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TEMPORAL VARIATION IN DORSAL PATTERNS OF JUVENILE GREEN-EYED TREE FROGS, *LITORIA GENIMACULATA* (ANURA: HYLIDAE)

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Abstract.—**Photo imaging of natural markings on amphibians is a non-invasive method of identifying individuals, but is only applicable to species that have persistent, distinguishable patterns. Few studies have investigated whether amphibian dorsal patterns are permanent or change during growth. We photographed juvenile Green-eyed Tree Frogs (*Litoria genimaculata*; n=20) for nine weeks after metamorphosis to determine whether individual frogs can be distinguished using dorsal pattern, and whether dorsal pattern changes over time. We found no detectable dorsal pattern change during the nine-week period. Each individual had a distinct dorsal pattern, which remained distinguishable, although the majority of the froglets (80%) changed color (brown to green or vice versa). We conclude that the photographic identification method can be used for at least moderately sized samples of individual *L. genimaculata* juveniles and possibly other species, although studies similar to ours should be conducted to verify this.**

Key Words.—amphibian; color pattern; Green-eyed Tree Frog; juvenile; *Litoria genimaculata*; photographic identification method; PIM

INTRODUCTION

Approximately 48% of anurans have polymorphic color patterns, and color variation among individuals from different regions and sexes has been extensively investigated (reviewed by Hoffman and Blouin 2000). However, little information is available on ontogenetic variation in color and pattern within individuals (Doody 1995; Bradfield 2004). Knowing whether color and marking patterns change through ontogeny is essential for determining whether polymorphic anurans are suitable for the photographic identification method (PIM). To date, PIM is the only non-invasive technique for individual identification of amphibians (Hagstrom 1973; Bradfield 2004). Doody (1995) detected no change in the patterns of adult *Ambystoma opacum* over a one-year period and suggested that PIM should be applicable to juveniles, although it is unclear whether results on the pattern stability of adult animals apply to individuals still growing and developing. We designed the present study to determine whether individually-identifiable elements of the patterns of a frog species remain identifiable during postmetamorphic ontogeny, making PIM applicable for following juvenile individuals.

Litoria genimaculata, the Green-eyed Tree Frog, occurs in rainforest areas in northern Queensland, Australia, between Townsville and Cooktown. Adult dorsal coloration and pattern vary among individuals, usually consisting of a broad russet/brown pattern, and a bi- or tri-lobed band with irregularly dispersed patches of green and copper (Barker et al. 1995). Juvenile *L. genimaculata* also possess a mottled dorsal pattern (Barker et al. 1995) but its persistence through ontogeny has not been examined. Ontogenetic pattern changes could render PIM unsuitable for studies of juveniles and sub-adults. Our aim was to determine whether the dorsal pattern of *L. genimaculata* froglets can be used to distinguish among individuals, and whether individuals remain identifiable over a substantial portion of the juvenile period.

MATERIALS AND METHODS

We raised *Litoria genimaculata* in the laboratory from eggs that were collected at Birthday Creek, Paluma Range National Park, Queensland, Australia (S18°58′54″ E146°10′02″). We housed tadpoles and froglets in aquaria (250 x 350 x 150 mm) at 24°C. We maintained tadpoles in aerated water that was partially changed each week, and we fed them *ad libitum* using a mixture of alfalfa pellets, fish food flakes and microserum fish powder suspended in agar in petri dishes. Froglets received two crickets every second day; once a week 0.5 mL of a liquid supplement mixture (2mL Calcivet/100mL rainwater) was applied dorsally to each frog. Calcivet (Vetafarm, Wagga Wagga, New South Wales, Australia) contains 33g/L calcium borogluconate, 2g/L magnesium sulphate and 25,000 i.u./L vitamin D_3.

