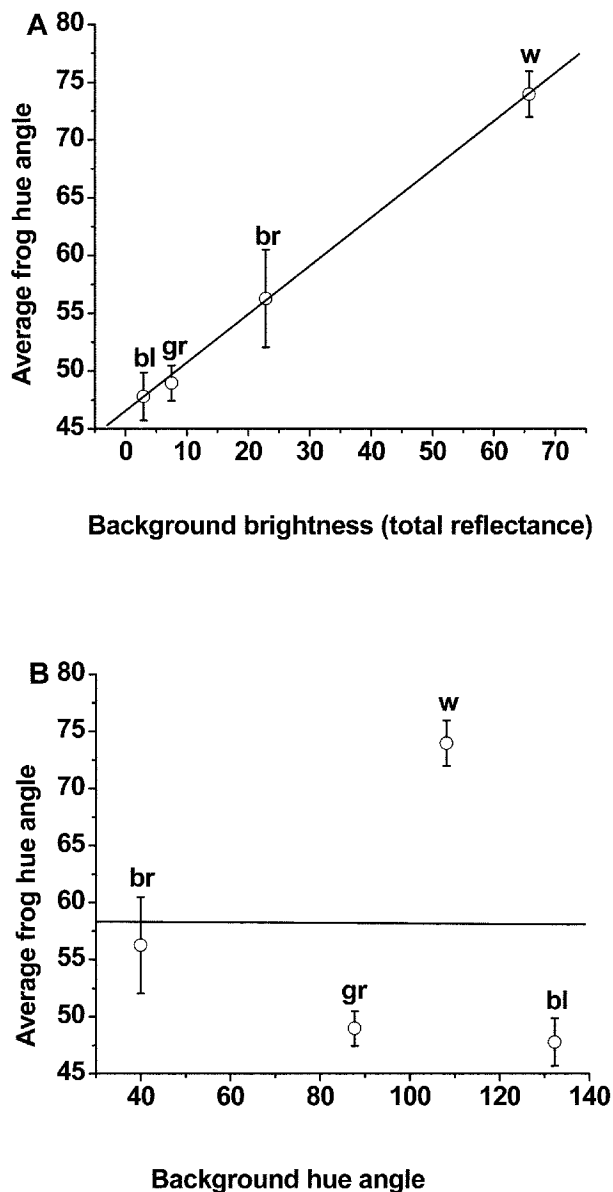


Figure 3: Green/brown background test results for known color changers. Frog hue angle exhibited a significant positive relationship with background brightness (total reflectance; A) but not with background hue angle (B). Points indicate the mean frog hue after each treatment, and error bars indicate SEM. Labels indicate background treatment values for hue or brightness: *gr* = green, *br* = brown, *bl* = black, and *w* = white.

greater ability to change hue when compared to the combined nonchanging group of frogs, including both nonchanging greens and nonchanging brown individuals (Wilcoxon-Mann-Whitney test, $z = 8.18$, $P \ll .001$, one-tailed). These results demonstrate that changers are not simply part of a continuous distribution of hue-change

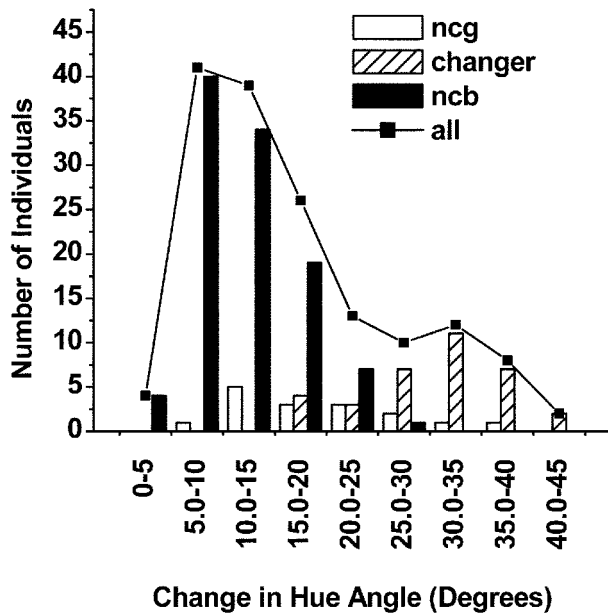


Figure 4: Total hue change for each individual during the course of the black/white experiment. Black bars represent nonchanging browns ($n = 105$), hatched bars represent changers ($n = 34$), and white bars represent nonchanging greens ($n = 16$). The solid line represents the total for all frogs in each hue angle category ($n = 155$).

ability. Differing color-change ability between the two morphs (nonchangers and color changers) was also supported by results of the principal component analysis (see app. B in the online edition of the *American Naturalist*).

