Range reduction of the Oblong Rocksnail, *Leptoxis compacta*, shapes riverscape genetic patterns

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Abstract

Many freshwater gastropod species face extinction, including 79% of species in the family Pleuroceridae. The Oblong Rocksnail, *Leptoxis compacta*, is a narrow range endemic pleurocerid from the Cahaba River basin in central Alabama that has seen rapid range contraction in the last 100 years. Such a decline is expected to negatively affect genetic diversity in the species. However, precise patterns of genetic variation and gene flow across the restricted range of *L. compacta* are unknown. This lack of information limits our understanding of human impacts on the Cahaba River system and Pleuroceridae. Here, we show that *L. compacta* has likely seen a species-wide decline in genetic diversity, but remaining populations have relatively high genetic diversity. We also report a contemporary range extension compared to the last published survey. *Leptoxis compacta* does not display an isolation by distance pattern, contrasting patterns seen in many riverine taxa. Our findings also indicate that historical range contraction has resulted in the absence of common genetic patterns seen in many riverine taxa like isolation by distance as the small distribution of *L. compacta* allows for relatively unrestricted gene flow across its remaining range despite limited dispersal abilities. Two collection sites had higher genetic diversity than others, and broodstock sites for future captive propagation and reintroduction efforts should utilize sites identified here as having the highest genetic diversity. Broadly, our results support the hypothesis that range contraction will result in the reduction of species-wide genetic diversity, and common riverscape genetic patterns cannot be assumed to be present in species facing extinction risk.

Introduction

Freshwater gastropods of the United States suffer one of the highest imperilment rates of any taxonomic group in North America (Johnson et al., 2013). Despite being critical components of many freshwater ecosystems, freshwater gastropods are grossly understudied compared to freshwater fish, mussels, and crayfish (Covich, Palmer & Crowl, 1999; Huryn, Benke & Ward, 1995; Strong et al., 2008). This creates a situation where desperately needed conservation efforts are hindered by a lack of information (Johnson et al., 2013). For example, data on the current range of many freshwater gastropods is lacking (Lydeard et al., 2004), but conservation assessments and effective management plans require detailed historical and contemporary range data (Potter & Thomas, 1983; USFWS, 2018). Population genetics data on freshwater gastropods are also needed to inform management efforts and provide basic understanding of freshwater ecosystems (Lysne et al., 2008).

The freshwater gastropod family Pleuroceridae is one group that suffers from a high imperilment rate (79%) and little research attention (Brown, Lang & Perez, 2008; Johnson et al., 2013; Perez & Minton, 2008). Pleurocerids are found east of the Rocky Mountains in North America, with most of their diversity concentrated in the southeastern United States (Lydeard & Mayden, 1995; Strong & Köhler, 2009). Pleurocerids lack a highly vagile veliger larval stage seen in many aquatic gastropod groups, and they are thought to move large distances only when washed downstream (Whelan et al., 2019; Whelan, Johnson & Harris, 2015). Only one study has been published on landscape and conservation genetics of pleurocerids, and that study focused exclusively on a single species, *Leptoxis ampla* (Whelan et al., 2019). Many freshwater species, including *L. ampla*, display common riverscape genetic patterns such as increased genetic diversity in downstream populations and isolation by distance (Hughes, Schmidt & Finn, 2009; Paz-Vinas et al., 2015). However, few studies have tested for such patterns in riverine species that have undergone drastic range reduction, and no such study has been done for a range restricted pleurocerid.

One pleurocerid in need of more research is the Oblong Rocksnail, *Leptoxis compacta* (Figs. 1,2). This species is a narrow range endemic known historically from the middle Cahaba River and a single tributary in central Alabama, USA (Fig. 2; Goodrich, 1922). Until recently, *Leptoxis compacta* was considered extinct as it had not been collected, or at least identified correctly, from 1935 to 2011 (Goodrich, 1941; Johnson et al., 2006; Whelan, Johnson & Harris, 2012). As early as 1941, the decline of *L. compacta* was documented (Goodrich, 1941), and the species now occupies less than 5% of its historical range (Fig. 2; Whelan, Johnson & Harris, 2012). As a narrow range endemic with few historical collections, little is known about the species aside from recent survey efforts and limited life history data (Whelan, Johnson & Harris, 2012). Yet, the rediscovery of *L. compacta* in 2011 resulted in an emergency petition to list the species under the US Endangered Species Act (Kurth, 2017). For management agencies to assess the status of *L. compacta* and design effective conservation plans, detailed survey work and population genetics research are required. Modern population genomic tools such as restriction site associated DNA sequencing (RAD-seq) can provide data that will enhance *L. compacta* management options (Andrews et al., 2016). As a result of having a narrow range along a single river path, an effective recovery strategy for *L. compacta* will likely require reintroduction efforts to previously occupied habitat(s). Maintaining genetic diversity of imperiled species is important for mitigating extinction risk (Frankham, 2005; Frankham, 2010), and reintroduction efforts will require detailed population genetics data to inform broodstock selection for maximizing genetic diversity of captively reared offspring.

In this study, we generated a dataset of thousands of single nucleotide polymorphisms (SNPs) to answer questions about conservation and riverscape genetics of *L. compacta*. Given the drastic range decline suffered by *L. compacta*, we set out to test the following hypotheses: 1) *Leptoxis compacta* has undergone a severe genetic bottleneck and 2) genetic diversity of *L. compacta* is considerably lower than *L. ampla*, a sympatric and wider ranging species. We also examined how genetic diversity of *L. compacta* varies across its current range, specifically assessing whether broad patterns seen in many other riverine taxa like isolation by distance and strong genetic structure are seen in *L. compacta*.

Materials & Methods

Sample Collection

Leptoxis compacta was collected during two trips to the Cahaba River in June 2018 and June 2019. We performed survey work at four sites, and all sites except Cahaba River above Shades Creek were outside the previously documented contemporary range of *L. compacta* (Fig. 3; Whelan et al. 2012). At each location, individuals were collected by hand and identified in the field. Despite being a narrow range endemic that has undergone distributional decline, *L. compacta* was locally abundant where found. Based on qualitative observations, we sampled less than 1% of the population, making our sampling negligible to species survival. Twenty specimens from each site were transported live to the lab, sacrificed following Fukuda et al., (2008), and placed in 96-100% ethanol until tissue clips could be taken. Specimens were collected under an Alabama Department of Conservation and Natural Resources Educational Scientific Collections Permit (License #2019100990068680) or as an agent of the state (P.D. Johnson). All shells have been cataloged separately to correspond to associated molecular data and deposited at the Auburn University Museum of Natural History (AUMNH 45652-45690; Table 1, Supplementary Table 1).

Molecular data generation

Tissue clips from the foot of 20 individuals per collection site were taken and subjected to a standard proteinase K digestion. DNA was extracted with the Qiagen DNeasy Plant Mini Kit with a minor modification to allow for tissue digested with proteinase K. We used a plant kit because it works well on freshwater gastropods that produce large amounts of mucus polysaccharides (Whelan et al., 2019). The integrity of whole genomic DNA was checked on a 1% agarose gel and quantified with a Qubit DNA assay. Extracted DNA was standardized to 120 ng/µL for 2bRAD library prep.

A reduced representation genomic library was generated for genotyping using the AlfI enzyme and the 2bRAD library prep protocol of Wang et al. (2012). This RAD-seq approach uses a type IIB restriction enzyme that has two recognition sites. AlfI recognizes two sites separated by six base pairs and makes two cuts that each have a one base pair overhang 12 base pairs from the 5' and 3' ends of the restriction sites. Following Whelan et al. (2019), we did a $1/16^{th}$ genomic reduction by using adaptors in the ligation step that had an "NC" overhang, thus only binding to AlfI RAD-loci that had a G base pair at the first base pair of each restriction cut overhang. For more details, see Wang et al. (2012) and the lab protocol on the FigShare repository for this study (DOI: 10.6084/m9.figshare.12014619).