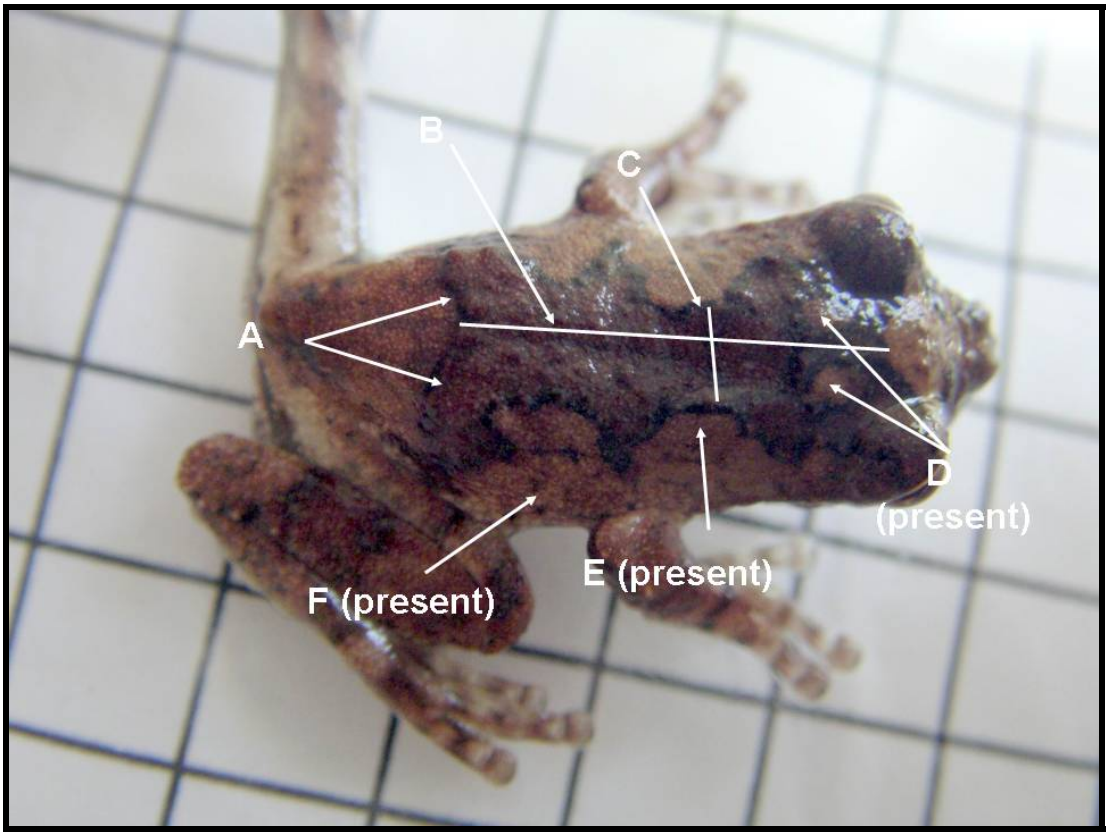

FIGURE 1. Aspects of Green-eyed Treefrogs **(***Litoria genimaculata*) dorsal patterns that we used to create binary state variables presented in Fig. 2. A) shape of the posterior line of the dorsal hourglass, B) length of the dorsal hourglass along mid-dorsal line, C) the narrowest part of the dorsal hourglass, D) dots between eyes, E) straight line within dorsal hourglass, F) dorsal dark lined circles.

We digitally photographed each individual froglet (N = 20) against a 0.5 cm^2 grid using a Pentax Optio 33WR camera to allow snout-vent length (SVL) to be measured. Initial photographs were taken within two weeks of metamorphosis and then weekly for nine weeks to allow us to examine the dorsal pattern during ontogeny. We measured six characteristics of the dorsal pattern (Fig. 1): (A) the shape of the posterior line of the dorsal "hourglass" marking (categorized as either an "S" or "V" shape), (B) greatest length of dorsal hourglass, (C) minimum width of dorsal hourglass, (D) the presence or absence of dots between the eyes, (E) the presence or absence of a straight line of the dorsal hourglass, and (F) the presence or absence of dorsal dark lined circles. Characteristics A and D-F are naturally dichotomous, occurring in only one of two possible states. So that we could conduct an ordination of these characters on a common scale, we used measurements B and C to create three additional dichotomous variables; one which had a value of 1 if the ratio C/B was less than or equal to 0.25, and 0 otherwise; one which was 1 if C/B was between 0.25 and 0.30, 0 otherwise; and one which was 1 if C/B was greater than or equal to 0.30, 0 otherwise. Examination of the data set showed that the features of each individual remained constant throughout the period of the experiment. To illustrate differences among individuals, we ordinated the Euclidean distances among them using a non-metric multidimensional scaling analysis performed using the PROXSCAL procedure in SPSS version 14 (SPSS, Chicago, Illinois, USA). We used Euclidean distances because shared zeros are informative. In addition to measuring quantitative characters, each week we categorized the background color of the dorsal pattern as either predominantly green or predominantly brown. The differences between individuals we categorized as green and brown were large and obvious. Individuals we categorized as green had dorsal backgrounds at or near 2.5G 5/6 on the Munsell scale (Munsell 1970), while those we categorized as brown were at or near 2.5YR 4/4 on that scale. To determine whether changes in background color were correlated among frogs across time, we estimated mean rates of transition from the data and used these to calculate expected numbers of individuals with each background color in each week from four to nine. We calculated an overall chi-squared statistic ($\alpha = 0.05$) across weeks that compared observed numbers to expected numbers with each color pattern in each week.

