Journal of Heredity
doi:10.1093/jhered/esq044

Quantitative Genetics of Pigmentation Development in 2 Populations of the Common Garter Snake, *Thamnophis sirtalis*

Michael F. Westphal and Theodore J. Morgan

From the Division of Biology and The Ecological Genomics Institute, Kansas State University, Manhattan, KS 66506. Michael F. Westphal is now at the Bureau of Land Management, 20 Hamilton Court, Hollister, CA 95023.

Address correspondence to Michael F. Westphal at the address above, or e-mail: mwestpha@blm.ge.

Abstract

The evolutionary importance of ontogenetic change has been noted since Darwin. However, most analyses of phenotypic evolution focus on single landmark ages. Here, we present an inheritance study that quantifies genetic variation in pigmentation across early-age (i.e., birth to 180 days) development in 2 populations of the common garter snake, *Thamnophis sirtalis*. The populations are phenotypically distinct and geographically isolated (Manitoba, CA and Northern California, USA). There were highly significant differences between populations for the developmental trajectory of mean pigmentation, with the Manitoba population exhibiting a mean pigmentation level that increased across ontogeny, whereas the California population exhibited mean pigmentation that was invariant across ontogeny. Subsequent quantitative genetic analyses revealed heritable variation at all ages in Manitoba but low levels of phenotypic and genetic variation in California at all ages. A quantitative genetic decomposition of the longitudinal genetic variance–covariance matrix for the age-specific pigmentation phenotypes in the Manitoba population revealed 2 primary orthogonal axes that explained most (~100%) of the pigmentation variation across ontogeny. The primary axis, explaining 93% of the genetic variation, is an axis of genetic variation whose principal value loadings change from positive to negative across development, suggesting that the most rapid evolutionary response to selection on pigmentation variation will occur in the direction characterized by a tradeoff in early-age versus late-age pigmentation phenotypes. Pigmentation is known to be ecologically important and subject to rapid evolution under selection. Our study shows that significant differences exist between these 2 populations for their capacity to respond to selection on pigmentation which is not only influenced by the population of origin but also by the developmental process. We suggest that developmental timing may be a potential explanatory mechanism for the difference between the populations.

Key words: *coloration, development, pigmentation, quantitative genetics,* Thamnophis sirtalis

A major goal of evolutionary biology is to understand how genetic variation within and among populations is translated into the phenotypic variation on which natural selection acts (Endler 1986). This transformation of standing genetic variation into phenotypic variation is influenced by a complex set of interactions between the genotype and the environment, the stage in development, or even the maternal environment (Gould 1977; Falconer and Mackay 1996). Thus, to completely understand the genetic basis of phenotypic variation and ultimately predict evolutionary responses to natural selection, it is essential to describe how the genotypic expression of phenotypes changes across environments and/or development (Falconer 1956; Kirkpatrick and Heckman 1989; Gomulkiewicz and Kirkpatrick 1992; Parichy 2006a).

Quantitative genetic theory has provided a strong theoretical foundation for studies linking developmental phenotypic variation with standing genetic variation (Kirkpatrick and Heckman 1989; Cowley and Atchley 1992; Kirkpatrick and Lofsvold 1992). The central model in quantitative genetics is the genetic variance–covariance matrix or **G**-matrix (often denoted as **G**) (Falconer and Mackay 1996). The **G**-matrix represents the genetic architecture underlying phenotypes and is especially useful in characterizing the genetic component of quantitative traits, that is, traits affected by many genes. Phenotypic traits

must be heritable if they are to experience population-scale evolution. The **G**-matrix provides the heritability component that allows prediction of the next-generation response to selection on suites of phenotypic traits (Lande and Arnold 1983) and has become a powerful tool for evolutionary geneticists (McGuigan 2006).

For ontogenetic traits, the general approach is to analyze the age-specific phenotypic measurements as if they were truly different phenotypic characters (Falconer 1956). With this approach, genetic variances and covariances (the components of the **G**-matrix) can be easily estimated within and between ages from a basic quantitative genetic breeding design. The magnitude and sign of the genetic variance and covariances describe how much genetic variation exists for the phenotype at each age as well as how much integration in the phenotype exists among ages (Morgan et al. 2003). This quantitative genetic framework is especially useful for identifying the possible phenotypic directions in which various forms of natural selection will change the developmental phenotypes across generations (Schluter 1996). For example, if natural selection influences an ontogenetic phenotype in a direction with abundant genetic variation, the evolutionary response will be rapid; however, if natural selection favors a pattern of ontogenetic change that has limited or nonexistent genetic variation, the evolutionary response will be limited or constrained (Schluter 1996; Kingsolver et al. 2001). Thus, by taking a quantitative genetic approach to the analysis of developmental change, it is possible to identify the probable directions of future evolutionary response.

