Molecular Phylogenetics and Evolution 60 (2011) 445–454

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Speciation at the Mogollon Rim in the Arizona Mountain Kingsnake (*Lampropeltis pyromelana*)

Frank T. Burbrink [a,b,*], Helen Yao [c], Matthew Ingrasci [d], Robert W. Bryson Jr. [e], Timothy J Guiher [b], Sara Ruane [b]

[a] *Biology Department 6S-143, 2800 Victory Blvd., College of Staten Island/CUNY, Staten Island, NY 10314, United States*
[b] *Biology Doctoral Program, City University of New York, Graduate Center 365 Fifth Avenue, New York, NY 10016-4309, United States*
[c] *Science, Math and Engineering, Staten Island Technical High School, 485 Clawson Street, Staten Island, NY 10306, United States*
[d] *University of Texas at Arlington, Biology Department, Box 19498, Arlington, TX 76019-0498, United States*
[e] *Ecology and Evolutionary Biology Section, School of Life Sciences, University of Nevada, Las Vegas, 4505 Maryland Parkway Las Vegas, Nevada 89154-4004, United States*

ARTICLE INFO

Article history:
Received 14 January 2011
Revised 6 May 2011
Accepted 13 May 2011
Available online 26 May 2011

Keywords:
Speciation
Taxon delimitation
Lampropeltis pyromelana
Coalescent
Mogollon Rim
Pleistocene

ABSTRACT

Studies of speciation and taxon delimitation are usually decoupled. Combining these methods provides a stronger theoretical ground for recognizing new taxa and understanding processes of speciation. Using coalescent methods, we examine speciation, post-speciation population demographics, and taxon delimitation in the Arizona Mountain Kingsnake (*Lampropeltis pyromelana*), a species restricted to high elevations in southwestern United States and northern Mexico (SW). These methods provide a solid foundation for understanding how biogeographic barriers operate at the regional scale in the SW. Bayesian species delimitation methods, using three loci from samples of *L. pyromelana* taken throughout their range, show strong support for the existence of two species that are separated by low elevation habitats found between the Colorado Plateau/ Mogollon Rim and the Sierra Madre Occidental. Our results suggest an allopatric mode of speciation given the near absence of gene flow over time, which resulted in two lineages of unequal population sizes. Speciation likely occurred prior to the Pleistocene, during the aridification of the SW and/or the uplift of the Colorado Plateau, and while these species occupy similar high-elevation niches, they are isolated by xeric conditions found in the intervening low deserts. Furthermore, post-speciation demographics suggest that populations of both lineages were not negatively impacted by climate change throughout the Pleistocene. Finally, our results suggest that at least for this group, where divergence is old and gene flow is low, Bayesian species delimitation performs well.

1. Introduction

The causes of speciation have been investigated in numerous organisms inhabiting diverse regions of the Earth. Evolutionary biologists have defined, modeled and studied the possible modes of speciation over the last century, and since the Modern Synthesis, advances in computational methods have helped determine if general processes drive the formation of lineages (Coyne and Orr, 2004; Hey, 2009; Mayr, 1942, 1963). Diversification likely occurs along a geographic spectrum, where on one end allopatric speciation is marked by having no gene flow due to a physical barrier, and on the other end sympatric speciation shows a complete lack of geographic isolation (Bolnick and Fitzpatrick, 2007; Gavrilets, 2004; Mayr, 1963; Nosil, 2008). Of the many possible avenues of diversification, allopatric speciation is one of the most widely cited (Coyne and Orr, 2004; Wiens and Graham, 2005) and is commonly implicated in many phylogeographic studies (Pyron and Burbrink, 2010).

