Pollination Biology of the Endemic *Erigeron lemmonii* A. Gray, and its Insect Visitor Networks Compared to two Widespread Congeners *Erigeron arisolius* G.L. Nesom and *Erigeron neomexicanus* A. Gray (Asteraceae)

by

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ABSTRACT

POLLINATION BIOLOGY OF THE ENDEMIC *ERIGERON LEMMONII* A. GRAY AND ITS INSECT VISITOR NETWORKS COMPARED TO TWO WIDESPREAD CONGENERS *ERIGERON ARISOLIUS* G.L.NESOM AND *ERIGERON NEOMEXICANUS* A.GRAY (ASTERACEAE)

Pamela Bailey **Advisors: Dr. Peter Kevan**
University of Guelph, 2013 **Dr. Paul Voroney**

This thesis is an investigation of network analysis to understand differences between how three congeners have adapted pollination network strategies to ensure adequate gene flow. This study will focus on three species of *Erigeron* (Fleabanes), one endemic species adapted to cliff wall habitat in one canyon fragmented by topography (*E. lemmonii*) at Fort Huachuca Military Reserve, and two others (*E. arisolius and E. neomexicanus*) adapted to more diverse habitat conditions in a larger range in Arizona, USA. Sustainability of military lands and protected species are primary concerns for Army land management, and these findings will be made available to the U.S. Army, and U.S. Fish and Wildlife Service resource managers to use in their respective management plans.

UCINET software was used to construct the insect flower visitor networks for the three *Erigeron* species by recording insect visitors / plant interactions and comparing their visitation networks to each other. *Erigeron arisolius* and *E. neomexicanus* have redudndant network architecture, compared to *E. lemmonii* which has a fragile network supporting a unique insect community. If the *E. lemmonii* population disappears, a collapse of its dependent insect visitors may also occur. Other new botanical information was discovered and recorded for *E. lemmonii.* It has a xenogamous mating system, and can also reproduce by vegetative means. An individual capitulum has a three week flowering period, and the population has individual plants blooming over a six month flowering season with May being the peak. It grows in highly organic soil on cliff face crevices in the Scheelite Canyon. Another aspect was to develop and publish genetic microsatellite markers for the three species of *Erigeron,* which are the first microsatellite markers to be identified for this genus. This included determining the chromosome number of *E. lemmonii,* before the genetic markers could be identified for this species. Eight microsatellite markers were identified for *E. lemmonii* and nine markers were identified for *E. arisolius.* However, no markers for *E. neomexicanus* were identified because of confounding results. *Erigeron lemmonii* has less genetic diversity, lower mean heterozygosity and fewer alleles, than *E. arisolius*.

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I acknowledge and thank Ms. Denise Lindsay, Dr. Christine Edwards, and Dr. Richard Lance, geneticists involved in the project from the Environmental Laboratory, US Army Engineer Research and Development Center, responsible for the development of the microsatellite markers and genetic analysis. They prepared and published the manuscript for the technical note on the microsatellites for *E. lemmonii* and *E. arisolius*, and published the microsatellites in GenBank®. Special thanks go to Dr. Neil Cobb, professor at Northern Arizona University Flagstaff, Arizona, (NAU), and three students who diligently worked on the insect collecting, and identification: Ms. Kim Whitley, an outstanding field assistant, Mr. Eric Kortenhoeven, and Mr. Jacob Higgins who was also primarily responsible for the insect identification, and the NMS and indicator insect analyses. Neil Cobb is also the curator of the Colorado Plateau Biodiversity Center, NAU, oversaw the proper identification of all insects and was responsible for the curation of the insect collection.

I thank Dr. Deborah Shafer for friendship and assistance with the Origin Pro 8.6 software; Dr Dave Nelson for review, friendship and positively motivating me; Dr. Richard Noyes for his help in obtaining the chromosome number, advice, sharing literature, lab and greenhouse facilities, and his expertise of *Erigeron*; Dr.David Bogler of the Missouri Botanical Garden for access to the lab, and use of the microscopes with camera attachments, and the electron scanning microscope. I acknowledge and thank Mr. Dick Kasul and Mr. Dale Magoun for their assistance and review of statistics. I also thank my friend Mr.Timothy Cary for the use of his lab to analyze the root architecture at the Cold Regions Research and Engineering Laboratory, N.H.

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List of Symbols and Abbreviations

ERDC Engineer Research and Development Center
DoD Department of Defense
USFWS United States Fish and Wildlife Service
CANPOLIN, Canadian Pollination Initiative

PERMISSIONS

This section describes permission for articles published, or articles in formal journal review, and the roles of co- authors of each article/chapter are described herein. This was a large funded research project and I had others involved in various aspects of the research project work.

CHAPTER 1: Literature review, goals, general methods and hypotheses. Pamela Bailey

CHAPTER 2: Pollination Attributes, Flowering period, and Mating System of *Erigeron lemmonii* A. Gray, Lemmon's Fleabane (Asteraceae)

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Roles: I lead the research project, collected and analyzed the data. I am the primary author and will be publishing this chapter in the future (tentatively in *Botany*).

Kim Whitley collected insect data with me for two seasons, identified most of the insects in 2010, and was a great field assistant. She also taught me not to fear tarantulas!

Peter Kevan assisted in the development of the work described within this chapter, and provided review. He also provided guidance on the research project as it developed.

CHAPTER 3: Soil and Root Properties of a Rare Cliff-dwelling *Erigeron* species Compared to Two Other *Erigeron* Species

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Roles: I lead the research project, collected the soil samples, and after Extension Service Lab analysis, analyzed the data. I scanned the roots and analyzed this data set. I am the primary author and will be publishing this chapter in the future (tentatively in the journal, *Plant and Soil*). Paul Voroney provided guidance and review of this chapter, and support through-out the research. Peter Kevan provided review, and support through-out the research.

CHAPTER 4: Network analysis of floral insect visitors of the rare *Erigeron lemmonii* compared to more widespread species, *Erigeron arisolius* and *Erigeron neomexicanus* (Asteraceae), in Arizona, USA.

Pamela Bailey, Jacob Higgins, and Neil Cobb

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Roles of all authors: I was the Principle Investigator for the overall research project and botanist. My involvement in this work as the Principle Investigator was the development of the proposal, research design and plan, and responsibility for all financial decisions, and how to carry out the work. As the botanist, I did all fieldwork: set up the sampling plots, collected the plant data, and identified all plants to species. I also constructed the networks using UCINET, and was the primary author of the article. Jacob Higgins assisted in some of the insect collection, was responsible for the insect identification, and the NMS and indicator insect analyses. He is the secondary co-author. Jacob.Higgins@nau.edu. Neil Cobb is a faculty member and the curator of the Colorado Plateau Biodiversity Center, Northern Arizona University (NAU), Flagstaff, Arizona, USA. He oversaw the proper identification of all insects and was responsible for the curation of the collection at NAU. He also reviewed the NMS and indicator species analysis and co-authored the paper. Neil.Cobb@nau.edu.

CHAPTER 5: Isolation and characterization of microsatellite loci for a hyper-rare cliff endemic, *Erigeron lemmonii*, and a more widespread congener, *Erigeron arisolius* (Asteraceae).

This chapter reports the development of the microsatellite markers for two of the three species of *Erigeron*. An article was published for only *Erigeron lemmonii* and *E. arisolius* as:

Denise L. Lindsay, Pamela Bailey, Joseph L. Anderson, Michael G. Jung, Christine E. Edwards, and Richard F. Lance. 2012. Isolation and characterization of microsatellite loci for a hyper-rare cliff endemic, *Erigeron lemmonii*, and a more widespread congener, *Erigeron arisolius* (Asteraceae). Conservation Genetic Resources DOI 10.1007/ s12686-012-9657.

Published online 6 May 2012. The final publication is available at www.springerlink.com.

Roles: My involvement in this work as the Principle Investigator was the development of the proposal, research design and plan, and responsibility for all financial decisions, and how to carry out the work. As the botanist, I did all fieldwork: set up the sampling plots, collected the plant data, and all genetic samples. A contract was prepared with Genetic Identification Services (GIS) to do initial genetic investigation, and prepare genetic libraries for each of the three species in 2010. GIS sent the data to the geneticist at our Environmental Laboratory, U.S.Army Engineer Research and Development Center (ERDC) for further analysis and microsatellite development. Work was on hold until the unpublished chromosome number for *E. lemmonii* was known. In order for the project to move ahead, I worked with Dr. Richard Noyes, a plant geneticist at the University of Central Arkansas who specializes in the genus *Erigeron*, to obtain the chromosome number (to be published in the near future). I am a co-author on this published article with the ERDC geneticists, although I am not the primary author.

Denise Lindsay, Joseph Anderson, Michael Jung, Dr. Christine Edwards, and Dr. Richard Lance were the geneticists involved in the project from the Environmental Laboratory at ERDC responsible for the development of the microsatellite markers. They prepared and published the manuscript for the technical note on the microsatellites for *E. lemmonii* and *E. arisolius*, and published the microsatellites in GenBank® in 2012. *Erigeron neomexicanus* proved problematic in its development and further genetic work was cancelled for this species due to time and money constraints.

CHAPTER 6: Conclusion Pamela Bailey

CHAPTER 1

Literature review, goals, general methods and hypotheses.

INTRODUCTION

The loss and fragmentation of natural habitats by human activities are pervasive in terrestrial ecosystems across the Earth and the main forces behind current biodiversity loss. Habitat loss by fragmentation is increasing every year in the United States of America (USA) where two million acres of native habitat are lost annually, and that rate of loss increases every year (McKinney 2002). Numerous studies have documented declines of plant diversity in response to habitat loss in fragmented landscapes (e.g. Agular *et al*.2006, McEuen and Curran 2005, and Richards 2000). It is well recognized that habitat fragmentation has occurred because of development and invasive species (National Research Council of the National Academies 2006). Once the native plant communities have been altered by invasive species or destroyed altogether by development, the native insect communities and pollinators are the next to be threatened.