One developmental phenotype that can have pronounced effects on adaptive evolution in many species is pigment developmental variation. Across animal species, pigmentation phenotypes have many essential roles including predator avoidance, warning coloration, thermoregulation, and mate attraction (Cott 1940; Cooper and Greenberg 1992; Ruxton et al. 2004). Patterns of pigmentation are generally species specific but display abundant genetic variation within species, which can also be influenced by the age of the animal as well as the environment. Pigment development has been most thoroughly studied in model species such as *Drosophila* and *Mus* (see Carroll et al. 2005) but important studies have also focused on insects (Monteiro et al. 1994), fish (Mabee 1995; McClure and McCune 2003; Kelsh 2004; Quigley et al. 2004; Parichy 2006a, 2006b; Parichy et al. 2006; Mills et al. 2007), amphibians (Twitty 1966; Parichy 1998; Parichy et al. 2006), and birds (Badyaev and Martin 2000; Badyaev et al. 2001). However, despite its great importance to adaptive evolution in animals (Cott 1940; Norris and Lowe 1964), few studies have focused on pigmentation development in reptiles. This is surprising because dorsal body coloration is likely under strong natural selection, either stabilizing or directional (Norris 1965; Kettlewell 1973). If pigmentation is both genetically and developmentally controlled, the efficiency of natural selection will tend to change with age.

Most studies of snake color pattern development have focused on species with dramatic species-specific pigmentation trajectories (Hadley and Gans 1972; Bowen 2003; Creer 2005). Although these studies are interesting in a comparative sense, they provide little insight into the role of within-species genetic variation on the evolution of pigmentation development across widely distributed species. Thus, to better understand how pigmentation development evolves, it is much more informative to utilize species and pigmentation phenotypes that are not species specific but display abundant adaptive genetic variation in the pigmentation developmental trajectory.

The common garter snake, *Thamnophis sirtalis* is one of these species. Although *T. sirtalis* does display species-specific pigmentation patterns, they also have remarkable variation in the size and intensity of the red and white coloration expressed in the dorsolateral blotches (Figure 1) within and among populations across the range from Central to Western North America (Fitch 2001). The dorsolateral blotches are thought to function in both thermoregulation and predator startle response behavior (Shine et al. 2000; Bittner et al. 2002; Figure 1), and red pigment in the blotches has been shown to be under significant positive selection as a result of crow predation (Westphal 2007). Additionally, the dorsolateral blotches are also known to change during development such that newborn snakes are generally less pigmented than adult snakes, although anecdotal accounts suggest there is wide variation among populations in the rate of this pigmentation development.

In this study, we present an inheritance study that quantifies genetic variation in dorsolateral blotch pigmentation across early-age (i.e., birth to 180 days) development in 2 populations of the common garter snake, *T. sirtalis*. The populations are phenotypically distinct and geographically isolated (Manitoba, CA and Northern California, USA). We quantify and compare the expression of red pigment at different developmental stages in California and Manitoba newborns in a quantitative genetic breeding design and derive quantitative genetic parameters at each developmental stage to assess the potential response to selection in subsequent generations.

Materials and Methods

Sampling and Breeding Design

For this study, we obtained both family- and population-level data. Multiple litters of newborn snakes were used from female snakes collected from 2 populations within the western portion of the range of *T. sirtalis*: northeastern California, USA and central Manitoba, Canada. The Manitoba female sample was collected in May 2004 at a limestone-substrate hibernaculum near the town of Vogar in the Interlake region (lat 50°56′53″N, long 98°26′37″W). In order to ensure that they had not yet been mated, females were captured by intercepting them as they emerged from hibernation. New emergence was evident from the extreme (much lower than shade-ambient) coldness of the snakes and by a crusty mud coating, which quickly wears off snakes once they have emerged from hibernation. Males were collected by nonselectively grab sampling from aggregations

Figure 1. Behavioral display and variation in dorsolateral blotches of the common garter snake, *Thamnophis sirtalis*. (**A**) Two male adult snakes from the same den in Manitoba, both in antipredator display. (**B**) Same snakes from panel A in "typical" foraging/basking mode. Epidermal red pigment ranges continuously from total absence (**C**) to partial saturation of blotch (**D**) to complete saturation (**E**).

of snakes and placing the captured snakes (usually 10–20 per sample) into nylon sacks. Females were released from the grab sample. Males and females were removed to a laboratory facility operated by Manitoba Conservation in Chatfield, Manitoba, where the snakes were allowed to mate in outdoor arenas. Arenas were constructed of ripstop nylon and measured approximately 1.5 × 1.5 m with a height of 1 m. After mating, females were moved to rearing pens, and males were released into the wild.

Gravid females were housed in a uniform environment in outdoor pens designed to simulate the native environment. Pens included a constant water supply and access to shade and basking sites. Gravid snakes were fed twice weekly ad libitum on a mixed diet of live fish, (primarily *Notropis atherinoides* and *N. hudsonius*), frogs, and tadpoles (*Rana pipiens*, *R. sylvatica*, and *Hyla cinereus*), gathered from the surrounding countryside. The snake's diet was supplemented with commercially obtained earthworms (*Lumbricus terrestris*). In mid-July gravid females were transported to a dedicated snake husbandry facility in the Department of Zoology at Oregon State University (OSU), where they were maintained until their litters were born. Females were kept in a uniform environment on a natural light and temperature cycle and were fed ad libitum on commercially obtained earthworms (*L. terrestris*) and thawed salmon par (*Oncorhynchus tshawytscha*) obtained from a local fish hatchery.

