

Reconstruction of the climate envelopes of salamanders and their evolution through time

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Ecological niches evolve through time, but at different rates and to different degrees. An integrated approach using diverse databases, methods, and analytical tools is used to estimate climate envelopes for species of salamanders (family Salamandridae). These species, which range widely across the Holarctic and have a rich and long fossil record, are used to probe the evolutionary dynamics of niches studied in a phylogenetic context through time and across space. Climate data and statistical methods are used to estimate niche dimensions related to precipitation and temperature, in both a phylogenetic and ecogeographic context. Using phylogenetic methods, climate envelopes are estimated for segments of a time-calibrated phylogenetic tree of salamandrids, to explore how far back in time it is reasonable to make such estimates. Our research strategy illuminates some of the challenges and limitations of the available data and methods and identifies potential solutions, such as the need for physiological and behavioral data that may help to better define species' niches or the development of novel evolutionary models that account for paleoclimatic data. We explore and analyze limits to the application of currently available methodologies. Organisms known to have evolved slowly and conservatively, and which are ectotherms and likely to be profoundly affected by climatic variables, may be the most useful for studies of this kind.

Grinnellian niches | niche evolution | phylogenetic analysis | Salamandridae

The pioneering research on the ecological distribution of organisms by Joseph Grinnell, celebrated in this colloquium, led rather directly to the modern studies of ecological niche evolution and its relevance to studies of biogeography during a period of climate change. Grinnell's conceptualizations and remarkable vision inspired generations of researchers. A special burst of creativity in the last decade is related to the availability of digital databases documenting species distributions [e.g., the Global Biodiversity Information Facility (GBIF; www.gbif.org) and HerpNet (www.herpnet.org)], and databases containing climatic information (e.g., ref. 1). Equally important were the exponential increase in computer power and the development of analytical methods and programs to model species distributions (2). Genetic information about species from sequencing DNA and the rapid growth of fields such as phylogeography produced large volumes of data that have been analyzed to both generate hypotheses of phylogenetic relationships and document patterns of geographic variation at levels unimaginable by past workers. A major current trend is the development of climate or environmental envelopes for species, which are used to predict likely responses to climate change. In this article we explore the possibility of integrating climate envelopes, robust phylogenetic hypotheses, and calibrated time trees to look backwards in time, in an attempt to understand important aspects of the historical biogeography of clades and the evolution of ecological niches and establish a foundation for predicting responses to climate change.

We selected a taxon we know well, amphibians belonging to the family Salamandridae, for this exploration. Salamanders are an ancient clade, and living families have a common ancestor that lived 165 million to 200 million years ago (mya) (3, 4). The salamandrids originated ≈ 100 mya. The fossil record of salamandrids is good, and there is abundant evidence from fossils and patterns of distribution of living forms that salamanders in general have displayed long-term ecological niche conservation (5), and their morphological evolution has been conservative as well (6). Species formation has been predominantly vicariant, with build-up of species in a lineage displaying what has been termed nonadaptive radiation (7). Because species ranges are broken up through time, leading to allopatry and vicariant species formation, the derivative species remain ecologically generally similar, although now living in different, yet nearby, regions. It is this pattern of conservatism and the availability of robust phylogenetic hypotheses, detailed knowledge of the distribution and many aspects of biology of all of the species, and a calibrated time tree that has encouraged us to use the Salamandridae as a test case to explore how far back in time we can trace ecological niche evolution in branching lineages.

Salamandridae is the second largest family of salamanders, with ≈ 80 species placed in 20 genera distributed across much of the Holarctic region. For ease of communication we use informal names for clades as follows: spectacled salamanders (*Salamandrina*), true salamanders (*Chioglossa*, *Lyciasalamandra*, *Mertensiella*, *Salamandra*), primitive newts (*Echinotriton*, *Pleurodeles*, *Tylostotriton*), New World modern newts (*Notophthalmus*, *Taricha*), Corsica-Sardinia newts (*Euproctus*), Asian modern newts (*Cynops*, *Laotriton*, *Pachytriton*, *Paramesotriton*), and western Palearctic modern newts (*Calotriton*, *Ichthyosaura*, *Lissotriton*, *Neuregus*, *Ommatotriton*, *Triturus*) (3).

We use an integrative approach for reconstructing elements of the niches of clades through time, incorporating methods and datasets derived from several disciplines to explore the general problem. We identify challenges and limitations of current approaches and suggest some potential solutions and directions for future research.

Results

Phylogenetic Approach. A nearly complete species-level phylogeny built from a mitochondrial dataset (8) was the base for recon-

