Geography is more important than life history in the recent diversification of the tiger salamander complex

Kathryn M. Everson[a,1], Levi N. Gray[a,1], Angela G. Jones[a], Nicolette M. Lawrence[a], Mary E. Foley[a], Kelly L. Sovacool[a,b], Justin D. Kratovil[a,c], Scott Hotaling[a,d], Paul M. Hime[a,e], Andrew Storfer[d], Gabriela Parra-Olea[f], Ruth Percino-Daniel[g], X. Aguilar-Miguel[h], Eric M. O'Neill[a], Luis Zambrano[f], H. Bradley Shaffer[i], and David W. Weisrock[a,2]

[a]Department of Biology, University of Kentucky, Lexington, KY 40506; [b]Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI 48109; [c]Ecology and Evolutionary Biology Department, University of Connecticut, Storrs, CT 06269; [d]School of Biological Sciences, Washington State University, Pullman, WA 99164; [e]Biodiversity Institute, University of Kansas, Lawrence, KS 66045; [f]Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México 04510, Mexico; [g]Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México 04510, Mexico; [h]Centro de Investigación en Recursos Bióticos, Facultad de Ciencias, Universidad Autónoma del Estado de México, 50000 Toluca, Mexico; and [i]Department of Ecology and Evolutionary Biology, La Kretz Center for California Conservation Science, University of California, Los Angeles, CA 90095

Edited by Nils Chr. Stenseth, University of Oslo, Oslo, Norway, and approved March 18, 2021 (received for review July 14, 2020)

The North American tiger salamander species complex, including its best-known species, the Mexican axolotl, has long been a source of biological fascination. The complex exhibits a wide range of variation in developmental life history strategies, including populations and individuals that undergo metamorphosis; those able to forego metamorphosis and retain a larval, aquatic lifestyle (i.e., paedomorphosis); and those that do both. The evolution of a paedomorphic life history state is thought to lead to increased population genetic differentiation and ultimately reproductive isolation and speciation, but the degree to which it has shaped population- and species-level divergence is poorly understood. Using a large multilocus dataset from hundreds of samples across North America, we identified genetic clusters across the geographic range of the tiger salamander complex. These clusters often contain a mixture of paedomorphic and metamorphic taxa, indicating that geographic isolation has played a larger role in lineage divergence than paedomorphosis in this system. This conclusion is bolstered by geography-informed analyses indicating no effect of life history strategy on population genetic differentiation and by model-based population genetic analyses demonstrating gene flow between adjacent metamorphic and paedomorphic populations. This fine-scale genetic perspective on life history variation establishes a framework for understanding how plasticity, local adaptation, and gene flow contribute to lineage divergence. Many members of the tiger salamander complex are endangered, and the Mexican axolotl is an important model system in regenerative and biomedical research. Our results chart a course for more informed use of these taxa in experimental, ecological, and conservation research.

life history | phylogenetics | salamanders | population genomics | *Ambystoma*

Life history—the complement of traits affecting survival and reproduction over an organism's lifetime—affects ecology and dispersal and therefore plays a potentially important role in shaping population structure and speciation (1, 2). The Mexican axolotl (*Ambystoma mexicanum*; hereafter, "axolotl") and related salamander species of the North American *Ambystoma tigrinum* complex (Table 1) display enormous life history variation, particularly with respect to the completion of metamorphosis (3). Some species are considered obligate paedomorphs, in which sexually mature adults retain a larval, aquatic body plan that includes external gills and an enlarged tail fin (4). Others are considered obligate metamorphs, transforming from aquatic larvae to terrestrial juveniles, eventually returning to the water to breed as adults. Most populations are facultatively paedomorphic, transforming under certain genetic and/or environmental conditions (5–8). This lability in life history is believed to have played an important role in the diversification of the *A. tigrinum* complex, particularly in the Trans-Mexican Volcanic Belt (TMVB) region, where several paedomorphic species, including the axolotl, are currently recognized (9–11). Obligate paedomorphosis is estimated to have evolved in this region multiple times (11–13) in association with relatively large, permanent bodies of water. Presumably, restricted gene flow between these isolated, paedomorphic populations led to speciation as well as morphological adaptations to the aquatic lifestyle (14).

The term "species complex," while not a formal taxonomic category, is often used to describe groups of closely related lineages, sometimes arising through a burst of diversification. The tiger salamander species complex has been highlighted as a potentially valuable example of such a recent and rapid radiation (15) that could provide insight into the early mechanisms initiating and/or maintaining diversity (16–21). However, species complexes pose a number of challenges: phenotypic differences among lineages may be subtle or absent (i.e., cryptic species), ancestral polymorphisms may lead to incomplete lineage sorting that confounds molecular systematic studies, and reproductive barriers may be

Significance

Population structure and speciation are shaped by a combination of biotic and abiotic factors. The tiger salamander complex has been considered a key group in which life history variation has led to a rapid rate of speciation, driven in large part by the evolution of obligate paedomorphosis—a condition in which adults maintain an aquatic, larval phenotype. Using a large multilocus dataset, we present evidence of gene flow between taxa with different life history strategies, suggesting that obligate paedomorphosis is not a strong driver of speciation in the tiger salamander complex. Many of these nominal taxa are listed as critically endangered, and our genetic results provide information and guidance that will be useful for their conservation.

Author contributions: K.M.E., L.N.G., A.G.J., K.L.S., and D.W.W. designed research; K.M.E., L.N.G., A.G.J., N.M.L., M.E.F., K.L.S., J.D.K., S.H., P.M.H., R.P.-D., X.A.-M., E.M.O., L.Z., and D.W.W. performed research; A.G.J., K.L.S., A.S., and G.P.-O. contributed new reagents/analytic tools; K.M.E., L.N.G., A.G.J., N.M.L., M.E.F., K.L.S., J.D.K., S.H., P.M.H., H.B.S., and D.W.W. analyzed data; K.M.E., L.N.G., R.P.-D., H.B.S., and D.W.W. wrote the paper; and A.S., G.P.-O., X.A.-M., L.Z., H.B.S., and D.W.W. contributed samples.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

[1]K.M.E. and L.N.G. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: david.weisrock@uky.edu.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2014719118/-/DCSupplemental.

Published April 22, 2021.

Table 1. Currently recognized species in the *A. tigrinum* complex

Species	Habitat	Status	Life history
A. altamirani Dugès, 1895	Streams, occasional ponds	E	2
A. amblycephalum Taylor, 1940	Ponds	CE	2
A. andersoni Krebs & Brandon, 1984	Lakes/ponds	CE	5
A. bombypellum Taylor, 1940	Lakes/ponds, occasional streams	DD	2
A. californiense Gray 1853	Vernal pools	V	1
A. dumerilii Dugès, 1870	Lakes/ponds	CE	5
A. flavipiperatum Dixon, 1963	Lakes/ponds, occasional streams	E	3
A. granulosum Taylor, 1944	Lakes/ponds, occasional streams	CE	3
A. leorae Taylor, 1943	Streams	CE	2
A. lermaense Taylor, 1940	Lakes/ponds	E	3
A. mavortium Baird, 1850	Lakes/ponds	LC	3
A. mexicanum Shaw & Nodder, 1798	Lakes	CE	4
A. ordinarium Taylor, 1940	Streams	E	3
A. rivulare Taylor, 1940	Streams	E	2
A. rosaceum Taylor, 1941	Streams	LC	3
A. silvense Webb, 2004	Lakes/ponds	DD	3
A. taylori Brandon et al., 1982	Saline lake	CE	4
A. tigrinum Green, 1825	Lakes/ponds	LC	2
A. velasci Dugès, 1888	Lakes/ponds, streams	LC	3

Habitat preference, conservation status ("Status"), and life history strategy were compiled from AmphibiaWeb (2019) and the IUCN (2019). Conservation status abbreviations are the following: LC = least concern, V = vulnerable, E = endangered, CE = critically endangered, and DD = data deficient. Life history codes are the following: 1 = obligate metamorph (no paedomorphs documented in the field), 2 = strong bias toward metamorphosis with rare paedomorphs found in the field, 3 = both metamorphosis and paedomorphosis common in the field, 4 = strong bias toward paedomorphosis with rare metamorphs found in the field, and 5 = obligate paedomorph (no metamorphs documented in the field). In the text, "paedomorph" describes taxa scored as 4 or 5, while "facultative paedomorph" describes taxa scored as 2 or 3. For a full description of the geographic ranges of each species, see *SI Appendix*, Table S5.

incomplete (22–24). The reliance on phenotype for species diagnosis in the *A. tigrinum* complex may require particular scrutiny, as several phenotypic traits, including developmental state and adult color pattern, can be plastic and highly variable (25–27). Given these challenges, fundamental questions remain concerning the reality of component lineages within the tiger salamander complex, calling into question the current taxonomy as an accurate reflection of its underlying evolutionary history.

Previous research has produced mixed results regarding levels of genetic differentiation among lineages in the *A. tigrinum* complex, as well as the relative importance of paedomorphosis as a driver of diversification. While population- and species-level structure is evident, an important theme emerging across multiple studies has been that reproductive barriers are porous (11, 24, 28). Shaffer and McKnight (11) noted "the striking lack of differentiation among the 14 species of the tiger salamander complex" (p. 425). Some studies have found an elevated degree of population structure among paedomorphic populations relative to metamorphic populations (12, 29). However, others have shown a lack of effect of paedomorphosis on population genetic differentiation (30) and demonstrated that paedomorphic taxa and neighboring metamorphic populations interbreed (31). Furthermore, crosses between metamorphic and paedomorphic taxa can produce viable hybrid offspring under laboratory conditions (32, 33).

