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Cryptic Behavior Is Independent of Dorsal Color Polymorphism in Juvenile Northern Leopard Frogs (*Rana pipiens*)

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ABSTRACT.—Animals that are susceptible to predation sometimes engage in crypsis to conceal themselves from predators. Such behavior often consists of background color matching via substrate choice. Intraspecific color variation may result in differential substrate preference among color morphs. I investigated this idea using juvenile Northern Leopard Frogs (*Rana pipiens*), which exhibit either green or brown background color. Both color morphs spent more time on dark, concealing substrates than light, revealing ones and hopped less frequently when located on dark, concealing substrates. Frogs did not respond differently to green and brown fabric substrates, and they preferred brown natural substrates over green ones. Because green and brown frogs did not behave differently in any experiment, this study suggests that juvenile Northern Leopard Frogs exhibit a generalized cryptic behavior pattern independent of background body color.

To conceal themselves from predators, animals often exhibit cryptic behavior by changing their body color to match the background or seeking matching substrates (Wickler, 1968). Color change and background matching occur in several anuran species (see Hoffman and Blouin, 2000, and references therein) and have been interpreted as predator avoidance responses (e.g., Tordoff, 1980; Heinen, 1985; Morey, 1990; Fernandez and Bagnara, 1991). Heinen (1993) and Morey (1990) showed that frogs (*Bufo americanus* and *Pseudacris regilla*) located on nonmatching substrates were captured more frequently by gartersnakes (*Thamnophis* spp.), which use vision when hunting (Drummond, 1985). Heinen (1985) found that American Toads (*Bufo americanus*) moved less frequently when they were matched to their background, presumably diminishing their risk of detection by predators. Similarly, other studies have shown that habitat (i.e., substrate) choice in other taxa can affect predation risk (Gilliam and Fraser, 1987; Schlosser, 1987).

Predation may be a major selective pressure influencing the evolution of color polymorphisms in anurans (e.g., Nevo, 1973; Tordoff, 1980; Caldwell, 1982). Bird predators are sensitive to light in the visual and near-ultraviolet spectra (Chen et al., 1984; Gill, 1995) and likely use color as a major cue in choosing prey items (Cooper and Allen, 1994). Snake predators and their amphibian prey possess cones (Réperant et al., 1992; Zug et al., 2001), the photoreceptors responsible for color vision in mammals and birds, and are likely able to detect color and possibly UV light as well (Hailman and Jaeger, 1974; Jacobs, 1992; Przyrembel et al., 1995). Furthermore, Morey (1990) provided convincing evidence that Terrestrial Gartersnake (*Thamnophis elegans*) predators are able to distinguish between hues of green and brown. The closely related Common Eastern Gartersnake, *Thamnophis sirtalis*, is a known predator of Northern Leopard Frogs (Lagler and Salyer, 1945).

Differential selection on individuals because of a correlation between color and various fitness traits (e.g., length of larval period [Merrell, 1972] and fecundity [Jameson and Pequegnat, 1971]) may also act to maintain polymorphism in populations. Differences in developmental rates and disease resistance among morphs, seasonal selection, and developmental plasticity may also have contributed to the evolution of modern patterns of color variation (Merrell and Rodell, 1968; Nevo, 1973; Stewart, 1974; Corn, 1981; Travis and Trexler, 1984; Harkey and Semlitsch, 1988). Thus, to understand the evolution of color polymorphism, experimental studies are needed to assess the relative importance of these competing explanations.

Populations of Northern Leopard Frogs (*Rana pipiens*) exhibit dorsal color polymorphisms (Corn, 1981); some individuals have green dorsal background colors, whereas others have brown. These intraspecific color differences may affect predator avoidance behavior (i.e., crypsis). In particular, if predation has been an important selective pressure influencing this trait, green and brown frogs would be expected to vary in their responses to differentially concealing green and brown substrates. In this study, I address three questions: (1) Do juvenile Northern Leopard Frogs choose concealing substrates? (2) Do they move less frequently when located on these substrates to afford further concealment? (3) Do these behaviors vary between color morphs? (e.g., Do green frogs choose green substrates, and do brown frogs choose brown substrates?)

