ANIMAL BEHAVIOUR, 2005, **70**, 279–287
doi:10.1016/j.anbehav.2004.10.029

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Microhabitat selection by the Pacific treefrog, *Hyla regilla*

WENDY H. WENTE & JOHN B. PHILLIPS
Department of Biology, Indiana University

(Received 3 October 2003; initial acceptance 16 December 2003; final acceptance 13 October 2004; published online 13 June 2005; MS. number: A9716R)

Substrate colour choice can provide a means of predator avoidance through crypsis. We investigated background colour choice in the Pacific treefrog, *Hyla regilla*, a species that shows a complex colour polymorphism. Individuals from a single population can be fixed in body colour (the nonchanging green or brown individuals) or they can show an ability to change colour between green and brown (colour-changer morph). In the laboratory, we tested background choice behaviour of the nonchanging green and nonchanging brown frogs to determine whether these frogs have a preference for the matching background substrate. Nonchanging green frogs preferentially selected a matching background colour. Such a preference, however, was not observed in nonchanging brown frogs. When tested in the presence of a predator cue, small samples of both nonchanging green and nonchanging brown frogs showed a preference for the matching substrate. We also investigated whether colour-changers have a preference for a background that matches their own body coloration (phenotype matching) at the time of testing. Colour-changers did not select matching substrates. An alternative to phenotype matching is that substrate colour preference (i.e. that shown by nonchanging green frogs) could result from a sensory bias that is genetically linked to body colour. One possible cause of differences in spectral sensitivity and/or hue discrimination is a photopigment polymorphism in the retina. We used electroretinogram techniques to characterize the spectral sensitivity of the retina but found no evidence for differences in spectral sensitivity curves among the three morphs that would suggest a visual pigment polymorphism.

Cryptic behaviour enables a prey species to avoid visual detection by resembling the background environment (Wickler 1968; Endler 1988). Two methods of achieving crypsis include individual colour change to match the substrate and a behavioural preference for the substrate that most closely resembles the individual's own body coloration (Wickler 1968). These two methods can be considered forms of phenotype matching where an individual assesses its own phenotype (i.e. body coloration) and either selects the appropriate matching background or changes colour to match the background it currently inhabits. In this study we explored background colour selection in a species that includes, within a single interbreeding population, individuals that can change colour as well as individuals that remain fixed as one of two distinct body colours.

Correspondence and present address: W. H. Wente, 1606 SW Carson Street, Portland, OR 97219, U.S.A. (email: wendy@wente.com). J. Phillips is now at the Biology Department, Virginia Tech, Blacksburg, VA 24061, U.S.A.

The Pacific treefrog, *Hyla regilla*, shows a stable dorsal colour polymorphism with a percentage of individuals, depending on the population and time of year, displaying a green dorsal body coloration, while a majority of the remainder have a brown dorsal body colour (Jameson & Pequegnat 1971). We have recently discovered that, in addition to the frogs with a permanent dorsal colour of either green or brown, there is a third morph capable of changing colour (Wente & Phillips 2003). The colour-changing frogs can switch between green and brown dorsal coloration over a period of several weeks. The three colour morphs (i.e. nonchanging green, nonchanging brown, and colour changer), coexist in the same population and mate contemporaneously in the same breeding habitats.

Variation in body colour of *H. regilla* might be maintained by predation pressure that selects for the ability of individuals to cryptically match either green or brown substrates (Jameson & Pequegnat 1971). In laboratory experiments, Morey (1990) found individual brown and green Pacific treefrogs actively selected the background colour that most closely matched their own body coloration. Furthermore, he demonstrated that background

0003–3472/04/$30.00/0

colour matching reduced detection by a common predator of *H. regilla*, the garter snake *Thamnophis elegans*, which is known to use visual cues to locate prey at short range (Hays 1989).

Using methods similar to those developed by Morey (1990), we compared microhabitat preferences (background colour choice behaviour) in the nonchanging green, nonchanging brown, and colour-changing morphs of *H. regilla*. Based on Morey's results, which did not distinguish colour changers from nonchangers, we expected all individuals to choose a substrate most similar to their own body colour at the time of the test.

A visual polymorphism could be associated with differences in substrate colour preference among the colour morphs. Evidence suggests at least limited spectral discrimination ability in anurans (Muntz 1966; Reuter & Virtanen 1972; Hailman & Jaeger 1974; King et al. 1993). To match typical microhabitats, *H. regilla* would need a visual system that would allow it to discriminate between the different spectral characteristics of green and brown substrates (Fig. 1). Beyond the ability to distinguish green and brown colour substrates, green frogs might also benefit by having better hue discrimination in the middle of the visible spectrum where the reflectance spectra of green substrates, such as leaves and algae, change most abruptly (Fig. 1). Greater hue discrimination ability in this region of the spectrum would make the frogs better able to use subtle differences in the spectral reflectance of green substrates to maximize their crypticity. Similarly, brown frogs might benefit from enhanced hue discrimination at the long-wavelength end of the visible spectrum. Relative hue discrimination ability in different regions of the spectrum depends on the number, location and spacing of photopigments across the visible spectrum (for review see Gouras 1991). We attempted to detect a visual polymorphism by characterizing cone mechanisms present in the retinas of the three colour morphs of *H. regilla* with an electroretinogram.