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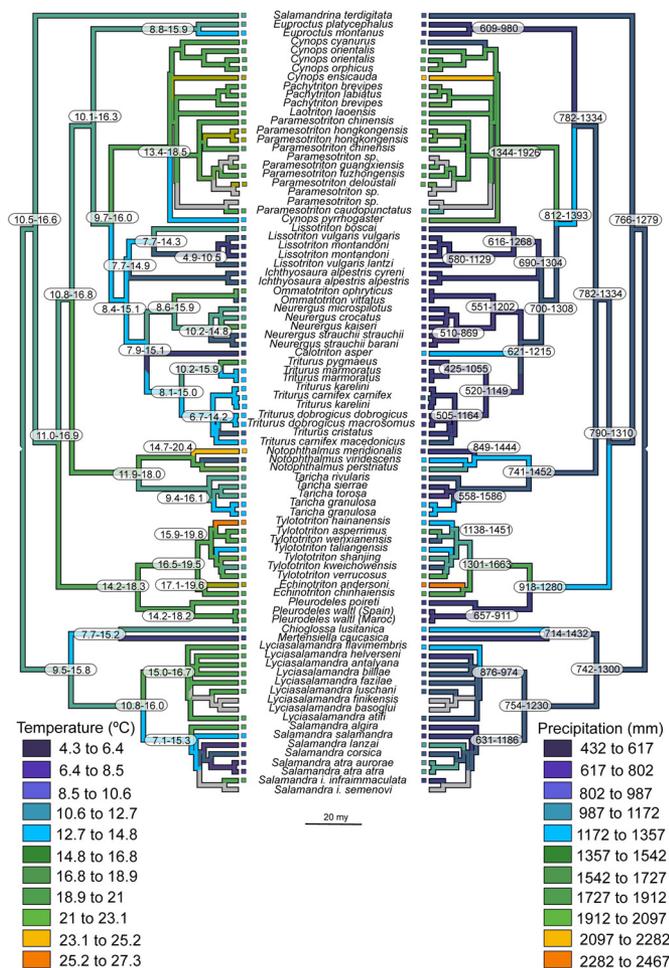


Fig. 1. Ancestral state reconstruction of AMT (Left) and AP (Right) for salamandrids, based on a nearly complete species-level phylogeny. To cover the ranges of precipitation and temperature values per species, reconstructions of the 95% confidence intervals of those values were performed, and ranges are shown for the major nodes. Branch lengths are proportional to time. Color scales represent temperature and precipitation ranges for the median climate values per species, going from lower values (dark blue) to higher values (red), gray representing missing data, which match the colors in the time tree.

structing ancestral states of climate niche dimensions. This phylogenetic hypothesis is well resolved, with some exceptions near the base of the tree. Divergence-time analysis (see Fig. 1) suggests an origin for most genera (*Chioglossa*, *Mertensiella*, *Pleurodeles*, *Taricha*, *Notophthalmus*, *Calotriton*, *Ichthyosaura*, *Triturus*, *Lissotriton*) in the Eocene-Oligocene. The splits between *Lyciasalamandra*-*Salamandra* and *Ommatotriton*-*Neuregus* occurred in the Miocene. The origins of several Asian newt genera are more recent: *Echinotriton*-*Tylostotriton* in the late Miocene and basal diversification of Asian modern newts in the mid-late Miocene. The basal split between true salamanders and newts is in the Cretaceous (≈ 75 mya), and the mean values for each node are within the confidence intervals obtained with other datasets.

To generate hypotheses of ancestral niche dimensions we integrated our timetree with climate data for species to perform maximum-likelihood reconstructions of phylogenetic ancestral states for annual mean temperature (AMT) and annual precipitation (AP) (Fig. 1). This approach assumes a Brownian motion model of character evolution, where the model scaling parameters (λ , κ , δ) are equal to one. We tested for directionality of

trait evolution on the reconstructions by comparing the likelihoods of a nondirectional versus a more complex directional model, as implemented in Bayestraits (9). These comparisons reveal no evidence of significantly better fitting of a directional model, because both models show similar log-likelihoods (L) for AMT (both $L = -221.48$) and AP (both $L = -575.6$). Likelihood ratio (LR) tests show that the model in which λ was set to its maximum-likelihood value for AP ($\lambda = 0.967$) performed statistically better than the default model with $\lambda = 1$ (LR = 23.4, 1 df, $P = 0.001$), and for AMT ($\lambda = 0.80$, versus $\lambda = 1$; LR = 105, 1 df, $P < 0.001$). The maximum-likelihood estimate for κ was 1.027 for AP; the model did not differ statistically from the default ($\kappa = 1$; LR = 3.16, 1 df, $P = 0.0755$). For AMT, with a κ of 1.148, the model performed better than the default with $\kappa = 1$ (LR = 35.8, 1 df, $P < 0.001$). The maximum-likelihood estimate of delta for AP was 2.84 and for AMT was 3, both models performing significantly better than the default with $\delta = 1$ (LR = 11.3, 1 df, $P < 0.001$, and LR = 21.8, 1 df, $P < 0.001$, respectively).

Because these results suggest that the evolution of selected climate parameters deviate from a Brownian model of evolution, we probed further to gain insight into the deviation. We performed the same analyses after eliminating a few taxa, outliers that differ substantially from climate parameters of phylogenetically close sister species. For example, by removing just two species that are very different from their close relatives for AMT, the maximum-likelihood values and likelihoods for two scaling parameters changed significantly ($\lambda = 0.998$, $L = -203.48$; $\kappa = 1.17$, $L = -202.9$). LR tests show no differences in comparisons with the default model for λ (LR = 1.28, 1 df, $P = 0.2586$) or κ (LR = 0.14, 1 df, $P = 0.7069$), but the model with δ at its maximum likelihood still performed slightly better than the default (LR = 5.45, 1 df, $P = 0.0196$). Because in this model λ and κ are not significantly different from one, they evolve as expected given the tree topology and a Brownian random walk model, and only δ deviates from the expected value under Brownian motion.

The models in which the scaling parameters (λ , δ , κ) were set to their maximum-likelihood values were also compared with null models in which these parameters were constrained to 0. For both AMT and AP, the models with λ set to its maximum-likelihood value performed significantly better than the null models (LR = 59.8, 1 df, $P < 0.001$, LR = 68.8, 1 df, $P < 0.001$), suggesting that traits do not evolve independently of the phylogeny and that the data have a phylogenetic signal. Models with κ set to its maximum-likelihood value also performed better than null models (AMT, LR = 24.5, 1 df, $P < 0.001$; AP, LR = 10.6, 1 df, $P < 0.001$), thus eliminating a punctuational evolution of these traits. Finally, the model with δ set to its maximum-likelihood value performed better than the null model for AP (LR = 118.2, 1 df, $P < 0.001$), but for AMT the null model performed better (LR = 24.6, 1 df, $P < 0.001$). In both cases, δ values were close to three, suggesting that there is a signature of accelerating evolution over time.

