THE GENETIC DIVERSITY AND POPULATION STRUCTURE OF *GEUM RADIATUM*: EFFECTS OF NATURAL HISTORY AND CONSERVATION EFFORTS

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by
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Abstract

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Geum radiatum is a federally endangered high-elevation rock outcrop endemic herb that is widely recognized as a hexaploid and a relic species. Little is currently known about *G. radiatum* genetic diversity, population interactions, or the effect of augmentations. This study sampled every known population of *G. radiatum* and used microsatellite markers to observe the alleles present at 8 loci. F-statistics, STRUCTURE, GENODIVE, and the R package polysat were used to measure diversity and genetic structure. The analysis demonstrates that there is interconnectedness and structure of populations and was able to locate augmented and punitive hybrids individuals within an augmented population. *Geum radiautm* has diversity among and between populations and suggests current gene flow in the northern populations. This information provides a greater understanding of the genetic sustainability of *G. radiatum* and what conservation efforts will most help this imperiled species survive.

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Foreword

Chapter Two of this thesis will be submitted to *The American Journal of Botany*, an international peer-reviewed journal owned by The Botanical Society of America and published by The Botanical Society of America; it has been formatted according to the style guide for that journal.

Chapter One

Natural history and past studies on *Geum radiatum*

Geum radiatum Michx, (Rosaceae), commonly known as Spreading Avens, is a rare Appalachian endemic perennial herb only found on high-elevation rock-outcrops and one grassy bald above 5000 ft (Weakley, 2015). It has been federally listed as Endangered under the Endangered Species Act since 1993 (Murdock, 1993). *Geum radiatum* grows in clumps and crevices in rocks in close association with several other rare mountaintop "pseudo-alpine" species, many of which are also considered in peril, including *Houstonia montana, Carex misera, Calamagrostis cainii, Juncus trifidus, Gymnoderma lineare, and Solidago spithamaea* (Weakley, 2015; Wiser, 1994). *Geum radiatum* is known from only 14 remaining sites in NC and TN, many of which are small and badly damaged from trampling or recreational activity and development. *Geum radiatum* is at a high risk of extinction from both global warming (Ulrey et al., 2016) and the continued residential and recreational development of high elevation sites in the Southern Appalachians (Godt et al., 1996; Wiser, 1998). All but one of the known sites (Tater Hill owned by Curtis Replogle) are protected, and one site (Phoenix Mountain) is only partial owned by The Nature Conservancy and the Margret J. Neil Trust. The federal status of *G. radiatum* guarantees pro-active management on State and Federal land, while under US law, private property owners own the plants, allowing them to do as they please. This makes Tater Hill and Phoenix Mountain the most at risk for destruction by development.

The main populations of *G. radiatum* are found in the Roan Mountain Massif, Grandfather Mountain, the Amphibolite Mountains (Bluff Mountain, Three-Top Mountain, The Peak, and Phoenix Mountain) in northwest NC and northeast TN. Other populations are found in the Craggy Mountains near Asheville, NC, two locations in the Balsam Mountains in southwest NC and one in the Smoky Mountains in TN (unpublished North Carolina Heritage Program (NCHP) Elemental Occurrence Reports; Murdock, 1993; Godt et al., 1996; Weakley, 2015).

Geum radiatum is thought to be a relic tundra/alpine species that was more widespread at the end of the last ice age and being unable to retreat further north it became stranded in the cooler mountain tops when the earth warmed (Weakley, 2015). Its closest relative*, G. peckii*, is known from alpine communities in the Presidential Range in New Hampshire and from one coastal community in Nova Scotia (Patterson and Snyder, 1999). Both *G. radiatum* and *G. peckii* are thought to be disjunct remnants from a more widely spread Pleistocene alpine species (Wiser, 1994). There has been debate in the past on whether or not *G. peckii* and *G. radiatum* are the same species (Wiser, 1998). Based upon a study using randomly amplified polymorphic DNA (RAPD) markers, *G. radiatum* and *G. peckii* appear different enough to justify recognition as separate species (Paterson and Snyder, 1999). Microsatellite markers have been isolated and characterized within *G. urbanum* and *G. repatans*, two European species. The markers have been tested and found to amplify across *Geum* (Arens et al., 2004; Hamann et al., 2014). Little work has been done on genetic diversity within or among populations of *G. radiatum*. The effects of gene flow and genetic drift among populations has not been investigated and very little is known about the evolutionary and natural history of this species.

There have been multiple attempts at introduction, reintroductions, and augmentation in different populations of *G. radiatum*, mostly without the consideration of any genetic information (Correspondence with USFS). Little information exists about what effects these conservation strategies have had on native populations and how they have changed the genetic makeup and viability of these populations. There is a lack of knowledge about how long different populations have been isolated from one another and how genetic material has moved within and among populations (Murdock, 1993; Godt et al., 1996).

A large amount of location information has been collected for *G. radiatum,* ranging from both rapid plant inventories by conservation agencies (United States Fish and Wildlife Service (USFW), Blue Ridge Parkway (BRP), Grandfather Mountain Inc., NCHP, and North Carolina State Parks (NCSP), to more extensive floristic studies that provide an understanding of community structure and type (Tucker, 1972; Jenkins, 2011). Most, or possibly all, populations of *G. radiatum* have been discovered and mapped. Population mapping and location data has been used for population prediction models for rare outcrop plants and comparison models made between populations of northern and southern disjucts considering multiple environmental and physical features (Wiser, 1994; Wiser et al., 1996). A similar study predicting tree-line in the Southern Appalachians includes *G. radiatum* as a floral component of what a Southern Appalachian alpine community would look like (Cogbill et al., 1997).

Though there has been a great deal of information collected on the location of *G. radiatum* populations, as well as morphological comparisons (Medford, 2001), there has been only one population genetic study conducted, using alloyzmes, which found very low diversity and very small genetic distances between individuals (Godt et al., 1996). The

allozyme study tested diversity richness within four different high elevation rock outcrop species, and found that within *G. radiatum* the level of diversity was lower than expected for an endemic plant, but that the diversity at the population level was comparable to other endemic plants. The genetic diversity index found (using Nei, 1972) across all populations of *G. radiatum* was 0.185, with a within population diversity of 0.119, showing very little difference. Only 1 'private allele' was detected in any population examined (Godt et al., 1996). There have been two studies that genetically address the taxonomic treatment *G. radiatum* and *G. peckii* through genetics. The first was a cytogenetic study using chromosome smashes of 22 different species of *Geum* that determined both species have the same ploidy number (6n) and that there are no morphological differences between the chromosomes of the two species (Raynor, 1952; Gajewski, 1957). The second was a comparison of Random Amplified Polymorphic DNA (RAPDs) amplified from *G. radiatum* and *G. peckii*, which found that there was enough genetic difference between the two species to justify the taxonomic distinction (Patterson and Snyder, 1999). The genetic distance between species (Nei, 1977), was 0.3472- 0.4976, with a within species the genetic distance was 0.0462 for *G. radiatum* and 0.0337 for *G. peckii* (Patterson and Snyder, 1999). This analysis demonstrated that most of the genetic variation is between the two species instead of it being shared by both species.

Rare plant management and the Endangered Species Act

By the time a plant is considered and officially recognized as threatened or endangered, it has often undergone a drastic population decline and/or loss of range. The

legal protections granted by governments to plants are often too late and only come into effect after the majority of damage to long-term survival is already done (Buza et al., 2000). Most of the time these protections just stop a plant from going extinct and do nothing before the bulk of the genetic and spatial diversity is lost. Plants have different legal protections and different measures must be used in the prevention of extinction. Plants are owned by the landowner, unlike animals that are owned by the government, and are regulated as such in most cases no matter how threatened or endangered a plant might be. The inability to compel private landowners, and the sessile nature of plant life demands that land conservation and protection, with proper management be the most important tool for threatened and endangered plant conservation. Threatened and endangered plants often are found in what is now rare habitat or undisturbed habitat and further loss and disruption of habitat will lead to extinction. In many places in the US rare plants are already on protected land, such as National Parks, National Forests, and Bureau of Land Management lands, but have been managed in ways that have led to plant declines. Management activities such as fire suppression and recreational activities like rock-climbing, without proper consideration of the effects on threatened and endangered plants, have created situations where plants that are only found on federal land and seem as though they should be protected, have been listed, like *Hudsonia montana* (Wells and Alexander, 2012).

Population declines can be caused by habitat loss, landscape modification, climate change, disease, poaching, etc. and can be drastic over short periods of rapid decline or a slow arduous march towards extinction over centuries (Maschinski et al., 1997; Traill et al., 2007). To reach a threshold where plants are rare enough to merit legal protection, plants must already be considered on the brink of extinction and the only way that the species can

survive would be through human management (Endangered Species Act of 1973). The Endangered Species Act of 1973 creates a framework in which species that are facing extinction are given special status and protection under that law and monies are made available with the goals of preventing extinction through immediately stabilizing current population decline and constructing a management plan for eventual removal from the jurisdiction of the Endangered Species Act (1973). Endangered Species Act (1973) management plans focus on stabilization and rehabilitation of threatened and endangered species by mitigating the factors that negatively affected past and current population declines and by increasing and encouraging factors needed for long term viability and security (Endangered Species Act of 1973). In order to achieve long term viability and security of a threatened and endangered species the habitat or, as the ESA refers, ecosystems that these species rely on must be preserved (Endangered Species Act of 1973). Habitat conservation through land acquisition and proper management of already protected lands are the key to stabilizing and insuring long-term survival of threatened and endangered plants.

In the face of rapid declines plants are particularly adept at preventing rapid extinction by surviving in small limited populations where they can be insulated from whatever has stimulated the decline (Maschinski et al., 1997; Traill et al., 2007). These pockets of insulated plants become increasingly more vulnerable to extinction and are easily extirpated by simple random events like a rock slide or the lack of events such as no fires on a landscape. As species decline, necessary steps must include protecting these limited populations and allowing if possible a range expansion with the addition or removal of factors that have led to the decline. Once a plant species is imperiled and found into disparate populations, other risks must be considered, including hybridization and the eventual

introgression of a rare species into a more common relative. Because the ESA uses the taxonomic rank of species for consideration for listing something as threatened and endangered, the way in which we taxonomically recognize and define a plant becomes central to its conservation. A change in rank of a threatened and endangered plant species can threaten and damage its legal protection and effort. Questions about what level of diversification and genetic difference are recognized as a species for the implementation of legal conservation mechanisms such as ESA are important and must be defined. The effects of species concepts on conservation and the legal mandates for threatened and endangered plants must be considered, fusing a rare plant's species concept into a common plant's species concept you can effectively remove it from the control of the ESA or, depending on perspective, you have rehabilitated the species from the brink of extinction.

Management plans for threatened and endangered plants often call for the immediate implementation of strategies that limit habitat destruction and protect niches that plants rely on to start to stabilize population number and size. If and once what is thought of as a sustainable or at least historic population size and number is reached, protected, actively managed, and there appears to be no new threats, USFW will start the process of delisting that species and declare that species secure and no longer in need of USFW protection under the ESA. All species, once they have been listed as threatened and endangered go through a management plan development process with the ultimate goal of delisting. An example success of land conservation and management is *Solidago albopilosa*, commonly known as White-Haired goldenrod. *Solidago albopilosa* through limiting human disturbance, procurement of almost all known populations, and establishment of long term management plans has been delisted (Floyd, 2016). *Solidago albopilosa* is native to the Red River Gorge

and surrounding sandstone cliffs and rock shelters in east central Kentucky with most sites falling within the Daniel Boone National Forest. By the time it was listed as threatened in 1988, many of it populations had been heavily damaged from human trampling, camping and fires, and archaeological looting (Shea, 1993). By 1990, 11 populations of *S. albopilosa* with 3422 individuals had been extirpated, with 75% of populations being heavily damaged with a loss of up to 96% of individuals within some populations (Shea, 1993). At this point, only 39 sites were protected out of the 69 known occurrences. In the couple of decades leading up to 1990, *S. albopilosa* was in steep decline. The USFW management plan highlighted different conservation strategies, including protecting more sites, closing off recreation at already protected sites, fighting illegal archeological digging for artifacts, and the discovery of new sites (Shea,1993). By the time of delisting for *S. albopilosa* in 2015, efforts on all of theses management strategies had been made, especially on the discovery of new populations and protecting more of the populations (Shea, 1993). By 2015 there were 115 known sites with an estimated 174,000 individuals. One hundred and eleven of the known sites are now under federal ownership and being protected and managed for *S. albopilosa* long-term survival (Floyd, 2016). In 2015, 81 sites were considered stable, while 46 of those sites were considered adequately protected and self-sustaining containing 131,000 individuals or around 75% of all known individuals (Floyd, 2016). In and around areas containing *S. albopilosa* populations trails have been moved, fencing has been added, and there has been a crackdown on illegal artifact digs (Floyd, 2016). With limiting recreational damage and by protecting more populations in perpetuity *S. albopilosa* numbers have stabilized and have started to recover. The most successful management strategies for threatened and endangered plants should strongly consider including land conservation and protection from human disturbance.

