

Research



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Evolutionary biology

Balancing selection and drift in a polymorphic salamander metapopulation

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Understanding how genetic variation is maintained in a metapopulation is a longstanding problem in evolutionary biology. Historical resurveys of polymorphisms have offered efficient insights about evolutionary mechanisms, but are often conducted on single, large populations, neglecting the more comprehensive view afforded by considering all populations in a metapopulation. Here, we resurveyed a metapopulation of spotted salamanders (*Ambystoma maculatum*) to understand the evolutionary drivers of frequency variation in an egg mass colour polymorphism. We found that this metapopulation was demographically, phenotypically and environmentally stable over the last three decades. However, further analysis revealed evidence for two modes of evolution in this metapopulation—genetic drift and balancing selection. Although we cannot identify the balancing mechanism from these data, our findings present a clear view of contemporary evolution in colour morph frequency and demonstrate the importance of metapopulation-scale studies for capturing a broad range of evolutionary dynamics.

Introduction

The growing recognition that evolutionary processes can occur on contemporary timescales and microgeographic scales compels the need to understand how evolution affects ecology [1–3]. In particular, this realization suggests that ecological and evolutionary processes interact in determining trait distributions, species interactions, population dynamics and ecosystem processes [4,5]. Oftentimes, ecological patterns cannot be fully explained without recourse to underlying evolutionary dynamics [1,6–8].

Concurrently, historical resurveys have emerged as an invaluable approach to understanding ecology and evolution [9–12]. Resurveys can expose dominant ecological processes underlying variation in population abundances [13,14], community composition [15,16] and geographic range [17] and can reveal phenotypic and genetic shifts in wild populations [18–22]. Knowing how and why traits change can provide insights into the fate of wild populations facing rapid global change [23,24]. However, if relegated to single or large populations, historical surveys might be biased in their revelations about underlying evolutionary mechanisms, particularly those operating in small populations such as drift.

Here, we take advantage of a historical survey (1990–1991) to assess contemporary evolution in a spotted salamander (*Ambystoma maculatum*) metapopulation. The focal trait is a visible polymorphism in egg mass coloration (figure 1*a,b*). Because microevolution has been demonstrated in spotted salamander behaviour, morphology and physiology [25–31], we resurveyed this metapopulation to evaluate if morph frequency had evolved over three decades. Based on natural history and theory, we generated a series of *a priori*

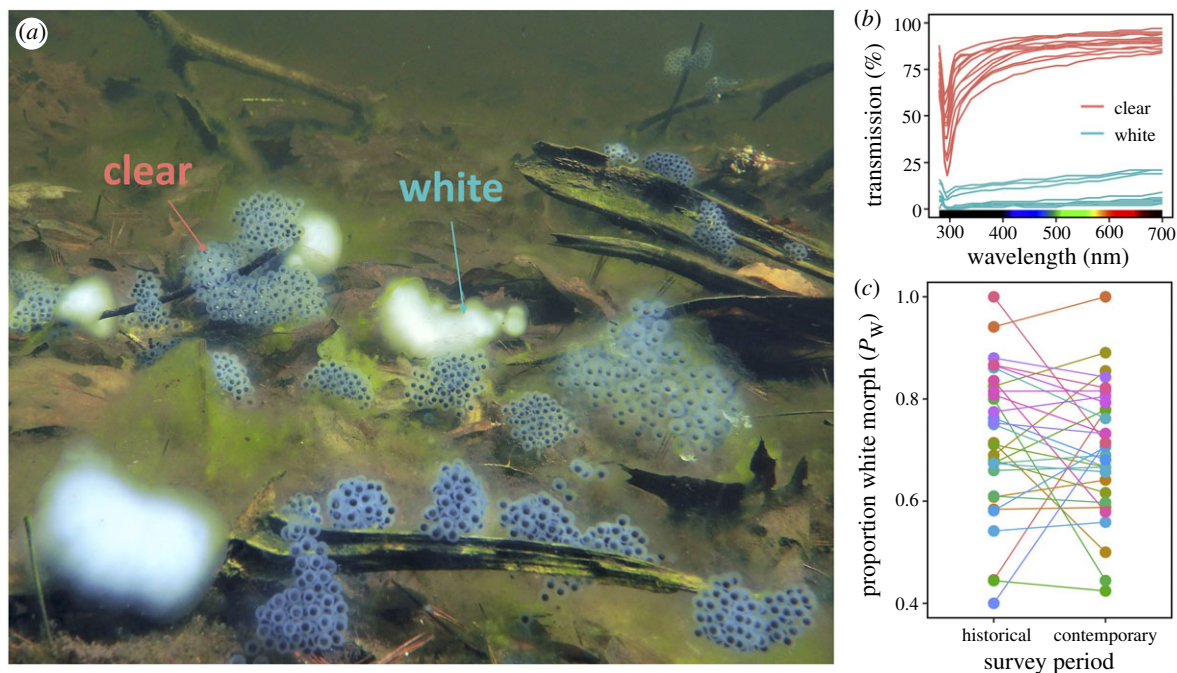


Figure 1. (a) Clear and white morphs of spotted salamander egg masses on the bottom of a temporary pond. (b) Colour variation is discrete, illustrated here by morph-specific transmission of incident light (280–700 nm) through 1 cm of jelly. (c) Morph frequency varied among populations and across time, exemplified by our resurvey of a spotted salamander metapopulation in central Pennsylvania. Each pair of coloured points linked by a line ($n = 31$) illustrates the varied direction and magnitude of population-level morph frequency change between surveys. Photo: Mark C. Urban.

predictions to identify possible modes of evolution: directional selection due to environmental change, balancing selection and genetic drift (table 1).