Examples of species that deviate from their close relatives can be observed in Fig. 1, which shows the current patterns for AMT and AP per species and their ancestral reconstruction for the whole family. The mean temperature range in the terminal tips is 5.6 ± 3.9 °C, with a maximum range of 16 °C, whereas for precipitation, the mean range is 519 ± 391 mm, with a maximum range of 1,700 mm (*Taricha granulosa*). There is a general pattern where both clades of Asian newts (*Echinotriton* + *Tylostotriton* and Asian modern newts) show higher AMT and AP values than European or North American clades and higher reconstructed values toward older nodes. There is a general homogeneity for the reconstructed AP within major clades, with the exception of *Tylostotriton* + *Echinotriton*. Within a particular clade, most species show closely similar values for AP, and these

remain relatively stable toward basal nodes. However, the nodes corresponding to the ancestor of two clades from different climates (e.g., western Palearctic and Asian modern newts) show values intermediate between them. The most basal nodes in the phylogeny show overall intermediate values in AP and AMT, considering the spectrum available from the species included in the phylogeny. The homogeneity observed in reconstructed precipitation values is less evident for the temperature reconstructions, and only Asian modern newts have similar temperature values. The temperature ranges for reconstructed nodes (Fig. 1) are between 5 °C to 7 °C and between 500 and 650 mm for precipitation, with the exception of some tropical clades (e.g., *Tylototriton-Echinotriton*), which display narrower ranges for both temperature and precipitation. The wide distribution of a single species can affect the reconstruction for the entire genus, as in the case of *T. granulosa*. The relatively high precipitation range reconstructed for the common ancestor of *Taricha* ($\approx 1,000$ mm) is likely an artifact, because three species have ranges of 660 to 930 mm, whereas *T. granulosa* has a greatly expanded range of 1,760 mm but it is nested within this clade.

Ecogeographical Approach. The position of all species of the family in the potential global environmental space is shown in Fig. S1. Salamandrids are mainly distributed in temperate climates (Fig. S1A); 95% of the localities occur between 4 °C and 20 °C and 200 and 1,600 mm of rainfall. Colder and hotter climates are avoided, and AP >200 mm does not seem to be a limiting factor, although the family does not occur in hot and wet tropical conditions. In the western Palearctic (Fig. S1B) species occur in temperate climates. Asian representatives of the family occupy a slightly different climatic envelope, with 95% of the point localities having values from 5 °C to 23 °C and 900 to 3,000 mm of precipitation, thus ranging from temperate to subtropical conditions. North American species occupy a temperate climatic space (Fig. S1D), although some species also occur in cool and wet conditions (e.g., *T. granulosa*), whereas others are present in hotter (≈ 23 –25 °C) and wetter conditions (e.g., *Notophthalmus meridionalis*).

The same outlier taxa (with climate ranges significantly different from their close relatives) that were detected with the phylogenetic approach are also identified by an ecogeographical approach, which provides a different and complementary perspective on the study of their niche evolution. For example, three of the four species of *Taricha* in western North America are broadly syntopic; they diverged relatively recently (Miocene-Pliocene; Fig. 1). The species overlap broadly in the two axes of a principal component analysis (PCA), but *T. granulosa* occupies a much wider climatic space. This species experienced a post-Pleistocene expansion (Fig. 2A), displaying a range that extends to the north, but also occupying an area of the climate space corresponding to wetter and cooler climates. Post-Pleistocene range expansions characterize some European species, such as *Salamandra salamandra*, which, however, does not range as far north as *T. granulosa*. The greatest climatic range in *Salamandra* is displayed by *S. salamandra*, which occupies an exceptionally wide climate envelope in both PCA axes, overlapping nearly all remaining species (Fig. 2B); congeners are much less climatically tolerant.

Common patterns of distributions and climate envelopes of species may have different historical origins, with consequences for the interpretation of patterns and processes of species formation. A clear example is *Triturus* (Fig. 2C), three species of which occur in Western Europe. The most northerly and widespread species, *T. cristatus*, ranging from Britain onto the European mainland from France to the Ural Mountains, overlaps geographically and hybridizes with *T. marmoratus*, a species distributed over most of central and southern France and the northern part of the Iberian Peninsula. South of the Duero River