To understand the processes underlying diversification in the tiger salamander complex, an important first step must be a range-wide assessment of population structure and the clarification of population- and species-level boundaries. With this groundwork,

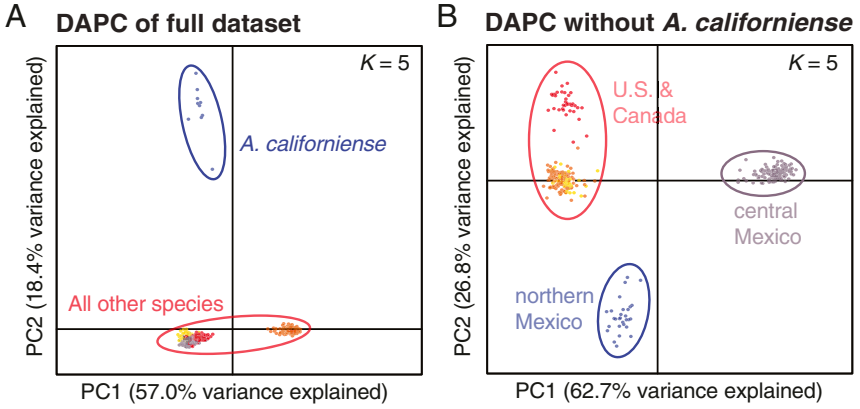

Fig. 1. DAPC on (*A*) the entire genomic dataset (without *A. opacum* and *A. texanum* outgroups) and (*B*) the genomic dataset without *A. californiense*. In both analyses, patterns of genetic differentiation stabilized at values of $K \geq 5$ ($K = 5$ is shown in both plots, with colors representing genetic clusters). Points represent individuals and ellipses show the groups identified by DAPC. The first and second principal components from the DAPC are on the *x* and *y* axes, respectively. Results from additional values of *K* are provided in *SI Appendix*, Figs. S3 and S4.

insights into the role of paedomorphosis and diversification can be addressed. For a study system of this geographic and taxonomic scale, robust inference of population structure requires information from multiple genes combined with thorough range-wide sampling. To meet these criteria, we expand on a large multilocus dataset containing 95 nuclear loci for 93 individuals (34) to produce a data matrix for 347 individuals across the full geographic range of tiger salamanders (*SI Appendix*, Fig. S1). Given the complex and somewhat checkered history of the group's taxonomy, we focus on a naïve approach, performing population structure analyses without a priori identification of taxa to resolve geographic genetic clusters and characterize patterns of admixture. We also overlay these results on the existing taxonomy to explore the correspondence between current taxonomy and genetic differentiation. We then use these data for a phylogenetic analysis to provide an updated working hypothesis for the evolution of the group. Finally, we test the hypothesis that life history evolution has driven speciation in the tiger salamander complex, particularly through the assumed isolation of paedomorphic species. In light of the life history variation in this species complex, we use natural history information to place taxa into one of five categories (Table 1) reflecting their propensity to metamorphose, then use model-based tests

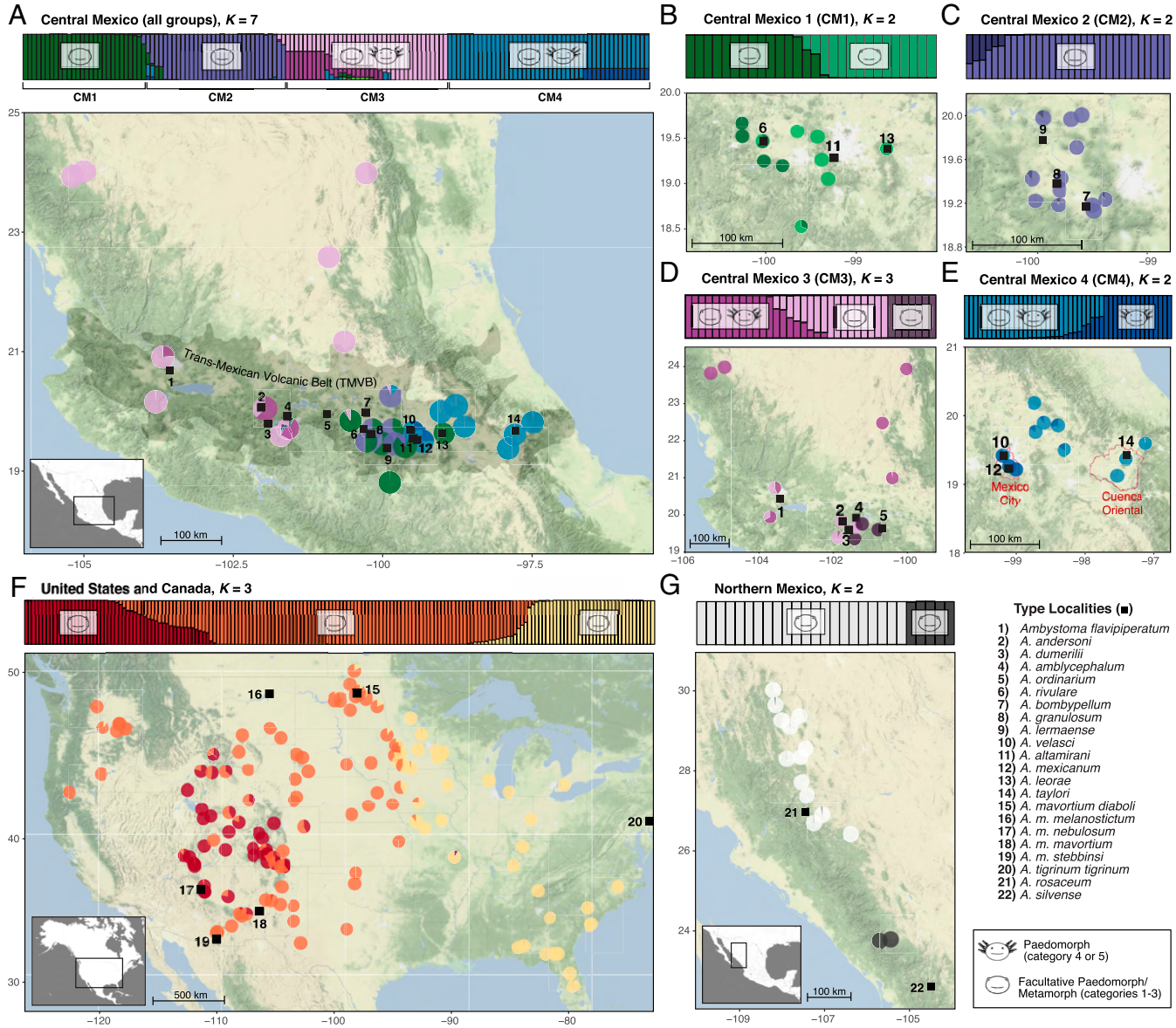

Fig. 2. Population genomic analyses of the *A. tigrinum* species complex. (*A*) STRUCTURE plot of central Mexico samples. The ref. 91 method identified $K = 7$, albeit with some admixture among groups. However, four major geographic genetic groups are apparent and were analyzed separately in subsequent analyses (*B*–*E*). In each plot, vertical bars represent individuals, while the *y* axis shows the membership probability for each group. Pie charts mapped below the assignment plot show the average group membership probability for that locality. Some localities were combined to a single pie chart to reduce visual clutter; see *SI Appendix*, Table S1 for specific coordinates of each sample. The range of the TMVB is identified by shading, data from ref. 107. The range of the Cuenca Oriental is outlined in red, data from ref. 31. Two geographic outliers placed in CM2 are thought to represent range introductions and are not shown here but are discussed in supplementary text and shown in *SI Appendix*, Fig. S9. (*F* and *G*) STRUCTURE results from analyses of the US and northern Mexico groups, respectively. Detailed results are shown in *SI Appendix*, Figs. S11 and S12. Black squares on the maps denote the type localities of all species included in this study. On each STRUCTURE membership plot, salamander face symbols denote the presence of salamanders with a life history category of 1 to 3 (the cartoon lacks gills) or 4 to 5 (paedomorphic, cartoon shows gills); groups labeled with both symbols contain a mixture of these life history categories.

of migration to ask whether paedomorphic populations (categories 4 and 5) exhibit greater levels of genetic differentiation relative to those that regularly metamorphose (categories 1 through 3).

Results and Discussion

Identification of Genetic Lineages and Phylogenetic Relationships. A principal component analysis (PCA) and a discriminant analysis of principal components (DAPC) (35) both recovered *Ambystoma californiense* Gray 1853 as genetically distinct from all other tiger salamanders (Fig. 1*A* and *SI Appendix*, Figs. S2 and S3), consistent with previous work (e.g., refs. 11, 36). Given that *A. californiense* is the only obligate metamorph in the complex (life history category 1, Table 1) and is genetically divergent and geographically isolated from all other tiger salamanders evaluated in this study, we focus the remaining analyses on the other members of the complex. After removing *A. californiense* from the dataset, DAPC supported the recognition of three primary genetic clusters: US (including samples from southern Canada), northwestern Mexico (primarily the Sierra Madre Occidental), and the central Mexican highlands (Fig. 1*B* and *SI Appendix*, Fig. S4). A PCA of these data produced similar clustering results, with ordination patterns largely mirroring geographic sampling (*SI Appendix*, Fig. S5). For a further discussion of the taxonomic implications of our results, see *SI Appendix*, *Supplementary Text*.