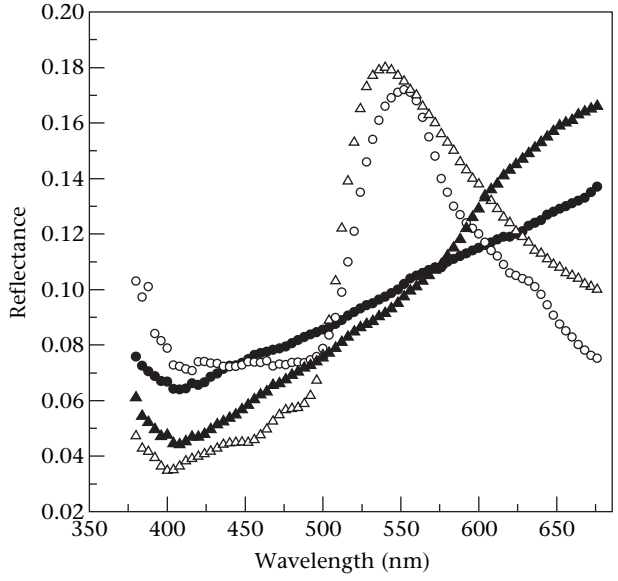

Figure 1. Reflectance spectra collected from brown and green *Hyla regilla* and natural backgrounds (green foliage and a brown stick). Triangles represent frogs: a nonchanging green frog (△) and a nonchanging brown frog (▲). Circles represent background substrates: a brown stick (●) and a blade of green grass (○). Green leaves typically have a distinct spectral reflectance peak around 530–550 nm. Soil, bark and dried leaves, which appear brown or reddish brown to the human eye, typically have a lower reflectance at 550 nm and a gradual increase in reflectance from shorter to longer wavelengths.

There are two likely sources of variation in spectral sensitivity in the retinas of the different frog colour morphs: differences in the protein ('opsin') portions of the photopigments, and differences in the light-absorbing chromophores. Visual pigment polymorphisms resulting from amino acid differences in the protein portion of photopigments have been described in fish (Archer et al. 1987), nonhuman primates (Bowmaker et al. 1985; Tovée et al. 1992; Jacobs 1996) and humans (Nathans et al. 1986; Neitz & Jacobs 1986; Neitz & Jacobs 1990). If brown frogs possess one or more classes of photoreceptors with opsins that produce absorption peak(s) at longer wavelengths than comparable photoreceptors in green frogs, then brown frogs might show greater sensitivity and better hue discrimination ability at longer wavelengths.

The photopigment chromophore provides an alternative source of variation in the spectral sensitivity of long-wavelength photoreceptors. In some species of frogs with aquatic larvae, the larval chromophore, 3-dehydroretinal vitamin A_2, shifts the sensitivity of the long-wavelength cones to longer wavelengths relative to the sensitivity of the adult chromophore, which is vitamin A_1 (Wilt 1959; Hoskins 1990). If adult brown *H. regilla* retain the larval Vit A_2 form of the photopigment chromophore in at least a subset of their long-wavelength cones, as occurs in adult bullfrogs *Rana catesbeiana* (Semple-Rowland & Goldstein 1981), these cones would show greater long-wavelength sensitivity. Interestingly, larval *H. regilla* are brown, making tadpoles cryptic when on long-wavelength reflecting substrates such as the dead leaves typically found on the bottom of streams and ponds (W. Wente, personal observation). Consequently, adult retention of multiple larval characteristics (i.e. body colour, chromophore type, and perhaps associated spectral processing mechanisms) could produce an adult phenotype consisting of a suite of physiologically linked traits that produce both increased fitness in and a preference for, microhabitats containing brown substrates.

METHODS

Study Animals

Hyla regilla were collected on Vandenberg Air Force Base in Santa Barbara County, California, U.S.A., during winter and spring from 1996 to 1999. This work was approved by the Bloomington Indiana Animal Care and Use Committee (BIACUC Protocol No. 99-057). Frogs were housed in environmental control rooms at Indiana University either singly in clear plastic containers or communally in terraria containing plants, and a soil and bark substrate. Frogs were maintained on a 12:12 h light:dark cycle throughout

the year and fed crickets (*Achetus* sp.) and house flies (*Musca* sp.) two to three times each week. Behavioural experiments were completed within about 6 months from capture, after which frogs were either transferred to other BIACUC-approved projects or euthanized.

Background Colour Matching

Frog dorsal surface and background substrate spectral reflectances were measured with a spectraradiometer (Photo Research SpectraScan SpectraRadiometer PR-714) following standard methods (Endler 1990). Determination of colour change ability involved alternate exposure to dark and light substrates for periods of 3–4 weeks during which spectral reflectance of the dorsal skin surface was regularly measured (Wente & Phillips 2003); the earlier study found no evidence of colour change when frogs were sequentially exposed to green and brown substrates. Following criteria established in the earlier work, a changer was operationally defined based on the amount of change in the ratio of mid- to long-wavelength reflectance during exposure to the light and dark substrates. Based on spectral reflectance of the dorsal skin surface, we assigned each frog to one of the three colour morph groups (nonchanger green, nonchanger brown, or colour changer; Wente & Phillips 2003).

The background choice test apparatus was a large, circular arena (32 cm high × 59 cm in diameter) divided into equal quadrants, two opposite quadrants were brown and the remaining two green. Brown and green inserts made of dowel rods and attached laminated artificial leaves provided vertical structure in the quadrants. Background substrate coloration, including the inserts, approximated measured spectral reflectance of brown and green dorsal frog coloration as well as naturally occurring background substrates (frogs and the typical substrates they would have access to under natural conditions have similar reflectance curves; Fig. 1). The test arena was housed in a light tight and vibration damped hood. In an attempt to replicate dawn and dusk lighting conditions, frogs were tested for background preference in a dim light environment (light from four 7.5-V incandescent bulbs passed through a white Plexiglas diffuser mounted in the top of the hood). Temperature and humidity were recorded during each test. Small, clear glass water bowls were placed in each quadrant and the arena was uniformly misted with water before each test. A video camera mounted in the top of the light-tight hood allowed remote monitoring and recording of the test. At the start of each test, an individual frog was held inside a remotely operated release device at the centre of the arena. After a 1-min delay, the release device was raised and the frog released into the arena. After an overnight acclimation period in the dark, a frog was scored for background matching every 5 min over a period of 330 min the following morning, starting when the lights came on. This resulted in the collection of 66 observations of the frog's location during each test. Frogs that did not choose a background in at least two-thirds (44/66) of the observations, either due to climbing on the sides of the arena (which could be indicative of escape behaviour, rather than substrate choice) or sitting on the release device, were excluded from further analysis. If a frog was located on the background most spectrally similar to its own dorsal coloration greater than 50% of the time it spent on the matching substrates, it was scored as demonstrating background choice behaviour. Since an individual was tested only once during an experiment, the time spent on each of the backgrounds, green or brown, was not independently measured. Therefore, for each group of frogs belonging to the same morph, we tested whether the number showing background choice behaviour deviated from random expectation (50% on each background) using a binomial test.

