Moving up:
Using climate, physiology, and gene flow to characterize current
and future geographic range limits in montane salamanders

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ABSTRACT

What causes, maintains, and changes species' geographic ranges are central

questions in ecology and evolution. Geographic ranges are a complex product of both

ecological and evolutionary processes, reflecting current biotic and abiotic conditions as

well as gene flow, drift, adaptation, and history. It is only through understanding the

factors that influence species past and present distributions that we can begin to

accurately predict how these distributions will change in the future. Anthropogenic

climate change poses a major threat to native biodiversity around the world, but

especially in montane systems. Understanding the dynamics at these lower elevation

range limits is of particular importance. My dissertation has sought to elucidate why

adaptation fails at the range edge and how that influences current and future species

distributions. For this work, I focused on mountaintop, terrestrial, lungless salamanders

of the genus *Plethodon.*

A commonly invoked hypothesis for the inhibition of range expansion centers

around the idea that asymmetrical gene flow from a densely populated range center

prevents local adaptation at the range periphery. In Chapter 1, I quantified gene flow and

effective population size along a bidirectional elevation transect in the Smoky Mountains,

for the species *Plethodon jordani.* I found evidence for downslope biased gene flow and

more dense mountaintop populations. In Chapter 3, I further explored the potential for

asymmetric gene flow to limit adaptation by assessing both gene flow and phenotypic

differentiation in the species *Plethodon ouachitae* in the Ouachita Mountains. Unlike my

findings in the Smoky Mountains, in the Ouachitas, there was no indication of

asymmetrically biased downslope gene flow, even though population density appears to

diminish at low elevation. On the majority of transects movement appeared to be biased upslope. Within a single mountain, I found sampling sites were connected by gene flow supporting a single panmictic population within a mountain. Between mountains, I found an overall signature of genetic structure with populations segregating by mountain, supporting prior work that indicated unique mitochondrial lineages on each mountain. Correlative niche models built on occurrence records for each individual mountain indicate that the abiotic conditions occupied by populations on each mountain are different. These same metrics have been used in other work to indicate niche divergence between species and as indication of niche adaptation. However, I found neither differentiation in metabolic rate thermal sensitivity nor differentiation in acclimation ability between populations on different mountains and populations at different elevations. These findings support that mountaintop endemic *Plethodon,* even in the absence of gene flow shows conservation in these ecophysiological traits.

In Chapter 2, I used this species-specific physiology to predict shifts in future distributions for four montane *Plethodon* in the Southern Appalachians. I was able to predict current range limits with high accuracy using both correlative and mechanistic distribution models for the three mountaintop species. Neither model was able to accurately predict the distribution of the one lower elevation generalist species, most likely because these limits are determined by biotic interactions as well as climate. As hypothesized the mechanistic model forecasted more suitable habitat under almost all future climate scenarios for the three mountaintop species. The choice of global circulation model had an order of magnitude influence on how much suitable habitat was

predicted for both distribution modeling methods. All models indicate that these animals

will be quickly contracting their distributions upslope.

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INTRODUCTION

What causes, maintains, and changes species' geographic ranges are central

questions in ecology and evolution. Geographic ranges are a complex product of both

ecological and evolutionary processes, reflecting current biotic and abiotic conditions as

well as gene flow, drift, adaptation, and history. For these reasons, species distributions

are an excellent testing ground for evaluating hypotheses concerning the ecological niche,

environmental thresholds, and the limits of adaptation (Holt and Keitt 2005). It is only

through understanding the factors that influence species' past and present distributions

that we can begin to accurately predict how these distributions will change in the future.

Anthropogenic climate change poses a major threat to native biodiversity around

the world, but particularly in montane systems (Parmesan 2006, Thuiller et al. 2008, La

Sorte and Jetz 2010, Gottfried et al. 2012). As the global climate warms, persistence of a

species *in situ* will depend on its tolerance of changed conditions via acclimation or

adaptation to them, or geographic shift of the range. For many montane species,

movement to track a latitudinal shift in their climatic niche would require passing through

inhospitable valleys, uniquely imperiling these species. Given that mountains are hotspots

for species richness and some montane species are already contracting their distributions

upslope (e.g. Parmesan and Yohe 2003, Wilson et al. 2005, Moritz et al. 2008,

Raxworthy et al. 2008, Rovito et al. 2009, Chen et al. 2011, Feeley et al. 2013, Freeman

and Class Freeman 2014), understanding the dynamics at these lower elevation range

limits is of particular importance.

My dissertation has sought to elucidate whether adaptation fails at the range edge

and how that influences current and future species distributions. I have focused my

research on a system of mountaintop terrestrial, lungless salamanders of the genus *Plethodon.* For this genus, there is evidence that its environmental niches have been conserved through past climatic fluctuations (Kozak and Wiens 2006, Shepard and Burbrink 2008, Kozak and Wiens 2010a). Species have tracked geographic shifts in abiotic conditions, moving downslope during periods of cooling and upslope during periods of warming. During periods of warming these populations have become "stuck" on the mountaintops, restricting gene flow between mountains, leading to diversification (Kozak and Wiens 2006, 2007, 2010a). Niche conservatism may result in the observed biodiversity pattern, but the processes that establish and reinforce environmental niches, maintaining stable climatic tolerance limits along environmental gradients, remain poorly understood.

Why might populations at the range edge fail to adapt to local environmental conditions and thus fail to expand into adjacent habitat? A commonly invoked hypothesis for the inhibition of range expansion centers around the idea that asymmetrical gene flow from a densely populated range center impedes local adaptation at the range periphery (Kirkpatrick and Barton 1997, Lenormand 2002). Genotypes from a more densely populated center are likely to be poorly adapted to conditions on the periphery. As a result of gene flow, these genotypes can swamp out alleles that confer higher fitness at the edge, thereby impeding local adaptation. Regions with high population density could determine which genotypes are most prevalent across the entire range, even if those genotypes are only adaptive in the center of the range. Alternatively, gene flow could enhance adaptation through the influx of beneficial alleles or through masking deleterious alleles in small peripheral populations (Whiteley et al. 2015, Fitzpatrick et al. 2017). Few

studies have empirically tested the predictions of asymmetric gene flow in vertebrate populations. My objective was to evaluate the interaction between ecological and evolutionary processes at the range limit of montane salamanders in the Appalachian and Ouachita Mountains.

In Chapter 1, I quantified gene flow and effective population size along a bidirectional elevation transect in the Smoky Mountains, for the species *Plethodon jordani*. In order to assess the role of gene flow in limiting adaptation at the range edge, it was necessary to set up multiple elevational transects while also testing for trait differentiation, which I was able to do in the Ouachita Mountains in chapter 3. I was able to test the role of gene flow in limiting adaptation by measuring gene flow and differentiation in key eco-physiological traits (metabolic rate thermal sensitivity and acclimation) for the mountaintop species *Plethodon ouachitae. Plethodon ouachitae* occupies six mountains in the Ouachita Mountains of Oklahoma and Arkansas. I leveraged this pattern of one species on multiple mountains to assess gene flow and differentiation for eight elevational transects on three mountains, while also quantifying gene flow, population structure, and differentiation between mountains, where movement is expected to be more limited.

Niche conservatism has implications for the current distributional patterns of mountaintop salamanders as well as for future distributions. The lack of evidence for adaptation in ecologically meaningful traits across different environments in current distributions suggests that these species may not adapt to future climate change. It appears more likely that they will shift their distributions, tracking the climatic envelope as it shifts upslope. These species thus risk running out of space on the top of the

mountain. Species distribution models, specifically correlative models, are the most commonly used methodology for predicting future habitat suitability under anthropogenic climate change. Correlative modeling tools, like MaxENT, use species' current presences to model where on the landscape there is suitable habitat under current environmental conditions; this can then be projected into the future to see where those combinations will exist under different climate change scenarios. For mountaintop *Plethodon* in the Appalachians, correlative species distribution models predict extinction for many species in the next fifty years. However, these methods have been criticized for their inability to extrapolate to non-analogous climates. Additionally, when forecasting future climates, researchers must make decisions on what future climate scenario to use. This involves choosing a representative concentration pathway (RCP), reflecting different pathways for how much CO_2 humans will emit in the future, as well as choosing a global circulation model (GCM). While the implications of RCP choice are straightforward, the differences between GCMs reflect the model complexity and parameters chosen by the earth science labs developing the models. Many studies using species distribution modeling pick one or two GCMs without justification of the choice. In Chapter 2, I sought to test whether this dire future of *Plethodon* extinction was strongly supported or the result of over-extrapolating a correlative model and use of certain global circulation models.

In Chapter 2, I compared the current and future suitable habitat predictions for four species of *Plethodon* in the Southern Appalachians, three mountaintop species and one low elevation generalist, using two methods for modeling distributions. For these distribution models, I used both MaxENT, a correlative approach, and a biophysical mechanistic model that was developed specifically for *Plethodon*. The mechanistic model

uses species-specific physiology to model where on the landscape individuals are in energy surplus based on foraging dynamics and the costs of growth and reproduction. Since this model is not built on occurrence locations but rather on the animal's biology, this method is not hindered by extrapolating into novel future climates. Whether there is enough suitable high elevation habitat for these species to persist will depend on the magnitude of future warming.

Chapter 1

Determinants of Range Limits in Montane Woodland Salamanders (Genus

***Plethodon*)[1]**

ABSTRACT

Montane regions are centers of endemism and species richness for many taxa, including plethodontid salamanders. The forces creating and maintaining species' elevational range limits have been extensively studied in members of the genus *Plethodon.* However, the mechanisms underlying these limits are still poorly understood. Prior work has often focused on range limits from a single perspective, testing ideas of niche conservatism and climatic sensitivity or interspecific competitive interference. Range limits are a complex interaction of both ecological and evolutionary processes. Biotic and abiotic factors may be interacting at different scales, regulating genetic drift, gene flow, and local adaptation. It is only through integrating these ideas across multiple systems that we will be able to begin addressing what limits species' elevational distributions.

INTRODUCTION

All species exist in a limited geographic range, but their distributions can vary greatly in size. For example, some species' ranges span continents whereas others are restricted to a single mountaintop. Despite extensive work characterizing species

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[1] A version of this chapter has been published with coauthors

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distributions, the processes that generate and maintain these ranges are still not well understood. The question of what limits the ranges of species has achieved heightened relevance in the face of climate change, especially in montane regions, which are centers of endemism and diversity for many plant and animal taxa (Myers et al. 2000, Parmesan 2006). A warming global climate is of particular concern for montane species because they can only track their niche as far as the top of the mountain (Parmesan 2006). Moreover, given that the intervening lowland regions between adjacent mountaintops seem to be climatically unsuitable for many montane specialists, migrating to higher latitudes may be impossible, even over small geographic distance. Understanding the mechanisms that generate and maintain species' elevational ranges is therefore essential for predicting how montane biotas will respond to climate change (Myers et al. 2000, Parmesan 2006, Graham et al. 2014).

What causes some species to be "stuck" on the top of a mountain? In landscapes like the sky islands in the desert southwest of the United States, the causes of montane endemism are obvious—species adapted to cool, mesic forests are unable to occupy the harsh desert habitats at lower elevations (Parmesan 2006, Knowles et al. 2007, Waltari and Guralnick 2009, Galbreath et al. 2009). However, in many montane regions, the elevational range limits of species are not associated with dramatic climatic barriers to dispersal. For example, the southern Appalachian Mountains harbor many high-elevation endemics (Stein et al. 2009). Yet, highland and lowland habitats are generally quite similar; both consist primarily of temperate deciduous forest (Delcourt and Delcourt 1980). Moreover, many species that are restricted to high-elevation habitats have close relatives that thrive at lower elevations (Wiens 2004). Why then do montane species fail

to occupy adjacent, lowland habitats?

Woodland salamanders (genus *Plethodon*) are an ideal system for studying the processes that drive species' elevational range limits. *Plethodon* are terrestrial salamanders that occur in forested habitats across North America (Highton 1995). As lungless ectotherms with direct development, activity, performance, and fitness are tightly linked to variation in temperature and moisture (Spotila 1972, Feder 1983). The elevational limits of *Plethodon* have been rigorously documented through extensive field collections over the past 50 years. Although a few species appear to thrive in lowland habitats, the clade reaches its greatest species richness in the southern Appalachian Mountains (Highton 1995, Kozak and Wiens 2010a, 2012). Many of the species that comprise this hotspot of woodland salamander diversity occur exclusively in high-elevation habitats (Highton 1995, Kozak and Wiens 2010a), and therefore provide "natural replicates" for testing whether similar processes limit the ranges of montane endemics, or alternatively, whether the causes of montane endemism are idiosyncratic and species-specific.

Given that their ranges often abut those of more widespread species, competition has long been hypothesized to limit the ranges of montane endemics (Hairston 1951, Diamond 1970, Jaeger 1971, Terborgh and Weskef 1975, Mayr and Diamond 1976). Many species of *Plethodon* display interspecific aggression and territoriality associated with interference competition (Jaeger 1971, Thurow 1976, Hairston 1980, Marvin 1998, Marshall et al. 2004), which would likely limit the ranges of inferior competitors. However, recent work on range limits of Appalachian *Plethodon* employing mechanistic- and distribution-based niche models suggested that elevational range limits of

mountaintop species are attributed to specialization to climates occurring at certain elevations (Arif et al. 2007, Gifford and Kozak 2012), and that competition with montane species prevents low-elevation species from occupying climatically suitable habitats at higher elevations.

Here, we review the current state of knowledge on the influence of biotic interactions, abiotic conditions, and population dynamics on the elevational range limits of North American woodland salamanders of the genus *Plethodon*. Next, we focus on the range limit of *Plethodon jordani* as this species has been studied extensively including behavioral trials and a mechanistic model, and to which we here add population genetics. Finally, we propose some promising future directions for investigating multiple drivers of range limits using recently developed molecular and geospatial tools.

Biotic interactions

Range limits due to biotic influences are expected when species interact in a way that drives down the population size of one or more of the interacting species. Biotic interactions are frequently invoked as the primary factor determining species' warm-edge (lower latitude) range limits whereas abiotic factors are considered to be more important in setting cold-edge (higher latitude) range limits (Dobzhasky 1950, MacArthur 1972). The general idea is that abiotic conditions are more favorable at lower latitudes for most species, but species richness, and thus the number of competitors, is also higher. In support of this idea, studies have found that biotic interactions more often determine species range limits in tropical systems than in temperate systems (reviewed in Schemske et al. 2009). Studies on species at higher latitudes have not widely supported biotic

interactions as drivers of warm-edge limits (reviewed in Cahill et al. 2013) and *Plethodon* may be less likely to be impacted by this phenomenon because of their extreme sensitivity to desiccation in warmer habitats (Peterman 2013). Prior work has approached warm range limits through the lens of either biotic or abiotic factors; however, competing species often come into contact in areas that experience additional pressures related to energy availability or habitat connectivity; many of these contact zones may be characterized by interactions among multiple factors.

Although biotic determinants of species ranges have primarily been studied in the context of competitive interactions, predation and parasitism could play a role in limiting species distributions, most likely through interactions with other determinants. Theoretical work has shown that predators have the potential to drive range limits of prey species, especially if prey occur along a gradient of productivity (reviewed in Gaston 2009). Predation on plethodontids has been studied primarily in the context of aposematic coloration (Hansel and Brodie 1976, Tilley et al. 1982, Fitzpatrick et al. 2009) and antipredator behavior (Feder and Arnold 1982). The pressures exerted by predatory snakes, birds, and mammals have not been extensively examined in *Plethodon*, but predation is unlikely to drive elevational range limits because distributions of most of these potential predators span the ranges of both montane and lowland species. Parasites have the potential to limit species ranges, but little is known about how parasites impact salamanders. *Plethodon* are subject to various parasites, but the natural history of these parasites is poorly understood. Some parasites such as the arthropod mites *Hannemania sp.* are found on *Plethodon,* but species vary widely in the frequency of infection (McAllister et al. 2002, Westfall et al. 2008). For example, *P. ouachitae* showed an 80%

infection rate while sympatric *P. albagula* were uninfected (Anthony et al. 1994). Species of *Hannemania* are found on other amphibians across the eastern U.S.; however, because the inter-dermal encapsulation stage happens during larval development, it has not yet been possible to characterize mites across salamander species. Like predators, parasites may exert varying levels of pressure on different species or in different habitats, and in this way predation and parasitism could interact with other range limiting factors, especially interspecific competition.

Species of *Plethodon* commonly exhibit elevational turnover where a mountaintop specialist is replaced at a lower elevation by a more widespread, generalist species; replacement occurs with minimal or no range overlap (Hairston 1951, Highton 1972). In contrast, other plethodontid genera generally do not show the sharp geographic turnover seen in *Plethodon*, and instead partition microhabitats within overlapping ranges (Hairston 1986). This pattern of sharp elevational species turnover in *Plethodon* has been used to support the hypothesis that the lower elevation range limit of the mountaintop species is a result of interspecific competition with the lowland species (Hairston 1951, Jaeger 1971). Interspecific competition has been demonstrated to influence species' geographic distributions in other montane systems (Jankowski et al. 2010, Price et al. 2011). Range limits could also form through interactions between biotic and abiotic factors, with species coming into contact in areas where there are habitat-inflicted energy constraints on one or both species that change population dynamics.

At least some of the narrow zones of sympatry between species of *Plethodon* have been associated with shifts in jaw morphology characters that may be driven by interspecific competition. Adams and Rohlf (2000) observed that populations of *P.*

hoffmani occurring in sympatry with *P. cinereus* displayed faster closing jaws when compared to *P. hoffmani* in allopatry. They also found that *P. cinereus* showed a shift toward a slower, stronger jaw morphology in sympatry, indicating character changes possibly caused by niche partitioning of prey items or increased aggression. Similarly, Adams (2004, 2010) found evidence for character displacement in contact zones between *P. jordani* and *P. teyahalee*. However, this pattern does not hold across all species or habitats, nor is it concordant with existing behavioral studies which suggest that montane endemics are actually more aggressive than lowland species with which they are narrowly sympatric (Anthony 1997, Nishikawa 1985). When examining head morphometrics in allopatric and sympatric populations of *P. cinereus* and *P. electromorphus*, Dietloff et al. (2013) did not recover a consistent shift in morphology across the species' overlapping geographic ranges. Shifts in head morphology due to competition may not be predictable if competition is mediated by other ecological and evolutionary factors.

Salamanders of the genus *Plethodon* have similar ecological requirements. All members of this genus have a similar life history and basic body plan. Plethodontid salamanders are gape-limited opportunistic predators with little dietary specialization (Powders and Tietjen 1974, Thurow 1976, Hairston et al. 1987), and species consume similar invertebrate taxa in zones of sympatry (Hairston 1981). There is little evidence that *Plethodon* directly compete for food resources (Fraser 1976, Hairston 1981, Hairston et al. 1987), although some species may compete for foraging territories (Thurow 1976). Many species seem to defend cover objects (rocks and logs), which provide refugia when conditions do not permit surface activity and foraging (Feder 1983, Jaeger et al. 1983,

Mathis 1990).

As terrestrial, cutaneous-breathing animals, *Plethodon* have restricted surface activity. *Plethodon* only emerge and forage when temperature and humidity levels prevent or severely impede desiccation (Feder 1983). During long periods of suboptimal temperatures (for example temperate winters or dry seasons) most *Plethodon* retreat to underground burrows; however, during shorter periods, salamanders take advantage of the moisture gradient created by large objects like logs and rocks on the forest floor (Heatwole 1962). Field studies on species of *Plethodon* rarely find more than one same-sex salamander under a single cover object (Mathis 1990, Anthony and Wicknick 1993, Marvin 1998), implying that local-scale distributions are driven by territoriality. Aggressive defense of a territory, which allows an individual to retain a suitable cover object like a rock or log on the forest floor, has been demonstrated in a number of *Plethodon* (Thurow 1976). Jaeger and Gergits (1979) demonstrated that two different species of *Plethodon*, *P. shenandoah* and *P. cinereus,* could detect heterospecific chemical cues, indicating that interspecific territoriality that may be common.

Interspecific and intraspecific competition have been extensively studied in many plethodontid systems. Agonistic behavior by resident salamanders has been shown to be an effective method for retaining territories and cover objects in lab experiments. The wide-ranging species, *P. cinereus,* has been the subject of many of the studies to date. Male and female *P. cinereus* show agonistic behavior in both laboratory and field trials (Jaeger et al. 1982). Larger and resident *P. cinereus* have the advantage for obtaining and retaining optimal cover objects (Jaeger et al. 1982, Mathis 1990). Additionally, when resident *P. cinereus* were removed from cover objects in field trials, other *P. cinereus*

moved in more often than when the resident was not removed (Mathis 1990). These studies, along with others conducted on *P. cinereus,* support territoriality as a dominant force driving intraspecific interactions and spacing.

Through aggressive defense of territories, when two similarly sized species of *Plethodon* occur in the same habitat, one species is expected to drive down the density of the other species through competitive interference. Interference competition has been invoked to explain the pattern of species turnover and lack of sympatry seen in many montane systems (Hairston 1983, Anthony et al. 1997, Marshall et al. 2004). Sympatry between similarly sized *Plethodon* can result from microhabitat partitioning, with the inferior competitor occupying less desirable habitat. For example, *P. cinereus* has been shown to restrict *P. shenandoah* to suboptimal habitats through competitive interference in nature (Jaeger 1971), with *P. cinereus* also displaying more aggression in laboratory trials (Wrobel et al. 1980). Competitive advantage of wide-ranging generalist species has been demonstrated through controlled laboratory aggression trials in *P. kentucki* and *P. glutinosus*, where *P. kentucki* was excluded from optimal cover objects by *P. glutinosus* (Marvin 1998). Similarly, *P. petraeus* is known to occupy rocky outcroppings in its restricted range on Pigeon Mountain in northwestern Georgia, with *P. glutinosus* occupying seemingly superior intervening woodland habitat. This corresponds with laboratory trials where *P. petraeus* was significantly less likely to win territorial disputes against *P. glutinosus* (Marshall et al. 2004).

