Morphological Color-Change in the Red-Backed Salamander (*Plethodon cinereus*) While Kept in Captivity

Andrew C. Kraemer[1], Jessica Kissner[2], and Dean C. Adams[1]

Coloration is a trait frequently cited as important to ecological, and thus evolutionary, processes in amphibians. To fully understand the impact of coloration, a consistent method of color measurement is required, which is often achieved by collecting color information in a captive, laboratory setting. However, results from the lab can only be generalized to the field if coloration remains consistent between the two. In this study we collected spectral data from two discrete color morphs of *Plethodon cinereus* (erythristic and striped) at two distinct time periods while in captivity to determine whether and how their coloration changed over time. We found that brightness in both color morphs decreased similarly through time, though the change was most pronounced in the erythristic morph. Additionally, we examined the overall coloration change over time using multivariate change vectors and found that the magnitude (amount) of multivariate color change was significantly greater in the erythristic morph as compared to the striped morph (for cheek color patches). Intriguingly, the direction of color change in color space was oriented similarly among measured regions within each morph, whereas the direction of color change differed between morphs, indicating that while coloration changed for all individuals, how that change occurred was morph-specific. We propose a physiological mechanism that may be responsible for the observed changes, and suggest several additional mechanisms that may influence labile coloration in these salamanders. Additionally, our findings suggest that quantifying color from individuals held in captivity should be used with caution, as color changes observed in a laboratory setting may not reflect what is seen in the field.

ANIMAL coloration is often tightly constrained by selection (Endler, 1980; Brodie, 1992; Andersson et al., 1998), and thus, patterns of coloration and color-change through time can be informative about the evolutionary processes that drive the generation and maintenance of phenotypic diversity (Bond, 2007). Amphibians in particular are diverse in coloration (e.g., Storfer et al., 1999; Santos et al., 2003; Kuchta, 2005), and this morphological diversity is in part due to the cellular diversity that underlies pigmentation in this vertebrate group. Unlike birds and mammals, which possess a single pigment cell type, most amphibians produce three pigment cell classes, each defined by the pigments they contain (Duellman and Trueb, 1986). Melanophores are the most ubiquitous pigment cell type and produce black to brown eumelanin. Xanthophores and erythrophores form the second pigment cell class and are generally yellow or red, respectively, and can contain dietary carotenoids or endogenous pterins. Finally, iridophores contain reflecting platelets that give many amphibians a shimmering or sparkling quality (Frost-Mason et al., 1994). Together, these three pigment cell types produce a spectacular diversity of coloration across Amphibia.

Individuals of many amphibian species are known to exhibit changes in coloration through time (Parker, 1943; Duellman and Trueb, 1986), and these changes are observed at distinct temporal scales. For instance, physiologically based color-changes, in which pigment-containing organelles are rearranged intracellularly, typically occur within minutes and can last several hours. For example, frogs are well known for their ability to shift the position of pigment-containing organelles within their chromatophores in response to environmental cues (such as light) and hormonal controls (e.g., alpha melanocyte-stimulating hormone, α-MSH; Fernandez and Bagnara, 1991). Conversely, color changes that occur throughout the lifetime of an individual are often morphologically based, where the process involves the synthesis or removal of pigments within the dermis (Bagnara and Hadley, 1969; Taylor and Bagnara, 1972; Duellman and Trueb, 1986). For example, in plethodontid salamanders, many species are thought to darken as they age, with juveniles possessing the most vivid colors and older individuals becoming more melanized (Highton, 1959; Pough, 1974). However, while changes in coloration throughout the course of an individual's lifetime have been documented in an axolotl (Barnett, 2011), examples of adult amphibian morphological color-changes on a smaller timescale, such as days to months, are less represented in the literature (Duellman and Trueb, 1986). Consequently, even though changes in coloration are thought to be common for amphibians kept in captivity, to our knowledge, this has never been rigorously quantified and reported.

To identify the extent of color change in captivity for an amphibian commonly used in evolutionary and ecological research, we collected spectral data from *Plethodon cinereus* (Eastern Red-backed Salamander) individuals of two discrete color morphs: striped and erythristic. The striped morph is laterally melanized with a red dorsal stripe containing mostly erythrophores with a few melanophores, while the erythristic morph replaces nearly all melanophores with red erythrophores (Bagnara and Taylor, 1970). Both morphs have a light-colored ventral surface populated by iridophores (Bagnara and Taylor, 1970). We hypothesized that coloration would change over time in captivity for both morphs. Additionally, we examined whether coloration changed similarly between morphs by measuring color change among anatomical points during the three captive months.