The California snakes were collected from multiple closely spaced stations at the California site. Of the 4 California collecting stations, 3 (Colman, Mahogany, and Nameless) are located within a 3 km radius and the fourth (Roney Corral) is situated 10 km west of the other 3 (see Manier and Arnold 2005 for coordinates). Rather than being captured as they emerged from dens, as in Manitoba, gravid females from California were captured by hand in wetland habitats where snakes aggregate to feed. All the snakes in the California sample were collected in 2005. Gravid females were immediately transported back to OSU and maintained under the same regime as described for the Manitoba sample above.

To study the genetic basis of postnatal development in pigmentation, we maintained 2 populations of full-sib litters in the laboratory after parturition. To characterize ontogenetic trajectories of pigmentation ratio, we scored the Manitoba neonates at 4 age-specific scoring periods: birth, 60, 120, and 180 days of age. In addition, we reared a sample of 29 California snakes. However, the California population displayed near-complete concordance of early-age (i.e., birth and 60 days) pigmentation with adult color traits. Additionally, pigmentation ratio (see below) was nearly saturated and invariant within the California population. Thus, we discontinued the ontogenetic experiment with the California population after 60 days.

Trait Scoring

We recorded snout-to-vent length (SVL), mass, and sex for all neonate snakes. To obtain estimates of red pigment expression, we focused on 3 adjacent dorsolateral blotches immediately anterior to a point on each snake located at one-quarter of the distance from the snake's snout to the snake's vent (estimated by dividing the SVL, measured in

millimeters, by 4). Dorsolateral blotches in common garter snakes consist of an unmelanized dermal region that appears white and an overlying epidermal aggregation of erythrophores of varying size that causes the blotch to appear red to a lesser or greater extent (Figure 1). We scored red pigmentation as the ratio of red-pigmented area within a blotch to the overall area of the blotch. Data were taken from the left side of each snake only. In order to remove the confounding effects of variation in individual snake size, we used each snake's own scalation as a sizing grid. We measured the width of each blotch at each scale row with respect to the scale nearest it. For example, we scored a blotch that was 1.2 times the length of the adjacent scale as a 12; a blotch 0.6 times the length of its adjacent scale earned a score of 6. We scored pigment at each scale row on a semidiscrete scale of 0–1: 0 indicated no trace of red pigment at that scale row; 0.5 indicated red pigment was present but did not completely saturate the blotch at that scale row; and 1 indicated complete saturation of the blotch at that scale row. Partially saturated regions of the blotch appear to the naked eye as mottled regions comprised of colonies of erythrophores separated by all-white regions. The final score for pigment area was obtained by multiplying the width of each blotch at each scale row by the corresponding pigment score at that scale row. We then calculated the pigmentation ratio by dividing the pigmentation area by the blotch area for each scale. The composite pigmentation ratios across the 3 scales were calculated by averaging over the 3 blotches to provide a ratio of red pigment area to total blotch area. Because the area of red pigment was always equal to or less than the total blotch area, the ratio was always a quantity between 0, an unpigmented (white) blotch and 1, a fully pigmented (red) blotch.

Statistical and Quantitative Genetic Analyses

The average developmental trajectories of pigmentation in the 2 populations were compared using a 2-way analysis of variance (ANOVA) in SAS (SAS Institute 2002), with fixed effects of population of origin (Manitoba vs. California) and age (birth vs. 60-day old), as well as the interaction of population and age.

We calculated age-specific (birth, 60, 120, and 180 days of age) quantitative genetic parameters (i.e., genetic variances and heritabilities within ages and genetic covariances between ages) from individuals from the Manitoba population. Estimates of the age-specific quantitative genetic parameters were calculated using the software package h2boot (Phillips 1998) with the full-sib ANOVA option and 10 000 bootstrap replicates across families to generate standard errors. The longitudinal **G**-matrix approach analyzes the age-specific phenotypic measurements as if they were different phenotypic characters (Falconer 1956). With this approach, the genetic variances within ages and covariances between ages describe how much genetic variation exists for the phenotype at each age as well as how much integration in the phenotype exists among ages (Morgan et al. 2003). This quantitative genetic framework is especially useful for identifying the possible phenotypic directions in which various forms of natural selection will change the developmental phenotypes across generations via the decomposition of the genetic variance–covariance (**G**) matrix into its orthogonal eigenvectors and associated eigenvalues (Schluter 1996). Eigenvectors represent the orthogonal axes of ontogenetic variation on which phenotypic responses to selection can act and the associated eigenvalues correspond to the amount of genetic variation associated with each independent axis of genetic variation (Kingsolver et al. 2001). The decomposition of the longitudinal **G**-matrix of the Manitoba population was done via an online matrix calculator (www.arndt-bruenner.de/mathe/scripts/engl_eigenwert.htm) resulting in a set of orthogonal eigenvectors and their respective eigenvalues (Kirkpatrick and Lofsvold 1992).