Within central Mexico, we identified $K = 7$ as the best-fit number of clusters in both DAPC and STRUCTURE analyses (Fig. 2*A* and *SI Appendix*, Fig. S6). However, several of these clusters were only represented as a small proportion of genetic assignments, rather than as meaningful groups of individuals, and visual inspection at multiple levels of K revealed four clear geographic clusters (hereafter referred to as CM1 through CM4) that captured the primary patterns of genetic differentiation and admixture across this region (Fig. 2*A* and *SI Appendix*, Figs. S7 and S8). Network analysis (37) also recovered groups that generally correspond to CM1 through CM4 but with evidence of many reticulations (Fig. 3). This starburst-like pattern indicates the presence of conflicting topologies within the dataset (37), which can be caused by biological processes including recent diversification with incomplete lineage sorting, current or evolutionarily recent gene flow, or both. Phylogenetic analyses (38–40) recovered monophyletic CM1, CM2, and CM4 clusters (see below for discussion of CM3) but often with low statistical support (Fig. 3).

Based on type locality and range information, the CM1 cluster includes three stream-breeding and facultatively paedomorphic taxa: *Ambystoma altamirani* Dugès 1895 (41), *Ambystoma leorae* Taylor 1943 (42), and *Ambystoma rivulare* Taylor 1940 (9). Subsequent analyses of this cluster identified two admixed populations associated with eastern and western portions of its geographic distribution (Fig. 2*B* and *SI Appendix*, Fig. S9). Phylogenetically, CM1 was monophyletic and sister to all other central Mexican groups (Fig. 3).

CM2 grouped *Ambystoma lermaense* (Taylor 1940) (9) with samples corresponding to the type localities and range of *Ambystoma bombypellum* Taylor 1940 (9) and *Ambystoma granulosum* Taylor 1944 (43). There was no evidence for genetic isolation among any of these three taxa (Fig. 2*C* and *SI Appendix*, Fig. S9).

Within CM3, population genetic analyses first separated samples of *Ambystoma ordinarium* Taylor 1940 (5) from the rest (with no admixture detected; Fig. 2*D* and *SI Appendix*, Fig. S9). Other samples clustered into northern and southern groups with some admixture in intermediate localities. The more southern cluster includes two obligately paedomorphic (category 5) taxa, *Ambystoma andersoni* Krebs & Brandon 1984 (44) and *Ambystoma dumerilii* (Dugès 1870) (45), endemic to Lakes Zacapú and Pátzcuaro, respectively. All samples that could not be assigned to *A. ordinarium*, *A. andersoni*, or *A. dumerilii* represent *Ambystoma amblycephalum* Taylor 1940 (9) and *Ambystoma flavipiperatum* Dixon 1963 (46).

CM4 included the axolotl, *A. mexicanum* (Shaw & Nodder 1798) (47), and *Ambystoma taylori* (10)—both category 4 paedomorphs—as well as samples assigned to the facultatively paedomorphic (category 3) *Ambystoma velasci* (Duges 1888) (48). Subsequent DAPC and STRUCTURE analyses of CM4 placed all but one *A. mexicanum* individual sampled from Lakes Xochimilco and Chapultepec (i.e., the remaining habitats of the axolotl) in a distinct cluster (Fig. 2*E* and *SI Appendix*, Fig. S9), with evidence of some admixture in samples to the northwest of Mexico City.

Within the United States and Canada (excluding *A. californiense)*, DAPC and STRUCTURE identified three genetic clusters ($K = 3$), with clear signs of admixed contact zones (Fig. 2*F* and *SI Appendix*, Figs. S10 and S11). Geographically, these clusters are associated with the eastern United States, the central and western United States, and the southwestern United States and Rocky Mountains (hereafter, we refer to the latter two groups as central US and Rocky Mountain US, respectively). Populations in the eastern United States are assignable to *A. tigrinum* Green 1825, while central US and Rocky Mountain US populations to *Ambystoma mavortium* Baird 1850. Phylogenetic analyses corroborated these results, albeit with extensive reticulations among clusters in the phylogenetic network (Fig. 3) and mixed support for species–tree relationships among the three major groups (Fig. 3 versus *SI Appendix*, Fig. S13). Further exploration of population structure within each of the three US clusters recovered additional patterns of differentiation (*SI Appendix*, Figs. S10 and S11), including northern and southern clusters in the eastern United States and central United States, and an allopatric cluster restricted to the Pacific Northwest. Overall, our US results are similar to results from previous studies based on mitochondrial DNA, which identified haplotype clades associated with the eastern United States, Great Plains and Rocky Mountains, and the Pacific Northwest (11, 49).

All analyses of the northern Mexico group identified two genetic clusters ($K = 2$, Fig. 2*G* and *SI Appendix*, Fig. S12). The northern cluster corresponds to *Ambystoma rosaceum* Taylor 1941 (50), while the southern cluster is likely *Ambystoma silvense* Webb 2004 (51, 52). Phylogenetic analyses recovered each of these two northern Mexico clusters as monophyletic, but not sister to one another, and with few reticulate nodes in the phylogenetic network (Fig. 3 and *SI Appendix*, Fig. S13).

Does Paedomorphosis Lead to Increased Genetic Differentiation? As a first step in addressing this question, we used Bayesian modeling to test for genetic isolation of paedomorphic taxa (i.e., species scored as life history category 4 or 5 in Table 1). We tested for gene flow between paedomorphs, *A. andersoni* and *A. dumerilii* in CM3 and *A. mexicanum* and *A. taylori* in CM4, and their surrounding facultatively paedomorphic populations. For both CM3 and CM4, the top-ranking models included migration among all populations, regardless of their degree of paedomorphosis (Fig. 4). Alternative models of no migration or restricted migration (*SI Appendix*, Fig. S14) were rejected with decisive Bayes factors (BF) (*SI Appendix*, Table S3). In CM3, migration rates entering the *A. dumerilii* population were higher than those leaving that population, while migration rates entering and exiting *A. andersoni* were approximately equal. In CM4, migration rates exiting the *A. mexicanum* population were much higher than those entering the population, while migration rates entering and exiting *A. taylori* were approximately equal (Fig. 4 and *SI Appendix*, Table S4). An important caveat to our migration and population structure analyses is that divergence time is not included as a model parameter, which prevents distinguishing between historical and contemporary gene flow. Thus, our ability to differentiate ongoing from historical gene flow in these tests is limited.

We also assessed whether a paedomorphic life history is associated with greater population genetic differentiation (calculated here as Φ_{ST}), which would be expected if paedomorphosis is an important reproductive barrier (12). Plots of Φ_{ST} against