Retest for Background Matching Behaviour

A subset of individuals from each of the colour morph groups was tested for background colour preference using the same protocol as the initial background choice test. Consistency in the behaviour of individual frogs between successive background choice tests was tested by calculating the Pearson correlation coefficient between the first and second test data sets for each morph group.

Background Choice Behaviour in Response to a Predator Cue

A small sample of previously tested nonchanging frogs (10 nonchanging brown frogs, six nonchanging green frogs) was tested for background choice in the presence of a predator cue. *Thamnophis sirtalis*, the common garter snake, is a known predator of adult *H. regilla* (Schaub & Larsen 1978). The test protocol was identical to the initial background choice test except a solution of *T. sirtalis* faecal matter (0.1 g of dried *T. sirtalis* excrement/100 ml of distilled water) was sprayed into the arena before each test. Background choice data were again analysed using a binomial test.

Measurement of Spectral Sensitivity

Electroretinogram (ERG) recording techniques (mass retinal response) (Goldsmith 1986) were used to measure the spectral sensitivity of individual frogs belonging to each of the colour morph groups. The spectral sensitivities of frogs of the same morph were averaged and compared to the other colour morphs to determine whether there were any differences in the shapes of the spectral sensitivity curves.

Dissection and preparation of the eye was carried out under dim and indirect white light to minimize selective adaptation of long-wavelength photoreceptors (Goldsmith 1986). After an animal was sacrificed by rapid decapitation, one whole eye and the surrounding cranial tissue was removed. We exposed the underlying retinal tissue by removing the upper half of the eye (cornea and iris) as well as the lens. To prevent folding, the open eye cup (intact retina and sclera) was supported and held open

by a portion of the skeletal orbit still attached to the sides of the eye cup tissue. Excess vitreous fluid was wicked out of the cup using a small, triangular piece of tissue paper (Kimwipe). The preparation was placed on a black cloth to minimize reflection of the stimulus light. To maintain the preparation we dampened the cloth with amphibian ringers (6.48 g of NaCl, 0.19 g of KCl, 0.33 g of $MgCl_2$, 0.15 g of $CaCl_2$, 1.8 g of D-glucose, 0.71 g of HEPES, 0.135 g of streptomycin in 1000 ml of water) while the recording chamber was aerated with a humidified mixture of 99:1 oxygen to carbon dioxide.

Monochromatic light stimuli were produced using a grating monochrometer (Oriel no.77250) and a quartz/halogen incandescent lamp that provided a peak output at the preparation of 15.24 log quanta per cm^2 per second at 570 nm. A pair of counter-rotating neutral density wedges (Melles Griot no.03-FDC-005) attenuated the test flash and a shutter set the flash duration at 50 ms. Stimulus flashes were carried by a liquid core fibre optic (Oriel no.77556) into the Faraday cage and exited the fibre optic 20 mm above the eye preparation. The light passed through a quartz window covering the recording chamber before reaching the eye cup. Methods for white-light adaptation were similar to those employed by Deutschlander & Phillips (1995). Eye preparations were white-light adapted using a 6-V incandescent lamp fitted with a heat filter and a single Schott BG34 filter to produce nearly equal quantal flux (10.75 log quanta per cm^2 per second) between 400 and 700 nm measured using a Photo Research SpectraScan spectraradiometer (model PR-714). The BG34 filter eliminated light wavelengths shorter than about 400 nm. The adapting light, which was carried to the eye via a trifurcated glass fibre optic, remained illuminated throughout the stimulus light series. The three outputs of the fibre optic were positioned symmetrically around the fibre optic carrying the stimulus light so that the adapting light uniformly illuminated the retina.

Spectral sensitivity of each retinal preparation was measured at 400, 430, 460, 490, 520, 550, 560, 570, 580, 590, 600, 610, 640, 650, 670 and 700 nm with a reference wavelength of 500 nm. Long-pass filters were used to eliminate any stray light at shorter wavelengths for the 600- and 610-nm stimuli (570 nm long-pass), the 640- and 650-nm stimuli (610 nm long-pass), and the 670- and 700-nm stimuli (640 nm long-pass). A chlorided silver wire electrode was placed on the surface of the exposed retina to detect the voltage response of the retina. The response was determined as the difference between the trough of the A wave and the peak of the B wave of the electroretinogram (Deutschlander & Phillips 1995). The sensitivity at each wavelength was defined as the quantal flux of a monochromatic flash required to elicit a criterion response. The criterion response level was held constant for any single preparation, but at different values (ranging from 15 to 45 μV) in successive experiments, depending on the sensitivity of the preparation being tested. The criterion level was chosen to fall near the centre of the most linear portion of the log intensity/response curve for that preparation.

Once the criterion response level was established, the eye cup was stimulated with a series of monochromatic flashes at each wavelength. Quantal flux of the monochromatic flash was varied by attenuating the light source until at least three responses were obtained that spanned the criterion response level and differed from the criterion level by less than 10 μV. The quantal flux necessary to elicit the criterion response was then estimated by fitting a line through the three (or more) responses using the least squares method. Sensitivity at each wavelength was then normalized with respect to a reference wavelength (in this case, 500 nm), which was given a sensitivity value of 0.0. Sensitivity of the preparation to the reference wavelength was checked repeatedly during the experiment. If the stimulus intensity necessary to elicit a criterion response at 500 nm drifted by more than 0.2 log units, the measurements obtained at other wavelengths sampled since the previous reference check were discarded and repeated. Once the measurements were completed, a spectral sensitivity curve was obtained by plotting the difference in the log quantal flux necessary to elicit a criterion response at each wavelength relative to that of the reference wavelength (500 nm). Spectral sensitivities were compared graphically between the different colour morph groups.

