Short Note

Postautotomy tail movement differs between colour morphs of the red-backed salamander (*Plethodon cinereus*)

Banan W. Otaibi, Quincey K. Johnson, Bradley J. Cosentino*

Abstract. Striped and unstriped colour morphs of the eastern red-backed salamander, *Plethodon cinereus*, vary in their pre-attack behavioural response to predators, but it is unknown whether the morphs vary in post-attack strategies. Both morphs employ tail autotomy, a post-attack defensive mechanism enabling an individual to release a portion of their tail to facilitate escape from predation. Postautotomy tail movement diverts attention of a predator away from the individual's body, so natural selection should favor vigorous tail movement in both colour morphs of *P. cinereus*. We compared the degree of postautotomy tail movement between morphs following simulated predation. Striped individuals exhibited substantially longer and faster tail movement than unstriped individuals. Divergence in postautotomy tail movement may be a direct evolved response to variable predation pressure between colour morphs. Alternatively, tail movement may be constrained in the unstriped morph due to a genetic correlation with colouration (e.g., pleiotropy).

Keywords: amphibian, behaviour, evolution, forest, physiology, Plethodontidae, polymorphism, predation.

Colour polymorphism can strongly affect individual fitness by modulating predation risk, mate selection, and thermoregulation (e.g., Kettelwell, 1956; Endler, 1978; Ducharme, Larochelle and Richard, 1989; Gray and McKinnon, 2007). The eastern red-backed salamander (*Plethodon cinereus*) has two primary morphs (striped and unstriped) that are mainly distinguished by colour (Moore and Ouellet, 2014) but also differ in other behavioural and physiological traits (e.g., Moreno, 1989; Anthony, Venesky and Hickerson, 2008; Reiter, Anthony and Hickerson, 2014; Paluh et al., 2015; Smith, Johnson and Smith, 2015). The colour morphs are genetically based (Highton, 1959) and vary in frequency among populations (Moore and Ouellet, 2015). Unstriped individuals tend to be associated with warmer, drier climates than striped individuals (Lotter

and Scott, 1977; Gibbs and Karraker, 2006; Anthony and Pfingsten, 2013; Fisher-Reid et al., 2013; Fisher-Reid and Wiens, 2015), but morph frequencies vary among populations within climate zones (Moore and Ouellet, 2015), indicating that colouration is linked to additional factors that affect fitness.

Predation may be important for maintaining colour polymorphism in *P. cinereus* (Lotter and Scott, 1977; Moreno, 1989; Venesky and Anthony, 2007). Field data on the frequency of tail breakage suggest that unstriped individuals experience greater predation pressure than striped individuals (Moreno, 1989; Venesky and Anthony, 2007). Striped and unstriped individuals vary strongly in pre-attack antipredator strategies, but these differences did not affect the probability of attack by a predator in an experimental setting (Venesky and Anthony, 2007).

Differences in post-attack antipredator behaviours may contribute to variation in fitness between *P. cinereus* colour morphs. Initial post-attack defense mechanisms used by striped and

Department of Biology, Hobart and William Smith Colleges, Geneva, NY 14456, USA

*Corresponding author; e-mail: cosentino@hws.edu

unstriped individuals include flipping, thrashing, and biting (Arnold, 1982; Venesky and Anthony, 2007). Like many amphibians and reptiles, striped and unstriped *P. cinereus* also employ tail autotomy, a defensive mechanism in which an individual releases a portion of their tail to increase the probability of escape after an attack (Venesky and Anthony, 2007). Once severed, the autotomized tail thrashes and produces a glutinous secretion thought to distract the predator, allowing the salamander to escape (Congdon, Vitt and King, 1974; Vitt, Congdon and Dickson, 1977; Dial and Fitzpatrick, 1983; Labanick, 1984; Beneski, 1989). In lizards, the degree of postautotomy movement of the detached tail increases the probability of escaping predation because tail movement attracts the predator and increases handling time of the consumed tail (Dial and Fitzpatrick, 1983). Tail movement may also increase the spread of chemical alarm cues used by conspecifics (Hucko and Cupp, 2001). Our objective was to test whether postautotomy tail movement differs between colour morphs of *P. cinereus* in order to provide insight into the role of predation in maintaining colour polymorphism.

We sampled 20 striped and 20 unstriped salamanders at two sites separated by 2.5 km in the Finger Lakes National Forest in Hector, New York (42.438, −76.812; 42.458, −76.798; datum WGS 84). We searched for salamanders by turning logs and rocks on July 11-13, 2016 as part of a broader study on population genetic structure of *P. cinereus*. Only individuals with full tails and no evidence of tissue regeneration were collected.