The decline in *S. albopilosa* was a product of human disturbance within its narrow habitat needs, sandstone rock shelters. Once habitat was protected through new land conservation and recreation strategies, *S. albopilosa* population numbers and individuals stabilized and have rebounded. Land conservation and management changes can be an incredibly powerful tool in to prevent plant species from going extinct and losing genetic and spatial diversity.

The reasons for declines in threatened and endangered plants can be in some cases caused by more entrenched and difficult to remedy landscape changes than just location of recreation trails and camping. When threatened and endangered plants depend on landscape features and process that humans have stopped and removed from the landscape, species declines can be difficult to stabilize and undo. The past common occurrence of fire in some habitats have allowed particular plants to specialize in post fire growth in areas sometimes devoid of competition from larger woody shading plants. One such plant, *Hudsonia montana*, commonly known as Mountain Golden Heather, is endemic to Linville Gorge and an adjacent cliff and ridge in Western North Carolina (Pendergrass, 1983; Wells and Alexander, 2012). *Hudsonia montana* is currently listed as threatened by USFW under the ESA and has been listed since 1980 (Pendergrass, 1983). *Hudsonia. montana* is a fire dependent, post fire colonizer found on rock outcrops, ledges, and slopes. Though fire dependent and found within an ecosystem with many fire dependent members, controlled burns have been fraught with legal fights and public protest by housing developments close to Linville Gorge, most notably the development Gingercake Acres. *Hudsonia montana* was first described in 1816 from Table Rock on the east rim of Linville Gorge and was said to be found in "extensive caespitose patches" in 1818. This was the only known population until 1978. For a period of time in the 1960s and early 1970s, *H. montana* on Table Rock became so rare that it couldn't

be located and was thought to be extinct till rediscovered in 1978 both on Table Mountain and on other sites on the east side of Linville Gorge (Pendergrass, 1983). In 1980 there was thought to be a little under 2,000 plants from 5 known populations all along the east rim of Linville Gorge. Decades of fire suppression allowed for woody species of *Kalmia* and *Rhododendron* to become dominant on cliffs and ledges shading out *H. montana* (Pendergrass, 1983). *H. montana* was in steep decline and though more populations has been discovered. There was nothing like the "extensive caespitose patches" described in 1818 by Nuttall, all sites were overgrown and almost shaded out with just a handful of individuals remaining. At the time of listing 1980 all known populations were within the Linville Gorge Wilderness Area and were protected from future development; in 1982 the total count of individuals was 2,901.

The focus of the management plans was to limit the amount of shade plants in *H. montana* habitat and back away from decades of fire suppression with the hope fire would stimulate in seedling growth as has been seen after a cliff ledge camp fire on Table Rock and in other member of the genus *Hudsonia*. The plan also focused on locating new population of *H. montana* and securing them if found. Through the 1980s different experimental uses of fire and mechanical clearing seemed to yield promising results, but overall the *H. montana* was still in decline. In 1990 USFW and NFS along with their NC partners decided to "switch efforts from research to active management" (Wells and Alexander, 2012) as *H. montana* continued to decline. This, coupled with the understanding and acknowledgment that trampling was greatly damaging several sites, new management strategies to combat trampling and shading were implemented. In 1993 another extensive inventory of *H. montana* was completed showing since 1982 there had been net loss of 36% of total known

individuals to 1,854 (Wells and Alexander, 2012). Throughout the 1990s extensive mechanical removal of shading brush and controlled burns continued and several large wildfires burned the east side of Linville Gorge clearing out overgrown habitat. Signs were added warning of rare plants and trails were altered to try to minimize trampling. Different sites were burned multiple times by both controlled burns and wildfires and where court cases had controlled burns mechanical removal was performed. By 2003 the number of individuals had increased drastically to the highest count ever, with 4,364 individuals across all populations, with the most drastic increase in the populations that had burned several times. The most spectacular increases occurred on the heavily burned Shortoff Mountain and Woods Mountain (Wells and Alexander, 2012). Even though *H. montana* population numbers have been stabilized and even started to increase, there still remains enough threat to justify the continued listing of *H. montana.* Threats still remain such as the return of the lack of fire and the continued increase in the foot traffic in Linville Gorge.

Rare plants in steep decline need management strategies tailored to the causes of their decline and to their biology. Strategies can include undoing landscape changes, preventing trampling, and securing from needed habitat from further development. Rare plants are often rare because of a dependence on rare or over utilized habitat and any conservation plan needs to start with protection if rare habitat and communities. Landscape changes such as the removal of fire can drastically change plant communities and push rare plants to extinction. Addition of fire back to these communities can help stabilize and restore these plants. Rare plants will always need special consideration and particular management to help them remain on earth.

Rare plant augmentations and conservation

Augmentations of rare plant populations have been commonly used as a management tool to attempt to offset and undo species declines and population damage that has occurred (Krauss et al., 2002, Godefroid et al., 2012). In the management of rare plant populations there has always been a considerable concern and worry about the effects of inbreeding depression and more recently on what is the necessary amount of genetic diversity needed to sustain a population or species into the future (Shaffer, 1981). Rare plants often have small population sizes and tend to be found in small pockets where they persist and are dependent on rare habitats and niches that can be easily damaged (Severns, 2003). The concern lies in when a breeding population declines to a size that is so small and with so little diversity that plants have a hard time reproducing. In these situations, the offspring that are produced have lower fitness and growth (Buza et al., 2000). Once all reproductive robustness is lost then it is only a matter of time before the population is extirpated or the species becomes extinct. Scientists and conservationists have struggled to create a metric of what is functionally enough diversity for populations to be "sustainable in the future", partly from the wide variety of reproductive methods from obligate clonal reproduction to obligate outcrossing (Traill et al., 2007).

Some of the issues with when and how augmentations are preformed arise from government conservation agencies needing a broad brush of what a viable population size is and species size is to be able to establish policy about when a species is on the brink of extinction. It is impossible to set a metric for the minimum number of individuals needed for a species to be sustainable, especially if it is supposed to apply to all species. Conservation

managers have a tough task of following broad governmental policies while trying to make decisions about conservation that are informed by science and the specifics of the rare plants they are trying to save from extinction. This is especially confounded by rapid changes in land use and a warming and drying climate with increased climatic uncertainty. Decisions have to be made fast while following policy prescriptions and on occasion the results can be regrettable (Krauss et al., 2002). Plants can be moved to places and in ways they should not have been without rigorous information beforehand, resulting in unintended consequences for the species of concern. Conservation and conservation management is often emotionally charged. Decisions to save a single rare but charismatic species can take precedence over an entire ecosystem based solely on how people feel. Governmental conservation agencies can be compelled to make scientifically unsound decisions based on the conservation community or the public at large. These actions can have unintended consequences that can be detrimental to the species they are trying to save or to the habitat and ecosystem that it relies on.

When rare plants are augmented most of the problematic issues arise from the source of the genetic material (Krauss et al., 2002). Is the source of plants or seeds native to the population being augmented or is the material from a different population? Local source augmentations take place when material is collected from a population and grown in controlled settings to maximize the amount of individuals. These are then planted back in the habitat where they originated to bolster the population. This is done to increases the population size without or minimally changing the genetic structure and diversity of the population.

Non-native augmentations are when material is taken from one population and moved to another population to bolster both numbers and genetic diversity of the site where the plants or material is added. This can be done by planting new plants at a site, by either moving whole plants or by growing seeds in a greenhouse or by collecting pollen and fertilizing plants at the site of interest. Either way artificial gene flow has occurred and local genetic diversity has been altered. Rare plant augmentations have mixed success in increasing population numbers, but are still performed regularly (Godefroid et al., 2011).

Rare plants can be hard to grow and be difficult to get established once in the habitat. Out of 135 rare plant augmentations reviewed, the authors described only 39 (29%) as a success (Godefroid et al., 2011). Plants may also have peculiar biology's that complicate augmentation efforts of either kind and these peculiarities are many times unknown to the augmenters even after the augmentation has been completed (Godefroid et al., 2011). Out of 39 different failed reintroduction, translocation, and augmentation events, 34 (87%) failed for an unknown reason (Godefroid et al., 2011).

Local source augmentations that have occurred at the Devil's Courthouse with *G. radiatum* have resulted in almost zero survivorship (personal communication with Chris Ulrey). *Geum radiatum* plants that were grown from seeds collected at the Devil's Courthouse were then planted on cliff faces that mostly eroded and washed away before the plants could become established. Today one can still see bare rock with blue tags where the augmented plants were placed. The ability for augmented plants to establish themselves once transplanted can really challenge conservation efforts. There have been 2 different failed augmentations of the endangered Sentry Milk-Vetch *Astragalus cremnophylax var.*

cremnophylax due to the inability for augmented plants to become established in arid conditions of the rim of the Grand Canyon (Maschinski et al., 1997).

Even with an understanding of the reproductive biology of a plant species and good demographic data, it is still incredibly difficult to determine what the minimum level of genetic diversity or number of individuals is required to have long-term security for a given plant. Once a minimum threshold of individuals or diversity has been established, and it is determined that augmentation is necessary, a decision about the location of the source material must be made. Is it important to preserve the native genotype *in situ* or is it appropriate to bring in genetic material from new locations? Is the existence of genetically different populations necessary? What are the goals of the conservation plan? These questions do not have necessarily scientific answers and data that has been collected to address these questions can be contradictory. Thirty-five different transplanting studies have experimentally demonstrated that locally adapted genotypes have higher survival rates, faster growth, and stronger fitness than non-native genotypes (Leimu and Fischer, 2008). The more diversity and the more differences in diversity the greater the likelihood that adaptive and disease resistant abilities exist in a species of plant; this effect of diversity has been demonstrated in crops like rice (Zhu et al., 2000).

Adaptive and disease resistant abilities are necessary for the long-term survival of rare, diversity poor plant species. But questions for example, what is the appropriate level of human intervention and what should be the extant of those actions? As well as, what is the importance for populations to be genetically unique? These are moral questions about human responsibility versus intervention. I personally do not believe such questions can be answered by scientific thought and process, but instead need to be made as a society about the value we

want to prescribe to maintaining biodiversity. Again understanding of reproductive biology and the recent history of a given species is imperative to constructing a conservation plan. If you move genetic material between locations, have these plants been recently connected and are now fragmented or have they been separated for thousands of years? If the goal is to increase genetic diversity, is the plant of interest clonal, an in-breeder, or an out-crosser?

When designing a conservation strategy that includes augmentation for a now rare, but once more widespread species, the negative effects of current inbreeding and loss of connectivity between populations needs to be considered. If a rare plant now found in scattered populations was once, in the recent past, part of a large widespread interconnected meta-population than artificial gene flow and movement of plants from different populations might be the best option (Severns, 2003). *Lupinus sulphureus ssp. kincaidii,* commonly known as Kincaid's lupine, now occupies 1% of its original range in the Willamette Valley in Oregon and is found only in small remnant pieces of the valley's once large upland prairie (Severns, 2003). It was found experimentally that with the movement of pollen and plants from different fragment sites, that seed set increased drastically; seed set increased with outcrossing from 0.6 seeds/fruit within site pollination to 1.6 seeds/fruit when out crossed to other sites (Severns, 2003). In cases of recent inbreeding of once widespread species, artificial gene flow through augmentations maybe necessary to conserve the species.

With species that have been rare with isolated populations for a long period of time, there is concern that non-native augmentation could create an outbreeding depression effect, causing an invasive population within the larger native population (Montalvo et al., 1997). If a species has adapted to inbreeding then the shock of hybridization with different genotypes long separated could cause reduced fitness, as locally adapted traits are broken (Storfer 1998).