All samples were dual-indexed for pooling. Sequencing occurred in multiple batches. The first batch had 48 *L. compacta* samples pooled in equimolar concentrations and sequenced on a single lane. The other individuals were pooled in equimolar concentrations with samples from

projects on conservation genomics of other pleurocerid species, and 87 individuals were sequenced per HiSeq 4000 lane. Although batch effects in RADseq data have been recently noted in studies that used different read lengths among sequencing runs (Leigh, Lischer & Keller, 2018) and in species introgression studies (Lambert et al., 2019), such issues were not relevant to our sequencing design or study objectives. Nevertheless, we took steps to limit potential batch effects by implementing strict filtering parameters during dataset assembly (see below). Pooled libraries were sequenced on an Illumina HiSeq 4000 with 1 X 75bp chemistry at University of Oregon Genomics and Cell Characterization Core Facility.

Raw Illumina reads were demultiplexed with the STACKS 1.48 (Catchen et al., 2013) module *process_radtags*, allowing for one mismatch per barcode. Demultiplexed reads were quality filtered with the script QualFilterFastq.pl (http://github.com/Eli-Meyer/sequence_processing) for any read that had five or more base pairs with Phred quality scores less than 20. Reads were processed with scripts from SHRiMP 2.23 (Rumble et al., 2009) and subsequently trimmed to AlfI RAD-loci with the script AlfIExtract.pl (http://github.com/Eli-Meyer/2bRAD_utilities). As this step removes any sequence that is not part of the RAD-locus, adaptor sequences and non-target sequences are removed from the sequencing reads. RAD-loci, defined as the stretch of DNA cut by the AlfI enzyme, were assembled with the STACKS 1.48 pipeline *denovo_map.pl* as no reference genome is available for *L. compacta*. For *denovo_map.pl* parameters, we set minimum stack depth to five (-m 5), distance allowed between stacks to three (-M 3), and distance between catalog RAD-loci to two (-n 2). These parameters were determined to be most appropriate for our data following Paris et al. (2017). All other *denovo_map.pl* parameters were set to defaults.

After assembly, RAD-loci were filtered for missing data using the STACKS program *populations*. In order to pass filtering steps, a RAD-locus had to be present in 75% of individuals from any given collection site and also present at three collection sites. RAD-loci that had a minimum minor allele frequency of less than 2.5% or heterozygosity higher than 50% were removed to limit the influence of paralogy and misassembly on final datasets. Sequencing coverage of RAD-loci with SNPs was measured with vcftools (Jombart & Ahmed, 2011). Kinship coefficients among individuals were inferred with KING (Manichaikul et al., 2010). Files output by STACKS were formatted for KING with PLINK 1.9 (Chang et al., 2015), and pairwise kinship coefficients were calculated with the KING flag "--kinship". No individuals were determined to be closely related by KING so no further dataset filtering was done.

After filtering, a dataset that included all SNPs per RAD-locus and a dataset with only one random SNP per RAD-locus were generated. We assume that RAD-loci are unlinked and that the one SNP per RAD-locus dataset had zero linkage disequilibrium. Analyses employed the one SNP per RAD-locus dataset, unless otherwise noted.

Population genetics analyses

Average observed heterozygosity (H_o), expected heterozygosity (H_e), nucleotide diversity (Π), and F_{IS} at each collection site were calculated by *populations*. The number of private alleles at each site was also reported by *populations*. Average allelic richness (A_r) was calculated with the R (R Core Team, 2020) package diveRsity (Keenan et al., 2013). An analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was done with the R packages poppr (Kamvar, Tabima & Grünwald, 2014) to test genetic structure among collection sites. AMOVA was implemented with the function "poppr.amova" using the ade4 method (Dray & Dufour, 2007) and 10,000 permutations.

We also tested for a pattern of isolation by distance by measuring the correlation between pairwise F_{ST} values and geographical distance between collection sites. Pairwise F_{ST} values were calculated using the Weir and Cockerham (1984) method with the R package hierfstat (Goudet, 2005). Stream distance was measured in Google Earth by tracing paths between collection sites along the Cahaba River (see Table 2). River distance was used, rather than straight line distances, because migration over land is impossible for gill breathing pleurocerids. A Mantel test was performed with the R package ade4 (Dray & Dufour, 2007), and significance was tested with 1,000 permutations. However, Mantel tests have been criticized as a method for testing isolation by distance (Legendre, Fortin & Borcard, 2015; Meirmans, 2015), so we also performed a multiple regression on distance matrices with 1,000 permutations using the R package ecodist and its MRM function (Goslee & Urban, 2007). In addition to a pattern of isolation by distance, past studies have shown that many freshwater organisms, including pleurocerids, display a pattern of increased genetic diversity in more downstream populations (Paz-Vinas et al., 2015; Whelan et al., 2019). Therefore, to better assess riverscape genetic patterns of *L. compacta*, we performed linear regression of distance from the most downstream site against H_o, H_e, A_r, and Π. Linear regressions were done in R.

We examined clustering of *L. compacta* genetic data with discriminant analysis of principal components (DAPC). We used the multiple SNPs per RAD-locus dataset and the R package adegenet (Jombart & Ahmed, 2011) to perform DAPC. We first used the adegenet function "find.clusters" testing up to 25 clusters and using Bayesian information criteria (BIC) to identify the best-fit number of clusters for our data. Using the number of clusters with the lowest BIC value, we performed a DAPC with the adegenet function "dapc" and plotted the results in R.

We inferred genomic admixture of *L. compacta* individuals with ADMIXTURE 1.3 (Shringarpure et al., 2016). ADMIXTURE assumes zero linkage disequilibrium, so we used the one SNP per RAD-locus dataset. ADMIXTURE analyses were run with the AdmixPipe pipeline (Mussmann et al., 2020). To determine the best-fit number of clusters (K) for our data, K values from 1 to 5 were assessed with 20% cross-validation. Twenty replicates of ADMIXTURE were run at each K, and the best-fit K was determined as the value that had the lowest average CV score across replicates. ADMIXTURE results were visualized with Clumpak (Kopelman et al., 2015).

Genomic co-ancestry among individuals was also assessed with fineRADstructure (Malinsky et al., 2018). Unlike ADMIXTURE, fineRADstructure can use linked SNPs and provides additional information on individual genomic background. Thus, the multiple SNPs per RAD-locus dataset was used for fineRADstructure analyses. First, a co-ancestry matrix was inferred with the script RADpainter. Subsequently, clustering was done with the Markov chain Monte Carlo method of fineRADstructure, running for 500,000 generations and sampling every 1,000 generations; the first 200,000 generations were discarded as burn-in (non-default parameters: -x 200000 -y 300000 -z 1000). We also inferred a tree for visualization with fineRADstructure using the tree-building algorithm of Lawson et al. (2012) with 10,000 attempts (non-default parameters: -m T -x 10000). fineRADstructure results were plotted with R scripts included in the fineRADstructure package.

Code and data availability

All bash and R scripts used for processing and analyzing data are available at github.com/nathanwhelan. Demultiplexed raw Illumina reads have been uploaded to NCBI under BioProject PRJNA631794. Assembled datasets in various file formats (e.g., vcf, genepop) and

the 2bRAD library prep protocol are available on FigShare (DOI: 10.6084/m9.figshare.12014619).

Results

Sample Collection

During survey work, we collected *L. compacta* from Cahaba River at old Marvel slab upstream to Cahaba River at Booth's Ford (Fig. 3). All sites except Cahaba River at Shades Creek are sites where *L. compacta* was not found during survey work over the least 30 years. Our collections represent a 1.83 km downstream range extension and a 4.76 km upstream extension compared to the previously documented contemporary range of *L. compacta* (Whelan, Johnson & Harris, 2012). While this study was ongoing, 3 putative *L. compacta* individuals were collected at Cahaba River at Belle Ellen Shoals (Fig. 3) during a general mollusk survey (Johnson, 2019). However, species identification was uncertain and *L. compacta* appeared exceedingly rare. Therefore, individuals from Cahaba River at Belle Ellen Shoals were not included in our analyses, and we consider this record unconfirmed without additional positive survey results.