Relative hue-change ability was not normally distributed (Kolmogorov-Smirnov test for normality, statistic = 0.128, $df = 155$, $P = .000$). Visual inspection of figure 4 reveals a bimodal distribution. Although these data indicate that color changers (*hatched bars*) exhibited a greater ability to alter hue than nonchangers (*black and white bars*), comparison of the hue distributions at the color changers' most extreme values indicates that they did not completely overlap the distributions of the nonchanging green and brown frogs (fig. 5). After exposure to the white surround, the hue distribution of changers approached, but did not completely overlap, that of nonchanging green frogs (fig. 5A). Likewise, when placed on the black background, the hue distribution of changers shifted to brown but did not completely overlap the hue distribution of the nonchanging brown frogs (fig. 5B).

Time Course of Hue Change

To characterize the time course of hue change in color-changing frogs, we collected dorsal reflectance measurements of known changers after 3 d of exposure to each

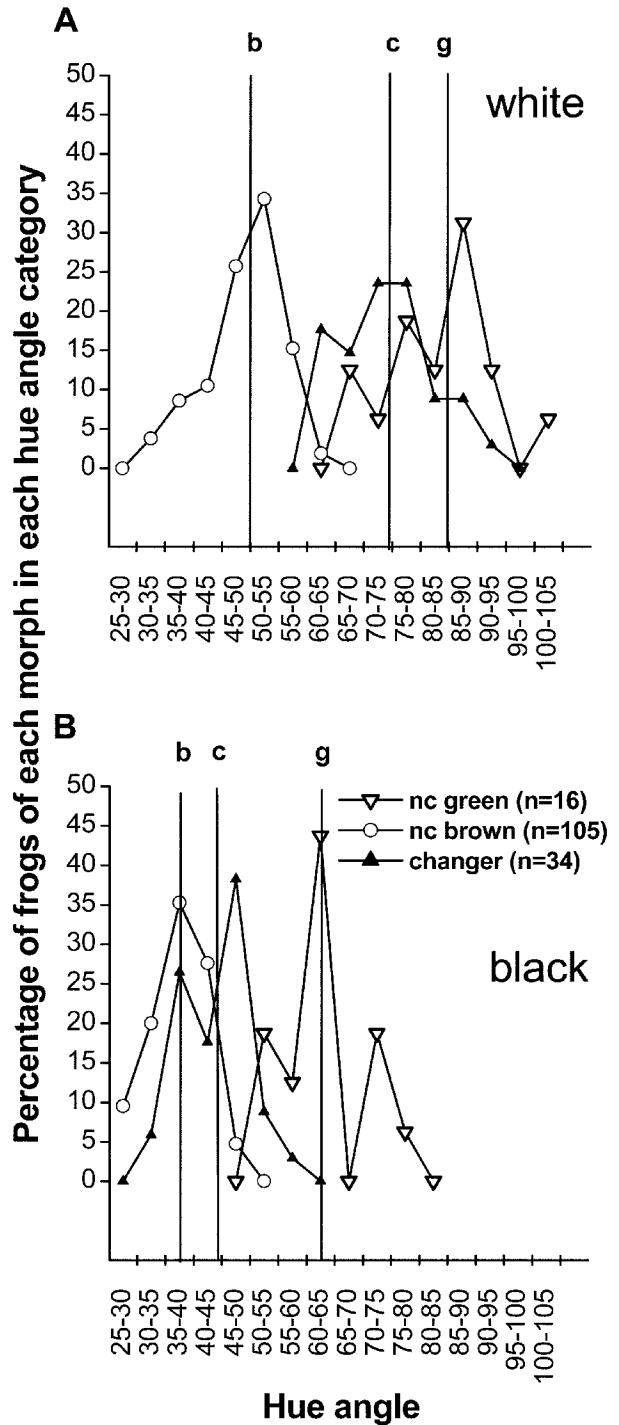


Figure 5: Hues of color changers do not completely overlap with hues displayed by the nonchanging morphs under the same background conditions. A, Distribution of hue angles after 4 wk on white background. B, Distribution of hue angles after 4 wk on black background. *Open triangle* = nonchanging green frogs, *filled triangle* = color changers, *open circle* = nonchanging brown frogs. Vertical lines indicate mean hue for nonchanging brown frogs (b), mean hue for color changers (c), and mean hue for nonchanging green morph (g).