The risk of this effect has not been well measured or studied and is hard to quantify in an already rare and struggling species (Godefroid et al., 2011). It has been shown that locally adapted individuals have higher fitness and growth (Leimu and Fischer, 2008), hybridizing non-native individuals into a population might dilute native adaptation thus lowering fitness and increasing the risk of extirpation (Storfer, 1999). Outbreeding depression has been shown experimentally to occur in rare plants such as *Grevillea scapigera,* commonly known as Corrigin grevillea, a rare shrub from Western Australia, where individuals from different populations with different genotypes combined together produced offspring with decreased fitness (Krauss et al., 2002). Long range gene flow in non-rare plants has also been shown to negatively affect fitness and growth. For example, in *Scleranthus annuus*, commonly known as German knotweed and native to North Africa, Europe, and Asia, F1 male offspring had a reduction in fertility of 36.5% between patches within the same population and a reduction of 90% between different populations as compared to within the same patch hybrids (Svensson, 1990). When establishing conservation plans involving non-native augmentation, hybrid vigor (heterosis) is not a guaranteed outcome of hybridizing two or more populations. There can be negative effects on fitness from these kinds of conservation oriented actions.

It has been demonstrated experimentally that through artificial combinations of multiple populations there is the potential to create more robust and reproductively active populations that are able to reproduce and grow faster (Godefroid et al., 2011). This could be a valuable conservation strategy to help reestablish and stabilize rare plants in decline. Hybrid vigor is the increased production by F1 or first-generation hybrids and this has been shown to occur in wild plant populations. *Arnica montana,* commonly called Wolf's Bane, a native herb to central Europe, has higher estimated biomass, mean percentage flowering, and

mean number of flower heads per plant with multi genotype plantings than it has with signal genotype plants in a single location (Vergeer et al., 2005). The use of multiple source populations for reintroductions in meta analyses of 135 different studies found that using multiple source populations was the most effective variable in determining the long-term success of the work (Godefroid et al., 2011). The use of artificial gene flow through non-native augmentations to create hybrid vigor in rare plant population maybe a short term effective way of increasing population size and stabilizing decline, though it is not without risk to overall species genetic diversity.

One can imagine the different scenarios that are possible based off of different forms of reproduction with different levels of genetic mixing within a population. Augmenting a clonally reproducing plant with non-native genotypes is essentially introducing new non-native competition to the habitat. If the species is an inbreeder, non-native genetic material might also be essentially adding new competition to the habitat and over time might lower genetic diversity if the new genetic material is more competitive. Even with the case of an out-crosser, moving genetic material may still decrease overall genetic diversity; if populations long separated are recombined genetically there still maybe an overall homogenization with the loss of location specific diversity generated from genetic drift. Overall, individual population diversity may increase but overall species diversity across all populations may decrease. Though if a species is an out-crosser and a population has so little diversity that it is no longer able to reproduce then bringing in new individuals may allow the population to have reproduction and new recruitment and allow for the population to rebound.

There is a massive lack of data about the effects of augmentations on the rare plant of interest and on the community where the augmentation occurs. Almost all studies on the

human mediated movement of rare plants focus on a narrow window of what is success. Success is defined as whether or not there is new recruitment of seedlings after the augmentation takes place (Godefroid et al., 2011). The conservation paradigm is that more plants is good and less is bad and as long as there are more plants, the augmentation was a success. There has been very little effort into research that complicates or challenges this idea. More research into the population genetic structure for augmented plants needs to occur. For example, studies like this project *G. radiatum* where plants were moved with the assumption that they would hybridize. But if that is not the case there may now be competitive genotypes on the same cliffs fighting for the same resources. Population admixture tests need to be performed to look at potential hybridizations post non-native augmentation to verify that hybrids are being formed. More work needs to be done on outbreeding depression and the effects of diluting locally adaptive genotypes in these rare plant populations. This could be done with common garden experiments, where different experimental crosses could be tested in different environments. Most rare plant conservation work is performed by government agencies and is designed without debate on the specific needs and realities of a given plant. Broad policy with little scientific input has been used to make decisions about augmentation of rare plants. Future work needs more rigor and must be accompanied with follow up genetic and demographic studies to find both the positive and negative consequences of the work. Up until now very little of this has been done and so very little is known about what is the most beneficial way to augment rare plants.

There is no golden rule or paradigm to govern and aid in rare plant augmentations with the diversity of biological and historical realities of rare plants and the unique conservation realities that make each species different. As more research is performed, and

there is more of an acceptance on both a policy and scientific level of the many factors that can affect rare plant augmentation success, there will be more positive conservation outcomes and a more targeted and individualized conservation strategy employed. Every plant is going to be different, so no augmentation plans should be the same. Until we know more about each species of rare plant and the effects of past augmentations we will not be able to guarantee the best outcome for the survival of a rare plant species.

Chapter Two

The genetic diversity and population structure of *Geum radiatum*: effects of natural history and past conservation efforts[1]

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The genetic diversity and population structure of *Geum radiatum*

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PREMISE OF THE STUDY: *Geum radiatum* is a federally endangered high-elevation rock outcrop endemic herb that is widely recognized as a hexaploid and a relic species. Little is currently known about *G. radiatum* genetic diversity, population interactions, or the effect of augmentations.

METHODS: This study sampled every known population of *G. radiatum* and used microsatellite markers to observe the alleles present at 8 loci. F-statistics, STRUCTURE, GENODIVE, and the R package polysat were used to measure diversity and genetic structure.

KEY RESULTS: The analysis demonstrates that there is interconnectedness and structure of populations and was able to locate augmented and punitive hybrids individuals within an augmented population. *Geum radiautm* has diversity among and between populations and suggests current gene flow in the northern populations.

CONCLUSIONS: This information provides a greater understanding of the genetic sustainability of *G. radiatum* and what conservation efforts will most help this imperiled species survive.

KEY WORDS: augmentation; cliff-face; conservation; endangered species; *Geum radiatum*; hexaploid; population genetics

INTRODUCTION

Geum radiatum Michx, (Rosaceae), commonly known as Spreading Avens or Appalachia Avens, is a rare Appalachian endemic perennial herb found on high-elevation rock-outcrops and a grassy bald above 1500 m in Western NC and Eastern TN (Weakley, 2015). *Geum radiatum* has a showy yellow flower and circular serrate leaves with venation that radiates from the base of the leaf. The plants grow in mats and clumps from a horizontal

rhizome attached in cracks and crevasses of rock outcrops. One population is also found in an open grassy area. The species has been federally listed as Endangered under the Endangered Species Act since 1993 (Murdock, 1993). *Geum radiatum* grows in close association with several other rare mountaintop "pseudo-alpine" species, many of which are also considered in peril, including *Houstonia montana, Carex misera, Calamagrostis cainii, Juncus trifidus, Gymnoderma lineare,* and *Solidago spithamaea* (Wiser, 1994; Weakley, 2015). *Geum radiatum* is known from 14 remaining sites in NC and TN, many of which are small and have been previously badly damaged from trampling or other recreational activity and development. *Geum radiatum* is at a high risk of extinction from both global warming (Ulrey et al., 2016) and the continued residential and recreational development of high elevation sites in the Southern Appalachians (Godt et al., 1996; Wiser, 1998). The federal status of *G. radiatum* guarantees some protection and management on State and Federal land, while under US law private property owners own the plants, making the remaining private sites the most at risk for destruction and eventual extirpation by landowners. Due to the sensitivity of location data for endangered plants, all population sites have been renamed population 1-14 from north to south along the range (Fig. 1)

Geum radiatum is thought to be a relic alpine species (disjunct remnant) that was more widespread at the end of the last ice age (Wiser, 1994). The species retreated to the cooler mountain tops of the southern Appalachians when the earth warmed and became stranded, unable to retreat further north (Weakley, 2015). Its closest relative*, Geum peckii*, is known from alpine communities in the Presidential Range in New Hampshire and from one coastal community in Nova Scotia (Patterson and Synder, 1999). The distribution of above timberline alpine comminutes like those found in the Presidential Range covered large areas

of the Southern Appalachians during the Pleistocene, supporting the hypothesis of a relic species (Delcourt and Delcourt, 1999). The habitat that *G. radiatum* occupies is the closest analog in the Southern Appalachians to an alpine habitat and is often found within spruce-fir mountain top forests; these rock faces have high sun exposure, low annual temperatures, and are some of the wettest high-elevation cliffs in the Southern Appalachians (Ulrey et al., 2016). Similar rock outcrops in the same region that are fire maintained are devoid of *G. radiatum*, suggesting moisture plays a key role in defining habitat preferences.

The current range of *G. radiatum* extends from Northwest North Carolina to Southwest NC close the border with Tennessee, with all populations within NC, except one population straddling the NC and TN border in the middle of the species range and another isolated population wholly located in TN near the Southern end of the range. These populations are found between 1341 to 2012 meters in elevation. Populations found at the lowest elevation are in the northern end of the range found within the Amphibolite Mountains Macrosite (AMM), (Poindexter and Murrell, 2008). These populations are also geographically close in proximity to each other compared to the Southern populations. There is also a conspicuous lack of spruce-fur forest and a relatively neutral pH of mafic mineral rich soil in these northern populations (Peet et al., 2003). Population 8 (see below) is one of the largest remaining populations found in the center of the range and is comprised of multiple subpopulations that are found on close but unconnected cliff systems.

Geum radiatum, its closest relatives *G. peckii* and *G. calthifolium* as well as many of the members of the genus *Geum,* are hexaploid (2N=6X=42) (Gajewski, 1957). The microsatellite markers used in this study where first isolated and characterized within *G. urbanum* (a circumboreal Avens), and *G. repatans* (a yellow flowering Avens from the Alps)

(Arens et al., 2004; Hamann et al., 2014). Microsatellite markers work well in diploids where there are only two sets of chromosomes and therefore a maximum of two alleles per genetic locus. Polyploid species such as *G. radiatum* have a higher possible number of alleles and there is a possibility of having multiple forms of heterozygous and homozygous genotypes within the same locus. This problem compounds itself as ploidy increases and complicates the ratios of the number of alleles at each site, known as allele dosage (De Silva et al., 2005). These higher order polyploids have the potential to carry more alleles per locus and therefore greatly complicate calculations and interpretations of genetic diversity.

There is a large amount of location information collected for *G. radiatum,* ranging from rapid plant inventories by conservation agencies such as United States Fish and Wildlife Service (USFW), Blue Ridge Parkway (BRP), Grandfather Mountain Inc., North Carolina Heritage Program (NCHP), and North Carolina State Parks (NCSP), to more extensive floristic studies that provide an understanding of community structure and type (Tucker, 1972; Jenkins, 2011). Most, or possibly all, populations of *G. radiatum* have been discovered and mapped. Population mapping and location data has been used for population prediction models for rare outcrop plants and comparison models made between populations of northern and southern disjucts considering multiple environmental and physical features (Wiser, 1994; Wiser et al., 1996). There has also been a similar study predicting tree-line in the Southern Appalachians that includes *G. radiatum* as a floral component of a Southern Appalachian alpine community (Cogbill et al., 1997) and most recently an extensive ten year demographic study and site climate change model of *G. radiatum* (Ulrey et al., 2016)

Demographic investigations suggest that *G. radiatum* is a very long-lived perennial plant with very high survivorship (in undisturbed habitats), and very low seedling

recruitment (Ulrey et al., 2016). Monitoring conducted over a period of ten years found that most populations had no seedling recruitment, but that most populations did not lose a single adult plant (Ulrey et al., 2016). *Geum radiatum* has been difficult to grow from seed in the past, even when air-conditioned greenhouses were used to get plants established (Johnson, 1995), demonstrating that *G. radiatum* is a species that may not naturally or quickly recover from anthropogenic damage. In some cases, it may take centuries for populations that are at their climatic limit to regenerate from even modest trampling (Johnson, 1995). Though there has been a great deal of information collected on the location of *G. radiatum* populations and morphological comparisons (Medford, 2001), there has been only one population genetic study conducted, using alloyzmes, which found very low diversity and very small genetic distances between individuals (Godt et al., 1996). The allozymes study tested diversity richness within four different high elevation rock outcrop species, and found that within *G. radiatum* the level of diversity was lower than expected for an endemic plant but the diversity at the population level was comparable to other endemic plants from previous studies.

Geum radiatum is a charismatic plant and has long been sought after by botanists such as Asa Gray (Gray, 1889). This fascination has led to over collecting of the species and has complicated the history of conservation actions. In past attempts to save struggling populations of *G. radiatum* some populations have been augmented with plants from different source populations, mostly without the consideration of any genetic information (Correspondence with USFS). The most notable augmentation was the movement of plants from population 1, in the north of the range to population 8 in the center of the range (Correspondence with USFS). In the present study, population 8 was heavily sampled across

all subpopulations to try to elucidate the genetic effect these actions may have had on genetic diversity. Little information exists about what effects these conservation strategies could have on native populations and how they have changed the genetic makeup and viability of these populations.