Molecular data and population genetics

DNA yields for two individuals were too low for library preparation so only 19 individuals were sequenced from Cahaba River at old Marvel Slab and Cahaba River at Booth's Ford. The number of demultiplexed raw reads per individuals varied from 930,062-10,146,649 (mean = 4,836,812). Much of the variation in raw reads can be attributed to whether the individual was sequenced on a HiSeq 4000 lane with 48 or 87 samples. Aside from raw read number, we saw no evidence of batch affects like individuals from one sequencing run all clustering together in analyses (see below). After initial raw-read filtering, the number of reads that passed quality filtering steps ranged from 865,314-9,838,187 (mean = 4,632,510). Assembly with the STACKS *denovo_map* pipeline resulted in 105,542 RAD-loci. Filtering with *populations*, including removal of 4,009 invariant RAD-loci that passed all filters, resulted in a dataset with 4,962 RAD-loci with at least one SNP. Per individual average sequencing coverage of filtered RAD-loci with at least one SNP, excluding missing genotypes, ranged from 31.7-343.2. Average sequencing coverage across variable RAD-loci, excluding missing genotypes, was 163.7. Kinship coefficients inferred with KING indicated that no individuals were closely related (i.e., half or full siblings).

The number of private alleles at each site ranged from 28-262 (Table 1). H_o at each collection site ranged from 0.0963-0.1568, and H_e ranged from 0.0980 to 0.1801 (Table 1). At each site, H_o was lower than H_e, except at Cahaba River at canoe launch where H_o was 0.001 greater than H_e (Table 1). The difference between H_o and H_e was largest at Cahaba River above Shade Creek and Cahaba River at Booth's Ford. A_r and Π ranged from 1.4511-1.8241 and 0.1010-0.1829, respectively (Table 1). F_{IS} values ranged from 0.0134-0.1934 (Table 1), with the highest values being at Cahaba River above Shades Creek and Cahaba River at Booth's Ford. Overall, genetic diversity was greatest at the most upstream site, Cahaba River at Booth's Ford, and lowest at the most downstream site, Cahaba River at old Marvel slab. All linear regressions of diversity statistics vs distance from the most downstream site were non-significant ($p \geq 0.169$).

Pairwise F_{ST} values among sites ranged from 0.0-0.055 (Table 2). We found no evidence of an isolation by distance pattern among sites (Mantel test, $p = 0.843$; multiple regression, $p =$

0.428). According to the AMOVA, significant genetic structure was present among collection sites ($p = 0.004$), but only 4.16% of variation was explained by collection site. In contrast, 81.8% of genetic variation was explained by within individual variation, further indicating high amounts of gene flow among collection sites.

DAPC indicated two genetic clusters were present in our data. Data were explained by a single discriminate function, and results are therefore presented as a frequency histogram (Fig. 4). ADMIXTURE analyses indicated that genetic diversity from two ancestral populations were present in our data ($K = 2$). Most individuals from across the range of *L. compacta* had a genomic admixture profile that was dominated by a genomic background from a single ancestral population, likely indicating that overall genomic diversity has been lost across the range of *L. compacta*. Nevertheless, 14 individuals had varying levels of admixture with a second ancestral population (Fig. 3). fineRADstructure analyses corroborated ADMIXTURE analyses as two semi-distinct groupings were recovered by fineRADstructure (Fig. 5, Supplementary Fig. 1). fineRADstructure groupings did not correspond to collection site or any other obvious variable, indicating gene flow among collection sites. Notably, six individuals with comparably high co-ancestry proportions (upper right of co-ancestry matrix in Fig. 5) correspond to individuals in ADMIXTURE analyses with a large proportion of genetic background from the less common ancestral population (represented by orange in Fig. 3).

Discussion

Our findings provide reasons to be optimistic about the survival of *L. compacta*. Despite a drastic range reduction in the last 120 years, we found *L. compacta* more widespread than documented in other recent surveys. Furthermore, the remaining sites where *L. compacta* occurs retain a relatively high amount of genetic diversity. Across its range, *L. compacta* had similar levels of H_o and Π to *L. ampla*, a species that is currently found across the historical range of *L. compacta* and in some tributaries like Shades Creek and Little Cahaba River (Whelan et al., 2019). The lowest genetic diversity values observed for *L. compacta* were greater than the lowest values determined for *L. ampla*. This observation rejects one of our main hypotheses that *L. compacta* would have lower genetic diversity than the more widespread *L. ampla*. Nevertheless, *L. compacta* is restricted to a 9.2 km stretch of river, and *L. compacta* has likely lost range-wide genetic diversity. This probable loss of evolutionary potential could be detrimental to the long-term survival of the species.

Observed *L. compacta* genetic patterns often conflicted with predictions made by broad-scale hypotheses about riverscape genetics. For example, we did not see an isolation by distance pattern, which is common among freshwater taxa (Hughes, Schmidt & Finn, 2009) and was documented in *L. ampla* (Whelan et al., 2019). We also did not uncover a pattern of increased genetic diversity in downstream populations, despite such a pattern being present in numerous plants and animals (Paz-Vinas et al., 2015), including *L. ampla* (Whelan et al., 2019). Patterns determined for *L. compacta* are likely explained by a drastic range reduction and the limited scale at which we performed the current study. That is, gene flow across the 9.2 km contemporary range of *L. compacta* explains observed patterns of riverscape genetic diversity.

Genetic diversity across a small landscape

The two most distant collection sites in this study were separated by a smaller distance (9.2 km) than all but two sites sampled for *L. ampla* in a previous study (Whelan et al., 2019). Therefore, it is difficult to make direct comparisons between genetic patterns of *L. ampla* and *L.*

compacta. However, we can leverage differences in geographical scale between the two studies to make inferences about fine-scale versus long-distance genetic patterns in pleurocerids. F_{ST} values among *L. compacta* collection sites (Table 2) were much lower than values determined for populations of *L. ampla* (F_{ST} 0.377-0.773; Whelan et al. 2019). Furthermore, even though AMOVA indicated significant genetic structure among *L. compacta* collection sites, the small amount of genetic variation that is explained by collection site probably limits its biological relevance. Overall, these data indicate that pleurocerid riverscape genetic patterns across small distances will not always follow common patterns such as isolation by distance and increased genetic diversity at more downstream collection sites. This is likely attributable to gene flow and random drift that prevent the establishment of genetic patterns typically seen across more geographically separated collection sites. From a historical standpoint, we hypothesize that *L. compacta* previously displayed an isolation by distance pattern across its range, similar to the patterns determined for *L. ampla* (Whelan et al., 2019). We think this scenario is likely given limited dispersal abilities of pleurocerids and patterns established for *L. ampla*, a species that retains a much larger portion of its historic range in the Cahaba River drainage than *L. compacta*. Whether or not there was a similar historical pattern of increased genetic diversity in downstream populations of *L. compacta* is more difficult to infer, as such a pattern may not be influenced solely by dispersal ability.

Given the well-documented decline of *L. compacta*, a small number of individuals with a less common genomic background suggests that the species has lost genetic diversity through bottleneck and drift. Patterns seen in DAPC, ADMIXTURE, and fineRADstructure were not driven by geography as individuals with the less common genomic background were not found in adjacent sites (orange in DAPC and ADMIXTURE plots and upper right corner of fineRADstructure plot; Figs. 3-5, Supplementary Fig. 1). Although individuals with some admixture from the uncommon ancestral population may be present in unsampled individuals at Cahaba River at old Marvel slab and Cahaba River at canoe launch, they would be uncommon. Recent migration is an unlikely explanation of observed co-ancestry profiles as it would indicate that a sizeable population of *L. compacta* exists elsewhere in the Cahaba River. The most likely hypothesis for explaining observed clustering and co-ancestry profiles (Figs. 3-5) is a genetic bottleneck resulting from species decline in the 20th century. In this scenario, *L. compacta* was genetically diverse across its historical range prior to decline, but range contraction caused a considerable loss of genetic diversity. In turn, genetic drift resulted in the observed coancestry pattern of one ancestral population being more common in extant individuals (Figs. 3, 5).