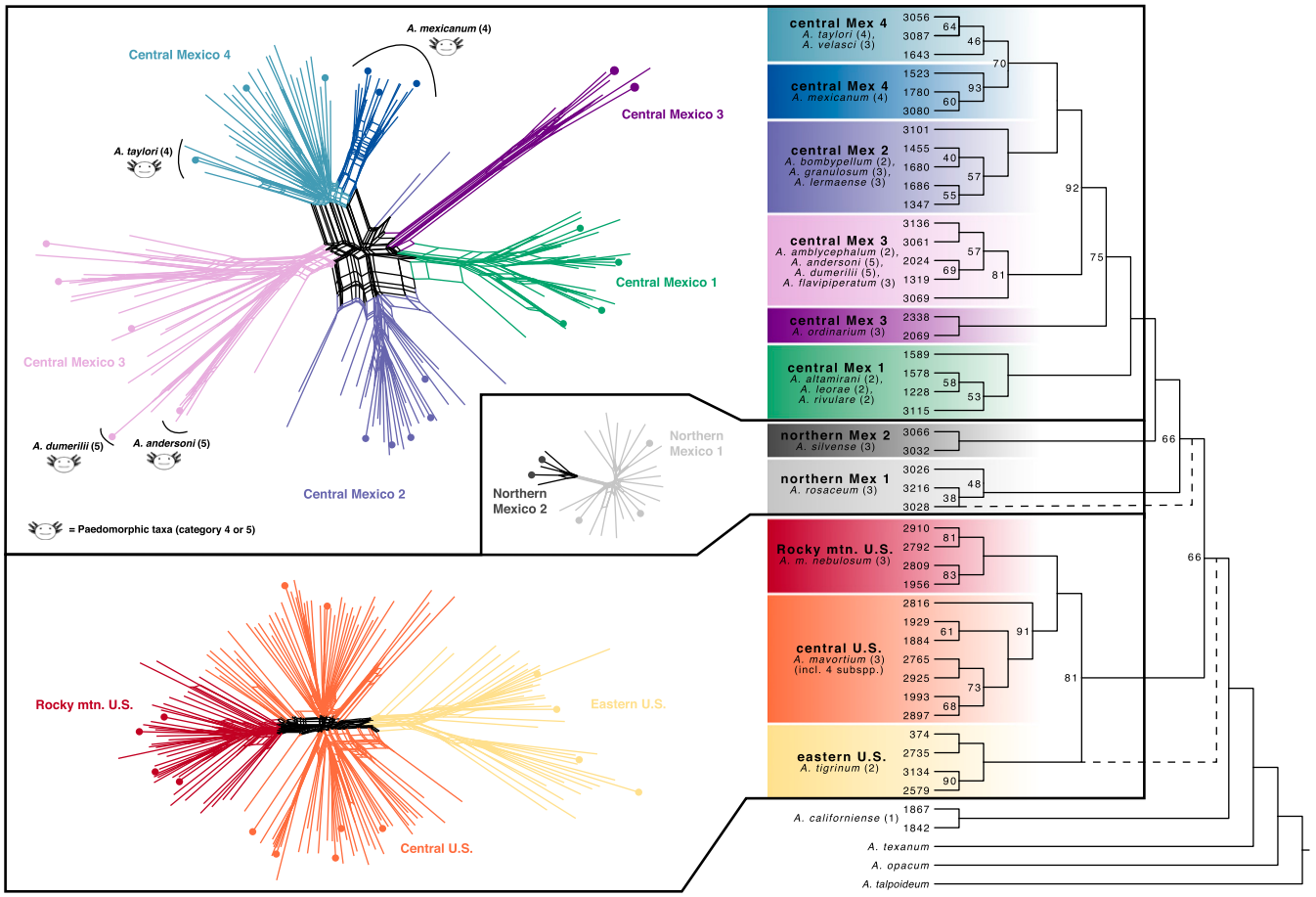

Fig. 3. Results of phylogenetic network analyses (*Left*) and the SVDquartets-based species tree analysis (*Right*) of the *A. tigrinum* species complex. Clades are colored according to their cluster assignments in population genetic analyses, as in Fig. 2. In phylogenetic networks, species names are only shown for paedomorphic taxa in categories 4 or 5 (Table 1). Life history categories for all taxa are given in parentheses in the species tree. Note that SVDquartet analysis used a subset of individuals, indicated by solid circles on the phylogenetic networks. Phylogenetic results from Bayesian and maximum likelihood concatenated analyses were largely concordant with the quartets tree (*SI Appendix*, Fig. S13), although two important differences are indicated by dashed lines. Bootstrap support (BS) values are indicated on each node of the quartets tree (BS values ≥ 95 are not shown), while tips are labeled with individual identification numbers (*SI Appendix*, Table S1). Branch lengths are not scaled to time or substitution rate.

geographic distance showed that comparisons involving paedomorphic populations were largely overlapping with those based on facultative–facultative comparisons, with no difference in slope or y-intercept (Fig. 4). In both CM3 and CM4, partial Mantel tests found no significant correlation between life history and pairwise Φ_{ST} after correcting for geographic distance (Fig. 4).

The Influence of Life History on Diversification. A popular perspective on diversification in this species complex is that paedomorphosis promotes reproductive isolation and, ultimately, speciation (10, 11). An important theme of our results is that taxa with different life history strategies commonly cluster together by geography in population genetic and phylogeographic analyses (Figs. 2 and 3) and that genetic differentiation is not significantly greater for paedomorphic populations relative to facultative populations (Fig. 4). Thus, the evolution of life history extremes does not appear to be the main driver of speciation in the tiger salamander complex. Rather, geography appears to have played a stronger role in diversification. Our model-based migration analyses also revealed gene flow among many populations with different life history strategies (Fig. 4) with only one exception: *A. dumerilii*, which is the only species not known to survive metamorphosis, even under laboratory conditions (53). That only one described taxon in the complex is demonstrably fixed for paedomorphosis and incapable of metamorphosis raises several questions: Are the rest of the primarily paedomorphic populations en route to fixation, rather than actually fixed? Do lineages fixed (or nearly fixed) for paedomorphosis tend to go extinct rapidly, making them short lived in evolutionary time? Do facultative populations fluctuate in paedomorph frequency over time, depending on environmental and/or demographic conditions (5)? Do transforming and nontransforming individuals interbreed in nature? While these remain open questions, below we provide some relevant context for exploration and future research.

Theory predicts that paedomorphosis in salamanders is most likely to become fixed in a population when ecological conditions are favorable in the larval, aquatic environment but unfavorable on land (e.g., when terrestrial habitats are arid or resource limited) (12, 54, 55). However, it is not known how long fixed populations persist over evolutionary time; obligate paedomorphic lineages may have arisen and gone extinct multiple times over tiger salamander evolutionary history (56). One situation that would result in complete speciation is if paedomorphosis became fixed in the population and migration with surrounding facultatively paedomorphic populations ceased due to strong assortative mating and/or selection against hybrids (57–59). However, assortative mating within life history morphs has not been demonstrated in other salamanders (60–62), and different tiger salamander morphs can interbreed (63). In fact, the only known instance of positive assortative mating in *A. tigrinum* is associated with tail length (64, 65),

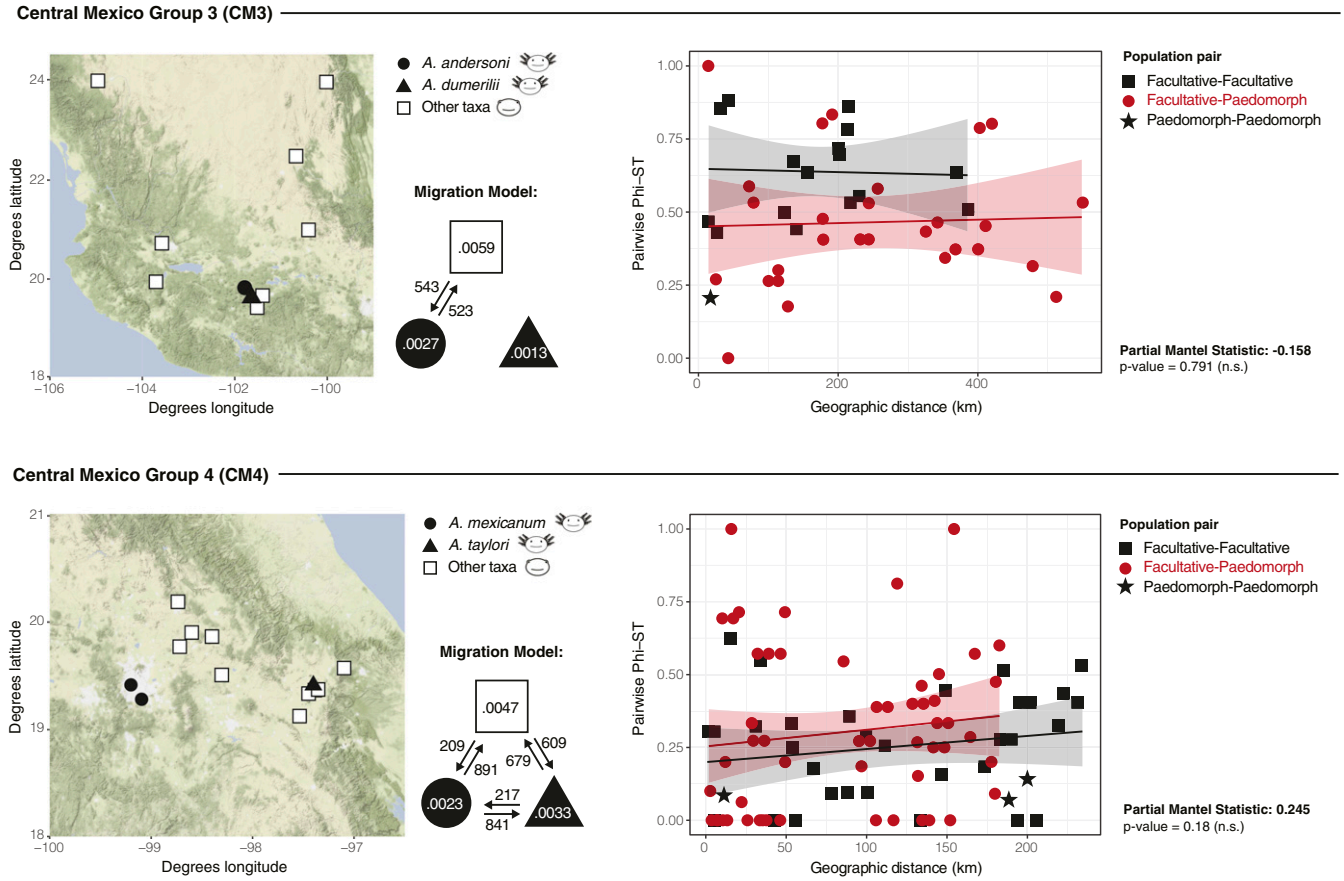

Fig. 4. Maps (*Left*) show localities for CM3 and CM4, the two genetic clusters containing paedomorphic taxa (life history categories 4 and 5). The top-ranked migration model is shown to the *Right* of each map, with median mutation-scaled effective population sizes (Θ) shown inside the shapes denoting populations. Arrows indicate the direction of migration ($M = m/\mu$, in which m is the fraction of immigrants in each generation and μ is the mutation rate per generation per site). and median migration rates are provided next to each arrow. Full model test results are provided in *SI Appendix*, Table S3, and full parameter estimates are provided in *SI Appendix*, Table S4. Scatterplots (*Right*) show pairwise geographic distances by pairwise ϕ_{ST} among all populations. Point colors and shapes denote the life history strategies of the pairwise comparison being made: black squares = both populations are facultatively paedomorphic, red circles = one population is facultatively paedomorphic and one population is obligately paedomorphic, and black stars = both populations are obligately paedomorphic. Solid lines denote the line of best fit, calculated separately for each life history comparison. Regressions were not performed on paedomorph–paedomorph comparisons due to low sample sizes. Shaded areas denote the 95% confidence interval for the regression. *P* values to the right of each graph were calculated using a partial mantel test, which tested for a correlation between ϕ_{ST} and life history while correcting for geographic distance (n.s. = not significant).

which might favor metamorphosed males as paedomorphic individuals can have relatively short tails (44). The evidence for selection against intermorph offspring is also scarce. Captive breeding using artificial fertilization has produced *A. mexicanum* x *A. tigrinum*, *A. dumerilii* x *A. tigrinum*, *A. andersoni* x *A. tigrinum*, and *A. dumerilii* x *A. rivulare* hybrids (7, 33, 66–68). This suggests that postzygotic barriers are weak, although the extent to which divergent lineages would interbreed in the wild remains a key question in need of investigation.