MATERIALS AND METHODS

Specimens and data collection.—We hand collected 79 individuals of *P. cinereus* from three locations in early June 2010: 1) Fisk Meadows State Wildlife Management Area,

[1] Department of Ecology, Evolution, & Organismal Biology, 251 Bessey Hall, Iowa State University, Ames, Iowa 50011; E-mail: (ACK) kraemer1@iastate.edu; and (DCA) dcadams@iastate.edu. Send reprint requests to ACK.
[2] Department of Natural Resources Management, Texas Tech University, Lubbock, Texas 79409; E-mail: jessica.kissner@ttu.edu.
Submitted: 10 January 2012. Accepted: 20 July 2012. Associate Editor: B. Stuart.
© 2012 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CH-12-006

Hampshire County, MA (42.39°N, 72.82°W; $n = 27$); 2) Elk State Park, Elk County, PA (41.60°N, 78.48°W; $n = 23$); and 3) Aurora Sanctuary, Portage County, OH (41.30°N, 81.31°W; $n = 29$). All sites were between 330 and 640 m in elevation and consisted primarily of hemlock and northern hardwood forest. Ambient temperatures at the time of collection ranged from 17°C at the Ohio site to 25°C at the Massachusetts site. We collected salamanders by searching under cover objects such as logs, flat stones, and bark. Of the salamanders collected, 50 individuals were the striped morph and 29 were the erythristic morph. After capture, we placed each individual within its own 14 × 1.5 cm circular petri dish accompanied by a wet paper towel and placed all individuals within a humid cooler kept at 15°C for transportation back to the laboratory at Iowa State University. In captivity, we maintained each individual in a 14 × 1.5 cm circular petri dish with a damp paper towel. Every three days, we fed the salamanders 4–6 live fruit flies or ants in a randomized fashion such that all salamanders were fed an equal mix of flies and ants during the captive period. No animals died during the course of this study. We placed all salamanders in a temperature-controlled chamber (15°C), and randomly rotated their positions within the chamber after each feeding. Light conditions are known to influence physiological color-change in many amphibians (Duellman and Trueb, 1986), but this has not been observed in *P. cinereus*. To control for any physiological color-change due to light in this nocturnal species, we kept animals in dark conditions for the entire captive period. Paper towels were changed as needed.

We quantified color using reflectance spectrometry from 18 anatomical points on each animal to ensure that all major body regions were measured (Fig. 1). Prior to obtaining spectral measurements, we anesthetized individuals in a tricaine methanesulfonate (MS-222) solution of 0.2 mg/mL. Although a procedure involving anesthetization could potentially influence physiological changes in coloration, we are not aware of any record of physiological color-change for any plethodontid salamander, nor did we observe any physiological color-change in our salamanders. We then measured spectral reflectance using a Jaz spectrometer (OceanOptics, Dunedin, FL) with a 100 μm entrance slit, LS-1 Tungsten Halogen lamp, and a QR400-7-UV-BX reflectance probe fitted with a handmade tip that restricted the measured patch to a 2 mm diameter circle. The probe was held 90° to the surface of the salamanders at a distance of 20 mm. A Spectralon white reflectance standard was used between each animal to correct for drift in lamp intensity. Each spectrum was measured at 1 nm intervals within the human visual spectrum (400 to 700 nm). The first set of spectral measurements was taken three weeks after collection (5–10 July 2010), and the second set of spectral measurements was taken three months after the first measurements (4–9 October 2010).

