Sweet Home Alabama: Hot Spot for Phylogeography

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Phylogeographers use molecular methods to map herpetological biodiversity in the heart of Dixie.

In the mid-1990s, the diversity of freshwater fishes, snails, mussels, and turtles endemic to the waterways of Alabama and neighboring states prompted biologists to call for more attention to the region. They noted that conservation measures there were not comparable to efforts being made in tropical locations, even though the aquatic systems of Alabama qualified as hot spots. Now, using molecular methods, phylogeographers are documenting and expanding the understanding of the amphibian biodiversity of the region—and continuing to call for conservation.

Conservation biologist Leslie Rissler has been at the University of Alabama in Tuscaloosa for five years. Well before she arrived, she knew that Alabama's rich biodiversity made it an excellent location for the molecular tools of phylogeography. "Alabama is special for many reason," says Rissler, an assistant professor of biology and curator of herpetology. She studies amphibians—both salamanders and frogs—that live in the state and asks questions about when, how, and why Alabama became home to so many. Rissler first learned to apply molecular studies to her research questions as a graduate student at the University of Virginia.

Conservation biologist Leslie Rissler searches for salamanders at Hurricane Creek in Tuscaloosa County, Alabama. She and her team caught Plethodon mississippi *that day. Photograph: Doug Gantt.*

"I started out as a community ecologist and then took a class at Mountain Lake Biological Station, 'Molecular Methods for Field Biologists,' and it totally changed my view of the world," she said. "I understood how many unique and interesting questions could be addressed in ecology using genetics and phylogenetics. And so that became a major tool for my work." Rissler joined the swelling ranks of evolutionary biologists engaged in phylogeography and comparative phylogeography.

Researchers in the field of phylogeography, founded two decades ago, use such strategies as ecological niche modeling and gene sequencing to map where species occur and to determine how they vary genetically across their range. Comparative phylogeographers then look for common genetic breaks across various species. By mapping breaks for many species across a landscape, researchers gain clues about when adaptations may have occurred and what may have prompted them.

The comparative phylogeography of various animals in Alabama, Rissler says, reveals many genetic breaks within the state. They are very likely related to the fall line, a physiographic slash across the eastern United States that, at its southern end, divides the Appalachian uplands (or Piedmont) from the Gulf of Mexico coastal plain. To understand what that might mean, Rissler and her colleagues stepped back and took a look at amphibians all across the United States. Amphibians are good for this type of work because they typically have low individual mobility coupled with high rates of philopatry (returning to natal sites). And, logistically, they are easier to sample than small mammals. Rissler and her team plotted the animals' whereabouts using geographic information systems (GIS) to identify locations of species breaks. They then partitioned samples of the

BioScience 59: 280–284. © Amy Mayer. ISSN 0006-3568, electronic ISSN 1525-3244. All rights reserved. doi:10.1525/bio.2009.59.4.3

same species by their genetic differences. When all of this is plotted on a map, Rissler says, Alabama emerges as a major hot spot.

"It's really striking," she says, of the as yet unpublished conclusions about Alabama's amphibian diversity. And it's consistent with others' work on mammals, birds, and even trees. The patterns vary, she says, but overall, it's clear that for many species Alabama is a suture zone—a place where hybrid zones, species contact zones, and phylogeographic breaks occur in the same area. Genetic analyses allow researchers to identify these regions, which represent populations that diverged from each other during ancient events, such as past sea-level changes that flooded now dry regions and made them inhospitable, or natural selection driven by different climatic conditions or soil environments.

Alabama's geography includes coastal plain and Appalachian ridges and plateaus, offering myriad habitats. With many endemic species and a huge variety of animal life, some biologists consider Alabama a hotbed for biodiversity. Figure courtesy of Joseph Apodaca, University of Alabama.

Mighty mtDNA

The ability to identify genetic breaks within species is one of the cornerstones of phylogeography. John Avise, now a professor at the University of California, Irvine, coined the term and founded the field after he devised techniques for using mitochondrial DNA (mtDNA) to look at genetic variations within species. The characteristics that make mtDNA useful for this type of study include its relatively rapid evolution and the fact that it gets passed down only matrilineally. Also, changes in mtDNA usually correlate with a mutation, meaning sequencing of mtDNA genotypes, called haplotypes, can be used to estimate the matrilineal histories of individuals and populations.