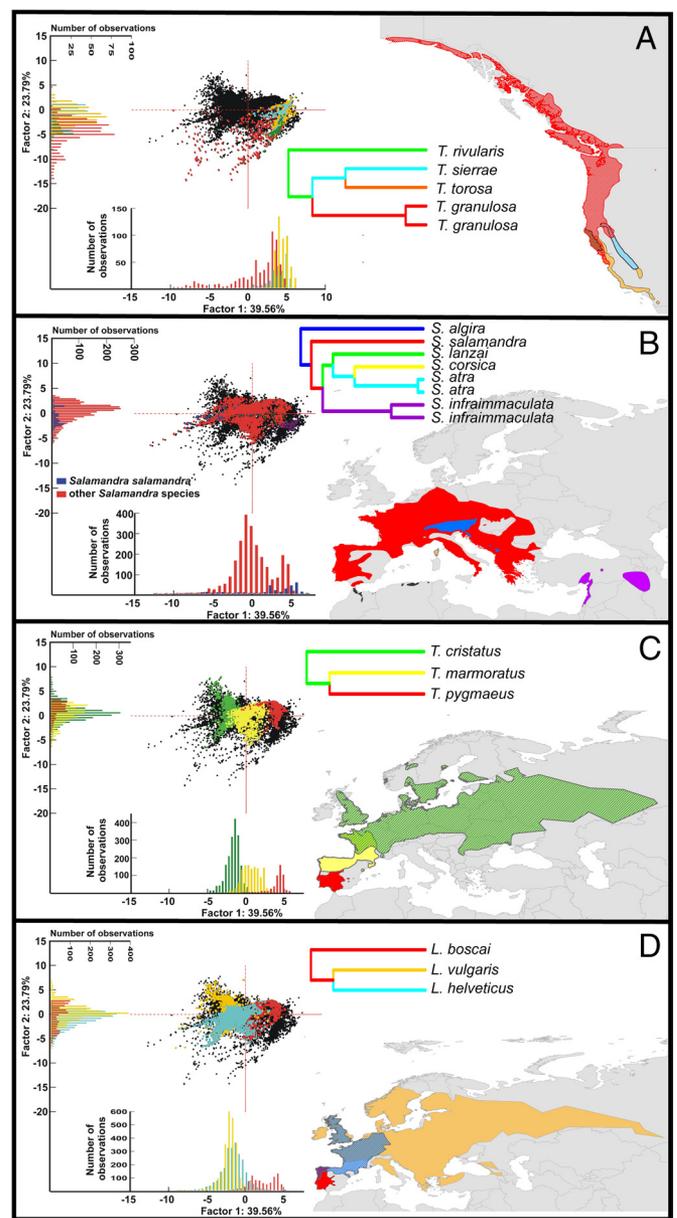


Fig. 2. PCA plots and distribution maps for selected case studies within Salamandridae. (A) PCA plot and distribution map for *Taricha*. (B) PCA plot and distribution map for *Salamandra*. (C) PCA plot and distribution map for selected species of *Triturus*. (D) PCA plot and distribution map for selected species of *Lissotriton*. Black dots represent the environmental space occupied by all species of the family Salamandridae.

on the Iberian Peninsula, *T. pygmaeus* replaces its sister taxon *T. marmoratus* to the west. The two species differ along the first PCA axis and widely overlap along the second, whereas *T. cristatus* overlaps *T. marmoratus* slightly both spatially and along both PCA axes. A Manova analysis, using the PCA axes as dependent variables and species as categorical variables, shows significant differences in the climatic envelopes of the three species (Wilks = 0.15, $P < 0.001$). Although statistically significant separation among species occurs along the two first PCA axes (x axis: $F_{2,3496} = 6922.7$, $P < 0.001$; y axis: $F_{2,3497} = 70.4$, $P < 0.001$), the percentage of variance explained by the first axis is 80%, whereas the second axis explains only 3.9%. Species of *Lissotriton* show a deceptively similar spatial and climatic pattern (Fig. 2D). *L. vulgaris* has a distribution generally similar to *T.*

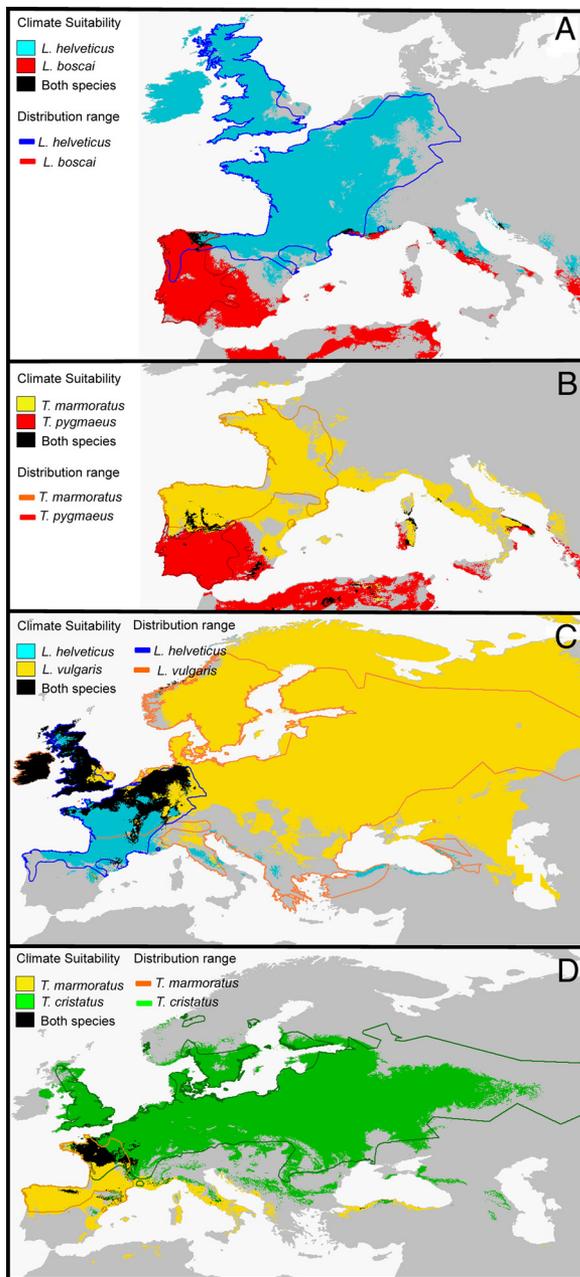


Fig. 3. Pairwise comparisons of modeled climatic suitability for European newts. (A) Comparison of *Lissotriton helveticus* and *L. boscai*. (B) Comparison of *Triturus marmoratus* and *T. pygmaeus*. (C) Comparison between *L. helveticus* and *L. vulgaris*. (D) Comparison between *T. marmoratus* and *T. cristatus*.