Pollination networks have been identified as critical to the survival of many native ecosystems (Kearns and Inouye 1998). In pollination networks, pollinators are more sensitive and quicker to respond than plants to habitat loss, however, the loss of pollinators is proposed to lead to eventual plant extinctions (Taki and Kevan 2007). In small populations, pollinator's abundance and diversity decrease with decreasing plant population size, and may drop to a point at which pollinator service deteriorates (Fritz and Nelson 1994). In small isolated populations, inbreeding and genetic drift have dominant influences on population genetic structure (Richards 2000, Pauw 2007). Plant inbreeding depression, can lead to eventual plant and pollinator extinction (Fritz and Nelson 1994). Gene flow between habitat fragments can be expected to decrease by total or partial loss of pollinators or the inability of the pollinator to carry pollen over large distances. This in turn may lead to increased rates of genetic drift. Genetic drift causes the loss of genetic variation (allelic richness) in populations. As a population becomes smaller, its gene pool becomes smaller within which there is a higher chance of a stochastic event where deleterious alleles become fixed (genetic drift, also known as inbreeding depression). In small isolated populations, inbreeding and genetic drift have dominant influences on population genetic structure (Richards 2000, Pauw 2007). If genetic diversity is lost, phenotypes that may be more adapted to particular environmental conditions and essential for adaption could be lost and therefore make the population more prone to local extirpation. That combination of processes can set in motion an interrelated cascade of extinction events of populations on a regional level (Richards 2000), or extinction vortices linked with plant/pollination losses within a pollination network (Pauw 2007).

Military bases and conservation lands may become fragmented genetic islands with increased development occurring outside many military bases and conservation areas. The loss of species and genetic diversity from restricted genetic pools that result from fragmentation is of crucial concern to military and conservation resource managers across the country. With increased fragmentation there are other associated impacts to the natural resources which challenge management and operations on Army bases. This concern has led to research priorities providing funding to reduce impacts caused by fragmentation.

My project has been funded by the U. S. Army's Basic Research Program to investigate the effects of fragmentation on a rare, endemic plant *Erigeron lemmonii* A. Gray, in comparison to two other more widely spread *Erigeron* species (Fleabane Daisies), *E. arisolius* G.L.Nesom and *E. neomexicanus* A.Gray, and to explore pollination relationships of all three species within a network science framework of small world networks (Jordano et al. 2006).

Region. The term 'sky island' was first used by Weldon Heald in 1967 to describe mountain ranges that are separated from each other by valleys of hot, dry grassland or desert (Austin 2010). There are about 20 sky island complexes on the planet, on all continents except Australia. Most of the literature focuses on the individual mountains, not the importance of valleys between them and little has been written about comparing the island ecosystems (Carlquist 1974, Warshall 1994). Configuations include: stepping stone archipelagos that are composed of mountains and valleys between 2 Cordilleras (4 sky island complexes), isolated massifs with outlying sky islands, linear chains of outlier mountains/ valleys at one end of a cordillera, and completely isolated groups of mountains. Some also include both oceanic islands and sky islands with both salt water and vegetative “seas” (Warshall 1994). The Madrean archipelago is a “stepping stone archipelago” between two mountain chains. Others complexes of this type are the Great Basin archipelago (USA), Altai/Tien Shan Basin (China), and the Messo-American massifs (Central America).

The Madrean archipelago is unique from a planetary perspective (Warshall 1994) for several reasons: it exists between two cordilleras (the Sierra Madre Occidental and the Rocky

Mountains); the archipelago contains approximately 40 Sky Islands between the Mongollon Rim to the Sierra Madre (Warshall 1994). The complex has a north- south orientation but contains an east-west valley bridge through the Deming Gap of the Rocky Mountains; the islands have a mixed geological composition unique in the world; the islands have a high relief (1,524 m); and it is the only sky island complex to straddle two major floristic and two major faunal realms as well as three major climatic zones (tropical, subtropical and temperate). The 181,300 square kilometer (70,000 sq.miles) Madrean Sky Islands region in southeastern Arizona, southwestern New Mexico, and northwestern Mexico is of particular interest to botanists because of its striking diversity of plant species and habitats, with more than 2,100 species of plants (McLaughlin 1994). This topography has created an area of high endemism within the flora and fauna inhabiting the various sky island mountain ranges.

This project was done at Fort Huachuca Army Base (Figure 1), Coronado National Historic site, and the Coronado National Forest within the Huachuca Range, in Arizona, U.S.A. In particular the Huachuca Mountain Range is exceptionally rich in plant species (Bowers and McLaughlin 1994) because it has a complex topography, a wide elevation range, a diversity of geographical substrates, aquatic habitats, and includes a large Apachian floristic district element.

Range and defining characteristics of Asteraceae, and *Erigeron.* Asteraceae is the largest plant family of flowering plants worldwide (Flora of N.A.Editorial Committee 2006) composed of 1500 genera, representing 23,000 species worldwide (418 genera, 2413 species within North America). This family is composed of annuals, biennials, perennial, subshrubs, shrubs, vine or trees, and is characterized by a indeterminate heads called capitula; with each head composed of a surrounding involucres of phyllaries, a receptacle, and 1-5-300+ florets consisting of a number of ray florets and disk florets, or just disk florets. Florets can be bisexual, pistillate, functionally staminate or neutral. Synapomorphies of the Asteraceae clade include: calyces modified to structures called pappi, anthers, connate (forming tubes) and styles modified to function as brushes in a specialized pollen presentation mechanism, ovaries each containing a single basal ovary, and has a production of sesquiterpene lactones (Flora of N.A. Editorial Committee 2006).

Figure 1.1. Looking southwest towards the Huachuca Range across grasslands at Fort Huachuca during a monsoon shower in July 2011. Photo by Pamela Bailey.

The genus of *Erigeron* is composed of annuals, biennials and perennials (subshrubs, shrubs, trees), and includes 390 species, nearly worldwide but primarily in temperate regions. *Erigeron* flower heads are bicolored, which seems to be associated with the dimorphic capitula of disc and ray florets. The disc florets are yellow and rays are white, pinkish or lavender. Defining characteristic of the genus are plants are rhizomatous, sometimes taprooted; heads are borne singularly or in corymbiform arrays; leaves are entire - dentate or pinnatifid; the disk corolla throats are usually tubular, sometimes strongly inflated-indurate; cypselae are glabrous, strigose or sericeous (Nesom 2006).

Range of the three *Erigeron* species chosen for this study. My study focused on three species of *Erigeron* that co-occur in the same region, but have very different life histories. The three species of *Erigeron* are *E. arisolius*, Dry Sun Fleabane; and *E. neomexicanus*, New

Mexico Fleabane (Figure 2); habitat generalists, growing in a broader range of environmental conditions; compared to *E. lemmonii*, a habitat specialist, growing in specific conditions (Figure 2).

Erigeron lemmonii is an endemic species found in one location in the rugged Scheelite Canyon, Huachuca Mountains (Cochise County), on Fort Huachuca Army Base, Arizona. It is adapted to cliffs in a naturally fragmented canyon landscape. *E. lemmonii* is a prostrate, perennial plant growing in dense clumps spreading 10-25 cm (occasionally up to 60 cm) growing on shady South, North and West facing cliff walls and vertical faces of large boulders along the canyon bottom (Nesom 2006), at an elevation between 1,900-2,200 m (6,300 to 7,300 feet) (AZ Game and Fish 2004). In those types of locations it is found in crevices and on ledges growing in highly organic soil (Bailey, unpublished data) on Escabrosa limestone substrate (Warren et al., 1991).

There are approximately 954 individuals (Malusa 2006) in the population, and it was protected as a Candidate Species by the US. Fish and Wildlife Service (USFWS 1975, 1980, 1993, 1996, 2006) until recently, when the USFWS partitioned to de-list *E. lemmonii* (2012). It is reported to bloom August through October, and possibly into December, depending on the temperature (Nesom 2006).

Dry Sun Fleabane (*E. arisolius*), a state-sensitive species, (Arizona Revised Statutes, 1999) has a wider distribution that includes Arizona and Mexico. *E. arisolius* is reported to be an annual or short –lived perennial (Nesom 2006), is 30-70 cm tall, taprooted, stems erect, with 20-50 flowering heads in loose corymibiform arrays. It grows in grassland habitats at an elevation range of 1300 to 1700 m (4,260-5,570 feet), and is reported to bloom (Nesom 2006) in June to August (May at earliest, and November at latest) (Nesom 2006). There is much of this species growing at Fort Huachuca Army Base.

New Mexico Fleabane (*E. neomexicanus*) grows in various natural habitats, including ridges, and has the largest range: Arizona, New Mexico, and Mexico (Nesom 2006). *Erigeron neomexicanus* is a perennial, is 30-70 cm tall, taprooted with caudices woody, erect stems, basal leaves with pinnatifid margins with (1-)5-15(-30) flowering heads in loose corymibiform arrays. It grows at an elevation range of 1500 to 2700 m (4,920-8,850 feet) in rocky substrates. It appears to be a ruderal species in that it is able to grow in a wide range of conditions including road cuts and stream beds. It is reported to bloom (Nesom 2006) in August to October (July at earliest, and December at latest).