Methods

Study system

The focal metapopulation is located in 70 km² of broadleaf forest in central Pennsylvania, USA (electronic supplementary material, appendix SI). Although terrestrial as adults, spotted salamanders depend on small, isolated wetlands for reproduction [41]. Adult salamanders migrate to breeding ponds in early spring to mate and lay eggs. Each female oviposits one or more globular clusters called egg masses which include up to several hundred eggs encased in jelly (figure 1a) [42]. After several months, larvae metamorphose and leave breeding ponds [41].

Spotted salamander egg masses are dimorphic in coloration (figure 1a). The clear morph is transparent whereas egg jelly proteins in the white morph [43,44] strongly attenuate light, rendering it almost opaque (figure 1b). For example, at its centre a clear egg mass with a radius of 3 cm receives 68% of incident visible light (400–700 nm). A white egg mass of the same dimensions receives 0.06% (electronic supplementary material, appendix SII). Such striking phenotypic differences make morph classification highly repeatable across surveys and surveyors (intraclass $r = 0.96$; electronic supplementary material, appendix SIII).

Polymorphic and monomorphic populations are found range-wide [43–52]. What, if any, selective agents drive this spatial variation is unresolved [44,48,49,51–55]. The original study of this metapopulation by Ruth [56] suggested a causative link between cation concentration and morph frequency. But while pondwater chemistry appears to have consistent explanatory power [54,56], a mechanistic link with morph frequency remains elusive, as does strong support for competing hypotheses: predation, oxygen limitation and ultraviolet radiation [49,53,55]. Although not conclusive, coloration is likely under genetic control: ovisac anatomy varies by polymorphism [43],

individual females lay the same egg mass morph through time [44], and morph-specific protein profiles are conserved across 1600 km [43,56]. Further, the demonstration of complementary patterns of morph-specific mortality and microgeographic variation is consistent with evolution by natural selection [48].

Data collection and analysis

Ruth [56] documented egg polymorphisms and water chemistry in 34 ponds in Rothrock State Forest in central Pennsylvania. We relocated 31 of them from annotated maps [57] and resurveyed them in April 2020 following original methods (electronic supplementary material, appendix SI: table S1). Briefly, one observer (STG) slowly waded ponds, counting each morph. Prior to each survey, the observer measured pH with an Oakton PCTS 35 probe and collected water for dissolved organic carbon (DOC) analysis [58]. DOC and pH were used as proxies for cation concentrations and environmental change: DOC covaries with sodium ($r = -0.54$, $p < 0.05$), and pH covaries with calcium, potassium and magnesium ($r > 0.6$, $p < 0.05$; electronic supplementary material, appendix SIV).

To assess ecological change since initial surveys, we compared population size (egg mass counts), DOC and pH from the two time periods (historical: 1990–1991; contemporary: 2020). The effect of survey period (t) on population size and DOC was tested with a linear mixed model with random intercepts for ‘population’ effects. Prior to analysis of population size, egg mass counts and DOC data were log-transformed to meet model assumptions (Shapiro–Wilk’s $W = 0.98$, $p = 0.2187$; $W = 0.98$, $p = 0.2419$). Acidity data did not fit Gaussian, lognormal, Poisson or gamma distributions, so the difference between time periods was evaluated with a pairwise Wilcoxon rank-sum test.

We next examined predictions in table 1 and followed model structures articulated there. Unless otherwise noted, all analyses were conducted in R v. 4.0.3 [59] using quantreg [60] and lme4 [61]. All data can be accessed in Dryad [62].

To compare morph frequencies between historical and contemporary surveys, we used a binomial generalized linear mixed model to test for metapopulation-wide shift in the proportion of

Table 1. Hypothesized modes of evolution and associated predictions based on differential effects of survey period (t), environment (e), population size (N) and initial morph frequency (P_{W90}) on morph frequency in 2020 (P_{W20}). Linear models used to evaluate predictions are also formulated: * indicates a significant effect without a specified direction, + and – indicate positive and negative effects, respectively, and ‘n.s.’ indicates no effect.