Once captured, individuals were held by the trunk with a Kimwipe (Kimberly Clark Professional), and 0.5 cm of posterior tail tissue was cut off using Metzenbaum scissors. Cutting tail tissue with scissors may cause the magnitude of postautotomy tail movement to vary relative to alternative approaches (e.g., grasping the tail with tweezers), but our approach should not bias the comparison of tail movement between morphs because we used the same method for both morphs. Following removal of tail tissue, the individual was placed in a plastic container with a moistened paper towel until collection of tail movement data was completed. Tail tissue remained attached to the scissors throughout all trials due to glutinous tail secretions. We recorded two metrics of tail movement: 1) Tail movement time was recorded as the time from cutting the tail until cessation of tail movement, and 2) Initial tail velocity was quantified as the number of oscillations of tail tissue within 10 s after the tail was severed. One oscillation was defined as movement of the

tail from a starting position, across to the opposite side, then back to its starting position. We measured snout-vent length (SVL) prior to releasing the individual at the point of capture.

We used Welch's *t*-tests to compare mean tail movement time and initial velocity between striped and unstriped morphs. Welch's *t*-test was used because the variance in each response variable differed between morphs. Conclusions based on the Welch's *t*-tests were identical to standard *t*-tests on log-transformed data. We also used linear regression to determine if tail movement time and initial velocity differed between morphs when including SVL as a covariate. All statistical analyses were conducted in R v3.3 (R Core Team, 2016).

Mean tail movement time was significantly greater for striped than unstriped individuals (fig. 1A; Welch's *t*-test, $t = 8.90$, $df = 20.67$,
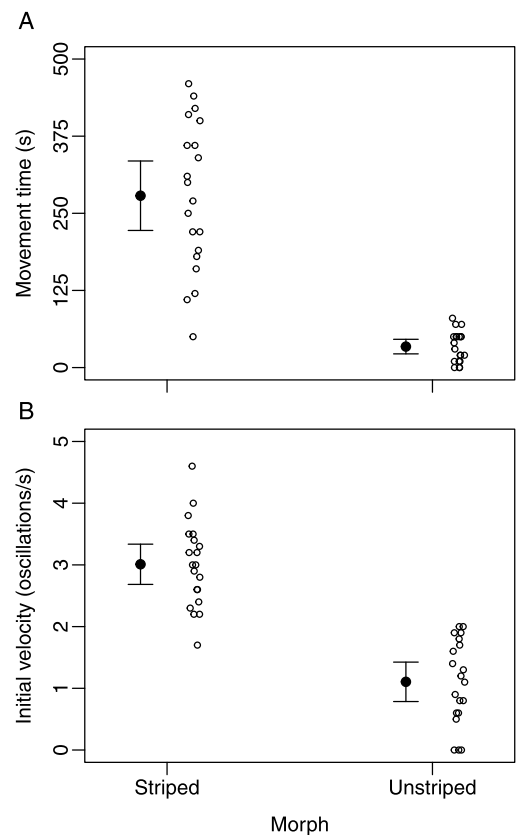

Figure 1. Total time of severed tail movement (A) and initial velocity (B) in striped and unstriped red-backed salamanders (*Plethodon cinereus*). Filled circles represent means, and error bars represent 95% confidence intervals. Unfilled circles represent individual observations ($N = 20$).

$P < 0.001$). Mean initial velocity was significantly greater for striped than unstriped individuals (fig. 1B; Welch's t-test, $t = 8.74$, df = 37.98, $P < 0.001$). We found the same pattern when examining the difference in initial velocity within the first 20 s and 30 s, indicating the results were not sensitive to choice of time interval ($P < 0.001$). Mean SVL was 3.76 cm (SD = 0.33 cm) for striped individuals and 3.46 cm (SD = 0.39 cm) for unstriped individuals. The differences in tail movement time and initial velocity between morphs were significant when including SVL as a covariate in linear regression (Movement time: $b = -251.80$, SE = 30.01, $P < 0.001$; Initial velocity: $b = -1.96$, SE = 0.24, $P < 0.001$). Tail movement time and initial velocity were not related to SVL (Movement time: $b = -24.74$, SE = 39.25, $P = 0.53$; Initial velocity: $b = -0.17$, SE = 0.31, $P = 0.58$), which indicates that differences in SVL between morphs cannot explain the results.