If biological barriers to reproduction are limited or absent, there are several potential mechanisms by which dispersal could mediate gene flow between paedomorphic and metamorphic populations. Paedomorphic individuals cannot survive in a terrestrial environment; however, metamorphosed individuals from facultatively paedomorphic populations could move between bodies of water containing paedomorphic populations. Our demographic analyses support this scenario, as they recovered immigration from facultative into paedomorphic populations (Fig. 4). However, demographic analyses also estimated emigration out of paedomorphic populations; thus, "obligate" paedomorphs might also occasionally metamorphose and disperse. Occasional metamorphosis has been documented in several putatively paedomorphic populations (9, 10, 44, 69–71) and may be more prevalent than currently recognized because rare, transformed individuals in nature are virtually impossible to verify (72). Importantly, even a low frequency of transformed adults is likely sufficient to maintain evolutionary cohesion with surrounding populations (10, 31, 73). Alternatively, gene flow may not necessarily be mediated across a terrestrial environment; genetic connectivity could also be maintained if populations have been forced into contact by water level fluctuations in the groundwater system of a region. The dynamic lacustrine history of the Cuenca Oriental (74), for instance, suggests that isolation of some paedomorphic populations has been punctuated by broader aquatic connections over short geological time scales, potentially facilitating gene flow. Finally, the dispersal of paedomorphs might be human mediated; this could partially explain the elevated emigration rates we observed in the axolotl (Fig. 4). Axolotls are known to be sold in local markets as food, bait, or pets and as a consequence are sometimes moved across central Mexico (75). Collectively, these mechanisms may explain the maintenance of geographic genetic groups, now or in the evolutionarily recent past, containing a range of life histories.

The genetic cohesion we detected across wide swaths of the TMVB is particularly striking given other evidence for local adaptation in this region. This scenario is perhaps best exemplified in the Cuenca Oriental (Fig. 2*E*), where some lakes contain paedomorphic

salamanders adapted to saline conditions, a very rare trait among amphibians (76). At one site, the saline Lake Alchichica, the population is currently considered a distinct species (*A. taylori*) (10). However, our results indicate that *A. taylori* is not genetically distinct from surrounding, putatively non-saline–adapted populations, a result consistent with a previous microsatellite-based study that found significant gene flow to and from *A. taylori* (31). While we cannot rule out the potential for localized selection and adaptation in the *A. taylori* genome, our results highlight that even populations adapted to aquatic conditions intolerable to most other *Ambystoma* have not reached a level of isolation identifying them as an independent evolutionary lineage (77).

Comparisons to Previous Work. This study included extensive geographic sampling of the tiger salamander complex, which provided a broad spatial context to more fully understand patterns of genetic variation. However, it is important to consider the possibility that our data are simply not sensitive enough to detect genetic differentiation associated with lineage divergence on an extremely recent time scale. The most recent common ancestor of *A. californiense* and the remainder of the species complex dates to approximately 5 million y (11, 36), and phylogenomic data indicate that speciation across the remaining tiger salamander lineages occurred within the last 1 million y (78). Such recent timing renders lineage boundaries difficult to detect (15). Our markers, developed from transcriptomic resources (34), may not have an overall substitution rate sufficient to detect such subtle genetic differentiation. We recommend that future research uses a larger genomic dataset or faster-evolving loci with particular focus on the sampling of paedomorphs in this system. To this point, perhaps the strongest evidence for the genetic divergence of paedomorphic populations comes from past microsatellite-based work indicating the genetic distinctiveness of *A. andersoni* and *A. mexicanum* from a select set of populations across central Mexico (29) and the genetic distinctiveness of *A. taylori* from neighboring populations in the Cuenca Oriental (31). Both of these studies recovered signatures of gene flow but identified greater overall population structure compared to this study, which could be due to numerous factors, including a faster microsatellite mutation rate and more limited locality sampling.

Conclusions

The extent to which populations within the tiger salamander complex exhibit phenotypic plasticity in life history traits is remarkable and is believed to have played a role in the rapid accumulation of lineages observed in the highlands of central Mexico (11, 59). While our results suggest that there is less species-level diversity in central Mexico than previously recognized, there is clearly more diversity in central Mexico than in the United States and Canada where there is 1) more geographic space and 2) less life history variation within and between lineages. While we cannot fully explain the greater diversity in central Mexico, our results suggest that major patterns of diversification are related to a complex history of geographic isolation and secondary contact, in which life history strategy has played a less important role, at least in the long (evolutionary) run. We agree with previous work (12) that the complex geological history of the TMVB, including montane uplift and fluctuating drainage connectivity since the Miocene, has been the cornerstone of the evolutionary history of this species complex and that the influences of geographic isolation and paedomorphosis may work synergistically to lead to the establishment of isolated populations. Lingering questions notwithstanding, this large-scale genetic and geographic study establishes a framework for understanding the evolutionary history of the *A. tigrinum* species complex. The results presented here will facilitate comparative studies of the axolotl and its allies, provide direction for conservation prioritization and management, and strengthen the use of the tiger salamander species complex as a model system in biology.

Materials and Methods

Geographic Sampling. We generated data from 254 individuals sampled from across the range of the *A. tigrinum* complex (*SI Appendix*, Fig. S1 and Table S1). These individuals were combined with 93 individuals sampled in O'Neill et al. (34) to produce a data set comprising 347 individuals. We sampled a large number of localities (188) with one to nine individuals sampled per locality (mean = 1.8). This sampling included 166 individuals from the US, two from Canada, and 178 from Mexico. *A. californiense* samples were primarily included as a close outgroup to the remainder of the species complex and were sampled from localities with limited to no impacts of introgression from invasive *A. mavortium* (49). Additional outgroup data were generated for two species outside of the *A. tigrinum* complex: *Ambystoma opacum* and *Ambystoma texanum*. Full details regarding the generation of these data can be found in *SI Appendix, Supplementary Material*. All *A. tigrinum* complex species were assigned to a life history category based on review of the published literature and private field records from authors of this study. These life history categories are as follows: 1 = obligate metamorph (no paedomorphs have been documented in the field), 2 = strong bias toward metamorphosis with rare paedomorphs found in the field, 3 = both metamorphosis and paedomorphosis common in the field, 4 = strong bias toward paedomorphosis with rare metamorphs found in the field, and 5 = obligate paedomorph (no metamorphs have been documented in the field). In the text, "paedomorph" describes taxa scored as 4 or 5, while "facultative paedomorph" describes taxa scored as 2 or 3.

Data Collection and Sequencing. We generated DNA sequence data from a panel of 95 nuclear loci developed specifically for the tiger salamander species complex. A more complete description of marker development can be found in O'Neill et al. (34) Genomic DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen). For a small number of DNA extractions, we increased DNA quantities using a Repli-g whole-genome amplification kit (Qiagen). We used an initial round of PCR in 96-well plate format to amplify all loci from an individual, followed by a smaller second round of PCR to amplify loci that did not amplify in the initial PCR. See O'Neill et al. (34) for the details of PCR conditions and primer sequences.

PCR products from all loci were pooled for each individual in roughly equal concentrations based on the intensity of amplification as visualized on an agarose gel. Indexed Illumina sequencing libraries were generated for each individual using an Illumina Nextera XT DNA Library Preparation Kit (Illumina). Subsequent to library preparation, indexed libraries were quantified using a Qubit Fluorometer, pooled in equimolar concentrations, and checked on an Agilent Bioanalyzer to assure proper fragmentation.

Sequencing was performed in four rounds using an Illumina MiSeq. We performed an initial round using a total of seven individuals to test the compatibility of the Illumina Nextera XT library kit with our PCR amplicons. Three subsequent rounds of library preparation and sequencing were performed on sets of 96 individuals each, and some individuals were sequenced in multiple rounds due to initially low read counts. All sequencing was performed with paired-end (PE) 150 bp reads. Overall, we generated a total of 29,426,894 PE reads across all newly sequenced individuals, with an average of 309,757 PE reads, 1,148X coverage, and 7.6% missing loci per individual (*SI Appendix*, Table S2).