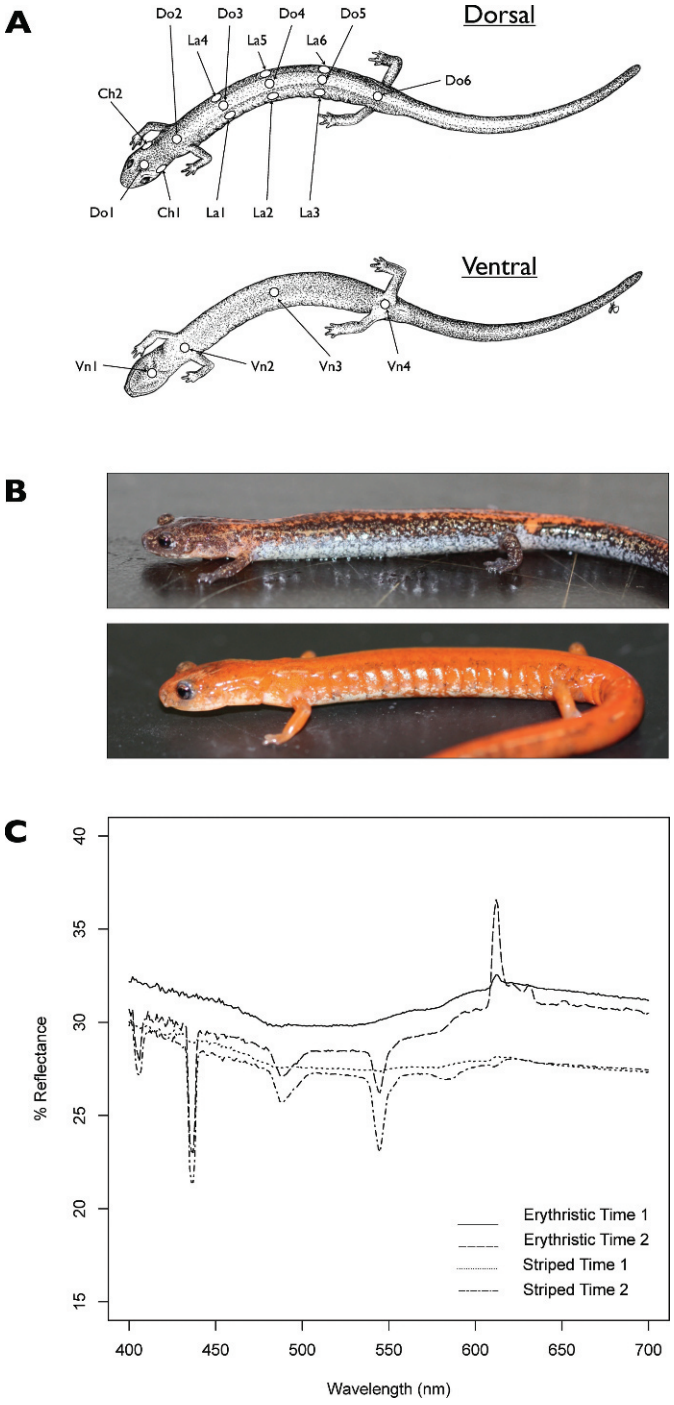


Fig. 1. (A) Locations of 18 regions where spectra were collected on each animal. Do1–Do6 correspond to the dorsal regions, Ch1 and Ch2 to the left and right cheek regions, La1–La6 to the lateral regions, and Vn1–Vn4 to the four ventral regions. (B) Representative images of each salamander morph: striped morph at top, with erythristic morph below. (C) Average spectra across all body regions for each morph and time point.

Statistical analyses.—We performed principal components analysis on the spectral data to obtain a set of color variables for statistical comparison (*sensu* Endler and Théry, 1996; Grill and Rush, 2000; Rosenblum, 2006). In this representation, PC1 is interpreted as brightness, or the total amount of light reflected from each patch, while the remaining PC-axes describe aspects of chroma and hue (henceforth, coloration: see Endler, 1990; Endler and Théry, 1996; Grill and Rush, 2000). In our case, PC1 was highly correlated with an independent measure of overall intensity (brightness = Q_t; *sensu* Endler, 1990, $r > 0.98$), indicating that interpreting PC1 as brightness was warranted for our data. The remaining principal component axes (PC_2–PC_{301}) described the shape of the spectral curve and thus the color that each spectrum represented.

To examine variation in brightness, we performed an analysis of variance (ANOVA) to determine whether brightness