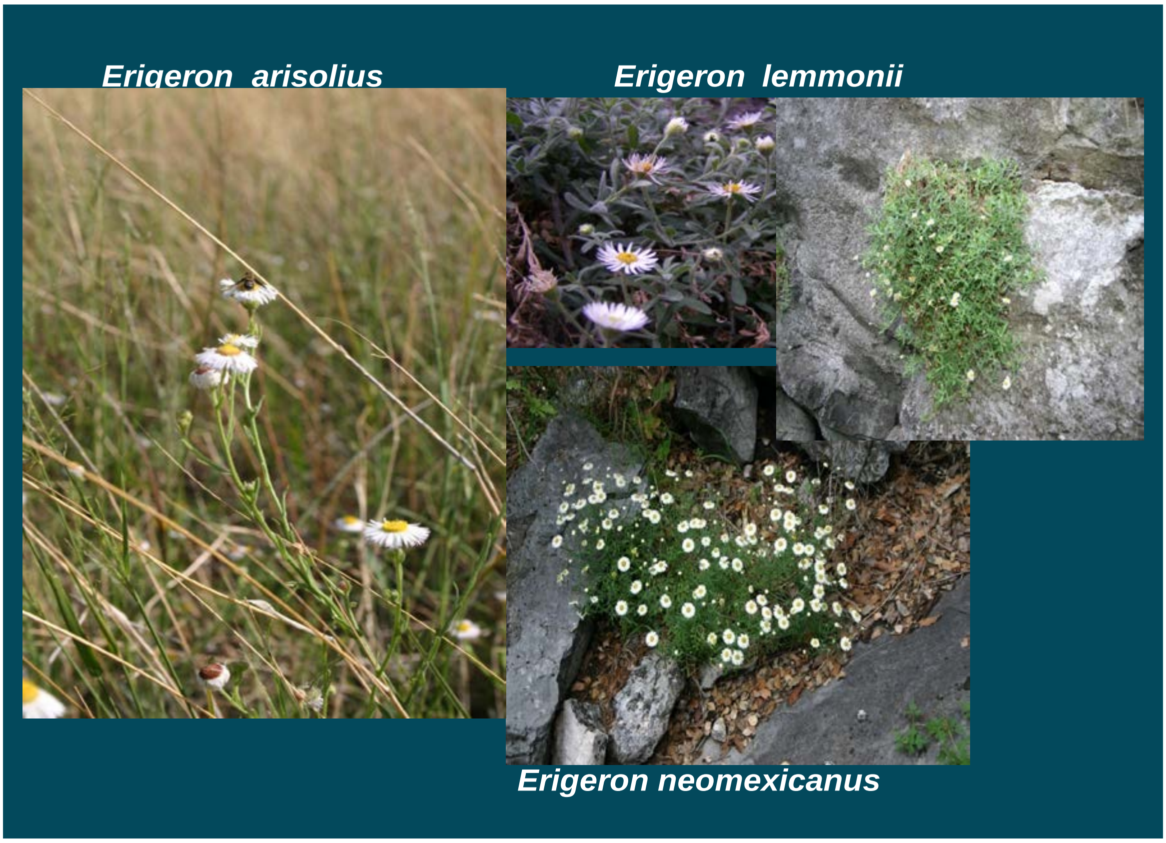

Figure 1.2. *Erigeron arisolius*, *Erigeron lemmonii*, and *Erigeron neomexicanus*.

Soil and Root analysis. Soil supports microbial communities, which are responsible for decomposing dead plant and animal matter, fixing nitrogen, and nutrient cycling and in facilitating growth of plants particularly under stress (Glick et al., 2007). A healthy soil is composed of living and dead organic matter, parent material from the rock substrate, water with solute nutrients, and air in pore spaces. While light, temperature and moisture determine what type of plant community grows in an ecosystem, the soil texture (sand, clay, loam) and the chemical nutrient composition of the soil determine plant growth. Root growth is important in plants because roots absorb water and solute nutrients, anchor the plant, store food, and convert inorganic nitrogen to organic nitrogen. Fine root hairs increase the surface of the root which increases the plant's ability to grow. Root analysis of each *Erigeron* species was conducted to determine the relationship between rooting structure and soil attributes, and to understand more about the environmental conditions and the various habitats of each of the three species.

Mating Systems. How genes are exchanged define the mating system. Richards (1997) defines mating systems in plants as different than in animals in three primary ways: First, plants not having a central nervous system like animals, are passive and cannot actively choose their sexual partners, as animals can. Second, plant mating can reproduce themselves in a modular fashion; a genetic individual (genet), formed by a single zygote, may be represented by many physiologically independent individuals (ramets); by contrast this asexual reproduction does not occur in complex animals. Third, all plants have an alternation of generations, so that sexual reproduction is achieved in the female gametophyte with the release of gametes from haploid gametophyte generation. The embroyo is the next generation diploid sporophyte. The significance of this system, is that any harmful mutants which are phenotypically expressed (within the pollen tube and embryo in flowering plants) are screened out by failure or gametophytic competition, and do not persist through to the sporophyte generation. These differences between plants and animals have many consequences, but the most obvious difference between plants and animals is the distribution of sexual function (Richards 1997); most complex animals have unisexual individuals, however most plants (95%) have hermaphrodite (co-sexual) individuals.

The following definitions are from Richards (1997). "Most seed plants have a wide range of reproductive options available for selection, and these may arise constantly, through genetic variation, or intermittently, through mutation, in most plants populations. These are as follows:

1) Hermaphrodity versus unisexuality; hermaphrodites may be able to self-fertilize whereas unisexuals can only cross-fertilize. Self fertilization tends to reduce genetic variability whereas crosspollination maintains genetic variability.
2) Self pollination versus cross-pollination in self fertile hermaphrodites. The amount of selfing from within-flower pollination (**autogamy**) depends on the degree of separation that occurs between pollen donation (from anthers) and pollen reception (on to stigmas) within the flower.

The amount of between -flower pollination (**allogamy**) that also results in selfing (**geitonogamy**) rather than outcrossing (**xenogamy**) depends on pollen travel between flowers, on the size of genets, and on the number of flowers open together on each genet.

3) Self-fertilization versus cross-fertilization; successful pollination does not necessarily imply successful fetilization. Many plants reject selfed pollen through a mechanism known as self-incompatibility (SI). Self-incompatibility will lead to more outbreeding and greater genetic variability than self-compatibility.
4) Sexuality versus asexuality; there are two main mechanisms by which plants can reproduce asexually. These are vegetative reproduction, and by producing seeds without sex (**agamospermy)** which is referred to as apomixis, or apomictic reproduction. Asexual reproduction maintains levels of variability already present, but does not create new genetic variability."

Most plants have evolved a mixed reproductive strategy, and in addition most perennial plants have the capability for at least some vegetative (asexual) reproduction (Richards 1997; Stebbins 1950). Many plant species in various regions include both sexually and apomictically reproducing populations (Asker & Jerling 1992; Grant 1982; Gustafsson 1946-1947). Apomixis is known for several genera in the Tribe Astereae and is associated with polyploidy (Brouillet et al. 2009). Apomictic reproduction may be favored in fragmented marginal habitats, as hypothesized by Haag & Ebert (2004) because it circumvents the deleterious effects of inbreeding depression and mate scarcity. However, another hypothesis, by Baker (1965) and (Lynch 1984), is that asexual lineages through polyploidy, hybridization, and genome level selection, evolve general–purpose genotypes that are capable of exploiting diverse habitats. Hybridization between sexual and apomictic plants can also be important as a source of novel apomictic genotypes (Stebbins 1950). The process of apomixes and the relationship among diploid and polyploid populations within apomictic taxa have been studied in *Erigeron compositus* (Noyes et al 1995) and in *Erigeron strigosus* (Noyes 2000a, b, 2006a,b, 2007, Noyes and Rieseberg 2000, Noyes and Allison 2005, and Noyes et al 2006).

Erigeron species are characterized by conspicuous rays and relatively numerous disc florets, features consistent with an outcrossing mating system (Noyes 2000b). "*Erigeron* is similar to other genera in being mostly obligate but some selfing is possible" (Semple, institution, personal communication, 2011). This has been confirmed with molecular techniques for at least one *Erigeron* species (Noyes et al., 1995). Self incompatibility (SI) is also important in promoting species within the genus of *Erigeron* (Noyes 2000b). However, Knuth (1908) reported that automatic self-pollination is possible in *Erigeron* during later stages by contraction of the stylar branches, which are brought into contact with pollen of the same flower. At least 20 different species of *Erigeron* in the *Olygotrichium* section have unknown reproductive modes.

Pollen quality is also diagnostic for mode of reproduction (Gustafsson 1946-1947). Sexual plants produce pollen of uniform sizes and high viability. By comparison, apomictic plants may produce pollen with high variation in size, and of low quality. It includes miniature aborted pollen grains, called micrograins (Noyes et al 2006). The sizes of pollen grains are also important: pollen grains of apomictic plants generally measure 17 µm (length) or greater compared to pollen grains produced by sexual plants which are usually between 12 and – 15 µm and lack micrograins (Noyes et al 2006).

Breeding Systems. Richards (1997) provides comprehensive coverage of plant breeding systems, and discusses how pollination biology, gene flow, incompatibility can be important components of a breeding system. He considers the breeding system as part of an adaptive syndrome that influences and is influenced by micro-evolutionary constraints and selection. Plants have a diversity of methods by which their breeding systems manipulate and control the genetic structure of their populations, and the patterns of their evolution (Richards 1997).

The different breeding systems within plants are described as follows: 1) a plant can be a hermaphrodite with bisexual flowers with pistils and stamen; 2) monoecious displaying male and female flowers on the same plant; 3) dioecious having unisexual male and female flowers on separate plants; 4) gynoecious with only female flowers,5) androecious with only male flowers, 6) gynomonoecious hermaphrodites with female flowers; 7) andromonoecious hermaphrodites with male flowers; 8) Trimonoecious with hermaphrodites, with female and male flowers. A species can have one breeding system in one part of its range and another breeding system in a different part of its range (Willmer 2011). Plants can also switch sex; alter its breeding system given local environmental conditions (Kevan 2010) to ensure reproductive success.