RESULTS

Background Colour-matching Behaviour

Based on the earlier work by Morey (1990), we hypothesized that both changing and nonchanging green and brown *H. regilla* would show a preference for the background substrate that most closely resembled their own body coloration. All of the frogs tested for background choice spent at least two-thirds of their time on one of the two coloured substrates. Nonchanging green frogs showed a significant preference for green backgrounds with 78% choosing the matching (green) background more than 50% of the time that they spent on one or the other of the coloured substrates (one-tailed binomial test: $k = 6$, $N = 27$, $P = 0.003$; Fig. 2). The average proportion of observations that individual nonchanging green frogs spent on matching substrates was 67.7%. Nonchanging brown individuals did not show a significant preference for the brown substrates (one-tailed binomial test: $k = 19$, $N = 44$, $P = 0.23$), although a slight majority (57%) of nonchanging brown frogs did show a preference for the matching brown background. The average proportion of observations that nonchanging brown frogs spent on the matching substrate was 51.2% (Fig. 3a).

The absence of background matching by nonchanging brown frogs could have resulted from a greater tendency to remain in one place after initially settling at random during the night when the lights were off. To investigate this possibility, we compared the activity of nonchanging green and nonchanging brown frogs during the matching tests. Specifically, we compared the rates of background switching between brown, green and other (e.g. sitting on release device in centre of arena). Nonchanging brown frogs switched an average of 3.45 times whereas nonchanging greens switched 3.64 times on average during a test (Mann–Whitney U test: $U = 606$, $N_1 = 28$, $N_2 = 44$,

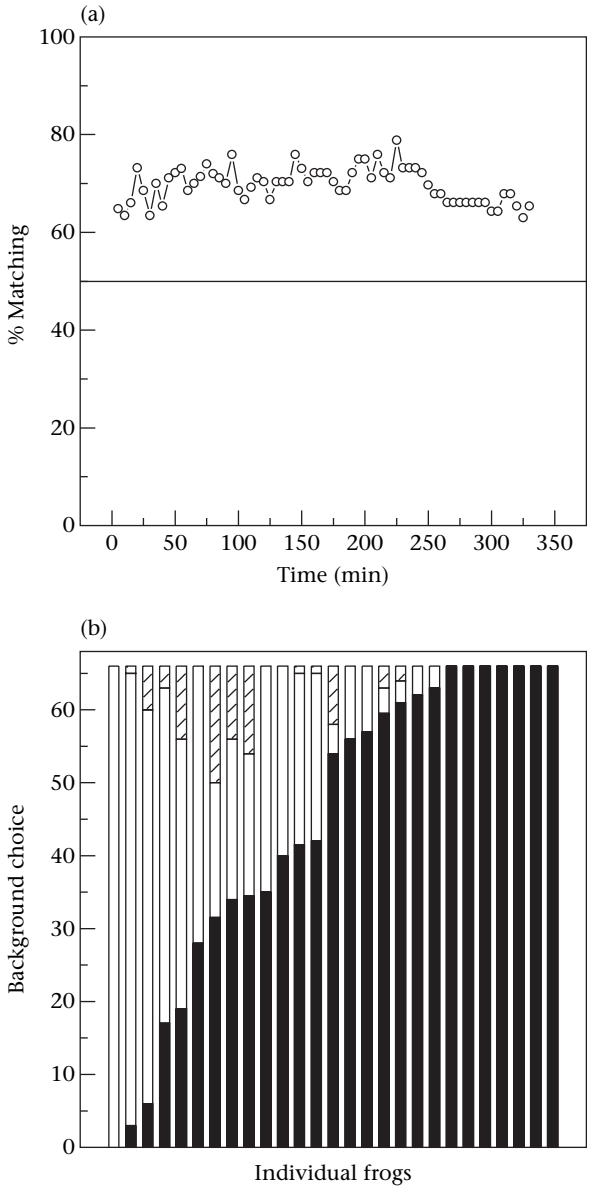

Figure 2. Performance of nonchanging green frogs ($N = 27$) in their first behavioural test for background preference. (a) Performance of the entire group of frogs over the period of the test. The location of each frog was recorded every 5 min for a period of 330 min. Each symbol represents the percentage of the entire group that was found on the matching substrate. (b) Performance of each frog during its test ($N = 66$ locations/frog). The black portion of a bar represents the proportion of time spent on the green background by a frog. The open portion of a bar represents time spent on brown and the hatched portion represents time spent either climbing or sitting on the release device during a test. Matching was assessed by determining whether a frog was on a matching background more than 50% of the time it was observed on either of the colour substrates. Time spent climbing or on the release device was not included in the assessment.