The goal of this study was to, 1) describe the genetic diversity contained within the 14 extant populations, 2) evaluate how this diversity is distributed within the species, and 3) to examine the ability to detect augmented plants. The role of these augmentations in the long-term survival of *G. radiatum* could be key to our understanding of rare plant conservation in the face of climate change.

MATERALS AND METHODS

Collection- *Geum radiatum* is a federally and state listed endangered species that is under the protection of multiple government agencies. The proper Untied States Fish and Wildlife endangered species permit was obtained under USFW agent Dale Suiter's permit (TE178876-1). A North Carolina Department of Agriculture's Plant Conservation Program Endangered species permit was also acquired (Permit #355). Site permits for North Carolina State Parks (R14-45), Great Smokey Mountains Nation Park (GRSM-2014-SCI-1184), and North Carolina Game Lands (14-ES00404) were also obtained. On other federal lands collections were made with agency personnel to ensure collection guidelines under our permit agreements, no specific locality data will be provided. All known extant populations of *G. radiatum* were sampled over a two-year period (2014-2016). An individual was determined with at least 25 cm separation between rhizomatous clumps. These clumps

may represent more than a single genetic individual. Ideally, twenty individuals were sampled at each population, except when fewer than 20 individuals were found. In those cases, all individuals found at the site were sampled. Larger populations with over 20 individuals were sampled at random intervals along the cliff face or topography of the population. Only plants that were deemed healthy and large enough to be sampled without long-term damage were collected. A small piece of leaf margin (approximately 100 mm^2) was harvested from each individual for DNA extraction and analysis. Plant tissue samples were stored in silica gel (Sigma-Aldrich 294316) with long-term storage in an -80 C freezer.

DNA Extraction- Dried tissue was disrupted and ground to a fine powder using a micro-pestle and sand. DNA was extracted from the powder using an Invitrogen PureLink Plant Total DNA Purification Kit (Invitrogen, Carlsbad, California, USA) or a Qiagen DNeasy Plant Mini Kit (Qiagen Valencia, California, USA) following the manufacture's protocol. DNA was quantified using a Nano-drop 1000 (Thermo Fisher Scientific, Waltham, MA, USA) and examined for quality using a 1% TBE agarose gel. Low concentration samples were concentrated with a traditional NaOAc and Ethanol precipitation.

Genotyping- DNA was diluted to 20 ng/ul and arrayed into multiple 96 well plates. Each 96 well plate array contained two individual samples chosen as controls that were included on each plate to ensure uniform scoring. Microsatellite markers from closely related species *G. urbanum* and *G. repatans* were tested for transferability to *G. radiatum* (Arens, 2004, Hamann et al., 2014). Five microsatellite markers were selected from *G. urbanum* (WGU5-12, WGU8-1, WGU6-23, WGU6-1, and WFU3-15) (Arens, 2004). Three were

selected from *G. repatans* (003651, 011534, and 14769) (Hamann et al., 2014). Each forward primer was modified to include a 5' M13 tag (5'-CACGACGTTGTAAAACGAC-3') to allow for fluorescent labeling of PCR products using a third primer labeled with FAM, VIC, NED, or PET (Life Technologies, Grand Island, NY, USA) (Schuelke, 2000). PCR reactions were prepared in 10μL volumes consisting of GoTaq Flexi Buffer, 2.5 mM $MgCl_2$, 800 μM dNTPs, 0.5 μM of reverse primer, 0.25 μM of tagged forward primer, 0.25 μM of a M13 fluorescent labeled primer, 0.5 units of GoTaq Flexi DNA Polymerase, and ~20ng of DNA (Promega, Madison, Wisconsin, USA). PCR was completed using a touchdown thermal cycling program on an Eppendorf Mastercyler thermal cycler (Eppendorf, Hauppauge, NY, USA). Initial denaturation was at 94 ˚C for 5 minutes, followed by 13 cycles at 94 ˚C for 45 seconds, 68˚C for 2 minute descending 1 ˚C in temperature per cycle, and 72 ˚C for 1 minute. These were then followed by 25 cycles at 94 ˚C for 45 seconds, 55˚C for 1 minute, and 72 ˚C for 1 minute, and a final extension of 72 ˚C for 5 minutes. Different fluorescently tagged PCR products from the same individual were combined to pseudo-multiplex 4 markers that were added to HI-DI (Applied Biosystems, Foster City, California, USA) with a GeneScan Liz 500 size standard (Applied Biosystems). Samples were shipped to Georgia Genomics (UGA, Athens, Georgia, USA) and were separated using an ABI 3730 Sequencer (Applied Biosystems). The Resulting chromatograms were scored in Geneious 9.1 using the microsatellite plug-in (Biomatters, Auckland, NZ). Individuals were scored with the potential of 6 distinct peaks, as *G. radiatum* is a hexaploid (Gajewski, 1957).

Statistics and Analysis- Basic descriptive statistics, including the number of alleles per locus, number of alleles per locus per population, total number of alleles, and the allelic ranges where calculated in Microsoft Excel (Redmond, Washington, USA).

The allelic frequency was estimated with the commands simpleFreq, which assumes partial heterozygosity, in polysat (version 1.6.0; Clark and Jasieniuk, 2011) in the R statistical language (version 3.3.2; R Core Team, 2016). Genetic distances were calculated using the Bruvo method (Bruvo et al., 2004), Jaccard and Dice similarity index (Lynch, 1990) and Nei's Interpopulation distance (Nei, 1972). These genetic distance tables were visualized with Principal Cordiant Analyses also in polysat. Deviations from Hardy-Weinberg equilibrium were tested in GENODIVE (version 2.0 b27; Meirmans and Van Tienderen, 2004) including total heterozygosity (Ht) and inbreeding coefficients of (Fis) and (Gis). Fixation and population structure where estimated with Wright's Fst statistics (Wright, 1943; 1965), Nei's Gst (Nei, 1973), and Jost's D (Jost, 2008) using polysat.

Population structure was investigated using the Bayesian clustering software STRUCTURE (version 2.3.4; Pritchard et al., 2000; Falush et al., 2003) assuming an admix model. A K-value analysis was run 100,000 times with a burn-in period of 25,000 in replicates of 5 from k = 1 to k = 13. The appropriate K-value was determined using the Evanno method (2005) in STUCTURE HARVESTER (version 0.6.94; Earl and von Holdt, 2012). Bar graphs of genetic clusters were generated using POPHELPER (version 2.2.0; Francis, 2016). A final STRUCTURE analysis was run 5,000,000 times with a burn-in period of 1,000,000 at K = 4. Mapping of ancestral genetic clusters was preformed using POPS R scripts (Jay, 2011).

Hybrid and Augmentation analysis- The populations known to have been augmented with plants from a distant population were tested in GenoDive using the Hybrid index function (version 1.2.3; Gompert and Buerkle; 2009) and a maximum-likelihood hybrid index method with an admix model, where the genotypes of non-augmented, "pure" plants were defined. The "pure" genotypes were identified using STRUCTURE results and prior knowledge of augmented plants nativity. The results of the hybrid index were mapped using the R package maps and mapplots (version 3.1.1; Becker and Wilks, 2016; version 1.5; Hans Gerritsen, 2014).

RESULTS

Genetic Diversity- A total of 141 alleles were identified across 8 microsatellite loci. The number of alleles per locus ranged from 6 to 27, with an average of 17.6 (Table 1). Removing those alleles calculated by the reciprocal of the expected homozygosity, adjusted the effective alleles per locus to range between 1.698 to 4.746, with an average of 2.997. The observed heterozygosity ranged from 0.571 to 0.948 with an average of 0.711 per locus (Table 1).

A total of 310 individuals were genotyped from all 14 of the extant populations of *G. radiatum,* representing the entirety of this species (Fig. 1). The populations are labeled with a number from north to south, to protect the location identification. The number of individuals scored per population ranged from 3 to 81, with an average of 22.143. The small number of individuals in populations 4 and 9 were the result of small population size. The eight-microsatellite markers were scored with an average allelic richness of 6.375, ranging from

2.138 to 9.875 (Table 2). The total number of alleles identified in a single population ranged from 30 in population 5 to 79 in population 8 with a mean of 51. The average number of alleles ranged from 2.571 in population 9 to 9.875 in population 8, and the effective number of alleles ranged from 2.138 in population 9 to 3.611 in population 8 (Table 2). None of the markers were monomorphic for any population. The total corrected heterozygosity was estimated to range from 0.489 to 0.783, with an average of 0.722. The estimated Fis values range from -0.231 to -0.592, with an overall Fis -1.391 across all populations. The estimated Gis values range from -0.199 to 0.104, with an overall Gis -0.046 across all populations (Table 2).

An individual pairwise Bruvo distance and a Jaccard and Dice similarity index were calculated to estimate pairwise genetic distance between individuals. A PCA was preformed using the pairwise table calculated for each distance. The first component of the Bruvo distance explained 21.5% of the variation and the second component explained another 15% of the variation (Fig. 2). The Bruvo distance clustered the northern populations (pop 1 through 5) and the southern populations (pop 10 through 14). A second PCA generated using the Lynch method of calculating genetic distance did not produce any discernable geographic or population clustering (Sup. Fig. 1). A Mantel's test using the Bruvo distance was run to assess the relationship of the genetic and geographic distances between the individuals. The analysis was run with 1000 replicates. The resulting r^2 value was 0.181 ($p = 0.001$).

Fst, Gst, and Jost's D pairwise values were calculated for each of the populations using a partial heterozygote model for inferred allele frequency (Clark and Jasieniuk, 2011). The three testes were run to provide cross validation of the results of each method and all three methods yielded similar results. The ranges for the Fst values are 0.011 to 0.022 (Sup.

Table 1). The Gst values ranged from 0.006 to 0.253 (Sup. Table 2). The Jost's D values ranged from 0.034 to 0.699 (Sup. Table 3). The population size had an effect on all three of the population differentiation values. The very small size of population 4 and 9 seems to influence the effect of increasing estimated fixation.

Population Structure- Analysis in the Bayesian clustering program STRUCTURE using the Evanno method strongly suggest 4 clusters (K=4) but also showed support for 2 clusters (K=2) and 7 clusters (K=7) (Fig. 3). The K = 7 analysis made little biological sense and the output had most individuals heavily admixed between multiple genetic clusters; it also has the weakest delta K among punitive K values calculated. As K value increases, southern populations are sub-divided into unique genetic clusters (Fig. 4). Maps inferring the ancestor coefficients from current population locations and genetic clusters were made for both STRUCTRE outputs K=2 (Supp. Fig. 2) and K=4 (Fig. 5). Population 8 had the largest number of samples collected (N = 81), because of it's known history of past augmentations. This population contained individuals of all genetic clusters and was further analyzed to identify hybrids (Fig. 4 and below).

Hybrid and Augmentation analysis- The STRUCTURE analysis and knowledge about past augmentations suggest the potential for hybrids within population 8. Those areas were sampled more thoroughly to attempt to elucidate the effects of these augmentations. The STRUCTURE analysis suggested potential hybrids between augmented plants and native plants (Fig. 4). Suspected augmented individuals were removed and the ancestor coefficients were remapped to show the effect on genotypes (Fig. 6). The only discernable

change was the increases in intensity of the clusters within population 8. To further test those individuals a hybrid index was run on all of population 8 with 4 potentially augmented sub-populations. The source populations for the augmented plants were known to include population 1 and an isolated sub-population (8e) of pop 8 was known to not be augmented. This analysis revealed 14 augmented plants with strong affinity for population 1, the original source population. A hybrid index also suggested 12 individuals consistent with hybrid origin (Table 3). These results were mapped showing the geographic relationships of the hybrids, augmented and native plants within population 8 (Fig. 7)

DISCUSSION

Geum raditaum has a complicated genetic structure due to its polyploidy, past conservation action, restrictive habitat requirements, and a natural history that includes widespread past alpine and tundra habitat. The Amphibolite Mountains Macrosite (AMM) populations (1-5) and population 8, due to various factors, each have unique genetic structure and different levels of connectivity across geographic space. The AMM populations have little differentiation among them, even over multiple mountain tops, while population 8 now, in part due to past augmentations, represents the most diverse population and contains all the genetic groups that where identified by the STRUCTUE analysis. The augmentations of population 8 have survived for ~24 years and yielded punitive hybrid descendants of mixed origin.