There are also secondary breeding system mechanisms, explained in detail in Richards

(1997): including gynodioecy, dioecy, heteromorphy, sporophytic and two-locus incompatibilities, monoecy, herkogamy and dichogamy. In plants with capitulate inflorences, as in Asteraceae, there is a division of labor between flowers, so those bearing less expensive gender energetically, expend more on secondary sexual characteristics such as showy petals, nectar etc (Yeo 1993).

Brief Historical Overview of Pollination Biology and Pollinator Syndromes. Just as there is a variety of plant mating and breeding systems, there are many different pollination systems. These are referred to as syndromes; mutualistic interactions between pollinators and associated plants (with different floral types). Books on this subject were written as early as 1733 by Joeseph Gottlieb Kölreuter who wrote about plant sexuality and types of pollination systems including self-pollination, wind pollination and insect pollination. In 1793, Christian Conrad Sprengel wrote a classic work (in German), describing structure and fertilization of flowers, in which he describes plant features such as nectaries and their guides, floral color, floral odor, and other themes within pollination biology. He also observed some flowers having many insect pollinators, while others flowers having only one pollinator (generalist vs specialist relationships). In his influential book, he tried to find an order within the diversity of flowering plants and the diversity of pollination relationships. Lloyd and Barrett (1996) give a historical perspective of Sprengel's work and more current conceptual problems in floral biology.

Charles Darwin was influenced by both Kölreuter's ideas of plant hybridization and Sprengel's study of plant sexuality. Darwin argued for adaptation through natural selection (1859) and placed pollination biology in its modern evolutionary framework. He described detailed observations of orchid pollination (1869) in the book entitled, *"On Various Contrivances by which British and Foreign Orchids are Fertilized by Insects".*

Darwin's contemporary, Hermann Müller, a botanist, wrote a compendium entitled, "*The Fertilization of Flowers*" (1883), describing flower species and their pollinators. He also describes the morphology and pollinating behavior of the major orders of pollinating insects. In the early 20th century, Paul Knuth expanded on Müller's work, by writing "*Handbook of Flower Pollination* (1906-1909). This three volume encyclopedia, describes many more plant species pollination mechanisms, and their pollinators.

Federico Delpino contributed by describing detailed accounts of pollination, including pollination by birds. He classified structural traits of flowers as co-evolved adaptations to flower visitor morphology of different animals, which eventually led to the idea of pollination syndromes (Faegri and van der Pijl 1979; Waser and Ollerton 2006). Vogel (1954) published another classification, which was refined by van der Pijl (1961), Baker and Hurd (1968) and Faegri and van der Pijl (1979). Stebbins (1970) formalized an approach, referred to as the most effective pollinator principle: different flowers have morphological traits that have evolved with certain pollinators, which promotes floral divergence and pollinator–mediated specialization. By contrast, a generalist pollinator pollinates more than one plant species, and a generalist plant has many pollinators.

In the 1990's many ecologists believed that pollination interactions were far more complex. There are many instances of a specialist being visited by generalist at different times, which Ollerton (1996) formalized as the "paradox". For example, some plants with a large spatial range, may have varying pollination visitation across the range; being generalist in some parts of the range, and specialist in a particular part of the range, or at different times of flowering (Herrera, Castellanos et al., 2006; Johnson 2006). Unless there is an adequate gene flow among populations, each isolated population becomes adapted to its local pollinators. However, this can be complicated by variation across space and time; for example there may be generalization at the species level, but specialization at the population level (Willmer 2011). It has also been suggested that where plant ranges are more affected by humans there tends to be more generalists, whereas in "natural", less disturbed ecosystems they are more likely to have a higher degree of specialization (Johnson and Steiner 2000).

Introduction to Pollinator vs. Anthrophiles. An anthrophile plant visitor may not be a true pollinator of that plant and can have multiple roles depending on the season, locality and other insects within the insect community of the plant (Kevan and Baker 1983, Barthelmess, et al. 2006). True pollinators are easier to recognize because of the anatomical features for gathering and transporting pollen grains, such as hairs on the legs and body, and adaptations of mouthparts and legs (Proctor et al.1996). There are also differences in the pollinator's ability to transport pollen, often associated with body size, form and foraging behavior (Horsburgh et al. 2011; Proctor et al.1996; MacKenzie 1994; Eckhart 1992; Motten et al., 1981).

In Asteraceae, the heads are habitually visited by a varied group of insects; including pollinating insects gathering pollen and nectar. There are also robbers that do not effect pollination, insects that seek shelter within the flower heads and their predators and parasites, and spore and fungus feeders (Mani and Saravanan 1999; Proctor et al.1996). Heteroptera and Homoptera are liquid feeders sucking sap from the plant or blood from other insects, and are believed to transfer pollen while crawling on disc florets (Knuth 1906-1908). Beetles (Coleoptera) are the most dominant insects on Earth, are also on Asteraceae (Mani and Saravanan 1999). Beetles have very little specialization for pollination; however, they may cross-pollinate flowers. They are more known to feed on spores, dead vegetative matter, and are predators of thrips, aphids and mites. Thrips (Thysanoptera) are not specialized for pollination, however they are quite common and breed in many different flowers at all stages, as sap and fungus feeders. If pollen is deposited by thrips on a receptive stigma of another floret within the same head, the self-incompatibility (SI) mechanism within the plant prevents pollen-tube germination (Mani and Saravanan 1999).

Flies (Diptera) are liquid feeders, sucking the plant or other insects. Flies are partly specialized and some species are known to be very effective pollinators of Composite heads. Hover flies (Syrphidae) suck juices of the flower heads and parasitize aphids. Syrhids are also considered important pollinators. Ants, wasps and bees (Hymenoptera) are among the most common flower visiting, social insects in the world. Bees are known lovers of sunshine and very important as pollinators of flowering plant during daylight hours. Bees are another important group of pollinators and are highly specialized for the collection of pollen and nectar. Ant-aphid mutualism introduces an important aspect to pollination; ants are attracted to honeydew secreted by aphids, but attack other damaging insects of the Composite heads and the aphids (Knuth 1906-8). Wasps are usually predators, but some species are also known pollinators (e.g. Masaridae).

Butterflies are diurnal and depend on nectar for carbohydrates and amino acids. Moths are crepuscular or nocturnal, often visiting white, strongly scented flowers that have long, narrow corolla tubes that cannot be reached by bees. Fermenting organic liquids are the primary food source and nectar–feeding is a phyletically recently acquired habit (Mani and Saravanan 1999). Butterflies are known to migrate in swarms up through the Madrean Sky Island complex, from Mexico into the United States (Felger and Wilson 1994). The Butterflies, moths and hummingbirds follow flowering plants as they flower and produce nectar and pollen resources, influencing flower pollination patterns within this corridor.

The trend in the flower heads of Asteraceae to develop yellow colors, either disk florets, ray florets, or both, favor butterfly pollination (Mani and Saravanan 1999). It has been reported by Mani and Saravanan (1999) that butterflies predominate, representing the most visitors to composite heads (75%), followed by solitary and social bees (15%), flies (8%), and thrips, beetles and bugs (2%). Knuth (1906-1908) reported on pollinators of *Senecio sylvaticus*, including butterflies (66%), flies (23%), while and only 11% were bees. A study conducted on *Symphyotrichum laceolatum* and *S. lateriflorum* (Horsburgh et al., 2011) reported both species of Aster being pollinated primarily by bees, wasps and flies, but slightly different specific assemblages.

Erigeron species are believed to be generalists, pollinated by many different types of insect pollinators (Semple, personal communication, 2011). Spongburg (1971) reported that Muscoid flies were very active in morning hours for *Erigeron simplex and E. grandiflorus* however, other insects visiting the flowers were sulphur butterflies, mosquitos, and a large presence of thrips in the disks. Butterflies and moths (Lepidoptera) have mouthparts consisting of a long proboscis and are important pollinators of the Composites, including *Erigeron* (Mani and Saravanan 1999). Lycaenidae, mostly small bluish or copper colored butterflies are known pollinators of several species of *Erigeron* (Mani and Saravanan 1999). Pleasants (1983) reported a diurnal separation of pollinators including visitation based on dehiscence at midday for the genus of *Erigeron*.

Introduction to Network Science. Network science is a relatively new branch of science. One aspect of network science uses graph theory for analyses of different types of data into “small world networks” (Newman et al. 2006). Network science originated from the social sciences (Barabasi 2003). Scientists have been utilizing network science for electrical power grids, the internet (Faloutsos et al.1999), cellular and metabolic networks (Kohn 1999). In ecology network science was initially applied to understanding food webs and predator-prey interactions (Cohen *et al*. 1990; Williams *et al*. 2000), but is increasingly being applied in other ecological contexts (Figure 3).