Broadly, genetic structure across the current range of *L. compacta* can be characterized by a single population with some subpopulation structure at Cahaba River above Shades Creek and Cahaba River at Booth's Ford (Figs. 3-5; Supplementary Figure 1). The subpopulation structure appears to be causing a Wahlund effect (Whalund, 1928). That is, the Wahlund effect predicts the lower H_o values compared to H_e values and the higher F_{IS} values seen in collection sites with inferred subpopulation structure (Fig. 3; Table 2). An alternative explanation for the observed pattern of F_{IS} and H_e is null alleles. However, null alleles are unlikely as they would increase pairwise F_{ST} values (De Meeûs, 2018) that are uniformly low across populations (Table 2). Despite the putative presence of a Wahlund effect, Cahaba River above Shades Creek and Cahaba River at Booth's Ford have greater genomic diversity than the two other sites (Table 1; Figs. 3-5). These sites may have better habitat suitability than the other two, allowing for *L. compacta* to persist with greater genetic diversity as the species declined in the 20th century.

Conservation of Leptoxis compacta

Leptoxis compacta suffered a massive decline during the 20th century, a period of intense mining, forestry, and urban development in the Cahaba River drainage (Onorato, Angus & Marion, 2000; Pitt, 2000; Shepard et al., 1994; Tolley-Jordan, Huryn & Bogan, 2015). The decline was so drastic that *L. compacta* was considered extinct less than a decade ago. Conservation efforts are needed to ensure the long-term survival of *L. compacta* as the species is at risk from both chronic habitat degradation and one-time catastrophic events. Two potential management strategies for *L. compacta* are habitat restoration and reintroduction with captively reared individuals.

In this study, we report an 8.26 km known range extension for *L. compacta*. One site, Cahaba River at old Marvel Slab, was previously the focus of intense habitat restoration through the removal of a low-level dam (Johnson et al. 2013). The site may have also benefited from improved water quality in Shades Creek (ADEM, 2007; ADEM, 2012) as the site is just below its confluence with the Cahaba River. Since removal of the low-level dam, increases in fish abundance and diversity have been reported (Bennett et al., 2015). Considering *L. compacta* was not found at this site by Whelan, Johnson & Harris (2012), we think habitat either improved from a point where *L. compacta* could not survive or from a point of considerably lower carrying capacity. As the only undammed, major river in the southeastern United States, the Cahaba River is much less modified than most other systems in the southeast. Our findings suggest that imperiled gastropods will benefit from water quality and habitat improvements even in relatively "pristine" river systems. Improving habitat, or identifying suitable habitat, will be a necessary starting point for *L. compacta* reintroduction efforts.

In addition to having a small range, *L. compacta* only exists along a single river path. This means that one catastrophic event such as a massive point source pollution event above Cahaba River at Booth's Ford could result in extinction of *L. compacta*. Such an event is not merely a hypothetical. In 2016, a gasoline pipeline spill came perilously close to the Cahaba River (Pillion, 2016). To mitigate the risks of a single catastrophic event, reintroduction efforts should emphasize range expansion outside the mainstem Cahaba River. Of course, reintroduction efforts also must be limited by the historical range of any given species. Thus, lower Buck Creek is potentially an ideal reintroduction site if habitat quality is sufficient for the persistence of *L. compacta*. Once a suitable reintroduction site is chosen, managers will need to choose a broodstock site. This decision should be informed with genetic data. The absence of an isolation by distance effect across the current range of *L. compacta* indicates that managers do not need to prioritize potential broodstock sites based on whether they are geographically proximate to reintroduction sites. Rather, sites with high genetic diversity and ease of access should be prioritized for broodstock. Therefore, the Cahaba River above Shades Creek is likely an ideal broodstock location. Moreover, *L. compacta* is easy to sample and relatively easy to distinguish from other sympatric species at Cahaba River above Shades Creek, making it ideal from both a genetic and sampling standpoint.

Conclusions

Even though *L. compacta* was considered extinct less than a decade ago, we now know more about this species than most other freshwater gastropods. This is helpful for conservation of *L. compacta* as the biggest barrier to effective management strategies for most freshwater gastropods is a lack of data. Future research efforts should focus on differences in dispersal dynamics among pleurocerids and causes of differences in riverscape genetic patterns seen

between *L. ampla* and *L. compacta*. As more population genomic data becomes available for pleurocerids, we will be better suited to develop strategies to conserve these critically important components of many North American riverine ecosystems.

Acknowledgements

We thank Lauren Allred, Jecca Shumante, and Living River: A Retreat on the Cahaba for providing access to field sites. Haley Dutton (Auburn University) assisted in the field. Jeffrey Garner (Alabama Department of Conservation and Natural Resources), Matthew Galaska (University of Washington), Kenneth Halanych (Auburn University) and Katherine Bockrath (U.S Fish and Wildlife Service) provided advice on multiple components of this work. Rodolfo Jaffé, Brian Hand, and an anonymous reviewer provided comments that improved the manuscript. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Funding

This was work funded by a reverted Section 6 grant from Alabama Department of Conservation and Natural Resources and by United States Fish and Wildlife Service. This study was also supported in part by NSF grant DBI-1658694, the Alabama Agricultural Experiment Station, and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture. This work was also made possible in part by a grant of high-performance computing resources and technical support from the Alabama Supercomputer Authority.

References

ADEM. 2007. 2007 rivers and streams monitoring summary report: Shades Creek at Jefferson County Road 6. Available at http://adem.alabama.gov/programs/water/wqsurvey/table/2007/2007ShadesCk.pdf (accessed 15 April 2020)

ADEM. 2012. 2012 rivers and streams monitoring summary report: Shades Creek at Parkwood Road in Jefferson County. Available at http://adem.alabama.gov/programs/water/wqsurvey/table/2012/2012ShadesCk.pdf (accessed 15 April 2020)

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* 17:81-92. DOI: i:10.1038/nrg.2015.28.

Bennett MG, Howell JH, Kuhajda BR, Freeman PL. 2015. New upstream records for fishes following dam removal in the Cahaba River, Alabama. *Southeastern Fishes Council Proceedings* 55:50-61. DOI:

Brown KM, Lang B, Perez KE. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. *Journal of the North American Benthological Society* 27:484-495. DOI: 10.1899/07-062.1.

Catchen JM, Hohenlohe P, Bassham S, Amores A, Cresko W. 2013. Stacks: an analysis tool set for population genomics. *Molecular Ecology* 22:3124-3140. DOI: 10.1111/mec.12354.

Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. 2015. Second-generation PLINK: rising to the challenge of larger and richers datasets. *GigaScience* 4:7. DOI: 10.1186/s13742-015-0047-8.