Results

Means

A total of 25 females, yielding 164 offspring, were obtained from the Manitoba population. After removal of individuals with missing data, 144 offspring were available for the analysis. Thirty-seven females yielding 234 offspring were obtained from the California population. Mean SVL for the Manitoba neonates was 158.3 ± 0.9 mm and was significantly smaller than mean SVL for the California neonates (177.2 ± 0.6 mm, 2-tailed *t*-test, $P < 0.0001$). Mean pigment area in the Manitoba sample (25.26 ± 1.67) was significantly lower at birth than blotch area (68.58 ± 1.98, $P < 0.001$, Figure 2A), and the phenotypic ratio of red pigment to blotch size in Manitoba snakes at birth was 0.366 ± 0.020 (Figure 2B). In the California population, dorsal blotches were maximally saturated with red pigment at birth in all individuals. Thus, the means of blotch area and pigment area were not significantly different in California newborns (blotch size = 64.41 ± 1.03, pigment = 63.99 ± 1.04, $P = 0.78$; Figure 2A). Consequently, the mean pigment ratio in California neonates is nearly fully saturated with a pigmentation ratio of 0.992 ± 0.002 (Figure 2B). Both blotch and pigment means showed normal distributions, allowing parametric tests. Mean blotch area did not differ significantly between the populations (2-tailed *t*-test, $P = 0.062$; mean MB = 68.6 ± 2.0, mean CA = 64.4 ± 1.0), but pigment area was significantly lower in the Manitoba population ($P < 0.001$; mean MB = 24.3 ± 1.7, mean CA = 64.0 ± 1.0), revealing pigment as the driving force behind the difference in pigmentation ratio between the Manitoba and California populations (Figure 2B).

The mean pigment ratio in the Manitoba population increased significantly with age (Figures 2B and 3, $P < 0.001$). Pairwise analyses between the ages revealed a pattern of pigmentation development that increased significantly from birth to 120 days of age. However, this increase in mean pigmentation ceased between 120 and

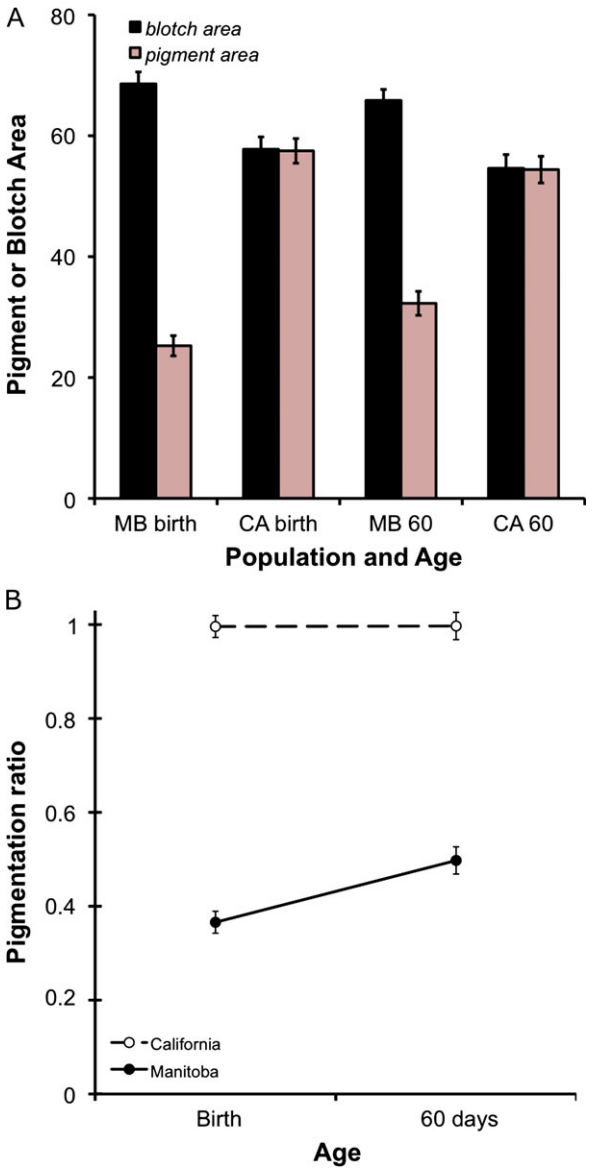

Figure 2. (**A**) Comparison of blotch area and pigment area means from samples of *Thamnophis sirtalis* neonates from Manitoba (MB) and California (CA) scored at birth and 60 days. (**B**) Sixty-day developmental trajectories of the ratio of pigment to pattern in the MB and CA populations of *T. sirtalis*.

180 days of age ($P = 0.20$; Figure 3). In contrast, the California population showed no significant change from mean pigmentation ratios between birth and 60 days of age ($P = 0.37$; Figure 2B), as a result of the nearly fully saturated dorsolateral pigmentation blotches present in the California population at birth and through development. Because of this invariant pattern of ontogenetic change, additional age-specific measurements (i.e., 120 and 180 days of age) were not collected for the California population.