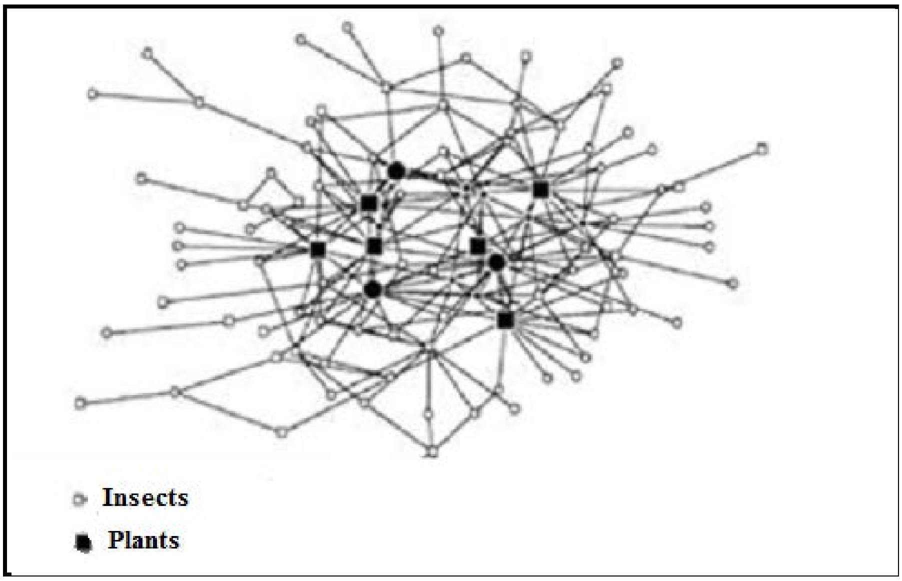

Figure 1.3. An example of a bipartite pollination network (Jordano *et al*. 2006).

More recently network science has been expanded to describe pollination community–wide patterns and pollination network studies have flourished (Alarcon *et al* 2008; Carvalheiro et al. 2008; Gonzalez et al 2010; Burkle and Alarcon 2011; Yoko et al. 2012). Networks of species interactions are the architecture of biodiversity because community dynamics rely on the way species interact (Jordano *et al*. 2006). Power law distributions of connectivity can be characterized by a high frequency of nodes with few connections, and by a few highly connected nodes (Jordano *et al*. 2006). This has implications for the evolution, stability, and resilience to perturbance in the network (Barabasi and Albert 1999).

Viewing plant/ pollinator relationships at a community level is important for several reasons. First, the evolution of pollination adaptations in floral traits most likely results from a community –level processes that involve interaction of a group of species. Second, robustness of plant/pollinator interactions depend on properties best viewed at a community level, such as invasion of exotic species, or resilience after extinctions. Third, how a rare species survives is based on a larger community of interactions (Jordano *et al*. 2006). Jordano et al. (2006) has provided some measures by which network characteristics can be assessed (see chapter 4).

Oceanic islands have been found to be particularly susceptible to disruption and loss of plant species (Ghazoul 2005), and the Southwestern Sky Islands of Arizona share many of these same characteristics including local isolation and high endemism. Phenological differences caused by mountain topography are also important in driving genetic divergence between populations (McEuen and Curran 2005), regardless of pollination syndrome. Pollination networks may be similarly susceptible to disruption and extinction, and desert (and island) ecosystems more susceptible to negative impacts from fragmentation because of the relative scarcity of species (= smaller network size). Smaller networks may lack redundancy (Bascompte *et al*. 2003) therefore be more impacted by fragmentation and less resilient.

Genetics using microsatellites. In eukaryotic organisms, such as seed plants, sexual reproduction has three major features that generate genetic variability: recombination, segregation, and sexual fusion (Richards 1997), and his definitions of these terms follow: 1) Recombination is the resorting of alleles of different loci linked on chromosomes, during meiosis (meiosis 1); 2) Segregation is the inclusion into a gametophyte, usually at random of any one of four chromatids. The chromatids of any chromosomes enter gametes randomly in respect to each other, and alleles of heterozygous unlinked loci are randomly resorted between the parent and the gamete, which generates genetic variability. 3) Sexual fusion or syngamy, is the fusion of a functionally haploid male and female gametes to form a diploid, or functionally diploid zygote. As a result of recombination, segregation and sexual fusion, creating gametic variation is immense, and it is very unlikely that any two offspring would be genetically identical (Richards 1997). Species with a low chromosome base number will create a much lower level of zygotic variability. For example, n=4 (as in the case of some higher plants) the potential number of zygote genotypes, ignoring recombination, is 256 (Semple and Watanabe, 2009).

Genes are arranged in a linear order within a chromosomes; the position of a gene is referred to as a locus. An allele is an alternative form of a gene or genetic locus. Genotype refers to the genetic makeup, whereas phenotype refers to the physical appearance. Genetic diversity is tied to observable phenotypic traits, for example different pigmentation. Organisms that have two sets of chromosomes are referred to as diploid, and have one copy of each gene (one allele) on each chromosome. If both alleles are the same, they are homozygotes, and if the alleles are different, they are heterozygotes. A species or population typically includes multiple alleles at each locus among various individuals (Weising et al. 2005). Polymorphism or allelic variation at a locus is measurable as the number of alleles present, or the proportion of heterozygotes in the population. (Weising et al.2005).

In the family of Asteraceae, large numbers of chromosome number reports for many species were determined between 1960-1990 (e.g. Raven et al. 1960; Anderson et al 1974). Solbrig (1977) reported x=9 was the ancestral base number of the family, and Cronquist (1981) reported that Asteraceae had a range of base numbers from x= 2 to x=19 and also suggested that perhaps x=9 was ancestral. Base numbers x=2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 occur in the family and the most frequent chromosome number, from mitotic counts, reported for Asteraceae is 2n=18 (Semple and Watanabe, 2009). Dysploidy is the change in the chromosomal base number through rearrangement of chromatin and loss or gain of a centromere (Semple and Watanabe, 2009), and in Asteraceae, dysploidy decreases are common in some clades, whereas losses are rare. Polyploidy is also common in Asteraceae and occurs in most clades.

The ancestral base chromosome number for *Erigeron* is x=9, and the frequent chromosome number reported for genus is 2n=18. This is reported for *E. arisolius* (Keil 1976; Sunberg 1990; Nesom 1990). *E. neomexicanus* is primarily reported as 2n= 18 in the majority of the accounts (DeJong and Longpre 1963; Schaack and Windham 1983; Sundberg 1983; Ward 1984, Semple and Chmielewski 1987 (2); Carr et.al.1999), however 2n= 36 was reported for this species in two accounts (Harms 1969; Pinkava and Keil 1977). *E. lemmonii* has not been reported in the literature.

Microsatellite markers. Microsatellite DNA loci are highly variable, informative genetic markers that can be used to evaluate allelic richness (≈ genetic diversity), heterozygosity (≈ population inbreeding), and population differentiation (both overall and between populations) over lesser distances and shorter time spans than most other genetic markers (Weising et al. 2005). A microsatellite has a short core of repeating units of 2 -9 base pairs, repeated at one or more places in the genome (Hartl 2000), compared to a minisatellite which has a larger repeated sequence (10-60 basepairs). They are generally more reliable and, because they are co-dominant, provide better information on breeding (Weising et al. 2005) than other, dominant-type, high-variation markers (e.g. AFLPs, RAPDs). In a search of GenBank ® (2009) no microsatellite markers have been developed for the genus *Erigeron*.

Some of the uses of microsatellite markers include (Hartl 2000):

- To estimate genetic variation in a population.

- Use the genetic differences as a means of DNA fingerprinting , to identify different individuals within a population.
- To understand the evolutionary mechanisms by which genetic variation is maintained and transformed into divergence of species.
- To analyze genetic differences between species in order to determine ancestral history (phylogeny).
- To monitor the genetic diversity in key indicator species in a community to evaluate habitats exposed to different physical stressors (chemical or biological).

Conservation issues for *Erigeron lemmonii.* *Erigeron lemmonii* is a G1 plant in NatureServe's Database (2012) which means it is critically imperiled globally because of extreme rarity (fewer than 1000 individuals) or because some factor(s) make it especially vulnerable to extinction. *Erigeron lemmonii* was listed as a Category 2 Candidate Species within the U.S. Fish and Wildlife System (USDI Fish and Wildlife Service 1975, 1980, 1993, 1996, 2006), and recently this agency petitioned to have it removed in October 2012 (USFWS 2012): "Lemmon fleabane, a candidate for listing under the Act since 1996, is being removed from the list of candidate species because the main threat (wildfire) does not rise to a level of significance such that the species is in danger of extinction or likely to become so in the foreseeable future. In addition to fire, recreational repelling which is not allowed in the canyon, might pose a minor threat, but would not threaten the entire population if it were to occur." Additionally in 2011, the Desert Botanical Garden collected hundreds of viable seeds for long term storage. The US Fish and Wildlife Service believe that seed collections by the Desert Botanical Garden may offset any impacts to the species in the event of fire or other catastrophic events.

Currently the Army Resource Manager monitors the population and is drafting a Cooperative Agreement with the U.S. Fish and Wildlife Service, as a result of the proposed change. Fort Huachuca Army Base maintains permanent photo-monitoring plots using a tripod and specific coordinates for future re-measurement of plots in an effort to survey for new plants within the population, and has monitored the *Erigeron lemmonii* population since 2008 (Stone, pers. comm.#1. 2010). This monitoring indicates the population has been stable during the time it has been monitored and may even be increasing slowly (Stone, pers. comm. #2. 2010). The cooperative agreement will ensure continued monitoring, 1) will promote adaptive management of this species, 2) continue to restrict recreational activities in its habitat, and 3) encourage further research into the life history and population biology.

GOALS

The goals include: 1) To provide greater understanding of the life history, flowering characteristics and phenology of *E. lemmonii*; 2) compare and contrast soil and root characteristics for the three species of *Erigeron*; 3) compare and contrast the pollination networks of three species of *Erigeron*; and 4) develop and publish microsatellite markers for *Erigeron lemmonii and E. arisolius;* 5) tie together the findings in a concise conclusion to summarize conservation issues for *Erigeron lemmonii*.

Phenology of *Erigeron lemmonii*. Very little is known about the rare, endemic plant species *Erigeron lemmonii* A. Gray, Lemmon's Fleabane. The first goal of this research is to improve botanical, habitat and flowering information for this rare species. New flowering data was obtained, including aspects of its flowering season and duration. The Huachuca Range has a monsoon season, which offers a bimodal flowering season. Flower and pollen microscopy and botanical study drawings have revealed more information about this species. This important information increases understanding of the basic life history for this plant species, which is important for its conservation.