Our results corroborate earlier studies indicating that *P. cinereus* colour morphs differ in their antipredator strategies (Moreno, 1989; Venesky and Anthony, 2007). Striped individuals tend to remain immobile before being attacked (Venesky and Anthony, 2007) and have autotomized tails that move longer and more vigorously after being attacked (this study), whereas unstriped individuals are mobile and have automized tails that move very little. Differentiation in antipredator responses between colour morphs suggests that predation pressure (e.g., types and abundances of predators) may help explain observed spatial variation in colour polymorphism.

Colour morphs in a variety of taxa differ in behavioural and physiological traits (Forsman et al., 2008), and these correlations are often genetically based (McKinnon and Pierotti, 2010). One mechanism that can maintain colour morphology and other traits in linkage disequilibrium is negative frequency-dependent selection (Gray and McKinnon, 2007). Colouration provides a visual signal to predators, and

rare colour morphs often have greater survival rates because predators form a search image for common morphs. The unstriped morph of *P. cinereus* often occurs at lower abundance than the striped morph (Lotter and Scott, 1977; Moore and Ouellet, 2015), including in our study area (B.J. Cosentino, personal observation). If predators form a search image for the common, striped morph, then the unstriped morph may be subject to weaker selection from predation (Fitzpatrick, Shook and Izally, 2009), and energy required for vigorous postautotomy tail movement may be directed to other life history traits. In this case variation in colour pattern and postautotomy tail movement would be maintained by selection. However, high rates of tail breakage suggest the unstriped morph does experience significant predation pressure even in populations dominated by striped individuals (Moreno, 1989; Venesky and Anthony, 2007). Furthermore, vigorous movement of an autotomized tail should be favored by selection regardless of an individual's colour morph because tail autotomy is primarily employed after an individual is physically attacked.

Pleiotropy is an alternative mechanism that can maintain genetic correlations between colour patterns and other traits (Ducrest, Keller and Roulin, 2008; McKinnon and Pierotti, 2010). For example, the melanocortin receptor genes (*MC1R*) in Eleonora's falcon (*Falco eleonorae*) affect colouration and immune responses to pathogens (Gangoso et al., 2015). These pleiotropic effects can constrain selection for optimal trait combinations. In lizards, the primary physiological mechanism supporting postautotomy tail movement is anaerobic oxidation of glycogen into lactate, and lactate concentrations differ between species with varying levels of tail movement (Dial and Fitzpatrick, 1983). It is possible that alleles for colour variation in *P. cinereus* also influence

physiological pathways that affect postauto-tomy tail movement, such that unstriped individuals have limited potential for anaerobic oxidation of glycogen. Unstriped individuals have lower metabolic rate (Moreno, 1989; but see Petruzzi, Niewiarowski and Moore, 2006) and greater hematological stress index compared to striped individuals (Davis and Milanovich, 2010), which may constrain an individual's allocation of energy to secondary defense strategies. Suboptimal antipredator behaviour could explain why the unstriped morph experiences greater predation pressure than the striped morph. In this case, geographic variation in selection imposed by predation may be particularly important for maintaining variation in colour morph frequencies among populations.

References

Anthony, C.D., Pfingsten, R.A. (2013): Eastern red-backed salamander, *Plethodon cinereus*. In: Amphibians of Ohio. Pfingsten, R.A., Davis, J.G., Matson, T.O., Lipps, G.J., Wynn, D., Armitage, B.J., Eds, Ohio Biological Survey, Columbus.

Anthony, C.D., Venesky, M.D., Hickerson, C.-A.M. (2008): Ecological separation in a polymorphic terrestrial salamander. J. Anim. Ecol. **77**: 646-653.

Arnold, S.J. (1982): A quantitative approach to antipredator performance: salamander defense against snake attack. Copeia **1982**: 247-253.

Beneski, J.T., Jr. (1989): Adaptive significance of tail autotomy in the salamander, *Ensatina*. J. Herpetol. **23**: 322-324.

Congdon, J.D., Vitt, L.J., King, W.W. (1974): Geckos: adaptive significance and energetics of tail autotomy. Science **184**: 1379-1380.

Davis, A.K., Milanovich, J.R. (2010): Lead-phase and red-stripe color morphs of red-backed salamanders *Plethodon cinereus* differ in hematological stress indices: a consequence of differential predation pressure? Curr. Zool. **56**: 238-243.

Dial, B.E., Fitzpatrick, L.C. (1983): Lizard tail autotomy: function and energetics of postautotomy tail movement in *Scincella lateralis*. Science **219**: 391-393.