Avise says the information gleaned from mtDNA, and the promise of what it could offer the field of evolutionary biology on a grand scale, helped change the way researchers perceived their studies of different populations. "Phylogeography has enriched the language and the perspective of what population genetics is all about," he says. When he was a graduate student in the 1970s, he says, the common knowledge was that there was no meaning to phylogeny below the species level; there were no branches within a given species. That assumption no longer exists.

"When we began studying mitochondrial DNA, it was a molecule that showed you could talk about genealogy at the intraspecifc level." Now, studies of different life histories within a given species are generally accepted. The implication of this is a broader understanding between biologists working from a macroevolutionary perspective and those studying questions on a microevolutionary level. Avise says more unified conversations about evolution, using a common language and common concepts, have followed and led to greater cooperation and collaboration across the subfields of evolutionary biology.

Phylogeography, Rissler says, brings together population genetics, systematics, and conservation, making it arguably the most integrative specialty within evolutionary biology.

Since the birth of phylogeography in this country, its use in examining whole regional biotas has expanded throughout the world. Across Europe, in Australia, and in South Africa, to name a few places, biologists are applying the techniques and strategies of phylogeography to create more robust and thorough inventories of animal populations. Antarctic researchers also follow developments in the field and look for ways to apply the concepts to more limited populations.

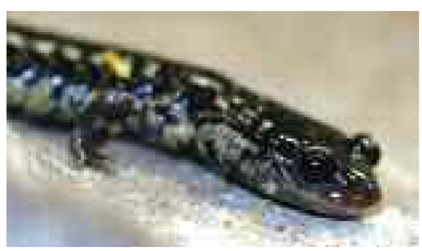
Field applications

Rissler says seeking detailed information about what's living in Alabama dovetails with her concerns about habitat preservation. Technology such as GIS and databases that contain information on latitude, longitude, and corresponding climate help researchers develop sophisticated algorithms from which they can form hypotheses about where certain species ought to be. Fieldwork follows, in which they attempt to find in those identified spaces the critters they expect to see. "And that can lead, obviously, to a lot of interesting questions, like if it's not where it should be based on the ecological niche of where it is now, why isn't it there? Is it because it's been extirpated from that area, potentially? Is it because maybe you're focusing on an invasive species and that's where it *can* go?"

To determine areas for conservation using phylogeography, you look for places that have significant populations of the animal, both in terms of large numbers as well as high genetic diversity, as that combination promises greater adaptability in the face of future changes. Though you look at a habitat currently suited to the species, you also need to consider how that habitat is changing and whether those changes will render it inappropriate for the species in question into the future. Using genetic information rather than just morphology gives scientists confidence in the diversity of what they're seeing. An area where populations exhibit deep divergences (representing high genetic diversity), particularly if those divergences prove to be ancient, may represent a more suitable spot for conservation than one in which species are more homogeneous.

In Rissler's lab, plethodontid salamanders are of particular interest. Among them are species that are imperiled by destructive land-use practices, especially coal mining along rivers, that have compromised habitat. *Phaeognathus hubrichti* (Red Hills salamander), the only member of its genus, is federally recognized as a threatened species. Rissler's lab received a state grant to study its genetics. Meanwhile, that salamander and other species endemic to the state, Rissler says, have prompted at least one major international conservation group to consider adding Alabama to its list of global hot spots of biodiversity, a distinction held by only one other American state, California.

Federally recognized as a threatened species, Phaeognathus hubrichti *(Red Hills salamander) has helped attract conservationists to Alabama. Photograph: Zach Riggins.*



This Plethodon mississippi *is visually very similar to* Plethodon glutinosus, *but the two different species of big, black, white-speckled salamanders are never found in the same location. Photograph: Heather Cunningham.*

Nearly identical to Plethodon mississippi *and found in bordering regions, this* Plethodon glutinosus *(slimy salamander) has genetically diverged from* P. mississippi. *Photograph: Heather Cunningham.*

Other salamander studies have also helped point to the importance of Alabama historically as a region where species survived during times of great environmental change. The distinctions between samples found in the southern part of the state versus those found to the north indicate that part of Alabama served as a refugium where species congregated when surrounding areas were inhospitable. When the environment changed again, animals were able to disperse and repopulate other areas.

Although exact locations of refugia haven't been identified, Rissler says, the fact that approximately 65 million years ago the ocean lapped at the shores of present-day Tuscaloosa, 200 miles farther north than it reaches today, means some terrestrial animals must have been isolated from their more northern relatives. Through time, they would have adapted to life in sandy substrates typical of the coastal plain, while their closest relatives became mountain dwellers. Again and again, she says, species show genetic divergence in the area of the fall line, supporting the hypothesis that Alabama is a region that has enhanced speciation events.