Soil and Root characteristics of 3 *Erigeron* species. The second goal was to investigate and analyze soil nutrients in relationship to root morphology for the *E. lemmonii*, *E. arisolius* and *E. neomexicanus*. Root analysis of each *Erigeron* species were conducted to see how the rooting structure and soil attributes were related. This chapter is essential in understanding more about the habitat conditions, soil characteristics influencing plant growth and their interrelated rooting response to adapt to environmental conditions of the various habitats of each of the three species.

Compare and contrast the floral insect visitor networks of three species of *Erigeron*. The network study will determine the structure of the plant and insect visitor network to gain insight to the underlying network architecture of each of the three *Erigeron* species. Some network measures have been made to be able to compare the three networks. A detailed analysis was made by applying Non-metric Multidimensional Scaling (NMS) to measure insect community composition, and comparing the dissimilarity of those communities between each *Erigeron* species.

Microsatellite markers for *Erigeron lemmonii and E. arisolius.* Another goal of this research project was to develop and publish novel genetic microsatellite markers for the three species of *Erigeron.* These will be the first microsatellite markers for the *Erigeron* genus. These microsatellites will be useful in characterizing the Erigeron populations in the future, and provide new opportunities for population genetic studies within the genus of *Erigeron.* They will be available at GenBank®, an annotated collection of all publically available DNA sequences available online at www.ncbi.nlm.nih.gov.

Conservation goals of *Erigeron lemmonii.* The goal of this concluding chapter is to interrelate and to connect various aspects important for the conservation of *E. lemmonii.* This investigation reports new information found about *E. lemmonii* including its mating system and genetic aspects, with some aspects identified in previous sections such as its phenology, insect visitors, habitat, soil, rooting characteristics, and ties them together to better understand what is important in the conservation of this rare plant. This information will be available to the Resource Manager at Fort Huachuca and the U.S. Fish and Wildlife Service.

METHODS

Monthly surveys were conducted at a number of sites on public lands in the Huachuca Range, Sky Island Region, Arizona, during the flowering season over the course of two years. Several sources for pollination techniques useful through-out the project were two books; Techniques for pollination biologists (Kearns and Inouye 1993), Practical Pollination Biology (Dafni et al.2005), and Kevan (2010) “Pollination Biology”, course through University of Guelph, CA.

Floral and pollen traits, flowering season and mating system of *Erigeron lemmonii*. New botanical information has been collected for the rare, endemic *Erigeron lemmonii.* Phenology aspects investigated and analyzed were the duration of the flowering season, and the time period of flowering for an individual plant from budding to senescence. Microscopy and illustrations are added to further define *E. lemmonii* and its pollen (see chapter 2 for a more detailed methods description). The duration of an individual flower and flowering season of *E. lemmonii* are also described and analyzed with SAS. The mating system for *Erigeron* *lemmonii* was unknown. In this research determination of the mating reproductive system was accomplished for *Erigeron lemmonii* using a bagging experiment methods as described by Dafni (1992) and Dafni et al. (2005), and results analyzed with SAS.

Soil and Root analysis of 3 *Erigeron* species. Soil samples were collected from the defined plot locations for the three Erigeron species. These soil samples were sent to the Soil, Water and Air Testing Lab in New Mexico for processing and analyzed using standard soil tests procedures. A standard soil analysis included pH, Organic C, total N, exchangeable NH4, NO3, plant available P, exchangeable K, (soluble Na, Ca, and Mg to calculate ESP) and texture (by feel). Cation Exchange Capacity (CEC) was calculated from the analytical data for each sample. A Munsell soil color (2000) was recorded in the field and the thickness of the surface soil horizon (A horizon) was measured. Statistics were applied to the results and a comparison was conducted between the three species of Erigeron.

Root analysis of each *Erigeron* species was conducted to see how the rooting structure and soil attributes interrelate, to understand more about the various habitats for each of the three species. Because of the scarcity of *E. lemmonii*, only one entire, midsized plant could be removed for this purpose, and to keep results consistent one plant for each species was collected and analyzed. Each plant was scanned using Regent instruments Winrhizo (2009) to measure the length of the plants roots, root distribution, root area, volume and number of tips (see chapter 3 for more detailed methods description).

Network typology characterization and analysis for 3 *Erigeron* species. Monthly floral insect visitor collections were made by vacuuming all flowering plants species, within all designated plots for the three *Erigeron* species, in a standardized time period. Monthly collections of insect visitors were conducted within plots at five distinct sites per plant species, with the exception of *E. lemmonii*, for which only one distinct Canyon location exists. Collections were made during the flowering season (April-October) in 2010 and 2011. Surveys were conducted using Fixed Sample Observations (Dafni et al. 2005) to understand the network relationships of the three *Erigeron* species to adjacent flowering plants and floral insect visitors within established plots.

In the survey, for each *Erigeron* species, adjacent plant species growing within the plots were sampled, and the number and diversity of insect visitors were recorded. Identification of all plants to species level, and all insects to the lowest taxonomic degree possible was accomplished. All identified plant and floral insect visitor species were used to construct, bipartite graphs and adjacency matrices of links (shared plants or anthrophiles) among plants and among insect visitors (Bascompte *et al*. 2003), and network features analyzed using the UCINET (Borgatti *et al*. 1999) software package. Some of these network features include: individual plant and animal species (nodes); and links between the nodes are interactions (k). More robust, meaningful measures, both between individual plant and pollinator species such as centrality (e.g., eigenvector centrality), and nestedness within networks also characterized specialization, and provided a richer understanding of network topology. The floral insect visitor networks between the three *Erigeron* species were compared once the networks for each *Erigeron* species were constructed. (see chapter 4 for more detailed methods description).

Microsatellite Development for *Erigeron lemmonii* and *E. arisolius*. Microsatellites were identified for *E. lemmonii* and *E. arisolius* following the method s of Glenn and Schable (2005), and primers were developed for these loci using Primer3 software (Rozen and Skaletsky 2000). At each study site 5-10 g of fresh leaf tissue was collected for 30 individual plants and fixed in RNAlater® (Ambion) for DNA extraction. For each species of *Erigeron,* a genetic library has been created and a subset of microsatellites were selected based on two criteria; 1) they exhibited polymorphism, and 2) could be consistently amplified through polymerase chain reaction (PCR) methods (*reviewed in* Weising 2005). The goal was to identify 7-15 microsatellite markers (polymorphic loci) from a genetic library, in order to characterize and compare genetic variation in the two species of *Erigeron*. The selected microsatellites were used to determine the allelic richness and average heterozygosity. The genetic markers have been given ascension numbers in Genbank® (JQ868414- JQ868430, 2012). Chapter 5 contains a more detailed description of the methods used in the development of the microsatellites.

Future research, not included in this dissertation may use these markers to determine levels of inter-population genetic differentiation. These novel microsatellites will be used in future studies to investigate gene flow within each population, one of the most influential aspects of plant population dynamics.

Conservation issues for *Erigeron lemmonii.* In this section (concluding thesis chapter), all aspects obtained in the research are interrelated, as to why they are important for the conservation of *Erigeron lemmonii*. Specimens of the three *Erigeron* species have been vouchered and deposited in the Missouri Botanical Garden's Herbarium, in St. Louis, MO. (Bailey, P. #301-#312, MO). Website for the herbarium is available at Tropicos.org. Missouri Botanical Garden. <http://www.tropicos.org>.

A synoptic collection insect species collected in this study are curated at the Colorado Plateau Museum of Arthropod Biodiversity, located at Northern Arizona University, Flagstaff, AZ. Additionally they have developed an image library of exemplars for many insect species. Websites to view insect collections are available at
http://bugguide.net/bgimage/user/28808,
http://bugs.nau.edu/BIO322/Fort_Huachuca_ERLE_Curc001.html,
http://www.bugs.nau.edu/Bio322/Fort_Huachuca_ERLE_HLasio.html,
http://www.bugs.nau.edu/Bio322/Fort_Huachuca_ERLE_Geron.html.

In February 2013, all final results were presented to the managers of the funding program, the U.S. Army Environmental Quality Technology Program (Project #09-03 [156334]). Results of this research will also be disseminated to the Resource Manager at Fort Huachuca Military Reserve, and to the U.S. Fish and Wildlife Service.

HYPOTHESES

There are five Hypothesis (***H1* – *H5***) important in this research project:

H1: There are differences between the soil nutrient analyses and organic matter content affecting the root morphology for the three species of *Erigeron.*

H2: Plants that evolved under naturally fragmented, highly specific habitat conditions (*Erigeron lemmonii)* have weakly linked nodes supporting a small number of pollinators in the pollination network.

H3: Plants that evolved under naturally fragmented, cliff habitat shared pollinators with relatively common plants that are pollinator generalists (and are nested in the pollination network structure).

H4**:** Plants that evolved as habitat generalists also supported more redundant, robust network relationships, and are relatively less impacted by fragmentation, showing relatively greater genetic similarity over the geographic distance than that found in habitat specialists.

H5: Plants that are generalists in networks are serviced by a greater diversity of insect visitors, they experienced a greater incidence of pollen exchange, resulting in more genetic variation and lower average inbreeding values compared to plants that are network and habitat specialists.