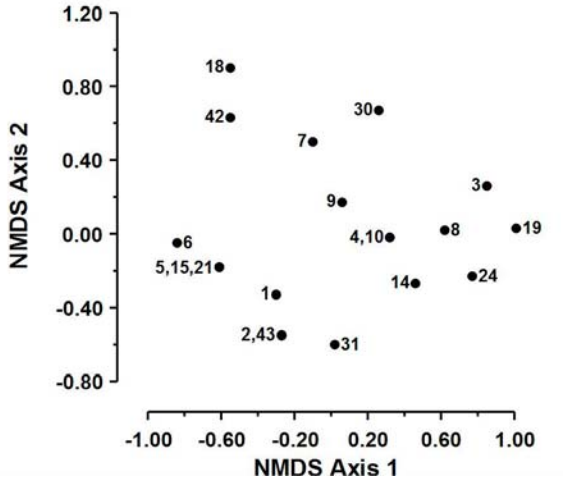

FIGURE 2. Results from non-metric multidimensional scaling ordination of Euclidean distances among individuals for dichotomized measurements of juvenile Green-eyed Tree Frog (*Litoria genimaculata*) dorsal patterns. Most individuals could be distinguished solely on these measurements, which did not change over the nine-week period of measurement.

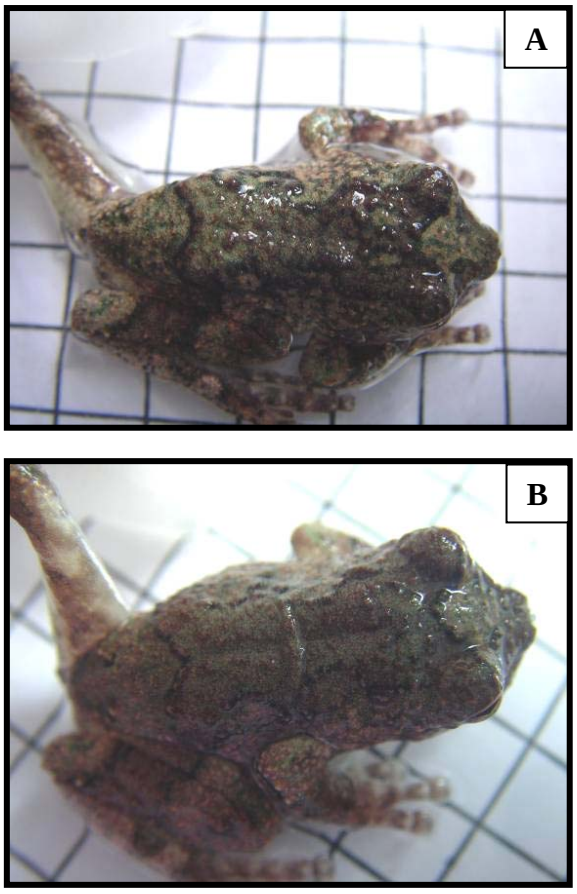

FIGURE 3. Dorsal pattern of two different Green-eyed Tree Frog (*Litoria genimaculata*) froglets - *Lg* 2 (A) and *Lg* 4 (B). In addition to the features that we quantified (Fig. 1), many distinctive ridges and tubercles are present.

RESULTS

The mean initial SVL of *L. genimaculata* metamorphs was 3.55 cm (range = 3.00–4.50 cm). During the nine-week study the 20 *L. genimaculata* grew an average of 0.84 cm (range = 0.5–1.0 cm). Thirteen of 20 froglets possessed a dorsal pattern distinct from that of any other individual in the group of 20 based on the character set we quantified; these individuals were thus unique in our data set and could not be misidentified. Two pairs of individuals and one set of three shared values of all of the characters we quantified (Fig. 2). The froglets that could not be distinguished quantitatively were easy to differentiate visually based on other aspects of their patterns, such as the locations of particular skin ridges and tubercles (Fig. 3). The dorsal pattern remained constant through time within individuals, but overall color varied considerably, changing from brown to green and the reverse in most individuals over the course of the experiment (Fig. 4, Table 1).

