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# A stable niche assumption-free test of ecological divergence

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## ARTICLE INFO

## ABSTRACT

Understanding the impact of geological events on diversification processes is central to evolutionary ecology. The recent amalgamation between ecological niche models (ENMs) and phylogenetic analyses has been used to estimate historical ranges of modern lineages by projecting current ecological niches of organisms onto paleoclimatic reconstructions. A critical assumption underlying this approach is that niches are stable over time. Using *Notophthalmus viridescens* (eastern newt), in which four ecologically diverged subspecies are recognized, we introduce an analytical framework free from the niche stability assumption to examine how refugial retreat and subsequent postglacial expansion have affected intraspecific ecological divergence. We found that the current subspecies designation was not congruent with the phylogenetic lineages. Thus, we examined ecological niche overlap between the refugial and modern populations, in both subspecies and lineage, by creating ENMs independently for modern and estimated last glacial maximum (LGM) newt populations, extracting bioclimate variables by randomly generated points, and conducting principal component analyses. Our analyses consistently showed that when tested as a hypothesis, rather than used as an assumption, the niches of *N. viridescens* lineages have been unstable since the LGM (both subspecies and lineages). There was greater ecological niche differentiation among the subspecies than the modern phylogenetic lineages, suggesting that the subspecies, rather than the phylogenetic lineages, is the unit of the current ecological divergence. The present study found little evidence that the LGM refugial retreat caused the currently observed ecological divergence and suggests that ecological divergence has occurred during postglacial expansion to the current distribution ranges.

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## 1. Introduction

Geographic range contraction, fragmentation, and expansion caused by Pleistocene glacial cycles all have had a major impact on the spatial distribution of genetic variation across many taxa (Avise et al., 1998; Hewitt, 2000; Zamudio and Savage, 2003; Steele and Storfer, 2006; Walker et al., 2009). The prominence of such geological events, particularly that of glacial retreat since the last glacial maximum (LGM), has led to questions regarding their influence on evolutionary diversification. Our ability to address the influence of geological processes on diversification has greatly improved with the recent development of methods that facilitate phylogenetic hypothesis testing coupled with the advent of ecological niche models (ENMs). For example, ENMs offer means to evaluate phylogeographic inferences based on simulated past distribution patterns (Knowles et al., 2007; Richards et al., 2007; Peterson and Nyari, 2008; Walker et al., 2009), as well as to test current niche divergence among closely related lineages (Graham et al., 2004; Rissler and Apodaca, 2007; Pyron and Burbrink, 2009).

Predicting past ENMs is an attractive and promising tool for the fields such as biogeography, evolutionary ecology, paleontology, phylogenetics, and systematics. However, the current method of developing historical ENMs relies on present-day species distribution data to predict past niches and thus rests on the assumption that ecological niches of target organisms are stable through time (Nogués-Bravo, 2009). This stable-niche assumption is problematic for at least three reasons. First, without abundant paleoecological

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and paleontological evidence, it is often difficult to corroborate the assumption of niche stability. Second, the increasing evidence for ecological speciation (Nosil et al., 2009; Schluter, 2009) suggests notable exceptions (but see Wiens, 2004; Wiens and Graham, 2005; Kozak and Wiens, 2010 for speciation via niche conservatism). Finally, there is no quantifiable definition of what constitutes a sufficiently "stable niche," wherein current niche variables can be appropriately used to predict past ENMs (Nogués-Bravo, 2009). As a consequence of the difficulty in assessing niche stability through time, there has been little attempt to test for an ecological niche shift between the past (e.g., LGM) and the present using ENMs. In response to environmental changes typical of large scale geologic events such as the LGM, populations must move, adapt, or suffer extinction.

Previous studies that integrate ENMs with phylogeographic analyses generate past ENMs based on the stable-niche assumption to evaluate inferences drawn from population genetic structures (Hugall et al., 2002; Carstens and Richards, 2007; Knowles et al., 2007; Waltari et al., 2007; Peterson and Nyari, 2008; Solomon et al., 2008; Jakob et al., 2009; Walker et al., 2009). While results from these disparate analyses are often in general agreement, Waltari et al. (2007) found that for 6 of 20 studied species, predicted historical distributions showed significant discordance between the predicted ENMs and phylogeographic analyses. Moreover, other studies found evidence of recent niche divergence between closely related taxa (Graham et al., 2004) and even within a single species (Rissler and Apodaca, 2007; Pyron and Burbrink, 2009). Furthermore, remarkably rapid niche shifts (less than 120 yrs) have been detected in a principal-component environmental space between the native and the invasive populations of spotted knapweed *Centaurea maculosa* (Broennimann et al., 2007) and Asian tiger mosquito *Aedes albopictus* (Medley, 2010). Indeed, there is increasing empirical evidence that adaptation to novel niches can occur rapidly, even potentially resulting in speciation events (see reviews in Rundle and Nosil, 2005; Nosil et al., 2009; Schluter, 2009). Thus, the assumption of stable ecological niches since the Pleistocene is not universal, and should rather be an empirical question. To this end, developing a framework for historical ENMs that are free from the stable-niche assumption would advance research that explores the importance of geological events in shaping ecological divergence of any species.