Genetic Diversity- Based on the eight microsatellite markers used in this study *G. radiatum* has considerably higher genetic diversity than previously reported based on limited allozyme data (Godt et al., 1996). This is likely due to the higher mutation rate of microsatellite loci, which have been shown experimentally to contain significantly more diversity then allozyme loci (Estoup et al., 1998). *Geum radiatum* is also a stable hexaploid (2N=6X=42), which could also influence the amount of diversity maintained by neutral (microsatellite) vs coding (allozyme) loci especially when inbreeding is occurring. It has been shown that polyploids lose coding sites more quickly than non-coding sites (Liu et al., 1998). In conjunction with its long life span (Ulrey et al., 2016) a single genetic individual can survive indefinitely via clonality allowing populations to maintain higher diversity at neutral loci. The number of effective alleles as calculated by GENODIVE was low in comparison to number of alleles with an average of 3 effective alleles per locus with many alleles only being found in low frequency, likely due to mutation in long-lived individuals.

The heterozygosity statistics and measures of Hardy-Weinberg equilibrium must be interpreted carefully in *G. radiatum* because of its polyploid nature. A heterozygous locus should be more common in a hexaploid than in a diploid species. It should also take more extended periods of inbreeding to create homozygous individuals where all six genomic locations carry the same allele. Therefore the high heterozygosity values reported in *G. radiatum* are likely a product of its ploidy state and possibly overestimate the true genetic diversity. Given the high observed heterozygosities, it is surprising that all populations have negative inbreeding coefficients for both Fis and Gis (Table 2), except for population 9 with a positive Gis, likely caused by its small population size (N=3). Our results suggest there is

diversity both within and between populations of *G. radiatum,* and that the species has not been reduced to a handful of genetic clones.

Genetic Structure- Different genetic tests across the extant populations of *G. radiatum* show that populations are differentiated from each other, but the structure changes from north to south across the range. Population differentiation was likely caused by the lack of long distance gene flow between the high elevation rock outcrop habitats, where extant populations are located, and the past geographic and genetic structure. Populations in the southern end of the range seem to contain strong genetic clusters with very little mixing of genotypes, a likely product of reduced geneflow and genetic drift. Seedling recruitment in many populations has been reported to be very low (Ulrey et al., 2016) and the time needed to integrate genotypic groups with such low seedling recruitment might not have been able to occur in the southern and most climatically stressed populations. The populations in the north of the range are very interconnected most likely because of gene flow between close mountaintops limiting genetic drift.

The genetic structure of the extant populations of *G. radiatum* are geographically divided generally into a northern AMM group, a central group between Boone, NC and Asheville NC, and a southern group south of Asheville, NC. There tends to be more diversity and overlap of different genetic clusters within a single population in the most southern populations and population 8, suggesting incomplete mixing of genotypes within the most stressed populations (Fig. 1). The northern AMM populations (1-5) consistently show the strongest relationship with genetic distance, Bayesian clustering, and Fst values reliably grouping these populations together. The southern and middle parts of the range (populations

6-14) contain different genetic clusters and greater differentiation between populations when compared to the northern AMM populations. These populations also tended to cluster in more complex ways with an east, west, and central breakdown (see below), but were always differentiated from the northern populations, except for the augmented/hybrid plants in population 8.

The Bruvo genetic distance clustered the northern populations together, illustrated by the circle (Fig. 2). The southern populations and those natural subpopulations of population 8 also clustered. Population 8 also has the largest spread of ordination of any population, suggesting it contains the greatest diversity. The PCA generated using these distances provides some ability to visualize groupings, but has significant overlap in the ranges of different populations. The Mantel's test, with a low r^2 value, suggests that the genetic diversity is not correlated within a simple linear geographic relationship, indicating a more complex isolation pattern than isolation by distance alone. In some cases, for example population 11 and 10, found in close geographic proximity, have distinctly different genotypes with strong affinities to populations at greater distances (Fig. 3).

Pairwise Fst, Gst, and Jost's D were generated for each population. Each approach at measuring population differentiation follows similar trends, where the strongest relationships seems to be between populations that fall into the same geographic group. Again the closest geographic populations do not always seem to be the most related, supporting the results shown by the Bruvo genetic distance approach and the Mantel's test (Fig. 2). One of the most notable treads in the data is how northern populations fall out as differentiated from the southern populations and middle populations with the exception of population 8, which has been augmented with plants from the north. Based on Fst values, the northern AMM

populations appear to be the most connected by gene flow. For example, Population 2 and 3 have pairwise values of over 0.1 and 0.095 respectively for every population south of population 6, but less than 0.1 and 0.09 for other northern AMM populations with as low as 0.011 when compared to each other while the average Fst is 0.07. The Fst values reiterate that the southern populations seem to be more differentiated from each other than the northern AMM populations, suggesting that there is more natural gene flow between the close peaks of the northern part of the range.

The northern AMM populations (1-5) have Fst values that are comparable to population 8's sub-population Fst values. The Fst values range in population 8 from 0.019 to 0.097, with a mean of 0.045 as compared to the northern AMM populations values, which range from 0.011 to 0.092 with a mean of 0.048 (Supp. Table 4). The northern AMM populations are approximately as interconnected via gene flow as the sub-populations of 8, with similar levels of differentiation. If population 8 is to be considered one population, than perhaps the northern AMM populations should genetically be described as a single population as well.

The Bayesian STRUCTURE analysis and Evanno correction's delta k values suggest 2, 4, or 7 genetic clusters. The k = 2 analysis fuses two clusters into one and therefore represents less of the diversity than k = 4 (Fig. 2), which is the more strongly supported K value. The results of the K= 4 indicates that the northern populations are the most differentiated and isolated populations and the STRUCTURE analysis clusters them into (blue) similar groups to the hierarchical Fst pairings and the Bruvno distance PCA groups (Fig. 4). The middle populations (6, 7, 9, 10, and 11) cluster and contain three different genetic groups (red, green, and purple). The southern populations (12-14) cluster and contain

two different genetic groups (red and purple). A map of the ancestor coefficients from the STURCTURE analysis suggests the affinities of these groups have a geographic origin (Fig. 1). The blue cluster is strongly associated with the northern populations and is only found within the northern AMM populations with the exception of the augmented plants within population 8. The red cluster has a central and southern affinity and may represent the ancestral high peak genetic group. The purple cluster has a western affinity and the green cluster has an eastern affinity, each becoming more common within populations moving father west or east. These ancestral coefficients have even stronger geographic affinities, when the augmented plants in population 8 are removed from the analysis (Fig. 5). These four different genetic clusters may represent ancient genetic partitioning from the Pleistocene when true above timberline alpine communities and permafrost existed more commonly in the Southern Appalachians (Delcourt and Delcourt, 1998).

The directional affinities of the genetic clusters may represent the remnants of diversity from a time when *G. radiatum* was more widespread with multiple large interbreeding populations. At the end of the Pleistocene, when the earth's climate was warming, the populations of *G. radiatum* may have retreated up mountains to the tops of the highest peaks where they became stranded on cliff faces. In the Southern Appalachians there is a fairly narrow band of peaks that reach over 1500 m, which is widest south of Asheville, NC where the Smokey Mountains and the Balsam Mountain have multiple high peaks around the same latitude. The width of the high Appalachian peaks narrows to a single mountain in Northwest NC. The population differentiation seen today may be a geographical condensed relic of past population structure where distinct genetic populations that were once

geographically separated by great distance retreated into the only remaining suitable habitat and are now close neighbors with their once distant relatives.

Hybrid Analysis- Subpopulation 8e from population 8 was thought to be free of augmented plants and did not contain any admixed genotypes or members from northern populations in the STRUCTURE analysis. These individuals were used as the native genotype for the hybrid index in GENODIVE. The individuals from population 1 were used for augmentation and therefore the source for the other parental genotype in the hybrid index analysis. This analysis suggested more punitive hybrids found among the subpopulations that were augmented (Fig. 7). These hybrids represent a unique artificial gene flow event that has increased the diversity within population 8 and lowered population 8's pairwise differentiation. This event also serendipitously captured all of the identified genetic clusters identified in this analysis, perhaps cementing the importance of population 8 to the conservation of *G. radiatum.*

The analyses conducted with STRUCTURE and GENODIVE reveals that past augmentations of 4 sub-populations within population 8 were successful and that F1 hybrids have been formed. This demonstrates that artificial gene flow by augmentation can be a successful strategy for conservation. In the early 1990s, augmented plants were added to various subpopulations of population 8 that had been substantially damaged from past human recreation (Fig. 1). The unique history of augmentation of Population 8 has lowered the average Fst values when compared to every other population of *G. radiatum* (Supp. Table 1). This is also shown in the STRUCTURE analysis where augmented plants from the northern (blue) cluster were added to population 8. Population 8 has the least consistent population

assignment of any of the STRUCTURE clusters and the least clustering in the Bruvo genetic distance PCA. This likely makes it the most important population for future conservation efforts, with the highest amount of diversity and the least differentiation from other population.

Conservation implication- *Geum radiatum* contains genetic diversity and population structure; preserving and protecting every single extant individual is the most reliable strategy to maintain diversity in the species as a whole. *Geum radiatum* is long lived and has a rhizomatous growth pattern but has exceptionally low seedling recruitment especially in the more southern populations and imperiled populations. Considering these factors, the loss of a single adult *G. radiatum* plant may take hundreds of years to regenerate, especially in the face of modern climate change, and the loss of single plant could mean the loss of a unique genetic make-up for that population or the species as a whole. The average population size of *G. radiatum* is very low, containing multiple different genotypes, where each individual may represent millennia of evolution. *Geum radiatum* as a species still has many different genetically unique individuals and has not yet been reduced to a handful of clones. To ensure long-term species survival, this genetic diversity should be maintained by continuing current protections and strictly limiting recreational development around populations.

If the end goal of conservation strategies is to increase local genetic diversity, then the past augmentations of *G. radiatum* have been successful. The authors highlight that the risk of augmenting populations with unknown genotypes can result in outbreeding depressions by breaking locally adapted traits (Storfer, 1999) or the introduction of non-hybridizing invasive genotype (Montalvo et al., 2007), can make genetic augmentation a

risky conservation action. The augmentations conducted in the early 1990s increased diversity but it is still unknown if they increased the chances of the long-term survival of the species. The geographic location, size of the population and sub-populations has generated a complex genetic structure within population 8, and the artificial gene flow event may have just finished the processes of geographic condensation to higher peaks. *Geum radiatum* has artificially migrated to a higher elevational point, which has been occurring naturally and may be beneficial to the long-term genetic survival of population 8 and the species as a whole as the earth warms.

The northern populations of the AMM in Northwest NC are interconnected and have as little differentiation as the sub populations of population 8. The AMM populations genetically are interbreeding and should perhaps be considered a single population. This should be done with caution, as it would reduce the known populations of this federally listed species to 10. The two most important sites genetically for the conservation of *G. radiatum* are the AMM populations (1-5) that are genetically unique and are interbreeding between mountain tops and population 8 which has five sub-populations and now contains the best overall representation of the genetic diversity within *G. radiatum* containing all 4 of the genetic clusters present in the species.

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Tables

Table 1. Descriptive statistics of the loci across all populations.

Locus	Author	N	A	Ae
003651	Hamann et al.	227	27	3.286
14769	Hamann et al.	219	6	1.934
WGU5-12	Arens et al.	272	16	4.746
WGU6-1	Arens et al.	290	15	2.622
WGU6-23	Arens et al.	307	22	3.574
WGU3-15	Arens et al.	214	24	2.999
011534	Hamann et al.	264	16	1.698
WGU8-1	Arens et al.	280	15	3.114
Mean		259.1	17.6	2.997

Note: N = the total number of genotyped individuals at each loci, A = the total number of alleles at all loci, Ae = the effective number of alleles at all loci calculated by reciprocal of the expected homozygosity.

Table 2. Genetic diversity of *Geum radiatum* as revealed by eight microsatellite markers.