No individual changed color prior to week four, and at least one individual changed from brown to green and back to brown within two weeks, indicating that at least after week three, color can change in either direction within one week. Under the null hypothesis that color changes occur in each frog at random times, the data for each week should reflect random alterations to the data for the preceding week. If color changes are constrained to occur at similar times among frogs, either by internal developmental mechanisms or by subtle changes in the light environment in the laboratory, changes between weeks should differ significantly from a random set of color shifts. Our comparison of observed versus random expected changes was significant ($\chi^2 = 12.66$, df = 6, $P = 0.05$), indicating that the timing of color changes was non-random.

DISCUSSION

Individual *L. genimaculata* froglets were visually distinguishable using their dorsal patterns, which did not change ontogenetically. We therefore suggest that PIM is suitable for distinguishing among juveniles of this species in short-term, laboratory and field studies. This method makes invasive marking methods such as toe-clipping unnecessary, at least for shorter-term studies examining limited numbers of animals of this species, and probably other species with similar degrees of pattern variation. Our results do not rule out the possibility that dorsal color patterns may change over a longer periods of time during juvenile growth. We recently found a minor pattern change over a two-month period in an adult *L. genimaculata* (Kenyon et al. 2009). We did not observe any pattern change on our sample of 20 individuals over nine weeks. Thus, it appears that both juvenile and adult *L. genimaculata* pattern tends to

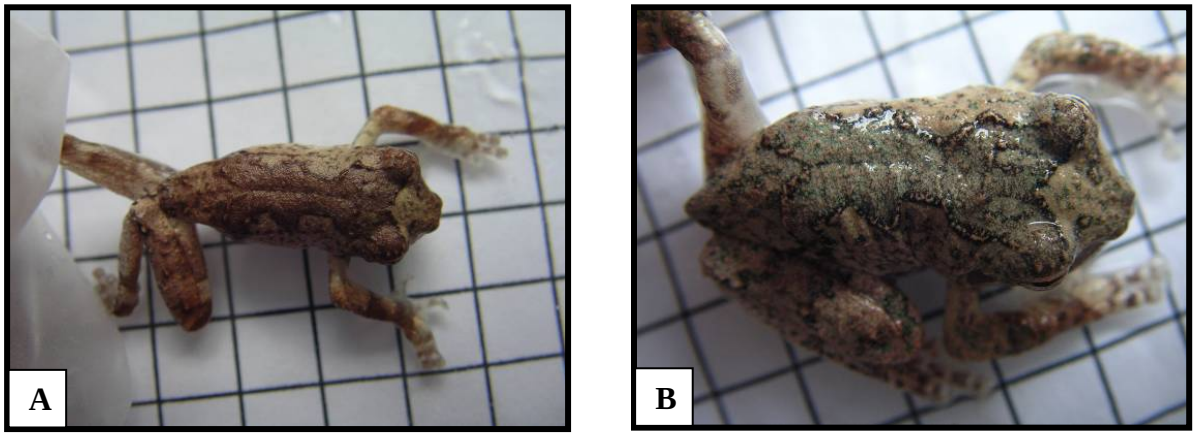

FIGURE 4. Constant dorsal pattern with temporal variation in color of a juvenile Green-eyed Treefrog (*Litoria genimaculata*; individual *Lg* 3) during (A) week two (2.5YR 4/4 in the Munsell scale), and (B) week nine (2.5G 5/6 in the Munsell scale).

be stable on a scale of months; however, longer-term studies may reveal more changes, rendering the PIM unsuitable for long-term studies in this species. We suggest that any study investigating pattern stability should occur over at least several months.

Hoffman and Blouin (2000) suggested that the darkening and lightening of existing skin tones is fairly common in anurans, but that actual color changes are rare, usually in one direction, and age related. However, the dorsal color of the majority of the froglets in our study changed in both directions (green to brown and vice versa) over the nine-week observation period. This was also observed by Hoffman and Blouin (2000) in laboratory populations of *Hyla regilla*.