of the treatments, as well as at the weekly intervals used in the earlier experiments (fig. 6). In a set of eight known changers that had been held in a "greening" environment (clear boxes) before the experiment, average hue change during the 3 wk on the black substrate was 27.2° . There was a negative relationship between average frog hue angle and time on the black substrate, although it was not significant ($r^2 = 0.704$, $F = 7.134$, $df = 1, 3$, $P = .076$). Frogs did turn brown on the black substrate since average hue changed from $75.6^\circ \pm 2.1^\circ$ (SEM) to $48.4^\circ \pm 2.0^\circ$. When a different sample of nine color changers that had been held in a "browning" environment (dark substrate terrariums) before the experiment was placed on the white background for a period of 3 wk, the average hue change was 19° . There was a significant positive relationship between frog hue angle and time spent on the white substrate ($r^2 = 0.816$, $F = 13.310$, $df = 1, 3$, $P = .036$). Frogs turned green on the white substrate. Note that under the conditions used in these experiments, (12L : 12D, 19.4° – 21.5° C), changers exhibited an intermediate hue for a period of a week or more (fig. 6B, 6C).

Discussion

Color-change ability in this population appears to be bimodal in distribution (see fig. 4), suggesting the existence of the three distinct morphs (color changers and non-changing green and brown frogs). Moreover, hue-change ability documented in this study is not simply a laboratory effect. Haugen (1992) noted at least some color change in 34% of individuals marked in September and recaptured in November during a field study of a southern California population, and hue change has been observed in marked individuals in a Washington state population (C. Straub, unpublished data).

Color Change in Response to Background Hue and Brightness (The Green/Brown Substrate Experiment)

The green/brown experiment tested whether frogs would respond with a color change to long-term exposure to green and brown substrates in addition to white and black substrates. Field studies of *Hyla regilla* have documented an increase in the frequency of green frogs from late winter through the early spring (Jameson and Pequegnat 1971; Schaub and Larsen 1978). This seasonal change in frog color frequency occurs simultaneously with a change in availability of different substrate colors as the environment becomes greener due to the effects of winter rains and increasing temperatures that stimulate plant growth. If color-change ability evolved because it enabled frogs to remain cryptic as they moved between different types of