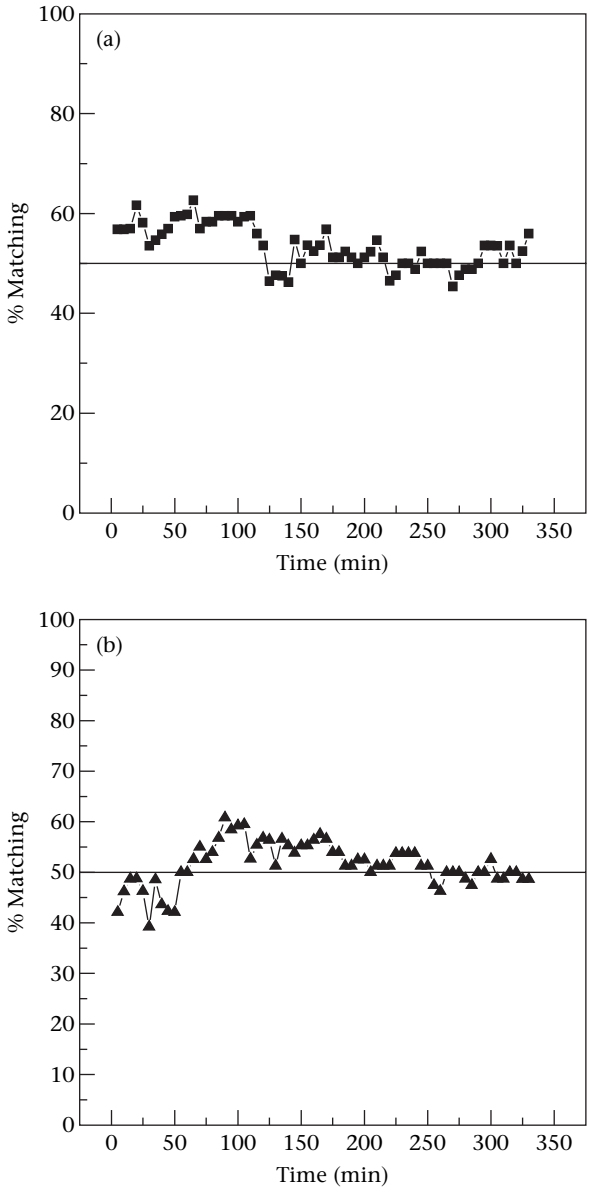

Figure 3. Performance of (a) nonchanging brown frogs ($N = 44$) and (b) brown phase colour-changing frogs ($N = 40$) in their first behavioural test for background preference. The location of each frog was recorded every 5 min for a period of 330 min. In (a) and (b), each symbol represents the percentage of the entire group that was found on the matching substrate.

$P = 0.911$, power = 0.53), suggesting that there was no difference in general activity levels between the two nonchanging colour morphs.

When retested for background colour choice, we expected nonchanging green frogs to maintain a preference for the matching substrate. Seventy-seven per cent of the nonchanging green frogs again showed a preference for the green backgrounds (one-tailed binomial test: $k = 3$, $N = 13$, $P = 0.046$; Fig. 4a). In nonchanging brown frogs, however, only 50% of the frogs showed choice behaviour for the matching brown substrate, which did not differ from random expectation (one-tailed binomial test: $k = 10$, $N = 20$, $P = 0.59$; Fig. 4b).

In the absence of initial test biases that might affect background choice behaviour (i.e. acclimation to the arena or learning during the initial test that could lead to a change in subsequent behaviour), we expected individual frogs to show similar background choice

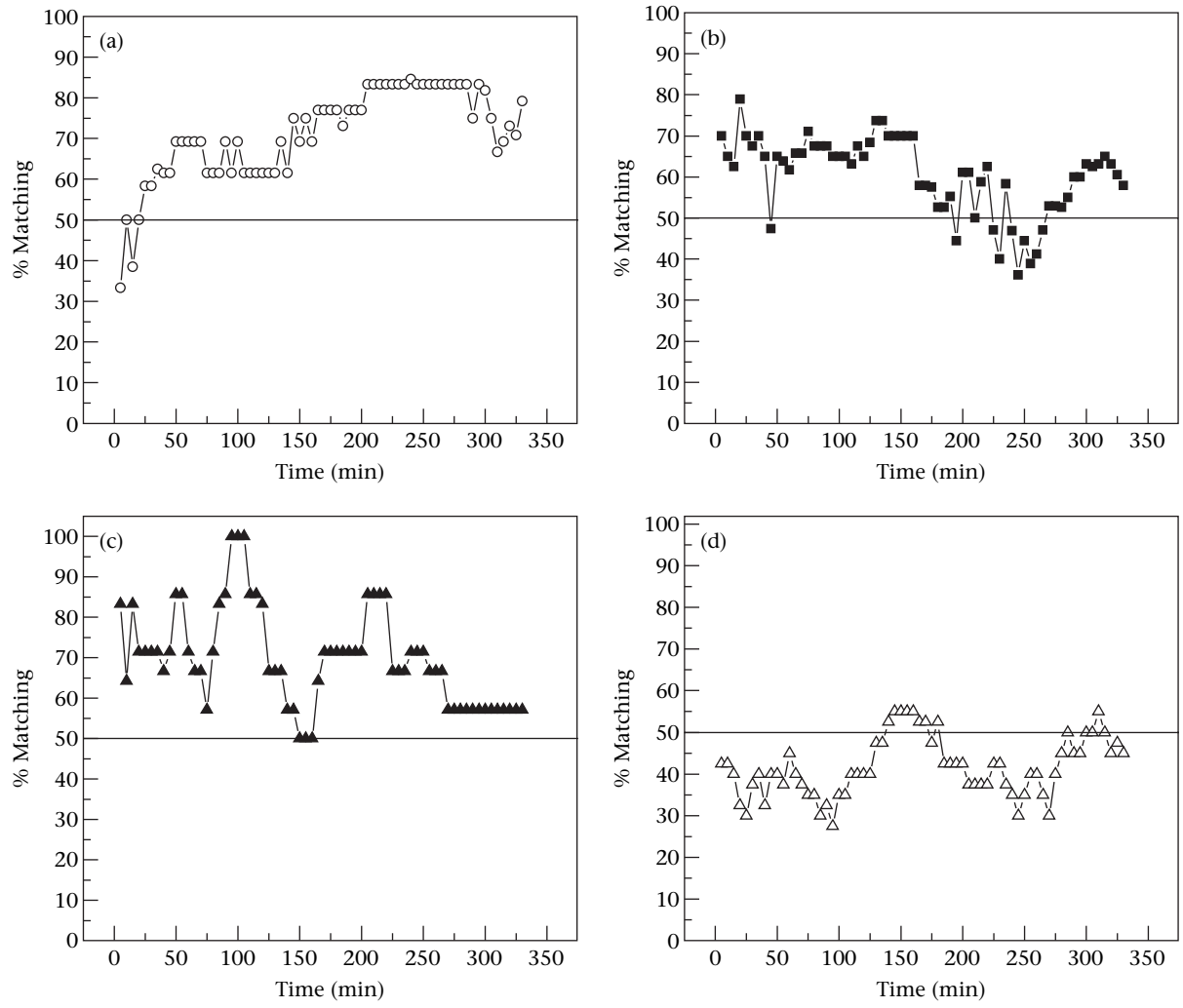

Figure 4. Performance of (a) nonchanging green frogs ($N = 13$) and (b) nonchanging brown frogs ($N = 20$) when retested for background colour choice behaviour. Performance of colour changers when retested in (c) the brown phase ($N = 10$) and (d) the green phase ($N = 19$).

performance between the initial and second background choice tests. In fact, individual background preference did not vary between the first and second tests for both the nonchanging green frogs (Pearson correlation: $r_{11} = 0.19$, $P = 0.529$) and the nonchanging brown frogs ($r_{18} = 0.44$, $P = 0.06$).