Many lowland species are slightly larger in body size compared to montane species (Kozak et al. 2009) and size seems to be an important factor in determining competitive advantage in intraspecific interactions (Mathis 1990). However, some

mountaintop species, like *P. ouachitae* have demonstrated that increased agonistic

behavior can overcome size differences during competitive interactions with the lowland

species *P. albagula* (Anthony et al. 1997). Anthony et al. (1997) demonstrated that this

aggressive advantage allowed the smaller *P. ouachitae* to exclude the larger *P. albagula*

and retain cover objects in both laboratory and field trials. These findings plus the

observation that *P. albagula* occur at high elevations in the Ouachita Mountains where *P.

ouachitae* are absent (DBS, unpublished data) indicate that *P. ouachitae* limits the

elevational distribution of *P. albagula*. What determines the lower elevation limit of *P.

ouachitae* remains unexplained. A similar result was seen in behavioral trials between

mountaintop *P. hubrichti* and the wide-ranging *P. cinereus*, where *P. hubrichti* was the

dominant competitor but has a more restricted geographic range (Arif et al. 2007).

Many of these competition studies have demonstrated the importance of

intraspecific aggression, with dominant species displaying higher levels of agonistic

behavior toward conspecifics rather than heterospecifics (Nishikawa 1985, Anthony et al.

1997, Marvin 1998). Combined with intraspecific studies (reviewed in Jaeger and

Forester 1993), these findings suggest that in some systems the observed aggression

toward heterospecifics is a byproduct of aggression toward conspecifics (Nishikawa

1987). Both intra- and interspecific territoriality would result in driving down population

density in areas where refugia are limited. The suitability of a cover object to serve as a

refugium will depend on local climatic conditions and refugia would be expected to be

more limited in warmer and drier environments, such as those found at lower elevations.

As a result, population dynamics in both contact zones and areas with suboptimal

climatic conditions would be different than dynamics at the core of the species' range,

and would potentially interact with other factors to restrict range expansion and local adaptation.

Abiotic conditions

Closely related species often have abutting or narrowly overlapping distributions along environmental gradients (Costa et al. 2008). Such a coincidence of two species' range limits along an environmental gradient makes it difficult to tease apart the relative importance of abiotic and biotic factors in determining species distributions. Climatic conditions on mountains vary with elevation and thus, mountains are characterized by environmental gradients in which the disparity in conditions depends on the elevational extent and the steepness of the gradient depends on the slope. Montane species typically occupy distinct elevational ranges, but explicit tests of whether they are constrained by abiotic or biotic factors are generally lacking.

Hypotheses based on abiotic constraints posit that montane species are physiologically specialized and are unable to persist in climatic conditions beyond the limits of their elevational ranges (Janzen 1967, Ghalambor et al. 2006). Under the abiotic constraints hypothesis, a species' range limit coincides with the limits of its fundamental niche (Buckley 2008, Costa et al. 2008, Kearney and Porter 2009). In contrast, hypotheses based on biotic constraints posit that interspecific competition at range margins compresses the ranges of species, resulting in the vertical replacement or zonation of species along elevational gradients (e.g. Jankowski et al. 2010). Under the biotic constraints hypothesis, competition restricts a species to a subset of the climatic conditions under which it can maintain viable populations, resulting in a mismatch

between the range limit and the spatial limits of the fundamental niche (Buckley 2008,

Costa et al. 2008, Kearney and Porter 2009). Therefore, discriminating between abiotic

and biotic drivers of range limits requires knowledge of the species' fundamental niche

and its spatial distribution on the landscape.

Estimating a species' fundamental niche is difficult because species often occupy

only a portion of their fundamental niche (i.e., the realized niche; Hutchinson 1957).

However, recent methods integrating species occurrence data and spatially explicit GIS

layers of abiotic variables have provided a means to estimate a species' fundamental

niche and project it onto geographic space (reviewed by Kozak et al. 2008). These

ecological niche models (ENM) can then be used to evaluate whether abiotic or biotic

factors set species' range limits by testing whether range limits coincide with the spatial

limits of their fundamental niche. ENMs for the Ouachita Mountain endemic species, *P.

ouachitae*, *P. fourchensis,* and *P. caddoensis*, showed that their distributions are predicted

well by climatic and geological variables (Shepard and Burbrink 2008, 2009, 2011).

Thus, the distributions of these species would appear to be determined primarily by

abiotic constraints, although the coarse resolution of climatic layers (~1 km^2) precluded

fine-scale examination of their lower elevational range limits. ENMs for *P. jordani* and

P. metcalfi in the southern Appalachians predicted that most of the range of *P. jordani* is

suitable for *P. metcalfi*, but most of the range of *P. metcalfi* is unsuitable for *P. jordani*

(Chatfield et al. 2010). The two species' distributions overlap only across a narrow zone

on Balsam Mountain that was predicted to be suitable for both species, suggesting that

the range limit of *P. jordani* is determined largely by abiotic factors whereas the range of

P. metcalfi is limited by biotic interactions with *P. jordani* (Chatfield et al. 2010). Kozak

and Wiens (2006) constructed ENMs for pairs of allopatric sister species of montane *Plethodon* and found that they occupy similar climatic niches and are separated by lowland habitats with unsuitable climatic conditions. In the same study, ENMs for parapatric sister species of *Plethodon* predicted suitable climatic conditions exist for both species across a broad zone, but species' distributions do not overlap (Kozak and Wiens 2006). Each species of a parapatric pair also had a portion of its distribution that was unique climatically, which was usually located distal to the contact zone with the other species (Kozak and Wiens 2006). Together these results suggest that the allopatric montane taxa examined are primarily limited by abiotic factors, but parapatric taxa are limited by biotic factors on one side of their range but abiotic factors on the other (Kozak and Wiens 2006).

The ENMs generated in the aforementioned studies were based on niche modeling methods that rely on presence-only distribution data and employ a correlative approach to infer a species' niche. The basic premise is that values for a set of environmental variables are extracted from locations where the species is known to occur and a model is constructed to predict where else the species is likely to occur because conditions are similar. Although these approaches have several shortcomings (Kearney and Porter 2009, Buckley et al. 2010), they are fairly easy to employ and have provided new insights into the relative importance of abiotic and biotic factors in shaping species' distributions. More recently, mechanistic approaches to modeling a species' niche have been developed that directly incorporate information about an organism's biology by linking functional traits with climatic data to model spatial variation in key fitness and range-limiting processes across a species range (Buckley 2008, Kearney and Porter

2009). Gifford and Kozak (2012) used a mechanistic approach to construct ENMs for *P. jordani* and *P. teyahalee* and test whether the lower range limit of the montane species, *P. jordani*, was set by abiotic factors or by biotic interactions with the larger, low-elevation species, *P. teyahalee*. They found that climatic, rather than biotic constraints, prevented *P. jordani* from successfully colonizing lower-elevation habitats. Specifically, elevational variation in climate appeared to constrain the time available for surface activity and foraging, such that *P. jordani* cannot obtain enough energy to offset the costs of metabolism and reproduction in locations just below their lower elevational range limit.

Plethodontid salamanders have long been an interesting study system for examining speciation and patterns of diversity. Given their biology, temperature and moisture are important determinants of where plethodontids can occur, and plethodontids, like many other taxa, reach their highest species diversity at mid-elevations (Spotila 1972, Kozak and Wiens 2010a). Plethodontids appear to have inhabited this mid-elevation climatic niche throughout their evolutionary history, with those species occupying the lower and higher elevations colonizing those climates much more recently (Kozak and Wiens 2010a). Although climatic niches have been conserved over evolutionary time, niches have shifted spatially, moving downslope during cold climates (e.g., glacial periods) and upslope during warm climates (e.g. interglacial periods). As species track their niches upward in elevation, they become isolated on mountaintops. Species continue occupying similar montane climatic niches as their ancestors and are unable to disperse through lowland habitats, leading to allopatric speciation. Sister taxa are often observed to inhabit similar ecological conditions on adjacent mountains (Kozak and Wiens 2006, Costa et al. 2008), supporting the idea that species' niches are

conserved through time and underlie the geography of speciation and elevational patterns of species richness. However, the mechanism preventing niche evolution and niche expansion over ecological and evolutionary timescales is still unclear and requires further study.

Population dynamics

The finding that specialization for montane climates has been conserved over evolutionary timescales in *Plethodon* (Kozak and Wiens 2006, 2010a) begs the question: Why have so many species failed to adapt to lowland habitats? Understanding why species fail to adapt to conditions at their range edge is essential to understanding what mechanisms prevent evolution of a species' niche over time (Wiens 2011).

Ecological and evolutionary models offer some insight into how population demography and connectivity could play a role in limiting local adaptation (Kirkpatrick and Barton 1997: Case and Taper 2000: Holt and Keitt 2005). Population density is expected to be highest at the center of a species' range, following the Abundant Center Hypothesis (Brown et al. 1995). Montane *Plethodon* appear to follow this pattern with the highest population density occurring on the mountaintop (Gifford and Kozak 2012). If a species' range occurs across an environmental gradient, then the area with the highest population abundance should coincide with the area of optimal environmental conditions, thereby defining the population center of the range. This abundant center not only affects population sizes, but also dispersal. Assuming purely random dispersal, if abundance is higher in the center, then the number of migrants from the center to other parts of the range is also expected to be higher in comparison to migrants from the less-populated

edge. Just as low levels of dispersal can prevent species from colonizing adjacent habitats, high levels of unidirectional dispersal can limit local adaptation by bringing in genotypes that are well adapted to conditions in the center of the range but are maladapted to conditions on the edge (Kirkpatrick and Barton 1997). On its own, asymmetrical gene flow may not be enough to create a stable range limit, but edge populations are also known to experience small effective population sizes, large amounts of genetic drift, habitat fragmentation, Allee effects, and in the case of many montane *Plethodon*, increased interspecific competition (Bridle and Vines 2007, Holt and Barfield 2011).

Theoretical work on dispersal, population size, and selection is often invoked to explain range limits, but these predictions have proven difficult to test in natural populations. The forces acting on range limits are expected to produce molecular genetic signatures that can be directly measured; however, different processes can create similar patterns. Source-sink dynamics and maladaptive gene flow are both expected to have an excess of common genetic variants at the edge, whereas recent colonization is expected to have an excess of rare variants. Sink populations should also have lower genetic variation although this could also be seen with a recent colonization event. Asymmetrical gene flow from center to edge is also expected in source-sinks, maladaptive gene flow, and recent colonization (Moeller et al. 2011). To fully assess the population dynamics at the range edge, it is necessary to measure more than neutral genetic variation in the edge population. Connectivity and gene flow among populations across a species' geographic range play an essential role in population dynamics and local adaptation; however, factors influencing movement across the landscape are poorly understood.

Population connectivity and gene flow have not been extensively studied in *Plethodon*. Mark-recapture studies along with observations of population structure have provided some indication of how little *Plethodon* move. Liebgold et al. (2011) observed that 44% of adult female *P. cinereus* moved a meter or less from their natal location. Males on average moved twice as far as females; nevertheless, sex was not the only important factor in determining salamander movement and dispersal. Given their sensitivity to microclimate, it is not surprising that local habitat features influence movement and thus gene flow in *Plethodon* (Spotila 1972, Peterman and Semlitsch 2013, Connette and Semlitsch 2013, Peterman et al. 2014). Peterman et al. (2014) used fine-scale environmental resistance surfaces to test hypotheses about the environmental factors influencing movement in *P. albagula* in the northern Ozarks of Missouri. *Plethodon albagula* were found to exhibit compensatory movement in which they moved farther in drier environments, presumably to get to superior (moister) habitats (Peterman et al. 2014). Thus, even if population density is highest in the most suitable habitat, patterns of gene flow may not be the result of neutral dispersal and may not be as asymmetrically biased downslope for montane species of *Plethodon*. Compensatory movement in poorer quality edge habitats could also result in more transient populations that do not occupy a given habitat long enough for selection to act on the edge populations.

CASE STUDY: *Plethodon jordani*

At mid-elevations in the Great Smoky Mountains, the montane species, *P. jordani,* has a narrow zone of contact with the lower elevation *P. teyahalee*. To test

hypotheses about factors limiting the distributions of both species, Hairston (1980)

removed one or the other species from plots within their contact zone. When *P. teyahalee*

were removed from an area, only the youngest age classes of *P. jordani* increased in

abundance. This finding was used to provide tenuous support to the hypothesis that *P.

teyahalee* limits population growth in *P. jordani*. In contrast, when *P. jordani* were

removed, abundance of all age classes of *P. teyahalee* increased substantially (Hairston

1980). These results provided strong evidence that interference competition with *P.

jordani* drives down population size of *P. teyahalee* at its upper elevational limit. In

laboratory encounters both species showed high but variable aggressive behavior

(Nishikawa 1985). *Plethodon jordani* from areas with higher interspecific competition

demonstrated the highest level of aggressive behavior toward heterospecifics, suggesting

that increased aggression is a result of competition with *P. teyahalee* (Nishikawa 1987).

In this same Smoky Mountain system, Gifford and Kozak (2012) created

mechanistic ENMs for both species based on energetic intake and consumption across the

mountains. Their mechanistic model estimated the annual energy budget for each

salamander species across 90-m grid cells based on microclimate conditions and lab-

measured standard metabolic rate and energy assimilation. The rationale of this approach

is that the mechanistic ENMs identify where in the landscape a species would be able to

acquire enough energy through surface foraging activity to offset metabolic demands for

growth and reproduction. Gifford and Kozak (2012) found that *P. jordani* was in energy

debt at their range edge, indicating that climatic conditions rather than biotic interactions

determine their lower elevation range limit. The model predicted suitable conditions for

P. teyahalee exist across the entire mountain, including the high elevations where this

species is not found but *P. jordani* occurs. These findings, along with the removal and aggression experiments conducted by Hairston (1980) and Nishikawa (1985) suggest that *P. teyahalee* is limited by competition with *P. jordani* whereas *P. jordani* is limited primarily by climatic conditions.

Although the mechanistic model supports the hypothesis that *P. jordani* is limited by climatic conditions, it does not explain the actual mechanism preventing range expansion over time. Edge populations are expected to adapt to local environmental conditions through natural selection, slowly expanding the species range limit over time. As discussed above, adaptation at the range edge could be limited by the influx of alleles that are optimized for environmental conditions at the range center but mismatched for conditions at the range edge. This "gene swamping" is hypothesized to occur based on neutral diffusion of genes from the more populated range center, causing asymmetrical gene flow between the range periphery and center.

As a preliminary test of these predictions, we used the energy budget model for *P. jordani* (Gifford and Kozak 2012) to classify 11 sampling localities into four groups (populations) along an elevational transect representing a gradient from core (high elevation, high energy surplus) to edge (lower elevation, high energy debt). We sequenced two anonymous nuclear loci (~540 base pairs each) for 30 individuals from these four populations and used Migrate-n version v.3.2.19 (Beerli and Felsenstein 1999 2001) to estimate migration rates between adjacent population pairs. We employed a Maximum likelihood method with 10 short heated chains with 500 recorded steps sampled every 100 steps for 4×10^4 post-burn in visited states and four long chains with 5×10^3 recorded steps again sampling every 100 steps. A stepping stone model was used

to estimate migration. Consistent with the gene swamping hypothesis, we found that migration was biased from the range center to the range edge (Fig. 1). Further, this asymmetric gene flow from core to edge was present in two opposing directions (Fig. 1). The decline in abundance with decreased elevation observed during surface counts of *P. jordani* (Gifford and Kozak 2012) also supports predictions that population sizes should decline from the range center to the range edge. In further support of those field observations, our genetic data indicated that populations from core areas with high energy surplus have higher Θ values ($\Theta = 4N_e\mu$ where N_e is effective population size) than populations on the edge that are in energy debt (Table 1). Measures of genetic diversity for the two loci were similar for the four populations; however, Tajima's D was near zero or slightly negative in three of four cases involving populations from areas in energy surplus compared to positive values in three of four cases involving populations from areas in energy debt (Table 1). These results are consistent with predictions that core populations will have higher rates of population growth and an excess of rare variants whereas edge populations are population sinks that consistently lose genetic diversity due to drift (Moeller et al. 2011).

FUTURE DIRECTIONS

Studies that examine the impact of a factor in determining range limits often find a significant effect; however, most of these studies only consider one or two variables and ignore a host of other potential determinants. Range limits are not necessarily a product of only biotic or abiotic forces. These factors may function at different scales, from fine-scale microhabitat and biotic interactions to broad-scale niche requirements,

and it is likely that these factors interact. Interactions with other closely-related species at a lower elevation range limit could create smaller effective population sizes, which are less capable of locally adapting due to lower standing genetic variation or genetic swamping from larger populations at the range center. Interspecific aggressive interactions will also impose a cost to other organismal processes, as energy is expended on antagonistic interactions instead of foraging and reproduction. The cost of a highly aggressive strategy, like that employed by *P. ouachitae* is still unclear. However, areas where most mountaintop endemics are encountering heterospecific *Plethodon* are the same peripheral habitats where mountaintop species already appear to be in energy debt (Gifford and Kozak 2012). According to the bioenergetic models that have been employed, these lower elevation populations do not appear to have enough time annually to forage on the surface in order to offset their energetic demands. Mechanistic bioenergetic models have not been employed in many systems so it is unclear to what degree this strong energy gradient occurs across mountaintop species of *Plethodon*.

Plethodon show a high degree of population differentiation for neutral markers across small spatial scales (Cabe et al. 2006, Shepard and Burbrink 2011 Liebgold et al. 2011). In part, this can be attributed to the extremely short distances that these salamanders move during their life (Liebgold et al. 2011). Given that mountaintop ranges occur along a steep gradient of environmental conditions correlated with elevation, it would not be surprising if local adaptation to differing environmental conditions between the range core and periphery occurred. To what degree lower elevation, edge populations are adapted to warmer and drier conditions could be important in predicting how species will respond to changing global temperatures. If edge populations are adapted to differing

conditions, these genotypes may be able to move upslope with temperature, preventing extinction of mountaintop species. How much populations show divergence in traits important to fitness is unknown.

To evaluate local adaptation, it is usually advisable to conduct reciprocal transplant experiments. Such studies are capable of considering multiple drivers simultaneously and have been successfully implemented at a small scale in the zone of range overlap between *P. glutinosus* and *P. mississippi* (Cunningham et al. 2009). Large, long-term reciprocal transplant experiments are difficult to implement with *Plethodon* because of their sedentary, partially subterranean life. Cunningham et al. (2009) looked at change in mass of transplanted individuals over the course of half of a year in paired competition enclosures. This study found that salamanders in interspecific pairs lost the most mass, and those pairings that were set up in the two species contact zone lost more mass than the pairings set up in core habitat, additionally individuals of the same species collected from the core and periphery performed differently in trials. These findings indicate that the abiotic conditions at the range edge influenced the biotic interactions and provide some evidence for local adaptation. Alternatively, local adaptation can be assessed in a laboratory setting by examining traits important to fitness under controlled, naturally occurring temperatures. This would involve collecting individuals from multiple populations within a species range, and ideally study subjects would be hatched and raised in a common garden setting to mitigate phenotypic plasticity in the traits being measured. Studies comparing adaptation to environmental conditions between species have been conducted (Spotila 1972, Bernardo et al. 2007), but rarely has this been done within species (Bernardo and Spotila 2006).

Local adaptation not only depends on the magnitude of adaptation and climate change, but also on movement and gene flow. Studies on movement and population structure of *Plethodon* are usually conducted in areas where salamanders are at a high density and easy to recover; however, movement and population dynamics may be substantially different at the range periphery. Dispersal may not be random and individual movement may be higher depending on habitat type, which would have different implications depending on complex landscape features and population sizes. Data on forest floor climatic conditions are becoming more available as researchers deploy sensors across many of these systems. These data will enable researchers to examine how population dynamics relate to the whole landscape and will also allow mechanistic models to be constructed for more species.

CONCLUSION

Geographic ranges are a complex product of both ecological and evolutionary processes, reflecting current biotic and abiotic conditions as well as gene flow, drift, adaptation, and history. Previous studies on geographic range limits have often been inconclusive because they have approached questions from a limited perspective rather than integrating ideas and methods from multiple fields such as physiology, morphology, behavior, ecology, and evolution. *Plethodon* offer an ideal vertebrate system for studying geographic range limits. The results of past studies should be used to develop testable hypotheses that integrate multiple drivers across different spatial scales and make use of recently developed molecular and geospatial tools.

Table 1. Genetic diversity parameters for populations of *Plethodon jordani* along two opposing elevational transects spanning from the core of the species' range to the range edge (see Fig. 1). N = the number of phased sequences for two anonymous nuclear loci (540 base pairs each). The mean maximum likelihood estimate of θ (= $4N_e\mu$) and associated 95% confidence intervals (in parentheses) were inferred using Migrate-n v.3.2.19 (Beerli and Felsenstein 1999, 2001). The number of segregating sites (S), nucleotide diversity (π), number of haplotypes (H), haplotype diversity (Hd), and Tajima's D were calculated using DnaSP v.5.0 (Librado and Rozas, 2009).