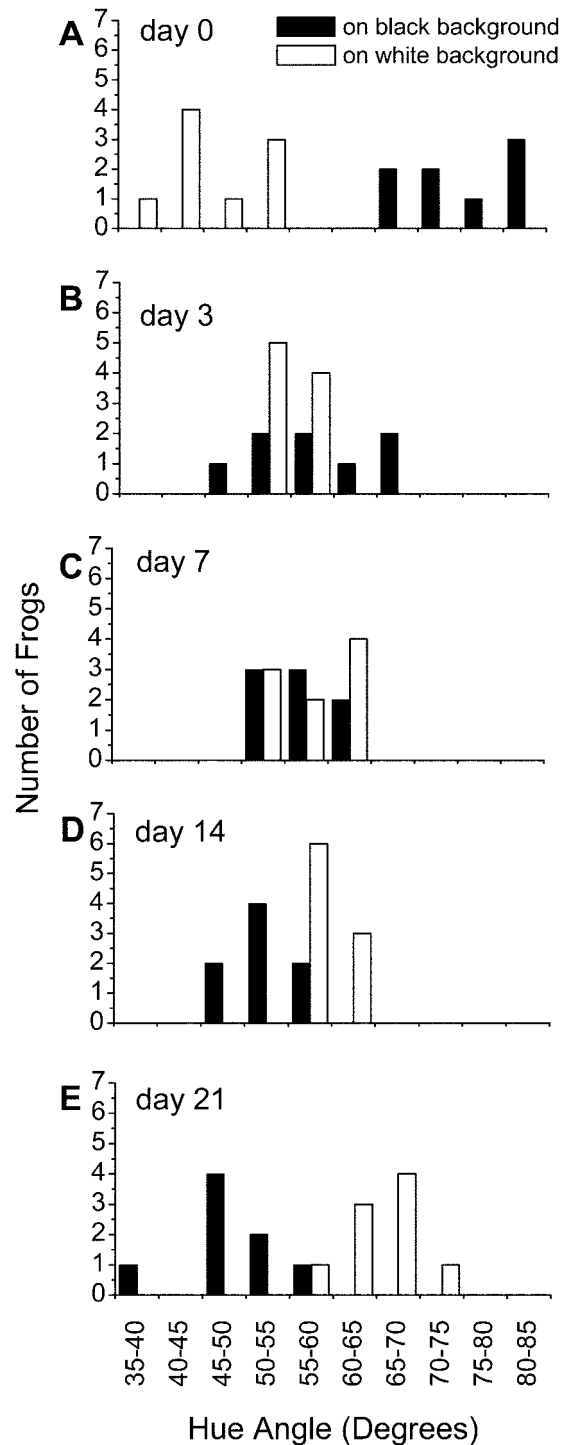


Figure 6: Time course of hue change for color-changing *Hyla regilla*. The white columns represent the white background treatment ($n = 9$). Dark bars represent the black treatment ($n = 8$). The time course progresses from (A) day 0 on the white or black treatment to (B) 3 d on treatment, (C) 7 d, (D) 14 d, and (E) 21 d. Notice there is a period of days to weeks where individuals exhibit an intermediate body coloration.

substrate, we would expect the frogs with an ability to change color to exhibit a shift toward larger (“greener”) hue angle values when placed on a green background and toward smaller (“brownier”) hue angles when placed on a brown background. The data from our green/brown test, however, suggest that *H. regilla* change color specifically in response to differences in background brightness rather than hue (fig. 3). Therefore, color change does not appear to be a direct response to the hue encountered by an individual frog as it moves about in the environment.

Time Course of Hue Change

The time course experiment offers more evidence that color change has not evolved as a means by which frogs can adopt cryptic phenotypes as they move from substrate to substrate. Even while calling at breeding ponds, *H. regilla* males are known to move at least small distances, although they usually remain at the same pond site between recaptures (Schaub and Larsen 1978). During the day, frogs apparently use an even larger activity area adjacent to ponds, perhaps for feeding, temperature regulation, and/or predator avoidance (Jameson 1957). Ponds and surrounding habitats often include a variety of both brown and green substrates (W. Wenthe, unpublished data). However, the color-change response of *H. regilla* is much slower (days to weeks) than the intervals at which *H. regilla* is likely to move between microhabitat types (fig. 6), implying at most a limited ability to change in response to different substrate types encountered during daily movements. Therefore, like in the green/brown experiment, the findings reported here strongly suggest that color change in the “changer” morph of *Hyla regilla* is not a direct response to substrate color.

Color Change and Microhabitat Use

An alternative explanation for the existence of color change exhibited by the changer morphs of *H. regilla* in response to background brightness is that color change is associated with a seasonal change in microhabitat use. As foliage changes over the season, so does frog behavior. It could be that frogs that are changers have a tendency to remain brown when they are in a period of inactivity, for example, spending most of their time in sheltered refuges, which are substantially different from the microhabitats used by frogs during the breeding season. Consistent with the possibility that color-changing frogs make more extensive use of refuges during the winter months, in a separate study we found that the percentage of color changers in samples collected in January was significantly lower than that in samples collected in March (Wenthe 2001). During refuge use, frogs would be more likely to encounter lower

ambient light levels and darker substrates (relative to surrounding microhabitats exposed to direct skylight or sunlight) regardless of background hue. Refuge use would also occur at a time of year when the substrate around the frog is more likely to be brown due to decreased plant activity. *Hyla regilla* is known to use refuges during the early winter months in southern California when temperatures are relatively cold (less than 10°C), and they seek shady refuges when temperatures increase to greater than 20°C (Brattstrom and Warren 1955). In Oregon, at the driest time of year (late summer and fall), frogs have been found occupying refuge sites under cover objects (Jameson 1957; W. Wenthe, unpublished data). When frogs emerge from refuges in the late winter and early spring and subsequently move toward mating ponds, they may change color due to exposure to higher light levels resulting from the use of brighter microhabitats. If so, color change in response to background substrate brightness and/or overall light levels would coincide with the presence of green foliage in the environment even if frog color change did not occur directly in response to the seasonal increase in the availability of green substrates.