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CHAPTER 2

Pollination Attributes, Flowering period, and Mating System of *Erigeron lemmonii* A. Gray, Lemmon’s Fleabane (Asteraceae)

ABSTRACT

Erigeron lemmonii, A. Gray, Lemmon's fleabane, is in the largest plant family, Asteraceae, the Aster family (Figure 1). The genus of *Erigeron* is composed of annuals, biennials, and perennials (subshrubs, shrubs, trees), which includes 390 species, nearly worldwide, primarily in temperate regions. *Erigeron lemmonii* is an endemic species found in one location in Scheelite Canyon, Huachuca Mountains (Cochise County), on Fort Huachuca Army Base, part of the Sky Island region of Arizona in the United States. It is a perennial; however, not much is known about its life history or pollination biology. It grows out of crevices and ledges of vertical cliffs and rock faces, at an elevation between 1900 and 2200 m (6,300 and 7,300 feet) above sea level.

Goals of this four-part study include: 1) investigation of the anatomy of *Erigeron lemmonii*; 2) investigation of *E. lemmonii* pollen; 3) examination of the flowering season of *Erigeron lemmonii*; and 4) analysis of *E. lemmonii's* mating system. This information is new for the species, and our findings will be useful in management and conservation of this rare plant species by the United States Army. This paper supports concurrent research within the same genus entitled, "A Comparison of Natural and Manmade Fragmentation Effects within Three Pollination Networks of *Erigeron."*

Key findings include an understanding of the morphology important in the pollination of *E. lemmonii* and its pollen. This includes structural aspects of the pollen grain that allow it to be transported by insect visitors, and its pollinators. *E. lemmonii* was found to have an extended flower progression of approximately 3 weeks from bud to senescence. The population takes advantage of environmental conditions over an extended six-month flowering period, with peak flowering occurring in May. *Erigeron lemmonii* has a xenogamous mating system, reliant on insect pollinators. It has a self incompatibility system, preventing fertilization by autogamy, or geitonogamy. *Erigeron lemmonii* does not reproduce by apomixis, but it can easily reproduce by vegetative means; plant rhizomes and stems produce roots easily from nodes when conditions are favorable.

INTRODUCTION

Range. Very little is known about the rare, endemic plant species *Erigeron lemmonii* A. Gray, Lemmon’s Fleabane, Asteraceae, growing in the rugged Scheelite Canyon at Fort Huachuca, Arizona, USA. This canyon is within the Huachuca Range, part of the “Madrean sky island region.” The term 'sky island' was first used by Weldon Heald in 1967 to describe mountain ranges that are separated from each other by valleys of hot, dry grassland or desert (Austin and Shaul 2010). The Madrean archipelago is unique from a planetary perspective (Warshall 1994) because it exists between two cordilleras; the Sierra Madre Occidental and the Rocky Mountains. The archipelago contains 40 Sky Islands and has the following characteristics: First, the complex has a north- south orientation but contains an east-west valley bridge through the Deming Gap of Rocky Mountains. Second, the islands have a mixed geological composition unique in the world. Third, the islands have a high relief (5,000 feet). Fourth, it is the only sky island complex to straddle two major floristic and two major faunal realms as well as three major climatic zones (tropical, subtropical, and temperate). Ecological and geographical isolation of a plant within the Sky Island complex is comparable to insular isolation, with the added dimension of elevation. This may be conducive to the forces of genetic drift and result in morphological differences within populations (Cronquist, 1947).

The 70,000-square-mile Madrean Sky Islands region of southeastern Arizona, southwestern New Mexico, and northwestern Mexico is of particular interest to botanists because of its striking diversity of plant species and habitats, with more than 3,000 species of plants (Austin and Shaul 2010). This topography has created an area of high endemism within the flora and fauna inhabiting the various sky island mountain ranges. In particular the Huachuca Mountain Range (Figure 2.1) is exceptionally rich in plant species (Bowers and McLaughlin 1994) because it has a complex topography, a wide elevation range, and a diversity of geographical substrates and aquatic habitats, including a large Apachian floristic district element.

Climate. A weather station is located on Fort Huachuca. Annual precipitation at Fort Huachuca is 37.1 cm (14.6 inches); about half falls as high-intensity “monsoonal” rains in July and August that originate as scattered thunderstorms. Winters are dry, with the wettest months being December and January, with an average of 4.5 cm precipitation (1.78 inches).

Summers and winters at Fort Huachuca are mild. The average temperature in January is 7.9°C (46° F) with average daily maximum and minimum temperatures of 14.7°C and 1.2°C (58.4° and 34° F). Summer temperatures are moderated by afternoon cloud cover. The average July temperature is 25.3°C (77.5°F), with average daily maximum and minimum temperatures of 31.4°C and 19.1°C (88.6° and 66.4° F).

Figure 2.1. Looking southwest towards the Huachuca Range across grasslands at Fort Huachuca during a monsoon shower in July 2011. Photo by Pamela Bailey.

Description of Asteraceae, *Erigeron,* and *Erigeron lemmonii.*

Asteraceae (the Aster family) is the largest family of flowering plants worldwide (Nesom

2006) and comprises 1500 genera, representing 23,000 species worldwide (418 genera, 2413 species within North America). *Erigeron lemmonii*, Lemmon's fleabane is in Asteraceae (Figure 1). Recent work has focused on both the morphologic (Nesom 1994, 2000) and molecular (Noyes and Rieseberg 1999) classification and phylogeny of the tribe Astereae. The seminal paper by Noyes and Rieseberg considers the North American genera as belonging to a single monophyletic clade, called the North American clade (Nesom 2006).

Erigeron is a large genera that originated in North America and spread to Eurasia and South America. It includes annuals, biennials, and perennials (subshrubs, shrubs, trees), with 390 species worldwide, primarily in temperate regions (Nesom 2006). Isolation affects speciation in this genus; 30 % of the North American species of *Erigeron* are narrow endemics (Cronquist 1947). Two early biosystematic studies have been completed in the genus of *Erigeron*; the first by Solbrig (1960) studied the "Andicola Complex" in South America. Nesom (1970) conducted a study on the variation and evolution in a group of North American western and southwestern species, centering on *Erigeron flagellaris* A. Gray, in the section *Olygotrichium*. Since then the *Erigeron* species have been divided into sections (Nesom 1990, 1994; Nesom and Noyes 1999; Noyes 2000b), emphasizing the variation in habit (especially tap rooted versus fibrous-rooted), arrangement of heads in arrays and orientation before flowering (erect, nodding, or arching pendent), behavior of ray corolla laminae (straight, reflexing, or coiling), and other morphological features. *Erigeron* is a paraphyletic group, as other genera are derived from within it (Nesom 2006); these are *Aphanostephus, Conyza, Neja,* and *Hysterionica* (Nesom and Robinson 2007).

Erigeron lemmonii is a member of the section *Olygotrichium*, and is closely related to *E. divergens and E. tracyi, E. piscaticus* and *E. lobatus.* (Nesom 2000; personal communication Nesom 2012). *Erigeron lemmonii* is a member of a genus that includes some of the rarest plants of Arizona (Warren *et al.* 1991) and has some of the most common species of fleabanes, such as *E. divergens* and *E. lobatus.* The *Erigeron pringlei* group are fleabanes like *E. lemmonii*, only known from one or a few mountain ranges in Coconino, Gila, Graham and Santa Cruz Counties, whose members, *E. pringlei, E. heliographis, E. anchana* and *E. saxatilis,* also live on vertical rock ledges and boulders (Nesom 1990). *Erigeron scopulinus* described by Nesom and Roth (1981) is also thought to be closely related to the *E. pringlei* group and grows on two isolated mountain ranges in the Southwest.

Erigeron lemmonii is an endemic species found in one location in the rugged Scheelite Canyon, Huachuca Mountains (Cochise County), on Fort Huachuca Army Base, Arizona. It is adapted to cliffs in a naturally fragmented canyon landscape (Figure 2.2). *E. lemmonii* is a prostrate, perennial plant growing in dense clumps spreading 10-25 cm (occasionally up to 60 cm) on shady south, north, and west-facing cliff walls and vertical faces of large boulders along the canyon bottom (Nesom 2006), at an elevation between 1,900 and 2,200 m (6,300 and 7,300 feet) (Arizona Game and Fish Department 2004). In those types of locations it is found in crevices and on ledges growing in highly organic soil (Bailey et al., in preparation) on Escabrosa limestone substrate (Warren et al. 1991). It is reported to flower from August through October, and possibly into December, depending on the temperature (Nesom 2006). Little is known about the pollination and reproductive biology of this rare, endemic plant species.

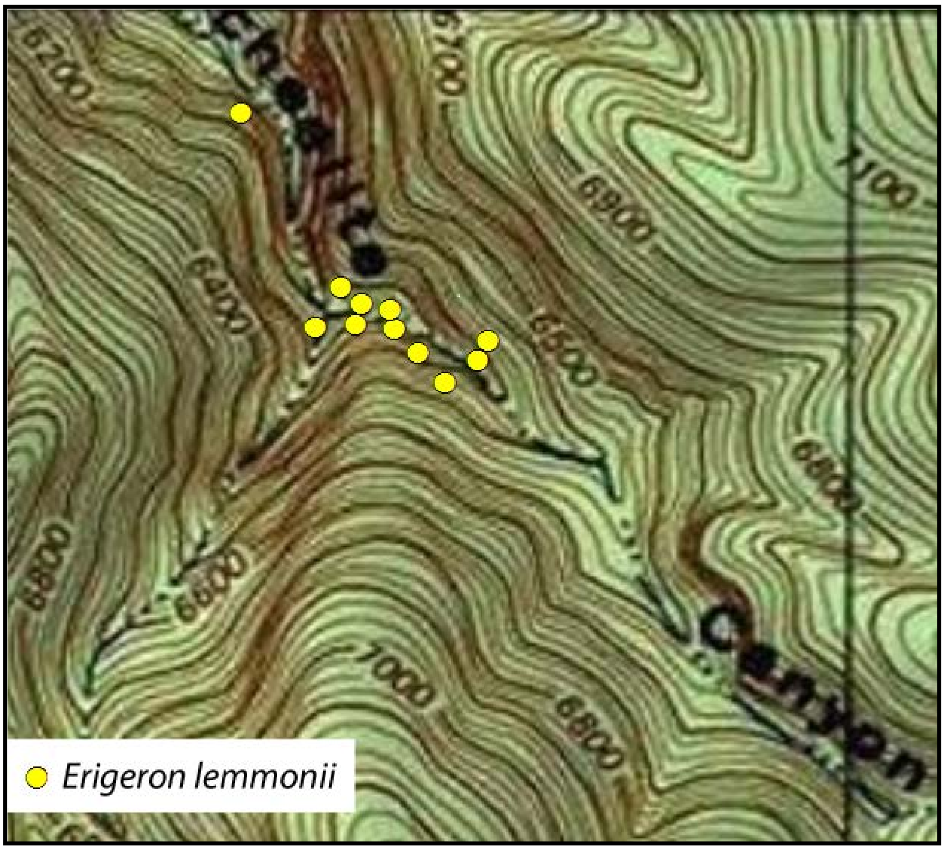

Figure 2.2. Approximate range of *Erigeron lemmonii* in Scheelite Canyon, Huachuca Range, Cochise County, Arizona. Circles indicate collection sites for *Erigeron lemmonii* plant data in an area of approximately one square kilometer. Map created by Jacob Higgins, 2012.