Population	N	θ	Locus	S	π	H	Hd	Tajima's D
North edge	14	0.0051 (0.0031–0.0092)	1	18	0.0123	7	0.846	0.355
			2	24	0.0149	10	0.945	-0.229
Core	26	0.0098 (0.0078–0.0162)	1	21	0.0113	10	0.8	-0.046
			2	20	0.0122	12	0.914	0.525
South middle	10	0.0096 (0.0059–0.0138)	1	14	0.0092	4	0.644	-0.646
			2	15	0.0107	7	0.867	-0.1
South edge	10	0.0045 (0.0004–0.0789)	1	13	0.0108	5	0.8	1.083
			2	10	0.0076	5	0.667	0.499
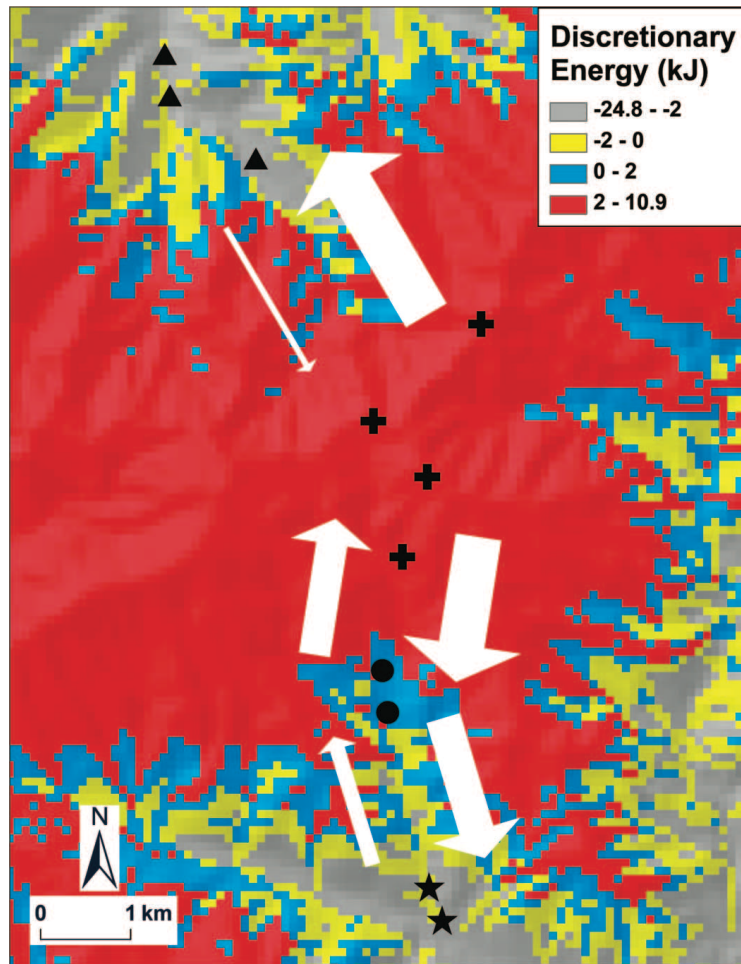
Fig. 1. Asymmetric migration along two elevational transects. Sampling points of *Plethodon jordani* overlaid on an energy budget model (Gifford and Kozak 2012) showing high elevation core areas in energy surplus and low elevation edge areas in energy debt. Based on this model, points were grouped into four populations indicated by different symbols and bidirectional migration rates between adjacent populations were estimated in Migrate-n. White arrows indicate the direction of migration and arrow widths are scaled by the number of migrants per generation.

Chapter 2

Vanishing islands in the sky? A comparison of correlation- and mechanism-based forecasts of range dynamics for montane salamanders under climate change

ABSTRACT

Species distribution models are an important tool for forecasting the effects of climate change on species and populations of conservation interest. These models, also known as niche models, predict where on the landscape there is suitable habitat for a species of interest and are usually developed using environmental data. Correlative niche modeling, the most commonly employed approach, relies on correlations between known species localities and current environmental data. This type of model could spuriously forecast less future suitable habitat both because species' current distributions may not adequately represent their niche and because conditions under future climate may not be analogous to current conditions. We compared the predicted distributions for four species of *Plethodon* salamanders in the southern Appalachian Mountains of North America using both a correlative modeling approach and a potentially more accurate mechanistic model. The mechanistic model incorporates species-specific physiology, morphology, and behavior to predict an annual energy budget for each species on the landscape. Both modeling approaches performed well predicting the current distributions for three montane-endemic species and predicted that all species could persist in habitats at higher elevation through 2055. By contrast, neither approach performed well predicting the current distribution of a lowland species of *Plethodon.* As hypothesized, the mechanistic model indicated more future suitable habitat than the correlative model for the three

montane-endemic species. Choice of global circulation model (GCM) contributed

significantly to niche model outcome, with up to a tenfold difference in future suitability

for each species based on GCM. Our results indicate that correlative models are over-

predicting habitat loss for montane species and that uncertainty from GCMs should be

included in models of species distributions under modern climate change.

INTRODUCTION

Anthropogenic climate change poses a major threat to biodiversity, especially in

montane regions (Parmesan 2006, Thuiller et al. 2008, La Sorte and Jetz 2010, Gottfried

et al. 2012). Some montane species have seemingly already responded to contemporary

warming by contracting their ranges upslope (e.g. Parmesan and Yohe 2003, Wilson et al.

2005, Moritz et al. 2008, Raxworthy et al. 2008, Rovito et al. 2009, Chen et al. 2011,

Feeley et al. 2013, Freeman and Class Freeman 2014). Given that mountains are centers

of endemism and species richness for many groups of plants and animals (Myers et al.

2000, Körner and Spehn 2002, Graham et al. 2014), the erosion of the geographic

distributions of montane taxa is particularly alarming. Moreover, recent studies suggest

that species inhabiting montane regions may have a limited capacity to adapt to changes

in climate, even over evolutionary timescales (Smith et al. 2007, Li et al. 2009, Kozak

and Wiens 2010a). Consequently, predicting the fates of species that make up montane

biodiversity hotspots has emerged as a major challenge for biologists.

Correlative niche models have become the primary tool for forecasting the range

dynamics of species under climate change (Thuiller et al. 2008). This class of models

uses two types of data, species' occurrence records and GIS-based maps of climatic

variation (e.g. temperature, precipitation), to build statistical models describing the association between the contemporary climate and the presence of a species (Elith and Leathwick 2009). These models are then projected onto maps of future climatic conditions to predict whether the geographic distribution of suitable habitat for a species will shift, contract, or remain stable (e.g. Peterson et al. 2002, Williams et al. 2003). Correlative niche models often predict extensive loss of climatically suitable habitats, especially for species inhabiting montane regions (e.g. Williams et al. 2003, Thuiller et al. 2005, Milanovich et al. 2010).

Correlative models make at least two important assumptions in the context of predicting species' range dynamics in response to climate change. First, they assume that a species' geographic distribution encompasses the full range of climatic conditions within the fundamental niche. Second, they assume that correlations between climatic variables and the processes setting range limits remain fixed across space and time (Pearson and Dawson 2003). Given that factors other than climate may limit the distributions of species (e.g. species interactions) and the correlations among climatic variables and range-limiting processes may change over time, some authors have argued that the range dynamics of species cannot be predicted from the current climate and species' distribution data (Kearney and Porter 2004, Williams and Jackson 2007, Kearney and Porter 2009). Mechanistic niche modeling is a potentially powerful alternative that does not use species' realized geographic ranges to forecast future distributions. The mechanistic approach links functional-trait and climatic data to model spatial variation in parameters that determine whether a species can persist in a given location (e.g. energetics, development time, population size, species interactions).

Because they focus on range-limiting biological processes, well-parameterized mechanistic models (i.e. those that include important mechanisms that limit species' ranges) may outperform correlative models in predicting the responses of species to climate change (Kearney and Porter 2009, Buckley et al. 2010).

Do existing correlation-based forecasts signal the impending collapse of montane faunas and floras? Alternatively, can limitations associated with projecting correlative models beyond current environmental conditions explain the predicted drastic loss of suitable climates for montane endemics? These questions are critical to conservation efforts aimed at sustaining montane diversity in face of climate change. Researchers have begun to compare the range dynamics forecast by correlative and mechanistic models (Hijmans and Graham 2006, Morin and Thuiller 2009, Buckley et al. 2010). However, no comparative studies exist for montane endemics, which correlative models predict are under threat of extinction as a result of climate change (Milanovich et al. 2010).

Here, we compare the range predictions of correlative and mechanistic niche models for four salamander species of the genus *Plethodon* that are restricted to the Appalachian Mountains of Eastern North America (Fig. 1). Three of the species (*P. jordani*, *P. montanus*, and *P. metcalfi*) are mountaintop endemics that have elevationally and climatically restricted distributions (Highton and Peabody 2000, Kozak and Wiens 2006) making them ideal species for studying the sensitivities of forecasting future suitable habitat. *Plethodon jordani* is only found within the Great Smoky Mountains National Park at elevation >1200 m: the most geographically restricted of species used in this study. Where it has been studied, climate appears to play the primary role in determining the lower elevation range limit for *P. jordani* (Gifford and Kozak 2012,

Lyons et al. 2016). *Plethodon montanus* and *P. metcalfi* have wider geographic

distributions, together spanning the states of Virginia, Georgia, and North Carolina.

However, both of these species are restricted in elevation, generally occupying forested

habitats above >1,000 m. *Plethodon teyahalee* occurs at lower elevations and has more

general climatic requirements (Kozak and Gifford 2012). The climatic niches of species

in the genus *Plethodon* have been conserved leading to allopatric speciation as

populations have tracked climate during periods of environmental change since the

Miocene, which seemingly has restricted many of the species in the clade from

colonizing lowland climates (Kozak and Wiens 2006, 2010b, 2012).

We forecast and compare range dynamics predicted by a widely used correlative

method (MaxENT), and a mechanistic model based on the climatic sensitivity of

metabolism, surface activity, and digestive efficiency. We find that the models perform

similarly well in predicting the current distribution of the mountaintop species and

similarly poorly at predicting the current range for the lowland generalist species.

However, the amount of range loss that is predicted differs markedly for the correlative

vs. the mechanistic model, especially for the two most elevationally restricted species.

We explore reasons for the causes of this discordance. We also examine how

methodological choices of future environmental data in the form of global circulation

models (GCMs) contribute to differences in predicted distributional extent.

METHODS

Ground Temperature

We generated high-resolution (90 m) layers of monthly maximum and minimum forest floor temperature using a model developed by Fridley (2009) for the Great Smoky Mountains National Park. All four species inhabit forested habitats within or in close proximity to the Great Smoky Mountains National Park, which allowed us to generate new temperature layers for present day and future models. Because of variability between global circulation models, we created future temperature layers for fifteen different global circulation models looking at two time periods, 2050-2060 and 2080-2090, resulting in thirty future models. This method estimates near-ground temperatures (~1 m) using data on incoming solar radiation, topographic shading, slope, aspect, and soil moisture along with model terms based on data gathered from ground level data loggers over the course of two years (Fridley 2009). The ground temperature model built by Fridley (2009) incorporated present air temperature through the use of lapse rates (degree temperature change per meter elevation change) calculated from the elevation and average monthly maximum and minimum temperatures recorded at weather stations within the park. We calculated lapse rates for present and future climates from random points placed at a density of 1 per 80 km^2 within a 10km buffered polygon of the species' current range. Climate data were then extracted to points using the ClimateNA v5.21 software package, available at *http://tinyurl.com/ClimateNA,* based on methodology described by Wang et al. (2016). All other inputs were generated using methodology described by Fridley (2009) and are detailed in the supplementary material (Appendix S3).

Climate Models

Creating future climate layers requires researchers to choose both a greenhouse gas scenario or representative concentration pathway (RCP) and GCM. The choice of RCP is based on predictions for how individuals and governments will attempt to mitigate warming and curb greenhouse gas emissions (IPCC, 2014). We used RCP4.5, which assumes greenhouse gas emissions peak in 2040 and then decline (IPCC, 2014). Many meteorological research centers have developed GCM that simulate climatic responses to increased greenhouse gas emissions. Because of large uncertainties in the interplay of physical processes, feedback processes, and parameterization, there is substantial variation in the predicted temperatures for different GCMs under the same RCP for a given future date (Buisson et al. 2010, Wright et al. 2014). We ran both the correlative and mechanistic models for 15 GCMs (Appendix S1, Table 1.2).

Mechanistic model

We modeled spatial variation in energetics to predict the potential geographic distribution of the four *Plethodon* species and their future range dynamics under climate change. Our model is based on a model developed by Buckley and Roughgarden (2005, 2006), modified to incorporate the hydric constraints on salamander surface activity (Gifford and Kozak 2012). This model is based on the premise that viable populations cannot persist in locations where energetic costs exceed energetic inputs. Energy budget was computed using the R package biophys (Peterman 2014), which was developed based on the model used in Gifford and Kozak (2012) for *P. jordani* and *P. teyahalee*, and is broadly applicable to organisms with available species-specific physiological,

behavioral, and ecological parameters. To parameterize the environmental variables in the mechanistic model, we used the high-resolution (90 m) model of temperature variation described above, and empirically derived estimates of relative humidity and wind speed. For details of the mechanistic model, please refer to the supplementary information (Appendix S2, Fig. 1.1 and Appendix S3).

Correlative model

We used MaxENT version 3.3.3 (Phillips et al. 2006) to quantify the correlation between climate and georeferenced occurrence locations (obtained from U.S. National Museum of Natural History). MaxENT is an approach for characterizing the probability that habitat is suitable at a given raster cell from incomplete information. In the context of modeling a species' geographic range, it computes a probability distribution that describes the relative suitability of each grid cell as a function of the environmental variables at the known occurrence locations for the species (Phillips et al. 2006). To facilitate direct comparison of the mechanistic and correlative models, we used the same ground temperature monthly maximum and minimum environmental layers at a 90m spatial resolution to construct bioclimatic variables (annual mean temperature, maximum temperature of the warmest quarter, and minimum temperature of the coldest quarter).

As with most correlative model studies, we were restricted to running presence only models based on the data available. In this study, we did have a set of locality points for other salamander species that we used as absence points for analyzing model performance. Because these absence points were sparse and studies rarely have access to absence points, we used a presence only model. The four species of *Plethodon* that we

focused on have robust locality data based on decades of fieldwork by R. Highton. Present and future niches were modeled within a 10 km buffer of a convex hull based on current locality information. This poses a problem of false pseudo absences at locations close to presence points (Wright et al 2014). To combat this we generated 10000 random background points for each species that were within the 10km hull buffer but not within 2km radius of known localities. We randomly selected 75% of the occurrence locations for model construction; the remaining 25% were set aside to test the model. Models were calibrated using quadratic features, 500 iterations, and a convergence threshold of 0.0001. Each model was run ten times using subsampling so a different 25% of points were set aside to test the model over 10 different runs and we used the average values across these runs.

Model performance

To examine the extent to which the mechanistic and correlative models discriminated among presence and absence localities, we used the sensitivity and specificity indices of Manel et al. (2001). The sensitivity index measures the proportion of true presences that are correctly predicted. The specificity index measures the proportion of absences that are correctly predicted. We also calculated model performance, which measures the proportion of all locations that were correctly predicted. We used the same presence points from running the MaxENT model. Absences were defined using localities from the National Museum of Natural History where R. Highton recorded finding species of salamanders, but not the species of interest. Because of the thoroughness and protracted time period over which Highton conducted

his surveys of plethodontid salamanders, we are confident that these points represent true absences. We did not use these data to build the correlative model as this type of data is rarely available for correlative niche modeling and it allowed us to use these absence points to test the model fit. Implementation of these indices required a threshold for presence vs. absence. For the mechanistic model, we considered any grid-cell with a discretionary energy value ≤ 0 unsuitable. MaxENT generates thresholding values according to eleven metrics relating to how training and testing locality points are used in generating cut off values, the choice of threshold can have a large impact on the resulting distribution estimation (Norris 2014). We evaluated all 11 available logistic thresholds from MaxENT. The four thresholds that were able to correctly predict on average at least 85% of current presences and absences for the three mountaintop species were the 10th percentile training presence, fixed cumulative value 10, maximum training sensitivity plus specificity, and maximum testing sensitivity plus specificity. The resulting suitable future habitat was not sensitive to the differences between these four thresholds so we used the 10th percentile of training presence. This is the suitability threshold associated with the top 90% of the training presence records, which is able to account for potential errors in the locality data.

Model differences

We projected the modeled ecological niche onto future climatic conditions using both approaches. For each model type, we calculated the average habitat suitability for each species across two years (2055 and 2085) for 15 GCMs. Habitat was considered suitable if the discretionary energy was > 0 for the mechanistic model and above the 10th

percentile training presence averaged across 10 replicate runs for the correlative model. To determine whether model types (mechanistic or correlative) and GCMs differed in their predictions, we fit a linear model using the stats package in R (R Core Team 2013). The percent suitable future habitat was the response variable with year, model type, and GCM as the predictor terms for each species.

RESULTS

The mechanistic and correlative models produced highly congruent predictions for contemporary range. For the three montane-endemic species, *P. jordani, P. metcalfi, and P. montanus,* we find little difference in the extent to which the climatic niche is over or under predicted based on the contemporary range. Both the correlative and mechanistic models had high overall prediction success, 79%-98%, high percentage of true positives correctly predicted with sensitivity at 77%-99% and high percentage of true negatives correctly predicted with specificity at 74%-100% (Table 1). The mechanistic model for *P. montanus* produces the lowest values for the montane species across all metrics. With the exception of *P. jordani* the mechanistic model has a slightly lower overall prediction success, but the differences are minor. For the lower elevation generalist species, *P. teyahalee*, both modeling approaches had low overall performance, resulting from low specificity. The models predicted only 21%-26% of the true negatives (Table 1). These results are in line with prior work showing that the contemporary range of *P. teyahalee* is at least partially the result of competition with another species (Gifford and Kozak 2012).

For most GCMs, the mechanistic model predicted more future suitable habitat compared to the MaxENT niche model for the three montane species (Fig. 2). This trend was not seen for *P. teyahalee*, where the two modeling approaches did not produce significantly different results. The average suitable habitat for *P. teyahalee* across both models by 2055 was 28%; this is not informative given the poor model performance for current range predictions. For the montane species, *P. jordani, P. metcalfi* and *P. montanus,* the mechanistic model predicted significantly more future suitable habitat when compared with that predicted using a MaxENT/correlative approach. While MaxENT predicted that *P. jordani* would persist in an average of 11% of its current habitat across the 15 GCMs by 2055, the biophysical model predicted 52% (Fig. 3). For *P. metcalfi* the mechanistic model predicted its persistence in 34% of its current range in 2055, whereas the correlative predicted 25% in 2055. *Plethodon metcalfi* currently occupies some moist microhabitats at lower elevation, with the result that MaxENT built habitat suitability correlations for more sites, projecting less range contraction. For *P. montanus*, MaxENT predicted that this species would lose all but 6% of its suitable habitat by 2055, with the biophysical model predicting an average retention of 19% (Appendix S2, Fig. 1.2), though some GCMs still predicted a loss of almost 100% of suitable habitat even using a biophysical model (Fig. 4, Table 2).

For each modeling approach and future time period, the predicted suitable habitat varied by at least an order of magnitude based on the GCM used (Appendix S1, Table 1.4). While the mean values for predicted range retention across tested GCMs was consistently higher for the mechanistic niche model, there was also more variation in the predicted suitable area among GCMs with the mechanistic model. The variation in

predicted suitable habitat between GCMs was higher in the mechanistic model compared to the correlative for the three montane-endemic species (Table 2 and Fig. 3). Overall, GCMs that predicted low or high future habitat retention in correlative model also predicted low or high habitat suitability in the mechanistic model, for example HadGEM2-ES consistently predicted very low future suitable habitat and INM-CM4 predicted high future suitability across species and niche models (Fig. 2, Appendix S1, Table 1.4).

DISCUSSION

Many montane species appear to be living near the upper thermal limits of their niches, making climate change one of the leading threats to montane floras and faunas (La Sorte and Jetz 2010). Correlative niche models predict that this upslope migration of species will continue throughout the 21st century, pushing many montane species to the brink of extinction (Williams et al. 2003, Thuiller et al. 2005, Milanovich et al. 2010). There is a need to explore the robustness of such forecasts by comparing them to those of mechanism-based models that explicitly model processes that limit the ranges of species. We found that in general species distribution models predict that the suitable habitat for the four *Plethodon* species we studied will shift upslope in the next 40 years, as predicted by other studies. As hypothesized, by employing a model that uses species-specific morphology and physiology to predict current and future suitable habitat, we found more suitable habitat retained under future climate than the traditional correlative modeling approach. Additionally, the choice of global circulation model had an order of magnitude influence on the predicted habitat suitability for both modeling approaches.

Niche model differences

Our results suggest that correlative models may overpredict the amount of range montane species will lose under climate change. Both MaxENT and the mechanistic model based on energetics predicted that the ranges of all four species will contract upslope in response to warming. However, the montane species, *P. jordani, P. metcalfi,* and *P. montanus*, will retain more suitable habitat, according to models that draw on species-specific physiology and morphology to forecast future niches.

One of the most widely-discussed explanations for the more extreme range changes predicted by correlative models is that they model the realized niche. By implicitly incorporating the impact of biotic interactions on a species' distribution, correlative models might exclude suitable climatic dimensions of the fundamental niche, and as a consequence, underpredict the geographic extent of suitable locations in the landscape. However, we found that the mechanistic and correlative models performed similarly well in predicting the current range of *P. jordani, P. montanus, and P. metcalfi* and were equally poor at predicting the range of *P. teyahalee*. Our results suggest that even when a species' range corresponds closely to the spatial extent of its fundamental niche, correlative models may still underpredict the availability of suitable habitats in response to climate change.

Correlative model under-prediction

Why then does the correlative model seemingly fail to predict many of the future locations that the mechanistic model deems climatically suitable? The mechanistic model

derives the climatic suitability of a grid cell by calculating the net energy obtained from

foraging when the nightly surface temperature is optimal for activity. Although the

temperature variables used to construct the correlative model are correlated with the

range limit, they do not strictly determine the underlying process that constrains the range

(limits and costs to surface activity). For example, two grid cells could have similar mean

temperatures, yet differ the overall length of time that they are thermally suitable for

surface activity and foraging, resulting in different forecasts of range dynamics. Our

comparisons demonstrate that correlative models can drastically overestimate range loss

because of such mismatches between bioclimatic variables (e.g. mean annual

temperature, maximum temperature of the warmest month) and the processes that

actually limit a species' range.

Our conclusion that the mechanistic modeling approach forecasts more realistic

future distributions is supported by paleoclimatic modeling of *P. jordani* distributions at

the last glacial maximum (LGM). Luxbacher (2014) used the mechanistic model from

Gifford and Kozak (2012) and MaxENT to produce niche models for *P. jordani* within

the Great Smoky Mountains National Park during the LGM. As with our future forecast,

the hindcast of the correlative model predicted significantly less suitable area with little

overlap to current distributions. By contrast, the mechanistic model predicted a

distribution at the LGM that was much more consistent with the population structure and

demographic history of the species inferred from population-genetic data (Highton and

Peabody 2000, Weisrock and Larson 2006). *Plethodon jordani* has deep phylogeographic

structure with population sizes inferred to have been stable dating back to refugia prior to

the LGM, which supports the mechanistic model's finding of large suitable mid-elevation

habitat at the LGM, and also is consistent with pollen records for the area. These multiple lines of evidence led the authors to conclude that the correlative model failed to predict past distributions and as a result is not reliable for forecasting future distributions.