Bioinformatics and Dataset Generation. All sequence reads were processed using a newly developed bioinformatic pipeline written for this project and available on GitHub (doi:10.5281/zenodo.3585970) that produces multiple sequence alignments for individual loci and genome-wide single-nucleotide polymorphism (SNP) matrices sampled from variable sites. This pipeline was developed using the Snakemake workflow management system (79), linking together multiple software tools to take sequence data from raw reads to phased sequence alignments for each locus. Demultiplexed PEIllumina fastq files were used as input, with separate forward and reverse read files for each individual. Sequence data were trimmed and filtered in Trimmomatic (80) using a sliding window of four base pairs and a minimum average quality score of 15. Filtered sequence reads were then aligned to reference sequences from the O'Neill et al. dataset (34); specifically, we used the clean sequences of an *A. ordinarium* sample that had high coverage and low amounts of missing data. The resulting aligned contigs were processed using SAMtools (81) to filter and prepare data for FreeBayes (82), which was used to call variable sites. Variants were filtered with VCFTools (83) by removing indels and setting a quality threshold of phred score > 20 and a minimum read depth of 30. The program WhatsHap (84) was used to perform read-based phasing of the data for each locus x individual contig. Finally, phased haplotypes from each individual (two copies, regardless of homo- or heterozygosity) were combined into an alignment of all individuals using MAFFT with the default auto parameter (85). We generated fasta files of

SNPs using the SNP-sites program (86) and created a SNP genotype matrix by sampling variable sites from a concatenated sequence alignment of all loci. While paralogs were not identified in O'Neill et al. (34), the expanded dataset in this study identified three loci (E12G1, E6A11, and E7G8) as potential paralogs based on high alignment error and high levels of heterozygosity for all individuals. These three loci were excluded from all analyses.

Across the remaining 92 loci, alignment lengths ranged from 124 to 631 bp (avg. = 269 bp) with a total concatenated alignment of 24,788 bp. For the full dataset (the *A. tigrinum* complex including *A. californiense*), single-locus alignments contained an average of 37 variable sites (min. = 9, max. = 93) and an average of 24 parsimony-informative sites (min. = 4, max. = 59). Following further filtering of nonbiallelic SNPs and minor allele counts ≥ 3, population genetic analyses were based on 2,360 SNPs.

Population Structure and Lineage Discovery. We developed hypotheses of population-level lineages across the range of the *A. tigrinum* complex ignoring the existing taxonomy, starting with the identification of major geographic patterns of differentiation and then performing a recursive set of analyses on more geographically restricted sets of individuals. In our initial round of analyses, we used two nonparametric methods: PCA and DAPC (35). While both analyses provide a multivariate summary of genetic data, DAPC is also used to assess the fit of data to varying numbers of population clusters. These analyses were applied to our full genotypic dataset including *A. californiense* and all remaining individuals from the *A. tigrinum* complex. The PCA was calculated using the function "prcomp" in the R package stats (87), while the DAPC was calculated using the package adegenet (88). The optimal number of principal components to retain for DAPC was identified using cross-validation via the xvalDapc function with default parameter values. DAPC was performed without prior assignment of individuals to groups across a range of cluster levels ($K = 1$ to 20). We used two metrics to identify the best estimate of the primary splits in our data. First, we used the BIC calculated in the DAPC analysis to assess the fit of the data to different levels of K. We note that the level of K with the absolute lowest BIC may not be a better explanation of the data than a K with a slightly higher BIC (89); therefore, we applied this measure for general guidance on a range of K that may describe the data well. We paired this assessment with visualizations of the first and second principal components (*SI Appendix*, Figs. S2 and S5) and DAPC ordination plots to identify the level of K at which similar clustering patterns could be observed with minimal change at successively higher levels of K. DAPC of the complete tiger salamander species complex identified a consistent pattern beginning at $K = 5$ for high differentiation of all *A. californiense* samples (*SI Appendix*, Fig. S3). Further DAPC analysis with *A. californiense* removed identified a consistent pattern beginning at $K = 5$ for differentiation between clusters of populations from northern and central Mexico and three clusters of US populations (two from the Western United States and one from the Eastern United States, *SI Appendix*, Fig. S4).

Using the clusters identified in the DAPC analysis of the total data set, we then used both DAPC and STRUCTURE v.2.3.4 (90) to analyze subsequent data sets comprising smaller numbers of individuals. Recursive rounds of DAPC analyses were stopped when BIC scores showed little improvement ($\Delta BIC < 2$) at values of $K > 1$. STRUCTURE analyses used an admixture model and 500,000 generations following a burn-in of 100,000 generations. Analyses were performed for $K = 1$ to 10 with 16 replicate analyses for each K. To help identify an optimal value for K, we calculated ΔK using the Evanno method (91) via the CLUMPAK web tool (92). A limitation of the Evanno method is that it cannot estimate the likelihood of $K = 1$ (91); thus, we also visually inspected individual group assignments and concluded a value of $K = 1$ if the corresponding DAPC cluster showed little improvement ($\Delta BIC < 2$) at values of $K > 1$ or when individuals were simply being split without any geographic or individual clustering. We also evaluated models where K was equal to the number of currently recognized species in that genetic cluster (e.g., $K = 3$ for CM1, which included the type localities for three described species: *A. altamirani*, *A. leorae*, and *A. rivulare*). We mapped individual species assignments onto these results to test the potential correspondence between naïve clustering and the existing taxonomy (*SI Appendix*, Fig. S9).

Phylogenetic Reconstruction. Given the high levels of admixture among groups, we used SplitsTree v. 4.14.8 (37, 93) to generate four phylogenetic networks: one with all tiger salamander individuals and one each for the U.S., central Mexico, and northern Mexico subgroups. Networks were constructed using uncorrected p-distances and the NeighborNet algorithm (94).

We also used three different analytical approaches to place hypothesized population lineages in a phylogenetic framework. For all analyses, we used a reduced data set containing the concatenated data for two to seven representative individuals from each population genetic cluster, which limited computation time and avoided violating the coalescent model assumption of zero gene flow. We first used PartitionFinder (95) to identify the number of preferred gene partitions and their substitution models. These partitions were used to infer a phylogeny using Bayesian Inference in BEAST v.1.8.3 (39). Analyses were run for five million generations, sampling every 1,000 generations after the first 500,000 generations were removed as burn-in. Run convergence was assessed with Tracer v.1.6.0 (96). Next, we inferred a maximum likelihood phylogeny using RAxML v.8 (40). Node support was assessed using a rapid bootstrap analysis with 1,000 replicates, which was summarized as a 95% rule consensus tree using the program SumTrees in the DendroPy python library (97). All of these analyses were performed on the CIPRES Science Gateway server (98). Finally, we inferred phylogenetic relationships using SVDquartets (38) implemented in PAUP* version 4.0a164 (99), sampling all possible quartets and assessing node support with 1,000 bootstrap replicates. For all phylogenetic analyses, trees were visualized using FigTree v.1.4.2 (100).

Tests of Migration and Population Differentiation of Paedomorphic Taxa. We used the coalescent-based program Migrate-N v.3.2 (101) to explicitly test for population structure and gene flow in each obligately paedomorphic species of *Ambystoma* (*A. andersoni*, *A. dumerilii*, *A. taylori*, and *A. mexicanum*). For each model (*SI Appendix*, Fig. S14), we treated paedomorphic species as distinct populations and the facultatively paedomorphic individuals from the same genetic cluster (CM3 or CM4) as another population. The best-fitting model was determined via BF, which were calculated using Bézier-corrected marginal likelihoods (*SI Appendix*, Table S3); the highest-ranking models have a BF = 0. Each Migrate-N analysis ran for 50 million steps, recording every 10 steps, with a burn-in of five million and the default heating scheme. Suitable upper bounds for priors on population size (Θ) and migration rate (M) were determined from an initial test run of 10 million steps for each analysis. Median values and 95% confidence intervals of Θ and M are in Fig. 4 and *SI Appendix*, Table S4.

Within CM3 and CM4, we also calculated pairwise Φ_{ST} (a metric of population differentiation) among all populations using the function "pairPhiST" in the R package haplotypes (102). These values were plotted against pairwise geographic distances (Fig. 4), which were calculated from locality latitude/longitude data (*SI Appendix*, Table S1) and converted to kilometers using the function "rdist.earth" in the R package fields (103). A life history matrix was also generated by assigning a value of 0 for pairwise comparisons of populations with the same life history and a value of 1 for populations with different life histories (i.e., facultatively paedomorphic versus obligately paedomorphic). Paedomorph–paedomorph comparisons were omitted to avoid confounding them with facultative–facultative comparisons. Finally, we used these matrices in a partial mantel test [function "mantel.partial" package vegan (104)] to test for a correlation between Φ_{ST} and life history while correcting for geographic distance. Significance of this test was determined using 999 permutations and an alpha level of 0.05.

Data Availability. Input files for all population genetic and phylogenetic analyses are available via figshare, https://figshare.com/projects/Life_history_strategy_does_not_reflect_genetic_differentiation_in_the_tiger_salamander_species_complex/74115 (105). Data pipeline is available on GitHub, https://github.com/kelly-sovacool/tiger_salamander_project (106). Sequence data are available on the NCBI Sequence Read Archive (BioProject accession PRJNA594660). All other study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. This project was supported by grants from the NSF (DEB-0949532, DEB-1355000, and DEB-1406876 [DDIG awarded to J.D.K.]) and by a University Research Fellowship awarded to K.M.E. from the University of Kentucky. We thank Chris Beachy, Jim Bogart, Ken Carbale, Sheri Church, Jeff LeClere, Carol Hall, Paul Moler, Nancy Staub, and Ken Wray for genetic samples and Jose Bocanegra, Ben Browning, Mackenzie Humphrey, Mary Virginia Gibbs, Ricky Grewelle, Alan Lemmon, Emily Moriarty Lemmon, Deborah Lu, Stephanie Mitchell, Alex Noble, Tolu Odukoya, Ben Tuttle, and Josh Williams for assistance with data collection and analyses.