Population	N	A	Rs	Ae	Ho	Fis	Gis
Population 1	20	51	6.375	3.436	0.675	-0.343	-0.038
Population 2	20	56	6.375	2.638	0.597	-0.41	-0.199
Population 3	19	61	7.625	2.873	0.605	-0.356	-0.127
Population 4	7	37	4.625	3.394	0.783	-0.39	0.104
Population 5	19	30	7	3.611	0.687	-0.316	0.051
Population 6	18	42	5.25	3.104	0.608	-0.397	-0.009
Population 7	14	41	5.125	2.924	0.628	-0.431	-0.097
Population 8	81	79	9.875	3.372	0.645	-0.404	-0.036
Population 9	3	19	2.571	2.138	0.685	-0.231	0.1
Population 10	12	34	4.25	2.467	0.537	-0.543	-0.107
Population 11	18	48	5.75	3.171	0.665	-0.382	-0.041
Population 12	23	38	4.75	2.709	0.573	-0.517	-0.135
Population 13	27	39	4.875	2.487	0.489	-0.472	-0.178
Population 14	29	39	4.875	2.744	0.597	-0.592	-0.101
Mean	22.1	51	17.25	2.661	0.635	-1.391	-0.046

Note: N = number of individuals genotyped per population, A = number alleles per population, Rs = allelic richness per population, Ae = effective number of alleles per population, Ho = observed heterozygosity per population, Fis = Wright's inbreeding coefficient, Gis = Nei's inbreeding coefficient

Table 3. Hybrid individuals identified in GENODIVE

Sub-population	h-value
8a	0.524
8a	0.51
8a	0.473
8a	0.428
8a	0.444
8b	0.571
8b	0.455
8c	0.59
8c	0.57
8c	0.513
8d	0.537
8d	0.486
8d	0.595

Note: h-vaule = percent identity that the individual shares with subpopulation 8e

Supplemental Table 1. Pairwise Fst values

	Pop 4	Pop 11	Pop 14	Pop 6	Pop 13	Pop 12	Pop 7	Pop 10	Pop 3	Pop 1	Pop 9	Pop 8	Pop 5	Pop 2
Pop 4		0.051	0.077	0.076	0.094	0.084	0.062	0.070	0.079	0.047	0.126	0.023	0.048	0.092
Pop 11	0.051		0.072	0.060	0.099	0.087	0.034	0.071	0.087	0.050	0.059	0.031	0.059	0.103
Pop 14	0.077	0.072		0.063	0.118	0.099	0.096	0.097	0.105	0.086	0.074	0.045	0.093	0.122
Pop 6	0.076	0.060	0.063		0.092	0.059	0.075	0.082	0.052	0.041	0.094	0.012	0.051	0.062
Pop 13	0.094	0.099	0.118	0.092		0.048	0.093	0.049	0.114	0.081	0.100	0.042	0.101	0.123
Pop 12	0.084	0.087	0.099	0.059	0.048		0.073	0.068	0.099	0.073	0.077	0.030	0.071	0.107
Pop 7	0.062	0.034	0.096	0.075	0.093	0.073		0.063	0.096	0.055	0.056	0.028	0.043	0.110
Pop 10	0.070	0.071	0.097	0.082	0.049	0.068	0.063		0.101	0.055	0.119	0.024	0.067	0.115
Pop 3	0.079	0.087	0.105	0.052	0.114	0.099	0.096	0.101		0.022	0.107	0.040	0.052	0.011
Pop 1	0.047	0.050	0.086	0.041	0.081	0.073	0.055	0.055	0.022		0.076	0.028	0.036	0.029
Pop 9	0.126	0.059	0.074	0.094	0.100	0.077	0.056	0.119	0.107	0.076		0.024	0.057	0.112
Pop 8	0.023	0.031	0.045	0.012	0.042	0.030	0.028	0.024	0.040	0.028	0.024		0.026	0.050
Pop 5	0.048	0.059	0.093	0.051	0.101	0.071	0.043	0.067	0.052	0.036	0.057	0.026		0.061
Pop 2	0.092	0.103	0.122	0.062	0.123	0.107	0.110	0.115	0.011	0.029	0.112	0.050	0.061	

Note: Pop =Population

Supplemental Table 2. Pairwise Gst values

	Pop 4	Pop 11	Pop 14	Pop 6	Pop 13	Pop 12	Pop 7	Pop 10	Pop 3	Pop 1	Pop 9	Pop 8	Pop 5	Pop 2
Pop 4		0.052	0.107	0.084	0.125	0.101	0.058	0.067	0.086	0.051	0.141	0.062	0.052	0.102
Pop 11	0.052		0.073	0.059	0.109	0.088	0.027	0.074	0.082	0.048	0.111	0.049	0.056	0.098
Pop 14	0.107	0.073		0.060	0.128	0.096	0.100	0.119	0.100	0.083	0.194	0.053	0.090	0.116
Pop 6	0.084	0.059	0.060		0.088	0.050	0.069	0.074	0.044	0.037	0.177	0.017	0.044	0.052
Pop 13	0.125	0.109	0.128	0.088		0.048	0.112	0.044	0.105	0.081	0.247	0.055	0.100	0.114
Pop 12	0.101	0.088	0.096	0.050	0.048		0.077	0.067	0.085	0.067	0.172	0.036	0.065	0.091
Pop 7	0.058	0.027	0.100	0.069	0.112	0.077		0.065	0.090	0.050	0.083	0.051	0.039	0.105
Pop 10	0.067	0.074	0.119	0.074	0.044	0.067	0.065		0.090	0.050	0.170	0.044	0.062	0.106
Pop 3	0.086	0.082	0.100	0.044	0.105	0.085	0.090	0.090		0.018	0.210	0.051	0.044	0.006
Pop 1	0.051	0.048	0.083	0.037	0.081	0.067	0.050	0.050	0.018		0.160	0.035	0.027	0.023
Pop 9	0.141	0.111	0.194	0.177	0.247	0.172	0.083	0.170	0.210	0.160		0.151	0.120	0.223
Pop 8	0.062	0.049	0.053	0.017	0.055	0.036	0.051	0.044	0.051	0.035	0.151		0.033	0.062
Pop 5	0.052	0.056	0.090	0.044	0.100	0.065	0.039	0.062	0.044	0.027	0.120	0.033		0.051
Pop 2	0.102	0.098	0.116	0.052	0.114	0.091	0.105	0.106	0.006	0.023	0.223	0.062	0.051	

Note: Pop =Population

Supplemental Table 3. Pairwise Jost's D values

	Pop 4	Pop 11	Pop 14	Pop 6	Pop 13	Pop 12	Pop 7	Pop 10	Pop 3	Pop 1	Pop 9	Pop 8	Pop 5	Pop 2
Pop 4		0.271	0.451	0.337	0.441	0.399	0.279	0.218	0.419	0.266	0.424	0.276	0.264	0.509
Pop 11	0.271		0.263	0.215	0.315	0.299	0.159	0.240	0.395	0.224	0.330	0.180	0.280	0.452
Pop 14	0.451	0.263		0.220	0.282	0.292	0.382	0.301	0.441	0.348	0.535	0.194	0.387	0.488
Pop 6	0.337	0.215	0.220		0.272	0.188	0.287	0.251	0.226	0.170	0.498	0.074	0.240	0.252
Pop 13	0.441	0.315	0.282	0.272		0.114	0.284	0.170	0.448	0.333	0.517	0.195	0.368	0.467
Pop 12	0.399	0.299	0.292	0.188	0.114		0.254	0.200	0.414	0.318	0.437	0.171	0.316	0.443
Pop 7	0.279	0.159	0.382	0.287	0.284	0.254		0.167	0.409	0.266	0.221	0.215	0.190	0.476
Pop 10	0.218	0.240	0.301	0.251	0.170	0.200	0.167		0.413	0.250	0.340	0.183	0.240	0.476
Pop 3	0.419	0.395	0.441	0.226	0.448	0.414	0.409	0.413		0.089	0.661	0.295	0.260	0.034
Pop 1	0.266	0.224	0.348	0.170	0.333	0.318	0.266	0.250	0.089		0.499	0.201	0.196	0.117
Pop 9	0.424	0.330	0.535	0.498	0.517	0.437	0.221	0.340	0.661	0.499		0.442	0.346	0.699
Pop 8	0.276	0.180	0.194	0.074	0.195	0.171	0.215	0.183	0.295	0.201	0.442		0.213	0.344
Pop 5	0.264	0.280	0.387	0.240	0.368	0.316	0.190	0.240	0.260	0.196	0.346	0.213		0.294
Pop 2	0.509	0.452	0.488	0.252	0.467	0.443	0.476	0.476	0.034	0.117	0.699	0.344	0.294	

Note: Pop =Population

Suppmental Table 4. Pairwise Fst values for Population 8 subpopulations

	8a	8e	8d	8b	8c
8a		0.041	0.020	0.058	0.097
8e	0.041		0.019	0.022	0.076
8d	0.020	0.019		0.028	0.052
8b	0.058	0.022	0.028		0.039
8c	0.097	0.076	0.052	0.039	

Figures

Figure 1 The geographic distribution of *G. radiatum*. The outline of North Carolina, Tennessee, and Virginia are shown. Populations are labeled with the sample name used in the study to protect the location information.

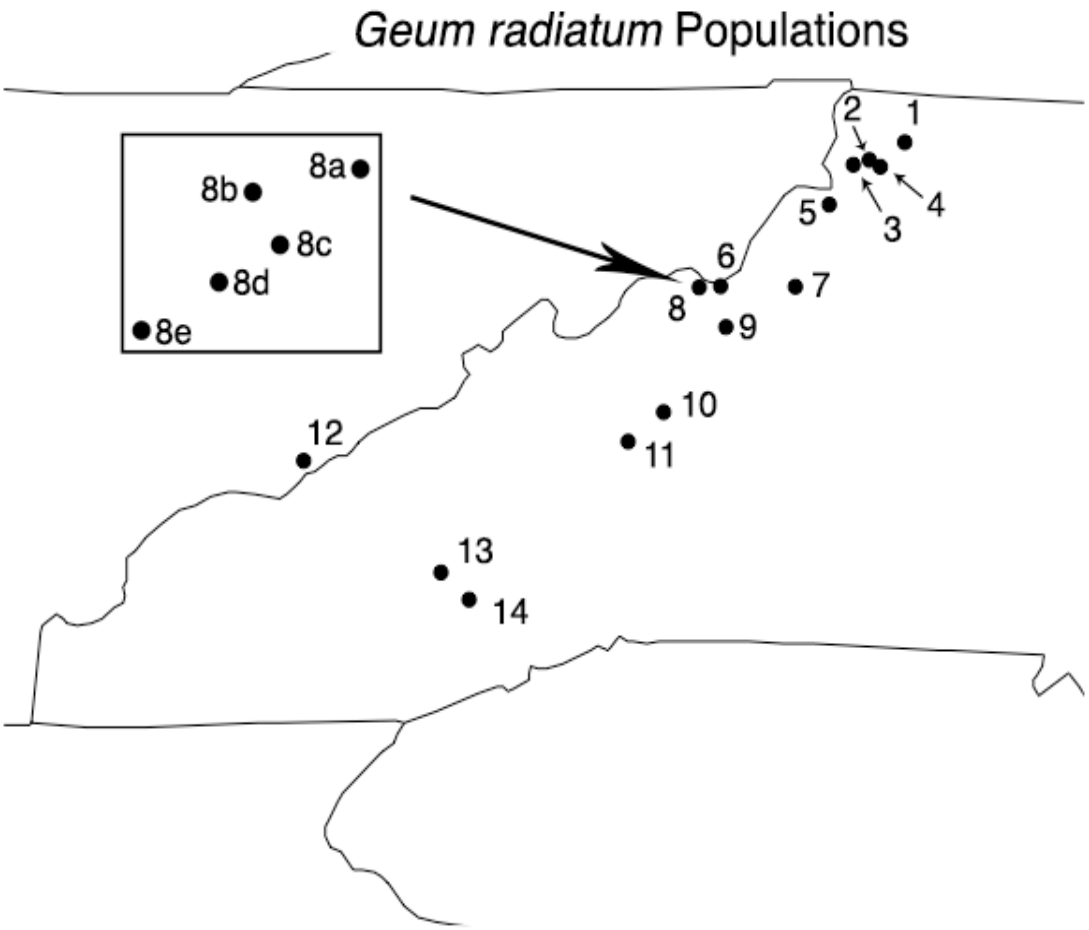


Figure 2 PCA using the Bruvno genetic distance calculation. The light and dark blue individuals highlighted by the circle are members of the AMM. Component 1 explains 21.5% of the variation and component 2 explains 15% of the variation.