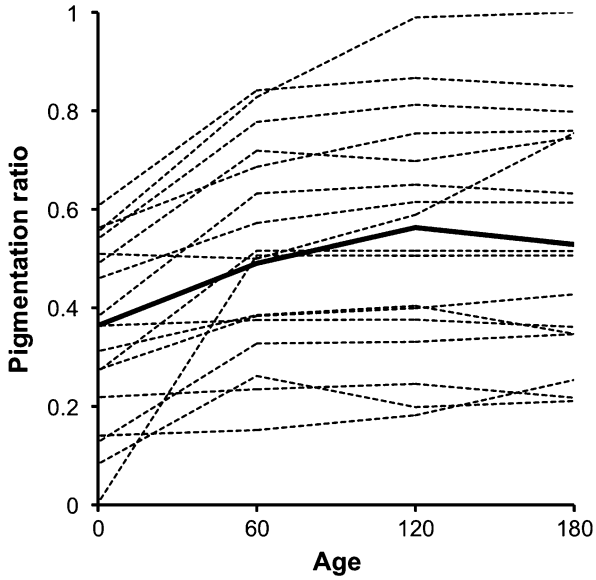

Figure 3. Ontogenetic trajectory of means of pigmentation ratios from 17 laboratory-born *Thamnophis sirtalis* litters from Manitoba. Bold line is the mean ontogenetic trajectory of the entire Manitoba population calculated at each age.

Quantitative Genetic Estimates for the Manitoba Population

SVL was included in initial analyses but was not found to significantly covary with any trait beyond a weak positive correlation with pigment (genetic correlation = 0.37 ± 0.16, $P \approx 0.05$) and was therefore excluded from further analysis. All estimates for within-age genetic variances and between-age covariances of the pigment ratio were significantly greater than zero over the 180-day scoring period (Table 1). Within-age variances ranged from 0.0466 ± 0.021 (birth genetic variance) to 0.0849 ± 0.038 (120 day genetic variance), and the age-specific heritabilities ranged from 0.572 ± 0.33 (180 day heritability) to 0.788 ± 0.28 (120 day heritability; Table 1). Decomposition of the longitudinal **G**-matrix for the Manitoba populations yielded 2 primary eigenvectors explaining nearly 100% of the genetic variation in pigmentation development (Figure 4). The first eigenvector of the longitudinal **G**-matrix explained 93% of the variance and showed a complex profile of principal component loadings over the 4 scoring periods from birth to 180 days of age. The pattern of principal components of this primary eigenvector across development changes sign from positive to negative between early and late ages (i.e., between 60 and 120 days of age), suggesting an axis of genetic variation consistent with a tradeoff between early-age and late-age pigmentation ratio (Figure 4). The second eigenvector explained only 7% of the variance and contained principal components that were positive across all 4 scoring periods (i.e., between 60 and 120 days of age), suggesting an axis of genetic variation consistent with positive covariation between age-specific pigmentation phenotypes across all 4 scoring periods. The third and fourth eigenvectors of the longitudinal **G**-matrix decomposition had associated eigenvalues of zero, suggesting

Table 1 Longitudinal **G**-matrix for the ratio of pigment to pattern in laboratory-reared *Thamnophis sirtalis*

Age (days)	1	60	120	180	
1	0.061 **0.009**	0.0466 **0.02** (**25**)	0.0535 **0.020** (**21**)	0.0570 **0.025** (**19**)	0.0432 **0.021** (**19**)
60	0.068 **0.010**	0.094 **0.011**	0.0677 **0.021** (**25**)	0.0787 **0.030** (**22**)	0.0625 **0.029** (**20**)
120	0.064 **0.009**	0.094 **0.013**	0.104 **0.016**	0.0849 **0.038** (**19**)	0.0918 **0.047** (**18**)
180	0.058 **0.010**	0.094 **0.013**	0.103 **0.018**	0.111 **0.017**	0.0661 **0.042** (**20**)
h^2	0.745 **0.28**	0.717 **0.20**	0.788 **0.28**	0.572 **0.33**	

Genetic variance and covariance estimates are given above the diagonal with their standard errors (in bold) and with numbers of litters in parentheses. Phenotypic variances and covariances are given below the diagonal with their standard errors in bold. All estimates were significant at $\alpha = 0.05$.

that these axes of variation do not contribute to the genetic variance in pigmentation ontogeny and thus represent evolutionary directions that are not attainable as a result of a lack of genetic variation.

Discussion

The expression of red pigment in *T. sirtalis* has a clearly observable temporal dimension in some populations but not others. Whereas neonate snakes from California parents express the full adult state at birth, a substantial proportion of snakes from Manitoba parents express low-pigment states that subsequently increase in pigmentation during ontogeny. Moreover, the significant heritability of pigmentation at each age class in the Manitoba sample, coupled with the significant genetic correlation of pigmentation among ages (Table 1), suggests that selection at any age can shape the phenotypic mean of future generations. However, the complex nature of the primary axis of genetic variation across ontogeny (i.e., the first eigenvector) implies that age-specific selection from predation or other such selective agent for an increase in pigmentation relative to the population mean at early ages will result in decreased pigmentation relative to the population mean at late ages (or vice versa). Furthermore, selection at multiple ages might influence the ontogenetic variation in the Manitoba population in an evolutionary direction not obtainable in the California population because of a lack of genetic variation in pigmentation.