cristatus, *L. helveticus* is similar to *T. marmoratus*, and *L. boscai* partly shares its distribution with *T. pygmaeus* in central and western Iberia. The PCA for *Lissotriton* displays a pattern similar to that of *Triturus*, but here the southern species, *L. boscai*, is sister to the other two species (10), whereas in *Triturus* it is the northern species (*T. cristatus*) that is sister to the other two. A Manova analysis shows statistical separation between the three species (Wilks = 0.31, $P < 0.001$). The species of *Lissotriton* overlap along the second PCA axis, which explains only 8% of the variance (y axis: $F_{2,5466} = 236.8$, $P < 0.001$), and only *L. boscai* differs from the other two along the first axis, which explains 55.1% of the variance (x axis: $F_{2,5466} = 3,361.8$, $P < 0.001$).

Climate envelope modeling results for *Triturus* and *Lissotriton* are shown in Fig. 3. All models have area under the curve (AUC)

values >90 and correspond well with the actual distribution of the species. The model for *L. helveticus* (Fig. 3A) predicts accurately the distribution of the species, with some areas of overprediction in northern Africa, Italy, Balkans, and Ireland, which the species never reached because of physical barriers. In northwest Iberia the species is predicted in small areas where it overlaps with *L. boscai*. The model for *L. boscai* (Fig. 3A) also predicts the actual distribution well, with some overprediction in southeastern Spain, where some old records of the species are not confirmed. The model for *L. vulgaris* (Fig. 3C) overpredicts the distribution in the north and underpredicts in the south, especially in central France where the species is present but the climate is apparently unsuitable. The models for *T. marmoratus* and *T. pygmaeus* (Fig. 3B) fit the distribution of both species well, with some overprediction in eastern Iberia and other southern European areas (e.g., Italy, Mediterranean islands, Balkans). The model for *T. cristatus* is a good fit with the current distribution of the species (Fig. 3D), with some overprediction in southern Europe.

Although *L. helveticus* and *L. vulgaris* are sister taxa that overlap widely in predictions of climatic suitability and extant distribution (Fig. 3C), *L. boscai* and *L. helveticus* show little overlap in their climate envelopes and they are only narrowly sympatric in northwest Iberia (Fig. 3A). The parapatrically distributed sister species *T. marmoratus* and *T. pygmaeus* overlap very little in their climate envelopes and distributions (Fig. 3B). Finally, *T. marmoratus* and *T. cristatus*, which are not sister taxa, display a pattern similar to that of *L. vulgaris* and *L. helveticus*, with an important overlap in their climate envelopes and distributions, being sympatric and hybridizing in central and northern France (Fig. 3D). The integration of phylogenetic and ecogeographical data show that the observed parallel geographical distribution patterns in those two clades, with little degree of climate overlap in most comparisons, have different evolutionary history and consequently the processes that gave rise to these patterns likely have been different.

Discussion

Our goal is to explore the potential for tracking climate envelopes backwards in time and examine how components of niches evolve in a phylogenetic context. Climate modeling usually has involved future predictions, but some studies have examined niche evolution in recent past history (see review in ref. 11). We extend climate envelopes into deeper time by integrating several perspectives. We focus on requisites for such studies, evaluate the degree of our success, and give reasons some approaches are more successful than others.

Overall Approach. Integration of diverse methods applied to several independent databases is essential for the success of our approach. The different parts of this study derive from distinct disciplines, with their own intrinsic assumptions, limitations, and requirements. Once an appropriate taxon has been selected (see below), a robust (well-supported statistically), species-level phylogeny is needed. A near-complete sampling of extant species is essential; our approach is ineffective on grossly incomplete phylogenies. To examine how niches evolve, a time tree is essential, so careful attention should be given to its calibration, ideally constrained by well-understood internal fossils. In such a tree, where branch lengths correspond to time, ancestral state reconstruction methods are used to analyze climate values for each species and reconstruct climate envelopes. Available methods assume a Brownian motion model of evolution, hence the data should be a close fit. Significant challenges are apparent. Among them are questions concerning how the range of continuous variables (e.g., climate) are to be coded, so as to incorporate variation within species or assess the degree of climate envelope conservation between species. Climate vari-

ables are unlikely to evolve under a strict Brownian motion model; for example, when niches are more conserved than random evolution (which we expect for salamanders), it is unclear how climate envelope reconstruction should proceed. Niche evolution must have been constrained by past available climates, and models alternative to Brownian motion that incorporate paleoclimate data await development. Niches are multidimensional, but neither the variation within a trait or multiple traits studied simultaneously are incorporated in the reconstructions, which pose challenges for the future of this field.