1. S. R. Palumbi, Marine speciation on a small planet. *Trends Ecol. Evol.* **7**, 114–118 (1992).
2. S. Mopper, S. Y. Strauss, *Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior* (Springer US, 1998).
3. S. J. Gould, *Ontogeny and Phylogeny* (Harvard University Press, 1977).
4. D. B. Wake, Paedomorphosis. *J. Herpetol.* **14**, 80 (1980).
5. H. H. Whiteman, Evolution of facultative paedomorphosis in salamanders. *Q. Rev. Biol.* **69**, 205–221 (1994).

6. R. B. Page *et al*., Effect of thyroid hormone concentration on the transcriptional response underlying induced metamorphosis in the Mexican axolotl (*Ambystoma*). *BMC Genomics* **9**, 78 (2008).
7. S. R. Voss, Genetic basis of paedomorphosis in the axolotl, *Ambystoma mexicanum*: A test of the single-gene hypothesis. *J. Hered.* **86**, 441–447 (1995).
8. S. R. Voss, H. B. Shaffer, Adaptive evolution via a major gene effect: Paedomorphosis in the Mexican axolotl. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 14185–14189 (1997).
9. E. H. Taylor, New salamanders from Mexico with a discussion of certain known forms. *Univ. Kans. Sci. Bull.* **26**, 407–439 (1939).
10. R. Brandon, E. Maruska, W. Rumph, A new species of neotenic *Ambystoma* (Amphibia, Caudata) endemic to Laguna Alchichica, Puebla, Mexico. *Bull. South. Calif. Acad. Sci.* **80**, 112–125 (1981).
11. H. B. Shaffer, M. L. McKnight, The polytypic species revisited: Genetic differentiation and molecular phylogenetics of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. *Evolution* **50**, 417–433 (1996).
12. H. B. Shaffer, Evolution in a paedomorphic lineage. I. An electrophoretic analysis of the Mexican ambystomatid salamanders. *Evolution* **38**, 1194–1206 (1984).
13. H. B. Shaffer, Phylogenetics of model organisms: The laboratory axolotl, *Ambystoma mexicanum*. *Syst. Biol.* **42**, 508–522 (1993).
14. H. B. Shaffer, Evolution in a paedomorphic lineage. II. Allometry and form in the Mexican ambystomatid salamanders. *Evolution* **38**, 1207–1218 (1984).
15. H. B. Shaffer, R. C. Thomson, Delimiting species in recent radiations. *Syst. Biol.* **56**, 896–906 (2007).
16. P. R. Grant, Speciation and the adaptive radiation of Darwin's finches. *Am. Sci.* **69**, 653–663 (1981).
17. O. Seehausen, Explosive speciation rates and unusual species richness in haplochromine cichlid fishes: Effects of sexual selection. *Adv. Ecol. Res.* **31**, 237–274 (2000).
18. M. Fegan, P. Prior, "How complex is the *Ralstonia solanacearum* species complex" in *Bacterial Wilt Disease and the Ralstonia Solanacearum Species Complex*, C. Allen, P. Prior, A. C. Hayward, Eds. (American Phytopathological Society Press, 2005), pp. 449–461.
19. J. Mallet, Hybrid speciation. *Nature* **446**, 279–283 (2007).
20. M. C. Fontaine *et al*., Mosquito genomics. Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science* **347**, 1258524 (2015).
21. J. Geml, G. A. Laursen, K. O'neill, H. C. Nusbaum, D. L. Taylor, Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*). *Mol. Ecol.* **15**, 225–239 (2006).
22. T. C. Giarla, J. A. Esselstyn, The challenges of resolving a rapid, recent radiation: Empirical and simulated phylogenomics of philippine shrews. *Syst. Biol.* **64**, 727–740 (2015).
23. H. Philippe *et al*., Resolving difficult phylogenetic questions: Why more sequences are not enough. *PLoS Biol.* **9**, e1000602 (2011).
24. D. W. Weisrock, H. B. Shaffer, B. L. Storz, S. R. Storz, S. R. Voss, Multiple nuclear gene sequences identify phylogenetic species boundaries in the rapidly radiating clade of Mexican ambystomatid salamanders. *Mol. Ecol.* **15**, 2489–2503 (2006).
25. R. Newman, Adaptive plasticity in amphibian metamorphosis. *Bioscience* **42**, 671–678 (1992).
26. H. H. Whiteman, S. A. Wissinger, W. S. Brown, Growth and foraging consequences of facultative paedomorphosis in the tiger salamander, *Ambystoma tigrinum nebulosum*. *Evol. Ecol.* **10**, 433–446 (1996).
27. P. J. Fernandez, J. P. Collins, Effect of environment and ontogeny on color pattern variation in Arizona tiger salamanders (*Ambystoma tigrinum nebulosum* Hallowell). *Copeia* **1988**, 928 (1988).
28. S. P. D. Riley, H. B. Shaffer, S. R. Voss, B. M. Fitzpatrick, Hybridization between a rare, native tiger salamander (*Ambystoma californiense*) and its introduced congener. *Ecol. Appl.* **13**, 1263–1275 (2003).
29. G. Parra-Olea *et al*., Conservation genetics of threatened Mexican axolotls (*Ambystoma*). *Anim. Conserv.* **15**, 61–72 (2012).
30. E. Routman, Population structure and genetic diversity of metamorphic and paedomorphic populations of the tiger salamander, *Ambystoma tigrinum*. *J. Evol. Biol.* **6**, 329–357 (1993).
31. R. Percino-Daniel, E. Recuero, E. Vázquez-Domínguez, K. R. Zamudio, G. Parra-Olea, All grown-up and nowhere to go: Paedomorphosis and local adaptation in *Ambystoma* salamanders in the Cuenca Oriental of Mexico. *Biol. J. Linn. Soc. Lond.* **118**, 582–597 (2016).
32. R. A. Brandon, Hybridization between the Mexican salamanders *Ambystoma dumerilii* and *Ambystoma mexicanum* under laboratory conditions. *Herpetologica* **28**, 199–207 (1972).
33. R. A. Brandon, Interspecific hybridization among Mexican and United States salamanders of the genus *Ambystoma* under laboratory conditions. *Herpetologica* **33**, 133–152 (1977).
34. E. M. O'Neill *et al*., Parallel tagged amplicon sequencing reveals major lineages and phylogenetic structure in the North American tiger salamander (*Ambystoma tigrinum*) species complex. *Mol. Ecol.* **22**, 111–129 (2013).
35. T. Jombart, S. Devillard, F. Balloux, Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genet.* **11**, 94 (2010).
36. H. B. Shaffer, G. B. Pauly, J. C. Oliver, P. C. Trenham, The molecular phylogenetics of endangerment: Cryptic variation and historical phylogeography of the California tiger salamander, *Ambystoma californiense*. *Mol. Ecol.* **13**, 3033–3049 (2004).
37. D. H. Huson, SplitsTree: Analyzing and visualizing evolutionary data. *Bioinformatics* **14**, 68–73 (1998).
38. J. Chifman, L. Kubatko, Quartet inference from SNP data under the coalescent model. *Bioinformatics* **30**, 3317–3324 (2014).
39. A. J. Drummond, A. Rambaut, BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214 (2007).
40. A. Stamatakis, RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).
41. A. A. Dugès, *Description d'un axolotl des montagnes de las cruces (Amblystoma [sic] altamirani, A. Dugès)* (Imprimerie du Ministère de "Fomento", 1895).
42. E. H. Taylor, Herpetological novelties from Mexico. *Univ. Kans. Sci. Bull.* **29**, 343–358 (1943).
43. E. H. Taylor, A new ambystomid salamander from the Plateau Region of Mexico. *Univ. Kans. Sci. Bull.* **30**, 57–61 (1944).
44. S. Krebs, R. Brandon, A new species of salamander (family Ambystomatidae) from Michoacan, Mexico. *Herpetologica* **40**, 238–245 (1984).
45. A. A. Dugès, Una nueva especie de ajolote de la Laguna de Patzcuaro. *La Nat.* **1**, 241–244 (1870).
46. J. Dixon, A new species of salamander of the genus Ambystoma from Jalisco, Mexico. *Copeia* **1**, 99–101 (1963).
47. G. Shaw, F. Nodder, *The Naturalist's Miscellany; or Coloured Figures of Natural Objects Drawn and Described Immediately from Nature* (Nodder & Co., ed. 9, 1798).
48. A. A. Dugès, Erpetología del valle de México. *La Nat.* **2**, 97–146 (1888).
49. J. R. Johnson, R. C. Thomson, S. J. Micheletti, H. B. Shaffer, The origin of tiger salamander (*Ambystoma tigrinum*) populations in California, Oregon, and Nevada: Introductions or relicts? *Conserv. Genet.* **12**, 355–370 (2011).
50. E. H. Taylor, Two new ambystomid salamanders from Chihuahua. *Copeia* **3**, 143–146 (1941).
51. R. Webb, Observations on tiger salamanders (*Ambystoma tigrinum* complex, family Ambystomatidae) in Mexico with description of a new species. *Bull. Maryl. Herpetol. Soc.* **40**, 122–143 (2004).
52. H. B. Shaffer, Biosystematics of *Ambystoma rosaceum* and *A. tigrinum* in northwestern Mexico. *Copeia* **1983**, 67 (1983).
53. R. A. Brandon, Spontaneous and induced metamorphosis of *Ambystoma dumerilii* (Dugès), a paedogenetic Mexican salamander, under laboratory conditions. *Herpetologica* **32**, 429–438 (1976).
54. H. M. Wilbur, J. P. Collins, Ecological Aspects of Amphibian Metamorphosis: Nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science* **182**, 1305–1314 (1973).
55. R. M. Bonett, M. A. Steffen, S. M. Lambert, J. J. Wiens, P. T. Chippindale, Evolution of paedomorphosis in plethodontid salamanders: Ecological correlates and re-evolution of metamorphosis. *Evolution* **68**, 466–482 (2014).
56. C. R. Marshall, E. C. Raff, R. A. Raff, Dollo's law and the death and resurrection of genes. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12283–12287 (1994).
57. M. J. West-Eberhard, Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**, 249–278 (1989).
58. M. J. West-Eberhard, Alternative adaptations, speciation, and phylogeny (A Review). *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1388–1392 (1986).
59. B. M. Fitzpatrick, Underappreciated consequences of phenotypic plasticity for ecological speciation. *Int. J. Ecol.* **2012**, 1–12 (2012).
60. M. Denoël, P. Poncin, J.-C. Ruwet, Sexual compatibility between two heterochronic morphs in the Alpine newt, *Triturus alpestris*. *Anim. Behav.* **62**, 559–566 (2001).
61. J. D. Krenz, P. A. Verrell, Integrity in the midst of sympatry: Does sexual incompatibility facilitate the coexistence of metamorphic and paedomorphic mole salamanders (*Ambystoma talpoideum*)? *J. Zool. (Lond.)* **258**, 435–440 (2002).
62. H. H. Whiteman, J. D. Krenz, R. D. Semlitsch, Intermorph breeding and the potential for reproductive isolation in polymorphic mole salamanders (*Ambystoma talpoideum*). *Behav. Ecol. Sociobiol.* **60**, 52–61 (2006).
63. H. Whiteman, J. Gutrich, R. Moorman, Courtship behavior in a polymorphic population of the tiger salamander, *Ambystoma tigrinum nebulosum*. *J. Herpetol.* **33**, 348–351 (1999).
64. R. D. Howard, R. S. Moorman, H. H. Whiteman, Differential effects of mate competition and mate choice on eastern tiger salamanders. *Anim. Behav.* **53**, 1345–1356 (1997).
65. D. H. Bos, R. N. Williams, D. Gopurenko, Z. Bulut, J. A. DeWoody, Condition-dependent mate choice and a reproductive disadvantage for MHC-divergent male tiger salamanders. *Mol. Ecol.* **18**, 3307–3315 (2009).
66. C. E. Nelson, R. R. Humphrey, Artificial interspecific hybridization among *Ambystoma*. *Herpetologica* **28**, 27–32 (1972).
67. S. R. Voss, H. B. Shaffer, Evolutionary genetics of metamorphic failure using wild-caught vs. laboratory axolotls (*Ambystoma mexicanum*). *Mol. Ecol.* **9**, 1401–1407 (2000).
68. S. R. Voss, K. L. Prudic, J. C. Oliver, H. B. Shaffer, Candidate gene analysis of metamorphic timing in ambystomatid salamanders. *Mol. Ecol.* **12**, 1217–1223 (2003).
69. J. B. Armstrong, G. M. Malacinski, *Developmental Biology of the Axolotl* (Oxford University Press, 1989).
70. J. M. Velasco, Anotaciones y observaciones al trabajo del Sr. D. A. Weismann sobre la transformación del ajolote Mexicano en Amblistoma. *La Nat.* **5**, 58–84 (1880).
71. H. M. Smith, The Mexican axolotl: Some misconceptions and problems. *Bioscience* **19**, 593–615 (1969).
72. L. L. Bailey, T. R. Simons, K. H. Pollock, Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecol. Appl.* **14**, 692–702 (2004).
73. C. L. Morjan, L. H. Rieseberg, How species evolve collectively: Implications of gene flow and selection for the spread of advantageous alleles. *Mol. Ecol.* **13**, 1341–1356 (2004).
74. J. L. Tamayo, R. C. West, "The hydrography of middle America" in *Handbook of Middle American Indians*, R. Wauchope, R. C. West, Eds. (University of Texas Press, 1964), pp. 84–121.