New frontiers

Frank Burbrink, an associate professor of biology at the City University of New York's College of Staten Island, also uses phylogeography to understand where species have been and where they might go. And he, too, looks for some of the answers in the Southeast. "Alabama is one of the key places," he says. The Mississippi River played a major role in dividing species, and Alabama offered a range of important habitats on the east side of the river, from the coastal plain and swamps in the south to uplands and ridges farther north.

Burbrink tries to employ as many phylogeograhic tools as possible in his studies, including ecological niche modeling, gene sequencing, and coalescent theory. Like Rissler, he makes predictions about where he expects to find certain species and then goes out to see how accurate his models are. In one field expedition, he traversed the Ouachita Mountains of Arkansas in search of the *Plethodon*

After niche models indicated Plethodon fourchensis *should be found in certain places, Frank Burbrink and his collaborators scoured the Ouachita Mountains to ground-truth the model. They found this one in Foran Gap, Arkansas. Photograph: Frank Burbrink.*

Snakes, such as this Coluber constrictor *(black racer) found in Tuscaloosa County, Alabama, help researcher Frank Burbrink explore the importance of southeastern habitats. Photograph: Frank Burbrink.*

fourchensis salamander, which niche modeling indicated should be there. The searching wasn't easy, Burbrink says, in part because some of the Forest Service and state gazetteer maps he used in creating his models were somewhat outdated. That can lead to models that predict a certain habitat in a place where that no longer exists or that has been greatly altered. If it turns out that the mountain you're searching on has been clear-cut, for example, "then you have to really, really look," Burbrink says. Eventually, he and his colleagues did find *P. fourchensis,* as well as other salamanders, in the area.

Long before he studied salamanders, Burbrink discovered his first love as a boy: "going out and flipping trash in the woods to catch snakes." As it turns out, phylogeographic tools also work well with reptiles. In Alabama, Burbrink has studied *Coluber constrictor* (black racer), a large snake species that, he says, "seems to show a major genetic break at the Mobile Bay and associated rivers draining into the bay." Alabama's importance for both snakes and salamanders becomes evident, he says, by looking at how geography has affected habitat historically. "Even if you look at [the Mobile Bay area] now and you think, well, it doesn't seem like these rivers are really all that impactful," he says, genetic differences indicate that at some point they clearly were.

Despite the arsenal developed for comparative phylogeographic studies—and the seemingly unlimited number of potential targets available—Burbrink is pushing into territory that Avise says presents a whole new set of challenges. Mitochondrial DNA offered the molecular insight that generated the field, but it has its limits. It's just one estimate of an organism's history, Burbrink says. While it may give the true history, it can also be wrong. Burbrink likens it to administering an intelligence test that evaluates only math skills. In some cases, the inability to do calculus may correlate with being unintelligent. But in other cases, the failure to evaluate other skills will result in a false conclusion. Still, mtDNA offers a fast, easy, inexpensive way to generate that one estimate. Moving forward, Burbrink hopes to employ nuclear genomic material, which has the potential to confirm inferences made from mtDNA. But it won't be easy.

"The biggest, baddest challenge is making sure that what you're getting out of the nuclear genome is a single gene," he says. With mtDNA, researchers can be more confident that when they identify a gene, it's a single copy. What makes nuclear genetic material so much more useful is also what makes it trickier to use. The material is passed both matrilineally and patrilineally, and because it's bigger, "the nuclear genome can't track the changes in population as quickly as the mitochondrial genome," he says. "It's mathematically a little more complicated."

Intragenic recombination, the nucleotide rearrangements occurring within a single hereditary unit during cell division, complicates the process of distinguishing genealogical signatures from the nuclear genome. But, as Avise notes, "it's what creatures do." It's a natural process that, although it may be an obstacle, is precisely the reason the nuclear genome is so enticing.

And it is most likely the future direction of the field. "Overcoming the technical hurdles (such as how to rapidly isolate nuclear haplotypes from diploid individuals) and the natural hurdles (especially how to deal with intragenic recombination) may be daunting, but any researcher broadly successful in such endeavors will be rightly considered a scientific pioneer," Avise writes in the January 2009 issue of the *Journal of Biogeography.*

Conservation

Meanwhile, in the context of conservation biology more generally, phylogeographers hope to make sound recommendations about critical habitats that should be preserved. If the number of species that call a place home is to be considered, then a whole new question is raised: the perennial dilemma of when to divide a species into more than one. Burbrink fears a "biodiversity crisis" in which disappearing habitats take with them unique, never-identified species. He hopes more work with lineages and genetic variation will help prevent such species loss.