For ecogeographical perspectives, we advocate using PCAs to determine which climatic variables are the most important and assess the degree of overlap (conservation) or divergence between species in PCA dimensions. Our analysis is based on point locality data, but whether climate envelopes derived from such data represent the real tolerances for the species is uncertain. Species are capable of using behavior to avoid climatic extremes. Differences in temperature, for example, thus might not be real, with respect to the practical impact on organisms. Mechanistic models of fundamental niches can be based on ecophysiological data, highly desirable as proxies and independent of ecological or biogeographical constraints (12, 13), but such data are scarce. Gathering such data are complicated and would require controlled experiments for many species in a phylogenetically comparative framework. The alternatives are models using a general assumption that species distributions are correlated with macroclimatic variables, so that conditions at the localities where species occur reflect their ecological niches (11). Modeling the distribution of species to determine the fit to the actual distribution is critical. If the models fit well, point locality data are in principle good predictors of the ecological niche of the species based on macroclimatic variables. To the degree this is true, the extent of geographical overlap of species can be assessed in relation to ecological niche models. This assessment, an alternative to PCA and ancestral state reconstruction, can be done by analyzing the models using the actual distributions of species based on point locality data. Whenever possible, physiological data should be used instead of point locality data, because such data may be the most appropriate way to investigate the evolution of fundamental niches and ancestral state reconstructions. In all of the above approaches, species interactions, such as competition, which are potential factors in shaping species distributions, typically are ignored. As an example, competition may be a significant factor setting range limits in some instances of parapatry in salamanders (14).

Integration is the last step of this approach. An ideal first level of integration would merge phylogenetic hypotheses with the results of the ecological niche models to explain patterns and infer processes (Fig. 3). The degree of overlap between species in climate space has been used to infer speciation processes, such as allopatric speciation (5, 15). However, our example of two clades (*Triturus*, *Lissotriton*) with similar distributional patterns, but arising from different evolutionary processes, challenges such an approach. Especially significant here is the impact of an unsampled species, for example one that has recently gone extinct, which could change the situation entirely. Had *T. granulosa*, which has a climate envelope that differs greatly from three other members of its clade, been the only survivor of that clade, our interpretation might have been very different. If ancestral state reconstruction is accomplished, a second level of integration is correlating the reconstructed climate components with real paleoclimatic data, to determine whether niches evolved in concert through geological time periods. A good fossil record demonstrating the historical presence of the clade in the study area is essential. A future challenge for the field will be to develop models that directly integrate (rather than correlate) paleo-climate into the reconstructions, to obtain more realistic estimates of niche evolution.

Salamandrids as a Case Study. Selection of taxa should be guided by several criteria: (i) clades of slowly evolving ectotherms likely to be more responsive to climate variables than endotherms; (ii) long-term (millions of years) residents in the study region with low dispersal abilities; (iii) availability of a species-level phylogenetic hypothesis and divergence time estimates for the period analyzed; (iv) high quality (georeferenced point localities) data on the distribution of extant species; (v) the physiological limits of species should be understood; without such data it may not be possible to assess how rapid niche evolution proceeds in a given clade; and (vi) climatic data for both current and past distributions must be available for the regions where the studied taxa occur. The Salamandridae fulfills most of these criteria, although less physiological data are available than we would like. The taxon is of manageable size (≈ 80 species) and is spread across the Holarctic. It originated in present-day Europe, has a good fossil record with continuous representation for the last 50 million years (16), and the climatic history of Europe throughout much of the relevant phylogenesis of the group is well known (17). The root of the family has been dated ≈ 100 mya in the Late Cretaceous (e.g., ref. 18, analysis A of 3), whereas in another analysis (ref. 18, analysis B of 3), a much younger age of 70 mya was obtained. Using new calibrations (*SI Text*) we obtain some younger and some older ages for the origin and diversification of different clades, but in general the results are similar.

Ancestral State Reconstruction of Salamander Climate Envelopes. We treat climate envelopes of species as evolving traits whose evolutionary history can be reconstructed through phylogenetic analysis. Species typically experience a range of climatic conditions across their geographic range, hence intraspecific variability should be taken into account. Averages may not adequately represent variation within a species and might be biased because of incomplete sampling across the species range (19). Alternatively, several coding strategies for continuous variables have been proposed (15, 19–22), and among them, MaxMin coding has been used to infer ancestral climatic ranges in a phylogenetic context (15, 19). This approach reconstructs the minimum and maximum values independently for each internal node in the topology, and those values are used to infer the climatic range for ancestral species. We used both the mean and the 95% confidence values instead of the minimum and maximum because climate values from point localities may not represent the fundamental niches of species and a single maximum or minimum can be a climatic outlier for such data (Fig. S1), expanding the climatic range of the species artificially. Should appropriate physiological data be available (e.g., temperature tolerance range), they should be used for each species instead.

We performed ancestral-state phylogenetic reconstructions of AMT and AP for salamandrids, which serve as a conservative proxy for estimating and reconstructing their fundamental niches. Ancestral-state reconstruction methods for continuous traits assume that each trait evolves according to a stochastic Brownian motion process, with a probability of change along a branch proportional to the length of the branch (21). Although many papers show ancestral-state reconstructions of environmental niche components using this model (15, 19, 23, 24), they fail to provide tests showing whether their data fit this model of evolution, whereas others do not provide an explicit model for niche reconstructions (25, 26). The different hypotheses tested with our model for scaling parameters showed a deviation from a Brownian model. However, we found that a few climate outliers have an important influence on ancestral-state reconstruction and strongly influence tests used to assess how data fit a particular model of evolution (e.g., Brownian motion). When a species is connected by short branch lengths to its close relatives, all showing similar climate ranges except this species,

the model tries to compensate, accordingly, thereby having a large effect on the fit of the whole model. As result of such compensation, λ and κ depart from 1. In our analytical approach, removal of only two outlier species changed λ and κ so that they were no longer significantly different from 1, as expected under Brownian motion (for the two species, λ and κ departed from 1). Removal of the two species also had an effect, although less profound, on δ .