75. J. McKay, "An evaluation of captive breeding and sustainable use of the Mexican axolotl (*Ambystoma mexicanum*)," Masters thesis, University of Kent, Canterbury, UK (2003).
76. G. R. Hopkins, E. D. Brodie, Occurrence of amphibians in saline habitats: A review and evolutionary perspective. *Herpetol. Monogr.* **29**, 1–27 (2015).
77. K. De Queiroz, Species concepts and species delimitation. *Syst. Biol.* **56**, 879–886 (2007).
78. P. M. Hime *et al*., Phylogenomics reveals ancient gene tree discordance in the amphibian tree of life. *Syst. Biol.* **70**, 49–60 (2020).
79. J. Köster, S. Rahmann, Snakemake–A scalable bioinformatics workflow engine. *Bioinformatics* **28**, 2520–2522 (2012).
80. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
81. H. Li, A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* **27**, 2987–2993 (2011).
82. E. Garrison, G. Marth, Haplotype-based variant detection from short-read sequencing. arXiv [Preprint] (2012). https://arxiv.org/abs/1207.3907 (Accessed 1 April 2021).
83. P. Danecek *et al*.; 1000 Genomes Project Analysis Group, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).
84. M. Martin *et al*., WhatsHap: Fast and accurate read-based phasing *bioRxiv* [Preprint] (2016). https://doi.org/10.1101/085050 (Accessed 1 April 2021).
85. K. Katoh, D. M. Standley, MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
86. A. J. Page *et al*., *SNP-sites*: Rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb. Genom.* **2**, e000056 (2016).
87. R Core Team, R: A Language and Environment for Statistical Computing (Vienna, Austria, 2020). https://www.R-project.org/. Accessed 1 April 2021.
88. T. Jombart, adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405 (2008).
89. T. Jombart, C. Collins, A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0. 0. (2015). adegenet.r-forge.r-project.org. Accessed 30 May 2019.
90. J. K. J. Pritchard, M. Stephens, P. Donnelly, Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959 (2000).
91. G. Evanno, S. Regnaut, J. Goudet, Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **14**, 2611–2620 (2005).
92. N. M. Kopelman, J. Mayzel, M. Jakobsson, N. A. Rosenberg, I. Mayrose, Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* **15**, 1179–1191 (2015).
93. D. H. Huson, D. Bryant, Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **23**, 254–267 (2006).
94. D. Bryant, V. Moulton, Neighbor-net: An agglomerative method for the construction of phylogenetic networks. *Mol. Biol. Evol.* **21**, 255–265 (2004).
95. R. Lanfear, B. Calcott, S. Y. W. Ho, S. Guindon, Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* **29**, 1695–1701 (2012).
96. A. Rambaut, A. J. Drummond, D. Xie, G. Baele, M. A. Suchard, Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **67**, 901–904 (2018).
97. J. Sukumaran, M. T. Holder, DendroPy: A Python library for phylogenetic computing. *Bioinformatics* **26**, 1569–1571 (2010).
98. M. A. Miller, W. Pfeiffer, T. Schwartz, "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in *2010 Gateway Computing Environments Workshop (GCE)* (IEEE, 2010), pp. 1–8.
99. D. Swofford, *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)* (Version 4.0 b10, Sinauer Associates, Sunderland, MA, 2002).
100. A. Rambaut, FigTree, Version 1.4.4. https://github.com/rambaut/figtree. Accessed 1 April 2021.
101. P. Beerli, M. Palczewski, Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics* **185**, 313–326 (2010).
102. C. Aktas, Haplotypes: Haplotype inference and statistical analysis of genetic variation. https://cran.r-project.org/web/packages/haplotypes. Accessed 1 April 2021.
103. D. Nychka, R. Furrer, J. Paige, S. Sain, fields: Tools for spatial data. https://cran.r-project.org/web/packages/fields. Accessed 1 April 2021.
104. P. Dixon, VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **14**, 927–930 (2003).
105. K. M. Everson, L. N. Gray *et al*., Data for: Life history strategy does not reflect genetic differentiation in the tiger salamander species complex (*FigShare*, 2020). https://figshare.com/projects/Life_history_strategy_does_not_reflect_genetic_differentiation_in_the_tiger_salamander_species_complex/74115. Deposited 7 October 2020.
106. K. L. Sovacool, Tiger salamander project data processing pipeline (GitHub, 2019). https://github.com/kelly-sovacool/tiger_salamander_project. Deposited 19 December 2019.
107. L. Ferrari, Slab detachment control on mafic volcanic pulse and mantle heterogeneity in central Mexico. *Geology* **32**, 77–80 (2004).