hypothesis	prediction	survey period (t)	environment change (Δe)	population size (N)	resurvey white morph (P_{W90})	reference(s)
frequency-independent selection	metapopulation-wide shift in mean morph frequency	$P_W \sim t$	*			[32]
	correlated change in morph frequency and environment	$\Delta P_W \sim \Delta e$	*			
	change in morph frequency is greater in large populations	$\Delta P_W \sim N$		+		[33–35]
	change in morph frequency due to environmental change is greater in large populations	$\Delta P_W \sim \Delta e + N$	*	+		[33–35]
balancing selection	morph frequency increases if historically uncommon	$P_{W20} \sim P_{W90}$			+	[36–38]
	no metapopulation-wide shift in morph frequency	$P_W \sim t$	n.s.			[32]
	absolute difference in morph frequency is greater in small populations	$ \Delta P_W \sim N$		–		[38–40]

white (P_W) egg masses with survey period (t) as the fixed effect and random intercepts for each population. Unless specified, we used linear regression for the remaining analyses. Given overlapping generations and the potential for skipped reproductive years in spotted salamanders, we estimated population size as the mean number of egg masses (N) observed across all surveys [63]. To characterize the change in environmental variables (Δe) and morph frequency (ΔP_W), we subtracted each population’s historical values (P_{W90}) from contemporary ones (P_{W20}). To test for balancing selection, we assessed the relationship between morph frequency in the historical period with the contemporary period: $P_{W20} \sim P_{W90}$. In this format, a slope (b) of less than 1 indicates that a balancing mechanism such as negative frequency-dependent selection could be stabilizing morph frequencies across the metapopulation [36,37,64,65].

If drift is driving morph evolution, frequency variance should increase with increasing population size. We used quantile regression, which tracks how the variance distribution of the mean varies, to analyse how the variance of absolute changes in morph frequencies ($|\Delta P_W|$) changed with population size. We also compared observed morph frequencies with Monte Carlo simulations designed to demonstrate sample size effects on drift. We simulated drift for each population, parameterizing each with corresponding field-collected data (initial proportion of white egg masses: P_{W90} , and population size: N). Briefly, samples of size N were drawn from a binomial distribution with replacement for five generations. Initial binomial probabilities were set to P_{W90} . Subsequent generations were initialized with frequencies from preceding iterations to produce a random walk in time. Each sampling event was replicated 100 times per population and included five generations, assuming a 6-year generation time [66–68] (additional detail in electronic supplementary material, appendix SIV). To compare simulated and observed morph frequency change, we analysed the simulated data as we did for the observed data.

Results

Overall, the metapopulation was stable across three decades. No populations became extirpated naturally, and population sizes were similar between survey periods at a mean of 88 egg masses per pond ($F_{1,51.5} = 1.57$, $p = 0.287$). Metapopulation morph frequency was also similar between surveys ($P_{W90} = 0.707$, $P_{W20} = 0.701$; $F = 0.365$, $p = 0.546$). Finally, biogeochemistry did not vary over time (pH = 4.6, $p = 0.32$; DOC = 3.95 mg l⁻¹, $F_{1,56.7} = 0.422$, $p = 0.519$; electronic supplementary material, appendix SI: figure S1)

Although the aggregate view of the metapopulation revealed no net change, morph frequencies in some populations increased while others decreased (ΔP_W : mean = -0.02 , range -0.36 – 0.33 ; figure 1c). Population size (N) did not explain observed morph frequency changes ($F_{1,29} = 0.909$, $p = 0.348$), nor did environmental change (Δ pH: $F_{1,29} = 0.588$, $p = 0.449$; Δ DOC: $F_{1,29} = 0.666$, $p = 0.421$; electronic supplementary material, appendix SI: figure S2). Including population size (N) in environmental change models did not alter the results (Δ pH: $F_{2,28} = 0.005$, $p = 0.967$; Δ DOC: $F_{2,28} = 0.162$, $p = 0.691$).

However, as predicted by balancing selection, we found a slope significantly less than 1 for the positive relationship between historical and contemporary morph frequencies ($b = 0.39$, 95% CI 0.09–0.69, $F_{1,29} = 7.1$, $p = 0.013$; figure 2a). To control for a possible effect of population size, we ran the same model again with N as a covariate; the results were similar ($b = 0.40$, 95% CI 0.09–0.71, $F_{2,28} = 6.9$, $p = 0.014$).