Recently evolved species that have experienced rapid change in a novel direction in their climate envelopes will have a strong impact. Detection and removal of such outliers may be possible. For example, after removing the two species in question, our model suggests gradual niche evolution in salamanders, with a signature of accelerating evolution as time progresses ($\delta > 1$). However, this also suggests that reconstructions of niches may be strongly affected by species for which climate data are not accurate or simply wrong, and by incomplete taxon sampling. The case of *T. granulosa* exemplifies another potential problem in reconstructing niches in a phylogenetic context from point locality data. The extreme climatic range of the species is the result of a Holocene range expansion to Alaska from California, but this wide distributional range influences the climate range reconstructed for the ancestor of the genus. The most parsimonious interpretation is that niche expansion evolved recently in *T. granulosa*, which is deeply nested in its clade; the ancestor of the genus likely displayed similar tolerances to the other species of *Taricha*. However, physiological preferences and limits may not correspond to the climate range extrapolated from point locality data, and *T. granulosa* could resemble its congeners and survive in northern latitudes because of behavioral plasticity. New data will be necessary to clarify these issues.

Evolution of Niches and Geographic Ranges: Patterns vs. Processes.

How niches evolve and their degree of conservation during lineage evolution are relevant for understanding both past diversification processes and the biogeographic history of clades (27). There are ongoing debates about the degree of niche conservatism and its phylogenetic scale (28–30), with some seeing great promise for understanding evolutionary patterns that niche conservatism may produce (29), for example, in vicariant (allopatric) species formation (5, 7). Numerous studies have examined the relationship between ecology and species formation in amphibians (reviewed in ref. 31). In the Salamandridae, vicariant species formation appears to be the rule, as exemplified in many cases studies: (32–36). Nonadaptive species formation, fragmentation of an ancestral species range with niche conservatism through time, was suggested to explain the ecological similarity of plethodontid salamanders in California (7). Information on observed spatial patterns in relation to climate envelopes is increasingly used in studies of species formation (5, 15, 36). For example, comparisons of the distribution and environmental suitability between sister pairs of species of montane plethodontid salamanders suggested that niche conservatism may promote allopatric species formation (5). Our results for ancestral-state reconstructions suggest an overall gradual evolution of salamander climate envelopes through time, and PCAs show a high degree of overlap in one dimension of the salamandrid species envelopes (precipitation variables), which is concordant with evolutionary conservatism. However, inferring how speciation happened from those patterns may be challenging, as exemplified by European newts (Figs. 2 and 3), which show how similar geographical and environmental patterns can be achieved through different evolutionary processes, where the same degree of environmental and spatial overlap, or lack thereof, is found between sister species and close nonsister relatives in spatially codistributed independent clades. Such patterns suggest that factors such as geographic range changes related to paleo-climatic conditions,

species interactions, or even niche evolution might be at least partially responsible for the detected patterns.

Because the origins of extant salamandrid species predate the Pleistocene (Fig. 1), species have persisted through several relatively recent (Pleistocene) glacial and interglacial cycles. Either species were tracking changing climates, contracting and expanding their ranges, or persisting in climatically stable areas, or some species may have a wide climatic tolerance that enables them to persist under changing climatic conditions, perhaps by behavioral plasticity. Salamandrids originated in Europe and dispersed at least twice to Asia and once to North America despite being ectothermic organisms with low dispersal capacity. Dispersal to other biogeographic regions may have been triggered by periods of global climatic change (4). Widely disjunct distributions of some closely related species raise questions concerning past climatic regimes that enabled spread from a place of common origin to the current ranges. The integration of paleo-climatic reconstructions, climate envelope modeling, and fossil and phylogenetic data show promise for resolving such questions.

Species interactions can affect geographic distributions. Sister species often have broadly overlapping climate envelopes and contiguous but largely nonoverlapping geographic ranges (Fig. 3B), which is evidence for the potential role of such interactions reflecting some combination of “competition” and/or perhaps a legacy of allopatric speciation. In some cases this situation can be extreme, with sharp contact zones of 100 m [as in torrent salamanders (37); in others, sister taxa coexist over much of their ranges (Fig. 3C)]. However, analyses should not be limited to sister species, because competition may be also important with phylogenetically distant species. Salamanders cannot expand in all directions of the global environmental space; during rapid climatic change most species are unlikely to be able to migrate to new areas sufficiently rapidly to be successful. Rapid climate changes probably will drive extinctions or major distributional changes in the future as in the past (4), yet amphibians survived past global extinctions (38). Behavioral and ecological plasticity (e.g., by reducing exposure to climate by displaying strong seasonality) and adaptations such as the multiple evolution of paedomorphosis as a way to escape conditions on land (39), or re-evolution of aquatic larvae in direct-developing terrestrial salamanders (40), may explain why species have been able to persist during periods of adverse climate. Niches may have evolved very rapidly in association with invasions of new ecological and geographic spaces, as in postglacial range expansions. In salamanders such expansions are known (*S. salamandra*, *T. granulosa*), but examples of rapid evolution of climatic niches are not. The first species is widely tolerant in all directions of its climate envelope (Fig. 2). *T. granulosa* also experienced a postglacial expansion, with northern populations genetically similar to southern ones (41). Range expansion in these species was probably enabled by physiological tolerance and higher dispersal capacity with respect to other species (42), rather than rapid evolution of their fundamental niches.

Prospects for Reconstructing Climate Envelopes in Geological Time.