Skin color in amphibians is due to the presence of dermal chromatophore units, containing layers of

Table 1. Temporal variation in dorsal pattern background color of 20 *Litoria genimaculata* froglets during the nine-week study period. Brown background indicated by 1, green by 0, missing data or not calculated by --. Expected proportions were calculated for weeks 4–9 by applying mean rates of transition from brown to green (0.2247) and green to brown (0.4138) calculated for the intervals between weeks three to four through eight to nine to the data from the preceding week.

Frog	Week								
	1	2	3	4	5	6	7	8	9
Lg 1	1	1	1	1	1	1	0	1	0
Lg 2	1	1	1	0	0	0	0	1	1
Lg 3	1	1	1	1	1	1	1	1	0
Lg 4	1	1	1	1	1	1	0	1	0
Lg 5	1	1	1	1	1	1	0	1	1
Lg 6	1	1	1	1	1	1	1	1	1
Lg 7	1	1	1	1	1	1	1	1	1
Lg 8	1	1	1	1	1	1	1	1	1
Lg 9	1	1	1	1	1	1	1	1	1
Lg 10	1	1	1	1	1	1	1	1	0
Lg 14	1	1	1	0	0	0	0	1	0
Lg 15	1	1	1	1	1	1	1	1	1
Lg 18	1	1	1	1	0	0	0	1	0
Lg 19	1	1	1	1	0	0	0	1	--
Lg 21	1	1	1	1	1	1	1	1	1
Lg 24	1	1	1	0	0	0	0	1	--
Lg 30	1	1	1	0	1	0	0	1	1
Lg 31	1	1	1	0	1	1	0	1	0
Lg 42	1	1	1	1	1	0	0	0	0
Lg 43	1	1	1	1	1	1	1	1	1
Observed green	0	0	0	5	5	7	11	1	8
Observed brown	20	20	20	15	15	13	9	19	10
Expected green	--	--	--	4.49	6.12	6.71	6.92	7.00	6.32
Expected brown	--	--	--	15.51	13.88	13.29	13.08	13.00	11.68
Contribution to chi-squared	--	--	--	0.07	0.29	0.02	3.68	7.90	0.69

xanthophores, iridiophores, and melanophores (pigment cells). Color changes occur when pigments are rearranged within the chromatophores, due to light, temperature, hormonal, and other stimuli (reviewed by Frost-Mason et al. 1994). The significant temporal correlations we found across individuals indicate that they were responding with some degree of synchrony to either internal or external cues. These could be part of a regular ontogenetic pattern initiated by hormonal changes occurring at fixed times after metamorphosis. However, changes in weeks three to four through six to seven were only moderately synchronous, while during the week seven to eight period there was a much higher degree of synchrony, with all brown frogs remaining brown and all but one green frog becoming brown. This suggests that the frogs were responding to an external cue, perhaps a subtle change in the light environment in the laboratory.

Although it is very useful to define and use a set of quantitative characters common to all individuals, our *L. genimaculata* required a more flexible approach. We could not differentiate two pairs of individuals and one set of three that shared values of all of the characters we quantified. If we had simply assigned individuals within these groups identities at random, on average 20% of the 20 animals would have been misidentified. This is an unacceptable error rate. One possible solution to this problem might be to simply add more quantitative characters. Particularly in a large-scale study, this would probably be inefficient and ultimately impossible to accomplish. Our characters were chosen for ease of quantification and measurement; any additional characters would be more difficult to measure and more prone to error, and would provide unnecessary, redundant, and potentially confusing information if measured for all individuals, as well as creating a rapidly increasing workload for the measurer. We suggest that the best approach is an adaptive one, as we used; select a set of quantitative characters that are repeatable, consistent, easy to measure, and provide a reasonable degree of discrimination among individuals, and adaptively use additional characters as necessary to reduce the error rate in separating the identities of individuals that cannot be discriminated using the basic character set.

Despite changes in color, the dorsal pattern remained distinctive, allowing accurate identification of individuals, indicating that the PIM should be suitable, for relatively short-term studies of *Litoria genimaculata* and may be applicable to juvenile frogs of other species although studies similar to ours should be conducted to verify which juveniles have distinctive patterns, and may be applicable to juvenile frogs of other species. However, studies like ours should be conducted *a priori* to verify this before the PIM is used for any other species.

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