GENETIC AND PHENOTYPIC DIVERSITY WITHIN AND AMONG ISOLATED POPULATIONS OF THE *ERIGERON STRIGOSUS* (ASTERACEAE) SPECIES COMPLEX IN WESTERN NORTH CAROLINA

A thesis presented to the faculty of the Graduate School of
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By

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ABSTRACT

GENETIC AND PHENOTYPIC DIVERSITY WITHIN AND AMONG ISOLATED POPULATIONS OF THE *ERIGERON STRIGOSUS* (ASTERACEAE) SPECIES COMPLEX IN WESTERN NORTH CAROLINA

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The *Erigeron strigosus* Muhl. Ex Willd (Asteraceae) species complex is composed of late spring to early summer herbs that are widespread in North America. Many populations of this complex are polyploid and apomictic, but studies have identified sexual diploid populations specializing on glade habitats throughout the southeastern United States, several of which have been described as new species. Two unusual *Erigeron* populations were recently discovered in Jackson Co., western North Carolina: one on an olivine outcrop that displays distinct morphological and phenological variation from other *E. strigosus*, and a second on a high-elevation, mafic rock outcrop that is taller, has more stems, and is rhizomatous. Plants from both populations were confirmed to be sexually reproducing diploids based on megagametophyte development and phylogenetically distinct from each other based on nrDNA ITS and ETS regions. However, additional genetic and environmental information was needed to determine whether these populations constitute different species from each other and from similar diploid populations elsewhere. The primary objective of this study was to determine the species status of two unusual populations of *Erigeron* in WNC by comparing them both to one another and to similar glade populations in Arkansas. Amplified Fragment Length Polymorphisms (AFLP) provide an efficient system for genetic fingerprinting, and AFLP fingerprints for each plant sample were generated for analysis to compare to diploid populations in AR and confirm initial sequence data results. Genetic distance data from AFLPs and morphological data collected in the field and from a greenhouse common garden experiment indicate distinct groupings of WNC populations. Additionally, we collected soil pH and nutrient data and measured reproductive and vegetative morphological traits on plants from populations of interest in western North Carolina to further analyze the variation found among populations in this complex. Variation in plant height, basal leaf size, and hair characters appear to be distinctive within this population. These methods have provided valuable insight into genetic, environmental, and morphological differences across populations and geographic localities, in tandem indicating an undescribed species of *Erigeron* in Webster, NC.

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CHAPTER ONE: INTRODUCTION

Introduction & Significance

The issue of delimiting geographically widespread species is often highly complex due to intrinsic genetic and morphological variation within taxa as the result of various factors including local adaptation, genetic isolation and drift, and migration (Albrecht et al. 2021, Sheth et al. 2020). While in some cases this variation may represent undescribed species (Nesom 2004, Weakly et al. 2017), taxa within species complexes display distinct variation in morphology, ploidy levels, and mode of reproduction that is not necessarily representative of taxonomic separation. One such genus is *Erigeron* (fleabane, Asteraceae), a large genus composed of 390 species worldwide, 173 of which occur in North America (Nesom 2004). *Erigeron strigosus* Muhl. ex Willd is a late spring to early summer herb that grows from a short rhizome producing one or more stems with several or many capitula and is one of the most widespread *Erigeron* species present in western North Carolina. This species is most often an annual, but can sometimes present as a weak perennial, with the rosette growing into the second year. Most populations of *E. strigosus* are polyploid and apomictic, but studies have identified sexual, diploid populations specializing on glade habitats throughout the southeastern United States (Stratton 1991, Noyes & Allison 2005, Noyes et al. 2006), several of which have been described as new species (Weakley et al. 2017).

Polyploidy in Flowering Plants

Because most populations of *E. strigosus* are polyploid, it can be assumed that polyploidy confers some evolutionary advantage over sexual reproduction. Polyploidy, a condition in which an organism possesses more than two sets of homologous chromosomes, is exceptionally common in flowering plants. Estimates on the frequency of polyploidy in angiosperms are highly variable depending on sample size of taxa considered as well as base chromosome number used. Early estimates of polyploidy in flowering plants range from 30-47% based on species counts available in 1955 and assuming polyploid origin of species with haploid chromosome numbers of $n = 14$ (Grant 1963, Grant 1981). Others have suggested these approximations are too conservative and estimate polyploidy in angiosperms at upwards of 70-80% assuming that taxa with chromosome numbers of $n = 9$ or $n = 10$ would have a polyploid ancestor at some point in their evolutionary history (Goldblatt 1980, Lewis 1980).

Autopolyploidy, or whole genome duplication, may instantly cause speciation and as such is a major driver of angiosperm diversification (Noyes & Wagner 2014). New gene pools can accumulate evolutionary advantages allowing the polyploid lineages to persist through time and occasionally outperform their diploid ancestors (Levin & Soltis 2018). The transitional period associated with the Cretaceous-Paleogene (K-Pg) boundary saw a mass extinction event that destroyed the over 60% of plant and animal species. Polyploidy may have enabled many species to survive this arduous period by creating various genetic traits that allowed polyploids to survive the extinction of their diploid ancestors (Fawcett et al. 2009). A number of whole genome duplication events in mosses, ferns, and flowering plants occurred during the K-Pg extinction, and it is likely that the long-term success of polyploids can be attributed to novel gene function (Fawcett et al. 2009). Furthermore, neofunctionalization, where one genetic copy maintains its ancestral function and the other develops a novel function, and subfunctionalization, where one copy maintains some, but not all, functions of the ancestral gene may have enabled new polyploids to fill ecological niches that their diploid progenitors could not (Force et al. 1999).

While early studies sought to determine the frequency of polyploidy in plants (Grant 1963, Grant 1981, Goldblatt 1980, Lewis 1980), actual whole genome duplication events are likely less frequent than chromosome numbers suggest. Examining the relationships between polyploid *Erigeron* species and their diploid progenitors may be better accomplished through genomic and phylogenetic analyses. Some of the first angiosperm genomes to be fully sequenced include *Oryza sativa* (rice, Poaceae; Paterson et al. 2004), *Arabidopsis thaliana* (Brassicaceae; Blanc et al. 2000, Paterson et al. 2000, Vision et al. 2000), *Populus trichocarpa* (poplar, Salicaceae; Tuskan et al. 2006), and *Vitis vinifera* (grape, Vitaceae; Jaillon et al. 2007, Velasco et al. 2007). Each species shows evidence of whole genome duplication. Evidence of whole genome duplication events is supported by the vast gene redundancy found within completely sequenced nuclear genomes, as well as expressed sequence tags (ESTs). In lieu of whole genome data, ESTs provide a “snapshot” into a genome, allowing researchers to assess individual lineages for evidence of whole genome duplication events. Additionally, the approximate time of these events can be estimated (Lynch & Conery 2000), providing valuable insight into how polyploidy may frequently facilitate speciation (Stuessy & Weiss-Schneeweiss 2019). The overwhelming occurrence of polyploidy in angiosperms indicates substantial evolutionary significance. In order to begin to unravel the evolutionary relationships between polyploid species, we must first characterize their diploid progenitors.

Although polyploidy may confer some evolutionary benefit, polyploid conditions may inhibit normal gamete formation. Under such conditions, polyploid plants may successfully reproduce through asexual means, such as apomixis. Apomixis is a type of asexual reproduction in which embryos develop from unfertilized eggs, therefore avoiding meiotic reduction (León-Martínez & Vielle-Calzada 2019). There are two main pathways in which an unreduced egg cell develops without fertilization: in adventitious embryony, embryos may develop from somatic tissues of the ovule. This type of apomixis is not linked to polyploidy and examples of taxa exhibiting adventitious embryony are usually diploid, e.g. Rutaceae, Celestraceae, Orchidaceae (Whitton et al. 2008). In gametophytic apomixis, an unreduced embryo sac may be formed via apospory or diplospory. Apospory occurs when the nucellus (a transitory tissue type providing support to the developing gametophyte) gives rise to an unreduced megagametophyte, while diplospory requires an unreduced megaspore mother cell to form an unreduced megagametophyte. In both cases, embryos develop without fertilization. Interestingly, gametophytic apomixis is strongly associated with polyploidy, and diplospory is particularly common within Asteraceae (Whitton et al. 2008). Freeling (2017) argues that apomictic reproduction combined with occasional sex provides plants with a superior reproductive fitness. During the first divisions of meiosis, polyploids have three or more homologous chromosomes, a condition referred to as multivalent. Most polyploids have developed genetic methods to ensure multivalents are not formed during meiosis. Multivalents cause chromosome non-disjunction, where chromosomes fail to properly disjoin, and in turn produce infertile gametes. This process of diploidization by an array of genetic mechanisms, which vary among species, help to ensure normal chromosome pairing during meiosis and create “diploid behavior” in the polyploid.

Variation in the *Erigeron annuus-Erigeron strigosus* Complex

The daisy fleabane complex of *Erigeron* includes *E. annuus* (L.) Pers., *E. strigosus*, *E. tenuis* Torr. & A.Gray, *E. allisonii* D.B.Poindexter, B.R.Keener, & Noyes, *E. dolomitica* (J. Allison) D.B. Poind., B.R. Keener, & Noyes, and *E. strigosus* var. *transversii* (Shinners) Noyes. This complex includes two distinct reproductive methods: *E. annuus*, *E. strigosus*, and *E. tenuis* are largely polyploid and apomictic, while other listed taxa represent sexual diploid populations

(Noyes 2006). Among sexual diploid *E. strigosus* plants in Missouri and Arkansas, there was conspicuous morphological variation in leaf shape and pubescence (Noyes & Groff 2011). Additionally, previous work indicates variation in the geographic distribution of *E. strigosus*, with apomictic polyploids broadly spread across the range of the species and sexual diploids restricted to specialized habitats. Herbarium data from Missouri and Arkansas show that sexual diploid plants are more likely to occur in glades or habitats with primarily shale or limestone substrates, while a smaller number of specimens were collected from roadsides, creek beds, and disturbed forests (Noyes & Groff 2011). Other sexual diploid *E. strigosus* populations have been discovered in Tennessee, Alabama, and Georgia, all of which occur on various types of glade habitats (Noyes 2006). While sexual diploid *E. strigosus* seem to prefer specialized habitats, they are not constrained by habitat type. It is likely that the primary contributing factor controlling geographic distribution of sexual diploid *E. strigosus* is soil composition and habitat type.

Many *Erigeron* species are edaphic specialists associated with glades or barrens. *Erigeron strigosus* var. *dolomiticola* is solely confined to dolomite glades in Alabama, and *E. strigosus* var. *calcicole* is primarily restricted to calcareous glades in Tennessee (Noyes & Groff 2011). Previous work (Noyes et al. 2006, Noyes 2007) found that *E. strigosus* var. *strigosus* prefers sandy substrates in the southeast (Texas, Georgia, and South Carolina) and glade habitats in Arkansas. It is currently unknown whether sexual diploid *Erigeron* represent the ancestral condition, or if apomictic polyploids well-adapted to xeric conditions permitted colonialization to more extreme environments such as glades and barrens, where soil is nutrient-poor and mineral-rich. A 1953 study of the Webster-Addie ultramafic ring in Jackson Co., NC revealed that the maximum amount of replacement of olivine rock by serpentinite was about 40% (Miller 1953). Although not definitively proven, the widespread geographic range of apomictic polyploid *Erigeron* considered in combination with evidence that polyploidy facilitates expanded ecological benefits could indicate that sexual diploid *Erigeron* represent the minority condition. Because whole genome duplication can instantly cause speciation, it is likely that sexual diploid glade specialist plants are progenitors to apomictic polyploids in the *E. strigosus* species complex (Noyes & Poindexter 2025). Soltis & Soltis (2000) hypothesize that polyploids may have better colonizing ability than their diploid relatives due to higher selfing rates and decreased inbreeding depression.

In addition to soil composition and habitat type, plants are subject to many other limitations affecting range distribution. The dispersal ability of a species directly affects its ability to colonize new landscapes and persist in fragmented habitats. Studies have indicated that plant height (Thomson et al. 2011) and life-history strategy (Beckman et al. 2018) are particularly important predictors of dispersal ability as measured by dispersal distance. Thomson et al. (2011) showed that taller plants have greater dispersal distance increasing with growth form (herb < shrub < tree), with plant height being a stronger predictor of seed dispersal than seed mass. Previous studies have considered seed mass as the primary indicator of dispersal ability within a species (Howe & Smallwood 1982, Venable & Brown 1988). Considering plant height and seed mass together for herbaceous, wind-dispersed species such as *Erigeron*, taller plants would therefore release seeds at greater heights presumably resulting in increased seed dispersal. It can be presumed that there is less likelihood of inbreeding with nearby siblings or parent plants as seed dispersal distance increases, therefore, a population with taller plants may disperse seeds at greater distances, in turn resulting in greater genetic variation within the population.

Glade Populations in North Carolina

Genetic, phenotypic, and geographic variation within *Erigeron* has been the focus of extensive research (Stratton 1991, Edwards et al. 2006, Noyes et al. 2006, Noyes & Groff 2015), but little work has been done on sexual diploid populations in North Carolina. Two unusual *Erigeron* populations were recently discovered in Jackson Co., western NC: one on an olivine outcrop in Webster, that displays distinct phenological and morphological variation from other *E. strigosus*, including earlier flowering time, shorter rhizomes, and disconnecting, over-wintering rosettes (Fig. 1a), and a second on another mafic rock outcrop in Cedar Cliff Mountain Preserve (Fig. 1b, 1c). Plants from both populations were confirmed to be diploid (K. Mathews & R. Noyes, unpubl. data) and were found to be phylogenetically distinct from each other based on the nrDNA ITS (ITS1, 5.8S, ITS2) and ETS regions thus confirming the populations to be in different haplotype groups (R. Noyes, K. Mathews, & D. Poindexter, unpubl. data). Additional diploid populations from Alleghany Co., NC fall into the same haplotype group as the Webster *Erigeron*.

Quantifying morphological differences among these populations has helped provide insight to the evolutionary relationships among apomictic polyploid populations and sexual diploid populations. Previous studies have confirmed that sexual diploid *E. strigosus* plants are glade specialists, preferring to grow on patches of soil in rock outcrops primarily composed of limestone substrates. Preliminary work indicated morphological and genetic variation between two populations of *E. strigosus* in western North Carolina, but more data were needed to confirm the species status of these populations (R. Noyes, K. Mathews, & D. Poindexter, unpubl. data).

Conducting a common garden experiment using seeds from North Carolina and Arkansas has allowed us to determine whether these sexual diploid populations are genetically distinct or if variation observed in the field is primarily due to environmental plasticity. Additionally, growing seeds from apomictic plants in a common environment has provided a contrast against sexual diploid plants. The results of this study can be used to improve conservation awareness and begin to normalize the practice of using a Unified Species Concept (de Queiroz 2007). Under this species concept, a species is defined as a separately evolving metapopulation lineage. Additional criteria are used as supporting evidence for species status including variation in morphology, geographic separation, environmental differences, and genetic distinction, but these criteria are not required. In this study, I have examined morphological, genetic, and environmental information to determine whether these NC populations constitute distinct species from each other and from diploid populations elsewhere (Noyes et al. 2006, Noyes 2011).