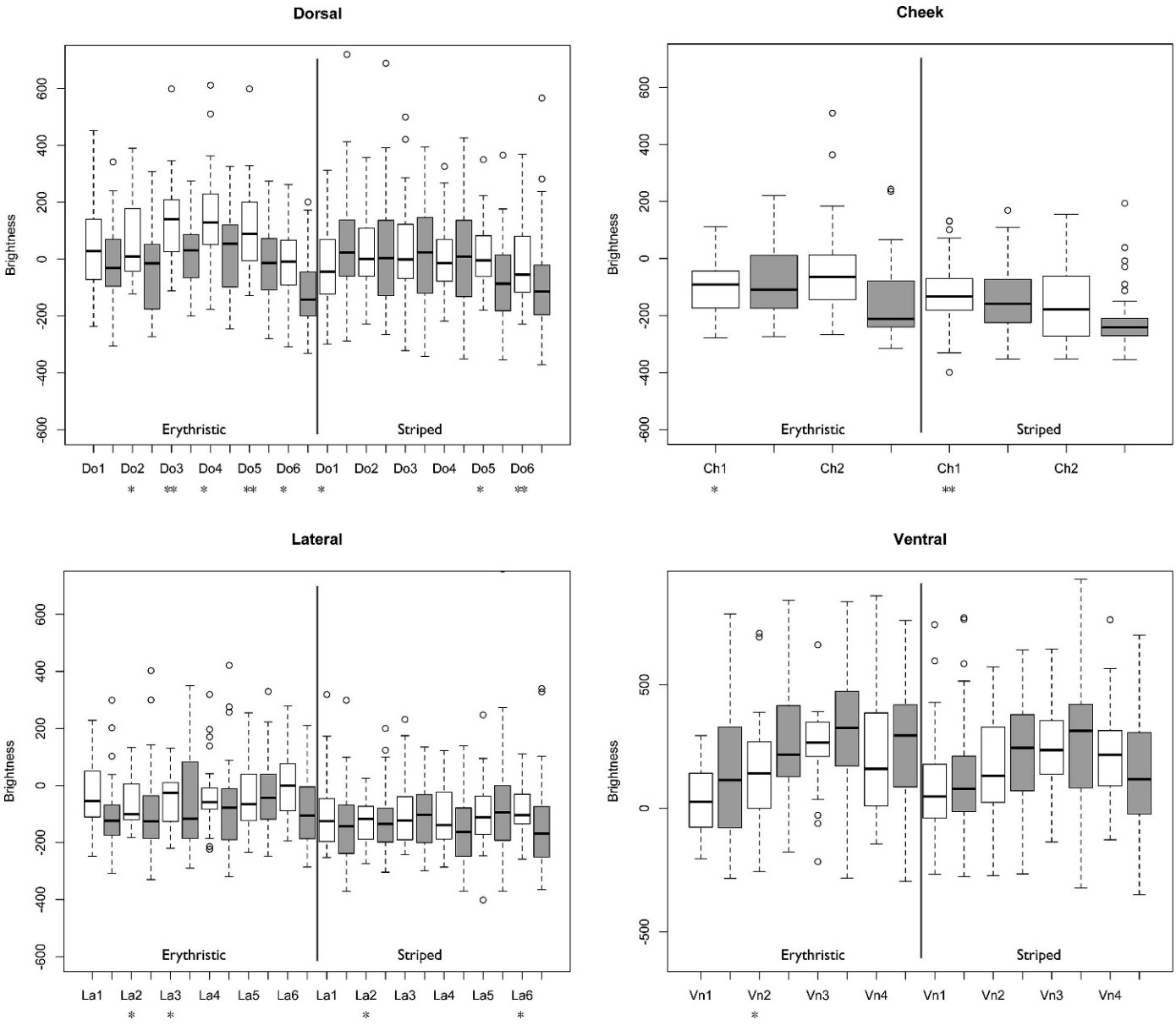


Fig. 2. Boxplots of brightness at each region and time point. White boxes correspond to the first measurement at each region, while the adjacent gray box corresponds to the second measurement at each region. Asterisks below each region label denotes the significance of the change associated with that region, where (*) $P < 0.05$ and (**) $P < 0.01$.

differed between groups (groups were defined as each measured point on each morph at each time point). To identify how brightness changed between time points for each group, we conducted a series of planned *post-hoc* comparisons between time points for each measured region using t-tests, as well as a Fisher's combined probability test on these values to obtain an overall estimate of whether changes in brightness were generally prevalent across the body in each morph.

Differences in coloration were examined using permutational multivariate analysis of variance (perm-MANOVA; Anderson, 2001) at each anatomical point, with morph and time as main effects, and including the morph × time interaction. Permutational-MANOVA was used in this case because the number of variables exceeded the number of specimens. Separate analyses were performed on coloration from each anatomical point. Additionally, we used multivariate vector analyses (Collyer and Adams, 2007; Adams and Collyer, 2009) to compare the magnitudes and trajectories of color change between groups and specifically to identify whether any observed changes in coloration were concordant between color morphs. Here, the magnitude and direction of color change were quantified from the least squares means for each group and statistically evaluated using residual randomization. Briefly, the observed pairwise differences between groups (measured as Euclidean distances) were first obtained from the least-squares (LS) means of the MANOVA model containing all factors (morph, time, morph × time). A reduced model lacking the interaction term was then calculated, from which predicted values and residuals were obtained for all specimens. Residual values were then randomized and added to the predicted values, the full model was recalculated, and pairwise distances between LS means were obtained. The proportion of randomly generated distances greater than the observed was then treated as the significance of each pairwise

Table 1. Results from the Vector Analysis of Coloration. The left columns describe the magnitude of color change for each morph at a particular region, while the right columns summarize the angle between the subsequent vectors. Single asterisks denote a significance level of 0.05>0.01, and double asterisks denote a significance level of 0.01>0.001.

	Magnitude				
Point	Erythristic	Striped	*P*	Angle	*P*
Do1	18.384	23.279	0.231	23.938	0.039 *
Do2	20.943	20.031	0.826	33.282	0.098
Do3	19.785	20.690	0.854	30.774	0.13
Do4	19.538	20.032	0.905	32.710	0.108
Do5	19.830	19.545	0.936	31.877	0.128
Do6	18.063	20.013	0.618	28.737	0.116
Ch1	26.407	16.254	0.010 *	45.347	0.011 *
Ch2	34.244	19.360	0.011 *	47.620	0.004 **
La1	25.656	17.565	0.107	35.572	0.016 *
La2	22.405	16.664	0.179	45.744	0.017 *
La3	21.407	19.587	0.685	38.493	0.018 *
La4	22.860	19.638	0.491	41.897	0.004 **
La5	19.600	21.120	0.759	37.707	0.011 *
La6	22.746	18.872	0.373	45.658	0.007 **
Vn1	30.712	22.479	0.122	9.706	0.828
Vn2	20.418	17.864	0.557	29.010	0.043 *
Vn3	19.449	14.376	0.484	23.068	0.510
Vn4	20.098	17.047	0.427	35.397	0.013 *

comparison (for statistical details see: Adams and Collyer, 2007, 2009; Collyer and Adams, 2007; also Anderson and Ter Braak, 2003).

Finally, for each group we calculated the multivariate dispersion in color space (using 'betadisper' in the Vegan library in R), which were compared among groups using permutation. All statistical analyses were performed in R 2.13 (R Development Core Team, 2011).

RESULTS

Over 98% of the total variation in spectra was explained by PC1, indicating that the vast majority of spectral differences among individuals were due to differences in overall brightness. The ANOVA identified significant differences between morph × anatomical point × time groups ($F_{72,2768} = 23.105$; $P < 0.001$), and a Fisher's combined probability test identified a significant concordance in the change of brightness between time periods among the measured points ($\chi^2 = 175.994$; $P < 0.001$). Furthermore, the t-tests conducted between time points demonstrated that brightness decreased at most measured points dorsally and laterally on both morphs, while the ventral surfaces tended to increase in brightness (Fig. 2).

We found significant differences in overall coloration between morphs and between time periods for most anatomical regions, as well as significant morph × time interactions (Table 1). These results were consistent for all dorsal, cheek, and lateral regions, though three of the four ventrally located points did not have significant morph × time interaction terms. Multivariate vector analysis revealed a significant color change in the cheek region of erythristic individuals between time points (Table 2). The direction of color change was consistent between dorsal and lateral regions within morphs, but diverged significantly between the two morphs (Fig. 3; Table 2). Finally, the individual tests of multivariate dispersion in color space for each group revealed that all measured points displayed a significant increase in multivariate dispersion through time with the exception of the dorsal region of the erythristic individuals (overall test of dispersion between time points: $F_{72,2768} = 9.0369$; $P < 0.001$; see Table 3 for individual group *P*-values).

DISCUSSION

Testing evolutionary hypotheses often requires that complex phenotypic traits (e.g., coloration) are quantified and compared to the selective environments in which they evolve. In this study, we obtained spectral measurements of two morphs of *Plethodon cinereus*. We observed a general trend of decreasing brightness of dorsal and lateral pigmentation over time, with captive salamanders losing reflectance relative to when they were recently caught, resulting in individuals that appeared darker after three months in captivity. Conversely, ventral reflectance increased in both morphs. These results are particularly intriguing considering that these salamanders were kept in dark conditions, as it is not well established how light can affect pigment synthesis in amphibians. Because iridophores are highly reflective relative to melanophores and erythrophores, we hypothesize that the changes in brightness we observed were the result of an upregulation of dermal pigments, which could result in a loss of reflectance in dorsal and lateral regions and an increase of reflectance in ventral regions. Future research focusing on the cellular aspects of dermal pigmentation in this system could address this hypothesis.

In addition to observations of overall brightness, we measured coloration at 18 anatomical points on every individual. First, we found that variation in coloration among individuals increased over time for all groups. Most intriguingly, while the coloration of each morph changed over time to a similar degree, the direction of change in color space differed significantly between morphs. The distinct distribution of pigments in the skin of each morph may explain the non-parallel changes of coloration between morphs. While the striped morph produces melanin

Table 2. Results from the Permutational MANOVA on Coloration between Morphs and Time Points at Each Anatomical Point. Single asterisks denote a significance level of 0.05>0.01, double asterisks denote a significance level of 0.01>0.001, and triple asterisks denote a significance level of >0.001.

Point	Factor	Sum Sq	*F* value	*P*
Do1	Morph	5500	20.812	0.001 ***
	Time	17554	66.426	0.001 ***
	Morph × Time	896	3.389	0.010 *
Do2	Morph	16120	30.7383	0.001 ***
	Time	15121	28.8328	0.001 ***
	Morph × Time	1271	2.4229	0.090
Do3	Morph	19299	40.845	0.001 ***
	Time	15314	32.411	0.001 ***
	Morph × Time	1066	2.255	0.100
Do4	Morph	19663	42.064	0.001 ***
	Time	14426	30.862	0.001 ***
	Morph × Time	1142	2.442	0.073
Do5	Morph	18601	38.771	0.001 ***
	Time	14087	29.362	0.001 ***
	Morph × Time	1070	2.230	0.093
Do6	Morph	5439	11.4933	0.001 ***
	Time	13892	29.3569	0.001 ***
	Morph × Time	852	1.8008	0.143
Ch1	Morph	25275	80.669	0.001 ***
	Time	13430	42.864	0.001 ***
	Morph × Time	3287	10.492	0.001 ***
Ch2	Morph	25061	77.029	0.001 ***
	Time	20375	62.627	0.001 ***
	Morph × Time	6000	18.442	0.001 ***
La1	Morph	22872	69.852	0.001 ***
	Time	15113	46.158	0.001 ***
	Morph × Time	2144	6.549	0.003 **
La2	Morph	26247	91.870	0.001 ***
	Time	11849	41.475	0.001 ***
	Morph × Time	2373	8.307	0.001 ***
La3	Morph	30596	111.749	0.001 ***
	Time	14534	53.084	0.001 ***
	Morph × Time	1703	6.220	0.005 **
La4	Morph	22154	74.774	0.001 ***
	Time	15017	50.687	0.001 ***
	Morph × Time	2202	7.432	0.003 **
La5	Morph	22502	77.023	0.001 ***
	Time	15115	51.736	0.001 ***
	Morph × Time	1608	5.505	0.002 **
La6	Morph	32356	114.970	0.001 ***
	Time	13897	49.382	0.001 ***
	Morph × Time	2510	8.919	0.001 ***
Vn1	Morph	1245	2.887	0.031 *
	Time	25507	59.140	0.001 ***
	Morph × Time	804	1.863	0.129
Vn2	Morph	1059	2.829	0.028 *
	Time	13124	35.066	0.001 ***
	Morph × Time	900	2.404	0.053
Vn3	Morph	1131	1.3916	0.217
	Time	10005	12.311	0.001 ***
	Morph × Time	647	0.7955	0.540
Vn4	Morph	2957	8.154	0.001 ***
	Time	11806	32.560	0.001 ***
	Morph × Time	1243	3.429	0.006 **