When predicting past and future distributions the correlative model may underperform because of extrapolation to non-analogous climates. Correlative approaches, like the one used in this study, are restricted to finding relationships based on current combinations of environmental variables. Future climate in the area is unlikely to mirror the exact climate that exists in the region now (Fitzpatrick and Hargrove 2009). Extrapolating to novel non-analogous climate is an often-cited concern with correlative models (Veloz et al. 2012, Araújo and Peterson 2012, Briscoe et al. 2016), but should present fewer challenges in a mechanistic model (Kearney and Porter 2009). Unlike correlative niche modeling, mechanistic modeling when carefully applied predicts niches based on the underlying biological processes that govern distributions and can thus incorporate novel climates.

Biotic interactions

While both modeling approaches were successful at predicting current distributions for the three montane-endemic species, in the case of the lowland generalist, *P. teyahalee*, the biophysical and correlative models had lower overall success. The inaccurate prediction of the current species range for *P. teyahalee* results from low specificity values, indicating that neither model correctly predicted absences for this species. *Plethodon teyahalee's* distribution appears strongly influenced by biotic interactions at the upper elevational limits of its distribution. Areas that are

environmentally suitable for *P. teyahalee*, as predicted by the models, lack this species because a competitor excludes it (Gifford and Kozak 2012). Competitive exclusion accounts for why the modeling approaches, especially the biophysical model, overpredict the current distribution of *P. teyahalee*. Correlative distribution models, like MaxENT, model a species' realized niche and thus should more closely predict the distributions of species determined by biotic and abiotic forces. In our study, the correlative approach only slightly improved the current prediction for *P. teyahalee* indicating that accounting for biotic interactions in niche models for current and future species distributions remains problematic.

Biotic interactions, especially due to new or increased contact between competing species, will probably increase as species distributions shift on the landscape (Cunningham et al. 2009). Future distributions for *P. teyahalee* are expected to be upslope from the areas where this species currently occurs, with future ranges restricted to mountaintops. These mountaintops are currently occupied by *P. jordani* and *P. shermani*, both of which have been found to competitively exclude and or be highly aggressive toward *P. teyahalee* (Hairston 1981, Highton and Peabody 2000). Even in areas where *P. teyahalee* coexists with other *Plethodon*, like *P. metcalfi*, its density is lower than in places where it does not overlap, though the biophysical model indicates that these mountaintops have higher available energy for *P. teyahalee* (Appendix S2, Fig. 1.2).

The interplay between biotic and abiotic forces, particularly at the range edge, has been largely ignored. This study focuses only on abiotic environmental factors, mapping current and future species distributions based on climate. For the three montane-endemic

species, the assumption that abiotic conditions will determine future range limits is based on work showing that abiotic conditions constrain current distributions (Gifford and Kozak 2012, Lyons et al. 2016) and further illustrated by the high performance of the biophysical model at predicting current presence and absence using only ground temperature data and physiology. However, biotic and abiotic forces have been shown to interact, and warming may alter the role of biotic interactions (Gilman et al. 2010, Urban et al. 2012, Blois et al. 2013, Dallalio et al. 2017). For example, Clay and Gifford (2015) found that in laboratory behavior trials between *P. montanus* and *Plethodon cylindraceus* (a species that occupies a similar niche to *P. teyahalee*) aggression was highly temperature-dependent, with the larger lowland species, *P. cylindraceus*, more aggressive under warmer test temperatures. These findings oppose previous work that has indicated that the small mountaintop species, like *P. montanus*, are superior competitors thus restricting species like *P. cylindraceus* and *P. teyahalee* from otherwise environmentally suitable habitat (Nishikawa 1985, Anthony et al. 1997, Gifford and Kozak 2012). One-on-one aggression trials in the lab may not be analogous to competitive interactions in nature where there is variation in body condition and species density. If montane species in the future are occupying mostly lower energy habitat as predicted by our mechanistic model, a reduction in their density could leave room for larger, warm tolerant species like *P. teyahalee*. Moskwik (2014) already found *P. teyahalee* to be expanding its range upslope, even though the montane species have yet to contract their range. Foothills and low elevational species, like *P. teyahalee,* may actually experience an increase in available habitat under climate change (Elsen and Tingley 2015).

Improving mechanistic models

The flexibility of biophysical models means that they can be easily altered as our understanding of species-specific physiology and behavior increases. In plethodontid salamanders, and other animals, there has been a recent surge in experimental work investigating local adaptation between populations and plasticity in physiological and behavioral traits under different environmental conditions (Riddell and Sears 2015, Clay and Gifford 2015, 2017). Resting and active metabolic rate are among the primary parameters determining energetics in most biophysical models, including the one used in this study. Other physiological traits like water loss resistance may also vary spatially or seasonally (Riddell and Sears 2015, Riddell et al. 2017a) and could interact with metabolic rate (Riddell et al. 2017b). Physiological studies in plethodontids have previously focused on metabolic rate, but are still limited in scope, sampling only a few species and mostly ignoring within-species variation and seasonal plasticity (reviewed in Gifford 2016).

Montane *Plethodon* like those used in this study exhibit phylogenetic conservatism in their climatic niches, so studies have not focused on variation in physiological traits in hard-to-find lower elevation populations. Evidence of seasonal plasticity or acclimation is lacking for narrow-range species like those examined here. Some mountaintop species have been shown to alter their metabolic rates under different acclimation temperatures through metabolic depression, which may be a sign of physiological stress (Markle 2015). Wider-ranging species, especially those in more seasonal and warm habitats may increase their yearly energy budget by altering their metabolic rate through acclimation to seasonal temperatures. Acclimation would be a

very simple addition to the biophysical model presented here by calculating energy budget per season using seasonal species-specific metabolic rates.

Environmental data

Beyond species-specific physiology and interactions, the environmental input data played a large role in the predicted distributions. Prior studies have found that GCMs induce as much variation in model results as RCPs (Wright et al. 2014), but without following a predictable outcome, so we chose to focus on differences in the GCMs. As others have previously found (McKenney et al. 2011, Tuanmu 2012, Thorne et al. 2013, Wright et al. 2014, Goberville et al. 2015), the choice of GCM had over a 10-fold impact on predicted range loss. The results from the mechanistic models differed more due to choice of GCM than with the correlative models, though both had substantial variation. The correlative results predicted more future outcomes with suitable habitat being close to 0 than the mechanistic, which contributed to the slightly smaller spread in the data. For each species there was at least one GCM-mechanistic model combination that resulted in almost a complete loss of suitable habitat. Certain GCMs, like HadGEM2, predict substantially more warming in the southern Appalachians. The degree of warming predicted by these circulation models is most likely intolerable for small montane *Plethodon*. However, with the mechanistic models there were many more instances with substantial range retention in comparison to the correlative niche model for the same GCM. This is most likely a result of the correlative models only being trained on current distributions that may not encompass a species entire niche along with non-analogous future climate. Some circulation models consistently predicted more future suitable

habitat (INM-CM4 and MRI-CGCM3), while predictions generated by the Hadley Center predicted the largest loss of habitat for all three montane-endemic species and substantial loss for *P. teyahalee*.

Global circulation models developed by the Hadley Center (HadGEM2 for CMIP5 and HadCM3 for CMIP3) are commonly used in studies predicting range shifts and habitat suitability for a variety of species in different parts of the world (Araújo et al. 2006, Milanovich et al. 2010, Tuanmu 2012, Vieilledent et al. 2013, Sutton et al. 2015, Saupe et al. 2015). In our findings, this GCM consistently predicted almost 100% loss of suitable habitat for the montane species. There is still much uncertainty in how the climate will change in the coming decades. It is important to include these dire predictions, but also to show the variation among predictions; we urge future researchers to include all reputable circulation models to incorporate climate uncertainty.

CONCLUSION

In this study, we have demonstrated how taking a mechanistic approach can dramatically alter the projected fate of narrow-ranging montane endemics. Mechanistic models should be improved to explicitly incorporate and consider potential changes in precipitation with altitude, especially for species in montane regions where variation in precipitation and temperature is decoupled (Crimmins et al. 2011, McCain and Colwell 2011). Mechanistic models that incorporate resistance to water loss are expected to further increase the forecasted future suitable habitat for the species used in this study (Riddell et al. 2017a). In the case of species with geographic range limits influenced by biotic interactions, models that directly project abundance and not just presence/absence

offer the best opportunity for accurate predictions of current and future distributions (Buckley 2008, Ehrlén and Morris 2015).

In the face of climate change, species must be managed as dynamic entities. Knowledge of where climatically suitable habitats will be retained and lost, along with size of those habitats, will be critical to the development of conservation plans that can preserve narrow-ranging montane endemics and the population processes that sustain them. The dramatic differences in predicted range loss and fragmentation that we report here suggest a critical need to incorporate mechanisms into forecasts of species range dynamics, and to cross-validate different models' accuracy in predicting range dynamics with independent data sources on species historical ranges and changes in population sizes.

Table 1. Model performance metrics for two niche modeling approaches. Data comparing the ability of the correlative and mechanistic models to predict known presence (sensitivity) and known absence (specificity) for four species of *Plethodon* salamander. Presence and absence localities obtained from U.S. National Museum of Natural History. Habitat suitability threshold for correlative model based on 10th percentile training presence averaged over 10 runs and for the mechanistic model values > 0 in annual discretionary energy.

Model	Sensitivity	Specificity	Performance
	(% Correct +)	(% Correct -)	(% Correct+&-)
Plethodon jordani			
correlative	90%	97%	91%
mechanistic	99%	91%	98%
Plethodon metcalfi			
correlative	91%	94%	91%
mechanistic	82%	100%	83%
Plethodon montanus			
correlative	89%	83%	83%
mechanistic	74%	71%	79%
Plethodon teyahalee			
correlative	90%	26%	65%
mechanistic	81%	21%	58%

Table 2. Mean and standard deviation of remaining suitable area from the results based on two niche modeling approaches for different future climates based on 15 GCMs. Values represent the percent of area still predicted to be suitable for each species within a 10km buffer of current distributions.

Species	Year	Model type	Mean (%)	sd (%)
Plethodon. jordani	2055	correlative	11.5	12.9
		mechanistic	52.0	23.2
	2085	correlative	8.7	13.6
		mechanistic	39.5	18.8
Plethodon metcalfi	2055	correlative	25.3	20.8
		mechanistic	33.7	25.5
	2085	correlative	15.4	11.8
		mechanistic	20.9	13.0
Plethodon montanus	2055	correlative	6.1	10.3
		mechanistic	20.0	21.0
	2085	correlative	3.3	6.2
		mechanistic	11.0	9.2
Plethodon teyahalee	2055	correlative	27.5	17.2
		mechanistic	29.2	21.1
	2085	correlative	19.5	14.3
		mechanistic	20.7	13.9
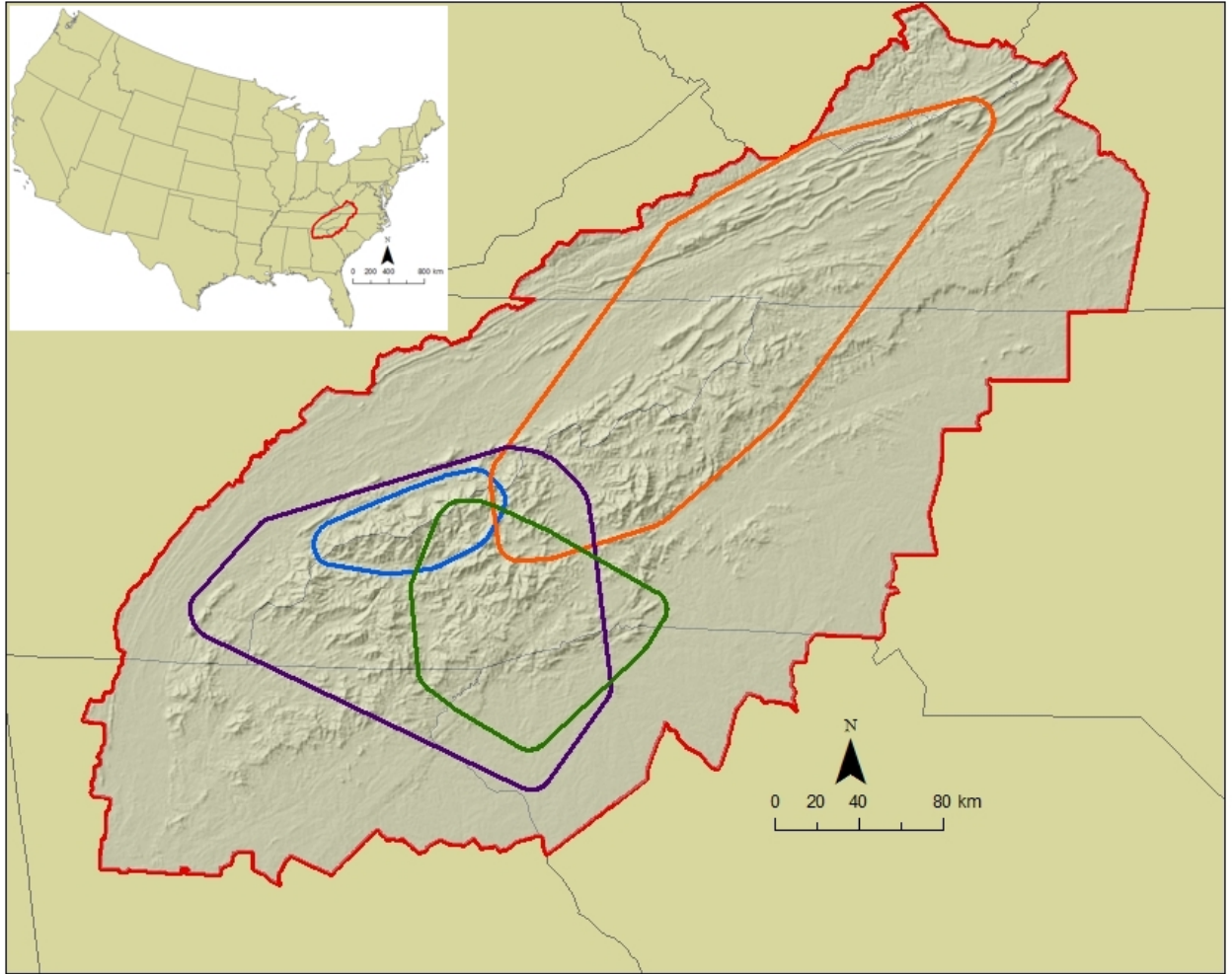
Figure 1. Focal salamander distributions in the Southern Appalachians. Species ranges depicted by a 10km buffered convex hull polygon based on localities obtained from U.S. National Museum of Natural History used in niche model training and projecting, *Plethodon jordani* (blue), *Plethodon metcalfi* (green), *Plethodon montanus* (orange) and *Plethodon teyahalee* (purple).
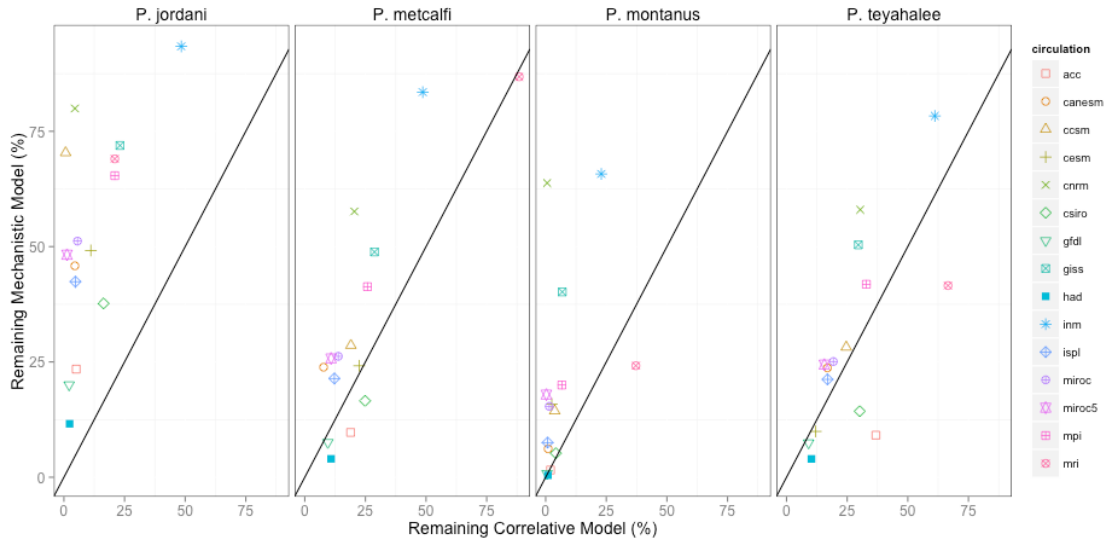
Figure 2. Comparison of predicted suitable habitat retention under correlative and mechanistic model for 15 GCMs for 2050-2060. Points represent global circulation models, colored by model, line for y=x, points above line represent instances where the mechanistic model predicts more suitable habitat than the correlative model.
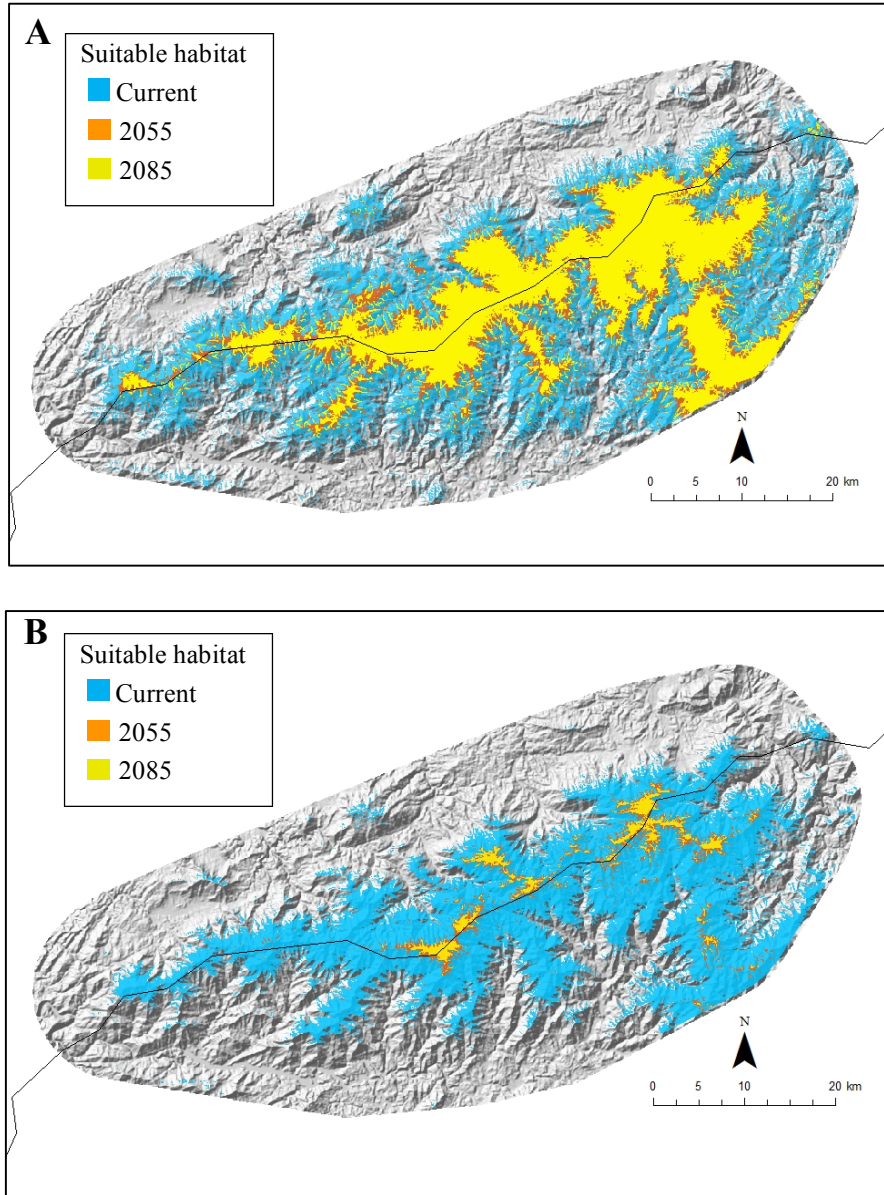
Figure 3. Maps predicting suitable area averaged across 15 global circulation models for *P. jordani* for correlative model (A) and mechanistic model (B). Grid cells are classified as unsuitable (grey), current distribution lost under future scenarios, suitable area lost between 2055 and 2085, and still suitable in 2085. Habitat suitability threshold for correlative model based on 10th percentile training presence averaged over 10 runs and for the mechanistic model values > 0 in annual discretionary energy.
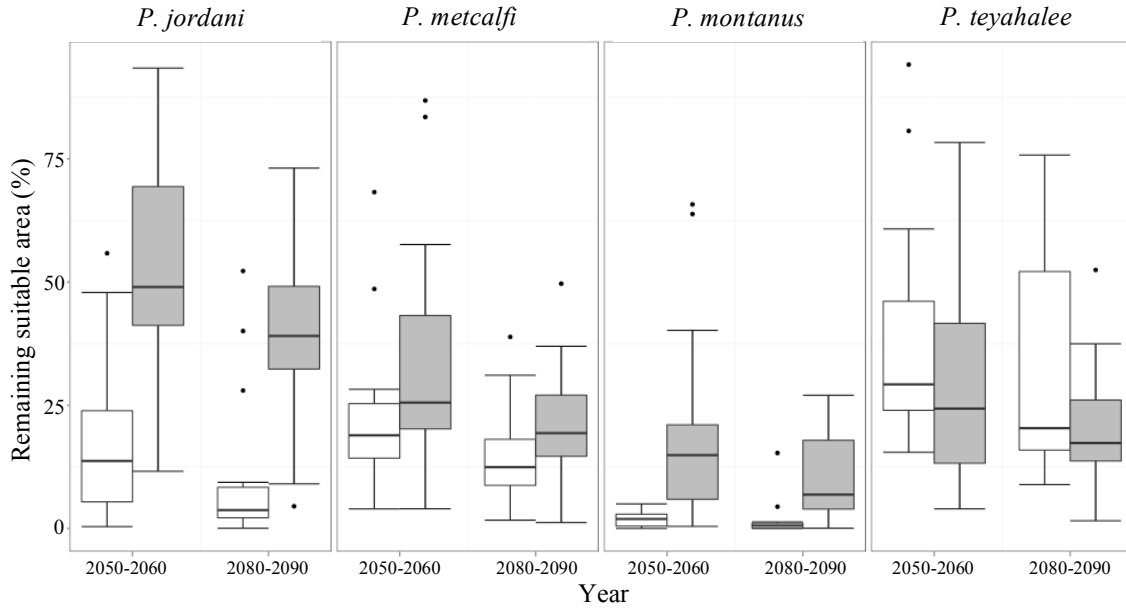
Figure 4. Proportion of total area predicted to remain suitable in 2050-2060 and 2080-2090. Variation represents differences between projected suitable area under different Global Circulation Models. Correlative model results in white and mechanistic model results in grey. Model (correlative vs. mechanistic), GCM, and year significant ($p < 0.05$) for all species except *P. teyahalee.*

Assessing the potential for gene flow to limit differentiation in ecophysiology

ABSTRACT

Understanding the mechanisms that constrain or promote the evolution of species' geographic range has emerged as a fundamental question at the intersection of evolution, ecology, and conservation. In this study, we use a combination of ecophysiology, population genetics, and niche modeling to evaluate whether asymmetrical gene flow from the range center toward the range edge swamps out differentiation in peripheral populations preventing local adaptation. Our focal species, the plethodontid salamander *Plethodon ouachitae*, is restricted to six mountains in the central highlands of the US, occurring in habitats that differ both along elevational gradients and between mountains. We found no evidence for differentiation in ecologically relevant physiological traits, and within mountains we found no population structure and high rates of gene flow. Although population density peaked at mid- to high-elevations, inferred gene flow was not uniformly biased from high- to low-density sites. Even so, high gene flow across elevations may limit elevational differentiation in physiological traits. We also found that this species occupies ecologically divergent mountains separated by low valleys that present a significant barrier to dispersal, and found significant genetic differentiation and environmental niche differences associated with these barriers. Despite the potential for adaptive divergence, we found that salamander populations showed no evidence for differentiation in ecophysiological traits between mountains. Our findings do not support asymmetric gene flow as a force restricting local adaptation and expansion at the range

edge, although uniformly high estimated gene flow within mountains could play such a role. However, even between mountain populations that differ in their environment and show restricted gene flow, we found that *P. ouachitae's* ecophysiology is conserved, suggesting a minor role for gene flow in local adaptation of this species.