MATERIALS AND METHODS

In July 1998, I collected juvenile *R. pipiens* from one population in a pond southeast of Pellston, Cheboygan County, Michigan. Experiments were performed within a week of collection in a cool basement laboratory at the University of Michigan Biological Station (UMBS). Prior to testing, frogs were housed in 40-liter aquaria. I used juvenile rather than adult frogs because juveniles are active diurnally and presumably more susceptible to local predators because of their small size. I chose frogs that were near the extremes of light green and dark brown because there was considerable variation in dorsal background color among individuals within each color morph. A population survey (D. A. Croshaw,

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TABLE 1. Number of green and brown frogs located on competing substrates after a 30-min acclimation period in Experiments 1 (light sand, dark topsoil), 2 (green fabric, brown fabric), 3 (light sand, dark sand), and 4 (green leaves, brown detritus). Experiments 1 and 2 consisted of individual and group trials. Experiments 3 and 4 only had group trials.

Color	Experiment 1				Experiment 2				Experiment 3		Experiment 4	
	Individual		Group		Individual		Group		Light sand	Dark sand	Green leaves	Brown detritus
	Sand	Soil	Sand	Soil	Green	Brown	Green	Brown				
Green	5	10	5	25	6	9	19	11	9	21	11	19
Brown	4	11	3	27	3	12	8	22	9	21	7	23
Totals	9	21	8	52	9	21	27	33	18	42	18	42

unpubl. data) and paint color chips allowed me to confidently collect samples of unequivocally discrete green and brown hues with minimal variation within each color morph.

I placed animals in experimental arenas (children's plastic swimming pools, ~124 cm diameter) containing alternating quarters of two substrates (e.g., soil, sand, cloth, plant material) of differing color. I augmented the heights of the pools to ~42 cm with wire screening covered by clear vinyl sheeting to assure that animals could not escape. Unless otherwise noted, the substrates were set at equal depths, saturated with water, and smoothed prior to each experiment. In all cases, experimental arenas were stored in the laboratory for at least one night prior to use, eliminating potential differences in temperature between substrates caused by differential heat absorption. Trials were conducted between 0900 and 1630 h under a 60-watt incandescent bulb and a 40-watt cool white fluorescent bulb. Arenas were placed between the two light fixtures. Minimal light entered the laboratory from three small windows. Temperature varied from 20° to 25°C but was most often near 22°C. After each trial, the arena was rotated 90° to control for substrate position.

To determine whether frogs prefer generally concealing substrates over revealing ones (Experiment 1), I added light beach sand (MCV = 5.0 in Munsell Soil Color Charts, 1975 ed., Munsell Color, Baltimore, MD, collected at the shore of Douglas Lake, UMBS) and dark topsoil (MCV = 2.0, collected in the upland hardwood forest of UMBS) to the arenas. To visually oriented predators, frogs should appear more cryptic on the topsoil. To determine whether brown and green frogs choose substrates that match their body color (Experiment 2), I placed them in arenas on a substrate of dry green and brown cotton fabric. To control for texture differences between topsoil and sand (Experiment 3), I gave frogs the choice between dry, light beach sand (MCV = 5.0) and dry, black sand (MCV < 2.0). To more accurately represent the natural habitat (Experiment 4), I used substrates located at the collecting site that most closely matched the color of the green and brown morphs (brown decaying cattail (*Typha latifolia*) material and green leaves of the aquatic plant, *Potamogeton amplifolius*).

In Experiments 1 (light sand, dark soil) and 2 (green fabric, brown fabric), I tested 15 animals of each color morph individually. I released frogs one at a time in the middle of the arena and allowed them 30 min to acclimate. I then recorded each frog's location and observed the frog for 30 min. During the observations, I remained motionless at a position ≥ 6 m from the

experimental arenas. To address the question of whether frog locomotor behavior is influenced by their concealment, I recorded all movements and the total time frogs were located on each substrate. When located on concealing substrates, frogs may move infrequently to remain inconspicuous to predators. I divided movements into two types, hops and crawls, as defined by Heinen (1985). Hops were faster, more noticeable, and resulted in considerably more travel distance than crawls. Escape attempts by jumping at the arena margins were recorded separately.