505 Covich AP, Palmer MA, Crowl TA. 1999. The role of benthic invertebrate species in freshwater
506 ecosystems: zoobenthic species influence energy flows and nutrient cycling. *Bioscience*
507 49:119-127. DOI: 10.2307/1313537.
508 De Meeûs T. 2018. Revisiting F_{IS}, F_{ST}, Wahlund effecs, and null alleles. *Journal of Heredity*
509 108:446-456. DOI: 10.1093/jhered/esx106.
510 Dray S, Dufour AB. 2007. The ade4 package: implementing the duality diagram for ecologists.
511 *Journal of Statistical Software* 22:1-20. DOI: 10.18637/jss.v022.i04.
512 Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from matrix
513 distances among DNA haplotypes: application to human mitochondrial DNA restriction
514 data. *Genetics* 131:479-491. DOI:
515 Frankham R. 2005. Genetics and extinction. *Biological Conservation* 126:131-140. DOI:
516 10.1016/j.biocon.2005.05.002.
517 Frankham R. 2010. Challenges and opportunities of genetic approaches to biological
518 conservation. *Biological Conservation* 143:1919-1927. DOI:
519 10.1016/j.biocon.2010.05.011.
520 Fukuda H, Haga T, Tatara Y. 2008. *Niku-nuki*: a useful method for anatomical and DNA studies
521 on shell-bearing molluscs. *Zoosymposia* 1:15-38. DOI: 10.11646/zoosymposia.1.1.5.
522 Goodrich C. 1922. The *Anculosae* of the Alabama River drainage. *University of Michigan*
523 *Museum of Zoology Miscellaneous Publication No 7*:63.
524 Goodrich C. 1941. Distribution of the gastropods of the Cahaba River, Alabama. *Occasional*
525 *Papers of the Museum of Zoology* 426:1-30.
526 Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological
527 data. *Journal of Statistical Software* 22:1-17. DOI: 10.18637/jss.v022.i07.
528 Goudet J. 2005. HIERFSTAT, a package for R to compute and test hierarchical *F*-statistics.
529 *Molecular Ecology Resources* 5:184-186. DOI: 10.1111/j.1471-8286.2004.00828.x.
530 Hughes JM, Schmidt DJ, Finn DS. 2009. Genes in streams: using DNA to understand the
531 movement of freshwater fauna and their riverine habitat. *Bioscience* 59:573-583. DOI:
532 10.1525/bio.2009.59.7.8.
533 Huryn AD, Benke AC, Ward GM. 1995. Direct and indirect effects of geology on the
534 distribution, biomass, and production of the freshwater snail Elimia. *Journal of the North*
535 *American Benthological Society* 14:519-534. DOI: 10.2307/1467538.
536 Johnson PD. 2019. Results of AABC freshwater mollusk survery Cahaba River historic shoal at
537 Belle Ellen Bibb County, AL-7 May 2019. Report Submitted to Alabama Department of
538 Conservation and Natural Resources. Marion, Alabama. 6 pp.
539 Johnson PD, Bogan AE, Brown KM, Burkhead NM, Cordeiro JR, Garner JT, Hartfield PD,
540 Lepitzki DAW, Mackie GL, Pip E, Tarpley TA, Tiemann JS, Whelan NV, Strong EE.
541 2013. Conservation status of freshwater gastropods of Canada and the United States.
542 *Fisheries* 38:247-282. DOI: 10.1080/03632415.2013.785396.
543 Johnson PD, Sides JD, McGregor SW, Ahlstedt SA, Garner JT, Butler RS, Novak SF. 2006.
544 Inventory of freshwater mollusks in the Cahaba River Basin, Alabama. Submitted to the
545 Alabama Department of Conservation and Natural Resources. 153 pp.
546 Jombart T, Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data.
547 *Bioinformatics* 27:3070-3071. DOI: 10.1093/bioinformatics/btr521.
548 Kamvar ZN, Tabima JF, Grünwald NJ. 2014. Poppr: an R package for genetic analysis of
549 populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281. DOI:
550 10.7717/peerj.281.

551 Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA. 2013. diveRsity: an R package for
552 the estimation and exploration of population genetics parameters and their associated
553 errors. *Methods in Ecology and Evolution* 48:782-788. DOI: 10.1111/2041-210X.12067.
554 Kopelman NM, Mayzel J, Jakobsson M, Rosebnberg NA, Mayrose I. 2015. Clumpak: a program
555 for identifying clustering modes and packaging population structure inferences across K.
556 *Molecular Ecology Resources* 15:1179-1191. DOI: 10.1111/1755-0998.12387.
557 Kurth JW. 2017. Endangered and threatened wildlife and plants; 90-day findings for five species.
558 *Federal Register* 82:60362-60366.
559 Lambert SM, Streicher JW, Fisher-Reid MC, Méndez de la Cruz FR, Martínez-Méndez N,
560 García-Vázquez UO, de Oca ANM, Wiens JJ. 2019. Inferring introgression using
561 RADseq and D_{FOIL}: Power and pitfalls revealed in a case study of spiny lizards
562 (*Sceloporus*). *Molecular Ecology Resources* 19:818-837. DOI: 10.1111/1755-
563 0998.12972.
564 Lawson DJ, Hellenthal G, Myers S, Falush D. 2012. Inference of population structure using
565 dense haplotype data. *PLoS Genetics* 8:e1002453. DOI: 10.1371/journal.pgen.1002453.
566 Legendre P, Fortin M-J, Borcard D. 2015. Should the Mantel test be used in spatial analysis.
567 *Methods in Ecology and Evolution* 6:1239-1247. DOI: 10.1111/2041-210X.12425.
568 Leigh DM, Lischer HEL, Keller LF. 2018. Batch effects in a multiyear sequencing study: false
569 biological trends due to changes in read lengths. *Molecular Ecology Resources* 18:778-
570 788. DOI: 10.1111/1755-0998.12779.
571 Lydeard C, Cowie RH, Ponder W, F., Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ,
572 Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE,
573 Thompson FG. 2004. The global decline of nonmarine mollusks. *BioScience* 54:321-330.
574 DOI: 10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2.
575 Lydeard C, Mayden RL. 1995. A diverse and endangered ecosystem of the southeast United
576 States. *Conservation Biology* 9:800-805. DOI: 10.1046/j.1523-1739.1995.09040800.x.
577 Lysne SJ, Perez KE, Brown KM, Minton RL, Sides JD. 2008. A review of freshwater gastropod
578 conservation: challenges and opportunities. *Journal of the North American Benthological*
579 *Society* 27:463-470. DOI: 10.1899/07-061.1.
580 Malinsky M, Trucchi E, Lawson DJ, Falush D. 2018. RADpainter and fineRADstructure:
581 population inference from RADseq Data. *Molecular Biology and Evolution* 35:1284-
582 1290. DOI: 10.1093/molbev/msy023.
583 Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. 2010. Robust
584 relationship inference in genome-wide association studies. *Bioinformatics* 26:2867-2873.
585 DOI: 10.1093/bioinformatics/btq559.
586 Meirmans PG. 2015. Seven common mistakes in population genetics and how to avoid them.
587 *Molecular Ecology* 24:3223-3231. DOI: 10.1111/mec.13243.
588 Mussman, SM, Douglas MR, Chafin TK, Douglas ME. 2020. AdmixPipe: population analyses in
589 ADMIXTURE for non-model organisms. DOI: 10.5281/zenodo.3270852
590 Onorato D, Angus RA, Marion KR. 2000. Historical changes in the ichtyofaunal assemblages of
591 the upper Cahaba River in Alabama associated with extensive urban development in the
592 watershed. *Journal of Freshwater Ecology* 15:47-63. DOI:
593 10.1080/02705060.2000.9663721.
594 Paris JR, Stevens JR, Catchen JM. 2017. Lost in parameter space: a road map for Stacks.
595 *Methods in Ecology and Evolution* 8:1360-1373. DOI: 10.1111/2041-210X.12775.