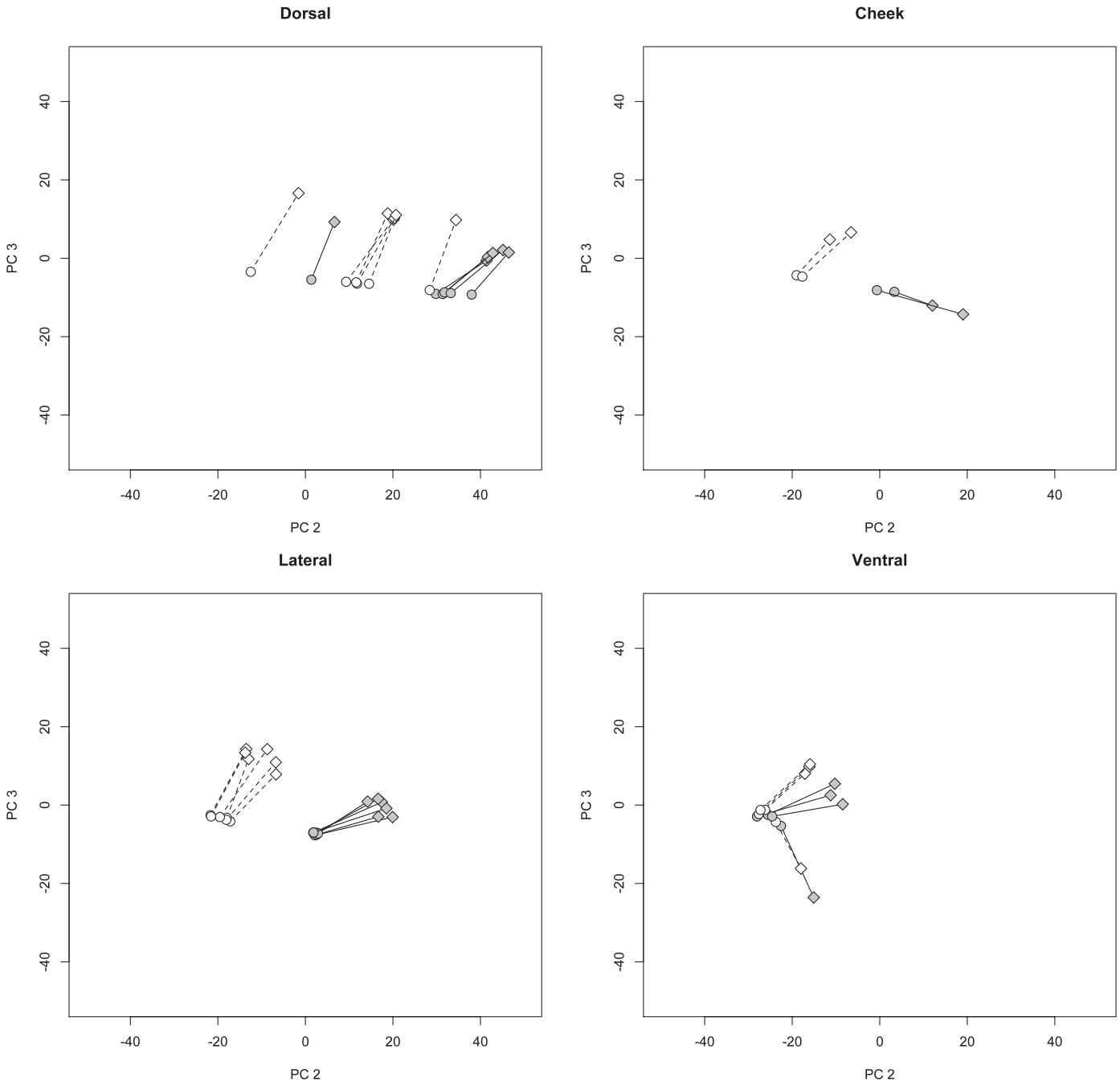


Fig. 3. Principal components plot of color variation of *Plethodon*. Data from erythristic individuals are shaded in gray, while open shapes represent striped individuals. In addition, circles represent individuals from the first time point, while diamonds depict the second measured time point. The least squares mean is displayed for each group. Dotted lines correspond to the striped morph vectors, while the solid lines correspond to erythristic vectors. All data are plotted in the second and third principal components, which represent approximately 79% of the remaining variation after the first principal component (brightness) is removed. Only group means are shown for clarity.

laterally on either side of the drosopterin-rich stripe region (Bagnara and Taylor, 1970), the erythristic morph produces melanin exclusively in the most distal regions of the tail, while replacing melanin with drosopterins across the rest of the body (Matthews, 1952). Conversely, the difference in ventral pigmentation between morphs appears minor. By placing our work in this context, we hypothesize that an increase in the synthesis of drosopterins, melanin, and reflecting platelets could account for the non-parallel color changes at the dorsal and lateral points, while color changed in a similar manner between morphs at the ventral points. An upregulation of dermal pigments, therefore, may result in both the changes in brightness that we observed as well as the changes in pigmentation, and therefore could be addressed together in future cellular-based research.