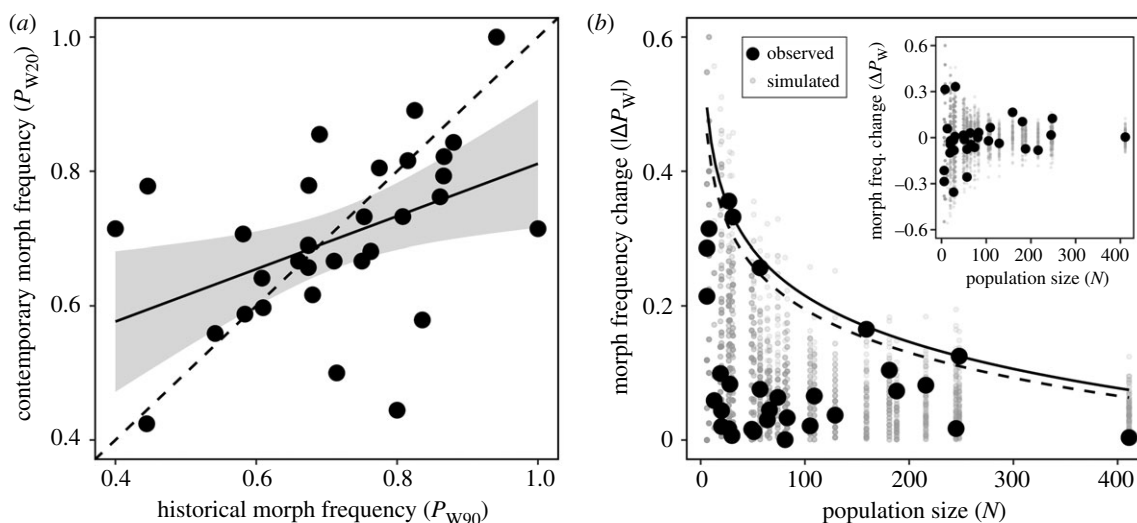


Figure 2. Evidence for multiple modes of contemporary evolution in a spotted salamander metapopulation. (a) A slope of less than 1 ($b = 0.39$) indicates a balancing mechanism such as negative frequency-dependent selection is stabilizing morph frequencies by driving high frequencies lower, and low frequencies higher. The dashed line indicates the 1:1 slope, where no change in frequency occurs between surveys. (b) There was no metapopulation-level difference in morph frequency between periods ($\Delta P_W = -0.017$ and -0.002 for observed and simulated data, respectively; inset). Using absolute change in morph frequency ($|\Delta P_W|$), quantile regression (95th quantile) fitted to observed data (solid line) indicates a population size effect driven by large-magnitude change in small populations. Observed data are a good qualitative match to a Monte Carlo simulation of evolution by drift alone; depicted here by quantile regression (95th quantile, dashed line) through the endpoints of simulated evolution by drift.

Also, as predicted by drift, we found that morph frequency change ($|\Delta P_W|$) was greater and more variable in small populations ($\tau = 0.95$, $t = -2.69$, $p = 0.0118$; figure 2b). Further supporting a role for drift, observed data fell within the 95% confidence intervals of the slope and intercept describing the 95th and 90th quantiles of the simulated drifting metapopulation (electronic supplementary material, appendix S1: table S2).

Discussion

We found no evidence of changes in metapopulation size, population size, biogeochemistry or morph frequencies between surveys. A closer inspection, guided by mechanistic predictions in table 1, indicated that genetic drift and balancing selection were ongoing during the three decades between surveys (figure 2). Our findings match a broad expectation that contemporary evolution is a multifarious process, in which the relative importance of drift and selection depends on population size [32,69–75].

This interplay is crucial for understanding the evolution and persistence of polymorphisms [38,76–79] and the conservation of small populations [32,80–82]. The observed, large-magnitude frequency change in small populations ($N < 100$) is consistent with contemporary evolution by drift—as our simulation demonstrates (figure 2b). However, our test for balancing selection (figure 2a) coupled with the persistence of the polymorphism in populations of all sizes suggests a broad influence of balancing mechanisms. Previous work suggested that spatial heterogeneity in selection could stabilize this polymorphism (e.g. [48,54]), but moderate-to-strong negative frequency-dependent selection could also overcome drift (electronic supplementary material, appendix SVI). For example, given that half of the populations in this metapopulation exceed 57, a selection differential greater than 0.018 would be required to stabilize the polymorphism in the majority of populations [83,84], which seems possible given

evidence for strong selection and evolutionary differences on other traits in this system [26–29]. Ultimately, our results provide support for one or more balancing mechanisms stabilizing morph frequency around an equilibrium frequency—presumably, the observed aggregate mean ($P_W \approx 0.7$; figure 2a). As a caveat, although our results are consistent with metapopulation-wide balancing mechanisms such as negative frequency-dependent selection, we rely solely on two time points spanning multiple generations. More robust inferences could be gained from data collected over shorter intervals.

To better understand this system, future work should confirm the genetic basis for jelly coloration, refine population size effects on drift–selection balance, experimentally probe potential balancing mechanisms [85,86], and examine the possibility that frequency variation can drive eco-evolutionary feedbacks [87–90]. Finally, although balancing selection could stabilize morph frequencies locally, the identity of the possible stabilizing selection on equilibrium morph frequency remains unknown. Given widespread evidence for clinal variation in polymorphism frequencies in other systems [91–93], regional differences in selection seem likely.

When combined with fine-scaled ecological data and replicate populations, historical resurveys can provide unique insights into evolutionary processes in nature. In this case, we leveraged metapopulation-scale resurveys to illustrate how contemporaneous drift and selection can drive microgeographic variation in a poorly understood polymorphism. Crucially, our ability to detect these dynamics depended on including the many small populations that constitute this metapopulation ($N < 100$ for most populations). Although populations of 1000 are often featured in studies (e.g. [51,68]), small populations are much more typical (e.g. [94]). For example, a survey of 405 breeding ponds (total $N = 29\,598$ egg masses) across Pennsylvania found that the median number of egg masses per pond was 33, with 78% of ponds having fewer than 100 (electronic supplementary material, appendix SVII). Given what we learned from our resurvey, drift is likely a significant evolutionary process in these

metapopulations, and for spotted salamanders in general. However, the metapopulation-wide influence of drift could be substantially under-represented if evolutionary dynamics are modelled on rare, large 'charismatic' populations.

Ethics. This research was permitted by the Pennsylvania Fish and Boat Commission (permit no. 2020-01-0102), the Pennsylvania Game Commission (permit no. 48892), and the Pennsylvania Department of Conservation and Natural Resources Bureau of Forestry (permit no. SFRA-2005). The Pennsylvania State University Institutional Animal Care and Use Committee does not regulate early stage, pre-hatching amphibian embryos. To minimize impacts to wild populations, we followed the American Society of Ichthyologists and Herpetologists guidelines for use of live amphibians in research, and disinfected field equipment between surveys to minimize the risk of spreading amphibian pathogens.