Background Tracking and Refuge Use as Complementary Mechanisms for the Maintenance of Fixed and Color-Changing Frogs

If the environment is complex, with many patches of colors, the color morph resembling the most abundant background color patch is likely to experience less predation (Endler 1978). A change in the frequency of a background patch type can lead to a situation where a color morph is more abundant than the matching background. Consequently, the overabundant morph will be conspicuous because it is no longer a “random sample of the background” (Endler 1978). When the availability of a matching substrate varies either spatially or temporally, selective predation on the conspicuous morph can result in prey morph frequency “tracking” the changes in background patch availability (Endler 1978; Cooper and Allen 1994). Frequency-independent selection against a morph that was more abundant than its matching background was demonstrated by Cooper and Allen (1994) in an experimental design using a two-morph prey system with a visual predator (a bird). Color, and not size or shape of the prey items, appeared to be a sufficient cue used in selection by bird predators (Cooper and Allen 1994). As hypothesized by earlier investigators (Endler 1978; Cook 1986), an equilibrium between background patch frequency and color-morph frequency was reached in Cooper and Allen’s study (1994) due to selection against the conspicuous morph, supporting the possibility of background tracking main-

tained by frequency-independent selection on fixed-color morphs by a visual predator.

In addition to frequency-independent selection against the morph that was more common than its matching background, frequency-dependent apostatic selection, where the predator prefers the most abundant morph (perhaps due to the use of a search image by the predator), may stabilize morph frequency by making it more difficult for one morph to reach fixation in the population if the opposite background temporarily becomes rare (Endler 1978; Cook 1986; Cooper and Allen 1994). Thus, in the case of a fixed two-color polymorphism, frequency-independent selection will tend to bring a population into equilibrium with its background environment (background tracking) while apostatic selection may serve to prevent the loss of the rare color morph.

Color change is an example of a plastic trait and involves sensitivity and phenotypic responsiveness of the organism to some environmental cue that will reduce the impact of a selective pressure (Moran 1992; van Tienderen 1997; DeWitt et al. 1998). Lively (1986a) presented a theoretical model demonstrating that both fixed and plastic morphs could evolve as alternative strategies within the same population. Color changers that exist with nonchanging individuals can also experience frequency-independent selection, but since individuals can experience more than one phenotype, individual fitness would be expected to be on average about the same as a nonchanging morph if changers actually utilize the matching background at least half the time (Lively 1986a).

If we apply these ideas to the *H. regilla* system, shifts in selection pressures that result in background tracking provide one possible explanation for the maintenance of the two nonchanging color morphs (green and brown). As the season progresses and the microhabitats (background patches) change in availability, frequencies of green and brown frogs active in the population would be expected to passively track the background patch availability due to frequency-independent predation. This, in conjunction with apostatic selection against the more common nonchanging morph (green or brown, depending on the population), could help to maintain the rare nonchanging frog morph in the population. There is observational evidence of background tracking in some other anurans (Jameson and Pequegnat 1971; Nevo 1973; Schueler and Cook 1980), lending support to the role of changing selection pressures in maintaining the *H. regilla* color polymorphism.

Refuge use could enable color-changing frogs to persist in the population by providing a mechanism for these frogs to more effectively utilize backgrounds that match their body coloration in the absence of a direct response to background hue. If, as discussed earlier, color changers

slowly turn green when they leave refuges in late winter and early spring to forage or visit a breeding pond and encounter brighter substrates, dorsal color change would coincide with a "greening" of the background vegetation, even though frogs are not directly responding to the increase in green backgrounds. Although drier conditions cause foliage throughout most of the habitat to turn brown in late spring and summer, green backgrounds continue to persist at water sources where frogs are active. Consequently, green color changers would still resemble a common background substrate found at the ponds during this time. As the ponds dry, we would expect activity levels to drop off, and the frogs would return to a brown coloration possibly in association with a return to refuges away from the ponds until winter rains again stimulate plant growth.