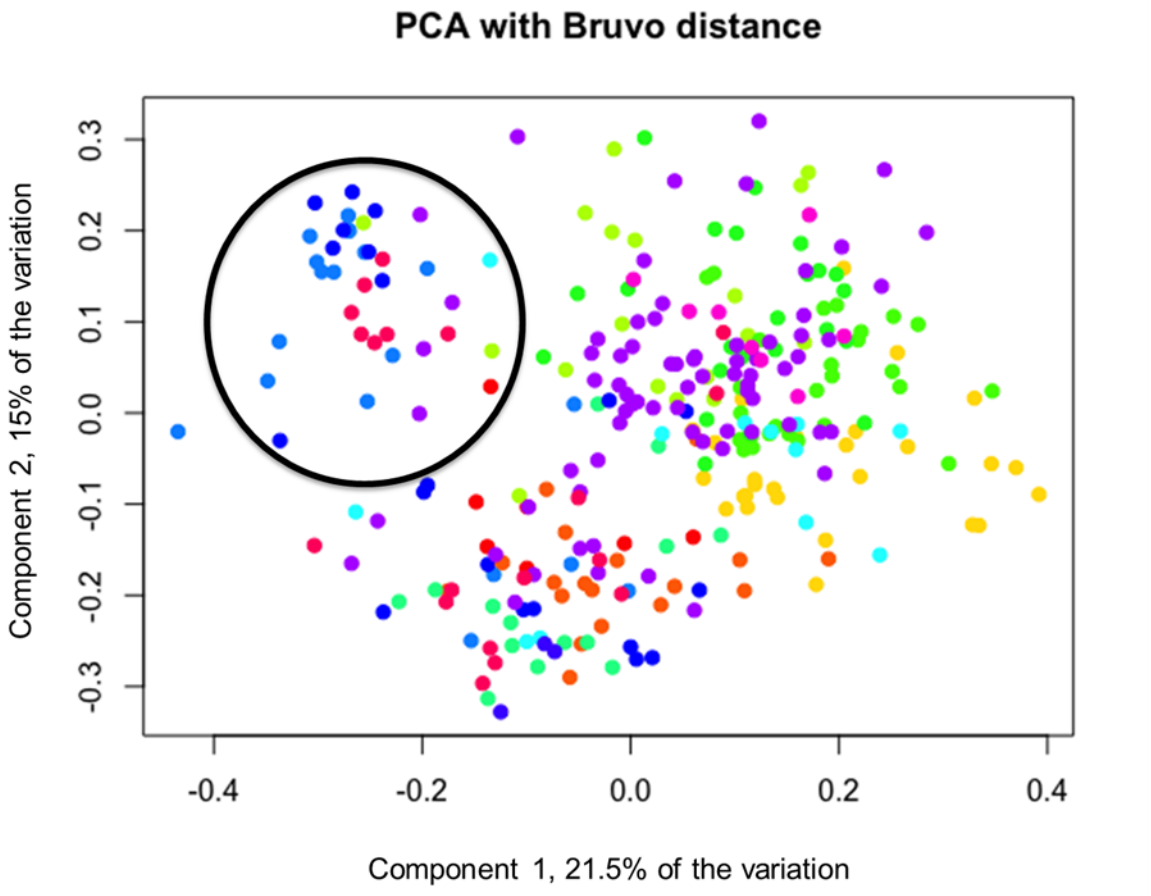


Figure 3 The delta K values for each possible K = 1-13, using the Evanno correction. K = 4 has the strongest support with a delta K of 87.771 and K = 2 has the second strongest support with a delta of 81.796

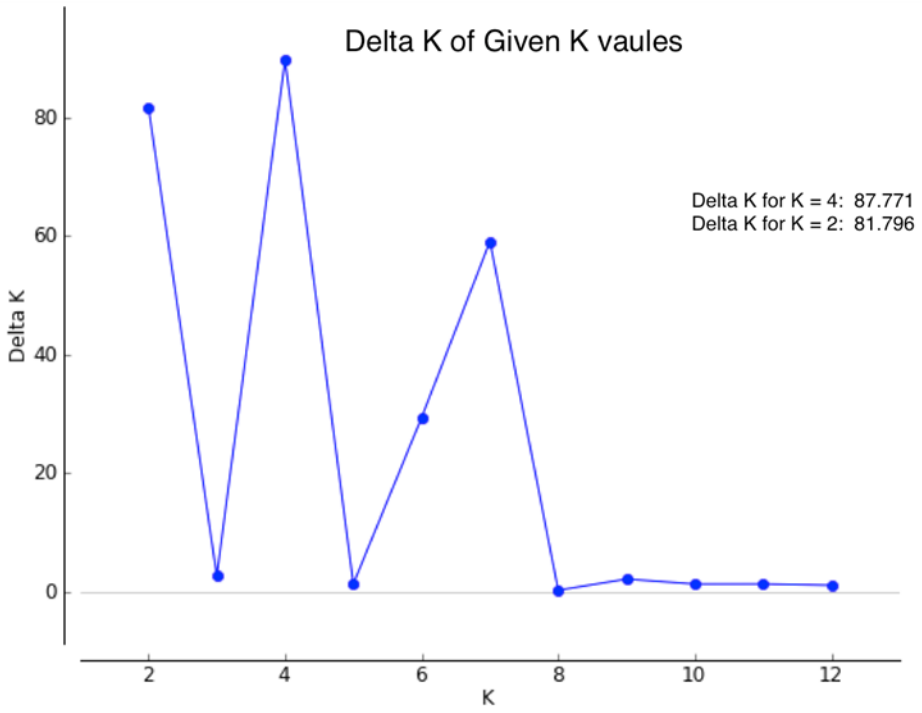


Figure 4 Bar plot output of analysis from STRUCTURE with both K = 2 and K = 4. K = 4 clusters are blue = northern AMM populations, green = eastern affinity cluster, purple = western affinity cluster, and red = central Appalachian high peak cluster. The populations are separated by a white dotted line and are numbered according to the population labels in Fig. 1.

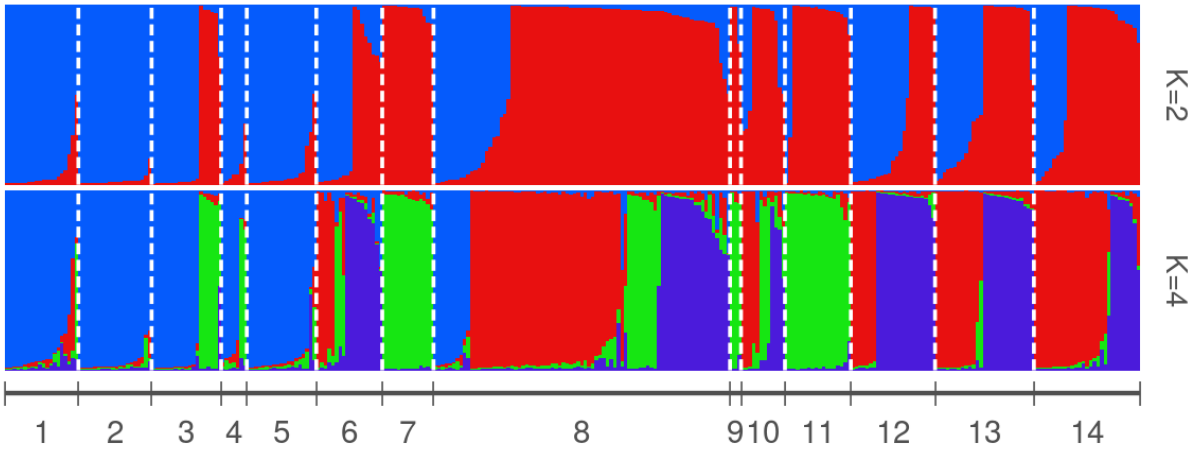


Figure 5 Map geographically displaying the predicted ancestor coefficients from the K = 4 STRUCTURE analysis. The colors correspond to Fig. 4

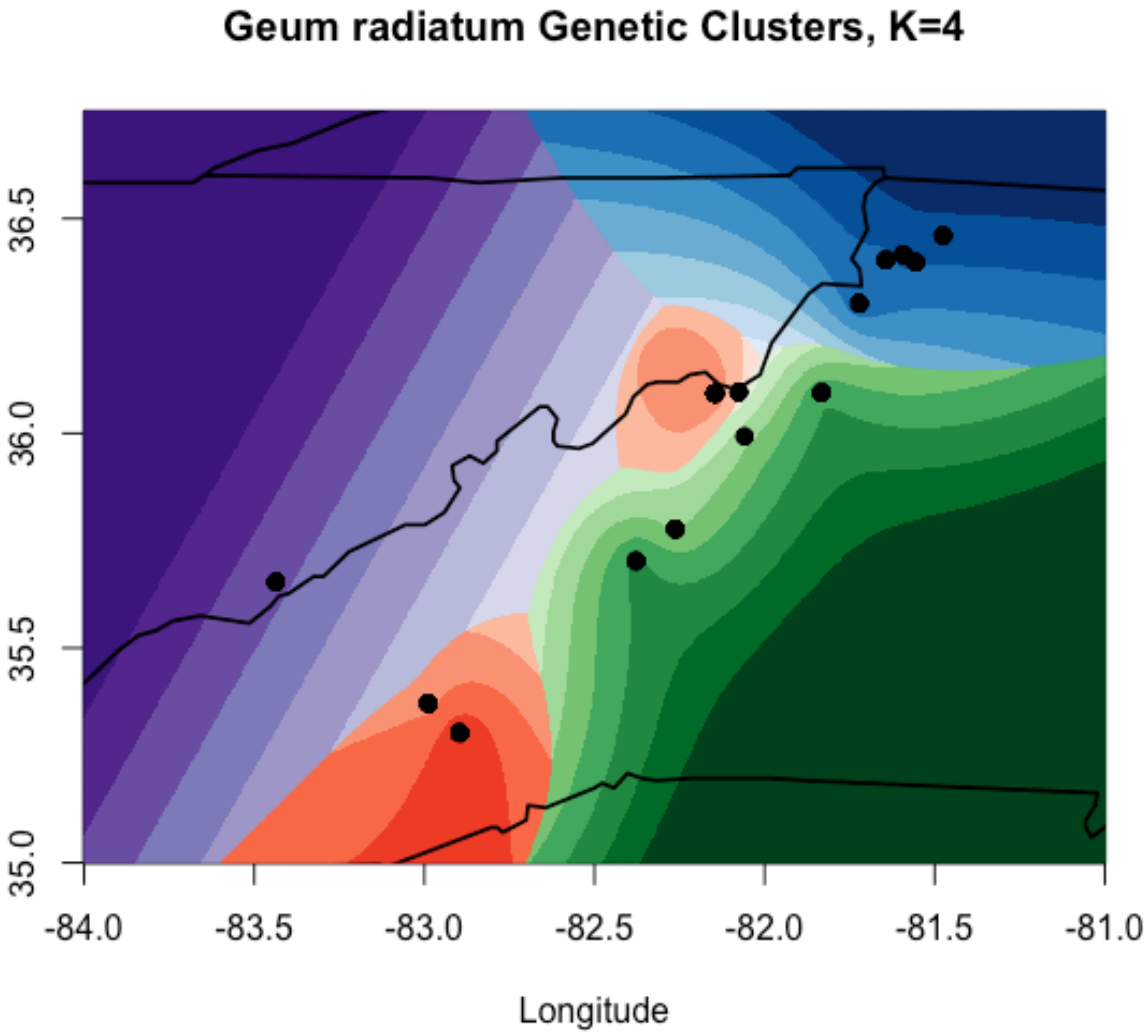


Figure 6 Map geographical displaying the predicted ancestor coefficients from the K = 4 STRUCTURE analysis with the augmented plants from population 8 removed. The colors correspond to Fig. 4.