We expected the colour-changing frogs to choose the background colour that most closely matched their own body coloration at the time of testing. Colour change requires from several days to several weeks in this species (Wente & Phillips 2003) and so was not a factor during these tests. Contrary to our predictions, however, colour-changing frogs showed no clear choice for background colour. Individuals were first tested during the brown phase and showed no background preference, since only 50% of the frogs chose the matching background (one-tailed binomial test: $k = 20$, $N = 40$, $P = 0.56$; Fig. 3b). The average proportion of observations that individual colour-changers in the brown phase spent on the matching substrates was 49.5%.

We retested colour-changing frogs in both the green and brown colour phases. When retested in the brown phase, 70% of the frogs showed a preference for the matching brown background but, due to small sample size, this was not statistically significant (one-tailed binomial test: $k = 3$, $N = 10$, $P = 0.17$; Fig. 4c). Colour-changers when retested in the green phase showed no preference for the matching background, with only 42% preferring the green substrate (one-tailed binomial test: $k = 8$, $N = 19$, $P = 0.32$; Fig. 4d).

Background Choice Behaviour in Response to a Predator Cue

We hypothesized an increase in background matching behaviour when frogs perceived the presence of a predator (*T. sirtalis*). Nonchanging frogs showed a preference for the matching background substrate when *T. sirtalis* waste was evenly dispersed throughout the arena (one-tailed binomial test: $k = 4$, $N = 16$, $P = 0.038$, power = 0.63). In this group, seven of the 10 nonchanging brown frogs (70%) and five of six nonchanging green frogs (83%) chose the matching substrate. The two groups of

nonchanging frogs did not differ in activity levels, measured by counting the number of times a frog switched between backgrounds, when tested in the presence of snake odour (Mann–Whitney U test: $U = 24.5$, $N_1 = 6$, $N_2 = 10$, $P = 0.533$ power = 0.147), nor did they differ from the group of nonchangers tested in the absence of snake odour ($U = 507$, $N_1 = 72$, $N_2 = 16$, $P = 0.448$, power = 0.432).

Visual Polymorphism

Comparing the average spectral sensitivity curves obtained from three nonchanging green frogs, three nonchanging brown frogs, and two colour-changing frogs provided no evidence for a difference in spectral sensitivity among the three groups (Fig. 5).

DISCUSSION

The goals of this study were to determine whether microhabitat selection (background colour choice) by *H. regilla* in a laboratory setting (Morey 1990) was replicable, and to determine how microhabitat selection by the newly described colour-changing morph compares to that of the nonchanging green and brown morphs. We also investigated a possible visual mechanism that could play a role in background colour matching by *H. regilla*.

Because of their demonstrated preference for a matching green background, nonchanging green frogs are the best candidates for a strong, perhaps genetically linked, association between dorsal colour and a behavioural preference for that colour. If there is an increased tendency for nonchanging *H. regilla* frogs to prefer matching substrates in the presence of *T. sirtalis* predator odour, the sample sizes used in the predator odour experiments were too small to detect this difference.

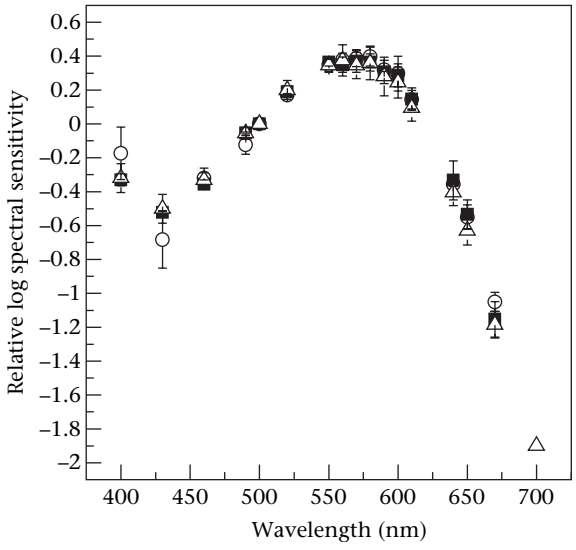

Figure 5. Spectral sensitivity for the three different *Hyla regilla* morphs (○: nonchanging green, $N = 3$; ■: nonchanging brown, $N = 3$; △: colour changers, $N = 2$). All three groups showed overlap in spectral sensitivity.

Nonchanging brown frogs might use an entirely different strategy for predator avoidance since they did not show a significant preference for a matching background colour even in the presence of a predator odour cue. This morph might rely on a hue-independent, substrate-brightness-based background choice behaviour similar to that of *Bufo americanus* (Heinen 1985, 1993, 1994). In the present study, we attempted to minimize differences in overall brightness between the green and brown substrates, making it unlikely that nonchanging brown frogs could use a brightness-matching strategy to distinguish between the different substrate types, hence we would expect to not see substrate matching.