Ecologically, several mechanisms may influence the changes in brightness and coloration that we observed. For example, dietary changes from the wild to captivity may make the precursors for pigments more readily available, or stress may influence the regulation of hormones important for pigment synthesis (e.g., α-MSH; Fernandez and Bagnara, 1991), potentially affecting the rate of pigment synthesis and thus concentration in the dermis. Conversely, the changes we observed may reflect natural seasonal changes in

Table 3. Summary of Multivariate Dispersion. Left columns summarize the average distance from all data points in each group to the centroid of that respective group for erythristic individuals, as well as the permuted *P*-value between the estimates of dispersion from time point 1 to time point 2 for each anatomical point. Right columns represent the same statistics for striped individuals.

	Erythristic			Striped		
Point	Time 1	Time 2	*P*	Time 1	Time 2	*P*
Do1	14.69	17.94	0.044	10.87	17.02	0.001
Do2	17.84	23.03	0.019	16.06	23.29	0.003
Do3	16.47	19.30	0.077	16.33	22.99	0.009
Do4	17.71	21.65	0.060	16.66	21.16	0.076
Do5	17.95	21.69	0.085	17.45	21.11	0.005
Do6	17.57	20.89	0.132	16.49	22.96	0.001
Ch1	13.40	23.18	0.001	11.88	17.34	0.001
Ch2	12.37	24.00	0.001	10.33	19.29	0.001
La1	12.91	19.36	0.001	11.06	20.12	0.001
La2	15.43	19.54	0.028	10.22	17.35	0.001
La3	15.71	20.54	0.003	10.20	16.23	0.001
La4	12.81	21.30	0.001	10.98	18.40	0.001
La5	13.82	21.69	0.001	10.27	17.14	0.001
La6	14.32	22.99	0.001	10.26	16.16	0.108
Vn1	11.78	24.58	0.001	14.13	22.74	0.001
Vn2	15.47	20.24	0.028	14.81	20.23	0.001
Vn3	16.29	20.42	0.033	14.63	24.44	0.034
Vn4	14.88	23.25	0.001	13.91	19.37	0.044

pigmentation. For instance, if selection for pigmentation varies seasonally, individuals with an ability to seasonally reallocate resources to and from pigmentation may have higher survival rates through hot summer months. This has already been hypothesized to affect the evolution of pigmentation in other amphibian species: some Pacific Treefrogs (*Pseudacris regilla*) have the ability to seasonally adjust their coloration to match the predominant background color (Wente and Phillips, 2005). Alternatively, if the selective environment for salamander coloration is highly variable, the ability to increase or decrease the production of dermal pigments in response to short-term environmental cues could be a valuable asset (for an example involving carotenoids and *Betta* fish, see Clotfelter et al., 2007). Future research on morphological color-change in amphibians should focus on teasing apart the ultimate mechanisms responsible for labile pigmentation.

Despite the clear patterns we observed in this study, a number of caveats must be considered when interpreting our findings biologically. First, our study only examined pigmentation changes in the visible spectrum (400–700 nm); the ultraviolet portion of the spectrum (300–400 nm) was not quantified. Therefore, while we observed clear changes in pigmentation over time in our study, any ultraviolet changes which may be detectable by avian predators (Hart and Hunt, 2007) cannot be addressed here. Second, any changes in pigmentation which may have occurred prior to the first quantification of color cannot be evaluated. Finally, because we did not quantify pigmentation in the field at the time of capture, any pigmentation differences between wild-caught and captive animals cannot be fully evaluated with these data. Future studies should investigate these aspects of pigmentation change in this group.

Finally, because we observed a temporal shift in coloration in captivity, our findings may also indicate that quantitative measures of coloration obtained in a laboratory setting may not approximate those obtained in natural conditions. While we did not directly measure color in the field, the observed shifts in a laboratory setting tentatively suggest that there may be differences in coloration between captive and wild amphibians. This is especially relevant for studies where the goal is to identify relative differences or similarities in coloration between organisms that differ only subtly in coloration. As a consequence, we suggest that studies aimed at comparing fine-scale variation in coloration among individuals or between groups of amphibians should directly measure wild-caught individuals. In conclusion, this study provides the first rigorous quantification of morphological color-change in a salamander. Consistent with common expectations of the effect captivity has on amphibian pigmentation, we found significant changes both in brightness and coloration. Surprisingly, we also observed that color change was morph-dependent. These findings suggest that selection on morphological color-change may affect color morphs in a different manner, which adds a new and unforeseen dimension to our understanding of the evolution of pigmentation in amphibians.