INTRODUCTION

What processes cause, maintain, and change species' geographic ranges are central questions in ecology and evolution. Geographic ranges are a complex product of both ecological and evolutionary processes, reflecting current biotic and abiotic conditions, as well as gene flow, drift, adaptation, and history. For these reasons, species distributions are an excellent testing ground for evaluating hypotheses concerning the ecological niche, environmental thresholds, and the limits of adaptation. Answering fundamental questions about range limits is essential to our understanding of many subdisciplines in ecology and evolution and is also of critical importance to conservation as we try to predict how species distributions will be altered by future changes in climate.

To what degree niches are conserved within and between species and to what degree species and populations diverge in their niches through local adaptation is the source of ongoing debate in evolutionary ecology (Pyron et al. 2015). Phylogenetic niche conservatism has been extensively studied in lungless salamanders (Plethodontidae) (Kozak and Wiens 2006, Shepard and Burbrink 2008, Kozak and Wiens 2010a). These studies have found that long term stasis in climatic niches explains patterns of biodiversity observed in the genera *Desmognathus* and *Plethodon* in temperate North America, with closely related species having similar climatic niches and biodiversity

peaking at midelevation in climates that these genera have occupied the longest (Kozak and Wiens 2010a). This is especially true in montane regions where sister species are often isolated in adjacent mountain ranges that are separated by inhospitable valleys (Kozak and Wiens 2006, 2007, 2010a). For these reasons, montane *Plethodon* are an excellent system for testing hypotheses about the ecological and evolutionary processes setting species distributional limits.

The processes that establish and reinforce conserved niches are still poorly understood. Why do populations on the edge of the geographic range fail to locally adapt to environmental conditions beyond their range? One hypothesis is that asymmetrical gene flow from the more densely populated range center may prevent local adaptation at the range edge (Haldane 1956, Slatkin 1978, Kirkpatrick and Barton 1997, Lenormand 2002). However, species' geographic distributions rarely meet the expectation of a single connected distribution with highest population density at the geographic range center (Dallas et al. 2017). For example, when considering the distribution of an elevationally restricted species that occupies multiple mountains, we must consider both the latitudinal and longitudinal geographic range limits, as well as the mountain-specific elevational limits. Within a mountain system, environmental elevation gradients are steeper than latitudinal. The close proximity of populations increases the potential for asymmetric gene flow to inhibit local adaptation and population differentiation. Conversely, populations occupying different mountains may experience more similar environmental conditions but have little to no contemporary gene flow. One method to assess whether asymmetric gene flow could be preventing adaptation to local environments is to examine the relative roles of environmental differences and gene flow on limiting local adaptation

in ecophysiology at both the elevational range limits and latitudinal limits between mountains.

One potential trait that could be the basis for ecophysiological adaptation to different thermal environments is metabolic rate. Metabolic rate determines how resources are allocated among growth, reproduction, and survival, making it a key contributor to fitness (Brown et al. 2004). Standard metabolic rate has also shown a high degree of within-individual repeatability and responded to selective breeding experiments in vertebrates (Burton et al. 2011, Careau et al. 2014). These factors make it a useful trait for assessing local adaptation. Within plethodontids, metabolic rate has been shown to vary among species in ecologically relevant ways (reviewed in Gifford 2016). In some cases, metabolic rate appeared to set lower-elevation boundaries for montane salamanders, with populations from lower-elevations displaying metabolic depression, which could indicate local adaptation or thermal stress (Bernardo and Spotila 2006, Markle 2015). Metabolic rate may also differ seasonally via a plastic temporary response, with a population manifesting a different metabolic rate depending on current conditions. For example, wide-ranging species of *Plethodon* that experience greater seasonal temperature variation have the capacity to acclimate, altering their metabolic rate at different times of the year (Vernberg 1952, Fitzpatrick 1973, Markle 2015).

 Here, we test whether asymmetrical gene flow could limit ecophysiological differentiation between populations of a montane terrestrial salamander. Within mountains, we predict that populations at the lower elevational limit will not exhibit ecophysiological differentiation from high-elevation populations. Though these habitats differ climatically, we predict that the swamping effect of asymmetrical gene flow from

denser high-elevation populations will prevent local adaptation. If elevational populations do show phenotypic differentiation, it could indicate that selection is a stronger force than the homogenizing effect of gene flow or that the traits we are measuring exhibit phenotypic plasticity under different environmental conditions. Between mountains, we predict that, if climate differs, then populations on different mountains will exhibit ecophysiological differentiation. If we do not find support for this but climate differs between mountains, this would indicate that populations on different mountains have a conserved ecophysiology even in the absence of contemporary gene flow. We combine ecophysiology, population genetics, and niche modeling to differentiate these alternative hypotheses.

METHODS

Study System

We conducted our study on the Rich Mountain salamander, *Plethodon ouachitae,* which occurs on the tops of six mountains in the Ouachita Mountains of Arkansas and Oklahoma. Previous work on mtDNA (Shepard and Burbrink 2008) and allozymes (Duncan & Highton 1979) found little to no contemporary gene flow occurring across the valleys between mountains. The Ouachita Mountains are oriented on an east-west axis (Fig. 1), which yields a steep environmental gradient on north-facing slopes. Additionally the Ouachita Mountains are highly seasonal (Pugh and Westerman 2014), especially at low elevation, which may select for seasonally plastic physiological responses compared to mountaintop *Plethodon* in the southern Appalachians, where previous work has focused. On Kiamichi and Round mountains, *P. ouachitae* co-occurs with the larger,

Plethodon kiamichi, whereas on the other four mountains, *P. ouachitae* comes into contact with *Plethodon albagula* at lower elevations (Spotila 1972) where *P. ouachitae* appears to aggressively exclude the larger species (Anthony et al. 1997, 2002). As *P. ouachitae* is the dominant species in these competitive interactions, we do not expect competition with congeners to set the lower-elevation range limit. Rather, elevational limits for *P. ouachitae,* like other mountaintop *Plethodon,* are most likely the result of climatic tolerance (Lyons et al. 2016), with valleys being too hot and/or dry for salamanders to be active.

Sampling

Salamanders were sampled during the day by looking under cover objects (*i.e.,* large rocks and logs) and at night by using a flashlight to find surface-active individuals. All sampling occurred in late March, early May, and late October between 2013 and 2015, during times when *P. ouachitae* is known to be active and after there was at least one substantial rainfall event in the prior week. To evaluate local adaptation within mountains, we focused sampling along eight elevational transects, six on Winding Stair Mountain, one on Round Mountain, and one on Kiamichi Mountain, attempting to collect 10 individuals both from high-elevation (>500 m) and from low-elevation (<400 m) sites on the north-facing slope of the mountains. Transects were areas where there was access to the top of the mountain either by nearby road or hiking trail and a low-elevation site along a straight downslope path. Upper-elevation sites were just off the mountain ridge on the north side of the mountain. For low-elevation sites, we started sampling at approximately 300 meters in elevation, if fewer than two *P. ouachitae* were located after

one person-hour of searching under suitable weather conditions, then we moved upslope approximately 50 meters in elevation and tried again. Our target number of individuals was 10 per site. We reached this goal at some sites in one visit whereas other sites required multiple visits either at day and night or on different days. When possible, one or two additional mid-elevation sites were also sampled along a linear transect between the high- and low-elevation sites. To evaluate adaptive differentiation in relation to climates on different mountains, we also sampled one to two sites on Round, Rich, and Black Fork mountains (Sampling site information available in Appendix S4, Table 2.1). We did not sample the smaller Buffalo Mountain, where *P. ouachitae* is also found, because this land is privately owned. At every site, we recorded the number of search hours, counts of all amphibians encountered, and the time of day, soil temperature, air temperature, humidity, and wind speed.

Salamanders collected for physiological measurements were separated by population and transported live with leaf litter in a cooler back to the University of Minnesota within one week of collection. Animals were sexed by presence of a mental gland (males) or visible egg sacks (females), if we were unable to make a determination, then the animal was sexed after euthanasia through dissection. Animals with a mass below two grams that could not be sexed were classified as juveniles. In order to examine geographic patterns of genetic differentiation on Winding Stair Mountain, we also collected tissue samples from some salamanders for which we did not take metabolic rate measurements. For animals where we did not take physiological measurements, mass and snout-vent-length were taken in the field along with a tail tip for tissue, which was preserved in 95% ethanol. These animals were then released at the point of collection

after processing.

Metabolic rate

All animals were maintained at 14º C in an environmental chamber under a 12L:12D photoperiod. Each salamander was housed in an individual plastic container containing moist paper towels for substrate and cover, and fed crickets weekly. Resting metabolic rate measurements were taken from 252 individuals at 15º, 20º, 25º, and 30º C. For 109 individuals, we also measured acclimation capacity by randomly assigning individuals to 14º C or 22º C treatments for the two weeks prior to measuring metabolic rate. After 14 days of acclimation at either 14º C or 22º C (for the seasonal acclimation treatment), standard metabolic rate measurements were taken over four consecutive days, with each individual experiencing test temperatures at 15º, 20º, 25º, and 30º C in a random order (one temperature per day). Salamanders were fasted for six days prior to the first test to ensure a post-absorptive state. We recorded the volume of oxygen consumed (VO_2) at rest using an automated closed-system respirometry set up (Sable Systems International, Hendersonville, NV). Each individual was placed in a 35-ml tube within a temperature-controlled cabinet, which kept the temperature within 1º C of the desired test temperature. For each test, measurements were taken from seven salamanders each in individual tubes fit with two-way stopcocks at either end, an additional tube was run with no animal to provide a baseline.

Because of our interest in standard rather than active metabolic rate, measurements were taken between 9am and 5pm, when nocturnal salamanders would usually be inactive. Oxygen consumption was measured eight times over a two-hour

period to estimate energy used and calculate standard metabolic rate. The first hour of data was not used, to allow time for the animal to adjust to the test temperature and recent handling. We averaged the lowest two runs per trial for each individual to calculate a standard metabolic rate for each individual based on the volume of O_2 consumed per hour at each test temperature.

These data were analyzed using a mixed-effect linear regression model in the lme4 (version 1.4, Bates et al. 2015) and lsmeans (version 2.2.7, Lenth 2016) packages of R (R Core Development Team 2016) to examine the effects on log-transformed oxygen consumption rate of temperature, body mass, population (elevational site, transect, and mountain), acclimation state as well as interactions between test temperature, population, and acclimation state. Because each individual was tested multiple times, we included individual as a random effect with all other variables as fixed effects. To test for transect-specific differentiation at different elevational sites we started with a model including mass, sex, age, and an interaction between test temperature, elevation, and transect. To test for physiological differentiation by mountain, we replaced the transect term with the mountain of each sampling site. To test whether any populations showed acclimation ability, we analyzed only those individuals that had been acclimated at both 14º C and 22º C and included acclimation (warm acclimated or cool acclimated) as an interaction with elevation, test temperature, and mountain. After an initial 35 individuals were measured for metabolic rate in 2013, we had the respirometry equipment serviced. This service revealed some leakage in our system. In order to account for the equipment alteration repairing this issue, we included a binary fixed effect term for the measurements taken before and after repairs (representing 18% of the

Winding Stair specific data). We stepwise tested nested models against each other using likelihood ratio tests and removed effects and interactions that did not significantly improve our model testing all combinations of 2 and 3 way interactions.

Collection of Genetic Data

Genomic DNA was extracted using the Blood and Tissue Genomic DNA Miniprep System (Viogene BioTek Corp, Taipei, Taiwan) following the manufacturer's protocol. We amplified sixteen microsatellite loci from markers developed for *Plethodon jordani* (Luxbacher 2014) and *Plethodon albagula* (Spatola et al. 2012), the majority of which were tetra-nucleotide repeats except for two tri-nucleotide and one penta-nucleotide repeat. Amplified fragments were multiplexed using 4 fluorescently-labelled M13 tags (6-FAM, NED, VIC, and PET). We followed the PCR amplification methods described by Spatola et al. (2013) and were able to pool up to 8 markers in each well of a 96-well plate (one long and one short fragment for each fluorescent tag). Products were sized by electrophoresis on an ABI 3730xl capillary electrophoresis platform (Applied Biosystems, Foster City, CA, USA) using a LIZ 500 internal size standard, at the University of Minnesota's Genomics Center. We called fragment sizes using the microsatellite plugin in Geneious v9.1 (Kearse et al. 2012), and all scores were visually checked. A random subset of individuals were reamplified for each marker, submitted for fragment analysis, and rescored to ensure that peaks were being scored consistently. Estimated fragment sizes were binned into alleles using TANDEM v1.09 (Matschiner and Salzburger 2009).

Microsatellite data were partitioned by mountain and each locus was tested for deviations from Hardy-Weinberg equilibrium using Fisher's exact test in the R package DiVersity (Keenan et al. 2013). Three loci did not conform to Hardy-Weinberg expectations. Two of these loci had also been flagged during fragment analysis for the presence of null alleles and after repeated attempts at amplification were removed from population genetic analyses.

Population Structure Analyses

Based on the asymmetrical gene flow hypothesis, we expected gene flow to be biased downslope, with more movement from higher density upper-elevation populations to less dense lower-elevation populations (Pope and Pope 1951), preventing local adaptation at the range edge. To quantify directionality of gene flow within the elevational transects, we employed two methods. First, we used BayesAss v. 3.0 (Wilson and Rannala 2003), which estimates recent migration between populations using MCMC, and second we used the coalescent-based program Migrate-N (Beerli and Felsenstein 2001, Beerli 2006). It is commonly assumed that BayesAss estimates more recent gene flow, while Migrate-N estimates historic gene flow and effective population sizes (Moeller et al. 2011, Paul et al. 2011, Wang and Shaffer 2017); however, BayesAss was unable to recover recent shifts in gene flow in a recent simulation study (Samarasin et al. 2016). We used Migrate-N to examine gene flow within elevational transects. Migrate-N was run using Bayesian inference with uniform priors θ (0, 200) and M (0, 200) with 1500 bins and a Brownian motion approximation of the stepwise mutation model. Each run consisted of one long chain and four heated chains (1,1.5, 3, 10^5), sampling every 150

steps, recording 5 x 10^4 steps and discarding the first 10^5 steps per chain. All runs were performed on the CIPRES supercomputing cluster (Miller et al. 2010). We compared models of asymmetric migration, symmetric migration, and a model that assumed all samples were from the same population (panmixia) using the Bezier approximation score to calculate Bayes factors and rank the likelihoods of each model (Beerli and Palczewski 2010).

BayesAss was then used to estimate the relative proportion of each sampling site made up of migrants from other sites along the same transect, which allowed us to quantify movement up and down the mountain in cases where sites were not divergent enough for Migrate-N to infer gene flow. BayesAss was run with 10^6 steps, a burn-in of 10^5 steps, and a sampling interval of 100 steps, with mixing parameters of 0.3 for m, 0.15 for inbreeding coefficient, and 0.15 for allele frequencies. We evaluated convergence in Tracer (v1.6.0, Rambaut et al. 2014) and replicated every run with multiple random number seeds.

We hypothesized that within mountains, gene flow would have a homogenizing effect, ultimately preventing phenotypic differentiation, while gene flow between mountains would be reduced, with drift being a more dominant force. We assessed this by testing for isolation by distance. We quantified genetic differentiation between all sites for which we had samples of five or more individuals using the pairwise F_{ST} value calculated in Genodive (v2.0b25, Meirmans and van Tienderen 2004). To examine how genetic differentiation varies with geographic distance, we calculated pairwise distances between localities (in meters) using latitude and longitude in the R package geosphere (Hijmans 2017) using the distm() command and a haversine method assuming a spherical

earth. With these data, we were then able to test for isolation by distance among all sampling sites, as well as within and between mountains using a Mantel test with 999 permutations in the program PASSaGE v2 (Rosenberg and Anderson 2010).

To further test the hypothesis that valleys would present a significant barrier to gene flow, we first assessed population structure between mountains using the program STRUCTURE v2.3.2 (Pritchard et al. 2000), which clusters individuals into populations based on the fit to linkage and genotype frequency expectations under Hardy-Weinberg equilibrium. We used all samples from Round, Kiamichi, Rich, and Black Fork Mountains. Because STRUCTURE has been shown to be unreliable when population sampling is uneven (Puechmaille 2016), we selected only one transect from the western end and one from the eastern end of Winding Stair Mountain, so that sample sizes would be more even across the five mountains. We ran STRUCTURE using an admixture model for 1 to 10 clusters with 5×10^4 MCMC steps after burn-in and 2.5×10^4 burn-in steps over 5 independent replicate runs. We determined the most likely number of clusters using Evanno et al.'s (2005) delta-K method.

Niche differentiation

To evaluate whether populations on different mountains occupied different niches, we used MaxENT v3.4.1 (Phillips et al. 2006) and ENMtools v1.4.4 (Warren et al. 2008, 2010). We gathered latitude and longitude locality information from our own sampling, VertNet (vertnet.org, retaining only localities with <100 m uncertainty), and previous publications (Shepard and Burbrink 2008, 2009). In addition to a data set including all locality points, we created five datasets by visually subsetting the locality

points by mountain using ArcGIS 10.1 (ESRI, Redlands, CA, USA). Locality details are available in Appendix S4 Table 2.2. We excluded locality points from Buffalo Mountain because it was not sampled for physiology or population genetics. We downloaded 19 bioclimatic variables at 30 second resolution from the CHELSA climate data set (Karger et al. 2017). These climate data are generated by quasi-mechanistic statistical downscaling of ERA-Interim temperature and precipitation estimates, and enable a more fine-scale examination of climate over complex terrain compared to WorldClim (Karger et al. 2017). The 19 bioclimatic layers were clipped to a region encompassing the Ouachita Mountains in Oklahoma and Arkansas, as well as parts of surrounding states, -92.2–-96.56º longitude and 33.18–36.93º latitude (Shepard and Burbrink 2008). To avoid overfitting the model (Phillips et al. 2006), we measured spatial correlation among all 19 BIOCLIM variables in ENMTools and removed all but one variable from groups of variables correlated at r > 0.7. This process resulted in retention of seven bioclimatic layers: annual mean temperature, mean diurnal range, temperature seasonality, mean temperature of the wettest quarter, mean temperature of the driest quarter, annual precipitation, and precipitation of the warmest quarter. We used MaxENT to characterize the environmental niche of each mountain's population. MaxENT was run with a 25% random test percentage 10,000 background points, 500 iterations, with a convergence threshold of 0.00001 over 10 subsampled replicates.

To quantify niche differences among populations on different mountains, we used our ENMs for each mountain generated in MaxENT with ENMTools (Warren et al. 2010) to calculate Schoener's D (Schoener 1968) and the I statistic, which is a derivative of Hellinger's distance (Warren et al. 2010). These statistics quantify overlap in niches

based on two ENMs and yield values between 0 (no niche overlap) and 1 (identical niches). To test for whether the observed differences in niche projections (D and I) are more different than niche projections of samples drawn randomly from pooled occurrence points we used the Identity test in ENMTools performing 100 replicates of every pairwise comparison, and took values falling below 97.5% of niche overlaps generated by randomizing identities as evidence for niche differentiation. To test whether mountain populations were more or less similar than expected based on their background environment, we used the Background test in ENMtools. This is done by comparing one mountain's occurrence points to new occurrence points created randomly within a minimum convex hull polygon of the known occurrence points of another mountain. We created 100 replicates comparing one mountain to the background of every other mountain separately. If our calculated D and I values fell below 97.5% of the overlaps generated through the background test this was indication for niche divergence of that mountain's occurrence points from the habitat of the other mountain; if the value was above 97.5% this indicated niche convergence (Warren et al. 2008).

RESULTS

As expected, we found no significant difference in metabolic rate based on transect, elevation, or an interaction between transect and elevation, we found no differentiation between high- and low-elevation sites on the eight transects. Against expectation, our analysis of the metabolic rate between the five sampled mountains did not reveal a significant effect. Although elevation and mountain had no effect, we found expected effects of test temperature, mass, age, and respirometer maintenance on

metabolic rate. Overall, metabolic rate increased both with temperature and with animal mass (Table 1). In the mixed effects model for individuals that went through acclimation treatment, test temperature and mass again had significant effects on the metabolic rate, though age was not significant. There was no significant interaction between test temperature and acclimation state on metabolic rate, indicating that the slope of thermal sensitivity does not show seasonal plasticity in this species. Acclimation did have a significant effect on metabolic rate (LRT $\chi^2 = 4.00$, df = 1, p = 0.045), however this was driven by a single sampling site (CG3), without that site there was no effect of acclimation.

Based on search effort required to find a single *P. ouachitae* (Fig. 2), population density appears to decrease at the lower-elevation range limit. No individuals were located below 360 meters in elevation, despite five hours of searching between four different sites. Within Winding Stair Mountain, where we had six elevational transects and six additional sites between transects, Migrate-N strongly supported a model of panmixia within every transect and among transects. Though runs individually converged for values of M and θ, replicate runs were inconsistent, however, there was consistently higher likelihood for models with a single panmictic population. As a result of the lack of evidence for genetic structure, it was not meaningful to quantify effective population sizes of high- and low-elevation sites separately. Though our results indicate that sampling sites within mountains should be considered one population, we were able to quantify the proportion of each sampling site made up of migrants from other sampling sites along the transect using BayesAss. We did not find consistent asymmetrically biased gene flow downslope to less dense sites, six out of eight transects had high-elevation sites

comprising more migrants than lower-elevation sites (Table 2).

The delta-K method revealed four genetic clusters (Fig. 3). These clusters corresponded closely to the mountains sampled, except for the Rich and Black Fork mountains, which STRUCTURE grouped together. There also appears to be more admixture among the neighboring mountains of Winding Stair, Rich, and Black Fork than between Kiamichi and Round Mountains. These findings were further supported by isolation-by-distance analysis (Fig. 4).

Genetic distance and geographic distance were positively related and Mantel tests indicated a general trend of isolation-by-distance between all sampling sites (R^2= 0.62, p=.001). Within mountains, the relationship between genetic and geographic distance was significant, but geographic distance explained less of the variation in pairwise genetic differences (R^2= 0.17, p=.001), analyzing only distances between mountains also resulted in less variation explained by geographic distance, though more than within mountains (R^2= 0.39, p=.001) (Fig. 4). Pairwise F_{ST} values within mountains ranged from 0 to 0.127 and pairwise F_{ST} between mountains ranged from 0.027 to 0.288. The largest F_{ST} value came from pairwise comparisons of neighboring Kiamichi and Round mountains, these mountains have most likely been inhabited by *P. ouachitae* the longest (Shepard and Burbrink 2009).

Ecological niches of populations occupying different mountains were also differentiated. Pairwise comparisons between the five mountains resulted in values for *D* ranging between 0.21 and 0.69, while values for *I* ranged between 0.43 and 0.84 (Table 3). Niche identity tests showed that 9 of the 15 pairwise comparisons were significantly different for *D,* and 10 out of 15 pairwise comparisons were different for *I* (Table 3). We

also tested whether each mountain's occurrence points differed significantly from the background environment of any other mountains, and found that 6 of the 20 comparisons showed divergence and 9 of the 20 comparisons showed convergence for *D*. For the metric *I,* 6 of the 20 comparisons showed divergence and 9 of the 20 comparisons showed convergence (Table 4).

DISCUSSION

Understanding the role of niche conservatism and niche divergence in speciation has emerged as a fundamental question at the intersection of evolution and ecology. Moreover, the underlying processes that constrain or promote evolution of the niche remain poorly understood. Asymmetrical gene flow has the potential to limit local adaptation at the range edge (Haldane 1956, Kirkpatrick and Barton 1997), however gene flow can also introduce beneficial alleles (Fitzpatrick et al. 2017) and mask deleterious alleles in small populations (Keller and Waller 2002, Whiteley et al. 2015). In this study, we compared populations occupying different environmental conditions but found no evidence for differentiation in the phenotypic traits for metabolic rate thermal sensitivity and seasonal plasticity in metabolic rate. This lack of phenotypic differentiation was observed not only between sites within mountains that are connected by contemporary gene flow, but also between mountain populations with little evidence of recent gene flow.

Within mountains

We hypothesized that though the hot, dry, and seasonal low-elevation habitats

would select for shifts in resting metabolic rate and the ability to acclimate for populations at the elevational range edge, asymmetrical gene flow from denser high-elevation populations would reduce local adaptation at the periphery. Within mountains, we found essentially panmixia with no downslope bias in gene flow and no evidence for local adaptation at lower elevations in metabolic rate thermal sensitivity or seasonal plasticity. Though the Ouachita Mountains are highly seasonal (Pugh and Westerman 2014), *P. ouachitae* did not demonstrate seasonal plasticity in metabolic rate, with the exception of one population, this species most likely minimizes susceptibility to seasonal changes behaviorally through regulation of surface activity in response to temperature and moisture avoidance, based on the influence of temperature and moisture on surface activity (Pope and Pope 1951).

Why do populations at the lower-elevation edge of this species' range fail to locally adapt? One hypothesis for why peripheral populations fail to adapt to local conditions is that asymmetrical gene flow from the more densely populated range center swamps out local adaptation at the less densely populated range edge (Kirkpatrick and Barton 1997). Using search effort as a proxy for estimated population density, we found evidence for population density being highest at mid- to high-elevations and decreasingly sharply at the low-elevation range limit. Though search effort supported a more abundant center compared to the low-elevation range edge, we did not see evidence for gene flow being biased downslope to sparser populations consistent with simple diffusion. In one analysis, we found that the majority of higher elevation sites actually contained more migrants from lower-elevations than vice versa, indicating if anything more movement upslope from less dense sites into more dense sites. We hypothesized that the lack of

physiological differentiation at the lower elevation range limit was caused by the asymmetrical flow of alleles moving downslope, as previously measured in the mountaintop Appalachian species, *Plethodon jordani* (Lyons et al. 2016). The high levels of gene flow within a single mountain could also prevent local adaptation to the environmental conditions in different parts of the mountain. However, local adaptation has been demonstrated in many systems that experience high levels of gene flow (Saint-Laurent et al. 2003, Hoekstra et al. 2004, Muir et al. 2014, Fitzpatrick et al. 2014, Moody et al. 2015).

As a whole, our system appears to be in drift-gene flow equilibrium (Hutchison and Templeton 1999), with F_{ST} and geographic distance being highly correlated and increasing linearly. However, the relationship within mountains was much weaker. There was support for isolation by distance, but distance explained little of the variation in pairwise F_{ST} values within mountains. Even over larger geographic distances, F_{ST} values are low; this trend is expected after recent colonization of an area or if gene flow remains the dominant force over drift, homogenizing populations and preventing differentiation at neutral loci (Hutchison and Templeton 1999). The collected findings on Winding Stair that gene flow is dominant to drift and that there are high rates of elevational gene flow predominantly upslope, suggesting that *P. ouachitae* is very mobile. Prior work on *Plethodon* found that individuals move little during their lifetime, resulting in low amounts of gene flow between populations (Cabe et al. 2006), with one study finding that female *Plethodon cinereus* stay within 1 m of their juvenile location (Liebgold et al. 2011). Our study shows that barriers like unsuitable valleys do reduce gene flow on a large geographic scale, but within an area of connected habitat even of varying

suitability, gene flow connects populations. The migration rates we calculated from the less dense, lower-elevation sites to upper-elevation sites may result from compensatory movement where individuals move more in suboptimal habitat (Peterman et al. 2014). This should be further assessed by testing resistance surfaces as hypotheses for the role of different environmental factors on gene flow in Winding Stair Mountain.

Between mountains

Based on the asymmetrical gene flow hypothesis, in the absence of gene flow, selection mediated by environmental differences should lead to differentiation in ecologically relevant traits. Gene flow between mountains was reduced, and the niches of the mountains significantly differed; however, even in this case, we saw no evidence for local adaptation. We found environmental niche differentiation between populations on different mountains that was greater than expected due to both chance choice of locality points and background environments. We also found multiple lines of evidence supporting reduced gene flow between mountains. Previous work on *Plethodon* has indicated that the intervening habitat between mountains is unsuitable for mountaintop species, restricting populations and preventing contemporary gene flow (Kozak and Wiens 2006, 2007, Shepard and Burbrink 2009). Our isolation by distance analysis between mountains did show a positive trend, but geographic distance only explained relatively little of the variance in F_{ST} value, and F_{ST} values for pairwise comparisons between mountains was higher than within mountains. The scatterplot between mountains shows a pattern associated with population isolation, and drift as an important process, with less gene flow between mountains (Hutchinson and Templeton 1999).

Though mountains differed in their ecological attributes and gene flow was restricted between mountains, there was no significant differentiation in any of the physiological traits measured.

Populations from different mountains were similar in ecophysiology, but some of these populations are differentiated in morphology, specifically body size. Shepard et al. (2011) found that *P. ouachitae* from Round and Kiamichi Mountains were significantly smaller than those from Buffalo Mountain (where we did not sample) and Black Fork Mountain, while salamanders from Rich and Winding Stair Mountains were not significantly different in size from any of the other mountains. The *P. ouachitae* on Round and Kiamichi Mountains represent the oldest lineages (Shepard and Burbrink 2009) of this species and occupy the same habitat space as a larger *Plethodon*, *P. kiamichi*, while on the other four mountains, *P. ouachitae* competitively excludes larger *P. albagula* through interspecific aggression. The larger size on the more recently colonized mountains may represent a competitive release from *P. kiamichi* or an adaptation to competitive interactions with *P. albagula*.

Body size in ectotherms can result from many complex processes relating to both biotic and abiotic influences (Peterman et al. 2016). Differences in size between mountains would not only affect competitive interactions, but also environmental tolerance. Smaller salamanders have reduced energetic needs while also being more prone to desiccation (Peterman et al. 2013), which would restrict surface activity time (Gifford and Kozak 2012). With finer-scale ground temperature data, a biophysical mechanistic niche model (Gifford and Kozak 2012, Riddell and Sears 2015) could be employed to test whether body size differences between mountains reflect an advantage

of the size of individuals in each population to their respective home environments. The niche differentiation quantified in this study does not correspond with expectations of the variation in body size; mountains with similar-sized salamanders had as much or more environmental differences than mountains with differentiated morphology.

Though we found no evidence for local adaptation in metabolic rate thermal sensitivity or seasonal acclimation in metabolic rate, this does not necessarily indicate that populations are not locally adapted. Besides body size and metabolic rate, selection could also act on other morphological, behavioral, and physiological traits such as water loss rate (Riddell and Sears 2015). We did not find evidence for the role of gene flow in limiting local adaptation, the lack of phenotypic differentiation we observed could result from constraints due to pleiotropy or lack of relevant genetic variation (Blows and Hoffmann 2005, Wiens and Graham 2005). Physiological traits are expected to be tightly linked in complex ways, tradeoffs between acclimation in metabolic rate and water loss rate have been observed in other mountaintop *Plethodon* (Riddell et al. 2017b). Additionally, these organisms are only experiencing a small portion of the climatic differences between sites, salamanders of the family Plethodontidae, like other ectotherms, are able to behaviorally thermoregulate adjusting surface activity based on moisture and temperature in order to optimize physiological processes (Heatwole 1962, Feder 1983, Strickland et al. 2016). Because *Plethodon* have low metabolic needs and large energy stores, they can remain inactive during challenging environmental conditions (Feder 1983), potentially avoiding the conditions that would select for physiological differences. For this reason, the environmental variables we used to quantify differences between mountains may not be the most relevant variables

determining *P. ouachitae* distributions. Precipitation and temperature are important

determinants for the amount of energy individual salamanders obtain and expend

(Gifford and Kozak 2012). However, environmental variables that average over the year

or quarter are most likely less important to organism fitness compared to temperature and

precipitation variation within shorter time periods (Clusella Trullas et al. 2011).

Our results also indicate that divergence in ENMs alone should not be used as

evidence for local adaptation or species' ecological divergence. The combination of

differentiation in occupied environmental niche and genetic differentiation has been used

to indicate niche divergence between and even within species (Graham et al. 2004,

Rissler and Apodaca 2007, Wooten et al. 2013). We found both genetic and realized

environmental niche differentiation in our study species corresponding to each mountain,

but no evidence for differentiation in two traits known to be important in the

environmental performance of salamanders (Bernardo and Spotila 2006) and

demonstrated to vary among species in ecologically relevant ways (Gifford 2016). Some

perceived environmental differences might be the result of unequal sampling. Winding

Stair and Rich Mountains are easily accessible and well sampled, while the other

mountains are difficult to access without extensive hiking. This does not fully explain our

findings because even the well sampled mountains differed significantly in environmental

attributes. Our findings also suggest that researchers should be cautious in interpreting

intraspecific variation in occupied niches as an indication for differentiation in

environmental tolerance. Recent work has promoted separating occurrence points in

correlative niche models by population or ecotype, arguing that species should not be

treated as single entities (Raxworthy et al. 2007, Valladares et al. 2014, Moran et al.

2015). Incorporating intraspecific variation that is actually or putatively linked to thermal tolerance has been shown to increase the amount of habitat predicted to be suitable under climate change as compared to models treating species as a single entity (Oney et al. 2013). For *P. ouachitae* genetic differentiation between mountains appears to be a product of restricted gene flow and time generating population structure. Our results demonstrate that caution should be used in conducting separate correlative niche models on population-level locality information and then combining this output in the absence of experimental tests for differentiation in fundamental niche space.

Climate change and niche conservatism

As global climate changes, organisms may adapt or acclimate in place, shift their distributions to track their climatic niche, or go extinct. Variation in environmental tolerance and performance within a species would demonstrate a physiological buffer not accounted for in current estimates of the impact of climate change on species and the potential to adapt to future changes. However, despite evidence for variation in the environmental niche occupied, we have not found evidence that populations differ in ecophysiological tolerances. Rather, we found that mountaintop *Plethodon ouachitae* may not have the capability to adapt in metabolic thermal sensitivity to changing climate, based on a lack of observed differentiation between populations occupying areas with presently different climates. We expect that these organisms will shift their distributions, tracking their climatic envelopes. These populations have survived past periods of climatic fluctuations through expanding and retracting, tracking their environmental niches, leading to the biogeographic patterns currently found in North America. Montane

species, currently restricted to high-elevation areas surrounded by inhospitable lowlands, will be unable to track their niches latitudinally, and distributions will most likely retract upslope. Whether there is enough suitable high elevation habitat for these species to persist will depend on the magnitude of future warming.

Table 1. Results of two mixed effects models with log volume O_2 consumed as the response and individual as a random effect. Only significant fixed effects determined by likelihood ratio tests of nested models were retained in these two final models. **A** is a model of the fixed effects of individual mass, age, test temperature and whether the respirometer set up had been serviced (Fix: Yes) on the oxygen consumed by 252 individuals over four test temperatures all housed at 14º C. **B** is a model of a subset of those 252 who were also acclimated at 22º C (Acclimation: Yes) and retested for oxygen consumption at the same four test temperatures. All testing of B occurred after equipment was serviced and juveniles were excluded so these terms were not significant/applicable.

	A (logvo2)			B (logvo2)		
	B	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts						
(Intercept)	-1.93	-1.99 – -1.87	<.001	-1.94	-2.01 – -1.87	<.001
Mass	0.11	0.09 – 0.12	<.001	0.13	0.11 – 0.15	<.001
Age (Juvenile)	-0.08	-0.12 – -0.05	<.001			
Temperature	0.04	0.04 – 0.04	<.001	0.04	0.04 – 0.04	<.001
Fix (Yes)	0.08	0.05 – 0.11	<.001			
Acclimation (Yes)				0.02	0.00 – 0.04	.046
Random Parts						
σ^2		0.018			0.021	
$\tau_{00, Num}$		0.003			0.004	
N_{Num}		252			109	
ICC_{Num}		0.140			0.173	
Observations		997			862	
R^2 / Ω_0^2		.825 / .825			.792 / .791	

Table 2. Fraction of each elevational site made up of migrants from the other sites sampled on that transect, with standard deviation, estimated using BayesAss. For six of eight transects gene flow is biased upslope, with higher elevation sites having more recent migrants than low elevation sites. All transects unless specified were on Winding Stair Mountain.

transect	low elevation (362-428 m)	mid elevation (445-603 m)	high elevation (508-709 m)
DMT	17% (9.3)		26% (6.7)
CG	13% (8.7)	26% (7.2)	30% (3.1)
DG	18% (5.7)	29% (4.0)	28% (4.9)
ELR	6.6% (4.4)		28% (4.4)
HTS	29% (3.3)	25% (8.1)	20% (9.4)
TCR	12% (6.9)		27% (6.7)
RM (Round)	6.8% (5.5)		27% (3.6)
PAT (Kiamichi)	26% (6.7)		11% (7.1)

Table 3. Identity test for niche overlap. Niche overlap measured with Schoener's *D* (upper matrix) and *I* (lower matrix) statistic values for *Plethodon ouachitae* grouped by mountain. Bold values indicate significantly non-overlapping niches (p < 0.05) based on comparison with a null distribution created by randomly reshuffling occurrence points and recalculating overlap for 100 replicates of every pairwise comparison (Identity test).

	Round	Winding Stair	Kiamichi	Black Fork	Rich	All
Round	--	0.34	0.43	0.52	0.37	0.50
Winding Stair	**0.55**	--	0.47	**0.23**	**0.49**	**0.69**
Kiamichi	0.72	0.72	--	**0.34**	**0.34**	**0.47**
Black Fork	0.83	**0.43**	**0.61**	--	**0.21**	**0.40**
Rich	0.64	**0.78**	**0.6**	0.46	--	**0.51**
All	0.76	**0.84**	**0.74**	**0.67**	**0.77**	--

Table 4. Background test for niche overlap. Niche overlap measured with Schoener's *D* (**A**) and *I* (**B**) for *Plethodon ouachitae* grouped by mountain, significant niche divergence or convergence calculated with replicates of occurrence points against background models for every other mountain. Bolded values indicate niche divergence (p < 0.05) and italicized values indicate convergence (p < 0.05).

A

	Round	Winding Stair	Kiamichi	Black Fork	Rich
Round background	--	*0.34*	*0.43*	*0.52*	0.37
Winding Stair background	*0.34*	--	*0.47*	*0.23*	*0.49*
Kiamichi background	*0.43*	**0.47**	--	**0.34**	0.34
Black Fork Background	*0.52*	**0.23**	*0.34*	--	**0.21**
Rich Background	0.37	0.49	**0.34**	**0.21**	--

B

	Round	Winding Stair	Kiamichi	Black Fork	Rich
Round background	--	0.55	*0.72*	*0.83*	0.64
Winding Stair background	*0.55*	--	*0.72*	*0.43*	*0.78*
Kiamichi background	*0.72*	**0.72**	--	0.61	**0.60**
Black Fork Background	*0.83*	**0.43**	*0.61*	--	**0.46**
Rich Background	0.64	0.78	**0.60**	**0.46**	--
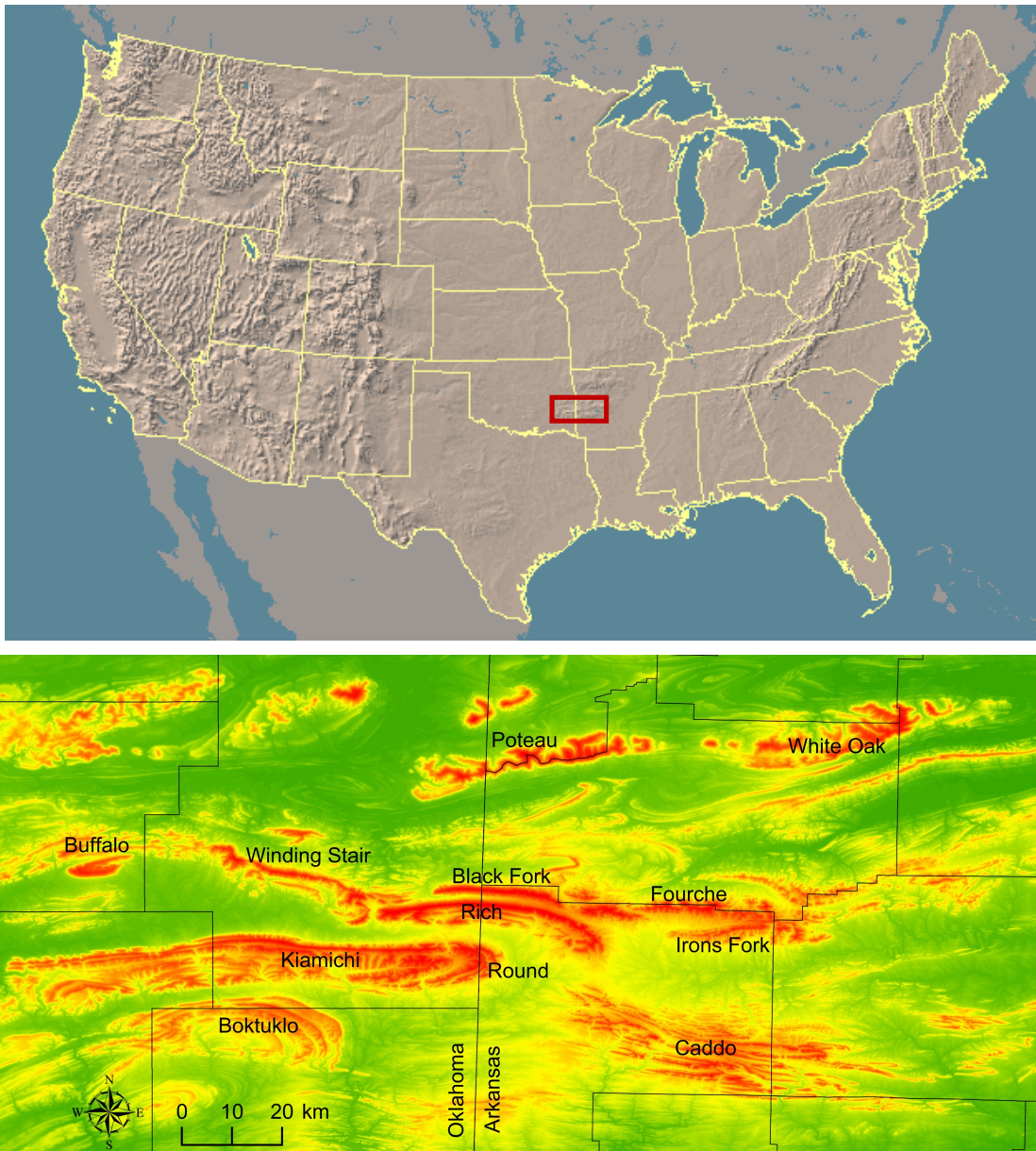
Figure 1. Digital elevation map of the Ouachita Mountains in Oklahoma and Arkansas, USA. Top panel of the area of focus, lower panel elevational map of the Ouachita Mountains. Elevation rages from a low of 120 m (green) to a high of 820 m (dark red). *Plethodon ouachitae* occurs on Kiamichi, Round, Rich, Black Fork, Winding Stair, and Buffalo Mountains above 330 m in elevation.
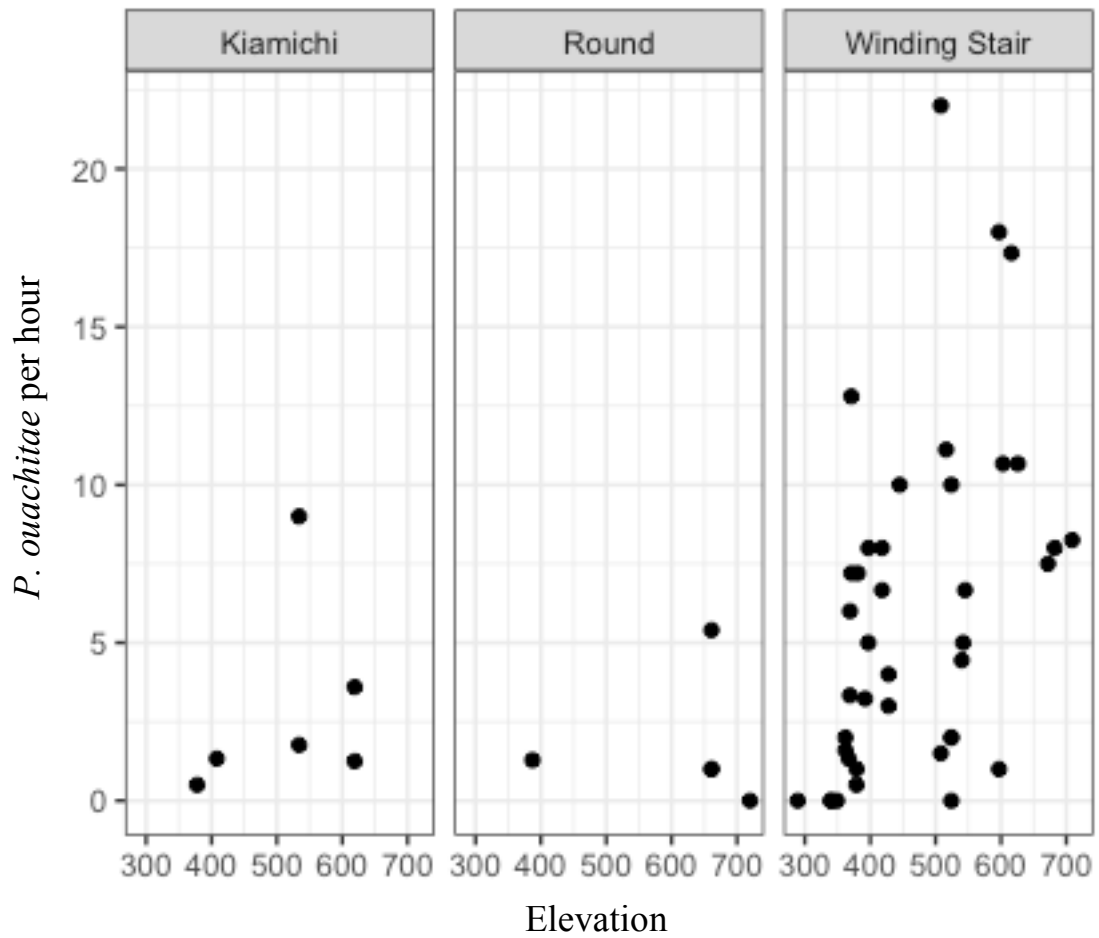
Figure 2. Number of *Plethodon ouachitae* collected per human hour of search effort by elevation (meters) for three mountains with more than one sampling site. Sampling occurring between 10:00 and 24:00, manually searching under cover objects during daylight and spotlighting for surface active salamanders after dark.
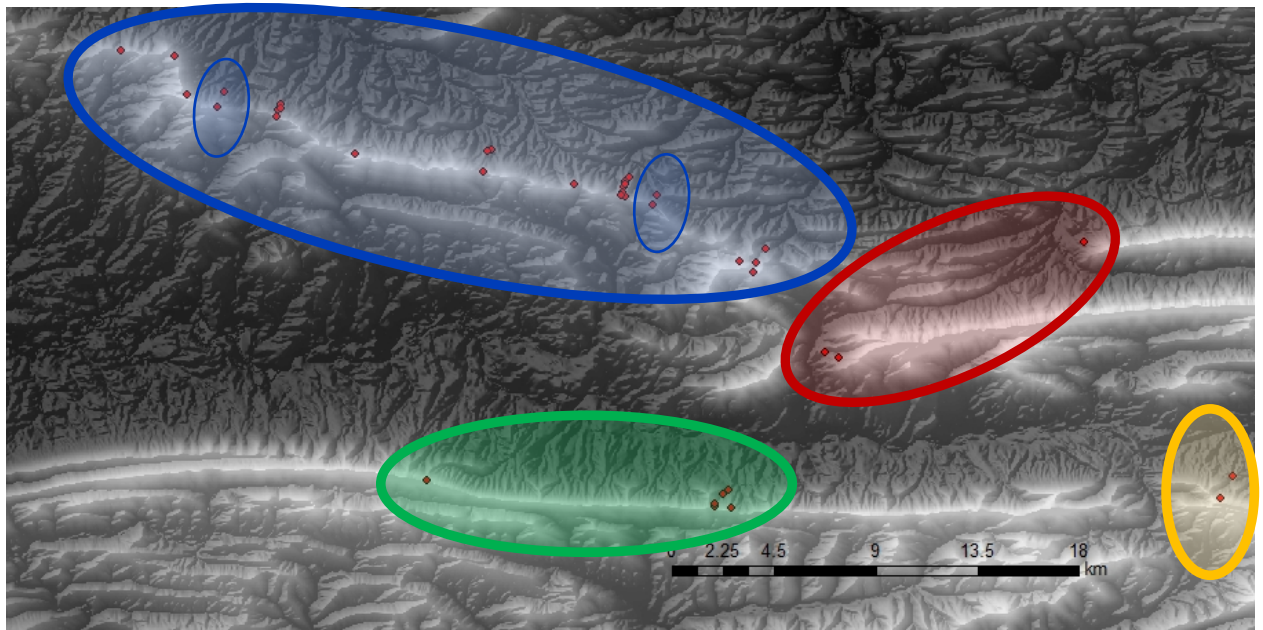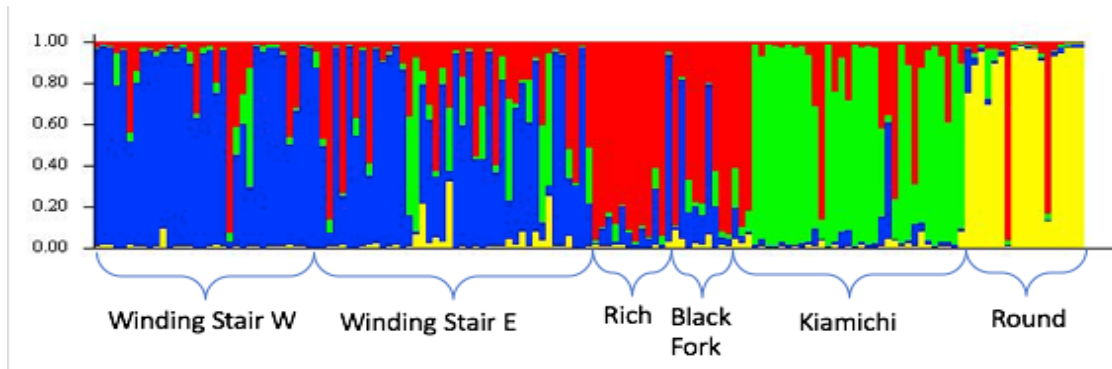
Figure 3. Population genetic structure for the six largest mountains within the geographic of *Plethodon ouachitae*. Top panel results of clustering analysis performed in STRUCTURE on 13 microsatellites for 149 individuals from 15 sampling sites for k = 4. Clusters conformed with the five mountains, except for Rich and Black Fork Mountains, which were clustered together, Pictured in the lower panel with sampling sites in red, only the sites within the inner circle were used for Winding Stair (blue).
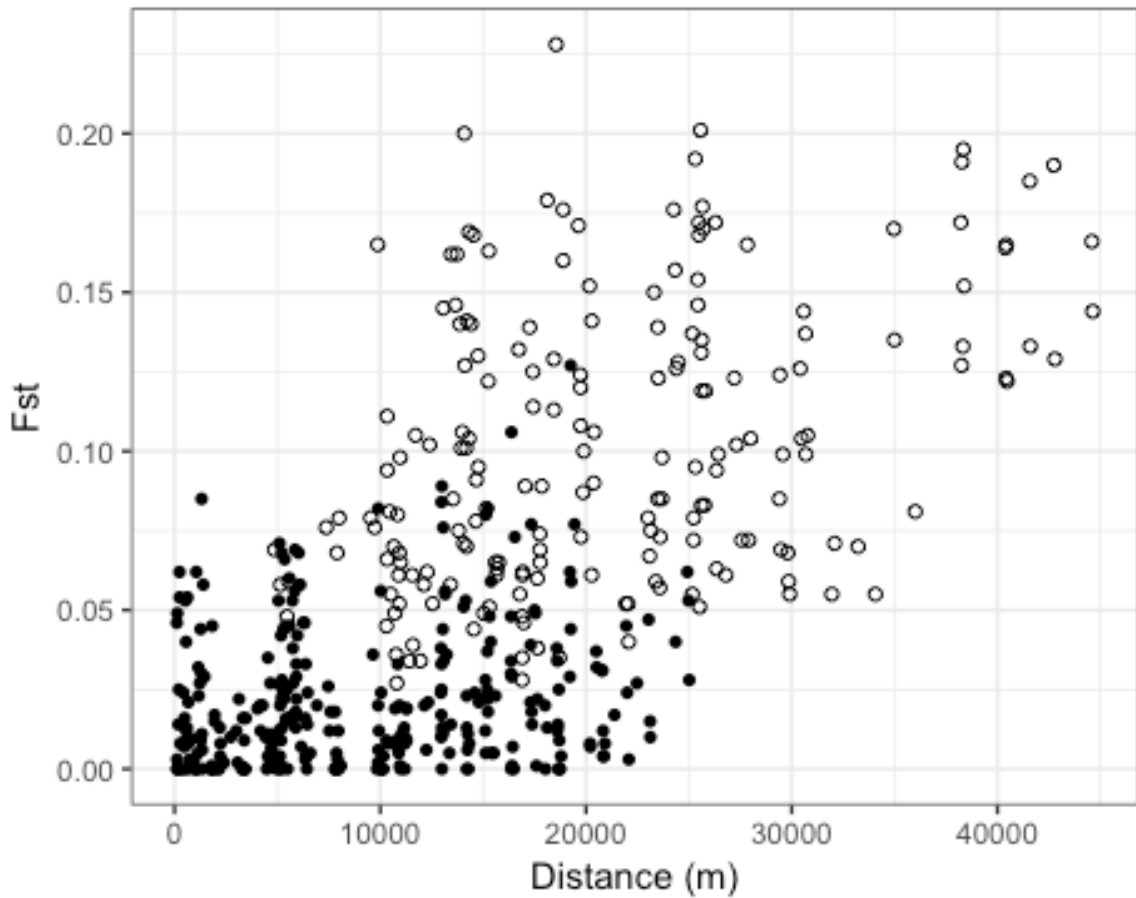
Figure 4. Isolation by distance plot. Scatterplot of F_{ST} estimates (calculated using GenoDive v 2.0) from 13 microsatellite loci against geographic distance (meters; determined using distm command in geosphere Hijmans 2017). Pairwise comparisons within a single mountain (filled circles) and pairwise comparisons of sites between differing mountains (open circles). Mantel test computed with 999 permutations in PASSaGE v2 (Rosenberg and Anderson 2010), for all pairwise comparisons (R^2= 0.62, p=.001), pairwise within mountains comparisons (R^2= 0.17, p=.001), and between mountains (R^2= 0.39, p=.001).

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APPENDICES

Appendix S1 Chapter 2

Table 1.1. Variables used for modeling ground temperature. Table and model adapted from Fridley (2009).

Variable	Description	units	Source
minSYN	Monthly average minimum temperature from elevation and temperature for random points	°C	Regression of minimum average monthly temperature and ELEV random points within 10 km buffered range hull
maxSYN	Monthly average maximum temperature from elevation and temperature for random points	°C	Regression of maximum average monthly temperature and ELEV for random points within 10 km buffered range hull
RAD	Daily shortwave radiation	W m^{-2}	r.sun function in GRASS
JDATE	Julian day	-	
TOTRAD	Annual shortwave radiation	W m^{-2}	sum of daily r.sun function in GRASS
ELEV	Elevation	m	90-m digital elevation model
STRDST	Stream distance (log transformed)	m	Euclidean distance from USGS stream network vectors
TCI	Topographic convergence index	-	r.topidx function in GRASS

Table 1.2. Global Circulation Models (GCM) used in future niche modeling projections. GCM point values were obtained from ClimateNA v5.21 software package, available at *http://tinyurl.com/ClimateNA* (Wang et al 2016) and entered in the ground temperature model as lapse rates.

Abbreviation	Model	Institution
ACC	ACCESS1-0	CSIRO (Commonwealth Scientific and Industrial Research Organisation, Australia), and BOM (Bureau of Meteorology, Australia)
Canesm	CanESM2	Canadian Centre for Climate Modelling and Analysis, Canada
CCSM	CCSM4	National Center for Atmospheric Research, USA
CESM	CESM1-CAM5	National Science Foundation, Department of Energy, National Center for Atmospheric Research
CNRM	CNRM-CM5	Centre National de Recherches Meteorologiques, France
CSIRO	CISRO-Mk3-6-0	Commonwealth Scientific and Industrial Research Organisation in collaboration with the Queensland Climate Change Centre of Excellence
GFDL	GFDL-CM3	Geophysical Fluid Dynamics Laboratory NOAA, USA
GISS	GISS-E2R	NASA Goddard Institute for Space Studies, USA
Had	HadGEM2-ES	Met Office Hadley Centre (additional HadGEM2-ES realizations contributed by Instituto Nacional de Pesquisas Espaciais)
INM	INM-CM4	Institute for Numerical Mathematics, Russia
IPSL	IPSL-CM5A-MR	Institut Pierre Simon Laplace, France
MIROC5	MIROC5	CCSR/NIES/FRCGC, Japan
MIROC	MIROC-ESM	Japan Agency for Marine-Earth Science and Technology, Atmosphere and Ocean Research Institute (The University of Tokyo), and National Institute for Environmental Studies
MPI	MPI-ESM-LR	Max Planck Institute fur Meteorologie, Germany
MRI	MRI-CGCM3	Meteorological Research Institute, Japan

Table 1.3. Relationships used to parameterize the mechanistic models and their sources.

Model Parameter	Value/Relation	Source
Salamander parameters		
Snout-vent length (mm)	55	Kozak and Wiens 2009
Mass, M (g) from snout-vent length, SVL (mm)	$M_{jordani} = 1.0 \times 10^{-5}(SVL^{2.53})$ $M_{teyahalee} = 2.0 \times 10^{-6}(SVL^{3.01})$	Gifford and Kozak 2011
Resting metabolic rate, from $\log_{10}$ mass, M, and temperature (°C) (O_2 consumption, mL O_2 h^{-1})	$\log_{10}MR_{jordani} = 0.036(T) + 0.57(\log_{10}M) - 1.95$ $\log_{10}MR_{teyahalee} = 0.035(T) + 0.59(\log_{10}M) - 1.83$ $\log_{10}MR_{metcafi} = 0.035(T) + 0.368(\log_{10}M) - 1.844$ $\log_{10}MR_{montanus} = 0.022(T) + 0.244(\log_{10}M) - 1.569$	Gifford and Kozak 2011
Salamander surface area (cm^2)	$9.62 \times M^{0.614}$	Whitford and Hutchinson 1967
Sprinting speed (m s^{-1})*	0.15	Austin and Shaffer 1992
Prey size, L (mm)	4.5	Mitchell and Taylor 1986
Digestive efficiency	$DE = -0.0094(T) + 0.99$	Bobka et al. 1981
Foraging intake (FI, cal g^{-1} day^{-1})	$FI = 0.015(T^3)-0.81(T^2)+12.76(T)-43.06$	Merchant 1970
Annual survival (%)	0.49	Hairston 1983
Age at maturity (years)	3	Hairston 1983
Mature ovum size, diameter (mm)	3.5	Hairston 1983
Ovum dry weight, DW (mg) from ovum volume (mm^3)	0.48	Kaplan 1980
Energy content of salamander ova (kJ mg DW^{-1})	0.025	Kaplan 1980
Foraging window parameters		
Activity temperature range (°C)	3 – 20	Feder et al. 1982
Potential foraging time, PFT (s) from water loss rate, WL, and mass, M	$PFT = (0.1*M) / WL$	Feder 1983 Feder and Londos 1984
Prey parameters		
Arthropod abundance, a (arthropods m^{-1} s^{-1})	0.029 (95% quantiles = 0.023–0.046)	Gifford and Kozak 2011
Prey dry mass, M (mg) from length, L (mm)	$M = 0.28 * L^{2.45}$	Schoener 1977
Prey energy content kJ mg^{-1} dry mass	0.02385	Reichle 1971

Table 1.4. Proportion of total area predicted to remain suitable in 2050-2060 and 2080-2090 for two niche modeling approaches and 15 global circulation models.

| GCM | Niche Model | Percent Remaining Suitable Area | | | | | | | |
| | | *P. jordani* | | *P. metcalfi* | | *P. montanus* | | *P. teyahalee* | |
		2055	2085	2055	2085	2055	2085	2055	2085
ACC	Correlative	5.1%	1.0%	18.9%	8.0%	2.0%	0.6%	36.9%	7.7%
	Mechanistic	23.4%	24.6%	9.7%	9.4%	1.6%	4.2%	9.1%	6.2%
CANESM	Correlative	4.5%	0.7%	7.7%	6.6%	1.0%	0.2%	16.9%	9.9%
	Mechanistic	45.8%	35.8%	23.9%	16.4%	6.2%	3.1%	23.7%	15.2%
CCSM	Correlative	0.7%	4.4%	19.0%	11.9%	3.7%	1.2%	24.6%	16.3%
	Mechanistic	70.4%	39.9%	28.6%	17.6%	14.4%	6.9%	28.2%	17.1%
CESM	Correlative	11.1%	3.2%	22.5%	12.9%	2.5%	0.7%	12.0%	16.0%
	Mechanistic	49.2%	34.9%	24.2%	19.3%	15.9%	6.8%	9.9%	16.2%
CNRM	Correlative	4.5%	1.7%	20.4%	11.7%	0.6%	0.4%	30.4%	16.9%
	Mechanistic	80.0%	41.5%	57.6%	20.8%	63.8%	11.7%	58.0%	20.0%
CSIRO	Correlative	16.4%	5.0%	24.9%	12.8%	4.2%	1.2%	30.2%	16.2%
	Mechanistic	37.7%	23.2%	16.6%	9.0%	5.2%	1.7%	14.3%	9.2%
GFDL	Correlative	2.1%	0.7%	9.5%	6.6%	0.6%	0.2%	9.1%	5.7%
	Mechanistic	20.0%	9.0%	7.5%	2.6%	0.8%	0.2%	7.4%	3.2%
GISS	Correlative	23.1%	14.2%	28.7%	21.0%	6.8%	3.5%	29.6%	35.3%
	Mechanistic	71.9%	60.1%	48.8%	37.0%	40.2%	26.4%	50.4%	37.5%
HAD	Correlative	2.3%	0.3%	10.8%	5.1%	0.9%	0.2%	10.3%	5.0%
	Mechanistic	11.6%	4.5%	4.0%	1.2%	0.4%	0.0%	4.0%	1.6%
INM	Correlative	48.4%	35.1%	48.6%	34.3%	22.9%	12.3%	61.2%	43.0%
	Mechanistic	93.4%	59.9%	83.5%	33.2%	65.8%	18.1%	78.3%	36.4%

ISPL	Correlative	4.7%	1.3%	12.2%	9.1%	0.8%	0.3%	16.9%	12.8%
	Mechanistic	42.4%	48.3%	21.4%	24.1%	7.5%	17.8%	21.2%	23.8%
MIROC	Correlative	5.6%	0.7%	13.8%	8.8%	1.5%	0.3%	19.3%	12.9%
	Mechanistic	51.2%	38.2%	26.2%	19.3%	15.4%	12.9%	25.1%	17.5%
MIROC5	Correlative	1.3%	2.0%	10.8%	10.8%	0.3%	0.4%	15.6%	15.7%
	Mechanistic	48.2%	50.9%	25.8%	26.9%	17.9%	21.5%	24.4%	25.3%
MPI	Correlative	21.0%	15.9%	25.8%	24.9%	6.7%	5.0%	32.9%	24.0%
	Mechanistic	65.4%	48.6%	41.3%	27.5%	20.0%	6.4%	41.8%	28.2%
MRI	Correlative	21.0%	44.6%	88.4%	47.1%	37.2%	22.7%	66.6%	54.9%
	Mechanistic	69.0%	73.1%	86.8%	49.7%	24.2%	27.0%	41.6%	52.5%

Appendix S2 Chapter 2
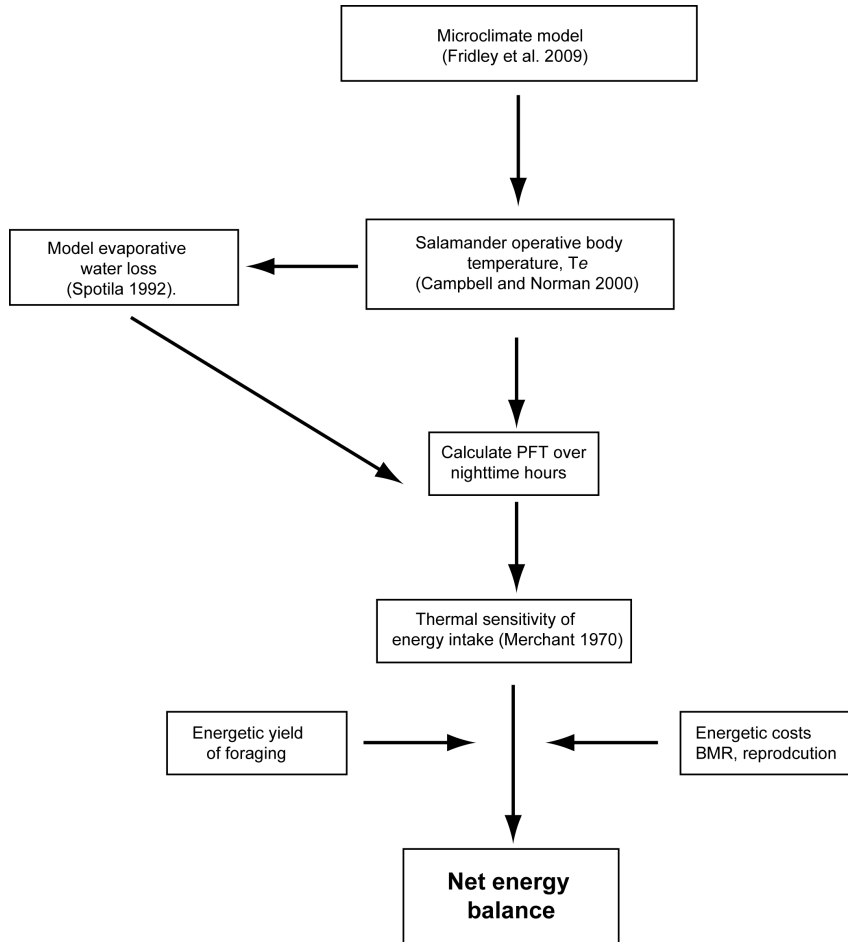
Figure 1.1. Diagram summarizing mechanistic model that estimate spatial variation in energetics.

Correlative Model	Mechanistic model
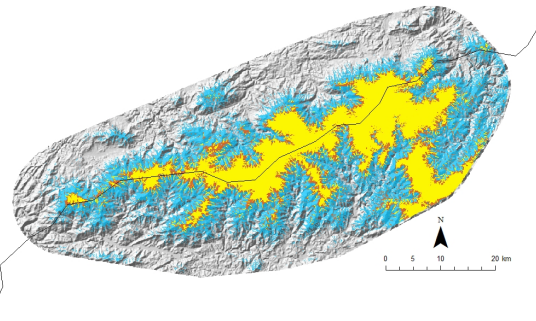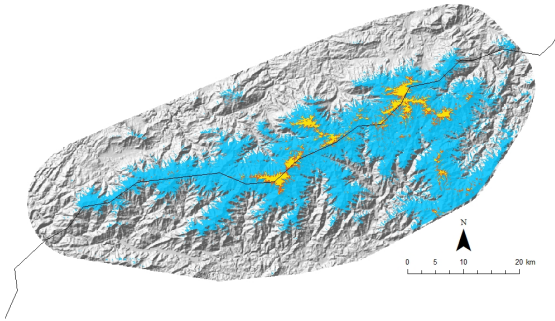
Plethodon jordani
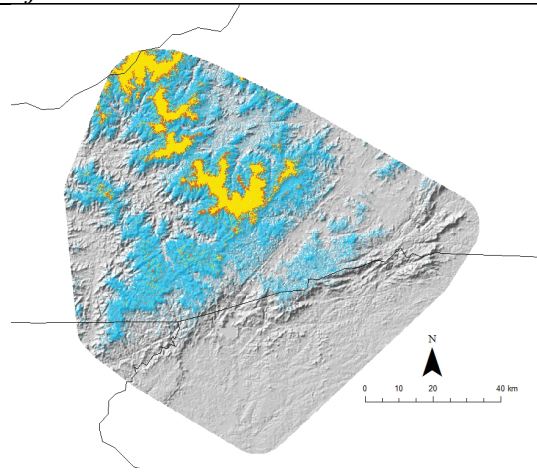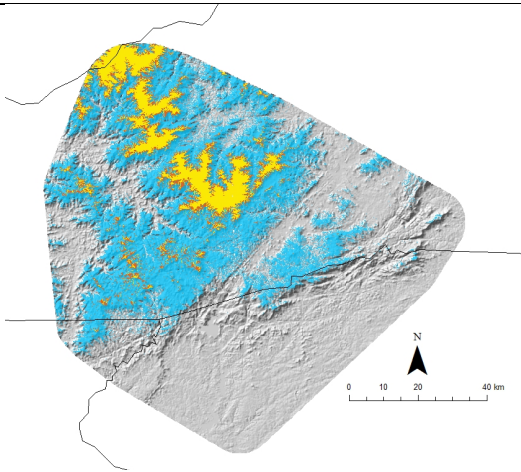
Plethodon metcalfi

Plethodon montanus

Plethodon teyahalee
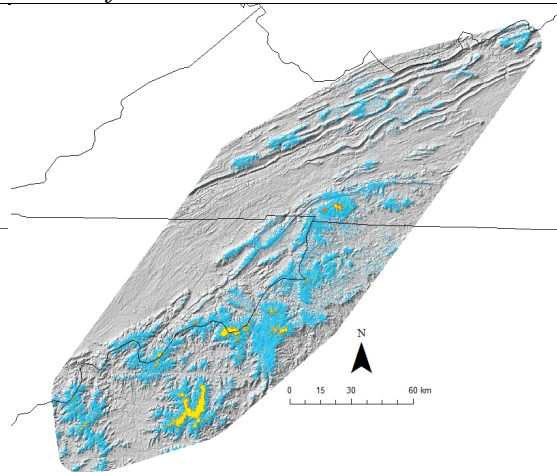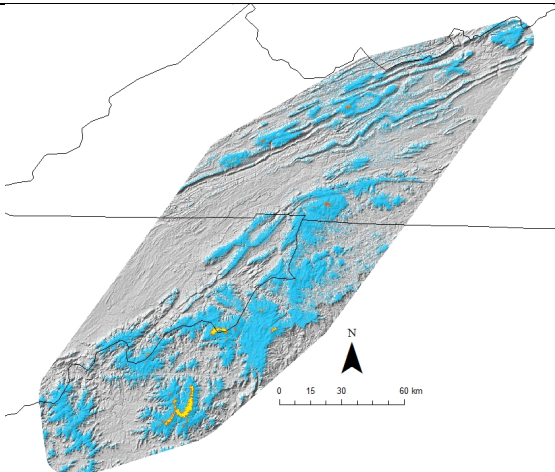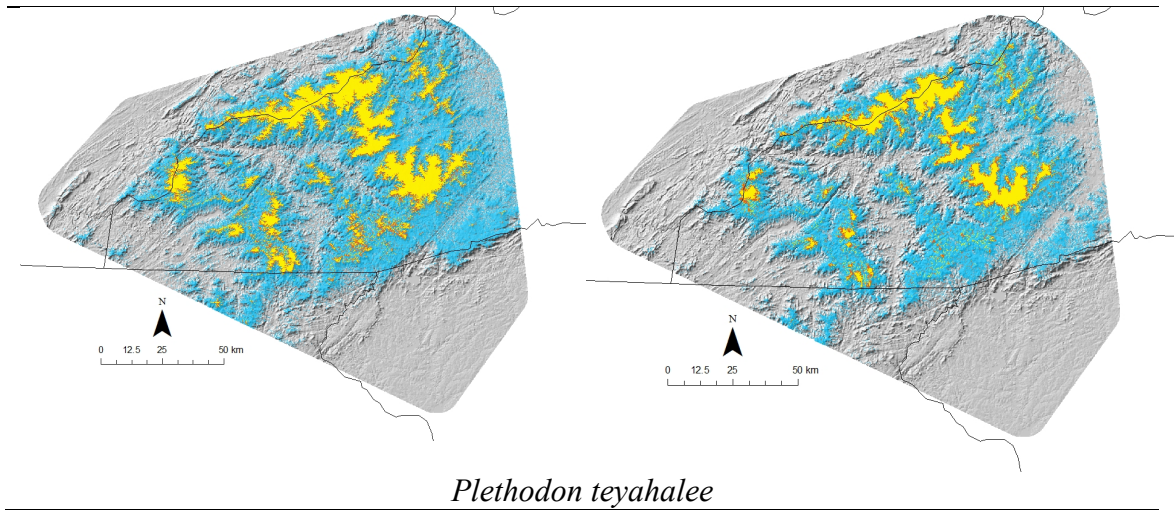
Figure 1.2. Maps predicting suitable area averaged across 15 global circulation models. Grid cells are classified as unsuitable (grey), current distribution lost under future scenarios (blue), suitable area lost between 2055 and 2085 (orange), and still suitable in 2085 (yellow). Threshold values described in text. Lines represent state boundaries.

Appendix S3 Chapter 2

MODEL DETAILS

Ground Temperature Model

Daily and yearly shortwave radiation was calculated using a 90m digital elevation

model in GRASS v6.7. Stream distance was mapped using Euclidean distance in ArcGIS

10.1 (ESRI, Redlands, CA, USA) from stream and river vectors (USGS National

Hydrology Dataset https://nhd.usgs.gov/) (Appendix S1, Table 1.1). These combined data

were then used estimate an average monthly maximum and minimum ground temperature

for each 90m grid cell according to the method described in Fridley (2009), R code

developed by B. Weinstein and available on request.

Energy Budget Model

This model was adapted from work done by Gifford and Kozak (2011) and

applied to additional species. The air near the forest floor is saturated during the

nighttime hours (relative humidity = 90% - 100%, (Hairston 1949, Petranka and Smith

2005). Therefore, we assumed that relative humidity was 95% when temperatures were

suitable for salamander surface activity (see below). We modeled wind speed at the level

of a salamander using average monthly wind-speed data (10' resolution, (New et al.

2002), and an attenuation coefficient for the canopy of a southern Appalachian deciduous

forest (Bolstad et al. 2001).

Salamander activity and foraging times were estimated based on temperature

variation during an "average" day for every month, using lapse rates calculated from

maximum and minimum temperatures averaged over the month and daily shortwave

radiation for the 15th day of the month. Biophys models hourly changes in temperature

using a sine approximation. Hourly temperature changes were used to estimate operative body temperature, *Te,* following the biophysical model of Campbell and Norman (2000). Ideally, variation in *Te* would have been calculated across each day of the year, and not averaged monthly. However, due to the fine-scale resolution of our models (90m), this approach is not computationally feasible. We assumed that salamanders could be surface active during nighttime hours when *Te* fell within the observed range of annual range field-body temperatures (3°C – 20°C, (Feder et al. 1982, Feder and Lynch 1982). We estimated potential foraging time as the proportion of the activity time during which a salamander could forage until it lost 10% of its total body mass through evaporative water loss (Appendix S1, Table 1.3).

Given *Te* and potential foraging time, biophys calculates daily energetic costs and energetic inputs for each grid cell in the 90 m environmental layers we used as input. We estimated daily energetic costs from laboratory measurements of the thermal sensitivity of salamander standard metabolic rate, and estimates of the quantity of energy contained in an average clutch of eggs (Appendix S1, Table 1.3. Daily energetic inputs were estimated using an empirically-derived prey-intake function for the thermal sensitivity of plethodontid salamander prey intake (Appendix S1, Table 1.3. We assumed that prey are sufficiently abundant that a salamander can obtain the quantity of the prey-intake function (Gifford and Kozak 2012). Prey mass and energetic content were calculated from prey size (Appendix S1, Table 1.3. Finally, daily energetic inputs and costs were summed to obtain annual estimates of discretionary energy (total energetic inputs minus total energetic costs).

Appendix S4 Chapter 3

Table 2.1. Sampling localities for *Plethodon ouachitae* in the Ouachita Mountains.
Localities of sites were salamanders were collected for phenotypic and genetic studies
along with the number of animals from each site used in the two analyses.

Sampling Site	Latitude	Longitude	Elevation	Transect	Mountain	N for phys	N for genetic
BF1	34.72026	-94.54285	345	--	Black Fork	9	9
BUD1	34.75507	-94.83271	618	--	Winding Stair	0	10
CG1	34.70788	-94.67441	727	CG	Winding Stair	10	10
CG2	34.71197	-94.67306	603	CG	Winding Stair	10	9
CG3	34.71722	-94.66942	418	CG	Winding Stair	16	20
DG1N	34.77006	-94.86349	524	DG	Winding Stair	10	20
DG2N	34.77262	-94.86346	428	DG	Winding Stair	0	10
DG2.5N	34.77334	-94.86224	392	DG	Winding Stair	6	6
DG3N	34.77452	-94.86208	379	--	Winding Stair	0	3
DMT1N	34.77383	-94.8875	672	DMT	Winding Stair	10	11
DMT2N	34.77995	-94.88452	371	DMT	Winding Stair	21	23
ELR1N	34.74784	-94.7818	597	ELR	Winding Stair	10	10
ELR2N	34.75614	-94.77982	358	ELR	Winding Stair	12	12
ELR3N	34.75655	-94.7785	347	--	Winding Stair	7	7
KT1N	34.62529	-94.80392	718	--	Kiamichi	0	2
MIR1	34.71234	-94.6796	636	--	Winding Stair	14	15
PAT1N	34.6148	-94.68971	619	PAT	Kiamichi	11	14
PAT2N	34.61592	-94.689767	534	PAT	Kiamichi	13	14
PAT3N	34.61969	-94.68629	413	PAT	Kiamichi	4	4
PO36	34.79635	-94.92547	556	--	Winding Stair	0	10
PV1	34.77834	-94.89912	672	--	Winding Stair	13	13
RG1	34.79406	-94.90434	626	--	Winding Stair	10	10
RIC1	34.67625	-94.64571	366	--	Rich	2	2
RIC2	34.67422	-94.64054	455	--	Rich	9	10
RM2N	34.61826	-94.48851	661	RM	Round	8	15
RM3N	34.62679	-94.48399	382	RM	Round	4	3
TCR1N	34.73475	-94.71423	542	TCR	Winding Stair	9	10
TCR2N	34.73858	-94.71237	369	TCR	Winding Stair	17	18
TH1	34.74292	-94.74553	547	--	Winding Stair	0	10
HTS0N	34.73836	-94.72706	508	HTS	Winding Stair	10	9
HTS1N	34.73822	-94.725	516	--	Winding Stair	0	10
HTS1.5N	34.73988	-94.72614	445	--	Winding Stair	0	10

HTS2N	34.74203	-94.72559	397	HTS	Winding Stair	0	10
HTS2.25 N	34.74325	-94.72545	385	HTS	Winding Stair	4	4
HTS2.5N	34.74424	-94.72515	362	HTS	Winding Stair	3	5

Table 2.2. Mountain specific occurrence localities used in niche comparisons. Locality information gathered from this study, prior studies, and VertNet (vertnet.org) database.

Mountain	Latitude	Longitude
Kiamichi Mountain	34.56381	-94.65093
Kiamichi Mountain	34.59387	-94.55838
Kiamichi Mountain	34.61364	-94.66984
Kiamichi Mountain	34.6139	-94.6625
Kiamichi Mountain	34.61433	-94.68319
Kiamichi Mountain	34.6148	-94.68971
Kiamichi Mountain	34.61508	-94.63116
Kiamichi Mountain	34.6153	-94.6311
Kiamichi Mountain	34.6153439	-94.9968055
Kiamichi Mountain	34.61592	-94.689767
Kiamichi Mountain	34.61858	-94.77128
Kiamichi Mountain	34.61969	-94.68629
Kiamichi Mountain	34.62529	-94.80392
Kiamichi Mountain	34.626972	-94.796896
Kiamichi Mountain	34.62747	-94.54755
Kiamichi Mountain	34.62747	-94.54755
Kiamichi Mountain	34.6278	-94.8119
Kiamichi Mountain	34.62824	-94.81229
Black Fork Mountain	34.689581	-94.299508
Black Fork Mountain	34.68976	-94.30044
Black Fork Mountain	34.690585	-94.302646
Black Fork Mountain	34.69059	-94.30265
Black Fork Mountain	34.69162	-94.30111
Black Fork Mountain	34.69226	-94.31475
Black Fork Mountain	34.69912	-94.32265
Black Fork Mountain	34.6992	-94.3219
Black Fork Mountain	34.70536	-94.45111
Black Fork Mountain	34.7058	-94.4369
Black Fork Mountain	34.70601	-94.33724
Black Fork Mountain	34.71	-94.55

Black Fork Mountain	34.7106	-94.45238
Black Fork Mountain	34.7165	-94.5347
Black Fork Mountain	34.7194	-94.5425
Black Fork Mountain	34.72026	-94.54285
Black Fork Mountain	34.72229	-94.53616
Black Fork Mountain	34.7294	-94.37607
Black Fork Mountain	34.7294	-94.37607
Black Fork Mountain	34.72989	-94.38028
Black Fork Mountain	34.73013	-94.44389
Black Fork Mountain	34.73567	-94.47664
Round Mountain	34.56962	-94.4466
Round Mountain	34.57135	-94.43921
Round Mountain	34.60366	-94.43076
Round Mountain	34.61528	-94.44688
Round Mountain	34.6153	-94.4972
Round Mountain	34.61537	-94.49725
Round Mountain	34.61745	-94.44709
Round Mountain	34.61826	-94.48851
Round Mountain	34.62679	-94.48399
Rich Mountain	34.58611	-94.23944
Rich Mountain	34.619868	-94.288615
Rich Mountain	34.61987	-94.28862
Rich Mountain	34.62677	-94.24461
Rich Mountain	34.62916	-94.2897
Rich Mountain	34.6537	-94.27376
Rich Mountain	34.65871	-94.28871
Rich Mountain	34.6629	-94.3303
Rich Mountain	34.66991	-94.63168
Rich Mountain	34.6715	-94.6301
Rich Mountain	34.67422	-94.64054
Rich Mountain	34.67554	-94.63972
Rich Mountain	34.67625	-94.64571
Rich Mountain	34.67955	-94.62919
Rich Mountain	34.679551	-94.629193
Rich Mountain	34.68108	-94.60857
Rich Mountain	34.6817	-94.4031
Rich Mountain	34.6831	-94.3711
Rich Mountain	34.685805	-94.631521
Rich Mountain	34.686067	-94.374043

Rich Mountain	34.686272	-94.352531
Rich Mountain	34.686686	-94.358302
Rich Mountain	34.6875	-94.62863
Rich Mountain	34.687502	-94.628629
Rich Mountain	34.68984	-94.62366
Rich Mountain	34.68985	-94.62366
Rich Mountain	34.69204	-94.42533
Rich Mountain	34.6937	-94.52264
Rich Mountain	34.69469	-94.46003
Rich Mountain	34.70536	-94.45111
Winding Stair Mountain	34.67974	-94.65552
Winding Stair Mountain	34.70788	-94.67441
Winding Stair Mountain	34.7097	-94.6778
Winding Stair Mountain	34.71197	-94.67306
Winding Stair Mountain	34.7123	-94.65454
Winding Stair Mountain	34.7125	-94.6792
Winding Stair Mountain	34.71339	-94.65913
Winding Stair Mountain	34.7142	-94.6603
Winding Stair Mountain	34.71474	-94.65899
Winding Stair Mountain	34.71490479	-94.67572784
Winding Stair Mountain	34.71500397	-94.68022919
Winding Stair Mountain	34.71504	-94.67884
Winding Stair Mountain	34.715043	-94.678841
Winding Stair Mountain	34.71722	-94.66942
Winding Stair Mountain	34.72803	-94.704528
Winding Stair Mountain	34.73475	-94.71423
Winding Stair Mountain	34.7374	-94.7265
Winding Stair Mountain	34.73822	-94.725
Winding Stair Mountain	34.73836	-94.72706
Winding Stair Mountain	34.73858	-94.71237
Winding Stair Mountain	34.73882	-94.7293
Winding Stair Mountain	34.73882	-94.7293
Winding Stair Mountain	34.73988	-94.72614
Winding Stair Mountain	34.74203	-94.72559
Winding Stair Mountain	34.74325	-94.72545
Winding Stair Mountain	34.74424	-94.72515
Winding Stair Mountain	34.74554	-94.72374
Winding Stair Mountain	34.74784	-94.7818
Winding Stair Mountain	34.748737	-94.800655

Winding Stair Mountain	34.748855	-94.800581
Winding Stair Mountain	34.74886	-94.80058
Winding Stair Mountain	34.753173	-94.8263747
Winding Stair Mountain	34.77006	-94.86349
Winding Stair Mountain	34.772149	-94.87778
Winding Stair Mountain	34.77262	-94.86346
Winding Stair Mountain	34.77334	-94.86224
Winding Stair Mountain	34.77383	-94.8875
Winding Stair Mountain	34.77452	-94.86208
Winding Stair Mountain	34.77477	-94.889
Winding Stair Mountain	34.775	-94.8958
Winding Stair Mountain	34.7762	-94.89785
Winding Stair Mountain	34.776409	-94.881603
Winding Stair Mountain	34.779799	-94.883953
Winding Stair Mountain	34.77995	-94.88452
Winding Stair Mountain	34.79631	-94.92545