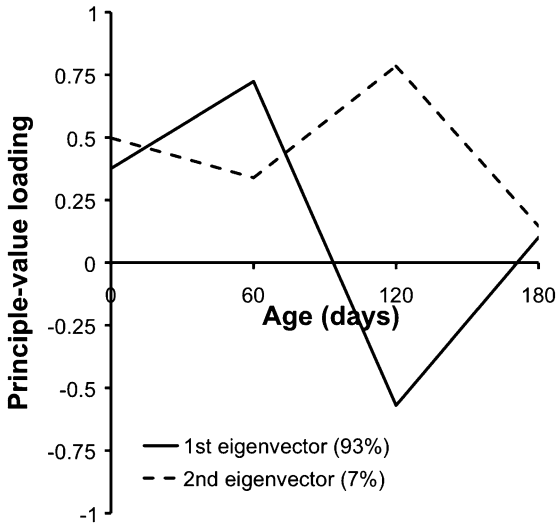

Figure 4. First 2 eigenvectors obtained from the longitudinal **G**-matrix of pigmentation ratio in a sample of laboratory-reared neonate *Thamnophis sirtalis*.

With one important exception, the results from the longitudinal study of Manitoba newborn *T. sirtalis* provide the first evidence for a reptile species that color traits can experience an increase in bright pigment during ontogeny (Booth 1990; Cooper and Greenberg 1992). The exception is for reptiles that express pigment as a secondary sexual characteristic (Martin and Forsman 1999). However, the more commonly noted ontogenetic trend in other studies of reptiles is for patterning to lose contrast and pigmentation to be reduced in brightness (Brodie 1993; Creer 2005).

Disruptive selection has been offered as a general explanation for juvenile–adult color variation in animals (Booth 1990) and has been proposed for a number of species of squamate. Neonates of the racer *Coluber constrictor* display cryptic patterns compared with the unpatterned adults, and their patterns are correlated with behaviors that maximize crypsis (Creer 2005).

Conversely, juvenile grass snakes, *Natrix natrix*, are more brightly colored than adults, possibly because their small size and vulnerability favors an aposematic phenotype (Madsen 1987). Many species of lizard capable of tail autotomy exhibit bright tails as juveniles (Vitt and Cooper 1986; Hawlena et al. 2006), which become unpigmented when lizards reach sexual maturity.

The ontogenetic and geographic variability in expression of red pigment reported may be a function of the timing of erythrophore differentiation and migration. Although erythrophore development has not been directly studied in *T. sirtalis*, erythrophores are known to readily migrate throughout the integument in poikilotherm vertebrates (Lyerla and Jameson 1968; Sherbrooke and Frost 1989; Zaccaria 1996; Ichikawa et al. 1998) and can experience chemotactic attraction or repulsion from other strata in the integument (Painter et al. 1999; Parichy 2006a). The difference between California and Manitoba *T. sirtalis* might therefore be described as a disparity in initial concentration of erythrophores. Given the complete colonization of the blotch regions by erythrophores at birth, there is apparently no more "room" for erythrophores and therefore no opportunity for ontogenetic change in California. In contrast, most individuals in Manitoba are born with incomplete erythrophore colonization of the dorsolateral blotch. Although it is unknown whether erythrophores migrate during development or differentiate in situ, aggregation of erythrophores in the blotch continues after parturition throughout the first year and proceeds from the ventral region upward across the dorsum. The rising slope of red pigment expression in Manitoba newborns stands out in sharp contrast to the flat line of the California sample.

Although *T. sirtalis* from our California site are born with complete red expression in their dorsal blotches, it is possible that erythrophores accrete in their blotches prenatally much as they do postnatally in snakes from the Manitoba site. We found evidence for the priority of white blotches in prenatal development in western *T. sirtalis* by dissection of embryos of *T. sirtalis* from a population in Benton County, Oregon in which all individuals express complete red blotches at birth (Westphal MF, unpublished data). We found that embryos of Zehr stage 36 (ca. 100-day old) express white blotches, and red erythrophores are only present at the ventral margin of the blotches (Zehr 1962). The California embryos, therefore, express the same low-pigment phenotype prenatally as Manitoba offspring express postnatally, suggesting that the difference between California snakes and Manitoba snakes is an outcome of some difference in timing of pigment development. One further clue might be inferred from the observation that midwestern neonates of *T. sirtalis* are significantly smaller at birth than western neonates (this study; Gregory and Larsen 1993, 1996), and the further observation from this study that size has a weak inverse correlation with pigmentation. These associations of lower pigment with smaller size at birth adds further weight to the question of whether a fundamental shift in developmental timing has contributed to the difference between the 2 populations in neonate phenotype. How developmental timing might interact with selection on pigmentation in *T. sirtalis* is therefore a question that merits further study.

Funding

US Department of Education through a Graduate Assistance in Areas of National Need fellowship to M.F.W.; Kansas State Ecological Genomics Institute through a postdoctoral fellowship to M.F.W. and a research seed grant to T.J.M.