Costs Associated with Nonchanging and Color-Changing Morphs

The maintenance of both color-changing and nonchanging frogs in the same population is most likely due to differential costs encountered by the color changers and the nonchanging frogs. Given the laboratory evidence that the *H. regilla* color polymorphism might be maintained at least in part by selection due to predation (Morey 1990), *H. regilla* probably qualifies as an example of a protective polymorphism (Roff 1996). If the nonchanging frogs are more similar in color to their respective microhabitats, a potential cost to color changing might be a reduced ability to match and be cryptic on a particular green (or brown) microhabitat. Figure 5 illustrates the inability of color-changing frogs to completely mimic the nonchanging frogs in minimum and maximum hue angles. For example, some green nonchangers exhibited more extreme values of green hues than were attained by the color changers. This suggests a potential cost of color-change ability if nonchanging green and brown frogs more closely match naturally occurring substrates, which might lead to a selective advantage by the nonchanging frogs under specific environmental conditions. On the other hand, nonchanging frogs are more limited in the range of backgrounds they can match. Even though there might be substrate colors that could only be matched by nonchangers exhibiting more extreme hues, these frogs would have a smaller proportion of the environment available to use over the course of a season.

There is evidence that nonchanging morphs prefer backgrounds that match the frog's skin coloration (Morey 1990; Wente 2001). Studies that we have carried out suggest that this behavioral preference is most striking in the nonchanging green frogs and appears to be absent in color-changing frogs (Wente 2001). Such a preference is likely to decrease the vulnerability of nonchanging green frogs

to visual predators, particularly at times of year when green substrates are rare. However, at these times of year the movements of nonchangers might be restricted due to an increased risk of predation because of their conspicuous color on the mismatched backgrounds they might encounter during daily movements (Morey 1990). In contrast, the apparent absence of a tendency for color-changing frogs to select matching backgrounds (Wente 2001) might indicate that they are maintained in the population even when they spend only a portion their time on matching substrates. Perhaps changers are not so conspicuous even when mismatched because they do not exhibit the more extreme hues of the nonchangers. A mismatched changer might not be as obvious as a mismatched nonchanger.

If visually based predation is a selective pressure affecting the *H. regilla* polymorphism, the most vulnerable period of time for color changers is likely to be when frogs are in the relatively slow process of changing color and, therefore, have a truly intermediate phenotype (fig. 6). If substrate colors that are intermediate between brown and green are rare under natural conditions, as suggested by the proliferation of distinct green/brown anuran polymorphisms (Hoffman and Blouin 2000), color changers that are intermediate in color are likely to be more vulnerable to predation. Alternatively, intermediate color changers might have an advantage if expression of this color coincides with an intermediate stage in the transition from winter to spring foliage when there is a broad mix of backgrounds available such that even a mismatched animal would blend in with the average background experienced by a predator. There are also likely to be backgrounds in the environment where an intermediate changer is cryptic (i.e., soil substrates or algal mats consisting of some dead and some live material). Vulnerability experienced by a frog that is intermediate in color might also be tempered by the normal activity patterns of the changer frogs. As the changer moves around in its environment it will likely spend some time on these backgrounds, reducing the effect of predation even when in a transitional stage of color change.

General Conclusions

The presence of both non-color-changing and color-changing morphs within the same population of *H. regilla* provides a unique opportunity to examine the costs and benefits of both body color polymorphism and plasticity as adaptations to a spatially and temporally heterogeneous environment. One possibility suggested by our findings is that changers and fixed green frogs represent alternative strategies for exploiting the limited seasonal availability of green substrates. Fixed green frogs preferentially select

green substrates (Morey 1990; Wente 2001) and, by doing so, remain cryptic even though the overall abundance of green substrates in the environment is reduced. The strategy exhibited by changers is to increase refuge use away from ponds, with an associated change to brown coloration, at times of year when green substrates are rare. Additional research on our study population and on other populations of *H. regilla* will be needed to investigate these and other possible explanations for the coexistence of the color-changing and nonchanging morphs.

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