Taxonomy. *Erigeron lemmonii* was first described and published by Asa Gray (1883) from the type specimen collected by John Gill Lemmon in 1982; Type: US-49942. J.G. Lemmon 2749, Aug 1882. The type locality is: Huachuca, Arizona, USA. Conquist's 1947seminal monograph described *Erigeron lemmonii*. However, he lumped *E. lemmonii* with what is now recognized as another species, *E. piscaticus* Nesom, so it then appeared that *E. lemmonii* had a broader range from the Mexican border to the Salt River, as reported in Kearny and Peebles (1960). However, the taxonomy of the genus has been clarified since by a combination of morphological and molecular analysis (Noyes 2000b; Nesom 1989).

The taxonomic classification for *Erigeron lemmonii* is as follows (US Department of Agriculture/Natural Resources Conservation Service (USDA/NRCS) 2004)

Kingdom	*Plantae* – plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnolipsida* – Dicotyledons
Subclass	*Asteridae*
Order	*Asterales*
Family	Asteraceae
Tribe	Astereae
Genus	*Erigeron* L. – Fleabane daisy
Section	*Olygotrichium*
Species	*Erigeron lemmonii* A. Gray – Lemmon’s fleabane

Population status. The most recent, comprehensive survey by Malusa (2006) examined the population at all elevations throughout the cliffs, and reported approximately 954 individuals of all ages on the cliffs and boulders at the bottom of the Scheelite Canyon. *Erigeron lemmonii* grows over an area of approximately 50 hectares (124 acres) and the habitat is contiguous with no more than 50 m (164 ft) between any two plants (Malusa 2006). An earlier survey (Gori et al. 1990) indicated a total of 108 plants counted from the ground (not from the top of the cliffs), within the southwest and southeast forks of the Scheelite Canyon. Census data taken indicated plants of all sizes were flowering and setting fruit, suggesting the population was healthy and reproducing (Gori et al. 1990). Other surveys taken in adjacent canyons indicated no other extant populations of *E. lemmonii* exist (Gori et al. 1990; Warren et al. 1991), with 100% of its population occurring in the Scheelite Canyon.

Protection. In October 2012, the U.S. Fish and Wildlife Service (USFWS) published a proposed rule change in the Federal Register: “Lemmon fleabane, a candidate for listing under the Act since 1996 (USFWS 1996a, 1996b), is being proposed for removal from the list of candidate species because the main threat (wildfire) does not rise to a level of significance such that the species is in danger of extinction or likely to become so in the foreseeable future”. In addition to fire, recreational repelling which is not allowed in the canyon, might pose a minor threat, but would not threaten the entire population if it were to occur. Additionally, in 2011, the Desert Botanical Garden collected hundreds of viable seeds for long-term storage. This and future planned seed collections by the Desert Botanical Garden may offset any impacts to the species in the event of fire or other catastrophic events.

Currently the Army Resource Manager monitors the population and is drafting a Cooperative Agreement with the U.S. Fish and Wildlife Service, as a result of the proposed change. Fort Huachuca Army Base maintains permanent photo-monitoring plots using a tripod and specific coordinates for future re-measurement of plots in an effort to survey for new plants within the population, and has monitored the *Erigeron. lemmonii* population since 2008 (Stone, personal communication 2010). This monitoring indicates the population has been stable during the time it has been monitored and may even be increasing slowly (Stone, personal communication 2010). The cooperative agreement will 1) ensure continued monitoring, 2) promote adaptive management of this species, 3) continue to restrict recreational activities in its habitat, and 4) encourage further research into the life history and population biology.

The Arizona state status at present is “Highly Safeguarded” (Arizona Revised Statutes (ARS), Arizona Native Plant Law (ANPL) 1999). In 1990 *Erigeron lemmonii* was listed as a Forest Service Sensitive Species by the US Forest Service (USDA Forest Region 3, 1990), but not listed as a Forest Service Sensitive Species by the US Forest Service since 1999 (USDA Forest Region 3, 1999). However, it is listed as a critically imperiled, G1 plant (Nature Serve 2012), which indicates the species has less than 1000 individuals in the population. G1 is defined as, “At very high risk of extinction to extreme rarity (often 5 or fewer populations), very steep declines, or other factors.”

Habitat description. The flora of the Huachuca Mountains is comparatively rich for an Arizona local flora, with 29% -39% more species than expected, based on its elevational range, the substrate complexity, and spring-fed, well-watered canyon habitats contributing to the high species diversity (Bowers and McLaughlin 1994).

Scheelite Canyon is a narrow canyon with steep, cliff topography providing shade and cold-air drainage. The canyon provides a more consistent habitat with higher humidity and cooler temperatures than the surrounding landscape. Malusa's survey (2006) discovered *Erigeron lemmonii* in only Scheelite Canyon, and no plants have been found in other canyons with similar exposures of Escabrosa limestone. Lemmon fleabane grows in the deep shade of the canyon bottom at 1,829 m (6,000 ft), and on cliff faces up to the most open, exposed, and sunny ridges at 2,118 m (6,950 ft) in elevation (Malusa 2006). Malusa defined "one plant" as individuals growing within 10 cm of each other, and plants growing out of the same crack in the rock (Figures 2.3 and 2.4).

Associated vegetation. The Scheelite Canyon cliffs are within the Madrean Evergreen Woodland Vegetation Association, with *Psuedotsuga menziesii,* Douglas Fir, and *Acer glabrum,* Rocky Mountain maple (Brown and Lowe 1974). Herbaceous plants found growing in association with *E. lemmonii* include: *Cheilanthes alabamensis,* Alabama lip fern; *C. arizonica,* Arizona lip fern; *Notholaena limitanea,* Southwestern false cloak fern; and *Petrophytum caespitosum* , Rock spiraea (Arizona Game and Fish Department 2004). Other frequent associates noted during this study include; *Petrophytum caespitosum*, Rockmat; *Huerchera sangrinea*, Coralbells; *Galium wrightii*, Wright's galium, *Fendlerella utahensis*, Utah Fendlerbush, and *Oxalis decaphylla*, Oxalis (Epple and Epple 1995, Kearney and Pebbles 1960). *Salvia lemmonii,* Lemmon's sage, and *Sedum cockerelli,* Cockerell's stonecrop, were also reported by Malusa (2006). *Erigeron neomexicanus* is found on the ground directly below many of the cliff areas.

Figure 2.3. Habitat of *Erigeron lemmonii*, 2010. Photo by P. Bailey.

Figure 2.4. *Erigeron lemmonii*, A. Gray, Lemmon’s Fleabane. Photo by P. Bailey.

Floral description. Throughout the Asteraceae, the floral unit is the capitulum composed of many small flowers (florets) packed together on a flat or convex receptacle, surrounded by involucre of bracts. Yeo (1993) describes the general morphology of Asteraceae: The capitulum contain tubular (actinomorphic) corollas in the center (constituting the disc), and enlarged, ligulate (zygomorphic) corollas round the edge (collectively the ray). The flowers are generally protandrous. The corolla tube is tubular at the base and typically three or five-lobed with numerous fine hairs attached to the base of the corolla, collectively known as the pappus, which allows for wind dispersal. There are two sets of pappi, one long set and a shorter set which are both attached to the top of the ovary. The ovary is inferior and single-seeded. Stamens are attached to the corolla tube. The style has two linear stylar branches, initially appressed so they form a continuation of the style and later diverging to expose the stigmatic surface on the adaxial side, extending all or part of the way to the apex. The stylar branches are partly visible even while the style is still appressed, and are open at the end of anthesis, but are not recurved in *Erigeron* (Knuth 1906-1908). The sequence of opening of disk flowers on the capitulum is centripetal (Yeo 1993).The nectar is at the base of the corolla tube. Nectar and pollen are both produced in the disc flowers.

The pollen presentation mechanism in Asteraceae is unique and has been described in detail by Small (1915, 1917) and others (Percival 1950; Lane 1994). First, the growth of the style upward through the anther column, carrying pollen with it, is a passive mechanism (Small 1915). Secondly a contraction of the filaments after stimulation of touch by visiting insects causes the anther cylinder to pull down over the stylar piston (Small 1917).

The *Erigeron* genus is characterized by dimorphic, bicolored capitula of disc and ray florets; the disc florets are yellow and rays are white, pinkish or lavender. