DETECTING AUGMENTATION IN A METAPOPULATION OF THE FEDERALLY ENDANGERED SOUTHERN APPALACHIAN ENDEMIC *GEUM RADIATUM* MICHX.

A Thesis
by
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Abstract

DETECTING AUGMENTATION IN A METAPOPULATION OF THE FEDERALLY ENDANGERED SOUTHERN APPALACHIAN ENDEMIC *GEUM RADIATUM* MICHX.

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Populations of rare and endemic plants are often small and fragmented, and they face numerous threats to their persistence. Many of these species exhibit lower levels of genetic diversity and are at risk of inbreeding and its downstream consequences. Rapid environmental change and subsequent habitat loss within dispersal ranges challenge conservationists' efforts to ensure species persistence. Managing such species necessitates an approach that incorporates genetic and demographic factors. *Geum radiatum* Michx. (Spreading Avens, Rosaceae) is a rare cliff-dwelling endemic restricted to fifteen fragmented populations above 1500 meters along the North Carolina and Tennessee border. This long-living perennial has been listed as federally endangered since 1990 and is considered imperiled (G2) in North Carolina and critically imperiled (G1) in Tennessee. Due to its restricted range and unique life history, this plant species is at risk of extinction within the century, with anthropogenic climate change and habitat loss exacerbating the threat. Twenty years following augmentation of a *G. radiatum* metapopulation, a population structure and genetic diversity analysis of all *G. radiatum* populations identified putative hybrid offspring of augmented and native plants in the metapopulation. This study

aimed to identify hybrids and detect the genetic structure of the augmentation. Although the study was limited by marker number and quality, it provides insights for future directions in identifying hybrids and later evaluating the impact of this historical augmentation on hybrid fitness.

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Dedication

I dedicate this thesis to all who have walked this scientific journey with me: Thank you for teaching me that the essence of science is not always about reaching the intended destination but about the growth we experience and the knowledge we gain along the way. In the words of Schwartz (2008), "Productive stupidity means being ignorant by choice. Focusing on important questions puts us in the awkward position of being ignorant. One of the beautiful things about science is that it allows us to bumble along, getting it wrong time after time, and feel perfectly fine as long as we learn something each time." Here's to every stumble that makes us better scientists.

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Introduction

Threats to Rare and Endemic Plant Species

Anthropogenic climate change is causing alarming downstream effects on biodiversity, with changes in species distribution, phenology, life history, and interspecific interactions altering ecosystems globally and locally (International Union for Conservation of Nature [IUCN], 2019; Walther, 2010; e.g., Munson & Sher, 2015). Climate change is causing the poleward and elevational shifts of bioclimatic envelopes, and suitable habitat ranges are responding by either shifting in a similar fashion or contracting (Christmas et al., 2016; Felde et al., 2012; Parmesan & Yohe, 2003; Wiens, 2016). Species must follow suitable habitats to avoid local extirpation and global extinction, but studies suggest that wide-reaching extinctions have already started occurring in response to habitat loss within dispersal ranges (Corlett & Westcott, 2013; Silva et al., 2019; Wiens, 2016).

According to a 2020 report by the Royal Botanic Gardens, climate change could lead to the extinction of two in five plant species within the next century (Antonelli et al., 2020). Species must respond by either migrating, exhibiting adaptive phenotypic plasticity, or evolving *in situ*. However, plants are limited by dispersal mechanisms, available habitat, and life history traits (Christmas et al., 2016; Gougherty et al., 2021; Lee-Yaw et al., 2016). Rare and endemic species with fragmented populations are particularly at risk, regardless of taxonomy or geography, and habitat specialization can make them more vulnerable (Chicorro et al., 2019).

Many habitats that harbor rare and endemic species serve as unique diversity hotspots and tend to be infrequent and spatially fragmented (Manes et al., 2021). For example, high-elevation cliffs and rock outcrops act as microrefugia that support local climatic conditions of botanical Pleistocene relicts (Dobrowski, 2011; Larson, 2000; Wiser et al., 1996; Ulrey et al.,

2016). Plants living in these exposed and barren landscapes maintain stable populations with high adult survivorship, relying on that cohort for population maintenance (Larson, 2000; Silva et al., 2016; e.g., Ulrey et al., 2016). Models suggest that longevity and population stability are positively correlated and might have played a role in adapting plants to harsh conditions similar to high-elevation cliffs and rock outcrops (García et al., 2008). These locally adapted traits benefit populations in equilibrium with their environment (Svenning & Sandel, 2013). However, with 84% of mountain species predicted to be at high risk of extinction, the longevity and reliance on adult survivorship might hinder their ability to track shifts in suitable habitat and decouple them from their locally adapted environment (Bellard et al., 2014; Kuparinen et al., 2010; Manes et al., 2021). This pattern is evident in the rare and endemic cliff-dwelling plant *Centaurea corymbosa*, where locally evolved traits are thought to have favored its fragmented cliff habitat while constraining its ability to disperse (Olivieri et al., 2016). However, phenotypic plasticity can produce adaptive responses to environmental changes.

If populations exist along an environmental gradient, selection can gradually couple them with their local environments, pulling them closer to their adaptive peaks. Over time, the genotypes and phenotypes of isolated populations will typically diverge. However, most plant species are phenotypically plastic, allowing them to respond to shifting environmental conditions without an intrinsic change in their genetic structure (Bakhtiari et al., 2019; Palacio-López et al., 2015). Adaptive phenotypic plasticity enables populations to adjust to changing biotic and abiotic factors, thus bringing them closer to optimum performance (Greenspoon & Spencer, 2021; Stotz et al., 2021).

The degree of phenotypic plasticity varies depending on the species, population, developmental stage, and trait (Boyd et al., 2022a). Moreover, phenotypic plasticity is more

strongly favored in populations that inhabit heterogeneous environments and experience variable phenotypic optima (Balaguer et al., 2001; Ogran et al., 2020; Wang et al., 2021). For instance, using a reciprocal transplant design, Baythavong and Stanton (2010) showed that *Erodium cicutarium* individuals from serpentine habitats with variable moisture exposure were more adaptively plastic than non-serpentine genotypes. These differences can lead to a genotype-by-environment interaction, causing mean population phenotypes to diverge and populations to express distinct reaction norms under the same conditions (Bakhtiari et al., 2019).

Rare plants adapted to specialized habitats are known to exhibit maladaptive plastic responses or become canalized and weakly respond to environmental change (Gougherty et al., 2021; Greenspoon & Spencer, 2021). For example, Boyd et al. (2022a) compared the phenotypic plasticity of the rare riparian plant, *Pityopsis ruthii*, to its more widespread and generalized species congener, *P. graminifolia*. They discovered that the rarer species experienced maladaptive plasticity under the varying temperature and light treatments but was more adaptively plastic to the water treatments than the generalist. The researchers hypothesized that these results reflect the specialized riparian environment of *P. ruthii*, in which the species is subject to fluctuating water levels. In a separate study, *Pelliciera rhizophorae*, an endemic mangrove species, was more sensitive to environmental stress than three widespread species in response to changing light and salinity levels (Dangremond, 2015). When exposed to new conditions, species that experience directional natural selection in narrow ranges and specialized habitats are susceptible to weaker phenotypic responses and maladaptation (Boyd et al., 2022b).

Neutral genetic diversity is a good predictor of a population's ability to adapt to environmental change (Chung et al., 2023; but see García-Dorado & Caballero, 2021 and Teixeira & Huber, 2021). Genetic adaptive potential, or a species' intrinsic ability adaptability, is

positively linked to genome-wide diversity (Kardos et al., 2021; Ørsted et al., 2019). Therefore, small and fragmented populations with high genetic loads are typically less able to adapt to changing environmental conditions due to the loss of allelic variation and fixation of harmful recessive alleles (Hedrick & García-Dorado, 2016; Kardos et al., 2021). Although natural selection and genetic purging can reduce the frequency of harmful recessive alleles in persistently small populations, such populations are still prone to the consequences of low genetic diversity (Hoffmann et al., 2021; Lynch et al., 1995; Pérez-Pereira et al., 2022). As genetic drift reduces genetic variation, offspring produced by related parents can experience inbreeding depression or decreased fitness due to breeding among related parents (Bucharova et al., 2019; Charlesworth & Willis, 2009). Furthermore, anthropogenic habitat loss has a more significant impact on genetic diversity in historically fragmented populations than in more recently fragmented populations, compounding the threat of extinction for relict species (Schlaepfer et al., 2018).

Intraspecific Hybridization and Conservation Augmentation

Gene flow can increase genetic diversity and lessen inbreeding depression by counteracting the harmful effects of genetic drift (Bucharova et al., 2019; Charlesworth & Willis, 2009; Richards, 2000). Infusing individuals from a genetically healthy population (i.e., a large population with low genetic load and high genetic diversity) into a small and isolated population can alleviate the effects of genetic load by introducing novel alleles and increasing heterozygosity (Bell et al., 2019). Intraspecific hybridization, or mating between genetically distinct populations, can promote adaptation by reestablishing gene flow and thus aid the persistence of populations (Allendorf et al., 2022; Frankham, 2015; Hoffmann & Sgrò, 2011; Newman & Tallmon, 2001;).

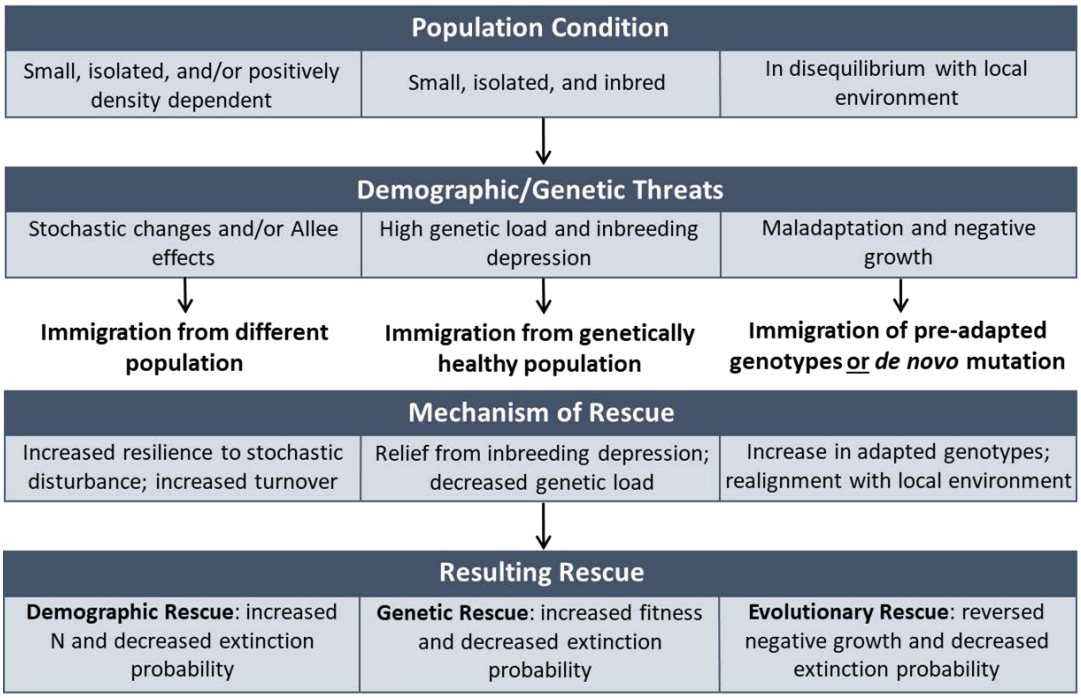
Conservationists face the challenge of preserving plant populations threatened by rapid habitat loss. The speed at which habitats are shifting is faster than the rate at which plant populations can respond, emphasizing the need for informed decision-making and the incorporation of genetic factors. Both migration and human-mediated translocations can lead to intraspecific hybridization. Translocations are intended to decrease a population's risk of extinction (IUCN-Species Survival Commission [SSC], 2013). These translocations may occur where a population was extirpated with a species' indigenous range (population 'restoration) or may involve individuals' movement into an existing population (population 'reinforcement' or 'augmentation') (IUCN-SCN, 2013). Augmentation is risky, as it can result in outbreeding depression and an increased extinction probability (Whitely et al., 2015). Despite its risks, augmentation offers a potential solution for fragmented populations faced with extinction (Bell et al., 2019; Ralls et al., 2017; Tallmon et al., 2017; Waller et al., 2015). This study focuses on population restoration and, specifically, augmentation.

Mechanisms of Population Rescue Via Augmentation

There are three main strategies to achieve the objective of augmentation. Demographic rescue involves increasing the effective population size to make the population more resilient to stochastic changes. Evolutionary rescue aims to reverse negative population growth by initiating adaptive genetic change. Lastly, genetic rescue attempts to counteract the detrimental effects of inbreeding depression on population fitness by introducing novel genetic variation (Figure 1; Rossum et al., 2020).

Figure 1

Different Types of Population Rescue



Note. The table describes the different circumstances that lead to the three types of rescue: demographic rescue, genetic rescue, and evolutionary rescue (adapted from Carlson et al., 2014).

"Rescue Effect" refers to the positive influence of immigration on extinction probability and is now recognized as demographic rescue (DR) (Brown & Kodric-Brown, 1977). Through immigration, DR can reverse negative growth in sink or isolated populations that cannot be sustained without the infusion of individuals or adaptive genetic material. An increase in size can fortify a population against stochastic change and reverse negative growth in positively density-dependent species (Bell et al., 2019; Carlson et al., 2014; e.g., Kramer et al., 2009). However, DR is merely a numerical response often insufficient for long-term population recovery. Other mechanisms are necessary to sustain population recovery, such as those that cause evolutionary and genetic rescue (Carlson et al., 2014).

Assisted gene flow (AGF), or the movement of existing genotypes into a population, can benefit small, isolated populations by counteracting genetic drift (Bell et al., 2019; Grummer et al., 2022). It can also aid locally adapted populations facing rapid environmental changes as they decouple from their environment (Gonzalez et al., 2013; Svenning & Sandel, 2013; Tomasini & Peischl, 2020). Evolutionary rescue (ER) aims to reverse negative population growth following rapid environmental change by introducing pre-adapted genotypes via AGF or *de novo* mutation (Bell & Gonzalez, 2009; Fulgione et al., 2022; Grummer et al., 2022; Hoffmann et al., 2021; Lewis et al., 2023).

Evolutionary Rescue is theoretically characterized by a U-shaped demographic trajectory with three phases (Carson et al., 2014; Gomulkiewicz et al., 1995; Gonzalez et al., 2013; Hufbauer et al., 2015):

Phase 1. Environmental stress and maladaptation cause a decline in population abundance.

Phase 2. The population falls below the minimum size, becoming increasingly susceptible to stochastic change.

Phase 3. Population abundance begins to recover.

Phase 3 corresponds to the increased frequency of adapted phenotype(s) (Carson et al., 2014); However, clear examples of ER in nature are rare due to complex demographic, genetic, and environmental interactions that can hinder evolutionary rescue effects. (Carson et al., 2014; Gonzalez et al., 2013; Tomasini & Peischl, 2020; but see Miller & Vincent, 2008 and Tinghitella, 2008).

Small and isolated populations can also suffer negative growth due to high genetic load and inbreeding depression (Bell et al., 2019). Genetic rescue alleviates genetic load and

inbreeding by infusing genetic variation and increasing heterozygosity (Bell et al., 2019; Carlson et al., 2014; Whiteley et al., 2015). While ER can occur in the absence of AGF if new mutations or standing adaptive genetic diversity leads to adaptation and the re-alignment of a population with its local environment, GR relies explicitly on AGF via intraspecific hybridization (Bell et al., 2019; Gonzalez et al., 2013).

Genetic Rescue

Following intraspecific hybridization between genetically distinct individuals, the first generation often expresses hybrid vigor or heterosis. The most common consequence of genetic rescue is heterosis, where the increased frequency of heterozygous loci masks the effects of deleterious recessive alleles, and the progeny outperform parents (Allendorf et al., 2022; Bell et al., 2019; Charlesworth & Willis, 2009). For example, intraspecific hybridization between populations of the annual wildflower *Clarkia pulchella* resulted in better-performing progeny when compared to their parental groups (Bontrager et al., 2019). Another study demonstrated that hybrid *Pinus torreyana* individuals were taller and more fecund than their inland and island parental groups (Hamilton et al., 2017). Furthermore, a meta-analysis of 156 cases of outcrossing in inbred populations found that fitness increased by 148% in stressful habitats and 45% in mild habitats, regardless of the taxon (Frankham, 2015).

Consequences of AGF: Genetic Rescue or Outbreeding Depression?

Heterosis is most potent in the first generation, and its effects can diminish in later generations following subsequent recombination and decreased heterozygosity (Allendorf et al., 2022; Bell et al., 2019; Charlesworth & Willis, 2009). Outbreeding depression, which is caused by genetic swamping and the breakdown of locally and coadapted gene complexes, often emerges in later generations (Allendorf et al., 2022; Edmands, 1999; Frankham et al., 2011;

Frankham, 2015; Whiteley et al., 2015). AGF can lead to increased extinction probability if it results in outbreeding depression. Historical genetic events and the current genetic health of populations play a fundamental role in the outcome of AGF, including population divergence, phenotypic plasticity, and genetic load (Pickup et al., 2013).

Population divergence relies on the combined effects of numerous genetic factors, such as historical and current gene flow, standing genetic variation, and locally adapted alleles and their epistatic interactions, making it a complicated but essential predictor of AGF outcomes. Leading range edge populations frequently display lower neutral genetic variation than their central conspecifics (Durka, 1999; Eckert et al., 2008; Pironon et al., 2017), reflecting historical successive founding events and their characteristically small and stochastically active populations (Pujol & Pannell, 2008; Vucetich & Waite, 2003). Therefore, AGF from genetically diverse central populations into leading range-edge populations could restore genetic diversity and adaptive potential (Sjölund et al., 2020). For example, Bontrager et al. (2019) demonstrated that AGF of *Clarkia pulchella* individuals from historically warmer climates enhanced genetic variability in less genetically diverse Northern-edge populations and concluded that differentiation positively correlated to increased fitness.

Suppose populations are distributed along an environmental gradient and are well-suited to their locally adapted habitats. The gene flow from large central populations to small range-edge populations might be harmful, wherein the centrally-adapted alleles swamp the locally adapted gene pool. This genetic swamping can hinder local adaptation and decrease fitness (Angert et al., 2020; Kirkpatrick & Barton, 1997). According to a study by Sexton et al. (2011), gene flow was beneficial between warm-edge populations of *Mimulus laciniatus* but harmful when performed from a central population to a warm-edge population. The researchers

concluded that gene flow was most effective between populations of the same range limit when significant genetic and environmental differentiation could introduce mal-adapted genotypes.

A population's ability to phenotypically respond to variable environmental conditions can also affect AGF outcomes. Natural selection, gene flow, and plasticity impact phenotypic divergence and, therefore, the downstream effects of AGF (Schmid & Guillaume, 2017). For example, plastic populations may mask adaptive genetic divergence and cause mutation accumulation, while populations with canalized ecotypes may experience heavier purging (Murren et al., 2015). Additionally, strongly plastic responses could slow adaptive divergence or increase genetic variability if maintained at the population level (Crispo, 2008; Draghi & Whitlock, 2012; Gomez-Mestre & Jovani, 2013; Sultan et al., 2017).

When augmenting for AGF, it is also essential to consider migrants' life stages during augmentation. Phenotypic responses are either labile and reversible throughout life, like biomass and phenology, or developmental and fixed during development, like anatomy and morphology (Crispo, 2008; Sultan et al., 2000; Sultan et al., 2017). Sobral et al. (2021) conducted a multi-generational common garden experiment to demonstrate the difference in these plasticity forms. They showed that wild *Raphanus raphanistrum* seedlings mobilized both physical and chemical defenses in response to herbivory, while mature plants only induced chemical defenses. Choosing juvenile versus adult migrants might impact their ability to respond to the target population's habitat, affecting their performance and ability to pass on genetic variation.

Plastic responses can also be transgenerational through epigenetic alterations (Harmon & Pfennig, 2021; Sultan et al., 2000). Sobral et al. (2021) revealed that the herbivory of mother plants significantly affected the physical defenses of their offspring throughout their lifetime. When site-specific variation exists among populations, epigenetic changes can cause phenotypic

divergence without mutation and persist in migrant offspring (Colicchio & Herman, 2020; Schmitz et al., 2011). Therefore, AGF might be ineffective if seedling migrants maintain energetically inefficient epigenetic changes from parental generations in different environmental conditions. On the other hand, migrants without transgenerational plasticity can hinder adaptive epigenetic effects by swamping a population where such alterations are adaptive. Therefore, it is crucial to consider how these factors could influence AGF outcomes (Greenspoon & Spencer, 2021).

Another significant factor when considering AGF effects is the genetic load of both recipient and donor populations. Large populations with high mutation loads could introduce deleterious alleles into smaller populations with a drift load (Pérez-Pereira et al., 2022). Migrant individuals from populations with large effective population sizes are expected to bear recessive deleterious alleles masked by the heterozygous state (Hedrick & García-Dorado, 2016; Pérez-Pereira et al., 2022). Gene flow from unpurged populations can unveil deleterious recessive alleles in target populations, leading to possible outbreeding depression and increased extinction risk (Charlesworth & Willis, 2009; Kardos et al., 2021; Kyriazis et al., 2021; Richards, 2000). Thus, minimizing the number of introduced deleterious alleles while maximizing adaptive genetic variation is crucial when performing AGF in a small and fragile population.

Measuring Genetic Rescue

Evidence for genetic rescue relies on limited indirect measurements. Bell and colleagues (2019) present a framework for evaluating genetic rescue that considers working with natural populations. The most robust evidence for genetic rescue is when population growth ($\lambda\lambda$) occurs after re-establishing gene flow (Allendorf et al., 2022; Bell et al., 2019). Increased vital rates or reproductive success may also indicate genetic rescue (Allendorf et al., 2022; Bell et al., 2019).

Additionally, vital rates, such as survival and fecundity, and reproductive success influence hybrid fitness relative to parents and can indirectly suggest that genetic rescue occurred (Allendorf et al., 2022). However, changes in population growth and vital rates depend on the demographic data available pre- and post-augmentation. Researchers can measure hybrid fitness relative to parental fitness to assess genetic rescue, but this should be done over multiple generations for the most robust assessment (Allendorf et al., 2022; Bell et al., 2019). Additionally, heterozygosity beyond neutral expectations may weakly suggest genetic rescue, but this is an inadequate indicator, necessitating more information on the demographic consequences of reestablished gene flow (Allendorf et al., 2022; Bell et al., 2019).

Identifying Hybrids

Underlying Theories for Detecting and Estimating Hybrid Identity

Offspring identity is commonly detected and summarized using ancestry indices or discrete classifications (i.e., parental, F_1, F_2, or backcross) (Allendorf et al., 2022; Fitzpatrick, 2012). Ancestry indices are the proportion of alleles in an individual's genome inherited from one of the two parents (Allendorf et al., 2022; Fitzpatrick, 2012; Frichot et al., 2014). Assigning individuals to genotypic classes is frequently done using interclass heterozygosity (HI), or the estimate of the proportion of alleles inherited from both parents, where parents have HI = 0, F_1 hybrids have HI = 1, and later hybrid generations have intermediate HIs (Allendorf et al., 2022; Fitzpatrick, 2012; Gompert & Buerkle, 2009). Discrete classification using interspecific heterozygosity is contingent on two criteria: it is only appropriate for classifying the first two generations of admixture, and it can only be used with codominant markers (Fitzpatrick, 2012; Rieseberg & Linder, 1999). Ancestry indices highlight the continuous spread of hybrid genotypes, while classification provides information needed to investigate differences among

earlier and later generations (Fitzpatrick, 2012). The input for these methods would ideally be derived from diagnostic bi-allelic loci where parental (e.g., native and augmented) genotypes have different fixed alleles (Allendorf et al., 2022). However, identifying fixed bi-allelic loci for previously developed highly polymorphic markers like microsatellites is rarely feasible. Several methods have been developed to reconcile this reality.

Buerkle (2005) developed a hybrid index for estimating ancestry indices using nondiagnostic codominant markers like microsatellites. This method expands on Rieseberg et al. (1999) and gives a maximum-likelihood estimation of ancestry. The output from the hybrid index (h) ranges from zero to one, where zero suggests none of the offspring's alleles were inherited from the reference parent, and one suggests all the offspring's alleles were inherited from the reference parent (Buerkle, 2005). When alleles are not fixed for parental groups, Buerkle's (2005) hybrid index accounts for the uncertainty of ancestry. Using nondiagnostic codominant markers, ancestry can also be estimated as a Bayesian admixture coefficient (Q) (Pritchard et al., 2000). While Q is not technically an ancestry index, it is roughly equivalent to the hybrid index (h) if two parental populations are assumed and genetically informative (Gompert & Buerkle, 2009).

Computational Approaches for Identifying Hybrids

Various software programs and R packages can estimate admixture, including NEWHYBRIDS (Anderson & Thompson, 2002), STRUCTURE (Pritchard et al., 2000), EIGENSTRAT (Patterson et al., 2006; Price et al., 2006), ADMIXTURE (Alexander et al., 2009), sNMF (Frichot et al., 2014), HINDEX (Buerkle, 2005), GenoDive (Meirmans, 2020), and the R packages *introgress* (Gompert and Buerkle 2009, 2010) and *HIest* (Fitzpatrick, 2012). NEWHYBRIDS and STRUCTURE use Bayesian models and are compatible with highly

variable codominant markers, with NEWHYBRIDS identifying hybrid or parental lineages and STRUCTURE predicting population structure. EIGENSTRAT employs PCA to determine ancestry and is better suited for conditions requiring more than a simple admixture model (Alexander et al., 2009; Frichot et al., 2014). ADMIXTURE, which uses the same likelihood model as STRUCTURE, and sNMF were explicitly designed to estimate ancestry from large multilocus SNP datasets. The R package *introgress* expands on Buerkle's (2005) hybrid index method and HINDEX software. The package provides functions for analyzing introgression between diverging groups and estimates genomic clines in hybrid zones to uncover deviations from expectations under neutral introgression.

Another extension of Buerkle's (2005) hybrid index is the *HIest* R package, developed by Fitzpatrick (2012), which jointly calculates hybrid index and interclass heterozygosity. By combining ancestry indices with classification, outputs capture similar information as discrete classification while allowing for more than two generations of admixture (Fitzpatrick, 2012). Rather than assigning individuals to the conventional genotypic classes, such as in NEWHYBRIDS, Fitzpatrick (2012) uses the same genomic proportions as Turelli and Orr (2000) to express genotypic probabilities. Like the other programs and software that estimate ancestry indices, *HIest* assumes that the study species is diploid.

According to Meirmans (2020), an increase in ploidy significantly increases the number of potential genotypes and poses computational challenges. Only a few programs are designed for polyploids, and currently, GenoDive (Meirmans, 2020) is the only software available for estimating Buerkle's (2005) hybrid index for polyploid datasets. GenoDive is an extension of Buerkle's (2005) hybrid index to enable the use of codominant markers and polyploid datasets. This study used GenoDive to estimate admixture in a *Geum radiatum* metapopulation.

Study System

Study Species: Geum radiatum Michx.

Geum radiatum Michx. (Rosaceae) is a long-living perennial that exists in 15 known locations along the North Carolina and Tennessee border and was listed as federally endangered in 1990 (ETWP, 1990; Weakley, 2020). Populations inhabit west-southwest to north-northeast facing cliffs and rock outcrops at elevations ranging from 1,400 to 2,000 meters (Weakley, 2020). There is also a single subpopulation that inhabits small and isolated boulders on a grassy bald. Sites tend to be cool and evenly moist, receiving direct sunlight for at least part of the day, and consistent moisture is a crucial environmental requirement for *G. radiatum* (Johnson, 1995; Ulrey et al., 2016).

The species grows as basal rosettes along a persistent horizontal rhizome nested between rock cracks and crevices and at the bases of steep and shallow talus slopes (Figures 2 and 3) (Weakley, 2020). Stems have a single large terminal kidney-shaped leaf with unevenly toothed margins and several smaller lateral lobes (Figure 4a) (Massey et al., 1983; Weakley, 2020). *Geum radiatum* flowers between July and September as an indeterminate cymose inflorescence with two to three bright yellow actinomorphic flowers and fruits between August and October as wind-dispersed hirsute achenes (Figure 4a and 4b) (Massey et al., 1983; Weakley, 2020). No studies have been conducted on the pollinators of this particular species, but insects in orders Diptera (Figure 5a) and Hymenoptera are known to visit the flowers (Figure 5b) (Massey et al., 1980; Morgan, 1980). Breeding patterns in *G. radiatum* are unknown; a related species, *Geum peckii*, can self-pollinate but produces higher seed yields when pollinated by flies (Murdock, 1993). *G. radiatum* does not appear to maintain a long-term seed bank, and the lack of suitable habitat limits successful establishment by dispersed seeds (Johnson, 1995). Adult survivorship is

high (97% annually), seedling recruitment is low (1-2 seedlings annually), and plants primarily reproduce by spreading along horizontal rhizomes (Johnson, 1995; Ulrey et al., 2016).

Hexaploidy is nearly ubiquitous within the genus. *Geum radiatum* is an allohexaploid, and it is hypothesized that its allohexaploid state is the product of two allopolyploidization events. The first event was likely between a diploid ancestral lineage of *Coluria* and *Waldsteinia* and an unknown diploid lineage (Gajewski, 1957; Smedmark, 2003). The second event was between the tetraploid hybrid *Geum heterocarpum* and another unknown diploid group, resulting in the extant hexaploid lineage (Gajewski, 1957; Smedmark, 2003). *Geum radiatum* exhibits moderate genetic diversity among and within populations, with a centrally located metapopulation harboring most diversity with the least differentiation (Hay et al., 2019). Its polyploid state likely contributes to genetic diversity, which is higher than expected for a post-Pleistocene relict with fragmented populations (Godt et al., 1996; Hay et al., 2019).

Figure 2

Early Geum radiatum Leaves

Note. Leaves sprouting from persistent rhizome at one of the northern populations. Photo taken by Morgan Gaglianese-Woody on 04/29/2021.

Figure 3

Flowering Geum radiatum Plant at One of the Metapopulation Sites

Note. Photo taken by Morgan Gaglianese-Woody on 07/29/2021.

Figure 4

Early- and Late-Season Geum radiatum Plants

(a) (b)

Note. (a) demonstrates characteristic kidney-shaped leaves and developing inflorescence and (b) shows newly formed achenes. Photos taken by Morgan Gaglianese-Woody on (a) 07/14/2021 and (b) 08/06/2021.

Figure 5

Insects in Orders Diptera (a) and Hymenoptera (b) Visiting Geum radiatum Flowers

(a) (b)

Note. Photos taken by Morgan Gaglianese-Woody on (a) 07/27/2021 and (b) 06/25/2021.

Augmented Metapopulation

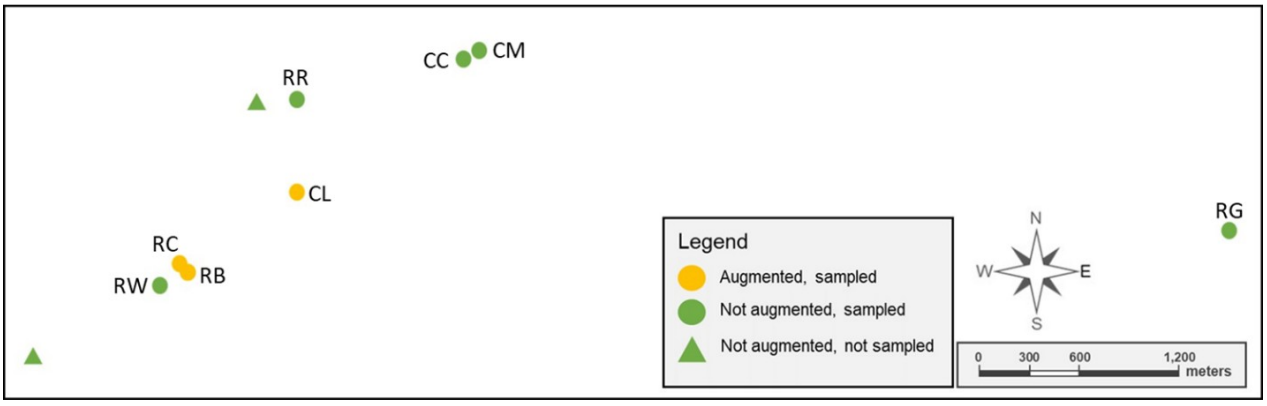
The study system is a metapopulation located near the center of the species distribution range. It straddles two national forests and several frequently trafficked areas, including one primary trail system and several smaller trails that weave in and around many *G. radiatum* sites. Several metapopulation sites underwent transplantations in the early 1990s to prevent population declines. Three of the thirteen sites were established or augmented with adult plants from the northeastern-most population in the mid-1990s; one of the sites, CL, had no *G. radiatum* plants prior to the augmentation, while the other two sites, RC and RB, did (Figure 6). There may have been additional transplantations before the 1990s in areas near CL, where a hotel was once located (personal communication with Gary Kauffman of the USFS; Johnson, 1995). One of those sites has since been extirpated, and neither is shown in Figure 6. Hay et al. (2019)

identified native, augmented, and possible F_1 individuals on the landscape and concluded that the "demographic rescue" introduced novel genetic diversity into the metapopulation.

The metapopulation still has traces of augmented and admixed genotypes more than two decades after the "demographic rescue." The introduction of novel genetic diversity through augmentation suggests that admixed genotypes might perform better than native and augmented genotypes due to heterosis. If F_1 individuals outperform parental generations, it could suggest a genetic rescue. By detecting genetic signatures of the historical augmentation, this study acts as a starting point to investigate genetic rescue as a possible conservation strategy for *Geum radiatum* and other long-lived and imperiled perennials.

Figure 6

Proximity of Site Locations



Note. Yellow circles correspond to augmented sites (RC, RB, CL), green shapes correspond to non-augmented sites (RW, RR, CC, CM, RG), circles correspond to sampled sites (RW, RC, RB, CL, RR, CC, CM, RG), and triangles correspond to unsampled sites.

Methods

Sampling and Tissue Collection

Plant collection was permitted under agent Dale Suiter's USFWS Endangered Species Recovery Permit (TE-178876-1), and a Protected Plant Permit (Permit #810) was also obtained from the North Carolina Department of Agriculture's Plant Conservation Program. Sampling occurred during the 2020 and 2021 summer monitoring seasons with National Park Service and U.S. Forest Service employees. Individual plants were defined according to the Rare Plant Management Plan for *Geum radiatum* (Blue Ridge Parkway [BRP], 2020), where rosettes greater than 25 centimeters apart were considered separate patches. All identifiable tagged individuals were sampled from monitored sites, with 203 tagged samples (CC = 10, CM = 45, RB = 12, RC = 51, RG = 21, RR = 45, RW = 19). Tagged samples were labeled with their unique monitoring number to connect them to the demography dataset. Four large plants were sampled at two ends of their clumps to test if they were single individuals or merged clumps. Individuals were also sampled from an unmonitored but known augmented subpopulation, CL (N = 27). Pre-sampled individuals from the previous genetic diversity and population structure study (Hay et al., 2019) were included as the source population, PM, for downstream analyses (N = 20). Approximately 150 mm^2 of green leaf tissue was collected per individual and placed in a collection tube with silica gel and indicator to dry. Dried samples were stored in a -80°C freezer. A total of 250 individuals were collected.

DNA Extraction and Genotyping

DNA was extracted from approximately 100 μg of dried green tissue per individual using a modified CTAB method (Doyle & Doyle, 1987). Extracted DNA was examined for concentration and purity using a NanoDrop™ 1000. The absorbance ratio of 260 nm to 280 nm

(A260/280) was measured to detect contaminants like proteins with an ideal ratio of 1.80. Conversely, the 260 nm to 230 nm (A260/230) ratio was used to identify contaminants such as phenol with an optimum ratio of 2.00 (Wilfinger et al., 1997). Deviations from optimal ratios were interpreted to indicate issues with the sample or the extraction procedure. Samples with A260/280 ratios below 1.15 were reextracted. Extracted DNA was also qualified on a 1% agarose gel with a Thermo Scientific™ GeneRuler™ 1 kb DNA Ladder. Seventeen individuals with DNA concentrations lower than 90 ng/μL and A260/280 ratios lower than 1.15 were removed from the study.

DNA from all 233 samples (CC = 8, CL = 26, CM = 40, RB = 11, RC = 46, RG = 21, RR = 42, RW = 19, PM = 20) was diluted to 30 ng/uL and randomly arrayed into two-and-a-half 96-well plates. Each plate contained two randomly positioned positive controls and one negative control (sterile OmniPur® Water) for uniform scoring and the detection of possible contamination. Twelve previously characterized microsatellite loci were amplified: WGU1-33, WGU2-10, WGU2-28, WGU3-15, WGU5-11, WGU5-12, WGU6-1, WGU6-23, WGU8-1 (*G. urbanum*: Arens et al., 2004), and 003651, 011534, 14769 (*G. reptans*: Hamann et al., 2014). A 5'M13 tag (5′-CACGACGTTGTAAAACGAC-3′) was added to each forward primer to label PCR products with FAM, VIC, NED, or PET fluorophores (Schuelke, 2000). Ten microliter PCR reactions were prepared with GoTaq Flexi Buffer, 2.5 mM $MgCl_2$ (1.0 μL), 800 mM dNTPs (0.8 μL), 0.5 μM reverse primer (0.5 μL), 0.25 μM tagged forward primer (0.25 μL), 0.25 μM fluorescently labeled primer (VIC, FAM, PET, or NED) (0.25 μL), 0.5 units GoTaq™ Flexi DNA Polymerase (0.1 μL), and 30 ng/uL DNA template (1.0 μL).

PCR was performed using a touchdown protocol (Korbie & Mattick, 2008). Initial denaturation occurred at 94°C for 5 min, followed by 13 cycles of denaturation at 94°C for 45 s,

annealing at 68°C for 2 min with a 1°C decrease each subsequent cycle, and extension at 72°C for 1 min. After 25 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 1 min, and extension at 72°C for 1 min, there was a final extension at 72°C for 10 min. Four differently labeled reaction products from all amplified loci were combined for each individual, for a total of three combinations per sample, and a GeneScan Liz 500 size standard (Invitrogen, Carlsbad, CA, USA) and HI-DI were added to the multiplexed arrays. Final products were sent to the WVU Genomics Core Facility (Morgantown, WV, USA) for genotyping. A maximum of six peaks were scored per individual using Geneious Prime 2022.2.1 (https://www.geneious.com).

Data Analysis

Polymorphic Information Content (PIC) was estimated for each locus in the R package *polysat* (version 1.7, Clark & Jasieniuk, 2011). A multilocus genotpye graph was produced in the package *poppr* (version 2.9.3, Kamvar et al., 2014) and visualized with *ggplot2* (Wickham, 2016) to estimate the power of the loci included in the analyses. Allele frequency was then estimated using the 'simFreq' function in *polysat*. Genetic distances were estimated using the Bruvo method (Bruvo et al., 2004) and visualized with a Principal Coordinates Analysis (PCA) in *polysat*. Site differentiation was also estimated with Wright's Fst (1969) in *polysat*. Differentiation was then visualized with a neighbor-joining tree in *adegenet* (version 2.0.0, Jombart, 2008).

Population structure was inferred and visualized first with a Discriminant Analysis of Principal Components (DAPC) in *adegenet* (Jombart et al., 2010) and then in the Bayesian clustering software STRUCTURE (version 2.3.4, Pritchard et al., 2000). Parameters for the STRUCTURE analysis were set under admixture and correlated allele frequency models with a Monte Carlo Markov Chain (MCMC) of 500,000, a burn-in period of 250,000 for K = 1 to K =

9, and ten replicates per K. Results from the STRUCTURE analysis were input into STRUCTURE Harvester (version 0.6.94, Earl & von Holdt, 2012) to calculate Delta K and estimate the strongest K-value using the Evanno Method (Evanno et al., 2005). A final STRUCTURE analysis was performed with the optimum Delta K value estimated in STRUCTURE Harvester, K = 4, with a burn-in period of 100,000 and 500,000 MCMC repetitions. The Q-plot was visualized in Microsoft Excel (2018).

Estimating Hybrid Index in GenoDive

Hybrid indexes were estimated in GenoDive (Meirmans, 2020) for all putative hybrid individuals. In the STRUCTURE analysis, most CM individuals clustered into one of the two genetic clusters that did not contain PM individuals. CM showed the most distinct structure and was not augmented, so it served as the Reference native group with 38 individuals. PM was the source of augmented individuals in 1990s, so it was set as the Alternative augmented group and comprised 19 of individuals. The putative hybrid group included 151 individuals from RW, RC, RB, CL, RR, CC, and RG. GenoDive generated maximum likelihood estimates, likelihood values, and 95% confidence intervals for estimated hybrid indices.

Results

DNA Quantity and Quality

DNA concentration ranged from 68.2 ng/µL to 747.2 ng/µL with a mean of 207.7 ng/µL and a median of 184.7 ng/µL. Most extracted DNA showed high molecular weight on the 1% agarose gel with possible protein contamination. A260/280 ratios ranged from 1.32 to 2.32 with a mean of 2.13 and a median of 2.16, and A260/230 ratios ranged from 1.15 to 2.26 with a mean of 1.56 and a median of 1.55. Due to time constraints, no cleaning protocol was employed to increase DNA quality before proceeding to downstream analyses.

Genotyping

Five of the 12 amplified markers could be consistently and confidently scored: WGU5-11, WGU5-12, WGU6-1, WGU6-23, and WGU8-1 (Arens et al., 2004) (Table 1). The remaining seven loci were removed due to difficulty scoring, inconsistent amplification, or contamination in the negative control. Of the 233 arrayed individuals, 215 were successfully genotyped. Seven pairs of two individuals shared multilocus genotypes and were identified as clones (RC554* & RC566; RC890PC1* & RC890PC2; RR719PC1* & RR219PC; CL010 & RR696*; RR692* & CM987; RC906 & RC197*; RR704* & RR705), so one clone from each pair was randomly removed from the dataset (indicated by *). Two hundred and eight individuals were included in the final data analysis: 16 from RW, 41 from RC, nine from RB, 24 from CL, 40 from RR, seven from CC, 38 from CM, 14 from RG, and 19 from PM.

Polymorphic information content (PIC) was then estimated in *polysat* for each of the included markers. PIC values range from 0 to 1, with 0 suggesting no capacity to detect polymorphisms and 1 suggesting a capacity to detect all polymorphisms. WGU5-12 was the least informative marker (PIC = 0.40), and WGU6-23 was the most informative marker (PIC = 0.81)

(Table 2). Then, a genotype accumulation curve was produced in *poppr* to estimate the number of multilocus genotypes (MLG) that can be detected as the number of loci increases. Four loci detected over 200 of the 208 MLGs with adequate confidence (Figure 7).

Site Differentiation

Site differentiation was calculated in *polysat* using the fixation index F_{ST}. The values range from 0 to 1, and values between 0.00 to 0.05 indicate little differentiation, 0.06 to 0.15 indicate moderate differentiation, and 0.16 to 0.25 indicate high differentiation between sites (Balloux & Lugon-Moulin, 2002). Fixation values ranged from 0.01 for CL and RW to 0.167 for PM and RB. The PM site had the highest differentiation values, with a mean of 0.108 and a median of 0.100. The PM site was the least differentiated from CL and the most differentiated from RG. There was little differentiation among sites RB, RR, RC, and RW (Table 3, Figure 8).

Genetic Structure

Several cluster analyses were performed to visualize structure and possible gene flow among sites. First, a PCA was built using Bruvo distance in *polysat* (Figure 9). A PCA reduces the number of variables to two principal components (PC1 and PC2) and plots individuals based on distances from each other. PM individuals (green) grouped in the upper left portion of the plot, but there was no apparent clustering. Next, a DAPC was performed in *adegenet* to visualize clusters (Figure 10). A DAPC is a multivariate analysis that infers genetic groups and assigns individuals to groups using a Bayesian K-means clustering method (Jombart et al., 2010). Most sites are grouped into a single central cluster, with PM vertically separated out and CM horizontally separated out.

Finally, a STRUCTURE analysis was performed to estimate site ancestry and admixture levels. STRUCTURE Harvester identified K = 4 as the optimum number of clusters (Figure 11),

and a Q-plot was built to visualize admixture under the assumption of K = 4 (Figure 12). Barring a single individual, only two of the four genetic groups were present in PM. At least three genetic groups were present in all metapopulation sites, with RR and CM showing the least admixture.

GenoDive Hybrid Index

CM individuals were input as the Reference Group in GenoDive's hybrid index. The hybrid index ranges from 0 to 1, and values below 0.5 indicates an augmented individual, above 0.5 indicates a native individual, and 0.5 indicates a hybrid individual. The average h-value for RW was 0.48, 0.57 for RC, 0.72 for RB, 0.47 for CL, 0.43 for RR, 0.56 for CC, and 0.88 for RG (Figure 13). Confidence intervals for individual h indices ranged from 0.000 to 1.000, with most confidence individuals artificially constrained by minimum and maximum possible hybrid index values. Individual genotypes could be confidently identified for hybrid indices with confidence intervals no less than 0.000 and no greater than 1.000 (Table 4).

Table 1

Information for the Five Loci Included in Analyses

Locus	Repeat motif	Motif size	No. of alleles	N genotyped
WGU5-11	$(CAA)(CAG)_{28}$	3	7	199
WGU5-12	$GTT_5(CGA\ TAA\ CAA)GTT_3$	3	4	153
WGU6-1	GAA_{15}	3	16	204
WGU6-23	AAG_{18}	3	10	207
WGU8-1	GAA_4, GAA	3	9	206

Note. The table includes the published locus name (from Arens et al., 2004), motif size, repeat motif, number of scored alleles, and number of unique individuals (N) genotyped out of 208 total individuals.

Table 2

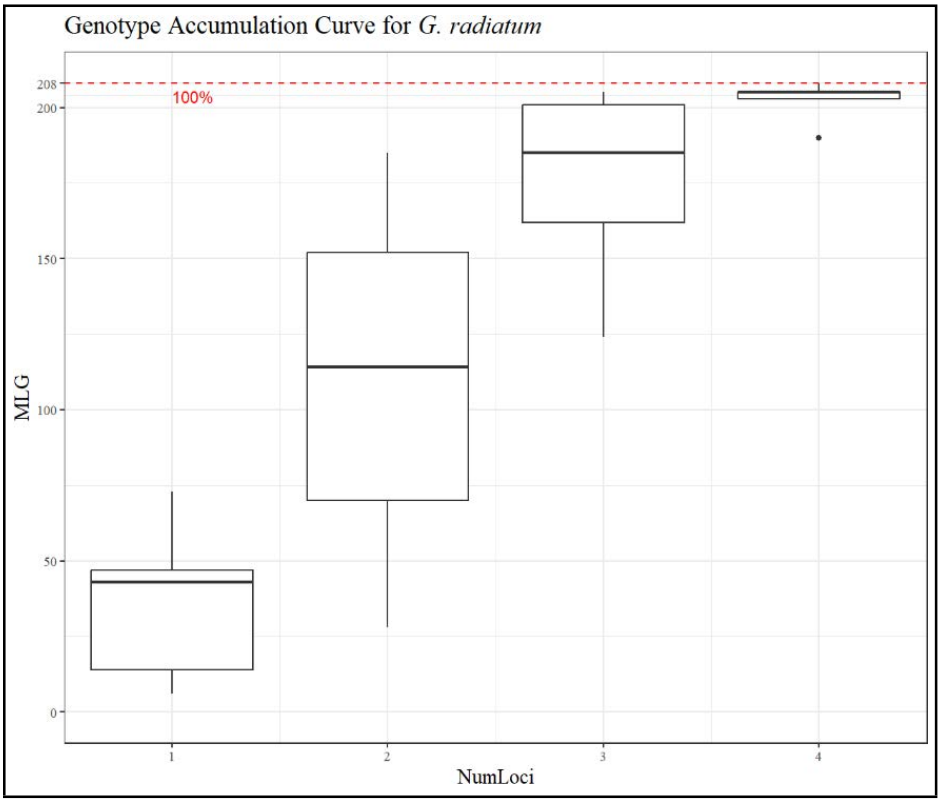
Polymorphic Information Content (PIC) for the Five Loci Included in Analyses

	WGU5-11	WGU5-12	WGU6-1	WGU6-23	WGU8-1
RW	0.64	0.38	0.76	0.78	0.50
RC	0.73	0.38	0.73	0.77	0.62
RB	0.69	0.43	0.54	0.77	0.59
CL	0.75	0.38	0.50	0.80	0.51
RR	0.70	0.42	0.79	0.75	0.49
CC	0.62	0.38	0.79	0.75	0.65
CM	0.75	0.38	0.59	0.75	0.65
RG	0.63	0.38	0.51	0.73	0.22
PM	0.68	0.51	0.32	0.63	0.48
Overall	0.78	0.40	0.71	0.82	0.56

Note. The table includes PIC values for each marker and site and the overall PIC value for all sites combined. Overall PIC values for this dataset range from 0.40 (WGU5-12) to 0.82 (WGU6-23).

Figure 7

Genotype Accumulation Graph



Note. The number of loci is on the x-axis, the number of MLGs is on the y-axis, and the red dashed line represents the point on the graph where all MLGs are detected. Four of the five loci are included in this particular analysis, with four loci estimated to detect nearly all of the 208 MLGs.

Table 3

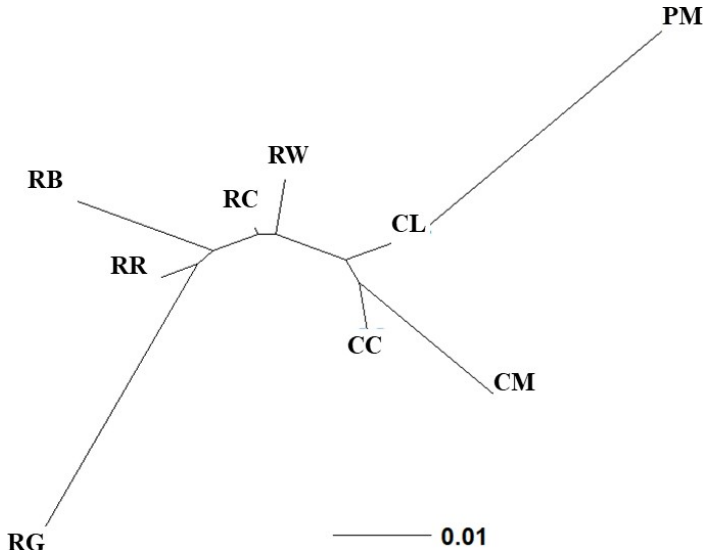
Pairwise Fixation Index (F_{ST}) for Each Site

	RW	**RC**	**RB**	**CL**	**RR**	**CC**	**CM**	**RG**	**PM**
RW		0.012	0.077	0.01	0.011	0.028	0.037	0.039	0.088
RC	0.012		0.046	0.028	0.026	0.025	0.025	0.022	0.105
RB	0.077	0.046		0.088	0.058	0.121	0.063	0.094	0.167
CL	0.010	0.028	0.088		0.017	0.021	0.045	0.043	0.071
RR	0.011	0.026	0.058	0.017		0.02	0.051	0.036	0.085
CC	0.028	0.025	0.121	0.021	0.02		0.035	0.082	0.093
CM	0.037	0.025	0.063	0.045	0.051	0.035		0.034	0.116
RG	0.039	0.022	0.094	0.043	0.036	0.082	0.034		0.138
PM	0.088	0.105	0.167	0.071	0.085	0.093	0.116	0.138	

Note. F_{ST} values represent differentiation between two sites. F_{ST} values range from 0.01 (CL and RW) to 0.167 (PM and RB).

Figure 8

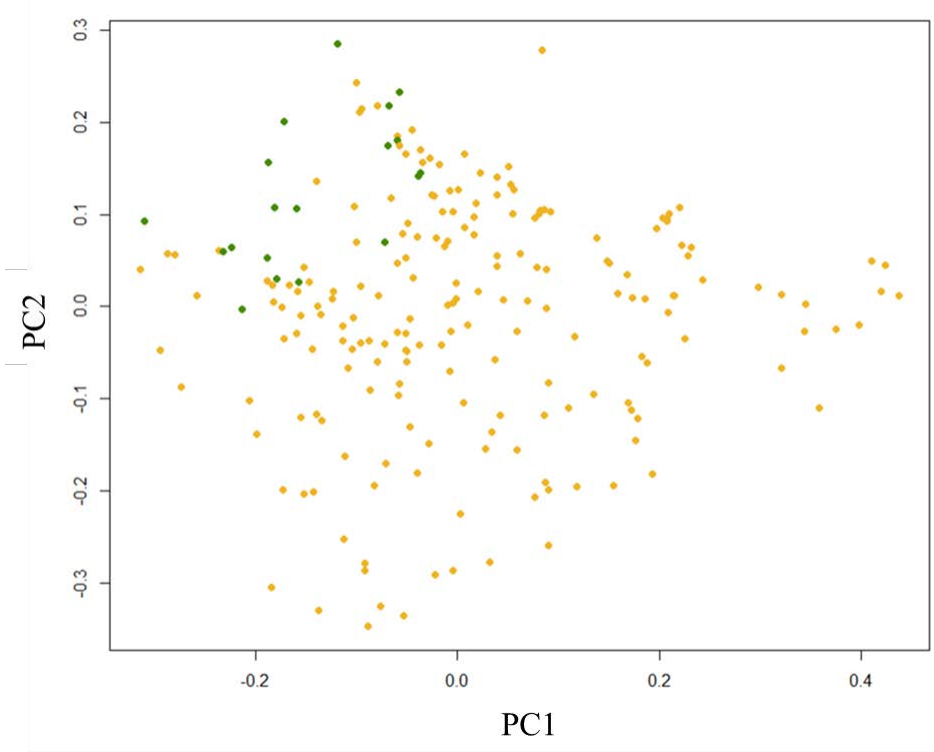
Unrooted Neighbor-Joining Tree



Note. The tree illustrates site differentiation (F_{ST}) among all sites and includes all nine sites and a scale that represents $F_{ST} = 0.01$.

Figure 9

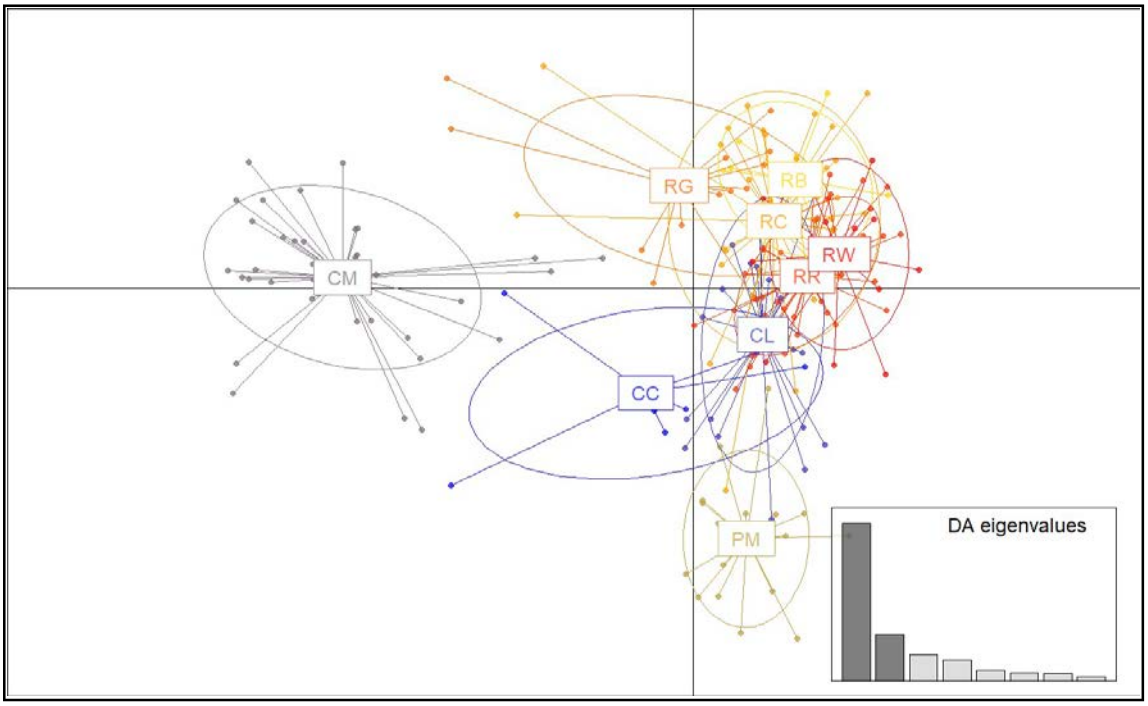
Principal Coordinates Analysis (PCA) Using Bruvo Distances



Note. The first principal component (PC1) is on the x-axis, and the second principal component (PC2) is on the y-axis. Green dots correspond to individuals from the source population (PM), and orange dots correspond to individuals from the targeted metapopulation (sites RW, RC, RB, CL, RR, CC, CM, and RG).

Figure 10

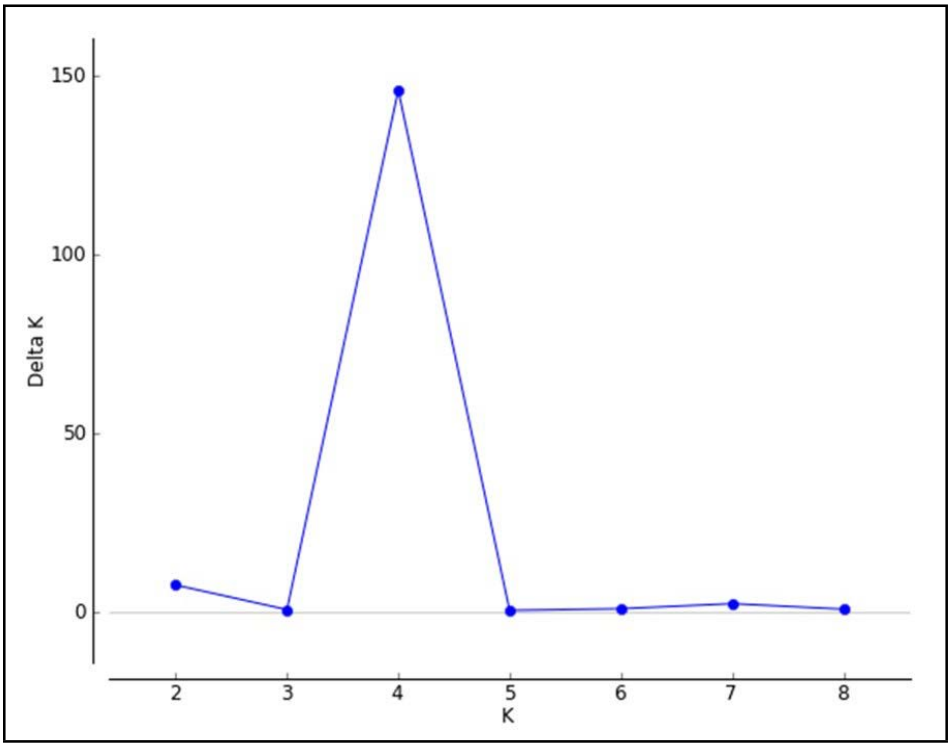
Discriminant Analysis of Principal Components (DAPC)



Note. The DAPC illustrates genetic clusters for all sites using a multivariate method.

Figure 11

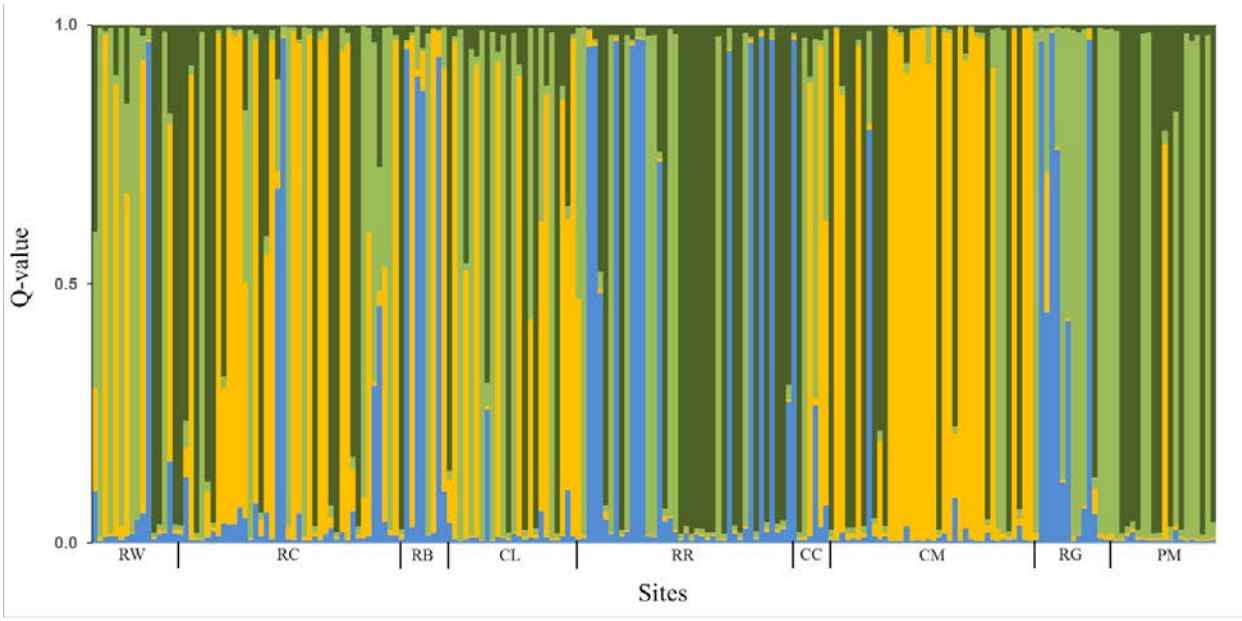
Delta K Values for K = 1 to 9



Note. Delta K values determine the optimum number of clusters that fit the dataset. K values are on the x-axis, and Delta K is on the y-axis. The optimum K is 4.

Figure 12

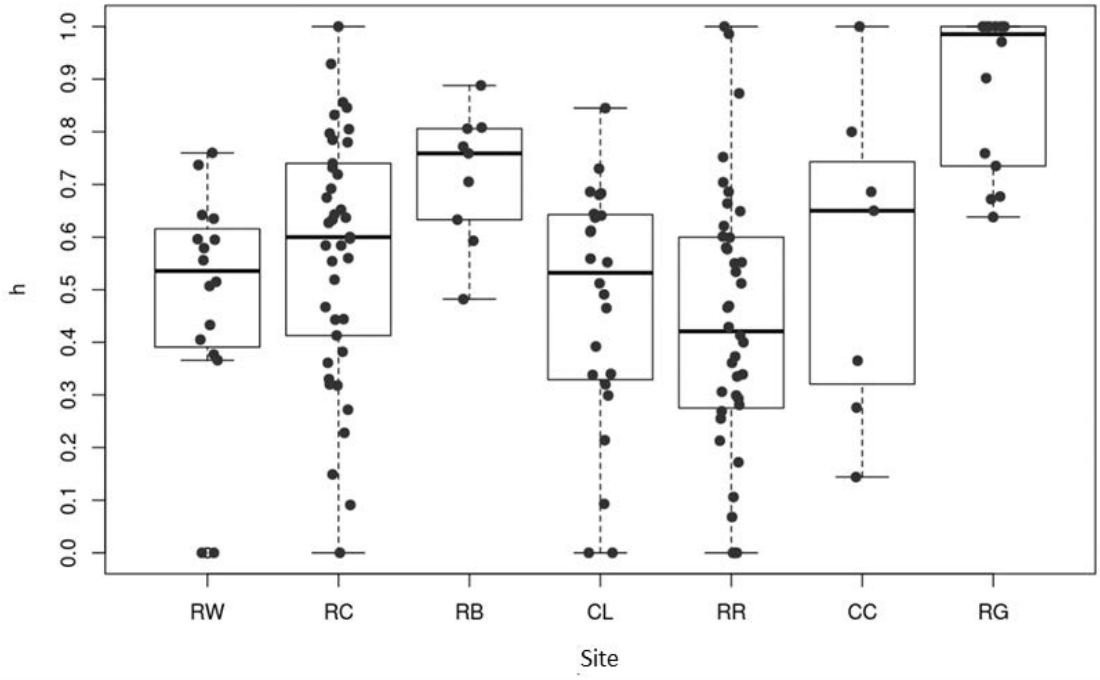
STRUCTURE Bar Plot Using K = 4



Note. Site codes are on the x-axis, and ancestry estimation (Q-value) is on the y-axis, which ranges from 0.0 to 1.0.

Figure 13

Boxplot of Hybrid Indices for Each Site



Note. The Y-axis represents the hybrid index (h) and ranges from 0.0 to 1.0, and the y-axis represents the site. All putative hybrid sites are represented in the plot. Each box depicts the range between the upper and lower quartiles, with the line inside the box showing the median h-value. The bottom whisker indicates the first quartile. The top whisker is the fourth quartile. Black dots represent individuals.

Table 4

Percent of Individuals Confidently Identified as Augmented or Native in Each Site

Site code	N	Augmented individuals (%)	Native individuals (%)
RW	16	18.8	0.0
RC	41	12.2	2.4
RB	9	11.1	11.1
CL	24	20.8	0.0
RR	40	35.0	0.0
CC	7	14.3	0.0
RG	14	0.0	0.0

Note. Hybrid indices with confidence intervals between 0.000 and 1.000 could confidently be used to identify individual genotypes.

Discussion

Study Limitations

Number of Markers

This study used only five polymorphic molecular markers, and marker scoring proved difficult due to stutter and inconsistent reads. It is crucial to have an adequate number of markers to enhance statistical power and minimize the likelihood of false positives. With fewer markers, there is a higher likelihood of observing chance similarities between individuals that are not truly related. For example, Wang and Scribner (2014) demonstrated that the accuracy of identifying full-sibship and parentage increased significantly as the number of loci increased. Using only five microsatellites, each with ten alleles, was insufficient to infer relationships accurately. However, with at least ten loci, over 90% of all possible relationships were identified correctly. Additionally, Wolfgang and colleagues (2017) demonstrated that with just eight loci, over 95% of *Drosophila nigrosparsa* individuals could be correctly identified, and two highly polymorphic loci still showed significant population structure. Regardless, increasing measurement resolution with a minimal number of markers is challenging. A larger number of markers provides more accurate and reliable results, allowing researchers to confidently distinguish between closely related individuals and accurately estimate population genetic parameters. Furthermore, more markers can detect subtle genetic differences between populations that might not be apparent with fewer markers.

Next-generation sequencing can greatly improve the identification of hybrids in plant populations by developing bi-allelic SNP markers. Although microsatellites are informative, using a high number of fixed diagnostic SNP alleles is more effective in identifying hybrids. SNPs have lower mutation rates, fewer alleles, and less homoplasy (Allendorf et al., 2022;

McFarlane & Pemberton, 2019). However, detecting and utilizing SNP markers in polyploid species can be difficult and may not yield the required accuracy to identify hybrids (Clevenger et al., 2018; Cuenca et al., 2013; McFarlane & Pemberton, 2019; Ottenburghs, 2021). Distinguishing between homologous and non-homologous chromosomes is challenging in polyploid organisms. Additionally, multiple alleles at a single locus can lead to ambiguity in identifying SNPs, and the high sequence similarity between homoeologous chromosomes can lead to the misidentification of SNPs (Bourke et al., 2018). Therefore, accurately identifying SNP markers in polyploid species requires sophisticated computational algorithms and experimental techniques (Kyriakidou et al, 2018).

Hybrid Identification

Choosing a suitable method to identify hybrids depends on several factors, such as the marker type, number of available loci, level of admixture, knowledge of parental individuals, and the study system's ploidy. The number of available loci is the most critical factor for species with pre-developed microsatellite markers. Confidence in identifying parents, F_1 individuals, and simple backcrosses requires fewer than six independent markers if completely fixed loci are available (Boecklen & Howard, 1997). However, the number of nondiagnostic loci can influence the power of estimation and accurate identification of hybrids. The hybrid index requires 35 to 45 loci for minimal confidence error between markedly different parents (an F_{ST} greater than 0.17). Robust detection of F_1 individuals needs up to 12 or 24 loci in STRUCTURE and NEWHYBRIDS, respectively, and sufficient genetic differentiation (F_{ST}) between parental groups (Vaha & Primmer, 2006). Separating parental individuals from backcrossed individuals in NEWHYBRID requires at least 48 loci for later generations (Vaha & Primmer, 2006).

Polyploidy is another crucial factor when selecting genetic tools for analysis. It is challenging to interpret the genetic data of polyploids due to unknown allelic dosage and allele frequency bias (Bourke et al., 2018). Polyploid organisms can be categorized as either autopolyploid or allopolyploid. Autopolyploids often display polysomic inheritance, while allopolyploids exhibit disomic inheritance. However, many polyploids are segmented allopolyploids that experience homeologous exchange and exhibit intermediate characteristics of autopolyploids and allopolyploids, further complicating genetic analysis (Bourke et al., 2018; Mason & Wendel, 2020).

Not surprisingly, tools designed for genetic analysis of polyploid datasets are limited, including those identifying hybrids (Meirmans et al., 2018). Statistical methods for analyzing population genetics often focus on diploids, which may lead to inaccuracies when applied to polyploid datasets (Dufresne et al., 2014; Jighly et al., 2019; Meirmans et al., 2018). Additionally, using genetic tools intended for diploids on segmented allopolyploids or autopolyploids is not advisable, as these organisms do not genetically behave like diploids (Bourke et al., 2018).

Combining methods can increase confidence in genetic data and is a common approach in hybridization studies (Gompert & Buerkle, 2016). For instance, Fitzpatrick et al. (2016) used a Bayesian model-based approach in NEWHYBRIDS v1.1 to assess the gene flow between two Trinidadian guppy populations with twelve microsatellite markers. They then generated datasets in HYBRIDLAB 1.0 and compared NEWHYBRID outputs for the simulated and actual datasets. In another study, Bersweden et al. (2021) used NEWHYBRIDS v1.1 and *introgress* to estimate admixture in hybridized orchid populations and then compared theoretical hybrid zones to the actual dataset by running hybrid simulations in *adegenet*. A combination of tools that can

identify admixture in polyploids can increase confidence and accuracy when a sufficient number of markers are available.

It is also feasible to transform co-dominant polyploid information into a pseudodiploid-dominant dataset, as demonstrated by Rodzen et al. (2004) and Wang & Scribner (2014). To achieve this transformation, each marker locus allele is considered an autonomous dominant locus, complete with two alleles, dominant and recessive, two phenotypes, band present and absent, and three genotypes, with two genotypes for the dominant phenotype, present/present or present/absent, and one genotype for the recessive phenotype, absent/absent. The dataset can then be analyzed using techniques intended for diploid dominant markers, such as hybrid identification in NEWHYBRIDS (Anderson, 2008; Sun & Lo, 2011). However, with this transformation comes a loss of information, such as heterozygosity (Hanson et al., 2008). Additionally, this method is better suited for autopolyploids with non-disomic inheritance and segmented allopolyploids with partial non-disomic inheritance than allopolyploids exhibiting disomic inheritance (Wange & Scribner, 2014).

Future Directions

Population Viability Analyses

PVA, or population viability analysis, is a modeling technique that considers diverse factors affecting population persistence to predict the risk of extinction or other measures of population viability (Allendorf et al., 2022). Current PVAs often incorporate stochasticity or chance events that affect the viability of natural populations, including demographic, environmental, natural, and genetic factors. PVAs have become more sophisticated in recent years, incorporating interactions between demographic and genetic effects. However, a recent

review of 160 PVAs found that only 25% of studies incorporated genetic stochasticity despite its importance (Chaudhary & Oli, 2020).

Researchers primarily incorporate genetics using inbreeding depression. In Vortex, a popular PVA software, inbreeding depression can be incorporated by assigning several lethal equivalents (LEs) that affect survival. Iwona and colleagues (2018) used Vortex v.10.2.7.0 (Lacy et al., 2017) to simulate their PVA and study the effects of supplementation, carrying capacity, inbreeding depression, and mortality rates in two populations of *Cerambyx cerdo*. Their study quantified the impact of inbreeding by assigning different lethal equivalents. If possible, including the inbreeding-stress interaction in viability modeling is crucial because as stress increases, the effect of inbreeding magnifies more than in outbred populations (Fox & Reed, 2010). The inbreeding-stress interaction can be especially important for populations of intermediate size that are relatively safe from environmental and genetic stresses acting independently.

Vortex was initially designed to predict the life history of mammals and birds (Lacy, 2000). Only 1% of PVA studies at that time included plant species, as stated in Menges' 2000 review. Davies et al. (2011) conducted a study to test the effectiveness of using vertebrate-based methods to estimate plant extinction risk, and they concluded that they are poor estimators of plant extinction risk. Zeigler and colleagues (2013) reviewed 223 published studies using plant population viability analyses (PVA) and assessed whether the authors followed the recommendations for improving PVA. They found that the most common model used in the publications was a matrix population model that relied on five years or fewer of demographic data. Few publications considered essential factors like genetics.

One way around this challenge is indirectly evaluating genetics and viability, such as in Kim et al. (2015). They compared genetic analysis results with the PVA outcomes for a population of *Asclepias lanuginosa*, a milkweed species threatened by a shift from sexual to asexual reproduction as its dominant reproductive mode. This study compared genetic analyses from a particular site to a population viability analysis incorporating 20 years of monitoring data. The aim was to compare the effectiveness of these two approaches and provide recommendations for a recovery plan for *A. lanuginosa*. Even though plant PVAs present unique challenges like seed banks, clonal growth, and periodic recruitment, they have proven helpful in guiding conservation and management (Menges, 2000).

In recent years, researchers have developed tools to integrate the eco-evolutionary traits of plants. RAMAS (Aiello-Lammens & Akçakaya, 2016) and HexSim (Schumaker & Brookes, 2018) are popular examples of spatially explicit individual-based models that can incorporate genetics and demography into plant PVAs. However, many PVA models are limited to diploid organisms and, therefore, necessitate creative integration of population genetic data for polyploids like *Geum radiatum*.

Since 2003, the National Parks Service (NPS) and Forest Service (FS) have been monitoring all *Geum radiatum* sites (BRP, 2020). During every monitoring season, they report individual plant patch areas, rosette counts, flowering stems per patch, and survival rates. This dataset could contribute to population viability analyses for *Geum radiatum* that consider genetic factors to provide conservation managers with a stronger understanding of the eco-evolutionary patterns in each site. Additionally, once the hybrid, augmented, and native genotypes can be confidently identified, they can be connected to the demography dataset, compare the performance of different genotypes, and examine the potential genetic rescue.

I analyzed the site-level response of the RG site using the NPS and FS demography dataset and a demographic model built with a Leftkovitch Transition Matrix. I used the R package *popbio* (Stubben & Milligan, 2007) to calculate the population growth rate, sensitivities, and elasticities. Population growth was 0.974, and the patch was dominated by small and large non-flowering plants and large flowering plants with close to no seedlings during stable conditions. Additionally, sensitivity and elasticity were calculated to estimate the proportional and absolute contributions of each matrix element. Among the changes observed, the most significant impact on λ was caused by changes in large flowering and non-flowering plants. Running demographic models similar to the RG *popbio* model that include genetic data could provide insight into site-level trends for conservation managers.

Future Performance Analyses. Several published examples provide methods for estimating performance using the demography dataset to analyze the impact of the historical *G. radiatum* augmentation. For example, Zavodna et al. (2015) studied the fitness of the perennial plant *Arenaria grandiflora* by analyzing its performance with demographic and genetic variables. They used the number of flowers per individual as an indicator of fitness and implemented a generalized linear mixed model (GLMM) in R, with the number of flowers as the dependent variable and individual age, heterozygosity, admixture level, and sampling year as independent variables. Site/population was included as a random intercept, and seven nested models were tested, with the final model selected using the AICc.

In another study, Rossum et al. (2020) assessed the effectiveness of plant translocations in restoring genetically viable populations of *Arnica montana* through genetic monitoring and measuring fitness quantitative traits across two generations. They evaluated heterosis by calculating Homozygosity by Loci (HL) values for each individual in each population using the

genhet R package. They then performed statistical tests such as two-way ANCOVA, pairwise Tukey HSD posthoc tests, and Pearson's correlation analyses to analyze differences in fitness variables relative to heterosis, inbreeding or outbreeding levels, site, seed source, maternal fitness, and cross-category. They also tested the effects of site, seed source, and their interaction on viable seed set, seed mass, seed germination, and cumulative fitness with maternal HL as a covariate.

This study's demography dataset tracks four fitness variables over 17 years in eight *G. radiatum* subpopulations. The performance variables include one continuous datatype, area, and three discrete datatypes, number of rosettes, number of flowering stems, and survival. Once hybrids can be confidently identified, the demography dataset can be connected to each genotype, and performance can be analyzed. I recommend generalized linear mixed models (GLMM) to analyze performance. The dependent variables should be set as the performance variables, while potential independent variables can include age, hybrid index, Q-values, and monitoring year. Subpopulation could be included as a nested factor to account for site-specific environmental effects. Finally, AIC can be used to determine the best-fit model. This approach is simple and can yield valuable insights into the effect of the historical augmentation.

Concluding Remarks

Rare and endemic plant species with fragmented populations like *Geum radiatum* are threatened by the rapidly changing climate. The subsequent loss of habitat within their dispersal range presents a unique challenge to conservation managers, and augmentation and genetic rescue offer a feasible solution. While this study faced numerous obstacles, such as marker number and quality and *G. radiatum*'s ploidy, it highlights the difficulties associated with identifying hybrid individuals and emphasizes the urgency for advanced genetic analysis tools for polyploids. Such urgency is magnified by the fact that more than 80% of plants are estimated to be polyploids (Kyriakidou et al., 2018; Meyers & Levin, 2006). Given the critical status and vulnerability of *G. radiatum*, conservation managers must take proactive measures to counteract the threats posed by habitat loss and human-induced climate change. Continuing this research will yield valuable insights for preserving *G. radiatum* and long-living rare endemics alike.

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Vita

Morgan Gaglianese-Woody was born in Charlotte, North Carolina, to Cynthia Gaglianese-Woody and Paul Woody on November 14th, 1995. They graduated from Providence High School in Charlotte in June 2014 and started at Appalachian State University that fall to study Biology. They were awarded their Bachelor of Science degree in May 2019. In spring 2020, they started their master's research at ASU. Morgan (also known as Jax) now resides in Asheville, N.C., with their twin and dog and is working at Highland Brewing in the quality control lab.