Data accessibility. Data are available in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.sf7m0cg5x> [62].

Authors' contributions. S.T.G. designed the study and collected the data. All authors made significant contributions to data analysis and writing, gave final approval for publication and agree to be held accountable for the work performed herein.

Competing interests. We declare we have no competing interests.

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References

- Hairston NG, Ellner SP, Geber MA, Yoshida T, Fox JA. 2005 Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* **8**, 1114–1127. (doi:10.1111/j.1461-0248.2005.00812.x)
- Kinnison MT, Hendry AP. 2001 The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* **112–113**, 145–164. (doi:10.1023/A:1013375419520)
- Schoener TW. 2011 The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* **331**, 426–429. (doi:10.1126/science.1193954)
- Hendry AP. 2017 *Eco-evolutionary dynamics*. Princeton, NJ: Princeton University Press.
- McPeck MA. 2017 *Evolutionary community ecology*. Princeton, NJ: Princeton University Press.
- Urban MC *et al.* 2020 Evolutionary origins for ecological patterns in space. *Proc. Natl Acad. Sci. USA* **117**, 17 482–17 490. (doi:10.1073/pnas.1918960117)
- Kinnison MT, Hairston Jr NG, Hendry AP. 2015 Cryptic eco-evolutionary dynamics. *Ann. N. Y. Acad. Sci.* **1360**, 120–144. (doi:10.1111/nyas.12974)
- Giery ST, Layman CA. 2019 Ecological consequences of sexually selected traits: an eco-evolutionary perspective. *Q. Rev. Biol.* **94**, 29–74. (doi:10.1086/702341)
- Tingley MW, Beissinger SR. 2009 Detecting range shifts from historical species occurrences: new perspectives on old data. *Trends Ecol. Evol.* **24**, 625–633. (doi:10.1016/j.tree.2009.05.009)
- Holmes MW *et al.* 2016 Natural history collections as windows on evolutionary processes. *Mol. Ecol.* **25**, 864–881. (doi:10.1111/mec.13529)
- Ozgo M, Liew T-S, Webster NB, Schilthuizen M. 2017 Inferring microevolution from museum collections and resampling: lessons learned from *Cepaea*. *PeerJ* **5**, e3938. (doi:10.7717/peerj.3938)
- Verheyen K *et al.* 2017 Combining biodiversity resurveys across regions to advance global change research. *BioScience* **67**, 73–83. (doi:10.1093/biosci/biw150)
- Skelly DK, Yurewicz KL, Werner EE, Relyea RA. 2003 Estimating decline and distributional change in amphibians. *Conserv. Biol.* **17**, 744–751. (doi:10.1046/j.1523-1739.2003.02009.x)
- Gibbs JP, Whiteleather KK, Schueler FW. 2005 Changes in frog and toad populations over 30 years in New York State. *Ecol. Appl.* **15**, 1148–1157. (doi:10.1890/03-5408)
- Moritz C, Patton JL, Conroy CJ, Parra JL, White GC, Beissinger SR. 2008 Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science* **322**, 261–264. (doi:10.1126/science.1163428)
- Stroud JT, Giery ST, Outerbridge M, Feeley KJ. 2019 Ecological character displacement alters the outcome of priority effects during community assembly. *Ecology* **100**, e02727. (doi:10.1002/ecy.2727)
- Freeman BG, Scholer MN, Ruiz-Gutierrez V, Fitzpatrick JW. 2018 Climate change causes upslope shifts and mountaintop extirpations in a tropical bird community. *Proc. Natl Acad. Sci. USA* **115**, 11 982–11 987. (doi:10.1073/pnas.1804224115)
- Zimova M, Giery ST, Newey S, Nowak JJ, Spencer M, Mills LS. 2020 Lack of phenological shift leads to increased camouflage mismatch in mountain hares. *Proc. R. Soc. B* **287**, 20201786. (doi:10.1098/rspb.2020.1786)
- Ozgo M, Schilthuizen M. 2012 Evolutionary change in *Cepaea nemoralis* shell colour over 43 years. *Glob. Change Biol.* **18**, 74–81. (doi:10.1111/j.1365-2486.2011.02514.x)
- Evans AE, Forester BR, Jockusch EL, Urban MC. 2018 Salamander morph frequencies do not evolve as predicted in response to 40 years of climate change. *Ecography* **41**, 1687–1697. (doi:10.1111/ecog.03588)
- DeLeo VL, Menge DNL, Hanks EM, Juenger TE, Lasky JR. 2020 Effects of two centuries of global environmental variation on phenology and physiology of *Arabidopsis thaliana*. *Glob. Change Biol.* **26**, 523–538. (doi:10.1111/gcb.14880)
- Des Roches S, Bell MA, Palkovacs EP. 2020 Climate-driven habitat change causes evolution in threespine stickleback. *Glob. Change Biol.* **26**, 597–606. (doi:10.1111/gcb.14892)
- Nadeau CP, Urban MC. 2019 Eco-evolution on the edge during climate change. *Ecography* **8**, ecog.04404. (doi:10.1111/ecog.04404)
- Pabijan M, Palomar G, Antunes B, Antol W, Zieliński P, Babik W. 2020 Evolutionary principles guiding amphibian conservation. *Evol. Appl.* **13**, 857–878. (doi:10.1111/eva.12940)
- Urban MC. 2007 Risky prey behavior evolves in risky habitats. *Proc. Natl Acad. Sci. USA* **104**, 14 377–14 382. (doi:10.1073/pnas.0704645104)
- Urban MC. 2008 Salamander evolution across a latitudinal cline in gape-limited predation risk. *Oikos* **117**, 1037–1049. (doi:10.1111/j.2008.0030-1299.16334.x)
- Urban MC. 2010 Microgeographic adaptations of spotted salamander morphological defenses in response to a predaceous salamander and beetle. *Oikos* **119**, 646–658. (doi:10.1111/j.1600-0706.2009.17970.x)
- Richardson JL, Urban MC. 2013 Strong selection barriers explain microgeographic adaptation in wild salamander populations. *Evolution* **67**, 1729–1740. (doi:10.1111/evo.12052)
- Urban MC, Richardson JL. 2015 The evolution of foraging rate across local and geographic gradients in predation risk and competition. *Am. Nat.* **186**, E16–E32. (doi:10.1086/681716)
- Giery ST, Drake DL, Urban MC. Submitted. Population density drives microgeographic evolution of metabolic physiology in a salamander metapopulation.
- Brady SP. 2012 Road to evolution? Local adaptation to road adjacency in an amphibian (*Ambystoma maculatum*). *Scient. Rep.* **2**, 235. (doi:10.1038/srep00235)
- Whitlock MC. 2004 Selection and drift in metapopulations. In *Ecology, genetics and evolution of metapopulations* (eds I Hanski, OE Gaggiotti), pp. 153–173. Burlington, MA: Elsevier Academic Press. (doi:10.1016/B978-012323448-3/50009-X)
- Willi Y, Van Buskirk J, Hoffmann AA. 2006 Limits to the adaptive potential of small populations. *Annu.*