We used the North American eastern newt, *Notophthalmus viridescens*, as a model to introduce a niche-stability free analytical framework for examining the role of Pleistocene refugia in post-LGM ecological niche divergence. Many wide-ranging species exhibit considerable intraspecific phenotypic variation (e.g., elk [*Cervus canadensis*], greenish warblers [*Phylloscopus trochiloides*], northern cricket frog [*Acris crepitans*] and common kingsnake [*Lampropeltis getula*]). Such phenotypic variation often reflects local adaptation and thus offers opportunities for testing the effects of the LGM on intraspecific ecological divergence. *Notophthalmus viridescens* comprises four subspecies based on morphological variation and ecological differentiation (Mecham, 1967; Fig. 1). The results from the common garden experiments suggest that the ecological differences among the subspecies observed in nature are genetically-based, rather than plastic; the subspecies differ in the expression of the life history traits such as larval period, body mass at metamorphosis, and life cycle polyphenism (i.e., paedomorphic vs. metamorphic adults; Takahashi and Parris, 2008; Takahashi et al., 2011).

We focus on the LGM and the subsequent postglacial warming as a potential catalyst of ecological niche divergence in *N. viridescens*. Hypotheses for the timing of recent niche divergence include: (i) refugial retreat during the LGM, (ii) postglacial range expansion to novel environments following the LGM, or (iii) a combination of both. When fragmented populations that have retreated into distinct refugia experience different selective pressures and become adapted to different refugial environments, ecological divergence can occur in allopatry during the LGM. The pattern of ecological divergence attained during the LGM may still be maintained at present even after postglacial expansion reconnecting the fragmented populations (i.e., secondary contact; Schluter, 2001). In such a scenario, the current ecological niches are predicted to be similar to those during the LGM and we should observe phylogenetic clustering in niche space. Alternatively, postglacial range expansion following the LGM may allow organisms to reach novel habitats and may facilitate adaptive divergence (e.g., ecological speciation in postglacial fishes, Schluter, 1996). This scenario predicts the ecological disparity between the LGM and the current niches, suggesting the violation of the niche stability assumption.

To test the hypotheses pertaining to the ecological niche divergence of *N. viridescens*, we first tested phylogenetic predictions resulting from the assumption that their subspecies are congruent with phylogenetic lineages. We then adopt the statistical method of Lemmon and Lemmon (2008) to estimate the geographic location of ancestral populations in conjunction with paleoclimatic reconstructions to create LGM niche models for refugial lineages. Next, we quantitatively assess niche divergence by comparing contemporary to historical ENMs through principal component analyses and niche overlap analyses. Our test of phylogenetic concordance between refugial lineages and subspecies delineation establishes a critical foundation for the subsequent construction of the LGM ENMs and the niche comparison between the LGM populations and the current subspecies. Additionally, we tested for niche shift between the refugial populations and the contemporary phylogenetic lineages of *N. viridescens* when the current subspecies designation was not congruent with refugial lineages.

## 2. Materials and methods

### 2.1. Study organism

The subspecific patterns of life-history variation within *N. viridescens* are associated with the environmental conditions of the subspecies distributional ranges (Takahashi and Parris, 2008; Takahashi et al., 2011). Larvae of the most terrestrial subspecies, *N. v. viridescens* (the red-spotted newt), typically metamorphose rapidly to leave ponds as terrestrial juveniles (called efts) and return to aquatic habitats as adults in 3–7 years. This subspecies, which has two rows of distinct red spots on its dorsum, is predominantly distributed within the Appalachian Highlands (Fig. 1) where permanent wetlands are scarce, but ephemeral vernal pools are common (Babbitt and Groat, 1998). These areas also offer ideal woodland habitats for terrestrial salamanders as evidenced in the abundance and diversity of terrestrial plethodontid salamanders (Wilbur and Collins, 1973; Petranka, 1998). In contrast, *N. v. piaropicola* (the peninsula newt), which lacks any dorsal patternings, typically completes its entire life cycle in aquatic habitats. This almost exclusively aquatic subspecies is distributed in the Florida peninsula (Fig. 1) where relatively permanent wetlands are abundantly available. The sandy and hot terrestrial environment of Coastal Plain in Florida likely offers unsuitable habitats for terrestrial salamanders. *Notophthalmus v. dorsalis* (the broken-striped newt) with discontinuous two red lines on its dorsum and *N. v. louisianensis* (the central newt) with small inconspicuous red spots on its dorsum are distributed along the Interior and Coastal Plains (Fig. 1) where wetlands are relatively abundant, but have widely varying hydroperiods. These two subspecies have life-history characteristics intermediate between *N. v. viridescens* and *N. v. piaropicola*. Both subspecies exhibit life-cycle polyphenism (i.e.,