596 Paz-Vinas I, Loot G, Stevens VM, Blanchet S. 2015. Evolutionary processes driving spatial
597 patterns of intraspecific genetic diversity in river ecosystems. *Molecular Ecology*
598 24:4586-4604. DOI: 10.1111/mec.13345.
599 Perez KE, Minton RL. 2008. Practical applications for systematics and taxonomy in North
600 American freshwater gastropod conservation. *Journal of the North American*
601 *Benthological Society* 27:471-483. DOI: 10.1899/07-059.1.
602 Pillion D. 2016. Alabama pipeline leak: what we know so far about the spill, gas shortages and
603 more. *Available at*
604 *https://www.al.com/news/birmingham/2016/09/how_alabama_pipeline_leak_led.html*
605 (accessed March 19, 2020).
606 Pitt RE. 2000. Water quality conditions in the Cahaba River and likely pollutant sources.
607 Birmingham, Alabama: Department of Civil and Environmental Engineering, University
608 of Alabama at Birmingham.
609 Potter JJ, Thomas WL. 1983. Endangered and threatened species listing and recovery priority
610 guidelines. *Federal Register* 48:43098-43105. DOI:
611 R Core Team. 2020. R: A language and environment for statistical computing. Vienna, Austria:
612 R Foundation for Statistical Computing.
613 Rumble SM, Lacroute P, Dalca AV, Fiume M, Sidow A, Brundo M. 2009. SHRiMP: accurate
614 mapping of short color-space reads. *PLoS Computational Biology* 5:e1000386. DOI:
615 10.1371/journal.pcbi.1000386.
616 Service NMF. 2018. Interim Endangered and Threatened Speices Recovery Planning Guidance
617 Version 1.4. In: Administration NOaA, editor. Silver Spring, Maryland. p 122.
618 Shepard TE, O'Neil PE, McGregor SW, Harris SC. 1994. Water-quality and biomonitoring
619 studies in the upper Cahaba River drainage of Alabama. *Geological Survery of Alabama*
620 *Bulletin* 160:1-118. DOI:
621 Shringarpure SS, Bustamante CD, Lange K, Alexander DH. 2016. Efficient analysis of large
622 datasets and sex bias with ADMIXTURE. *BMC Bioinformatics* 17:218. DOI:
623 10.1186/s12859-016-1082-x.
624 Strong EE, Gargominy O, Ponder W, F., Bouchet P. 2008. Global diversity of gastropods
625 (Gastropoda; Mollusca) in freshwater. *Hydrobiologia* 595:149-166. DOI:
626 10.1007/S10750-007-9012-6.
627 Strong EE, Köhler F. 2009. Morphological and molecular analysis of '*Melania jacqueti*'
628 Dautzenberg and Fischer, 1906: from anonymous orphan to critical basal offshoot of the
629 Semisulcospiridae (Gastropoda: Cerithioidea). *Zoologica Scripta* 38:483-502. DOI:
630 10.1111/j.1463-6409.2008.00385.x.
631 Tolley-Jordan LR, Huryn AD, Bogan AE. 2015. Effects of land-use change on a diverse
632 pleurocerid snail assemblage. *Aquatic Conservation: Marine and Freshwater Ecosystems*
633 25:235-249. DOI: 10.1002/aqc.2474.
634 Wahlund S. 1928. Zusammensetzung von population und korrelationserscheinung vom stand-
635 punkt der vererbungslehre aus betrachtet. Hereditas 11:65-106. [English translation. In:
636 Weiss KM, Ballonoff PA, editors. 1975. Demographic Genetics. Dowden, Hutchinson
637 and Ross, Stroudsburg. p. 224–263]
638 Wang S, Meyer E, McKay JK, Matz MV. 2012. 2b-RAD: a simple and flexible method for
639 genome-wide genotyping. *Nature Methods* 9:808-810. DOI: 10.1038/nmeth.2023.
640 Weir BS, Cockerham CC. 1984. Estimating *F-statistics* for the analysis of population structure.
641 *Evolution* 38:1358-1370. DOI: 10.1111/j.1558-5646.1984.tb05657.x.

Whelan NV, Galaska MP, Sipley BN, Weber JM, Johnson PD, Halanych KM, Helms BS. 2019. Riverscape genetic variation, migration patterns, and morphological variation of the threatened Round Rocksnail, *Leptoxis ampla*. *Molecular Ecology* 28:1593-1610. DOI: 10.1111/mec.15032.

Whelan NV, Johnson PD, Harris PM. 2012. Rediscovery of *Leptoxis compacta* (Anthony, 1854) (Gastropoda: Cerithioidea: Pleuroceridae). *PLoS One* 7:e42499. DOI: 10.1371/journal.pone.0042499.

Whelan NV, Johnson PD, Harris PM. 2015. Life-history traits and shell morphology in the genus *Leptoxis* Rafinesque, 1819 (Gastropoda: Cerithioidea: Pleuroceridae). *Journal of Molluscan Studies* 81:85-95. DOI: 10.1093/mollus/eyu058.

Tables

Table 1: Summary statistics and AUMNH catalog numbers of *L. compacta* at each collection site.

Collection Site	Private alleles	H_o (sd)	H_e (sd)	A_r (sd)	Π (sd)	F_{is}	AUMNH #
Cahaba River at Booth's Ford	43	0.1568 (0.1349)	0.1801 (0.1222)	1.8241 (0.2742)	0.1855 (0.1261)	0.1319	45691-45709
Cahaba River at canoe launch	32	0.1046 (0.1552)	0.1045 (0.1421)	1.4511 (0.4518)	0.1075 (0.1459)	0.0134	45709-45729
Cahaba River above Shades Creek	262	0.1363 (0.1334)	0.1779 (0.1245)	1.8072 (0.2883)	0.1829 (0.1281)	0.1934	45671-45690
Cahaba River at Old Marvel Slab	28	0.0963 (0.1400)	0.0981 (0.1343)	1.4606 (0.4387)	0.1010 (0.1382)	0.0226	45652-45670

Table 2: Pairwsie F_{ST} and distances (km) between sites. F_{ST} below diagonal and distances above diagonal

	Booth's Ford	boat launch	above Shades Creek	old Marvel slab
Cahaba River at Booth's Ford	-	4.62	5.55	9.2
Cahaba River at canoe launch	0.04	-	0.98	4.57
Cahaba River above Shades Creek	0	0.05	-	3.64
Cahaba River at old Marvel slab	0.03	0.04	0.03	-

Figures

Figure 1: Photograph of live *L. compacta*. Photo Credit: Thomas Tarpley, ADCNR.

Figure 2: Shells of representative individuals that we sequenced. A) Cahaba River at Canoe Launch, B) Cahaba River at Booth's Ford, C) Cahaba River above Shades Creek, D-F) Cahaba River at old Marvel slab. Scale bar = 1 cm

Figure 3: Map of known historical and current range of *L. compacta*, collection sites, and other landmarks. Lines from collection sites lead to ADMXITURE plots with K = 2 for each site. Each column is an individual with ADMIXTURE proportions of the two inferred ancestral populations.

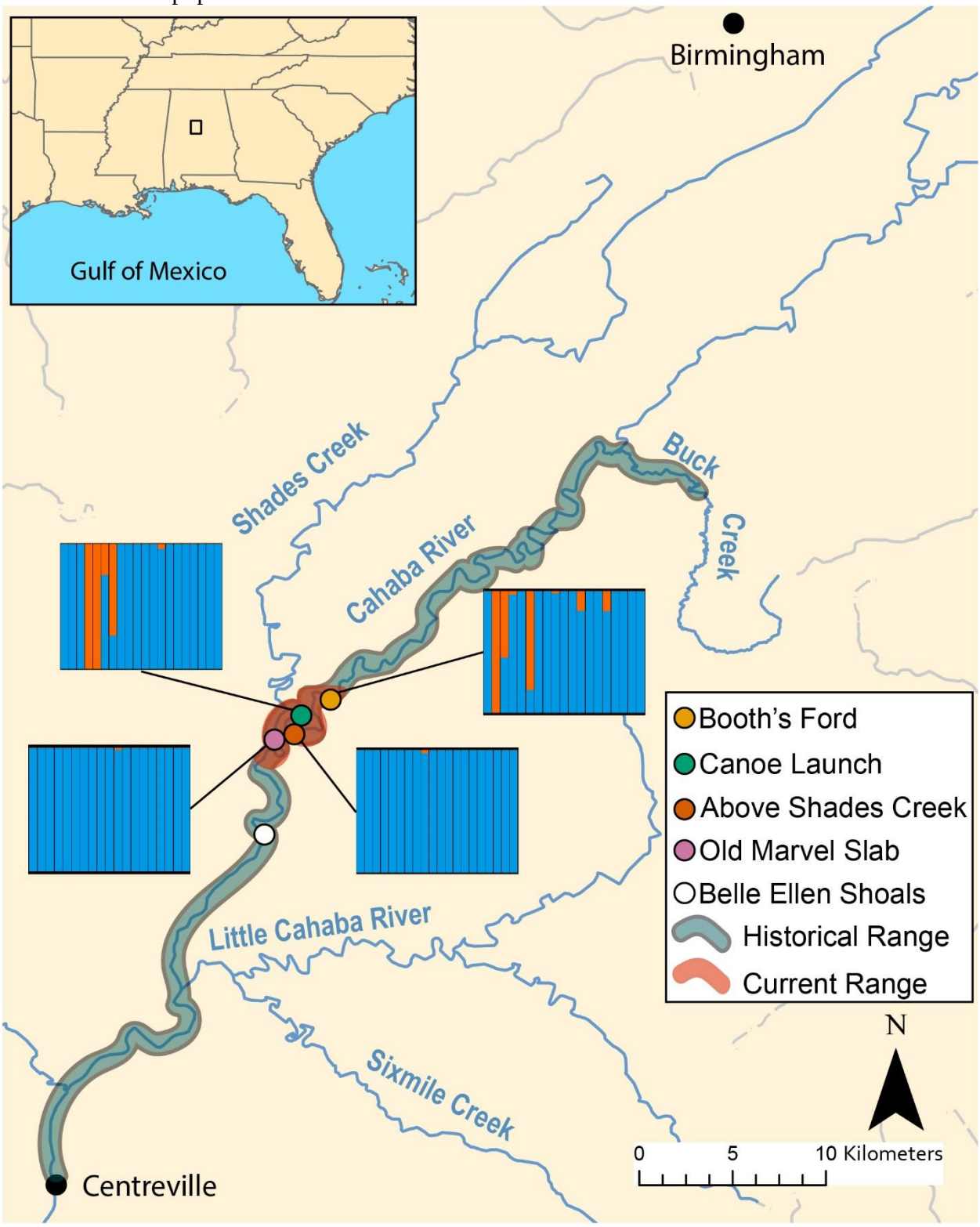


Figure 4: DAPC plot colored by genetic cluster. Tick marks on x-axis represent individuals.

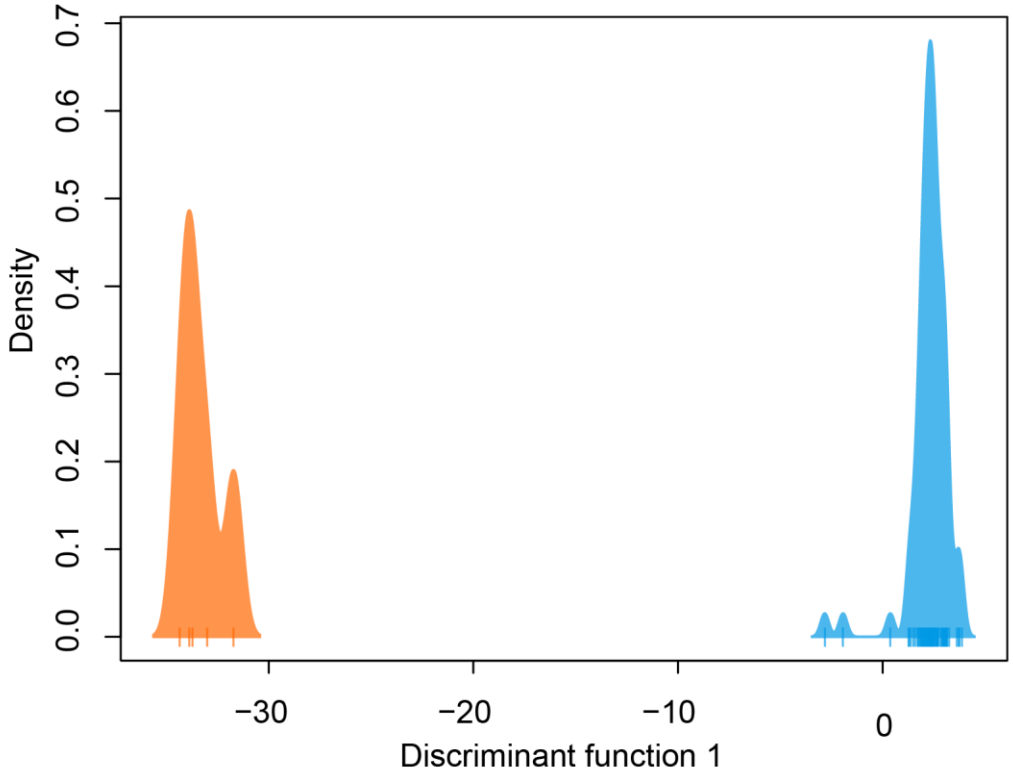
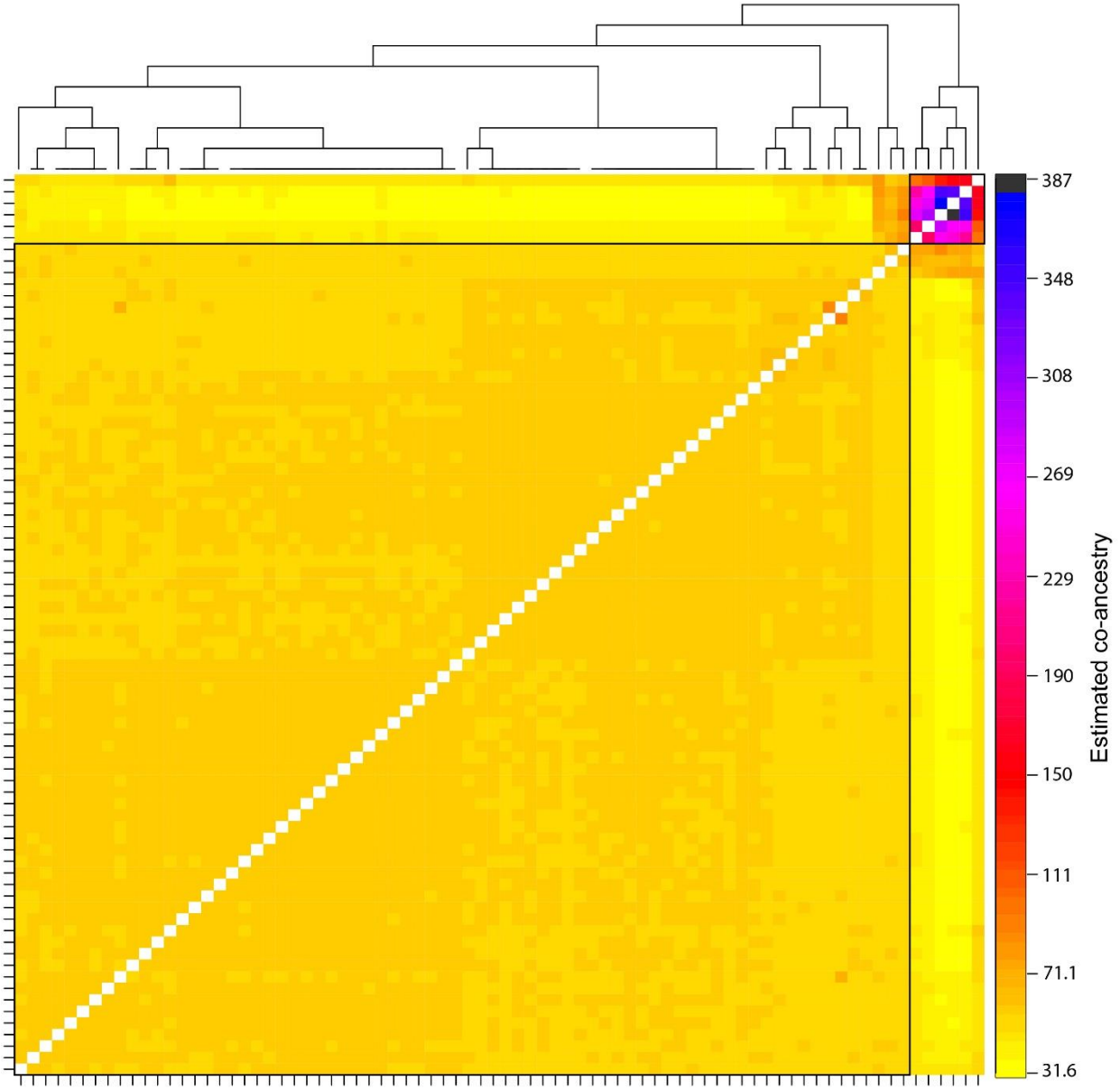


Figure 5: Pairwise co-ancestry matrix and simple tree inferred with fineRADstructure. Boxes surround the two main groupings. Tick marks represent individuals, but labels have been removed for visualization. For a figure with full taxon labels, see Supplementary Figure 1.