Acknowledgments

We would like to thank S. J. Arnold for his mentorship and for making his snake rearing facility freely available. R. T. Mason provided invaluable assistance and encouragement, as did J. B. Westphal, D. Roberts, R. Shine, and R. P. O'Donnell. All snakes were collected under authority of collecting permits to M.F.W., R. T. Mason, and S. J. Arnold and were maintained according to stipulations in Institutional Animal Care and Use Committee animal care permits.

References

Badyaev AV, Hill GE, Dunn PO, Glen JC. 2001. Plumage color as a composite trait: developmental and functional integration of sexual ornamentation. Am Nat. 158:221–235.

Badyaev AV, Martin TE. 2000. Individual variation in growth trajectories: phenotypic and genetic correlations in ontogeny of the house finch (*Carpodacus mexicanus*). J Evol Biol. 13:290–301.

Bittner TD, King RB, Kerfin JM. 2002. Effects of body size and melanism on the thermal biology of garter snakes (*Thamnophis sirtalis*). Copeia. 2002:477–482.

Booth CL. 1990. Evolutionary significance of ontogenetic colour change in animals. Biol J Linn Soc. 40:125–163.

Bowen KD. 2003. Ontogenetic changes in the coloration of the northern watersnake, *Nerodia sipedon sipedon*. J Herpetol. 37:729–731.

Brodie ED III. 1993. Consistency of individual differences in anti-predator and colour pattern in the garter snake, *Thamnophis ordinoides*. Anim Behav. 45:851–861.

Carroll SB, Grenier JK, Weatherbee SD. 2005. From DNA to diversity. Molecular genetics and the evolution of animal design. 2nd ed.. Malden (MA): Blackwell Publishing.

Cooper WE, Greenberg N. 1992. Reptilian coloration and behaviour. In: Gans C, Crews D, editors. Biology of the reptilia. New York: Alan R. Liss, Inc. p. 298–422.

Cott HB. 1940. Adaptive coloration in animals. London: Methuen 508 pp.

Cowley DE, Atchley WR. 1992. Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. Evolution. 46:477–494.

Creer DA. 2005. Correlations between ontogenetic change in color pattern and antipredator behavior in the racer, *Coluber constrictor*. Ethology. 111:287–300.

Endler JA. 1986. Natural selection in the wild. Princeton (NJ): Princeton University Press (Monographs in population biology 21) 336pp.

Falconer DS. 1956. The genetics of litter size in mice. J Cell Comp Physiol. 56(Suppl 1):153–167.

Falconer DS, Mackay TFC. 1996. Introduction to quantitative genetics. 4th ed. New York: Longman 464pp.

Fitch HS. 2001. Further study of the garter snake, *Thamnophis sirtalis*, in northeastern Kansas. Univ Kansas Nat Hist Mus Sci Pap. 19:1–6.

Gomulkiewicz R, Kirkpatrick M. 1992. Quantitative genetics and the evolution of reaction norms. Evolution. 46:390–411.

Gould SJ. 1977. Ontogeny and phylogeny. Cambridge (MA): Harvard University Press.

Gregory PT, Larsen KW. 1993. Geographic variation in reproductive characteristics among Canadian populations of the common garter snake (*Thamnophis sirtalis*). Copeia. 4:946–958.

Gregory PT, Larsen KW. 1996. Are there any meaningful correlates of geographic life-history variation in the garter snake, *Thamnophis sirtalis*. Copeia. 1:183–189.

Hadley WF, Gans C. 1972. Convergent ontogenetic change of color pattern in *Elaphe climacophora* (Colubridae: Reptilia). J Herpetol. 6:75–78.

Hawlena D, Boochnik R, Abramsky Z, Bouskila A. 2006. Blue tail and striped body: why do lizards change their infant costume when growing up? Behav Ecol. 17:889–896.

Ichikawa Y, Ohtani H, Miura I. 1998. The erythrophore in the larval and adult dorsal skin of the brown frog, *Rana ornativentris*: its differentiation, migration, and pigmentary organelle formation. Pigment Cell Res. 11:345–354.

Kelsh RN. 2004. Genetics and evolution of pigment patterns in fish. Pigment Cell Res. 17:326–336.

Kettlewell HBD. 1973. The evolution of melanism. Oxford: Clarendon Press.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri N, Hill CE. 2001. The strength of phenotypic selection in natural populations. Am Nat. 157:245–261.

Kirkpatrick M, Heckman N. 1989. A quantitative genetic model for growth, shape, reaction norms, and other infinite-dimensional characters. J Math Biol. 27:429–450.

Kirkpatrick M, Lofsvold D. 1992. Measuring selection and constraints in the evolution of growth. Evolution. 46:954–971.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. Evolution. 37:1210–1226.

Lyerla TA, Jameson DL. 1968. Development of color in chimeras of Pacific tree frogs. Copeia. 1968:113–128.

Mabee PM. 1995. Evolution of pigment pattern development in centrarchid fishes. Copeia. 1995:586–607.