Ducharme, M.B., Larochelle, J., Richard, D. (1989): Thermogenic capacity in gray and black morphs of the gray squirrel, *Sciurus carolinensis*. Physiol. Ecol. **62**: 1273-1292.

Ducrest, A.-L., Keller, L., Roulin, A. (2008): Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trend Ecol. Evolut. **23**: 502-510.

Endler, J.A. (1978): A predator's view of animal color patterns. Evol. Biol. **11**: 319-364.

Fisher-Reid, M.C., Wiens, J.J. (2015): Is geographic variation within species related to macroevolutionary patterns between species? J. Evol. Biol. **28**: 1502-1515.

Fisher-Reid, M.C., Engstrom, T.N., Kuczynski, C.A., Stephens, P.R., Wiens, J.J. (2013): Parapatric divergence of sympatric morphs in a salamander: incipient speciation on Long Island? Mol. Ecol. **22**: 4681-4694.

Fitzpatrick, B.M., Shook, K., Izally, R. (2009): Frequency-dependent selection by wild birds promotes polymorphism in model salamanders. BMC Ecol. **9**: 12.

Forsman, A., Ahnesjö, J., Caesar, S., Karlsson, M. (2008): A model of ecological and evolutionary consequences of color polymorphism. Ecology **89**: 34-40.

Gangoso, L., Roulin, A., Ducrest, A.-L., Grande, J.M., Figuerola, J. (2015): Morph-specific genetic and environmental variation in innate and acquired response in a color polymorphic raptor. Oecologia **178**: 1113-1123.

Gibbs, J.P., Karraker, N.E. (2006): Effects of warming conditions in eastern North American forests on red-backed salamander morphology. Conserv. Biol. **20**: 913-917.

Gray, S.M., McKinnon, J.S. (2007): Linking color polymorphism maintenance and speciation. Trend Ecol. Evolut. **22**: 71-79.

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

Hucko, J.A., Cupp, P.V., Jr. (2001): Avoidance of chemical alarm cues released from autoomized tails of ravine salamanders (*Plethodon richmondi*). Ethology **107**: 315-322.

Kettelwell, H.B.D. (1956): A resume of the investigations of the evolution of melanism in the Lepidoptera. Proc. R. Soc. B **145**: 297-303.

Labanick, G.M. (1984): Anti-predator effectiveness of autotomized tails of the salamander *Desmognathus ochrophaeus*. Herpetologica **40**: 110-118.

Lotter, F., Scott, N.J., Jr. (1977): Correlation between climate and distribution of the color morphs of the salamander *Plethodon cinereus*. Copeia **1977**: 681-690.

McKinnon, J.S., Pierotti, M.E. (2010): Colour polymorphism and correlated characters: genetic mechanisms and evolution. Mol. Ecol. **19**: 5105-5125.

Moore, J.-D., Ouellet, M. (2014): A review of colour phenotypes of the eastern red-backed salamander, *Plethodon cinereus*, in North America. Can. Field-Nat. **128**: 250-259.

Moore, J.-D., Ouellet, M. (2015): Questioning the use of an amphibian color morph as an indicator of climate change. Global Change Biol. **21**: 566-571.

Moreno, G. (1989): Behavioral and physiological differentiation between the color morphs of the salamander, *Plethodon cinereus*. J. Herpetol. **3**: 335-341.

Paluh, D.J., Eddy, C., Ivanov, K., Hickerson, C.-A.M., Anthony, C.D. (2015): Selective foraging on ants by a terrestrial polymorphic salamander. Am. Midl. Nat. **174**: 265-277.

Petruzzi, E.E., Niewiarowski, P.H., Moore, F.B.-G. (2006): The role of thermal niche selection in maintenance of colour polymorphism in redback salamanders (*Plethodon cinereus*). Front. Zool. **3**: 10.

R Core Team (2016): R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reiter, M.K., Anthony, C.D., Hickerson, C.-A.M. (2014): Territorial behavior and ecological divergence in a polymorphic salamander. Copeia **3**: 481-488.

Smith, G.R., Johnson, T., Smith, W.O. (2015): Effects of colour morph and season on the dehydration and rehydration rates of *Plethodon cinereus*. Amphibia-Reptilia **36**: 170-174.

Venesky, M.D., Anthony, C.D. (2007): Antipredator adaptations and predator avoidance for two color morphs of the eastern red-backed salamander, *Plethodon cinereus*. Herpetologica **63**: 450-458.

Vitt, L.J., Congdon, J.D., Dickson, N.A. (1977): Adaptive strategies and energetics of tail autotomy in lizards. Ecology **58**: 326-337.