Alternatively, the primary defences of nonchanging green and brown frogs against predation might differ as a consequence of the types of predators they are likely to encounter. Even primarily visual predators may use very different strategies to capture prey. Jays (*Perisoreus* spp.) perch and look for frogs that are stationary but mismatched by colour, whereas robins (*Turdus* spp.) can hunt frogs by actively flushing them, apparently focusing more on movement than mismatch with the background (Tordoff 1971). If brown frogs are more subject to predators using movement-based search strategies rather than spectral contrast to localize prey, nonchanging brown frogs might evolve a predator avoidance strategy that relies on reduced movement when exposed to predator cues, regardless of the substrate colour or brightness. There is precedence for this strategy since some larval anurans, including *H. regilla*, are known to show changes in behaviour specifically in response to a predator cue (McCollum & Buskirk 1996; De Vito et al. 1999). Garter snakes prey upon moving Pacific treefrogs at much higher rates than upon nonmoving frogs, irrespective of whether they match the hue of the underlying substrate (Morey 1990). However, in our study there was no detectable difference in the rates of movement between nonchanging green and nonchanging brown frogs in the presence of snake odour.

Since frogs of the colour-changer morph did not show a preference for a background colour, this morph might rely on an entirely different strategy of predator avoidance than the nonchanging green frogs that actively choose a matching background. One possibility is that changers can facultatively change their colour to match green and brown substrates, as has been shown to occur in other treefrog species (e.g. *H. arborea*; Nielsen 1980). If so, there would be a corresponding reduction in the advantage of selecting a matching substrate colour. In a related paper (Wente & Phillips 2003), however, we have demonstrated that colour-changers alter body colour in response to background substrate brightness, rather than hue, and require from several days to as much as 2–3 weeks for colour change to occur. Consequently, changers do not appear to have the ability to rapidly match substrate colour, making this an unlikely explanation for the absence of a behavioural preference for matching substrate colours.

The finding that background brightness, rather than hue, triggers colour change in colour-changing individuals, and the relatively slow time course of this response

(Wente & Phillips 2003), suggests that an explanation for the absence of a substrate colour preference should be sought in the context of more long-term (seasonal, rather than daily) changes in behaviour. Exposure to differences in light intensity, and corresponding changes in substrate brightness, could be related to a shift in activity patterns that occurs as the spring breeding season begins. *Hyla regilla* are known to use winter refuges, including cavities underneath rocks and piles of dead vegetation near water sources, crevices in soil or boulders, and rodent burrows (Brattstrom & Warren 1955; W. Wente, personal observation). The substrates found in such refuges tend to be brown (soil, dead vegetation, etc.), and the interiors of such refuges are dark relative to more exposed substrates. Emergence from winter refuges would coincide with exposure to both higher light levels and more reflective substrates and, thus, could be the proximal factor triggering colour change under natural conditions.

Since the rate of colour change is slow, changing frogs emerging from winter refuges would be brown in colour when the majority of the background substrates are brown (winter foliage). They would gradually shift to green as the prevalence of green substrates increased with the growth of spring foliage. Colour changers would pass through an intermediate coloration, possibly a time of greater vulnerability to predation (Wente & Phillips 2003), but they would also track the seasonal changes in the availability of differently coloured substrates, even if the proximal cue triggering change was background brightness, rather than hue. Consequently, an overall tendency towards crypsis might result, not from a behavioural preference, but from activity patterns that expose frogs to elevated light intensities and/or substrate brightnesses that coincide with seasonal increases in the availability of green substrates. A final possibility that warrants further investigation is whether colour changers in the green phase (as well as nonchanging green frogs) are more arboreal than either type of brown frog, or are more likely to be found in exposed locations like emergent vegetation in breeding ponds. Substrate colour matching in such exposed locations would be especially important during daylight hours (including times near dawn and dusk) when the danger from visual predators is greatest. Such sites would coincidentally be associated both with higher light levels (which could trigger a switch to green body colour in the changer morph) and a greater prevalence of green substrates, in particular frog-sized patches of green substrate, such as leaves, that would enhance crypsis by increasing the match between the frog and both the size and colour of surrounding patches of substrate.

Our attempts to understand the sensory mechanism(s) contributing to substrate colour matching in *H. regilla* were unsuccessful. We should emphasize, however, that our failure to find differences among the morphs in the spectral sensitivity of the retina does not rule out the possibility that there is a polymorphism in the central nervous system of the frog including the sensory mechanisms that process inputs from the photoreceptors.

Our findings also failed to provide evidence for phenotype matching. If frogs were capable of phenotype matching, we would have expected colour-changing frogs to show a preference for substrate colours that matched their body colour at the time of testing. The absence of matching by colour-changing frogs does not, however, rule out the possibility that phenotype matching plays a role in colour matching by nonchanging green frogs. An understanding of the sensory basis of colour matching, therefore, will have to await the results of future research.

In summary, the findings presented here, along with those reported earlier (Wente 2001; Wente & Phillips 2003), indicate that there are at least three colour morphs of *H. regilla* that differ in body colour, in ability to change colour, and in the presence or absence of a correlated substrate colour preference. Further studies of the sensory basis, ecological correlates, and fitness consequences of colour variation and related behaviours in this species will undoubtedly contribute to a better understanding of the many processes and interactions that help to maintain phenotypic variation in natural populations.

Acknowledgments

We thank Jen Kwasny for her assistance with the snake odour tests as well as Mark Deutschlander and Chris Borland for their technical advice and assistance with the physiological portion of the project. This work was funded by the Indiana University Center for the Integrative Study of Animal Behavior, the Indiana University Department of Biology, a Sigma Xi Grant-in-Aid of Research, a Society for the Study of Amphibians and Reptiles Research Grant, the Indiana University Graduate School Doctoral Student Grant-in-Aid of Research, the Indiana Academy of Science, and J. Bart Culver. Collections were completed under California Scientific Collecting Permits 802008-01 and 802030-01 with special permission from Vandenberg Air Force Base.

References

Archer, S. N., Endler, J. A., Lythgoe, J. N. & Partridge, J. C. 1987. Visual pigment polymorphism in the guppy *Poecilia reticulata*. *Vision Research*, **27**, 1243–1252.