"We are low-balling our diversity of organisms," he says, "and you can imagine that's a conservation nightmare." If you don't know what the different species are, then you can't possibly identify their unique environments nor isolate those locales for protection. But there is hope. Burbrink predicts the future of phylogeography will include the twin developments of more extensive and complete genomic sequencing capabilities along with a drop in cost, which at present is prohibitively expensive.

Over the next two decades, he expects to see greater computational automation, which will allow faster sequencing of more genomes. Applying that voluminous data to major evolution models, and incorporating it into ecology studies, he says, will lead to the ability to develop a "co-phylogeography" of 10 or 20 species "to give you a really strong picture, or breadth, of what's going on historically in a particular area." Did the same environmental change in the Mississippi River basin affect a thousand species at the same time? Or were the effects random through time?

For this to transpire, he emphasizes, every piece of the process has to get cheaper, not only because current costs are so high but also because phylogeography as a field struggles for adequate funding. Meanwhile, with the tools widely employed today, Rissler says, critical decisions can be made about habitat preservation in the face of climate change.

"We hope that if we can pinpoint [for conservation] populations or geographic regions with high genetic diversity and enough land area, for terrestrial species at least, that some populations will be able to move to track the environment. Of course we hope, but are not optimistic, that adaptation will occur fast enough, because much of Earth's land area either has been destroyed or divided so much that species are unable to disperse into more suitable areas as the climate changes," she says.

The urgency to identify species and preserve appropriate habitats is driven in part, Rissler says, by biologists' understanding of humans' role in climate change and the fear that we may be embarking on the sixth mass extinction. "In each of the past five extinctions, well over 50 percent of all life was destroyed; we don't want that to happen now, especially because this extinction is driven by humans," Rissler says.

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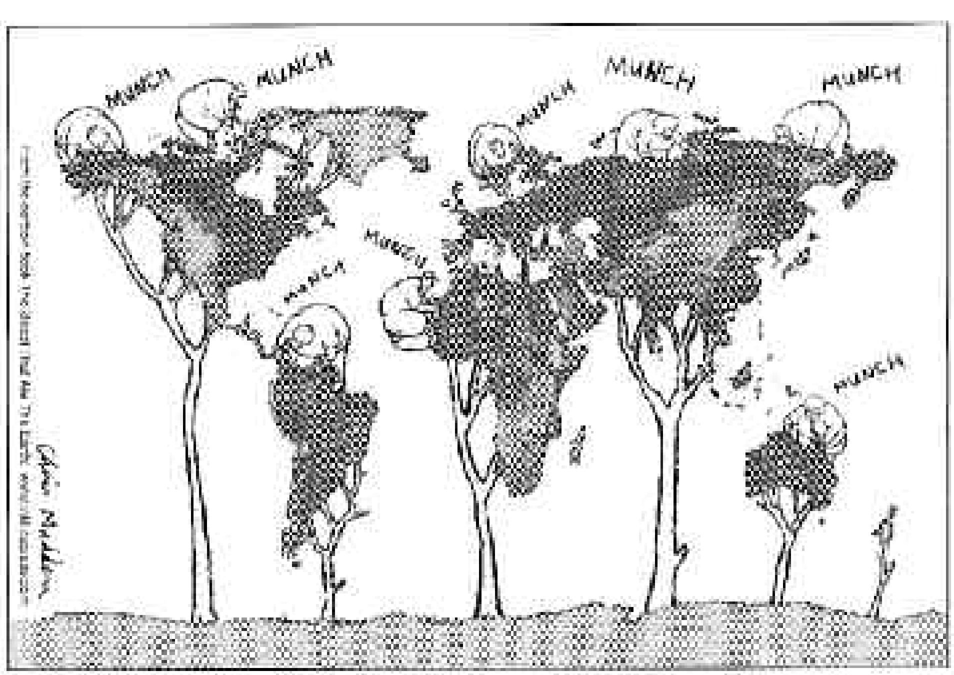
For more information, visit these Web sites:

http://web.mac.com/ljrissler/lab/Research.html

http://163.238.8.180/~fburbrink/Research/index.htm

http://ib.berkeley.edu/labs/moritz/research/historic_dna.html



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