In addition to the individual trials, I performed group trials in Experiments 1 and 2. Four separate groups of 15 animals were chosen randomly without replacement from a stock of 60 frogs (30 green and 30 brown), placed together in the arenas, and given a 30-min acclimation period after which the number located on each substrate was recorded. Each group consisted of a random mixture of green and brown frogs.

I also conducted group trials in Experiments 3 (light sand, black sand) and 4 (green leaves, brown detritus). However, individual trials were not performed in these experiments. Group trials were done exactly as in Experiments 1 and 2, with the same 60 frogs used in all four experiments.

Because a Chi-square test for independence showed that there was no difference in the results of the individual and group trials (Table 1) for Experiment 1 ($\chi^2 = 3.6$, $df = 1$, $0.10 > P > 0.05$) or Experiment 2 ($\chi^2 = 1.9$, $df = 1$, $P > 0.10$), I considered social effects to be negligible and treated each frog's choice in the group trials as an independent observation. Because these experiments involved substrates of vastly different color and texture and frogs showed no tendency toward learned responses, I considered the results of the experiments independent of one another. Statistical analyses were performed with SAS statistical software (vers. 8.01, Statistical Analysis Institute, Inc., Cary, NC).

RESULTS

Contingency table analyses (2×2) indicate that brown and green frogs did not show a significantly different response in any of the experiments (Experiment 1: $\chi^2 = 0.2$, Experiment 2: $\chi^2 = 1.4$, Experiment 3: $\chi^2 = 0.0$, Experiment 4: $\chi^2 = 1.3$, $df = 1$, $P > 0.05$ in all cases, Table 1). Based on their initial location after the 30-min acclimation, frogs preferred the dark-colored soil substrate over the lighter beach sand ($\chi^2 = 4.8$, $df = 1$, $P < 0.05$) and the dark sand over the light sand ($\chi^2 = 9.6$, $df = 1$, $P < 0.005$). Brown frogs