Attempts to reconstruct the evolution of niches in a phylogenetic context are few, although the number of studies is increasing (8, 15, 24–26, 43–45). A special challenge is extending such reconstructions into geological time, which requires integration of phylogenetic, fossil, climatic, and ecological datasets. The first step in the integration would be to correlate phylogenetic reconstructions of climate variables with paleoclimate, in areas where there is a high degree of certainty of historical presence of the study clade (e.g., with a good fossil record). This is the case of salamandrids in Europe, with a continuous fossil record for the last 40 million years (16). Data on paleotemperature for central Europe

during the last 48 million years, inferred from a chronostratigraphic study of fossil paleofloras, show a gradual cooling, while AP was relatively stable (17). Our reconstructions show a closely similar trend, with stability toward older nodes, which may be in agreement with paleoclimate. However, stochastic models (e.g., Brownian motion) may be inaccurate for environmental characters thought to be subject to directional or stabilizing selection or adaptive radiations that implicate rapid and significant amounts of character change (46–49). A further step in the integration will be to develop new models that are appropriate for such data. The climate in which species evolved is not today's climate, and because ancestral climate envelopes were constrained by past climates, these models would benefit by incorporating such temporal climate constraints into the reconstructions. Physiological data will provide closer estimates to fundamental niches, and because niches are multidimensional, methods that allow reconstructing several variables at a time (e.g., precipitation + temperature) need to be developed.

New implementations, such as those outlined above, will enable investigation of how niches evolve and how far back those can be reconstructed. Doubtless such efforts will be specific to clades, and appropriate modifications of the approach will necessitate detailed knowledge of the biology of members of each clade. The degree of niche conservatism will depend on the phylogenetic scale of the study, the time of origin of the clades, and in particular the capacity of the taxa studied to evolve their niches. One expects organisms such as salamanders, slowly evolving, highly philopatric ectotherms heavily dependent on their environment, to differ from the more rapidly evolving, endothermic birds and mammals in degree of niche conservatism. We believe that prospects for future developments in this field are good, but we are far from understanding how niches evolve, how they responded to past climate changes and how they are likely to respond to future climates.

Materials and Methods

A detailed explanation of phylogenetic reconstructions and divergence time estimates can be found as *SI Text*.

Modeling Climate Envelopes of Species. A comprehensive locality database for all species of the Salamandridae was assembled from different sources, including voucher specimen data from museum collections mainly available on Herpnet, global databases like GBIF, literature searches, and unpublished records. Available data on existing databases were carefully evaluated and many records were discarded, mainly because of imprecision or lack of coordinates, species misidentifications, species occurring outside their current ranges, and record duplication. In many instances, records did not reflect current taxonomy for some species' and they were evaluated and corrected individually when possible. Almost 18,000 species locality records were included in the database. We extracted the climatic values for each locality in the database from a set of 19 climatic Geographic Information Systems (GIS) layers from the WorldClim database version 1.4 (1). To investigate how much of the

environmental space available on Earth is occupied by representatives of the Salamandridae, we plotted AMT and AP data from occurrences of all salamandrid species available to us into a 2D plot together with 10,000 randomly generated background points that cover all climates available on Earth (Fig. S1). We performed a PCA in Statistica 6.0, including all salamandrid species for which molecular data are available to examine the levels of divergence in climatic space among these taxa.

Climate envelopes were modeled for a subset of species by using MaxEnt version 3.3.1, a maximum entropy method for predicting species distributions (45). Models were developed for each species using 70% of the data randomly selected as training data and the remaining 30% for testing the model. To estimate real absences we used the entire locality dataset for Palearctic Salamandridae and their distribution ranges, instead of random pseudo-absences that could be far outside the climatic tolerance of salamanders. As predictive variables we selected the environmental variables that contributed the most to the PCAs (Table S1). Models were evaluated by using the area under the receiving operation characteristic curve, known as AUC, to estimate the accuracy of the distribution models. For the output maps, we used the logistic format that gives a probability estimate of presence. Those maps were reclassified, from continuous probabilities to binary maps of presence and absence, using the sensitivity-specificity sum maximization. For selected pairs of species, we calculated the degree of overlap between their climate envelopes in GIS.

Ancestral Reconstructions of Climatic Variables. We used Mesquite (ref. 20 and <http://mesquiteproject.org>) to reconstruct ancestral characters under a Brownian motion model of trait evolution. For each species we calculated the mean and 95% confidence intervals of the values for the AMT and AP from all of the localities available per species. Those values were used as input data for the reconstructions. Ancestral climate ranges for each major node were estimated following an approach similar to the MaxMin coding (15, 19). We used the program Bayestraits (ref. 9 and www.evolution.rdg.ac.uk/Bayestraits.html) to assess whether traits evolve under a Brownian motion model of evolution. If they evolve under such model, the values of the scaling parameters (λ , δ , κ), should be equal to one (the default model in Bayestraits). We used LR tests to statistically compare the default model to models in which each parameter is allowed to take its maximum-likelihood value (9). We also tested whether trait evolution was independent of the phylogeny (no correlation between traits and phylogenetic relatedness) by setting $\lambda = 0$ and compared the likelihood of this model with λ equal to its maximum likelihood. The value of κ indicates whether traits evolve with a punctuational mode of change ($\kappa = 0$) versus gradual evolution of characters on the phylogeny ($\kappa = 1$). We tested this by comparing a null model with $\kappa = 0$ to the model setting κ to its maximum-likelihood value. Finally, if $\delta < 1$ indicates an adaptive radiation, then values >1 indicate species-specific adaptations. We tested these alternatives as before, setting $\delta = 0$ and compared the models using LR tests. Because strong directionality during character evolution may cause bias in character reconstruction (9), we also tested whether the data better fit a standard constant-variance random walk model versus a directional random-walk model, to detect any general trend in the direction of evolutionary change.

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