Supplementary Table 1: Collection localities, Auburn University Museum of Natural History, and SRA accession numbers.

Lab Number	Locality	AUMNH Catalog Number	NCBI SRA accession
Lcom_pop01	Cahaba River at old Marvel slab	45652	SRR11773571
Lcom_pop02	Cahaba River at old Marvel slab	45653	SRR11773570
Lcom_pop03	Cahaba River at old Marvel slab	45654	SRR11773559
Lcom_pop04	Cahaba River at old Marvel slab	45655	SRR11773548
Lcom_pop05	Cahaba River at old Marvel slab	45656	SRR11773537
Lcom_pop06	Cahaba River at old Marvel slab	45657	SRR11773604
Lcom_pop08	Cahaba River at old Marvel slab	45658	SRR11773593
Lcom_pop09	Cahaba River at old Marvel slab	45659	SRR11773582
Lcom_pop10	Cahaba River at old Marvel slab	45660	SRR11773573
Lcom_pop11	Cahaba River at old Marvel slab	45661	SRR11773572
Lcom_pop12	Cahaba River at old Marvel slab	45662	SRR11773569
Lcom_pop13	Cahaba River at old Marvel slab	45663	SRR11773568
Lcom_pop14	Cahaba River at old Marvel slab	45664	SRR11773567
Lcom_pop15	Cahaba River at old Marvel slab	45665	SRR11773566
Lcom_pop16	Cahaba River at old Marvel slab	45666	SRR11773565
Lcom_pop17	Cahaba River at old Marvel slab	45667	SRR11773564
Lcom_pop18	Cahaba River at old Marvel slab	45668	SRR11773563
Lcom_pop19	Cahaba River at old Marvel slab	45669	SRR11773562
Lcom_pop20	Cahaba River at old Marvel slab	45670	SRR11773561
Lcom_pop21	Cahaba River at above Shades Creek	45671	SRR11773560
Lcom_pop22	Cahaba River at above Shades Creek	45672	SRR11773558
Lcom_pop23	Cahaba River at above Shades Creek	45673	SRR11773557
Lcom_pop24	Cahaba River at above Shades Creek	45674	SRR11773556
Lcom_pop25	Cahaba River at above Shades Creek	45675	SRR11773555
Lcom_pop26	Cahaba River at above Shades Creek	45676	SRR11773554
Lcom_pop27	Cahaba River at above Shades Creek	45677	SRR11773553
Lcom_pop28	Cahaba River at above Shades Creek	45678	SRR11773552
Lcom_pop29	Cahaba River at above Shades Creek	45679	SRR11773551
Lcom_pop30	Cahaba River at above Shades Creek	45680	SRR11773550
Lcom_pop31	Cahaba River at above Shades Creek	45681	SRR11773549
Lcom_pop32	Cahaba River at above Shades Creek	45682	SRR11773547
Lcom_pop33	Cahaba River at above Shades Creek	45683	SRR11773546
Lcom_pop34	Cahaba River at above Shades Creek	45684	SRR11773545
Lcom_pop35	Cahaba River at above Shades Creek	45685	SRR11773544
Lcom_pop36	Cahaba River at above Shades Creek	45686	SRR11773543
Lcom_pop37	Cahaba River at above Shades Creek	45687	SRR11773542
Lcom_pop38	Cahaba River at above Shades Creek	45688	SRR11773541
Lcom_pop39	Cahaba River at above Shades Creek	45689	SRR11773540

Lcom_pop40	Cahaba River at above Shades Creek	45690	SRR11773539
Lcom_pop41	Cahaba River at Booth's Ford	45691	SRR11773538
Lcom_pop42	Cahaba River at Booth's Ford	45692	SRR11773536
Lcom_pop44	Cahaba River at Booth's Ford	45693	SRR11773613
Lcom_pop45	Cahaba River at Booth's Ford	45694	SRR11773612
Lcom_pop46	Cahaba River at Booth's Ford	45695	SRR11773611
Lcom_pop47	Cahaba River at Booth's Ford	45696	SRR11773610
Lcom_pop48	Cahaba River at Booth's Ford	45697	SRR11773609
Lcom_pop49	Cahaba River at Booth's Ford	45698	SRR11773608
Lcom_pop50	Cahaba River at Booth's Ford	45699	SRR11773607
Lcom_pop51	Cahaba River at Booth's Ford	45700	SRR11773606
Lcom_pop52	Cahaba River at Booth's Ford	45701	SRR11773605
Lcom_pop53	Cahaba River at Booth's Ford	45702	SRR11773603
Lcom_pop54	Cahaba River at Booth's Ford	45703	SRR11773602
Lcom_pop55	Cahaba River at Booth's Ford	45704	SRR11773601
Lcom_pop56	Cahaba River at Booth's Ford	45705	SRR11773600
Lcom_pop57	Cahaba River at Booth's Ford	45706	SRR11773599
Lcom_pop58	Cahaba River at Booth's Ford	45707	SRR11773598
Lcom_pop59	Cahaba River at Booth's Ford	45708	SRR11773597
Lcom_pop60	Cahaba River at Booth's Ford	45709	SRR11773596
Lcom_pop61	Cahaba River at Lebron canoe launch	45710	SRR11773595
Lcom_pop62	Cahaba River at Lebron canoe launch	45711	SRR11773594
Lcom_pop63	Cahaba River at Lebron canoe launch	45712	SRR11773592
Lcom_pop64	Cahaba River at Lebron canoe launch	45713	SRR11773591
Lcom_pop65	Cahaba River at Lebron canoe launch	45714	SRR11773590
Lcom_pop66	Cahaba River at Lebron canoe launch	45715	SRR11773589
Lcom_pop67	Cahaba River at Lebron canoe launch	45716	SRR11773588
Lcom_pop68	Cahaba River at Lebron canoe launch	45717	SRR11773587
Lcom_pop69	Cahaba River at Lebron canoe launch	45718	SRR11773586
Lcom_pop70	Cahaba River at Lebron canoe launch	45719	SRR11773585
Lcom_pop71	Cahaba River at Lebron canoe launch	45720	SRR11773584
Lcom_pop72	Cahaba River at Lebron canoe launch	45721	SRR11773583
Lcom_pop73	Cahaba River at Lebron canoe launch	45722	SRR11773581
Lcom_pop74	Cahaba River at Lebron canoe launch	45723	SRR11773580
Lcom_pop75	Cahaba River at Lebron canoe launch	45724	SRR11773579
Lcom_pop76	Cahaba River at Lebron canoe launch	45725	SRR11773578
Lcom_pop77	Cahaba River at Lebron canoe launch	45726	SRR11773577
Lcom_pop78	Cahaba River at Lebron canoe launch	45727	SRR11773576
Lcom_pop79	Cahaba River at Lebron canoe launch	45728	SRR11773575
Lcom_pop80	Cahaba River at Lebron canoe launch	45729	SRR11773574

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Supplementary Figure 1: Pairwise co-ancestry matrix and simple tree inferred with fineRADstructure.
Tick marks represent individuals. Lcompop_01-20: Cahaba River at old Marvel slab; Lcompop_21-40: Cahaba River above Shades Creek; Lcompop_41-60: Cahaba River at Booth's Ford; Lcompop_61-80: Cahaba River at canoe launch