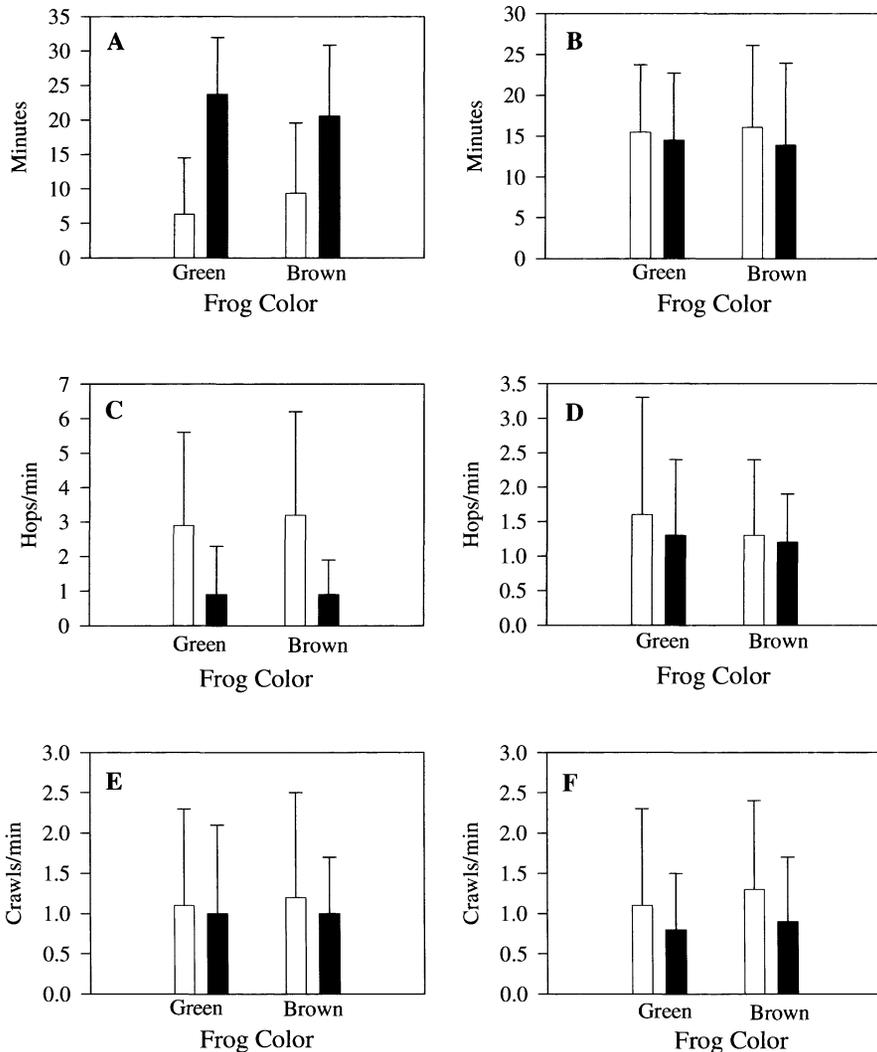


FIG. 1. Minutes, hop rate, and crawl rate (\pm SD) on each substrate in Experiments 1 and 2 for green and brown frogs. Dark bars represent dark or brown substrates. Light bars represent light or green substrates. (A), (C), and (E) Experiment 1: light sand, dark topsoil. (B), (D), and (F) Experiment 2: green fabric, brown fabric. See text for description of statistical tests.

preferentially chose the brown fabric over green ($\chi^2 = 5.4$, $df = 1$, $P < 0.05$), whereas green animals did not prefer either substrate ($\chi^2 = 0.6$, $df = 1$, $P > 0.05$). When data from the two colors were pooled, frogs chose brown cattail matter over green leaves ($\chi^2 = 9.6$, $df = 1$, $P < 0.005$).

Two-factor ANOVA indicated that frogs spent significantly more time on soil than sand ($F_{1,28} = 17.98$, $P = 0.00$, Fig. 1) but showed no preference between green and brown fabric ($F_{1,28} = 0.23$, $P = 0.64$, Fig. 1). The insignificant substrate by color interaction in Experiment 1 ($F_{1,28} = 0.83$, $P = 0.37$) and Experiment 2 ($F_{1,28} = 0.04$, $P = 0.84$) indicated that green and brown morphs did not respond differently to the experimental stimuli.

I analyzed behavioral data using a two-factor MANOVA with hops per min (hop rate) and crawls per min (crawl rate) as the response variables and frog color and substrate as factors (Table 2). In Experiment 1, frog color had no effect alone, but there was a significant substrate main effect and substrate by response variable interaction. Post hoc comparisons showed that hop rate was higher than crawl rate on sand and that hop rate on sand was higher than hop rate on soil (Fig. 1). I used the Bonferroni correction to adjust α to 0.006 for each hypothesis of interest resulting in an experimentwise α of 0.05. Experiment 2 showed no significant main effects or interactions, and post hoc comparisons were not performed. Frogs that remained on one of the two substrates for the

TABLE 2. Results of MANOVA for Experiments 1 (light sand, dark topsoil) and 2 (green fabric, brown fabric). Response designates the movement measures, hop rate, and crawl rate. No post hoc comparisons were performed for Experiment 2. The hop rate and crawl rate effects are pairwise comparisons between the dark and light substrates. * Significant based on $\alpha = 0.006$.

Effect	Experiment 1			Experiment 2		
	F	df	P	F	df	P
Substrate	9.87	1,16	0.006*	2.43	1,23	0.132
Substrate \times color	1.06	1,16	0.318	0.01	1,23	0.906
Substrate \times response	10.57	1,16	0.005*	0.35	1,23	0.561
Color	0.00	1,16	0.973	0.00	1,23	0.965
Hop rate	12.85	1,16	0.003*			
Crawl rate	0.02	1,16	0.904			
Hop rate vs. crawl rate, sand	11.37	1,16	0.004*			
Hop rate vs. crawl rate, soil	0.11	1,16	0.749			

duration of the trial were excluded from these analyses.