- Rev. Ecol. Evol. Syst.* **37**, 433–458. (doi:10.1146/annurev.ecolsys.37.091305.110145)
34. Lanfear R, Kokko H, Eyre-Walker A. 2014 Population size and the rate of evolution. *Trends Ecol. Evol.* **29**, 33–41. (doi:10.1016/j.tree.2013.09.009)
 35. Hoffmann AA, Sgrò CM, Kristensen TN. 2017 Revisiting adaptive potential, population size, and conservation. *Trends Ecol. Evol.* **32**, 506–517. (doi:10.1016/j.tree.2017.03.012)
 36. Ayala FJ, Campbell C. 1974 Frequency-dependent selection. *Annu. Rev. Ecol. System.* **5**, 115–138. (doi:10.1146/annurev.es.05.110174.000555)
 37. Gromko MH. 1977 What is frequency-dependent selection? *Evolution* **31**, 438–442. (doi:10.1111/j.1558-5646.1977.tb01024.x)
 38. Wright S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97–159. (doi:10.1093/genetics/16.2.97)
 39. Lenormand T, Roze D, Rousset F. 2009 Stochasticity in evolution. *Trends Ecol. Evol.* **24**, 157–165. (doi:10.1016/j.tree.2008.09.014)
 40. Lande R. 1988 Genetics and demography in biological conservation. *Science* **241**, 1455–1460. (doi:10.1126/science.3420403)
 41. Petranka JW. 1998 *Salamanders of the United States and Canada*. Washington, DC: Smithsonian Institution Press.
 42. Salthé SN. 1963 The egg capsules in the Amphibia. *J. Morphol.* **113**, 161–171. (doi:10.1002/jmor.1051130204)
 43. Hardy LM, Lucas MC. 1991 A crystalline protein is responsible for dimorphic egg jellies in the spotted salamander, *Ambystoma maculatum* (Shaw) (Caudata: Ambystomatidae). *Comp. Biochem. Physiol. A Physiol.* **100**, 653–660. (doi:10.1016/0300-9629(91)90385-P)
 44. Ruth BC, Dunson WA, Rowe CL, Hedges SB. 1993 A molecular and functional evaluation of the egg mass color polymorphism of the spotted salamander, *Ambystoma maculatum*. *J. Herpetol.* **27**, 306–314. (doi:10.2307/1565152)
 45. Banta AM, Gortner RA. 1914 A milky white amphibian egg jelly. *Biol. Bull.* **27**, 259–261. (doi:10.2307/1536185)
 46. Brodman R. 1995 Annual variation in breeding success of two syntopic species of *Ambystoma* salamanders. *J. Herpetol.* **29**, 111–113. (doi:10.2307/1565093)
 47. Cunnington DC. 1998 Breeding site suitability and optimal egg size in the spotted salamander, *Ambystoma maculatum*. PhD thesis, The University of Guelph.
 48. Petranka JW, Rushlow AW, Hopey ME. 1998 Predation by tadpoles of *Rana sylvatica* on embryos of *Ambystoma maculatum*: implications of ecological role reversals by *Rana* (predator) and *Ambystoma* (prey). *Herpetologica* **54**, 1–13. (doi:10.1643/ce-04-037r1)
 49. Starnes SM, Kennedy CA, Petranka JW. 2000 Sensitivity of embryos of southern Appalachian amphibians to ambient solar UV-B radiation. *Conserv. Biol.* **14**, 277–282. (doi:10.1046/j.1523-1739.2000.98596.x)
 50. Shearin AF. 2012 Influence of landscape arrangement and wetland condition on breeding dynamics of *Ambystoma maculatum* (spotted salamander) in Maine, USA. PhD thesis, University of Maine.
 51. Urban MC, Lewis LA, Fučíková K, Cordone A. 2015 Population of origin and environment interact to determine oomycete infections in spotted salamander populations. *Oikos* **124**, 274–284. (doi:10.1111/oik.01598)
 52. Hale RE, Kennedy C, Winkelman D, Brown C. 2017 An advantage of clear over white egg mass morphs in metabolically demanding microhabitats suggests a role of symbiotic algae in the maintenance of a polymorphism in the spotted salamander (*Ambystoma maculatum*). *Evol. Ecol. Res.* **18**, 637–650.
 53. Rowe CL, Sadinski WJ, Dunson WA. 1994 Predation on larval and embryonic amphibians by acid-tolerant caddisfly larvae (*Ptilostomis postica*). *J. Herpetol.* **28**, 357–364. (doi:10.2307/1564535)
 54. Pintar MR, Resetarits WJ. 2017 Persistence of an egg mass polymorphism in *Ambystoma maculatum*: differential performance under high and low nutrients. *Ecology* **98**, 1349–1360. (doi:10.1002/ecy.1789)
 55. D'Errico M, Kennedy C, Hale RE. 2020 Egg mass polymorphism in *Ambystoma maculatum* is not associated with larval performance or survival, or with cell density of the algal symbiont *Oophila amblystomatis*. *Evol. Ecol.* **34**, 981–997. (doi:10.1007/s10682-020-10083-9)