Bowmaker, J. K., Jacobs, G. H., Spiegelhalter, D. J. & Mollon, J. D. 1985. Two types of trichromatic squirrel monkey share a pigment in the red-green spectral region. *Vision Research*, **25**, 1937–1946.

Brattstrom, B. & Warren, J. 1955. Observations on the ecology and behavior of the pacific treefrog, *Hyla regilla*. *Copeia*, **1955**, 181–191.

De Vito, J., Chivers, D. P., Kiesecker, J. M., Belden, L. K. & Blaustein, A. R. 1999. Effects of snake predation on aggregation and metamorphosis of Pacific treefrog (*Hyla regilla*) larvae. *Journal of Herpetology*, **33**, 504–507.

Deutschlander, M. E. & Phillips, J. B. 1995. Characterization of an ultraviolet photoreception mechanism in the retina of an amphibian, the axolotl (*Ambystoma mexicanum*). *Neuroscience Letters*, **197**, 93–96.

Endler, J. A. 1988. Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of the Royal Society of London, Series B*, **319**, 505–523.

Endler, J. A. 1990. On the measurement and classification of color in studies of animal color patterns. *Biological Journal of the Linnean Society*, **41**, 315–352.

Goldsmith, T. H. 1986. Interpreting trans-retinal recordings of spectral sensitivity. *Journal of Comparative Physiology A*, **159**, 481–487.

Gouras, P. 1991. Color vision. In: *Principles of Neural Science* (Ed. by E. R. Kandel, J. H. Schwartz & T. M. Jessell), pp. 467–480. Norwalk, Connecticut: Appleton & Lange.

Hailman, J. P. & Jaeger, R. G. 1974. Phototactic responses to spectrally dominant stimuli and use of colour vision by adult anuran amphibians: a comparative survey. *Animal Behaviour*, **22**, 757–795.

Hays, F. E. 1989. Antipredator behavior of recently metamorphosed toads (*Bufo a. americanus*) during encounters with garter snakes (*Thamnophis sirtalis*). *Copeia*, **1989**, 1011–1015.

Heinen, J. T. 1985. Cryptic behavior in juvenile toads. *Journal of Herpetology*, **19**, 524–527.

Heinen, J. T. 1993. Substrate choice and predation risk in newly metamorphosed American toads *Bufo americanus*: an experimental analysis. *American Midland Naturalist*, **130**, 184–192.

Heinen, J. T. 1994. The significance of color change in newly metamorphosed American toads (*Bufo a. americanus*). *Journal of Herpetology*, **28**, 87–93.

Hoskins, S. G. 1990. Metamorphosis of the amphibian eye. *Journal of Neurobiology*, **21**, 970–989.

Jacobs, G. H. 1996. Primate photopigments and primate color vision. *Proceedings of the National Academy of Sciences, U.S.A.*, **93**, 577–581.

Jameson, D. L. & Pequegnat, S. 1971. Estimation of relative viability and fecundity of color polymorphisms in anurans. *Evolution*, **25**, 180–194.

King, R. B., Douglass, J. K., Phillips, J. B. & Baube, C. L. 1993. Scotopic spectral sensitivity of the optomotor response in the green treefrog *Hyla cinerea*. *Journal of Experimental Zoology*, **267**, 40–46.

McCollum, S. A. & Buskirk, J. V. 1996. Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysosceles*. *Evolution*, **50**, 583–593.

Morey, S. R. 1990. Microhabitat selection and predation in the Pacific treefrog, *Pseudacris regilla*. *Journal of Herpetology*, **24**, 292–296.

Muntz, W. R. A. 1966. The photopositive response of the frog (*Rana pipiens*) under photopic and scotopic conditions. *Journal of Experimental Biology*, **45**, 101–111.

Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B. & Hogness, D. S. 1986. Molecular genetics of inherited variation in human color vision. *Science*, **232**, 203–210.

Neitz, J. & Jacobs, G. H. 1986. Polymorphism of the long-wavelength cone in normal human color vision. *Nature*, **323**, 623–625.

Neitz, J. & Jacobs, G. H. 1990. Polymorphism in normal human color vision and its mechanism. *Vision Research*, **30**, 621–636.

Nielsen, H. I. 1980. Color and color adaptation of the European tree frog, *Hyla arborea*. *Journal of Experimental Zoology*, **211**, 143–151.

Reuter, T. & Virtanen, K. 1972. Border and color coding in the retina of the frog. *Nature*, **239**, 260–263.

Schaub, D. L. & Larsen, J. H., Jr. 1978. The reproductive ecology of the Pacific treefrog (*Hyla regilla*). *Herpetologica*, **34**, 409–416.

Semple-Rowland, S. L. & Goldstein, E. B. 1981. Segregation of vitamin A_1 and vitamin A_2 cone pigments in the bullfrog retina. *Vision Research*, **21**, 825–828.

Tordoff, W. 1971. Environmental factors affecting gene frequencies in montane populations of the chorus frog, *Pseudacris triseriata*. Ph.D. thesis, Colorado State University, Fort Collins.

Tovée, M. J., Bowmaker, J. K. & Mollon, J. D. 1992. The relationship between cone pigments and behavioral sensitivity in a New World monkey (*Callithrix jacchus jacchus*). *Vision Research*, **32**, 867–878.

Wente, W. 2001. Microhabitat choice in the Pacific treefrog (*Hyla regilla*): characteristics of a complex color polymorphism and implications for the development of assortative mate choice within an interbreeding population. Ph.D. thesis, Indiana University, Bloomington.

Wente, W. & Phillips, J. 2003. Fixed green and brown color morphs and a novel color-changing morph of the Pacific tree frog, *Hyla regilla*. *American Naturalist*, **162**, 461–473.

Wickler, W. 1968. *Mimicry in Plants and Animals*. Toronto: World University Library of McGraw-Hill.

Wilt, F. H. 1959. The differentiation of visual pigments in metamorphosing larvae of *Rana catesbeiana*. *Developmental Biology*, **1**, 199–233.