DISCUSSION

Collectively, the four substrate choice experiments did not support the idea that dorsal color polymorphism affects cryptic behavior in juvenile *R. pipiens*. Rather, these results show that juvenile Northern Leopard Frogs exhibit generalized cryptic behavior regardless of their dorsal background color. Green and brown frogs did not respond differently in any experiment. In Experiments 1 and 3, significantly more frogs chose the dark-colored substrate, and frogs spent more time there. This result was independent of frog color. Also, in Experiment 4 in which frogs were presented with concealing green and brown substrates, significantly more frogs chose the darker brown background, again regardless of their color. Although brown frogs chose the brown cotton fabric in Experiment 2, green frogs did not prefer either substrate, and choice was statistically independent of frog color. Thus, this study suggests that cryptic behavior occurs in *R. pipiens* but is independent of dorsal color.

The experimental design was sufficient to detect substrate preference. Frogs uniformly chose darker substrates in Experiments 1 and 3. Although substrate texture may have confounded the results of Experiment 1 because particle size could be important in water absorption, frogs preferred black sand over light sand in Experiment 3. Also, frogs were probably fully hydrated because they were provided with water ad libitum prior to testing and were housed, before and during experiments, in a dark, damp, and cool basement laboratory (Heinen, 1985). These observations implicate crypsis rather than texture and/or particle size as the causal agent in substrate choice.

Frogs hopped less frequently when located on the concealing dark topsoil substrate in experiment 1. This result supports the hypothesis that cryptic behavior has been influenced by predation pressure. *Thamnophis sirtalis*, presumably an important predator of juvenile Northern Leopard Frogs, use motion as a cue in hunting (Drummond, 1985). Heinen and Hammond (1997) reported that leopard frogs decreased motion frequency in the presence of garter snakes, rendering them less vulnerable. Morey (1990) found that moving frogs (*Pseudacris regilla*) were equally likely to be caught by *Thamnophis elegans* when on matched or unmatched backgrounds. If frogs choose cryptic microhabitats to escape detection by predators, they should move less frequently when located on these substrates to ensure concealment. The higher hop rate on sand, compared to hop rate on soil and crawl rate on sand, is consistent with this prediction because hops were more noticeable and resulted in greater travel distance than crawls (pers. obs.; Heinen, 1985). Frogs should be expected to quickly travel away from revealing substrates such as light sand.

Wente and Phillips (2003) provided evidence of a color-changing morph in *P. regilla*. Some individuals can apparently change between green and brown background color over a period of several days. Although preliminary observations do not suggest that this trait occurs in *R. pipiens* (D. A. Croshaw, unpubl. data), the idea cannot be fully assessed until more data are collected. Generally, because of its slow progression, the ability to change hue should not substantially change patterns of cryptic behavior that serve as antipredator mechanisms, especially in the context of short (from 30 min to 1 h) experimental trials as reported here.

Morey (1990) found that green and brown morphs of *Pseudacris regilla* chose areas in steel arenas that were painted green and brown, respectively. However, Brattstrom and Warren (1955) did not find these color preferences when working with a much smaller sample of *P. regilla* and colored paper. My work with *R. pipiens* also conflicts with the findings of Morey (1990). Green and brown morphs both responded to substrates of varying hue with generalized cryptic behavior. Because of the diversity of anuran clades that exhibit color polymorphisms (Hoffman and Blouin, 2000) and the multitude of possible selection pressures influencing them, it is likely that frogs of distant lineages have been faced with different predation risks and refuge availability, generating inconsistencies in cryptic behavior patterns.

Although my study provides no evidence that predation has been important in the evolution and maintenance of color polymorphism in *R. pipiens*, it does suggest that predation has influenced generalized cryptic behavior in these frogs regardless of dorsal color. Thus, my results implicate predation as an important evolutionary force and cannot eliminate it as a potential factor contributing to color polymorphisms. For example, it is possible that green morphs are more frequently taken by visually oriented predators, especially birds that have acute color vision, because of their tendency to choose dark brown rather than green microhabitats. Further experiments with varying shades of green and brown substrates and direct predation as a factor influencing frog response

are needed before we can fully understand predation's effects on background color phenotypes in this and other frog species.

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