ACKNOWLEDGMENTS

The authors would like to thank the Massachusetts Division of Fisheries and Wildlife (#145.10SCRA), the Pennsylvania Department of Conservation and Natural Resources (#492), the Pennsylvania DCNR-Bureau of Forestry (SFRA-1016), the Ohio Division of Wildlife (#11-281), and the Ohio Division of Natural Areas and Preserves (#RP-10-06) for the required permits to conduct this research. C. Kraemer graciously helped collect salamanders for the project. T. Robinson and T. French were instrumental in locating appropriate collection sites in Ohio and Massachusetts, respectively. We also thank J. Gassman for the salamander illustration in Figure 1. A. Alejandrino, J. Deitloff, A. Kaliontzopoulou, J. Serb, and A. Worthington provided valuable comments on earlier versions of this manuscript. This research was conducted under Iowa State University Institutional Animal Care and Use Committee permit number 2-10-6881-J.

LITERATURE CITED

Adams, D. C., and M. L. Collyer. 2007. Analysis of character divergence along environmental gradients and other covariates. Evolution 61:510–515.

Adams, D. C., and M. L. Collyer. 2009. A general framework for the analysis of phenotypic trajectories in evolutionary studies. Evolution 63:1143–1154.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Anderson, M. J., and C. J. F. Ter Braak. 2003. Permutation tests for multi-factorial analysis of variance. Journal of Statistical Computation and Simulation 73:85–113.

Andersson, S., J. Ornborg, and M. Andersson. 1998. Ultraviolet sexual dimorphism and assortative mating in blue tits. Proceedings of the Royal Society: Biological Sciences 265:445–450.

Bagnara, J. T., and M. E. Hadley. 1969. The control of bright colored pigment cells of fishes and amphibians. American Zoologist 9:465–478.

Bagnara, J. T., and J. D. Taylor. 1970. Differences in pigment-containing organelles between color forms of red-backed salamander, *Plethodon cinereus*. Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie 106:412–417.

Barnett, J. 2011. Pigmentation loss and regeneration in a captive wild-type axolotl, *Ambystoma mexicanum*. Herpetological Bulletin 115:17–18.

Bond, A. B. 2007. The evolution of color polymorphism: crypticity, searching images, and apostatic selection. Annual Review of Ecology and Systematics 38:489–514.

Brodie, E. D., III. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. Evolution 46:1284–1298.

Clotfelter, E. D., D. R. Ardia, and K. J. McGraw. 2007. Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. Behavioral Ecology 18:1139–1145.

Collyer, M. L., and D. C. Adams. 2007. Analysis of two-state multivariate phenotypic change in ecological studies. Ecology 88:683–692.

Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. Johns Hopkins University Press, Baltimore, Maryland.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76–91.

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

Endler, J. A., and M. Théry. 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. The American Naturalist 148:421–452.

Fernandez, P. J., and J. T. Bagnara. 1991. Effect of background color and low-temperature on skin color and circulating alpha-MSH in two species of leopard frog. General and Comparative Endocrinology 83:132–141.

Frost-Mason, S., R. Morrison, and K. Mason. 1994. Pigmentation, p. 64–97. *In*: Amphibian Biology. Volume 1: The Integument. H. E. Heatwole and G. T. Barthalmus (eds.). Surrey Beatty & Sons, Chipping Norton.

Grill, C. P., and V. N. Rush. 2000. Analysing spectral data: comparison and application of two techniques. Biological Journal of the Linnean Society 69:121–138.

Hart, N. S., and D. M. Hunt. 2007. Avian visual pigments: characteristics, spectral tuning, and evolution. The American Naturalist 169:7–26.

Highton, R. 1959. The inheritance of the color phases of *Plethodon cinereus*. Copeia 1959:33–37.

Kuchta, S. R. 2005. Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of pacific newts. Copeia 2005:265–271.

Matthews, E. 1952. Erythrism in the salamander *Plethodon cinereus cinereus*. Copeia 1952:277.

Parker, G. H. 1943. Animal color changes and their neurohumors. The Quarterly Review of Biology 18:205–227.

Pough, F. H. 1974. Comments on the presumed mimicry of red efts (*Notophthalmus*) by red salamanders (*Pseudotriton*). Herpetologica 30:24–27.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.r-project.org/

Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the White Sands ecotone. American Naturalist 167:1–15.

Santos, J. C., L. A. Coloma, and D. C. Cannatella. 2003. Multiple, recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences of the United States of America 100:12792–12797.

Storfer, A., J. Cross, V. Rush, and J. Caruso. 1999. Adaptive coloration and gene flow as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri*. Evolution 53:889–898.

Taylor, J. D., and J. T. Bagnara. 1972. Dermal Chromatophores. American Zoologist 12:43–62.

Wente, W. H., and J. B. Phillips. 2005. Seasonal color change in a population of Pacific treefrogs (*Pseudacris regilla*). Journal of Herpetology 39:161–165.