 56. Ruth BC. 1992 Possible adaptive significance of the visible polymorphism of spotted salamander egg masses. Master's thesis, The Pennsylvania State University.
 57. Sadinski WJ. 1991 Direct and indirect effects of low pH on the communities of temporary ponds. PhD thesis, The Pennsylvania State University.
 58. Giery ST, Layman CA. 2017 Dissolved organic carbon and unimodal variation in sexual signal coloration in mosquitofish: a role for light limitation? *Proc. R. Soc. B* **284**, 20170163. (doi:10.1098/rspb.2017.0163)
 59. R Core Team. 2020 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <https://www.R-project.org/>.
 60. Koenker R. 2020 *quantreg: Quantile Regression. R package version 5.61*. See <http://CRAN.R-project.org/package=quantreg>.
 61. Bates D, Mächler M, Bolker BM, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48. (doi:10.18637/jss.v067.i01)
 62. Giery ST, Zimova M, Drake DL, Urban MC. 2021 Data from: Balancing selection and drift in a polymorphic salamander metapopulation. Dryad Digital Repository. (doi:10.5061/dryad.sf7m0cg5x)
 63. Vucetich JA, Waite TA, Nunney L. 1997 Fluctuating population size and the ratio of effective to census population size. *Evolution* **51**, 2017–2021. (doi:10.1111/j.1558-5646.1997.tb05123.x)
 64. Ayala FJ. 1972 Competition between species: the diversity of environments in which most organisms live permits the coexistence of many species, even when they compete for the same resources. *Am. Scient.* **60**, 348–357.
 65. Knoppien P. 1985 Rare male mating advantage: a review. *Biol. Rev.* **60**, 81–117. (doi:10.1111/j.1469-185X.1985.tb00418.x)
 66. Whiteley AR, McGarigal K, Schwartz MK. 2014 Pronounced differences in genetic structure despite overall ecological similarity for two *Ambystoma* salamanders in the same landscape. *Conserv. Genet.* **15**, 573–591. (doi:10.1007/s10592-014-0562-7)
 67. Semlitsch RD, Anderson TL. 2016 Structure and dynamics of spotted salamander (*Ambystoma maculatum*) populations in Missouri. *Herpetologica* **72**, 81–89. (doi:10.1655/herpetologica-d-15-00049)
 68. Homan RN, Holgerson MA, Biga LM. 2018 A long-term demographic study of a spotted salamander (*Ambystoma maculatum*) population in central Ohio. *Herpetologica* **74**, 109–116. (doi:10.1655/herpetologica-d-17-00067.1)
 69. Clegg SM, Degnan SM, Moritz C, Estoup A, Kikkawa J, Owens IPF. 2002 Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird. *Evolution* **56**, 2090–2099. (doi:10.1111/j.0014-3820.2002.tb00134.x)
 70. O'Hara RB. 2005 Comparing the effects of genetic drift and fluctuating selection on genotype frequency changes in the scarlet tiger moth. *Proc. R. Soc. B* **272**, 211–217. (doi:10.1098/rspb.2004.2929)
 71. Yeaman S, Otto SP. 2011 Establishment and maintenance of adaptive genetic divergence under migration, selection, and drift. *Evolution* **65**, 2123–2129. (doi:10.1111/j.1558-5646.2011.01277.x)
 72. Grueber CE, Wallis GP, Jamieson IG. 2013 Genetic drift outweighs natural selection at toll-like receptor (TLR) immunity loci in a re-introduced population of a threatened species. *Mol. Ecol.* **22**, 4470–4482. (doi:10.1111/mec.12404)
 73. Funk WC *et al.* 2016 Adaptive divergence despite strong genetic drift: genomic analysis of the evolutionary mechanisms causing genetic differentiation in the island fox (*Urocyon littoralis*). *Mol. Ecol.* **25**, 2176–2194. (doi:10.1111/mec.13605)
 74. Johnson MTJ, Prashad CM, Lavoignat M, Saini HS. 2018 Contrasting the effects of natural selection, genetic drift and gene flow on urban evolution in white clover (*Trifolium repens*). *Proc. R. Soc. B* **285**, 20181019. (doi:10.1098/rspb.2018.1019)
 75. Husband BC, Barrett SCH. 1992 Effective population size and genetic drift in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* **46**, 1875–1890. (doi:10.1111/j.1558-5646.1992.tb01175.x)
 76. Ford EB. 1945 Polymorphism. *Biol. Rev.* **20**, 73–88. (doi:10.1111/j.1469-185X.1945.tb00315.x)
 77. Hedrick PW. 1986 Genetic polymorphism in heterogeneous environments: a decade later. *Annu.*