While divergence between species often happens in isolation with no gene flow, one alternative, parapatric speciation, suggests that low levels of gene flow may occur as populations diverge (Coyne and Orr, 2004; Hey, 2006; Nosil, 2008). Although it is expected that divergence in the face of gene flow is generally unlikely to occur, numerous examples have demonstrated the plausibility of the phenomenon (Hey, 2006; Niemiller et al., 2008). Alternatively, populations may diverge allopatrically, but recurrent gene flow from subsequent changes in geographic barriers and climate may create the illusion of parapatric speciation. Estimating the timing of migration (m) can help differentiate between allopatric speciation with secondary gene flow (m = late) from parapatric speciation (m = early; Slatkin, 1989; Slatkin and Maddison, 1989; Won and Hey, 2005), but changes in m and population structure through time can obscure these patterns. Finally, peripatric speciation, where differences in effective population size (N_e) may occur at the initial split of lineages is less likely to happen given that genetic drift would have to break up coadapted ancestral gene

---

* Corresponding author.
E-mail address: frank.burbrink@csi.cuny.edu (F.T. Burbrink).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.05.009

complexes in order for intrinsic reproductive isolation to occur (Losos and Glor, 2003; Mayr, 1954; Templeton, 1980).

The first step to studying the geographic context in which speciation occurs usually requires the identification of independently evolving lineages. Phylogeographic methods have often relied on identifying species using single gene-tree estimates or previous morphological designations of taxa (Avise, 2000; Edwards, 2009). For most phylogeographic studies, identifying patterns of phylogeographic structure takes precedence over understanding the processes that gave rise to those patterns (Edwards and Bensch, 2009; Zink and Barrowclough, 2008). However, the application of coalescent methods using multiple unlinked genes can aid in both delineating species and understanding the processes of speciation (Knowles and Carstens, 2007; Yang and Rannala, 2010). Going beyond the standard gene-tree paradigm, these methods assess species-tree uncertainty due to the coalescent using multiple gene trees to identify independently evolving lineages. These new methods offer a substantial improvement over traditional gene-tree techniques because they account for lineage sorting processes due to N_e size and assess the probability of speciation in a quantifiable manner (Carstens and Knowles, 2007; Leaché and Fujita, 2010; Yang and Rannala, 2010). At least for newly arisen taxa, coalescent species-delimitation techniques can be used in conjunction with spatial information to identify biogeographic barriers responsible for lineage divergence. Moreover, when augmented with other coalescent tests of migration between lineages, divergence time, and changes in N_e, a clear picture can be constructed about the process of speciation and post-divergence dynamics of population demographic changes in relationship to the environment (Heled and Drummond, 2008; Hey, 2006; Hey and Nielsen, 2004). With respect to lineage demographics that follow divergence, populations may expand as new habitat is colonized, or alternatively, shrink as habitable area decreases (Burbrink and Castoe, 2009; Hewitt, 2000). Therefore, the trajectories of N_e could be quite different between newly formed species given the geological history of the areas occupied by the lineages and the initial sizes of new species.

Phylogeographic structure has been examined in numerous organisms that inhabit the southwestern deserts and associated montane regions of North America (SW). These areas have been shown to yield deep phylogeographic structure in a wide range of organisms, including plants, insects and vertebrates (Barber, 1999; Bryson et al., 2010, 2011; Castoe et al., 2007; Devitt, 2006; Hafner and Riddle, 2008; McGuire et al., 2007; Mulcahy, 2008; Pyron and Burbrink, 2009a; Riddle and Hafner, 2006). This area is interesting to phylogeographers because of the wide variety of habitat changes associated with temperature, precipitation, and topography. Separation among deserts, sky islands and mountain ranges, as well as changes in climate, have been implicated in the formation of species, which occurred as early as the Miocene and as late as the Pleistocene (Barber, 1999; Downie, 2004; Lomolino et al., 1989; Masta, 2000; McCormack et al., 2010). However, for many organisms with clear phylogeographic structure in this region, tests have not been performed to elucidate modes of diversification, levels of gene flow, or historical population demography.

Within the SW, areas of high-elevation habitats are sharply isolated by the intervening low desert habitats. These highlands mainly include the Colorado Plateau north of the Mogollon Rim in Arizona and New Mexico, isolated Madrean sky islands and the continuous range of mountains along the Sierra Madre Occidental in Mexico (Lomolino et al., 1989; Stevens and Polhemus, 2008). These mesic areas of high elevation from Utah to central Mexico are separated by low-elevation desert habitats at the interface of the Chihuahuan and Sonoran Deserts in southeastern Arizona and southwestern New Mexico (Barber, 1999; Lomolino et al., 1989; Masta, 2000). Although not directly impacted by glaciers, these highlands have been affected by climate changes throughout the Pleistocene. Cooler mesic montane habitats sink to the valleys during glacial maxima, and then recede to higher elevations during warmer interglacials. Globally, these glacial processes have impacted diversification as well as changes in population size through time in several organisms (Betancourt, 1990; Comes and Kadereit, 1998; Ditto and Frey, 2007; Hewitt, 2000, 1996). Across the SW, however, some taxa have highly structured lineages that formed prior to major Pleistocene climate changes (Barber, 1999; Bryson et al., 2010; Masta, 2000; Smith and Farrell, 2005), whereas other species show little to no population structure currently (Downie, 2004).

Snakes have been used in a wide variety of studies to examine the impacts of biogeographic barriers and climate on lineage formation and population demography (Burbrink and Castoe, 2009; Burbrink et al., 2008; Devitt, 2006; Pyron and Burbrink, 2009a). Here, we address mechanisms of speciation in the brightly colored Arizona Mountain Kingsnake (*L. pyromelana*), an organism absent from low desert habitats. This snake is found at high elevations (1400–2734 m) intermittently from Utah to northwestern Mexico (Fig. 1; Ernst and Ernst, 2003; Tanner et al., 1982; Tanner, 1953). At lower elevations they occur in chaparral and piñon-juniper pine-oak, and at higher elevations these snakes are found in evergreen woodlands. Because of its particular habitat requirements and fragmented distribution, this species should be useful for investigating the effects of biogeographic barriers and climate on lineage formation and population demography.

Using three independent loci, we examine phylogeographic structure in the Arizona Mountain Kingsnake to address several hypotheses. First, is population structure of this high-elevation snake influenced by uninhabitable low elevation habitat? If so, this prediction would suggest that distinct lineages should be found on the separate areas of high elevation (i.e., the Colorado Plateau, the Madrean sky islands and the Sierra Madre Occidental). Phylogeographic structure has been found on each of these areas of high elevation in a variety of organisms (Barrowclough et al., 2006; Goldberg et al., 2004; Haenel, 2007; Lamb et al., 1997). Second, using coalescent species delimitation models, we ask if the three unlinked loci provide support for the existence of more than one species. Using gene trees and estimates of N_e and timing of divergence, these models assess the probability that multiple species can be delineated (Yang and Rannala, 2010). Third, if multiple independent lineages exist, then when did divergence occur, and was it associated with major climatic changes during the Pleistocene? Fourth, did limited migration accompany speciation, or did these lineages form in strict allopatry? Fifth, did diversification produce lineages of equal population size, or is there evidence that indicates the newly formed species were of vastly different N_e sizes? Sixth, did the population sizes of distinct lineages fluctuate throughout the Pleistocene? Providing tests for all of these hypotheses allows us to understand modes of speciation, impacts of climate change, and diversity in the SW.

2. Methods and materials

2.1. Data collection

We obtained 45 samples of *L. pyromelana* collected throughout their range (Fig. 1; Appendix A) and used *Lampropeltis triangulum* as the outgroup (Pyron and Burbrink, 2009b). DNA was extracted using the Qiagen DNeasy kits (tissue protocol) to obtain genomic DNA from samples of shed skin, liver, muscle tissue or whole blood. Three loci (one mtDNA and two nDNA) were amplified using GoTaq Green MasterMix (Promega Corp.) according to the manufacturer's specifications, with a 90 s extension time. The