Madsen T. 1987. Natural and sexual selection in grass snakes, *Natrix natrix*, and adders, *Vipera berus* [PhD thesis]. Lund (Sweden): University of Lund.

Manier MK, Arnold SJ. 2005. Population genetic analysis identifies source-sink dynamics for two sympatric garter snake species (*Thamnophis* elegans and *T. sirtalis*). Mol Ecol. 14:3965–3976.

Martin J, Forsman A. 1999. Social costs and development of nuptial coloration in male *Psammodromus algirus* lizards: an experiment. Behav Ecol. 10:396–400.

McClure M, McCune AR. 2003. Evidence for developmental linkage of pigment patterns with body size and shape in Danios (Teleostei: Cyprinidae). Evolution. 57:1863–1875.

McGuigan K. 2006. Studying phenotypic evolution using multivariate quantitative genetics. Mol Ecol. 15:883–896.

Mills MG, Nuckels RJ, Parichy DM. 2007. Deconstructing evolution of adult phenotypes: genetic analyses of *kit* reveal homology and evolutionary novelty during adult pigment pattern development of *Danio* fishes. Development. 134:1081–1090.

Monteiro AF, Brakefield PM, French V. 1994. The evolutionary genetics and developmental basis of wing pattern variation in the butterfly *Bicyclus anynana*. Evolution. 48:1147–1157.

Morgan TJ, Garland T Jr, Carter PA. 2003. Ontogenies in mice selected for high voluntary wheel-running activity. I. Mean ontogenies. Evolution. 57: 646–657.

Norris KS. 1965. Color adaptation in desert reptiles and its thermal relationships. In: Milstead WW, editor. Lizard ecology: a symposium. Columbia (MO): University of Missouri Press. p. 162–226.

Norris KS, Lowe CH. 1964. An analysis of background color-matching in amphibians and reptiles. Ecology. 45:565–580.

Painter KJ, Maini PK, Othmer HG. 1999. Stripe formation in juvenile *Pomacanthus* explained by a generalized Turing mechanism with chemotaxis. Proc Natl Acad Sci Biol. 96:5549–5554.

Parichy DM. 1998. Experimental analysis of character coupling across a complex life cycle: pigment pattern metamorphosis in the tiger salamander, *Ambystoma tigrinum tigrinum*. J Morphol. 237:53–67.

Parichy DM. 2006a. Evolution of *Danio* pigment pattern development. Heredity. 97:200–210.

Parichy DM. 2006b. Homology and the evolution of novelty during *Danio* adult pigment patten development. J Exp Zool. 306B:1–13.

Parichy DM, Reedy MV, Erickson CA. 2006. Regulation of melanoblast migration and differentiation. In: Nordland JJ, Boissy RE, Hearing VJ, King RA, Ortonne JP, editors. The pigmentary system and its disorders. 2nd ed. New York: Oxford University Press.

Phillips PC. 1998. H2boot: bootstrap estimates and tests of quantitative genetic data. Eugene (OR): University of Oregon Available from: www.uoregon.edu/~pphil/software.html.

Quigley IK, Turner JM, Nuckels RJ, Manuel JL, Budi EH, MacDonald EL, Parichy DM. 2004. Pigment pattern evolution by differential deployment of neural crest and post-embryonic melanophore lineages in *Danio* fishes. Development. 131:6053–6069.

Ruxton GD, Sherratt TN, Speed MP. 2004. Avoiding attack—the evolutionary ecology of crypsis, warning signals and mimicry. Oxford: Oxford University Press.

SAS Institute. 2002. SAS/STAT software user's guide. Rel. 9.1. Cary (NC): SAS Institute, Inc.

Schluter D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution. 50:1766–1774.

Sherbrooke WC, Frost SK. 1989. Integumental chromatophores of a color-change, thermoreulating lizard, *Phrynosoma modestum* (Iguanidae: Reptilia). Am Mus Novit. 2943:1–14.

Shine R, Olsson MM, LeMaster MP, Moore IT, Mason RT. 2000. Effects of sex, body size, and location on the antipredator tactics of free-ranging gartersnakes (*Thamnophis sirtalis*, Colubridae). Behav Ecol. 11:239–245.

Twitty VC. 1966. Of scientists and salamanders. San Francisco (CA): W.H. Freeman and Company.

Vitt LJ, Cooper WE. 1986. Tail loss, tail color, and predator escape in *Eumeces* (Lacertilia, Scincidae) age-specific differences in costs and benefits. Can J Zool. 64:583–592.

Westphal MF. 2007. On the evolution of correlated color traits in garter snakes. Unpublished discussion. Corvallis (OR): Oregon State University. p. 180.

Zaccaria RA. 1996. A laboratory classroom exercise: cell migration in cutaneous wound healing and pigmentary pattern formation in the red-spotted newt. Int J Dev. Biol. 40:897–899.

Zehr DR. 1962. Stages in the normal development of the common garter snake, *Thamnophis sirtalis sirtalis*. Copeia. 2:322–329.

Received November 22, 2009; Revised January 29, 2010; Accepted April 1, 2010

Corresponding Editor: Robin Waples