- Rev. Ecol. Syst.* **17**, 535–566. (doi:10.1146/annurev.es.17.110186.002535)
78. Hedrick PW. 2006 Genetic polymorphism in heterogeneous environments: the age of genomics. *Annu. Rev. Ecol. Syst.* **37**, 67–93. (doi:10.1146/annurev.ecolsys.37.091305.110132)
79. Mitchell-Olds T, Willis JH, Goldstein DB. 2007 Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nat. Rev. Genet.* **8**, 845–856. (doi:10.1038/nrg2207)
80. Franklin IR. 1980 Evolutionary change in small populations. In *Conservation biology: an evolutionary-ecological perspective* (eds ME Soulé, BA Wilcox), pp. 135–149. Sunderland, Mass: Sinauer.
81. Caughley G. 1994 Directions in conservation biology. *J. Anim. Ecol.* **63**, 215–244. (doi:10.2307/5542)
82. Gaggiotti OE, Hanski I. 2004 Mechanisms of population extinction. In *Ecology, genetics and evolution of metapopulations* (eds I Hanski, OE Gaggiotti), pp. 337–366. Burlington, MA: Elsevier Academic Press. (doi:10.1016/B978-012323448-3/50016-7)
83. Lowe WH, Kovach RP, Allendorf FW. 2017 Population genetics and demography unite ecology and evolution. *Trends Ecol. Evol.* **32**, 141–152. (doi:10.1016/j.tree.2016.12.002)
84. Li W-H. 1978 Maintenance of genetic variability under the joint effect of mutation, selection and random drift. *Genetics* **90**, 349–382.
85. Svensson EI. 2017 Back to basics: using colour polymorphisms to study evolutionary processes. *Mol. Ecol.* **26**, 2204–2211. (doi:10.1111/mec.14025)
86. McKinnon JS, Pierotti ME. 2010 Colour polymorphism and correlated characters: genetic mechanisms and evolution. *Mol. Ecol.* **19**, 5101–5125. (doi:10.1111/j.1365-294X.2010.04846.x)
87. Svensson EI, Connallon T. 2019 How frequency-dependent selection affects population fitness, maladaptation and evolutionary rescue. *Evol. Appl.* **12**, 1243–1258. (doi:10.1111/eva.12714)
88. Forsman A. 2016 Is colour polymorphism advantageous to populations and species? *Mol. Ecol.* **25**, 2693–2698. (doi:10.1111/mec.13629)
89. Mills LS *et al.* 2018 Winter color polymorphisms identify global hot spots for evolutionary rescue from climate change. *Science* **359**, 1033–1036. (doi:10.1126/science.aan8097)
90. Forsman A, Ahnesjö J, Caesar S, Karlsson M. 2008 A model of ecological and evolutionary consequences of color polymorphism. *Ecology* **89**, 34–40. (doi:10.1890/07-0572.1)
91. Hantak MM, Page RB, Converse PE, Anthony CD, Hickerson CM, Kuchta SR. 2019 Do genetic structure and landscape heterogeneity impact color morph frequency in a polymorphic salamander? *Ecography* **42**, 1383–1394. (doi:10.1111/ecog.04534)
92. Matthews G, Goulet CT, Delhey K, Atkins ZS, While GM, Gardner MG, Chapple DG. 2018 Avian predation intensity as a driver of clinal variation in colour morph frequency. *J. Anim. Ecol.* **87**, 1667–1684. (doi:10.1111/1365-2656.12894)
93. McLean CA, Stuart-Fox D. 2014 Geographic variation in animal colour polymorphisms and its role in speciation. *Biol. Rev. Camb. Phil. Soc.* **89**, 860–873. (doi:10.1111/brv.12083)
94. Egan RS, Paton PWC. 2004 Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. *Wetlands* **24**, 1–13. (doi:10.1672/0277-5212(2004)024[0001:WPA0BW]2.0.CO;2)