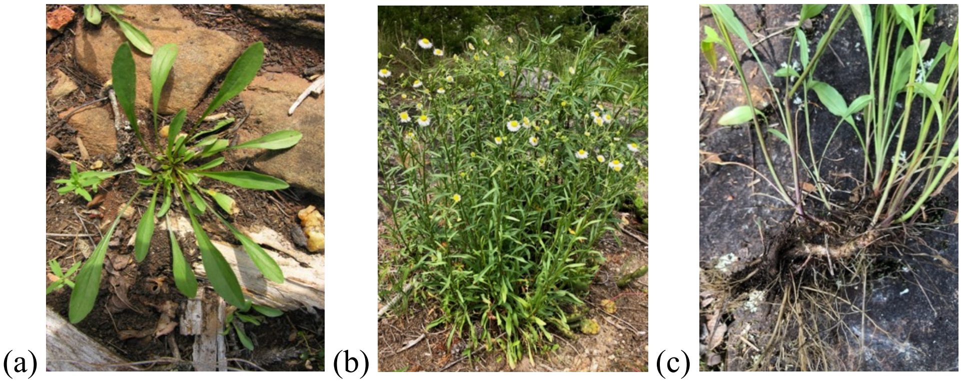

Figure 1: *Erigeron* plants from western North Carolina diploid populations. (a): Webster *Erigeron* non-flowering rosette. (b): Cedar Cliff *Erigeron* clump of flowering stems. (c): Cedar Cliff *Erigeron* excavated rhizome. Photos by Katherine Mathews.

Species Diagnosability

Conservation research and policy is centered around species, and species richness and evenness are widely accepted as valid methods for measuring important components of biodiversity (Maclaurin & Sterelny 2008, Freudenstein et al. 2017). Species richness is a strong indicator of community stability, and high species richness facilitates the ability of ecosystems to withstand disturbances related to weather events, human interference, and disease (Albrecht et al.

2021). Because approaches to defining species vary widely among biologists, various lines of evidence are needed when describing new species (Baum & Donoghue 1995, de Queiroz 2007, Freudenstein et al. 2017). Systematists often disagree on whether to focus on characters or history of a given taxon, subsequently leading researchers to utilize a more integrative approach with broader requirements by including both morphological and historical data (Hillis 1987, de Queiroz 2007). The issue of defining species by their method of reproduction is especially important for plants which often hybridize and reproduce asexually through apomixis and parthenogenesis (Noyes 2022, Noyes et al. 2006, Edwards et al. 2006).

Whereas many biologists have focused on the differences among rival species concepts, some authors have attempted to integrate key components of accepted concepts (de Queiroz 2007, Freudenstein et al. 2017). Recognizing that alternative species concepts fall short, de Queiroz defined species as “separately evolving metapopulation lineages” where *lineage* represents ancestors-descendants. This Unified Species Concept focuses on the similarities among previous alternative species concepts rather than their differences. Instead of requiring specific criteria that some biological entities may not meet, it provides a more generalized method of species delimitation. Several species concepts have been created to meet the needs of various subgroups of biologists. Under the Unified Species Concept, species are not required to be phenotypically unique, monophyletic, geographically isolated, or ecologically distinct. Rather, a species only requirement is to evolve separately from other lineages. De Queiroz states that all lineages are species, regardless of the amount of time under which it has diverged from other lineages. Other authors have discussed the need for species concepts using theoretical definitions as opposed to specific operational criteria, including Simpson’s Evolutionary Species Concept (1951) and Freudenstein’s recharacterized species concept (2017). The Evolutionary Species concept focuses on lineages through evolutionary time and views species as historical entities. Freudenstein views species as segments of evolutionary lineages that are not independent biological entities but rather exist as a convenient classification boundary for biologists. When attempting to draw boundaries between species, especially for plants which frequently hybridize and reproduce asexually through apomixis and parthenogenesis, systematists would benefit from operating under the Unified Species Concept. Instead of focusing on the boundaries set by other species concepts, the Unified Species Concept allows for species delimitation using morphological, molecular, and historical data.

Objectives

The primary goals of this study were to determine whether two North Carolina sexual, diploid, glade populations of *Erigeron strigosus* are morphologically and genetically distinct from each other and from sexual diploid populations found elsewhere and to clarify the species status of these populations based on these data. To achieve these goals, (1) I collected and analyzed morphological data from plants growing in the field in NC, (2) collected and analyzed morphological data from plants grown in a common garden from seeds from multiple populations of *E. strigosus*, and (3) generated and analyzed Amplified Fragment Length Polymorphism (AFLP) data from NC plants compared to other populations.

Common garden experiments may compare traits across populations found along elevational gradients, latitudinal gradients, or environmental gradients (Berend et al. 2019). The results of common gardens allow researchers to investigate local adaptation, ecotypes, and phenotypic plasticity by controlling for certain environmental factors. When used in tandem with analysis of morphological variation observed in nature, common gardens can help researchers deduce the primary source of morphological differences observed in the field.

Additionally, AFLP data are especially useful for botanical studies as AFLPs are predominantly nuclear markers and many plants often lack sufficiently variable markers in the nuclear genome or other organelles, especially in polyploids where many alleles may be present at each locus (Holland et al. 2008). AFLPs selectively amplify a subset of digested DNA fragments to compare unique genetic fingerprints in genomes of interest. This method is particularly useful because it does not require any prior information regarding the target genome. AFLPs are particularly powerful for studying plant genomes because they allow for the assessment of genetic diversity within or among closely related species. AFLPs have been widely used to study phylogeny within *Erigeron* (Noyes 2005, Noyes 2022) and were therefore selected as the DNA fingerprinting method for this study.

CHAPTER TWO: METHODS

Field Sites

Morphological measurements were made in the field from the following NC sites. Leaf material, pollen samples, and seeds were also collected from these sites.

1) Webster olivine outcrop: an olivine outcrop on private property, not protected by a conservation easement. The Webster outcrop sits on a private property adjacent to the Tuckasegee River in Webster, Jackson Co., North Carolina (35.34084°N, -83.21880°W, 636 m). At approximately 12-acres, the *Erigeron* population grows relatively contiguously within and along the edges of an olivine rock outcrop. This rock outcrop represents a small portion of the Webster-Addie ultramafic ring which is elliptical in shape and approximately 10 km long and 5 km wide (Miller 1953, Pittillo 1994). The Webster-Addie ultramafic rocks are composed of dunite, websterite, and enstatite pyroxenite. These soils are nutrient-poor, heavy metal-rich and are poor substrates for many plants. *Silene virginica* and *Smilax* spp. grow abundantly among the *Erigeron*, and *Pinus virginiana* dominates the surrounding habitat. This population occurs in highly disturbed habitat as the outcrop sits in a private homeowner’s backyard and has been mowed irregularly.

2) Cedar Cliff Mountain Preserve: (North Carolina Plant Conservation Program; permit # 936) protected through the North Carolina Plant Conservation Program. Cedar Cliff is an exposed area of mafic rock approximately 17 acres (35.25065°N, -83.07728 °W, 1,034 m) in Tuckasegee, Jackson Co., North Carolina. The outcrop is primarily composed of hornblende gneiss or schist, resulting in slightly basic soils. *Erigeron* patches grow sporadically among soil

patches in the exposed rock often alongside *Opuntia humifusa*, *Juniperus virginiana*, *Sedum glaucophyllum*, and other base-preferring plants.

3) Cullowhee (WCU Hike and Bike Trail): land open to the public owned by Western Carolina University in Jackson County, North Carolina. *Erigeron* grows sparsely alongside open, sunny trails within a powerline cut in this highly disturbed habitat alongside *Aquilegia canadensis*, *Rubus* sp., and *Solidago* spp. This population is made up of ~50-100 individuals and occurs at approximately 35.305224° N, -83.205106° W.

In addition, Dr. Richard Noyes collected seeds used in this study from populations at the following sites in Arkansas. Site data are being protected from the public at this time.

4) Snakeskin population, Arkansas – Izard Co., AR

5) Culp Road population, Arkansas – Stone Co., AR

Field Data Collection and Analysis

All data and material collection at the Webster population was conducted in May and June of 2023. Morphological data and material collection (capitula and leaves) were collected May 12 and May 14 of 2023. One capitulum per plant was collected for pollen staining to ensure we collected from diploid individuals. 3 leaves per plant were placed in paper envelopes and the envelopes were stored in Ziploc bags with silica gel dessicant for DNA preservation. Achenes were collected in June of 2023 by placing whole, fruiting capitula in paper envelopes. Envelopes were cold stratified in a refrigerator at approximately 3°C for at least 30 days prior to germination.

Morphological characters were measured with a ruler and by observations made with a 14x hand lens on each of 24 individuals per site (Table 1). At the Webster site, where the plants grow relatively contiguously in soil along the edges of the rocks, a 50 m transect tape was placed parallel to the long edges of the rock outcrop throughout the densest part of the population. The individual plants nearest to the transect line every 2 meters were marked and numbered with a flag and tape for measuring.

Table 1. Morphological characters measured on *Erigeron* specimens. Asterisks indicate whether the character was measured in the field, in the greenhouse, or both.

Character	Field	Greenhouse
Plant height (cm)	*	*
Rosette present/absent	*	
Number of flowering stems	*	*
Stem hair length (mm), average of 3		*
Stem hair density (# of hairs in 1x1 mm)		*
Total number of capitula, including buds	*	*
Capitulum diameter (mm)	*	*
Involucre height (mm)	*	*
Basal leaf margin (entire, dentate, denticulate)	*	*
Basal leaf length (mm), average of 3 rosette leaves	*	*
Basal leaf width (mm), average of 3 rosette leaves	*	*
Basal leaf pubescence (puberulent, strigillose)	*	*
Basal leaf hair length (mm), average of 3		*
Stem leaf length (mm), average of 3		*
Stem leaf width (mm), average of 3		*
Stem leaf hair length (mm), average of 3		*

In contrast to the Webster population, plants at the Cedar Cliff populations were not uniformly distributed. Clumps of plants were isolated in soil patches separated by bare rock, making a transect unsuitable. Instead, we selected five patches of plants with mature heads and collected morphological data from 2-5 flowering plants per patch at least 1 m apart.

In Cullowhee, mature, fruiting capitula were collected for achenes from plants found growing along the WCU Hike and Bike Trail at a powerline cut. Since the plants were growing linearly along the trail, we collected heads from the first 24 plants seen.

The ploidy of each individual plant sampled was confirmed in the field (or by bringing flowering capitula back to the lab in the case of the Cullowhee population) following the method of Noyes & Allison (2005) and Noyes et al. (2006) in which pollen grains are stained with cotton blue stain in lactophenol. The stain was prepared in a lab setting prior to field use, and was composed of 20 ml phenol, 40 ml glycerol, 20 ml lactic acid, 20 ml ddH_2O, and 5 ml 1% aqueous cotton blue. Using a micropipette, I placed a 20µl drop of the stain solution in the middle of a glass microscope slide. I removed at least 5 unopened disk florets from the mature capitula using forceps, placed them into the stain, and mashed them using a dissecting needle to release the pollen grains. Any floret debris were removed with clean forceps. A few more microliters of stain were added to the slide if needed to compensate for any stain that was lost upon removal of floret debris. I applied a coverslip and waited at least 15 minutes before viewing the slide under an AmScope™ M150 field microscope at 400x magnification. Slides were labeled with the sample number, sealed using clear nail polish, and stored for future reference. Later, slides were viewed in the lab using Motic® microscope under 400x magnification and photographed with a Moticam X® camera. Pollen grains of sexual diploid plants are uniform in size and stain evenly dark (Fig. 2a), while pollen grains of polyploid plants (Fig. 2b) are variable in size and stain lighter and inconsistently (Noyes et al. 2006).

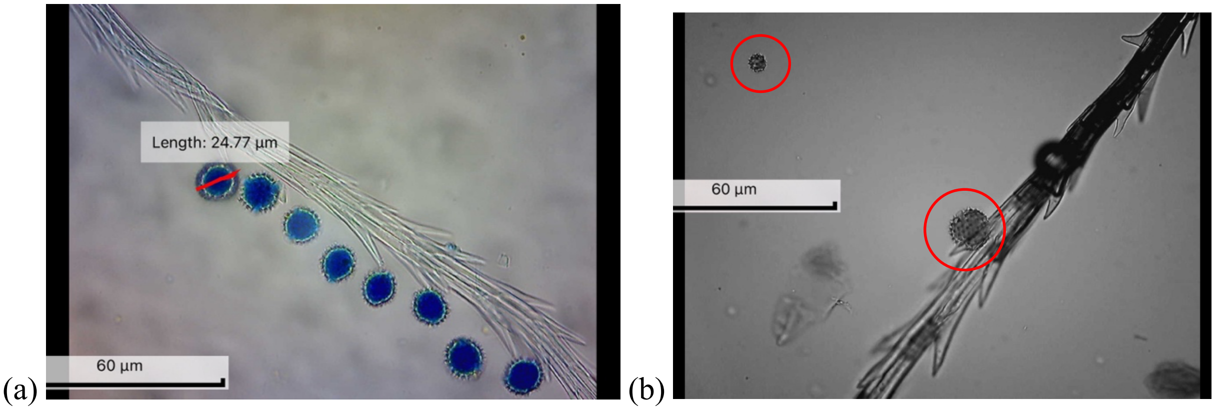

Figure 2: *Erigeron* pollen grains stained with cotton blue in lactophenol. (a): Uniformly-sized pollen of a diploid plant from the Webster site. (b): Variably-sized pollen of a polyploid plant (circled in red) from the Cullowhee site.

To raise plants in the common garden experiment, mature achenes were collected from 1-3 fruiting capitula from each tagged plant. Achenes were placed in labeled paper seed envelopes. For cold stratification, seed envelopes were placed in a refrigerator maintained at approximately 3°C for at least 30 days prior to germination.

Achenes from two populations in central Arkansas were obtained from Dr. Richard Noyes for comparison in the common garden experiment.

I collected soil samples from each NC site in July 2023 and sent them to Raleigh, NC, for composition and nutrient analysis by the North Carolina Department of Agriculture & Consumer Services Agronomic Division. Following protocol from the NC Department of Agriculture, I collected soil samples from a total of three different locations within each site using a hand trowel and uniformly mixed collections inside a cardboard sample collection box.

Common Garden Experiment

In order to examine whether local environment is driving the expression of morphological variation, I conducted common garden experiment with seeds collected from the Webster, Cedar Cliff, and central Arkansas populations of diploid *Erigeron*. Additionally, I grew seeds from apomictic *Erigeron* local to central AR and western NC for morphological comparison against sexual glade plants. Seeds from 24 mother plants per population were collected for germination in the summer of 2023. Seeds from individual mother plants were stored and sown separately. Cold stratification is not necessary, but a short, 30-day cold, moist stratification improves germination (Luna et al. 2008). Our pilot data were consistent with these results, and we employed this stratification method. Seeds from each population were cold stratified for at least 30 days then germinated in a petri dish with wet filter paper. This method allowed for easy separation of individual seeds, as the seeds are very small.

Sprouting seeds in petri dishes allowed for precise selection of healthy seeds at the expense of germination rate. Germination rates using this method were low and seeds took at least 7 days to sprout. Once seeds had germinated and cotyledons were visible, seedlings were carefully transferred to plastic flats filled with Miracle Gro© Seed Starting Potting Mix and placed in a grow tent with lights set to a 12:12 photoperiod. In order to minimize disturbance, seedlings were transferred to soil with their filter paper. Seeds were misted daily with double distilled water and flats were covered with a plastic lid to retain moisture. Though seedlings were carefully handled, viability using these methods was extremely low and only a small fraction of the 281 plants raised for the common garden were germinated in petri dishes. Seeds were directly sown into soil in plastic flats from this point forward, drastically improving both germination and survivability.

When the seedlings were large enough to emerge from the soil, they were transferred to the WCU greenhouse and watered uniformly via the greenhouse sprinkler system and rotated once every two weeks prior to installation of a drip irrigation system to mitigate differences in sunlight or temperature. Individual seedlings were transplanted to pots with Sta-Green® Potting Mix Plus Fertilizer (0.10-0.08-0.06) and provenance tags. During the summer of 2024, whiteflies were accidentally introduced to the greenhouse, likely through outside soil. The infestation was managed over the summer using a regimen of Spinosad, Safer Insect Killing Soap, and Evergreen. Beginning in fall of 2024, whiteflies were managed using lacewings as a biological pest control. All plants were uniformly affected by the infestation, and I did not notice any particular patterns between infestation level and population.

Key morphological characteristics (Table 1) were measured at flowering stage when possible, or on vegetative plants that did not flower during the experiment. To analyze vegetative morphology, I sampled three basal leaves, three stem leaves, a small section of stem, and one capitulum per plant grown in the WCU greenhouse. Basal and stem leaf measurements were averaged together for data analysis. I imaged leaves, stems, phyllaries, and capitula using a Leica M205 C microscope and Leica Application Suite X software. All continuous measurements of hair types, lengths, and densities were conducted in ImageJ (Schneider et al. 2012) from images containing scalebars to ensure accuracy.

Morphological Data Analysis

All multivariate analyses and data visualizations were conducted in R Studio (vers. 4.2.2; R Core Team 2022). I conducted principal components analyses (PCAs) using the *princomp*() function in the stats package (R Core Team 2022) to determine whether individuals form distinct clusters based on morphological characteristics (floral and vegetative), identify principal components that explain the greatest variation in datasets, and determine whether particular traits are associated with population.

Field data analyses: I conducted two PCAs using multivariate datasets of Webster and Cedar Cliff plants collected in the field 1) using all continuous floral and vegetative characters collected in the field and categorical data of leaf pubescence type and 2) using all continuous floral and vegetative characters with the addition of leaf margin type. To clearly show the distribution of each categorical variable across the samples, two separate PCAs were conducted. PCAs were plotted using the ggplot2 package (Wickham 2016), ggfortify (Tang et al. 2016), ggrepel (Slowikowski 2023), and factoextra (Kassambara & Mundt 2020). In order to statistically validate the PCAs, I utilized the PCAtest package (Camargo 2022). The PCAtest package tests for the overall significance of a PCA, the significance of each PC axis, as well as individual statistical contributions of each variable to each significant PC axis.

I also conducted a multivariate analysis of variance (MANOVA) for continuous floral and vegetative characters using the *manova*() function in the car package (Fox & Weisberg 2019). Floral and vegetative characters were analyzed separately for ease of interpretation. To analyze continuous floral characters, I used *manova*() and cbind to bind plant height and capitula diameter with population as the predictor: *manova*(cbind(height, capituladia), ~ Population, data = NCfieldcont). For vegetative characters, I used *manova*(cbind(leaflng, leafwd) ~ Population, data = NCveg). The car package utilizes F-distrubutions. Additionally, I used the emmeans package (Lenth 2023) to conduct post-hoc analyses using a Bonferroni test. I used *emmeans*(NCfloral, pairwise ~ Population, adjust = “bonferroni”). For count field data, I used the *glm*() function in Base R (R Core Team 2022). I used *glm*(Population ~ number of stems + number of capitula, family = binomial, data = NCfloralcount).

Greenhouse data analyses: I conducted a third PCA using the *prcomp*() function in base R (R Core Team 2022) to determine if the *Erigeron* samples grown in the WCU greenhouse as part of the common garden experiment can be discriminated based on the measured floral characters. Because this test was solely conducted on individuals that have flowered in the greenhouse, four populations were included: 1) APOAR, n = 3: an apomictic, polyploid population from Arkansas, 2) SNK, n = 14: a sexual, diploid population from Arkansas, 3) CC, n = 4: sexual, diploid plants from Cedar Cliff, NC, and 4) W, n = 12: sexual, diploid plants from Webster, NC. I used the *adonis2*() function in the vegan package (Oksanen et al. 2022) to conduct a permutational multivariate analysis of variance (PERMANOVA) using the continuous floral characters measured on greenhouse plants to determine if the Snakeskin, AR population differed significantly in morphology from the Webster, NC population. I created a distance matrix using *dist*(num_data, method = "euclidean"). To run the PERMANOVA, I used *adonis2*(dist_matrix ~ Population, data = floralcont, permutations = 999). Due to small sample sizes, Culp Rd., AR and Cedar Cliff, NC samples were excluded from this analysis. For each individual continuous trait, I used the *geom_violin*() function in the ggplot2 package (Wickham 2016) to create violin plots. For non-parametric analysis of individual traits, I ran Kruskal-Wallis tests using the *kruskal.test*() function from the stats package (R Core Team 2022), e.g. *kruskal.test*(Plant.height ~ Population, data = floralcont). To analyze greenhouse count data, I used the *glm*() function in Base R (R Core Team 2022) with *glm*(Population ~ stem number + capitula number, family = binomial, data = floralcount).

Because of strong collinearity between population and mode of reproduction, I separated datasets accordingly and ran separate analyses with population and reproductive mode as predictor variables. I used the *adonis2*() function in the vegan package (Oksanen et al. 2022) to conduct a PERMANOVA using the continuous vegetative characters measured on greenhouse plants to determine if apomictic polyploid plants significantly differed from sexual diploid plants. I created a distance matrix using *dist*(num_data, method = “euclidean”) and *adonis2*(dist_matrix ~ Rep.mode, data = vegrep, permutations = 999). I created violin plots using the *geom_violin*() function in the ggplot2 package (Wickham 2016). I then conducted the same analyses using the aforementioned packages on continuous vegetative characters measured on greenhouse plants with population as the predictor variable for sexual populations only. Again, I created a distance matrix using *dist*(num_data, method = “euclidean”) and conducted the PERMANOVA using *adonis2*(dist_matrix ~ Population, data = vegsex, permutations = 999). Because this dataset consisted of four total sexual populations, I utilized the *pairwise.adonis*() function from the pairwiseAdonis package (Martinez 2017) to determine which groups varied significantly from one another using pairwise comparisons. I used *pairwise.adonis*(dist_matrix, factors = vegsex$Population, perm = 999). I also utilized Bonferonni correction as a post hoc pairwise comparison test after the PERMANOVA tests.

Molecular Data Collection and Analysis

While in the field, 3-4 of the largest basal leaves per tagged individual were collected at each site and preserved in silica gel desiccant for DNA extraction and molecular analysis. The leaves were bagged in acid-free herbarium packets, which were in turn stored in plastic bags with silica gel desiccant. One mature capitulum was also collected from each plant sampled and stored similarly for use in DNA extraction in the event that there was not enough starting leaf material, though we did not end up needing to use them.

To isolate DNA, I used the DNeasy® Plant Pro Mini Kit by Qiagen© starting with approximately 100 mg of tissue. Individual leaves were cut into multiple pieces before loading into 2 mL bead tubes per the manufacturer's recommendation. The razor blade was cleaned in between samples using ethanol. In order to homogenize the tissue, I added a small amount of liquid Nitrogen and thoroughly crushed the leaves with a micropestle. Following the manufacturer's protocol, I added 700 µL of Solution CD1 and vortexed the tubes briefly to mix. Tissue disruption tubes were centrifuged at 12,000 x g for 2 minutes. The supernatant was then transferred to 1.5 mL collection tubes. I added 2000 µL Solution CD2 and vortexed samples for 5 seconds. Samples were then centrifuged at 12,000 x g for 1 minute at room temperature. Avoiding the pellet, I transferred the supernatant to 1.5 mL collection tubes. Next, 500 µL Buffer APP was added to the supernatant and vortexed for 5 seconds. I added 600 µL of the lysate onto MB Spin Columns and centrifuged at 12,000 x g for 1 minute. The flow-through was discarded and the previous step was repeated to ensure that all lysate has passed through the MB Spin Column. Next, the MB Spin Columns were placed into clean 2 mL collection tubes. I added 650 µL of Buffer AW1 to the MB Spin Columns and centrifuged at 8,000 x g for 1 minute. The flow-through was again discarded and MB Spin Columns placed back into their respective 2 mL collection tubes. To the MB Spin Columns, I added 650 µL Buffer AW2 and centrifuged at 12,000 x g for 1 minute. The flow-through was discarded once more and the Spin Columns placed back into their respective 2 mL collection tubes. Collection tubes were centrifuged at 16,000 x g for 2 minutes. The MB Spin Columns were then placed into new 1.5 mL collection tubes. Finally, 50 µL of Buffer EB was added to the center of the white filter membranes in the collection tubes. Collection tubes were centrifuged at 8,000 x g for 2 minutes. MB Spin Columns were discarded. DNA concentrations were quantified using a NanoDrop® One Microvolume UV-Vis Spectrophotometer© by Thermo Fisher Scientific Inc.

Extracted DNA was diluted appropriately with ddH_2O to a concentration of 10 ng/µl in 30 µl of solution, except in the case of two samples with extremely low DNA concentrations (W4: 6 ng/µl, W11: 8 ng/µl). Starting dry weight of leaf material from both individuals was very low (W4: 0.008 g, W11: 0.020 g).

We employed Amplified Fragment Length Polymorphism (AFLP) analyses using isolated DNA to assess diversity within and between populations in this study. AFLP uses PCR to selectively amplify regions of interest from digested DNA fragments using two restriction endonucleases, one with a 4-bp and one with a 6-bp recognition site (Paun & Schönswetter 2012, Avise 2004). Standard AFLP protocol includes four main steps: 1) restriction of genomic DNA and ligation of adaptors to the restricted fragments, 2) preamplification of a subset of restricted fragments, 3) selective PCR amplification to further reduce restriction fragment number, and 4) separation and visualization of amplified DNA fragments (Paun & Schönswetter 2012). Ligation of adapters, preamplification, and selective amplification followed standard protocols (Noyes & Rieseberg 2000). Necessary enzymes and buffers were purchased from New England Biolabs (Beverly, MA) provided by Dr. Richard Noyes. Two 96-well plates were prepared using *Eco*RI and *Mse*I adaptors/primers.

Adapter Preparation

For a single plate, adapters were prepared in two parts: for *ECO*RI, I mixed 9.35 µL *ECO*RI adapter 1, 8.25 *ECO*RI adapter 2, 16.5 µL 10X NEB#2, and 296.0 µL ddH_2O for a total volume of 330 µL divided into 3 PCR tubes. For *Mse*I, I mixed 88 µL *Mse*I adapter 1, 77 µL *Mse*I adapter 2, 16.5 µL 10X NEB#2, and 149.0 ddH_2O for a total volume of 330 µL divided into 3 PCR tubes. Each 96-well plate was placed in the Theremocycler set to the AFLP-AD program to heat the mixtures to 95°C and allowed the tubes to slowly cool to room temperature afterwards for about 25 minutes. PCR tubes were stored in a -20°C freezer.

40 µL Restriction Digest

For each sample, we used a Master Mix consisting of 566.5 µL H_2O, 440 µL NEB#2, 44 µL BSA, 33.0 µL *Mse*I, and 16.5 µL *ECO*RI. I aliquoted 10 µL to each well of a 96-well plate. Additionally, I added diluted, genomic DNA to each well. We incubated the plates at 37°C for 1 hour, followed by 70°C for 15 minutes to irreversibly denature the restriction enzymes. I used the Thermocycler Program: AFLPDIGLIG—1.5 hours at 37°C, 15 minutes at 70°C, 3.0 hours at 37°C to successfully conduct digestion and ligation.

Ligation of Adapters

After 1.5 hours of incubation, I added the following to each 40 µL restriction digest: 3.0 µL *ECO*RI DS adapter, 3.0 µL *Mse*I DS adapter, 1.0 µL 10mM ATP, 2.0 µL 10X NEB#2, 0.5 µL T4 Ligase, 0.1 µL BSA, and 0.4 µL ddH_2O for a total volume of 10 µL. The reaction was continued for 3 additional hours at 37°C. At this point, the total volume was 50 µL and the ligation reactions were stored in a -20°C freezer.

Pre-amplification

Pre-amplification was conducted by Dr. Richard Noyes in his lab at UCLA in Conway, Arkansas. Each sample was performed in a 25 µL volume with PCR run for 1 hour using Thermocycler Program NEWAFLP1. For each sample, Dr. Noyes mixed 1 µL template DNA (from 50 µL ligation reaction, undiluted), 2.5 µL Taq Buffer [10X NEB ThermoPol Reaction Buffer; provided with Taq], 2.5 µL *ECO*+1 primer, 2.5 µL *Mse*+1 primer, 16.1 µL sterile ddH_2O, 0.2 µL Taq (NEB #M0267L), and 0.2 µL 25mM dNTPs.

Pre-amplification Dilution

Dilution was also conducted by Dr. Richard Noyes. Each pre-amplification sample was diluted with 100 µL of sterile ddH_2O and stored at -80°C.

Selective Amplification

Selective amplification was conducted by Dr. Richard Noyes. Each sample was performed in a 10 µL volume. Each reaction consisted of 7.12 µL sterile ddH_2O, 1.0 µL Taq buffer, 0.5 µL M+3 primer, 0.1 µL E+3 primer, 0.08 µL dNTPs, and 0.2 µL Taq. Using a multi pipettor, 9.0 µL of the mixture was aliquoted into 12 wells and 1.0 µL of the diluted, pre-amplification template DNA was added to each well. The samples were run in a Thermocycler using the program NEWAFLP2 for 3 hours.

AFLP Analysis

AFLP samples were analyzed at the University of Missouri, Columbia, DNA Core Facility, which employs an Applied Biosystems (Foster City, CA, USA) 3730xl 96-capillary DNA Analyzer. Peaks were analyzed using GeneMarker v3.0.1, SoftGenetics, LLC, State College, PA 16803. Peak data were extracted as binary code (presence/absence of alleles on a given loci) and analyzed in Excel using GenAlEx Version 6.5, Australian National University (Peakall & Smouse 2006, 2012). Allele data were subject to Principal Coordinates Analysis (PCoA) and cluster analysis using GenAlEx (Peakall & Smouse 2006, 2012). Genetic distance analyses, including a PCoA via covariance matrix with data standardization using binary distance data for within population analysis (Webster and Cedar Cliff, NC) and a PCoA for among population analysis (apomictic polyploid *E. annuus* from AR, apomictic polyploid *E. strigosus* from AR, sexual diploid *E. strigosus* from AR, sexual diploid *E. strigosus* from AL and GA, and Webster and Cedar Cliff, NC) were also conducted in GenAlEx.

CHAPTER THREE: RESULTS

Field Site Results

The first six parameters describing the soil and its degree of acidity reveal notable differences between Webster and Cedar Cliff field sites (Table 2). Cedar Cliff contains a much higher percentage humic matter (3.10%) than Webster (0.27%). Humic matter increases nutrient availability and is linked to improved soil quality and microbial diversity (Li et al. 2019). Because humic matter is primarily composed of negatively charged humic acids, the negatively charged sites attract cations, increasing soil cation exchange capacity (Ampong et al. 2022). A higher percentage of humic matter therefore is normally associated with a higher CEC. Additionally, higher percent CEC occupied by basic cations indicates higher nutrient availability and often coincides with more fertile soils (Saha 2008). Interestingly, Webster has a neutral soil pH of 6.8 while Cedar Cliff has a more acidic pH of 5.2. Because Cedar Cliff sits on parent rock composed of hornblende gneiss or schist, we would expect the resulting soil pH to be much more basic.

Other notable difference in soil nutrient levels include percent CEC occupied by calcium, which is highly different between sites (Table 2). Webster has a much lower percent CEC occupied by calcium (12%) as compared to Cedar Cliff (49%). The Webster population sits on an olivine rock outcrop, which typically has low calcium levels, while the soil at the Cedar Cliff population has eroded from mafic parent rock that is calcium rich. Both olivine and mafic rock are characterized by high magnesium levels, though Webster has much higher percent CEC occupied by magnesium (81%) as compared to Cedar Cliff (12%). Other highly notable differences among sites include the sulfur index (Webster: 10, Cedar Cliff: 46) and copper index (Webster: 99, Cedar Cliff: 29).

Table 2. Summary of soil and nutrient analysis data for Webster and Cedar Cliff field sites. Units: W/V in g/cm^3; CEC and Na in meq/100 cm^3

Test Results	Abbreviation	Webster	Cedar Cliff
HM%	percent humic matter	0.27	3.10
W/V	weight per volume	0.99	0.69
CEC	cation exchange capacity	18.2	12.0
BS%	% CEC occupied by basic cations	94	63
Ac	exchangeable acidity	1.0	4.5
pH	current soil pH	6.8	5.2
P-I	phosphorus index	12	21
K-I	potassium index	57	35
Ca%	% CEC occupied by calcium	12	49
Mg%	% CEC occupied by magnesium	81	12
S-I	sulfur index	10	46
Mn-I	manganese index	788	470
Zn-AI	zinc availability index	86	105
Cu-I	copper index	99	29
Na	sodium	0.0	0.0

Morphological Analysis Results

Field data: A Principal Components Analysis (PCA) of continuous morphological data for sexual diploid plants in Webster and Cedar Cliff shows distinct clusters for floral and leaf characters measured in the field (Fig. 3, Fig. 4). With the exception of two individuals being minor outliers, Webster plants are smaller in total plant height, leaf length and width, and have fewer total capitula (mature capitula and unopened buds) as compared to Cedar Cliff specimens. Additionally, floral characters measured including involucre height and capitulum diameter are uniformly smaller in the Webster population (Fig. 3, Fig. 4).

The majority of variation in the morphological dataset can be attributed to PC1 (59.49%). PC2 accounts for the second-most variation in this dataset (16.38%). Involucre height and capitulum diameter show a strong, positive association with PC1, while total capitula shows a strong, negative association with PC1. Plant height, leaf length, and leaf width contribute similarly to PC1. There is little overlapping of Webster and Cedar Cliff individuals on PC1, indicating significant morphological variation between these sites (Fig. 3, Fig. 4).

Pubescence type differs between Webster and Cedar Cliff plants observed in the field (Fig. 3). Basal and stem leaves on Webster individuals are mostly strigillose (hairs with stiff, slender bristles, relatively uniformly distributed), while Cedar Cliff leaves are equally puberulent (hairs with short, uneven, pubescent bristles) or strigillose (Fig. 3). Similarly, basal leaf margin type is distinctive between populations: Webster individuals are either entire or denticulate, while Cedar Cliff plants are primarily dentate (Fig. 4).

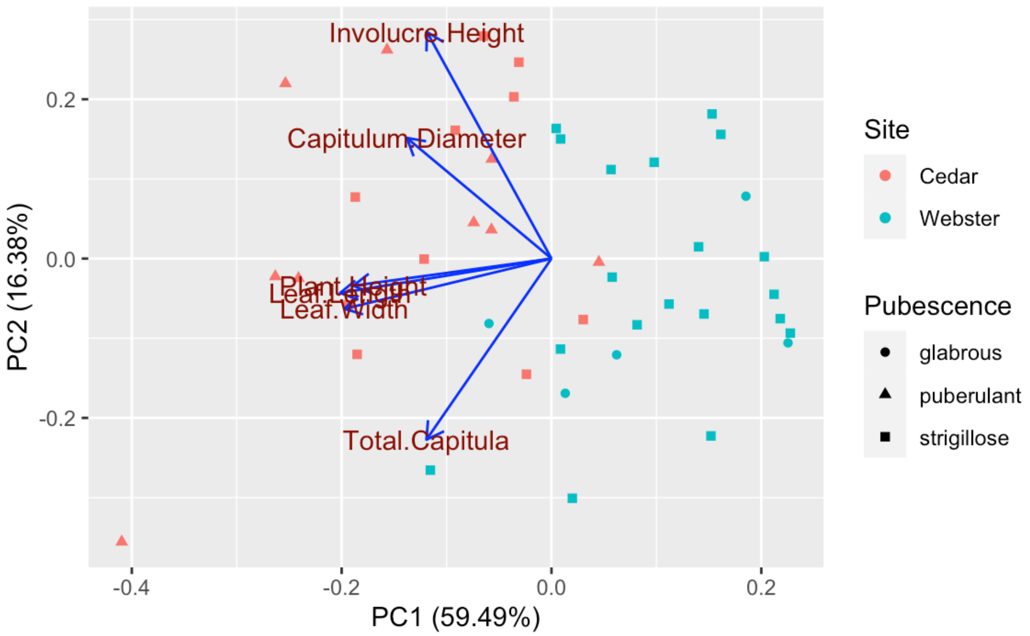

Figure 3. Principal Components Analysis (PCA) of *Erigeron* specimens from Webster and Cedar Cliff based on morphological data from leaves, flowers, plant height, and pubescence type.

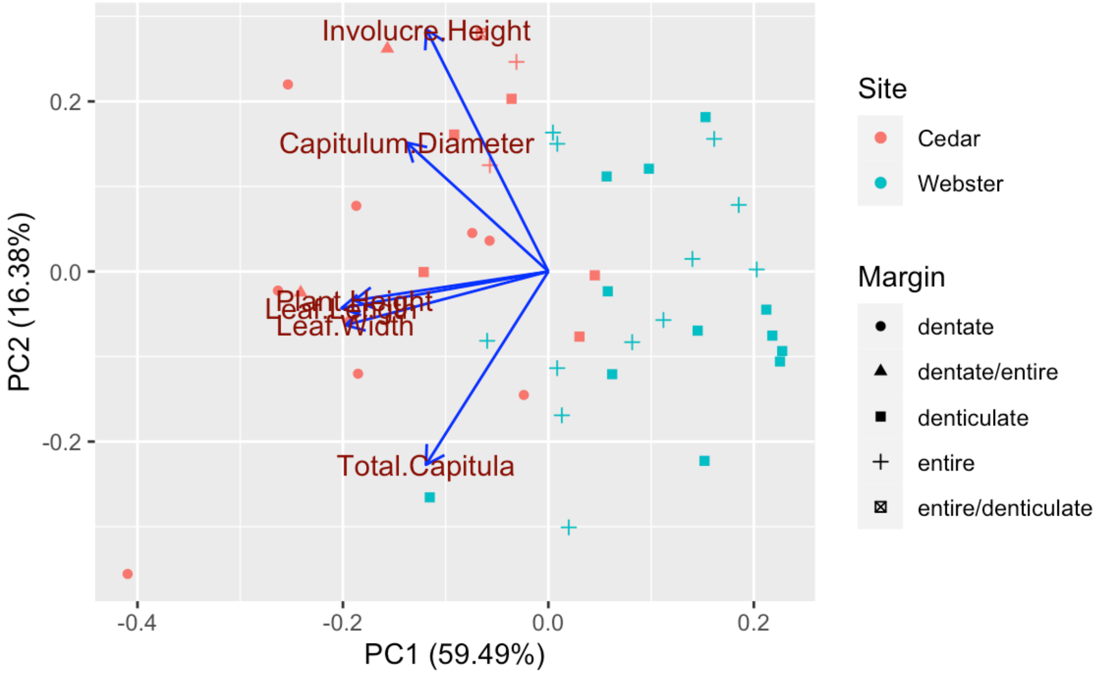

Figure 4. Principal Components Analysis (PCA) of *Erigeron* specimens from Webster and Cedar Cliff based on morphological data from leaves, flowers, plant height, and margin type.

PC1 is associated with overall plant size. Plant height, leaf length, and leaf width were strongly associated with PC1 (Table 3). Webster plants are smaller than Cedar Cliff plants measured by plant height of the tallest flowering stem and leaf size. PC2 is associated with reproductive characters. Particularly, involucre height and capitulum diameter have strong positive loadings (PC2). Total number of capitula is strongly associated with PC2 (Table 3). Plants with high loading values along the PC2 axis have fewer total capitula but larger heads, while plants with low loading values along PC2 have more capitula and smaller heads.

Table 3. Factor loadings for characters used in a Principal Components Analysis of *Erigeron* from Webster and Cedar Cliff.

Trait	PC1	PC2
Plant Height	-0.4685357	-0.08451365
Total Capitula	-0.2956191	-0.56372454
Capitulum Diameter	-0.3418920	0.37567202
Involucre Height	-0.2945716	0.70569958
Leaf Length	-0.4996684	-0.10697204
Leaf Width	-0.4896453	-0.15648544

There was significant variation in floral and vegetative characters among Webster and Cedar Cliff plants measured in the field. Floral characters including plant height as measured by the tallest flowering stem and capitula diameter were significantly different among Webster and Cedar Cliff (MANOVA, df = 1, F = 18.727, *P* = 1.209e-06). A post-hoc examination of individual response variables using the Bonferroni correction method revealed significant variation in plant height (df = 1, F = 34.759, *P* = 4.15e-07, Fig. 5) but not capitula diameter (df = 1, F = 0.9905, *P* = 0.3248, Fig. 6). Webster and Cedar Cliff plants had similar average means of capitula diameter, though Cedar Cliff plants had greater variation overall (Fig. 6). A post-hoc test using the Bonferroni correction method returned significant results (df = 46, SE: 1.58, t ratio = 4.169, *P* < 0.001).

Leaf measurements including basal leaf length (Fig. 7) and width (Fig. 8) varied significantly among populations (MANOVA, df = 1, F = 27.487, *P* = 1.584e-08). A post-hoc test using the Bonferroni correction method confirmed significant results (df = 1, SE: 2.45, t ratio = 7.285, *P* < 0.001). For floral count data including number of stems (GLM, negative-binomial distribution, z = -2.996, *P* = 0.00273) and total number of capitula including buds (GLM, negative-binomial distribution, z = 2.611, *P* = 0.00902), we observed significant variation among populations. Webster plants have fewer stems and fewer capitula as compared to Cedar Cliff plants in the field, consistent with PCA results (Fig. 3, Fig. 4).

Figure 5. Plant height was significantly different (df = 1, F = 34.759, *P* = 4.15e-07) in *Erigeron* plants measured in the field at two sexual diploid populations in North Carolina: Cedar Cliff (CC) and Webster (W).

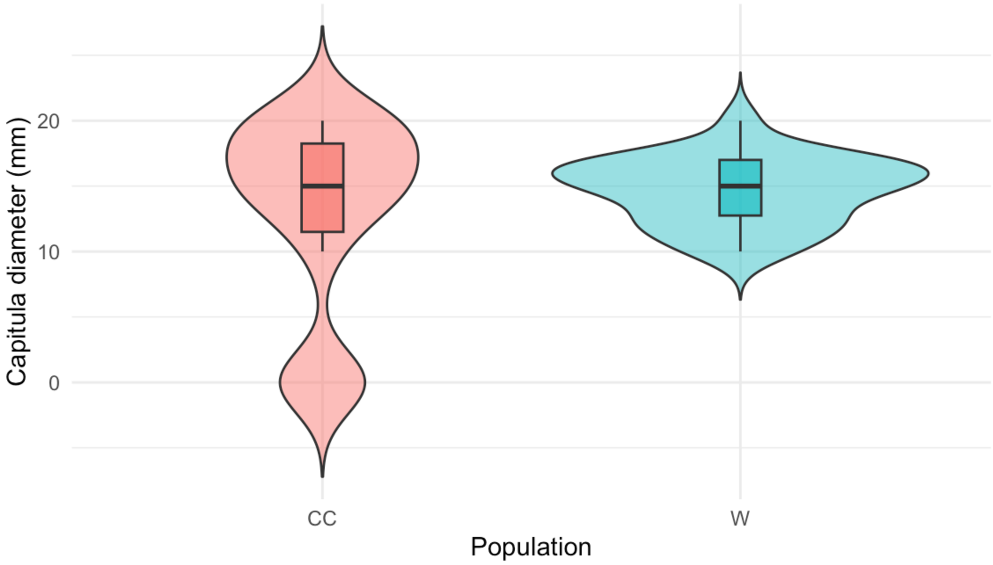

Figure 6. Capitula diameter was not significantly different (df = 1, F = 0.9905, *P* = 0.3248) in *Erigeron* plants measured in the field at two sexual diploid populations in North Carolina: Cedar Cliff (CC) and Webster (W).

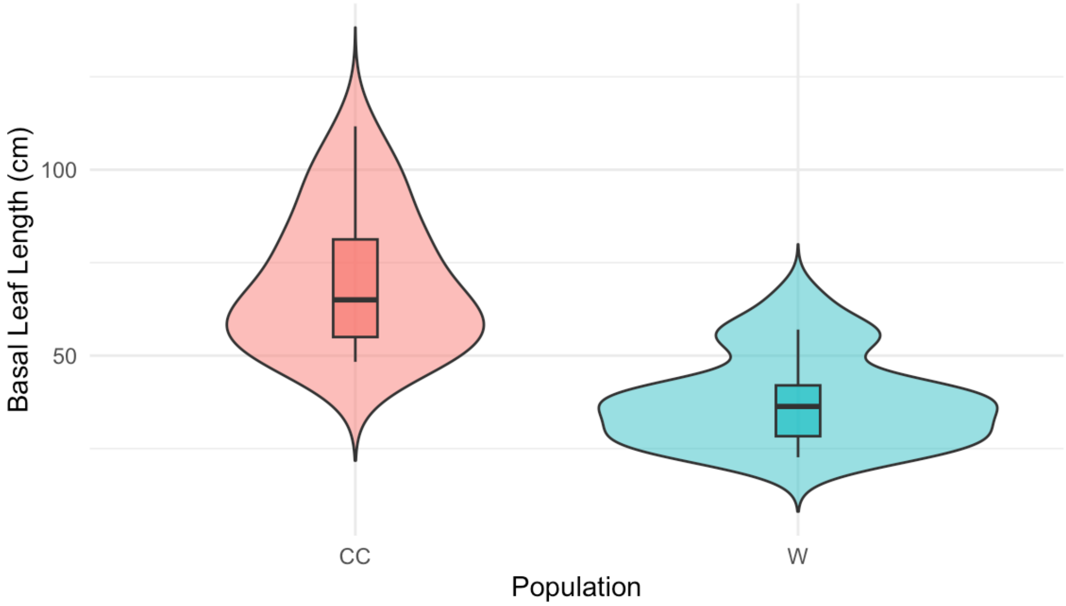

Figure 7. Basal leaf length was significantly different (df = 1, F = 51.466, *P* = 4.9994e-09) in *Erigeron* plants measured in the field at two sexual diploid populations in North Carolina: Cedar Cliff (CC) and Webster (W).

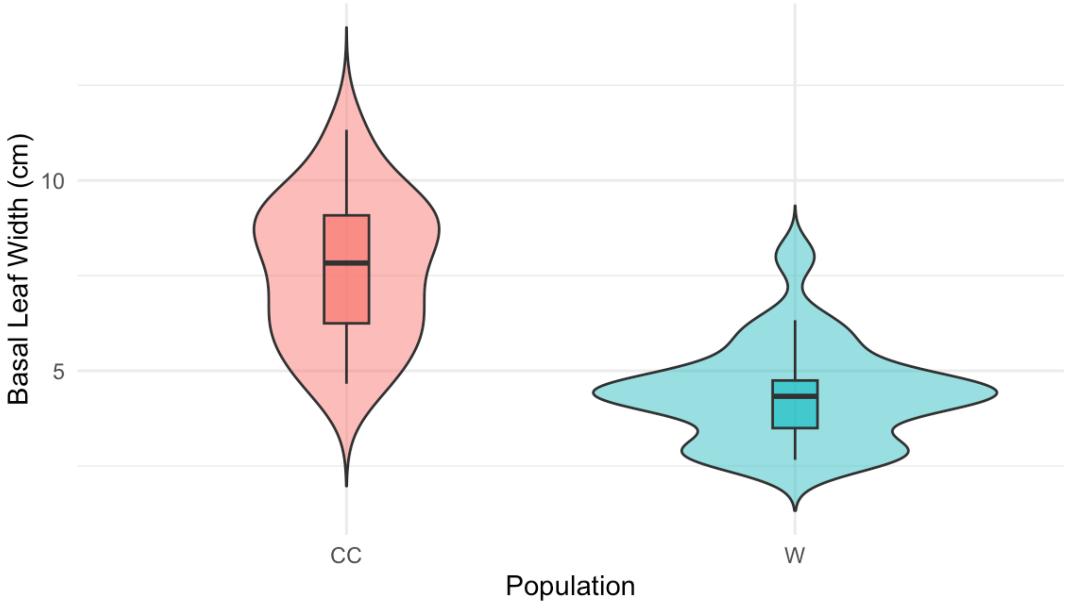

Figure 8. Basal leaf width was significantly different (df = 1, F = 50.054, *P* = 7.036e-09) in *Erigeron* plants measured in the field at two sexual diploid populations in North Carolina: Cedar Cliff (CC) and Webster (W).

Greenhouse data: A Principal Components Analysis (PCA) conducted on floral and vegetative traits of specimens grown in the WCU greenhouse from four populations in the common garden experiment shows some separation along PC1 between populations, particularly Webster (Fig. 9). PC1 is primarily associated with size characters like leaf and hair measurements, while PC2 mostly separates individuals on reproductive characters including plant height, stem number, and capitula number (Table 4).

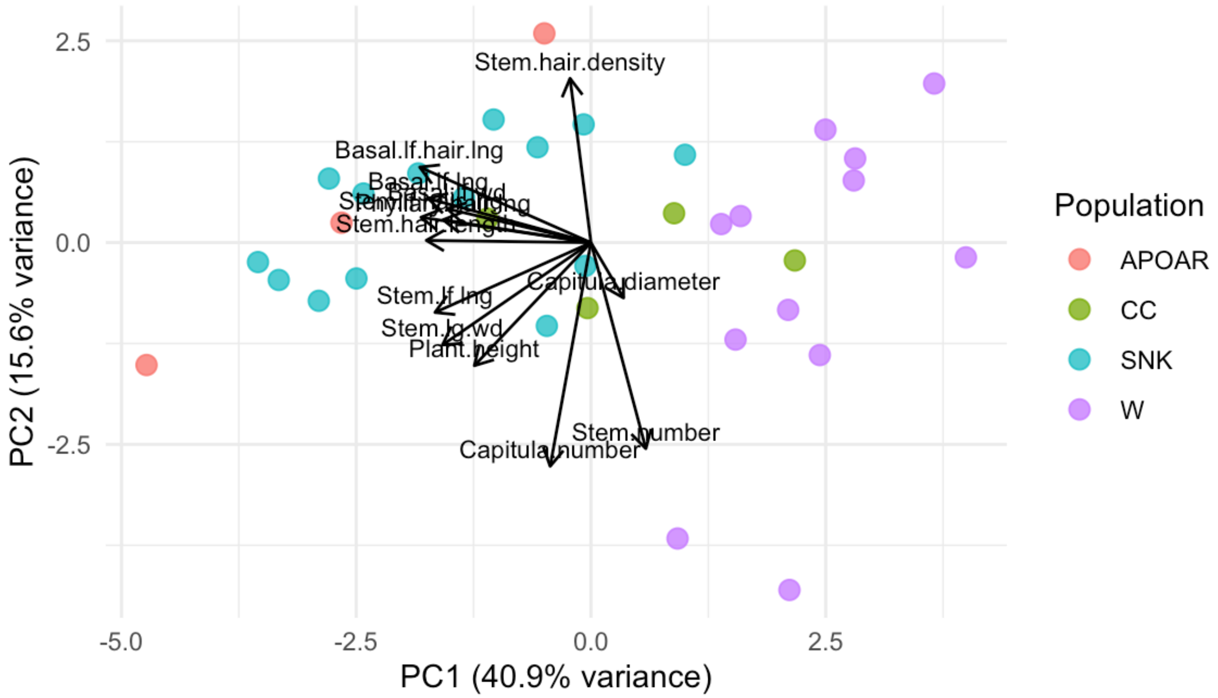

Figure 9. Principal Components Analysis (PCA) of floral and vegetative traits from *Erigeron* grown in the WCU greenhouse as part of a common garden experiment. Data were collected on flowered plants from 4 populations. APOAR: apomictic polyploid plants from Arkansas, SNK: sexual diploid plants from an Arkansas glade population, CC: sexual diploid plants from Cedar Cliff, North Carolina, and W: sexual diploid plants from Webster, North Carolina.

Table 4. Factor loadings for each character used in a Principal Components Analysis from *Erigeron* grown in the WCU greenhouse.

Trait	PC1	PC2
Plant Height	-0.248	-0.305
Stem Number	0.117	-0.510
Stem Hair Length	-0.351	0.006
Stem Hair Density	-0.045	0.407
Total Capitula	-0.087	-0.554
Phyllary Hair Length	-0.315	0.057
Basal Leaf Length	-0.346	0.109
Basal Leaf Width	-0.306	0.084
Basal Leaf Hair Length	-0.365	0.187
Stem Leaf Length	-0.332	-0.173
Stem Leaf Width	-0.316	-0.253
Stem Leaf Hair Length	-0.362	0.063

Due to strong collinearity between population and mode of reproduction, we analyzed populations using sexual mode as the predictor variable. A PERMANOVA revealed significant variation in basal leaf length, basal leaf width, and basal leaf hair length between apomictic polyploid and sexual diploid plants (df = 1, $R^2 = 0.03534$, F = 5.1293, *P* = 0.032, Fig. 10). We also analyzed sexual diploid populations only using population as the predictor variable. This revealed significant variation in vegetative traits (df = 1, $R^2 = 0.3793$, F = 23.628, *P* = 0.001, Fig. 11). Post-hoc pairwise comparisons (Table 5) returned significant variation between Webster, NC and Snakeskin, AR (*P* adjusted = 0.006), Cedar Cliff, NC and Snakeskin, AR (*P* adjusted = 0.006), and Snakeskin, AR and Culp Rd., AR (*P* adjusted = 0.006). Vegetative traits on plants grown in the greenhouse were significantly different between Webster, NC and Cedar Cliff, NC, but only prior to the adjusted p-value in a pairwise test (*P* = 0.046, Table 5).

Table 5. Post-hoc pairwise comparisons of PERMANOVA results on vegetative traits of sexual diploid *Erigeron* grown in the WCU greenhouse as part of a common garden experiment. Populations include Webster, NC (W), Cedar Cliff, NC (CC), Culp Rd., AR (CULP), and Snakeskin, AR (SNK).

Pairs	R^2	*P*	*P* adjusted
W vs CC	0.06529466	0.046 *	0.276
W vs SNK	0.38678105	0.001 *	0.006 *
W vs CULP	0.01886432	0.370	1.000
CC vs SNK	0.20498851	0.001 *	0.006 *
CC vs CULP	0.06345092	0.116	0.696
SNK vs CULP	0.29452928	0.001 *	0.006 *

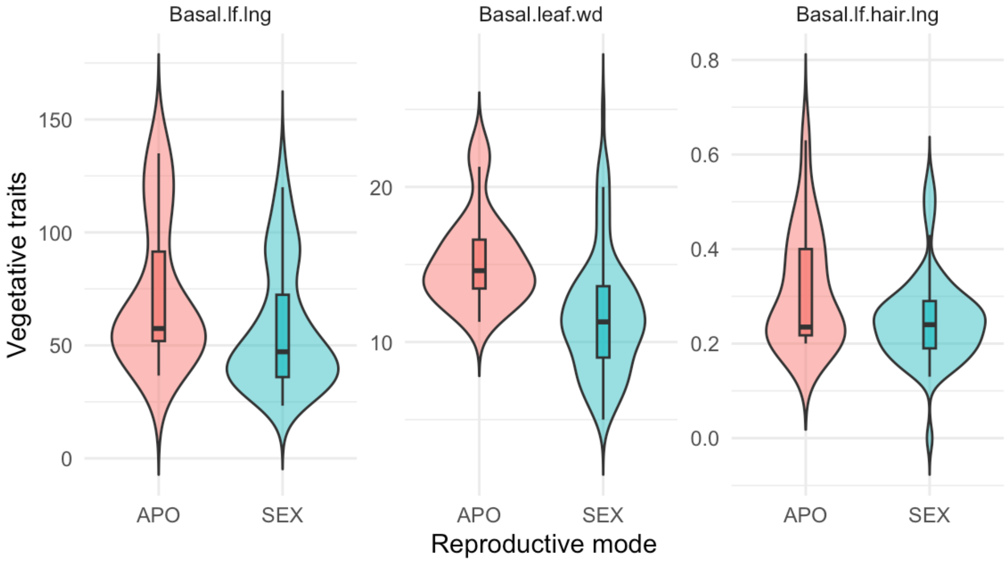

Figure 10. A PERMANOVA conducted on vegetative traits of apomictic polyploid *Erigeron* and sexual diploid *Erigeron* grown in the WCU greenhouse revealed significant differences in basal leaf length, basal leaf width, and basal leaf hair length (df = 1, $R^2 = 0.03534$, F = 5.1293, $P = 0.032$).

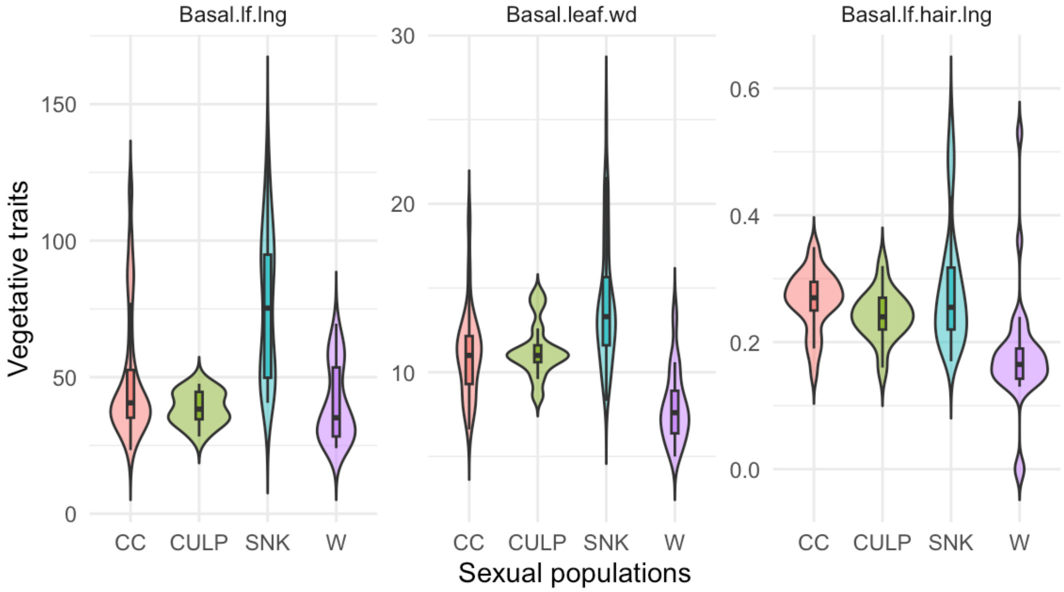

Figure 11. A PERMANOVA conducted on vegetative traits of sexual diploid *Erigeron* grown in the WCU greenhouse revealed significant differences in basal leaf length, basal leaf width, and basal leaf hair length (df = 1, $R^2 = 0.3793$, F = 23.628, $P = 0.001$).

Additionally, we conducted a PERMANOVA on reproductive traits (Table 1) of flowering *Erigeron* grown in the WCU greenhouse from two sexual diploid populations (Webster, Jackson Co., NC and Izzard Co., AR). We found a statistically significant difference between populations (df = 1, $R^2 = 0.40818$, F = 16.553, *P* = 0.001, Table 6).

From these data, individual characters were analyzed using Kruskal-Wallis tests and means of individual traits were visualized using violin plots. Plant height ($\chi^2 = 3.3408$, df = 1, *P* = 0.06758, Fig. 12) and capitula diameter ($\chi^2 = 1.31$, df = 1, *P* = 0.2524, Fig. 13) did not significantly vary between populations. Stem hair length significantly differed between Webster and Snakeskin populations ($\chi^2 = 12.783$, df = 1, *P* = 0.0003498, Fig. 14). While stem hair density shared more overlap in distribution between populations, differences were significant ($\chi^2 = 6.881$, df = 1, *P* = 0.008712, Fig. 15). Additionally, other floral characters were significantly different between populations, notably hair characters including phyllary hair length ($\chi^2 = 8.8152$, df = 1, *P* = 0.004223, Fig. 16) and stem leaf hair length ($\chi^2 = 17.357$, df = 1, *P* = 0.00003097, Fig. 17). Stem leaf length was significantly different between populations ($\chi^2 = 11.703$, df = 1, *P* = 0.000624, Fig. 18). Similarly, stem leaf width was notably different between populations ($\chi^2 = 7.0746$, df = 1, *P* = 0.007819, Fig. 19). For floral count data, number of stems was significantly different among Webster, NC and Snakeskin, AR (GLM, *P* = 0.0369). Interestingly, there was no significant difference in total number of capitula (GLM, *P* = 0.1150). Analysis of vegetative characters revealed significant differences in both basal leaf length ($\chi^2 = 15.286$, df = 1, *P* = 0.00009242, Fig. 20) and basal leaf width ($\chi^2 = 15.158$, df = 1, *P* = 0.00009889, Fig. 21).

Table 6. PERMANOVA results for floral characters measured on sexual diploid *Erigeron* in the WCU greenhouse from Webster, Jackson Co., NC (n = 12) and Snakeskin, Izzard Co., AR (n = 14).

	d.f.	R^2	F	*P*
Population	1	0.40818	16.553	0.001

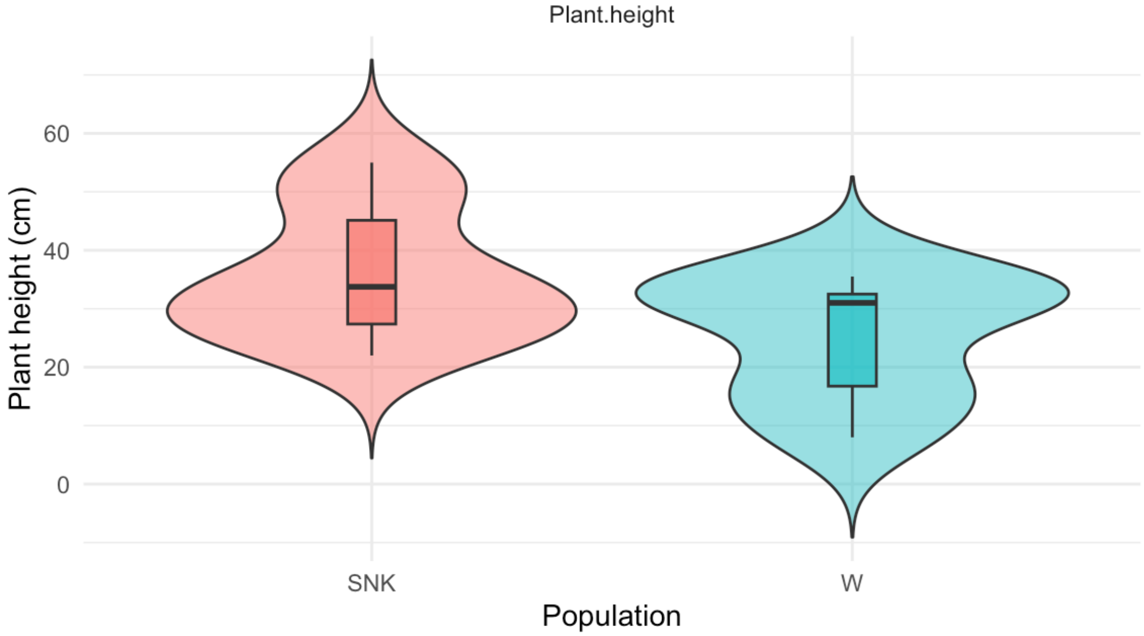

Figure 12. Plant height was not significantly different ($\chi^2 = 3.3408$, df = 1, $P = 0.06758$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

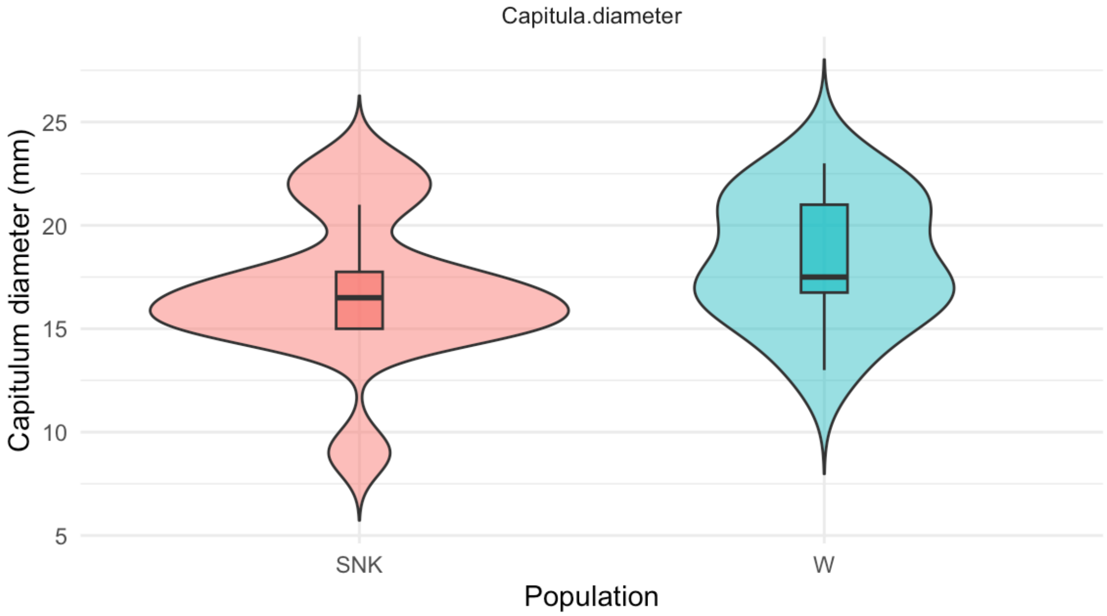

Figure 13. Capitulum diameter was not significantly different ($\chi^2 = 1.31$, df = 1, $P = 0.2524$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

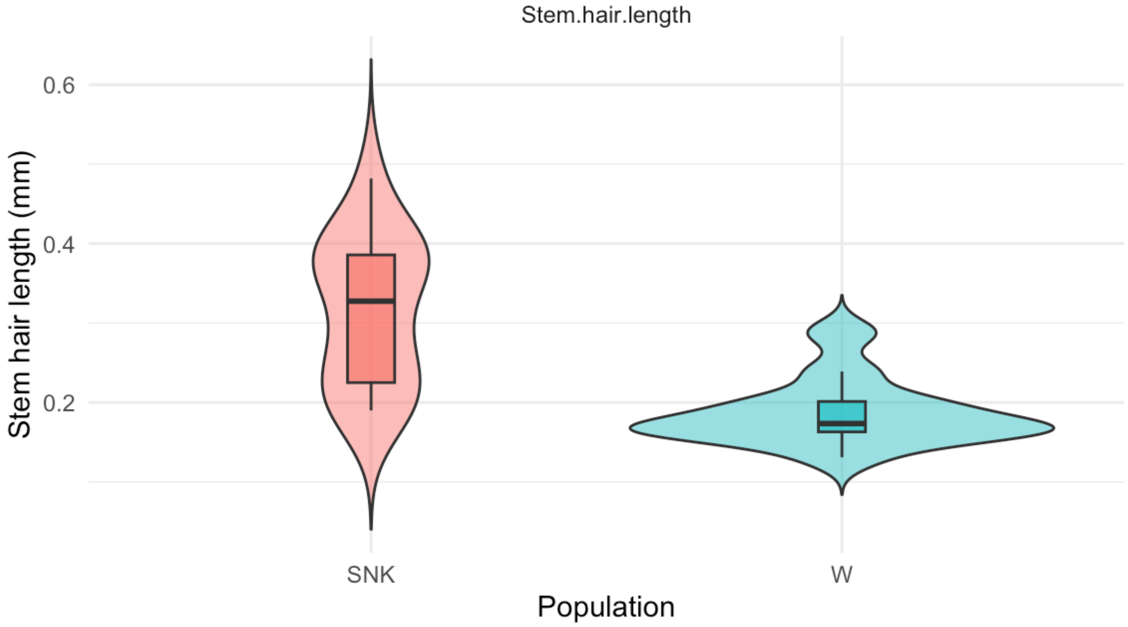

Figure 14. Stem hair length was significantly different ($\chi^2 = 12.783$, df = 1, $P = 0.0003498$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

Figure 15. Stem hair density was significantly different ($\chi^2 = 6.881$, df = 1, $P = 0.008712$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

Figure 16. Phyllary hair length was significantly different ($\chi^2 = 8.8152$, df = 1, $P = 0.004223$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

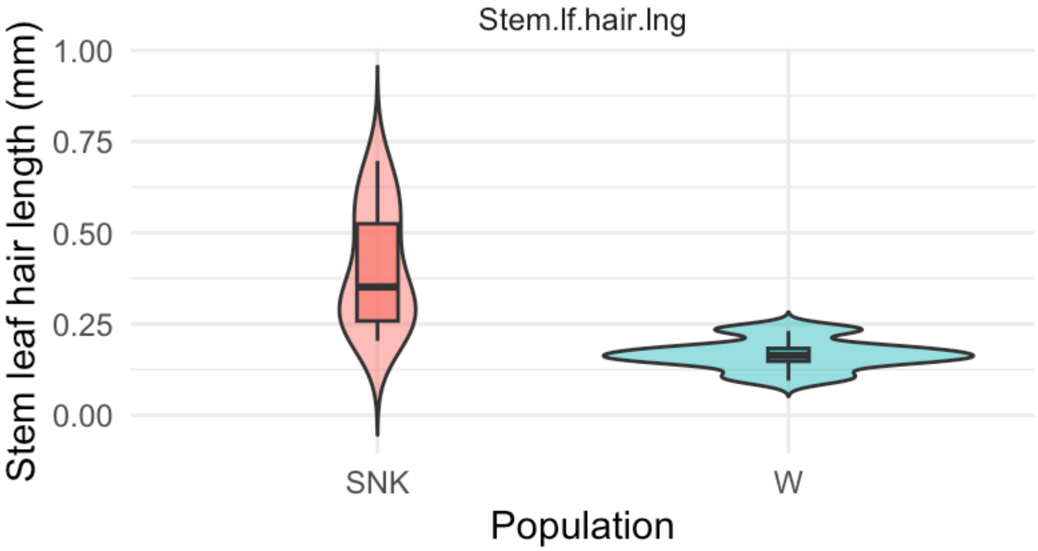

Figure 17. Stem leaf hair length was significantly different ($\chi^2 = 17.357$, df = 1, $P = 0.00003097$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

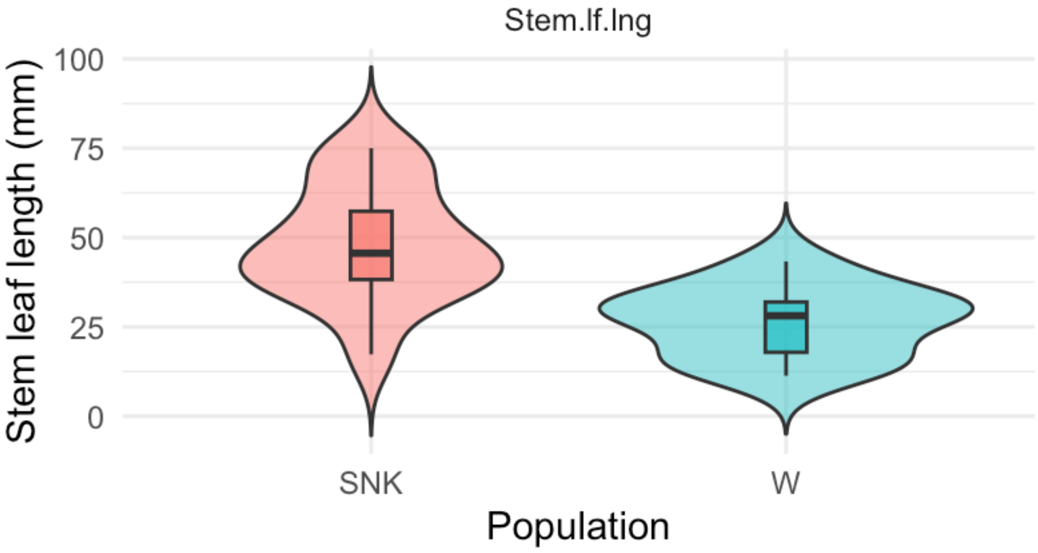

Figure 18. Stem leaf length was significantly different ($\chi^2 = 11.703$, df = 1, $P = 0.000624$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

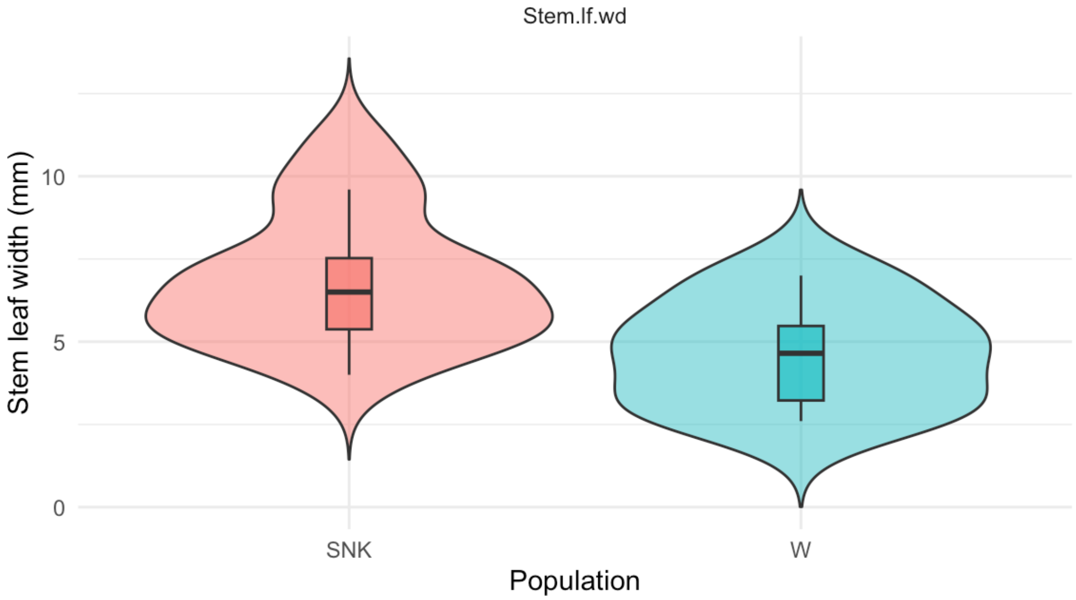

Figure 19. Stem leaf width was significantly different ($\chi^2 = 7.0746$, df = 1, $P = 0.007819$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

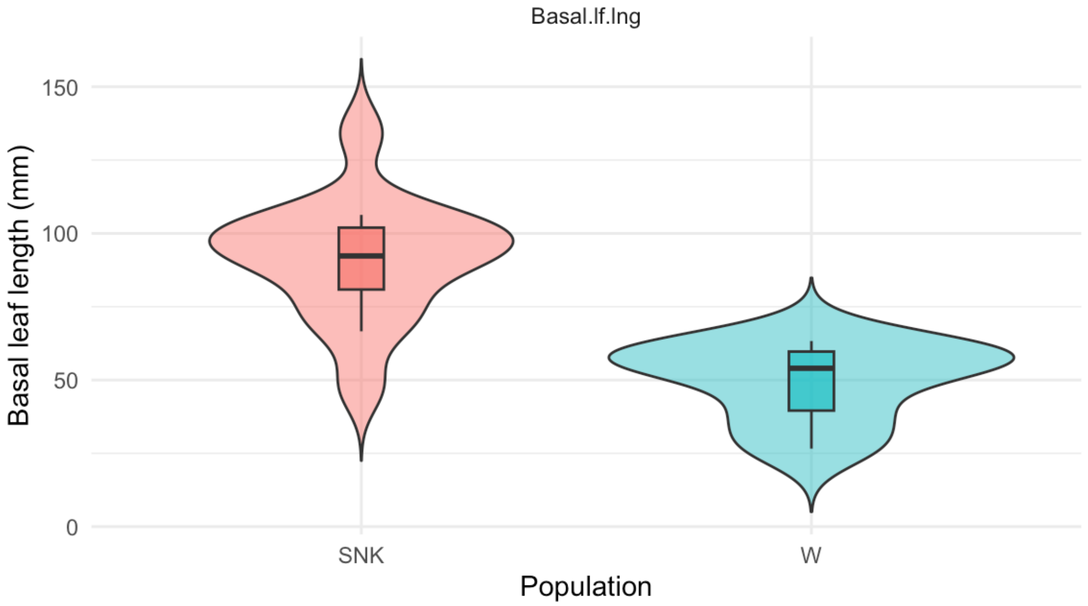

Figure 20. Basal leaf length was significantly different ($\chi^2 = 15.286$, df = 1, $P = 0.00009242$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

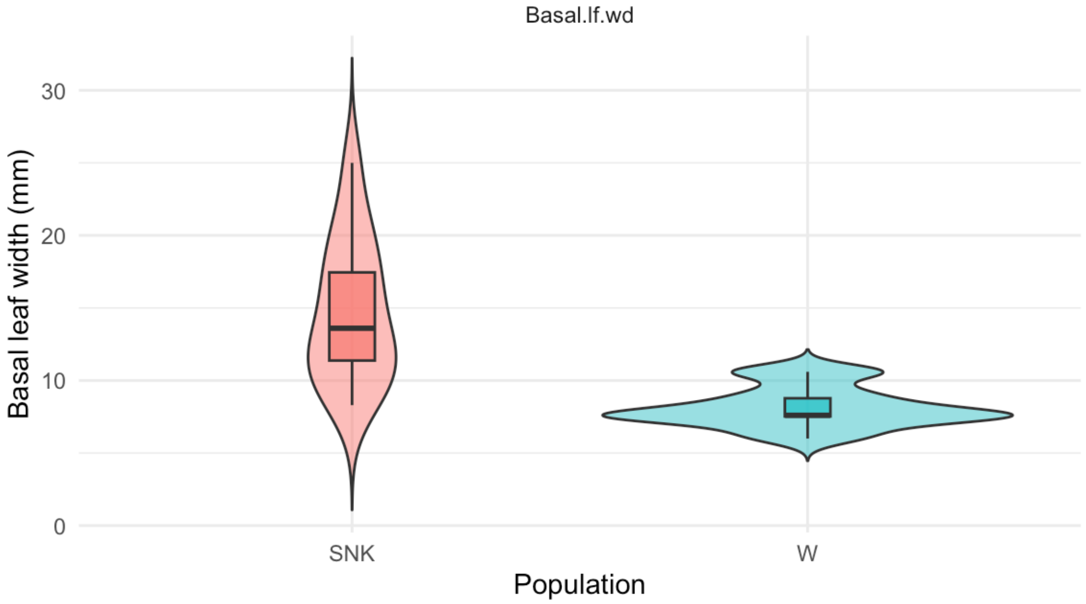

Figure 21. Basal leaf width was significantly different ($\chi^2 = 15.158$, df = 1, $P = 0.00009889$) in *Erigeron* plants measured in the greenhouse for two sexual diploid populations: Snakeskin (SNK) and Webster (W).

Genetic Analysis

A Principal Coordinates Analysis (PCoA) conducted with *Erigeron* samples from Webster and Cedar Cliff shows clear distinction between populations (Fig. 22). The PCoA also shows greater genetic variation within the Cedar Cliff population than within the Webster population.

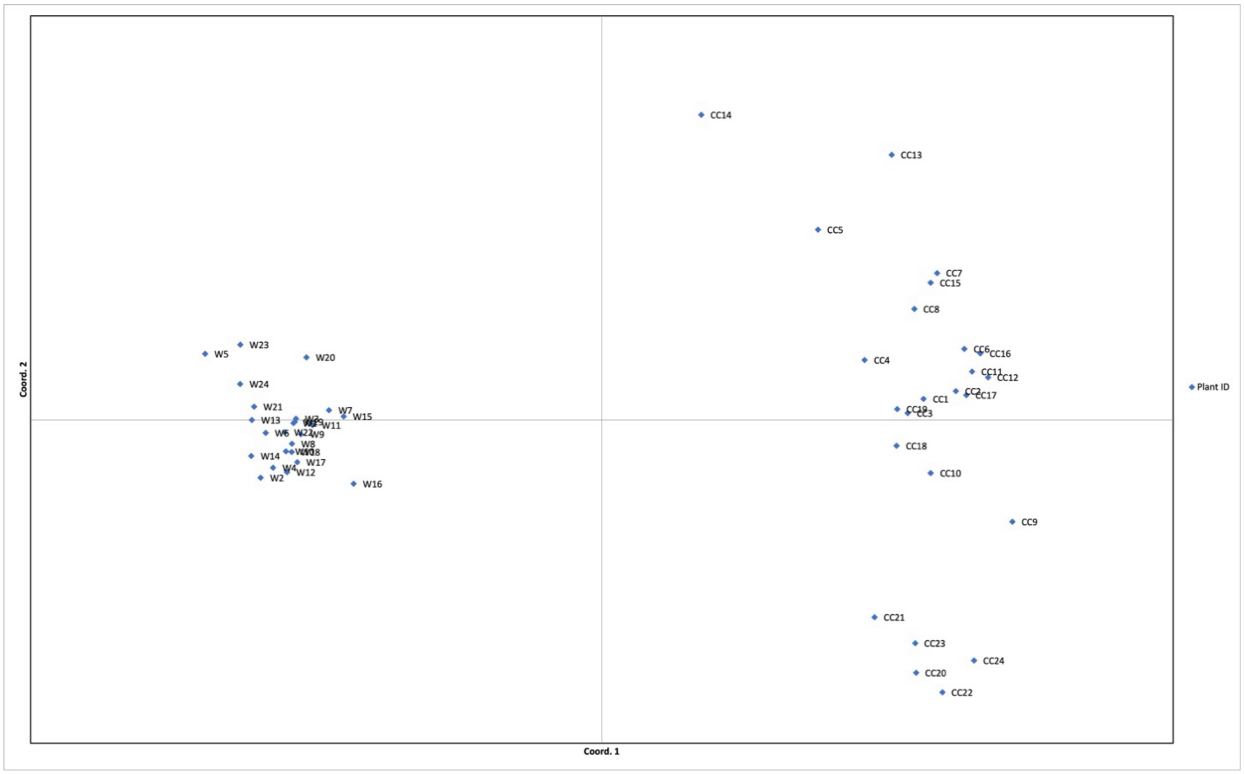

Figure 22. Principal Coordinates Analysis of genetic distances for 4 AFLP primers spanning 2,225 loci. W: sexual diploid individuals from Webster, Jackson Co., North Carolina, and CC: sexual diploid individuals from Cedar Cliff Nature Preserve, Jackson Co., North Carolina.

Additionally, genetic distance data of 254 loci from 1 AFLP primer imply species distinction of North Carolina populations as compared to both sexual diploid and apomictic polyploid populations in Arkansas (Fig. 23). This PCoA shows Webster and Cedar Cliff populations as genetically distinct from one another and from other populations within the *Erigeron* species complex. For comparison against another species within this complex, genetic data for a population of apomictic polyploid *Erigeron annuus* (Pop1) and a population of apomictic polyploid *Erigeron strigosus* (Pop2) from Arkansas were included for comparison against sexual diploid *Erigeron*. Sexual diploid *Erigeron strigosus* populations from Arkansas (Pop4, Pop5, Pop6, and Pop8) and Alabama and Georgia (Pop7) were included for additional comparison.

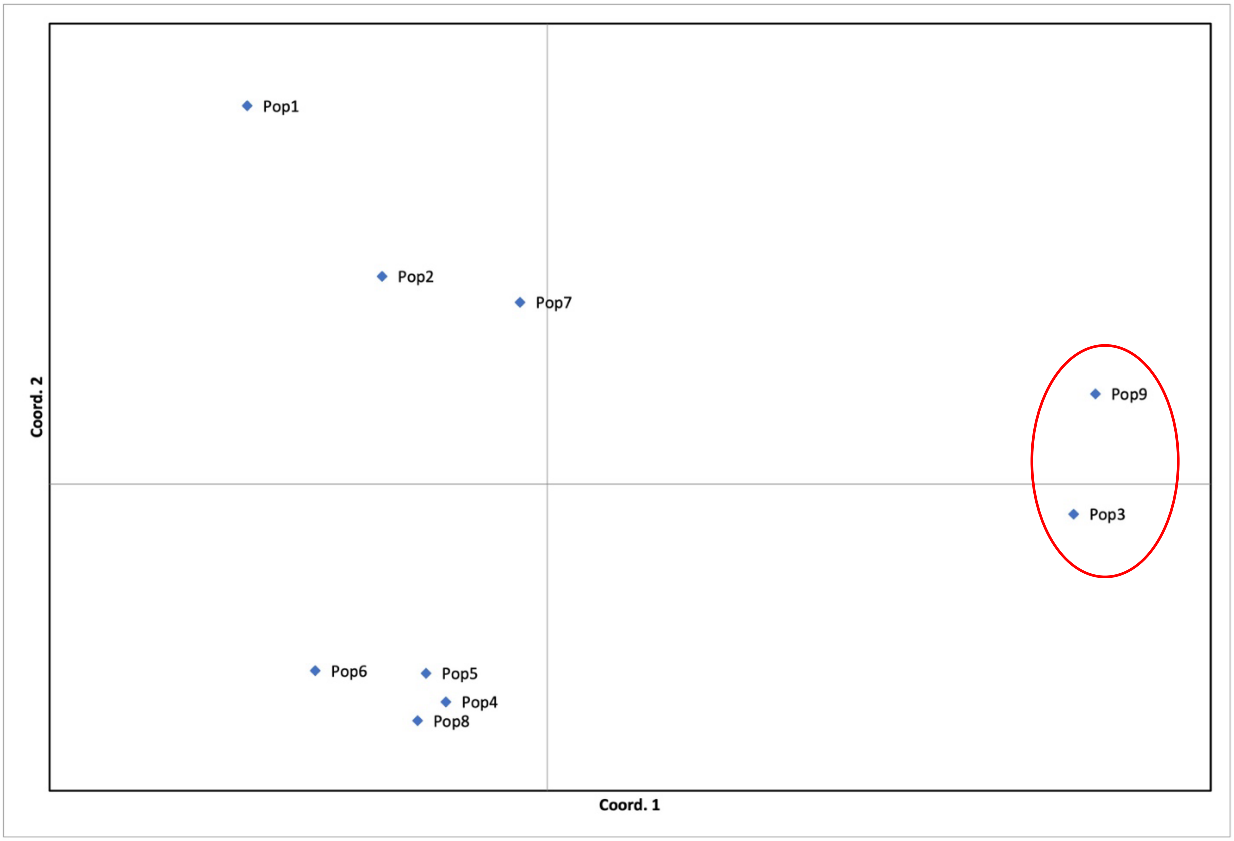

Figure 23. Combined genetic distance data for 1 AFLP primer where Pop1: apomictic *E. annuus* (AR), Pop2: apomictic *E. strigosus* (AR), Pop3: sexual *Erigeron* (Cedar Cliff, NC), Pop4, Pop5, Pop6: sexual *E. strigosus* populations (AR), Pop7: sexual *E. strigosus* populations (AL, GA), Pop8: sexual *E. strigosus* (AR), Pop9: sexual *Erigeron* (Webster, NC). Jackson County, NC populations circled in red.

CHAPTER FOUR: DISCUSSION

The goals of this study were to 1) determine whether two North Carolina sexual, diploid, populations of *Erigeron strigosus* were morphologically and genetically distinct from each other and from sexual diploid populations found elsewhere and 2) to clarify the species status of these populations based on these data. Morphological data in the field and greenhouse in conjunction with genetic AFLP data indicate that the Webster population likely represents an as yet undescribed species of *Erigeron*. Although the *E. strigosus* species complex is geographically widespread and highly variable in morphology, ploidy, and mode of reproduction, there is enough evidence to indicate species distinction in this population. Field-collected data on Webster and Cedar Cliff populations revealed distinct morphological variation in both floral and reproductive characters. Furthermore, both multivariate analysis of quantitative traits measured in the field and traits on living specimens grown in the common garden experiment indicate significant variation between the Webster population and sexual diploid populations in Arkansas.

For the Cedar Cliff population, it is difficult to say whether this same trend will appear in the common garden experiment because very few plants from this population have flowered at this time. The few Cedar Cliff plants that have flowered have not been included in multivariate analyses, other than a PCA (Fig. 9), due to small sample size. However, based on vegetative traits alone, Cedar Cliff plants more closely resemble similar sexual diploid glade populations from Arkansas when grown in a common environment (Fig. 11).

Moreover, genetic distance data reveal Webster and Cedar Cliff to be both distinct from one another (Fig. 22) and from apomictic polyploid and sexual diploid populations of *E. strigosus* in the southeast (Fig. 23). These Western North Carolina populations are genetically distinct from apomictic polyploid populations of *E. annuus*, another closely related species within this complex. Preliminary analysis of ITS and ETS regions revealed these populations to be representative of two different haplotype groups, supporting my conclusions from AFLP data (R. Noyes, K. Mathews, & D. Poindexter, unpubl. data). Including genetic data from other sexual diploid *E. strigosus* populations in Western North Carolina, as well as apomictic polyploids, would provide further insight into the rarity of these populations.

Analysis of morphological data of geographically separate populations in combination with greenhouse data in a common garden experiment has resulted in a cohesive picture of Webster and Cedar Cliff populations. Because only a handful of Cedar Cliff plants have flowered in the greenhouse, it was not possible to include these individuals in the majority of multivariate statistical analyses conducted in this study. Plant height as measured by the tallest flowering stem was significantly different between Webster and Cedar Cliff plants in the field, however, Webster plants were not different when compared to Snakeskin plants in the greenhouse. Webster plants grown in the greenhouse have comparable mean plant heights with plants in the field (30 cm, Fig. 5, Fig. 12). Capitula diameter did not vary significantly between Webster and Cedar Cliff plants in the field or between Webster and Snakeskin plants grown in the greenhouse, however, Webster plants grown in the common garden maintained a similar mean capitula diameter (Fig. 6, Fig. 13). Interestingly, the PCAs revealed an apparent trade-off between total number of capitula and capitula size in both Webster and Cedar Cliff plants in the field (Fig. 3, Fig. 4). Webster plants are smaller overall, so a higher reproductive output via increased capitula number could be indicative of a reproductive adaptation to compensate for reduced plant size.

Although the small sample size of Cedar Cliff plants greatly restricts comparisons in floral traits for greenhouse data, we were able to analyze vegetative traits on greenhouse plants from Webster, Cedar Cliff, Snakeskin, and Culp. Webster plants were significantly smaller than Cedar Cliff plants in the field as measured by basal leaf length and width (Fig. 7, Fig. 8). When Webster plants were compared against Snakeskin plants grown in the greenhouse, basal leaves were significantly smaller (Fig. 20, Fig. 21). Because much of the morphological variation observed in the Webster population in the field was maintained in the common garden experiment, I believe the Webster population constitutes a distinct species under the Unified Species Concept.

Other characters measured on plants grown in the common garden were not measured on individuals growing in wild populations included in this study. Micromeasurements including stem hair length, stem hair density, phyllary hair length, and stem leaf hair length were only measured on greenhouse plants. Additionally, stem leaf measurements were recorded for greenhouse plants, but not plants in the field. Including these data would provide further insight into which characters are associated with local adaptation in the Webster population. Length of stem hairs, phyllary hairs, and leaf hairs has been documented as a strong distinguishing character trait among species (Nesom 2004, Nesom & Murray 2004). Future studies should collect leaf samples and capitula from populations in nature to photograph for inclusion in analysis of these characters.

Previous floral surveys recorded peak flowering time at the Webster population from late April to late May (K. Mathews, D. Poindexter, J. Costa, R. Noyes, unpubl. surveys), while Cedar Cliff *Erigeron* reach peak flowering a few weeks later. In late May of 2023, many Cedar Cliff buds had yet to open, while we had already collected seed from Webster plants at this time. While the PCAs showed that Webster plants had a greater percentage of total capitula including buds at time of collection (Fig. 3, Fig. 4), phenology differences of a few weeks at different locations could easily be due to variation in local conditions. In the common garden experiment, phenology of the first growing period could not be accurately recorded due to plants being repotted at different life stages. We turned off the grow lights in the greenhouse for a 6-week period before turning them on again to mimic an over-winter dormancy period in attempt to induce flowering in more individuals. Using day one of the lights being turned on after the 6-week dormancy period as the first day of the growing "season," I have been recording the dates of new plants as they flower. These data will inform conclusions as to whether phenological variation observed in Webster and Cedar Cliff populations in the field will follow similar patterns when grown in a common garden. I noticed anecdotally that many of the first plants to flower in the common garden were Webster plants, but currently there is not enough data to accurately assess this pattern.

Genetic data for the Webster population indicate relatively lower genetic diversity as compared to Cedar Cliff plants. Plants at the Webster population grow abundantly and uniformly in soil patches among the exposed rock, so high levels of inbreeding can be expected. Conversely, AFLP data show high genetic diversity at the Cedar Cliff population likely due to the pattern in which plants grow at the site. Cedar Cliff plants are scattered in soil patches throughout large areas of bare rock. We sampled 5 patches at this population: Patch 1 included individuals CC 1-5, Patch 2 included individuals CC 6-10, Patch 3 included individuals CC 11-17, Patch 4 included individuals CC 18-22, and Patch 5 included individuals CC 23-24. With the exception of a small number of outliers including individuals 5, 13, and 14, the genetic distance data show distinctive clustering of genetically similar plants. Geographic distance analysis between patches will reveal whether patches closely together in space correspond to genetic similarity.

Notably, polyploid populations of *E. strigosus* are mostly geographically separate from the diploid populations, with little overlap (Noyes 2007). Under the Unified Species Concept, this separation is a strong indicator of a distinct evolutionary lineage. Sexual diploid plants well-adapted to the harsh conditions found in glades or barrens could have permitted colonization to the more diverse habitats occupied by apomictic polyploid plants. These sexual diploid lineages may have evolved unique ecological niche differences as compared to their polyploid relatives that could be used as additional evidence of speciation. Soil analysis of local apomictic polyploid populations could reveal patterns between soil composition and habitat suitability for polyploids as compared to diploids. While polyploids in this complex are triploid or tetraploid, polyploid *E. strigosus* are predominantly triploid (Noyes & Givens 2013). Triploid populations could represent the derived condition, as triploids would be expected from a tetraploid-diploid cross. Interestingly, studies have found that many apomictic plants retain the ability to reproduce sexually and are therefore facultative (Asker & Jerling 1992, Richards 2003). It is possible that plants may effectively "choose" the most beneficial mode of reproduction based on current environmental conditions. It has been hypothesized that the ability of plants to reproduce asexually with occasional sexual reproduction when needed could provide plants with superior reproductive fitness (Freeling 2017).

Based on consistent morphological differences in floral and vegetative morphology observed in the field and in the common garden in tandem with genetic data, I have determined that the Webster population of *Erigeron* is worthy of recognition as a distinct species within the *E. annuus*-*E. strigosus* species complex. After examination of Cedar Cliff plants grown in the greenhouse as compared to other sexual diploid populations included in this study, I did not find sufficient evidence to support Cedar Cliff as an additional undescribed species. I believe that the study design of this common garden experiment has minimized environmental variation and that differences observed between populations are reflective of real variation among plants. Future work should include a robust molecular analysis that could confirm independently evolving lineages. Additionally, crossing experiments could determine reproductive viability of triploid offspring between a tetraploid and diploid cross. AFLP data can complement other DNA sequencing markers, such as RADSeq, in phylogenetic studies by providing resolution in different parts of the tree, however, such inferences may become inaccurate when genetic divergence is too high. The recognition of at least one distinct species of *E. strigosus* in North Carolina has been previously overlooked and formal recognition of a new species status can provide further insight into the vast diversity of diploids within the *E. annuus*-*E. strigosus* species complex.

REFERENCES

Albrecht, J., Peters, M. K., Becker, J. N. *et al.* 2021. Species richness is more important for ecosystem functioning than species turnover along an elevational gradient. Nature Ecology and Evolution. 5:1582–1593. https://doi.org/10.1038/s41559-021-01550-9

Asker, S. E. and Jerling, L. 1992. Apomixis in plants. CRC, Boca Raton, FL.

Avise, J. C. 2004. Molecular markers, natural history, and evolution. 2nd edition. Sunderland (MA): Sinauer Associates, Inc. Chapter 3. Molecular Techniques; p. 94-95. ISBN: 0-87893-041-8

Ampong, K., Thilakaranthna, M. S., Gorim, L. Y. 2022. Understanding the role of humic acids on crop performance and soil health. Frontiers in Agronomy. 4: https://doi.org/10.3389/fagro.2022.848621

Berend, K., Haynes, K., MacKenzie, C. M. 2019. Common garden experiments as a dynamic tool for ecological studies of alpine plants and communities in northeastern North America. Rhodora. 121(987): 174-212.

Baum, D. A. and Donoghue, M. J. 1995. Choosing among alternative "phylogenetic" species concepts. Systematic Botany. 20(4):560-573.

Beckman, N. G., Bullock, J. M., Salguero-Gómez, R. 2018. High dispersal ability is related to fast life-history strategies. Journal of Ecology. 106(4): 1349-1362.

Blanc, G., Barakat A., Guyot, R., Cooke, R., Delseny, I. 2000. Extensive duplication and reshuffling in the *Arabidopsis* genome. Plant Cell. 12: 1093-1101.

Camargo, A. (2022) PCAtest: Testing the statistical significance of Principal Component Analysis in R. PeerJ 10: e12967. doi: 10.7717/peerj.12967

de Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology. Vol 56(6):879-886. https://doi.org/10.1080/10635150701701083

Edwards, P. J., Frey, D., Bailer, H., Baltisberger, M. 2006. Genetic variation in native and invasive populations of *Erigeron annuus* as assessed by RAPD markers. International Journal of Plant Sciences. 167(1):93-101.

Fawcett, J. A., Maere, S., and Van de Peer, Y. 2009. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. Proceedings of the National Academy of Sciences of the United States of America. 106(14): 5737-5742.

Force, A., Pickett, F. B., Amores, A., Yan, Y. L., Postlethwait, J. 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics. 151: 1531-1545.

Fox, J. and Weisberg, S. (2019). An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Freeling, M. 2017. Picking up the ball at the K/Pg boundary: The distribution of ancient polyploidies in the plant phylogenetic tree as a spandrel of asexuality with occasional sex. The Plant Cell. 29(2): 202-206.

Freudenstein, J. V., Broe, M. B., Folk, R. A., Sinn, B.T. 2017. Biodiversity and the species concept—lineages are not enough. Systematic Biology. 66(4): 644-656.

Goldblatt, P. 1980. Polyploidy in angiosperms: Monocotyledons. In W. H. Lewis [ed.], Polyploidy: Biological relevance, 219-239. Plenum Press, New York, NY, USA.

Grant, V. 1963. The origin of adaptations. Columbia University Press, New York, NY, USA.

Grant, V. 1981. Plant speciation, 2nd ed. Columbia University Press, New York, NY, USA.

Hillis, D. M. 1987. Molecular versus morphological approaches to systematics. Annual Review of Ecology and Systematics. 18:23-42.

Holland, B. R., Clarke, A. D., Meudt, H. M. 2008. Optimizing automated AFLP scoring parameters to improve phylogenetic resolution. Systematic Biology. 57(3): 347-366.

Howe, H. F., & Smallwood, J. 1982. The ecology of seed dispersal. Annual Review of Ecology and Systematics. 13: 201-228.

Jaillon, O., Aury, J., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., et al. 2007. The grapevine genome sequence suggests hexaploidization in major angiosperm phyla. Nature. 449: 463-467.

Kassambara A, Mundt F (2020). _factoextra: Extract and Visualize the Results of Multivariate Data Analyses_. R package version 1.0.7, <https://CRAN.R-project.org/package=factoextra>.

Lenth R (2023). _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.8.5, https://CRAN.R-project.org/package=emmeans.

León-Martínez, G. and Vielle-Calzada, J. P. 2019. Apomixis in flowering plants: Developmental and evolutionary considerations. Current Topics in Developmental Biology. Plant Development and Evolution. 131: 565-604. https://doi.org/10.1016/bs.ctdb.2018.11.014

Lewis, W. H. 1980. Polyploidy in angiosperms: Dicotyledons. In W. H. Lewis [ed.], Polyploidy: Biological relevance, 241-268. Plenum Press, New York, NY, USA.

Levin, D. A. and Soltis D. E. 2018. Factors promoting polyploid persistence and diversification and limiting diploid speciation during the K-Pg interlude. Current Opinion in Plant Biology. 42:1-7.

Li, Y., Fang, F., Wei, J., Wu, X., Cui, R., Li, G., Zheng, F., Tan, D. 2019. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: a three-year experiment. Scientific Reports. 9: 12014. https://doi.org/10.1038/s41598-019-48620-4

Luna, T, Corey, S, Evans, J, Wick, D, Hosokawa, J. 2008. Propagation protocol for production of container *Erigeron peregrinus* (Pursh) Greene plants. Native Plant Network [internet]. West Glacier(Montana): United States Department of Agriculture. [cited 2023 Feb 27]. Available from: https://npn.rngr.net/renderNPNProtocolDetails?selectedProtocolIds=asteraceae-erigeron-29&referer=wildflower

Lynch, M. and Conery, J. S. 2000. The evolutionary fate and consequences of duplicate genes. Science. 290(5494): 1151-1155. doi:10.1126/science.290.5494.1151.

Maclaurin, J. and Sterelny, K. 2008. What is biodiversity? 1st edition. Chicago (IL): The University of Chicago Press. ISBN: 978-0-226-50081-2

Martinez Arbizu P (2017). _pairwiseAdonis: Pairwise Multilevel Comparison using Adonis_. R package version 0.4.1.

Miller III, R. 1953. The Webster-Addie ultramafic ring, Jackson County, North Carolina, and secondary alteration of its chromite. *American Mineralogist* 38: 1134–1147.

Nesom, G. L. *Erigeron*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 20. http://floranorthamerica.org/Erigeron. Accessed [August 30, 2023].

Nesom, G. L. 2004. Taxonomic reevaluations in North American *Erigeron* (Asteraceae: Astereae). SIDA, Contributions to Botany. 21(1): 19-39.

Nesom, G. L. and Murray, D. F. 2004. Notes on North American arcitic and boreal species of *Erigeron* (Asteraceae: Asterae). SIDA, Contributions to Botany. 21(1): 41-57.

Noyes. R. D. 2006. Intraspecific nuclear ribosomal DNA divergence and reticulation in sexual diploid *Erigeron strigosus* (Asteraceae). American Journal of Botany. 93(3): 470-479.

Noyes, R. D. 2007. Reticulation and the evolution of apomixis in *Erigeron* sect. *Phalacroloma* (Asteraceae) In: Hörandl, E., U. Grossniklaus, P. Van Dijk, T. Sharbel, eds. Apomixis: evolution, mechanisms and perspectives. Regnum Veg. 147. Gantner Verlag, Ruggell, Liechtenstein. Pp. 337-358.

Noyes, R. D. 2022. Mendelian segregation for parthenogenetic embryo development at the diploid level in the flowering plant *Erigeron*. American Journal of Botany. 109(10):1-11. https://doi.org/10.1002/ajb2.16071

Noyes, R. D. and Allison, J. R. 2005. Cytology, ovule development, and pollen quality in sexual *Erigeron strigosus* (Asteraceae). International Journal of Plant Sciences. 166(1):49-59.

Noyes, R. D., Gerling, H, and Vandervoort, C. 2006. Sexual and apomictic prairie fleabane (*Erigeron strigosus*) in Texas: Geographic analysis and a new combination (*Erigeron strigosus var. traversii*, Asteraceae). Contributions to Botany. 22(1):256-276.

Noyes, R. D. and Givens, A. D. 2013. Quantitative assessment of megasporogenesis for the facultative apomicts *Erigeron annuus* and *Erigeron strigosus* (Asteraceae). International Journal of Plant Sciences. 174(9): 1239-1250.

Noyes, R. D. and Groff, D. V. 2011. Geography and phylogeny of sexual diploid *Erigeron strigosus* (Asteraceae) in Arkansas and Missouri. Journal of Botanical Research Institute of Texas. 5(2):577-586.

Noyes, R. D. and Poindexter, D. 2025. *Erigeron beyrichii* (Asteraceae), a new combination and redefinition of a widespread taxon in the southeastern United States. Journal of the Botanical Research Institute of Texas. 19(1): 27-33. https://doi.org/10.17348/jbrit.v19.i1.1389

Noyes, R. D. and Rieseberg, L. H. 2000. Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. Genetics. 155(1): 379-390.

Noyes, R. D. and Wagner, J. D. 2014. Dihaploidy yields diploid apomicts and parthenogens in *Erigeron* (Asteraceae). American Journal of Botany. 101(5): 865-874.

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022). _vegan: Community Ecology Package_. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.

Paterson, A. H., Bowers, J., Burow, M., Draye, X., Elsik, C., Jiang, C., Katsar., C. Lan, T., Lin, Y., Ming, R., Wright, R. J. 2000. Comparative genomics of plant chromosomes. Plant Cell. 12(9): 1523-1540.

Paterson, A. H., Bowers, J. E., Chapman B. A. 2004. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proceedings of the National Academy of Sciences. 101: 9903-9908.

Paun, O. and Schönswetter P. 2012. Amplified Fragment Length Polymorphism (AFLP) – an invaluable fingerprinting technique for genomic, transcriptomic and epigenetic studies. Methods in Molecular Biology. 862:75-87. doi: 10.1007/978-1-61779-609-8_7.

Peakall, R. and Smouse, P. E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6, 288-295.

Peakall, R. and Smouse, P. E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 28, 2537-2539.

Pittillo, J. D. 1994. Natural areas inventory for Jackson County, North Carolina. A report to the Conservation Trust for North Carolina, Jackson County Department of Planning and Development, State of North Carolina Natural Heritage Program.

Pringle's Fleabane. 2016. U.S. Forest Service [internet]. Washington(DC): United States Department of Agriculture. [cited 2023 Feb 26]. Available from: https://www.fs.usda.gov/wildflowers/beauty/Sky_Islands/climatechanges/erigeron_pringlei.shtml

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
Richards, A. J. 1997. Plant breeding systems. 2nd ed. Chapman & Hall, London.

Saha, U. 2014. Cation exchange capacity and base saturation. University of Georgia Extension. https://extension.uga.edu/publications/detail.html?number=C1040&title=cation-exchange-capacity-and-base-saturation

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 9(7):671–675. doi:10.1038/nmeth.2089

Sheth, S. N., Morueta-Holme, N., Angert, A. L. 2020. Determinants of geographic range size in plants. New Phytologist. 226(3): 650-665.

Simpson, G. G. 1951. The species concept. Evolution. 5(4):285-298.

Slowikowski K (2023). _ggrepel: Automatically Position Non-Overlapping Text Labels with 'ggplot2'_. R package version 0.9.3, <https://CRAN.R-project.org/package=ggrepel>.

Soltis, P. S. and Soltis, D. E. 2000. The role of genetic and genomic attributes in the success of polyploids. Proceedings of the National Academy of Sciences of the United States of America. 97(13): 7051-7057.

Stratton, D. A. 1991. Life history variation within populations of an asexual plant, *Erigeron annuus* (Asteraceae). American Journal of Botany. 78(5): 723-728.

Stuessy, T. and Weiss-Schneeweiss, H. 2019. What drives polyploidization in plants? The New Phytologist. 223(4): 1690-1692.

Thomson, F. J., Moles, A. T., Auld, T. D., Kingsford, R. T. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. Journal of Ecology. 99(6): 1299-1307.

Tuskan, G. A., DiFazio S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten U., Putnam, N., et al. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science. 313: 1596-1604.

Velasco, R., Zharkikh, A., Troggigo, M., Cartwright, D. A., Cestaro, A., Pruss, D., Pindo, M., et al. 2007. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE. 2: e1326, doi:10.1371/journal.pone.0001326.

Venable, D. L. & Brown, J. S. 1988. The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. The American Naturalist. 131: 360-384.

Vision, T. J., Brown, D. G., Tanksley, S.D. 2000. The origins of genomic duplications in *Arabidopsis*. Science. 290: 2114-2117.

Weakly, A. S., Poindexter, D. B., LeBlond, R. J., Sorrie, B. A., Karlsson, C. H., Williams, P. J., Bridges, E. L., et al. 2017. New combinations, rank changes, and nomenclatural and taxonomic comments in the vascular flora of the Southeastern United States. II. Journal of Botany Research Institute Texas. 11: 291-325.

Whitton, J., Sears, C. J., Baack, E. J., Otto, S. P. 2008. The dynamic nature of apomixis in the angiosperms. International Journal of Plant Sciences. 169(1): 169-182.

Wickham, H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Yuan Tang, Masaaki Horikoshi, and Wenxuan Li. "ggfortify: Unified Interface to Visualize Statistical Result of Popular R Packages." The R Journal 8.2 (2016): 478-489.