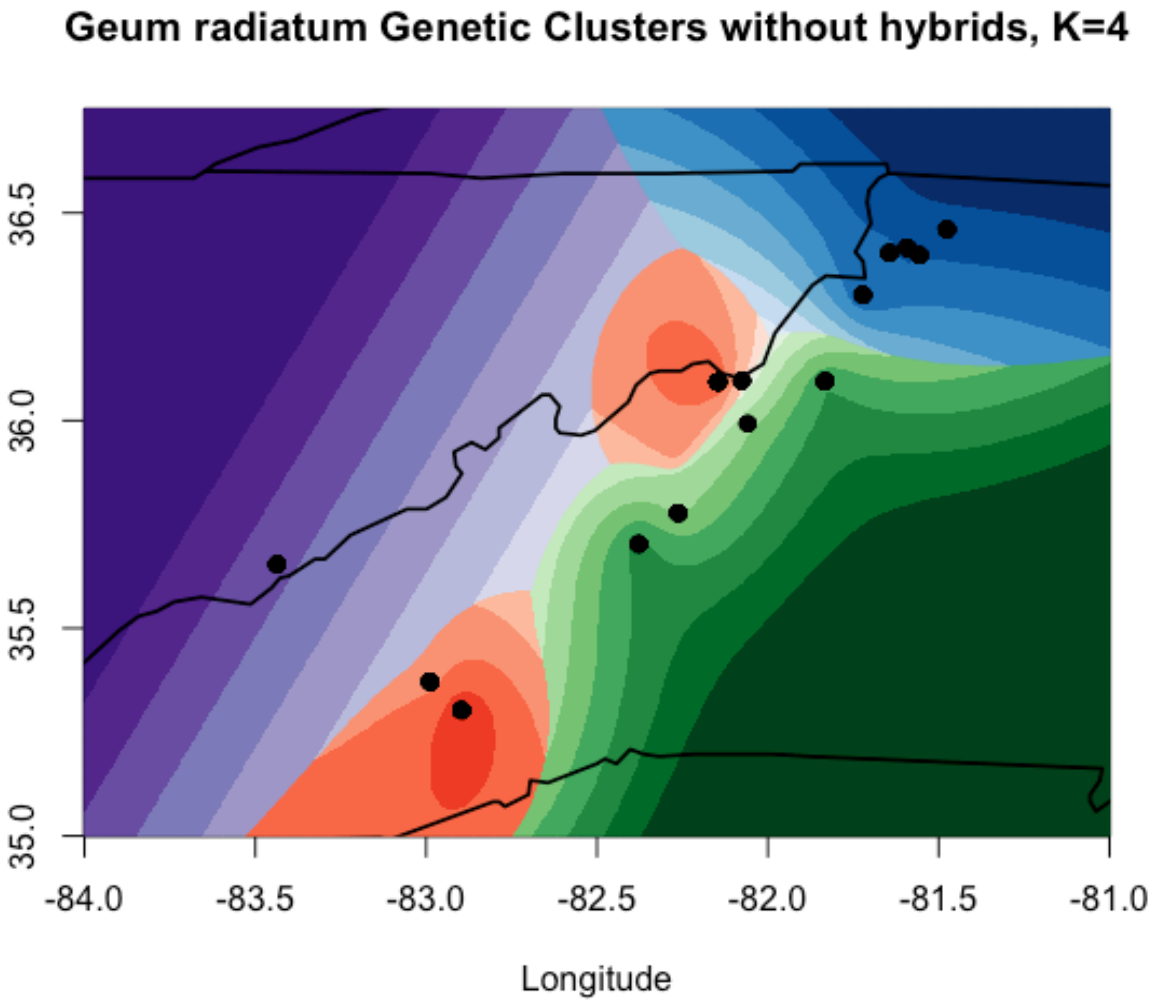
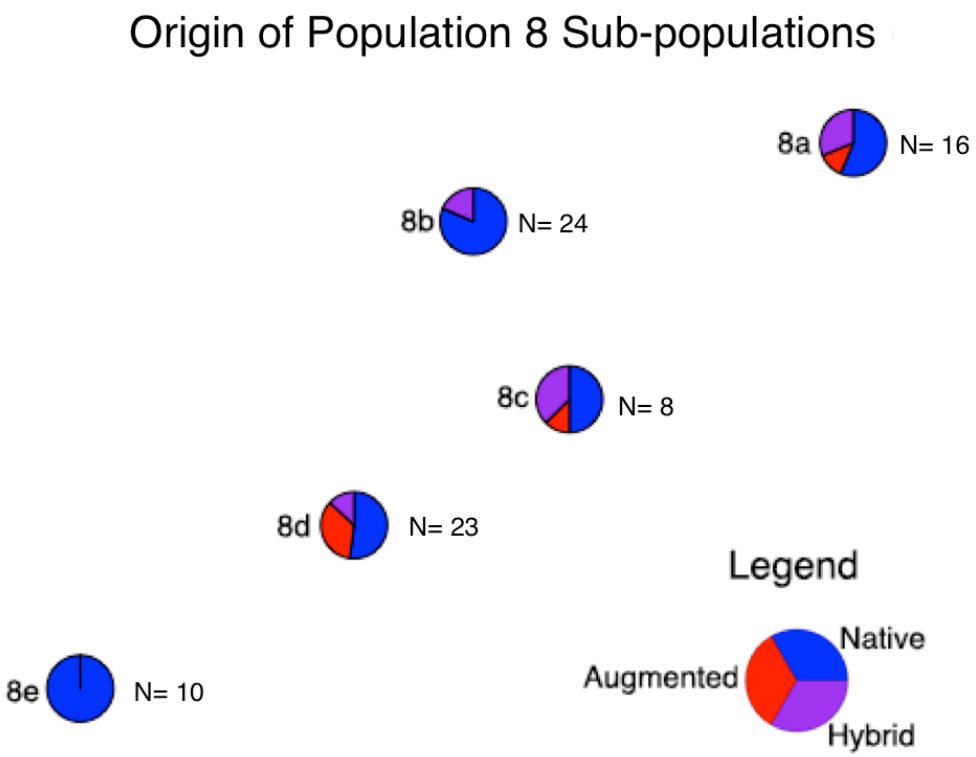
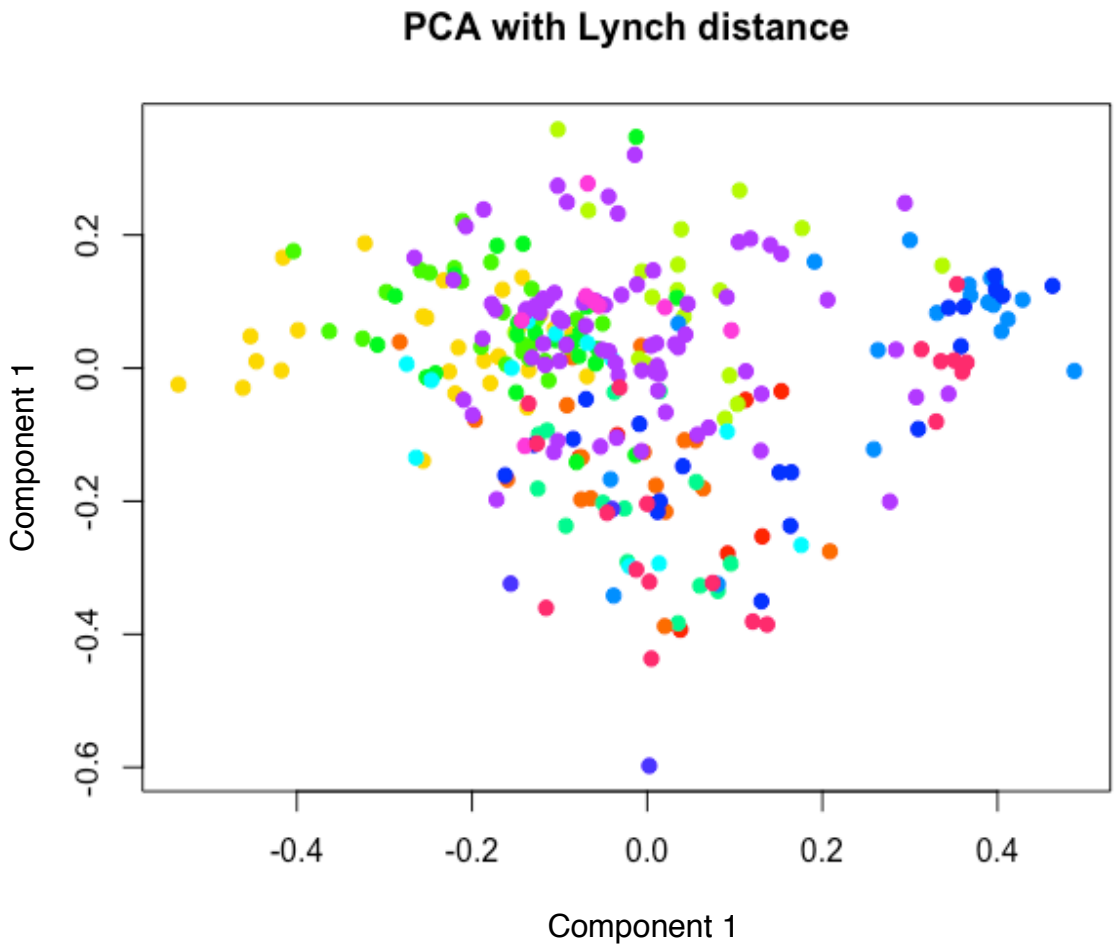


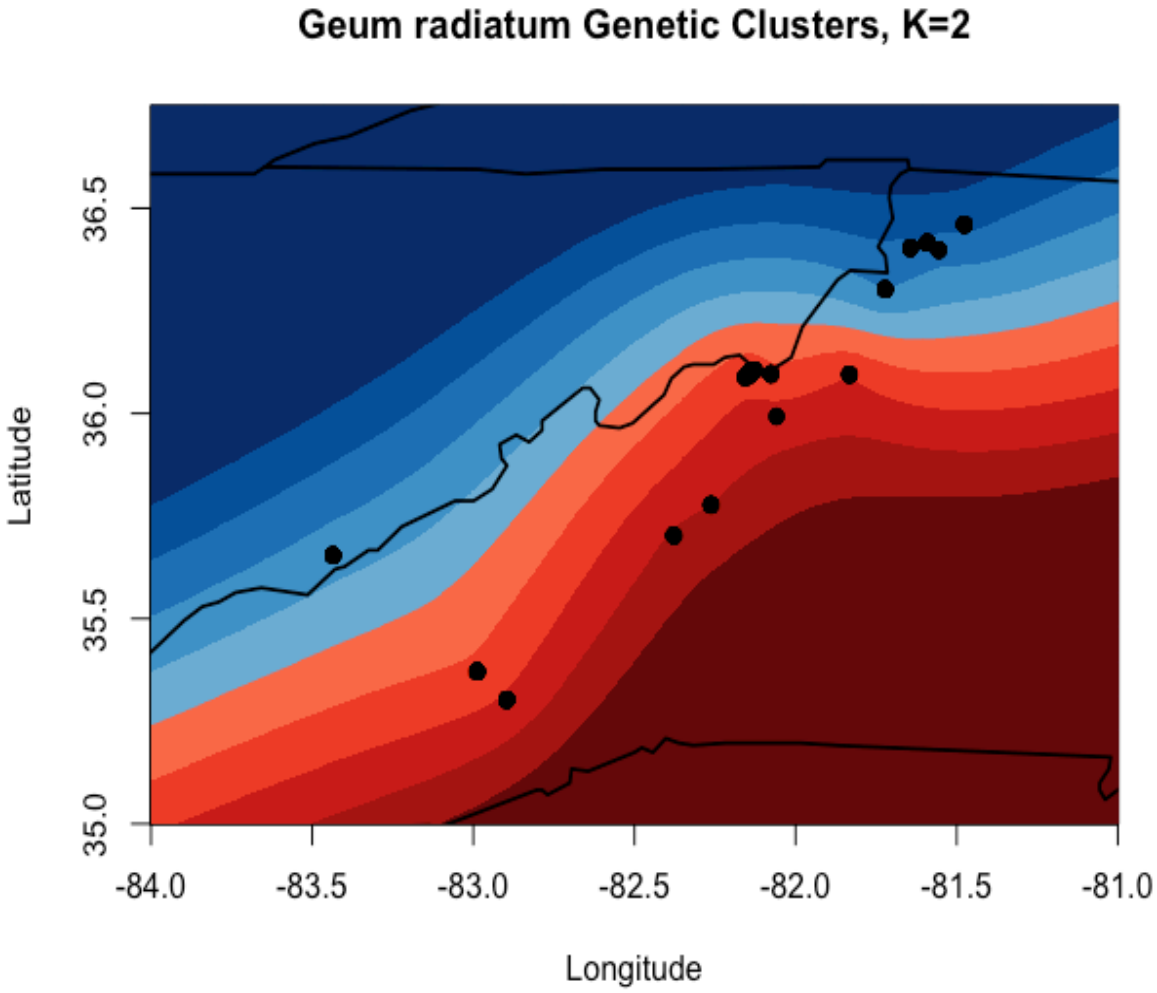
Figure 7 Map of the hybrid, augmented, and native plants within the subpopulations of population 8 as determined by GENODIVE hybrid index. The location of the pie charts is geo-referenced.



Supplemental Figure 1 PCA using the Lynch genetic distance calculation.



Supplemental Figure 2 Map geographically displaying the predicted ancestor coefficients from the K = 2 STRUCTURE analysis. The colors correspond to Fig. 4



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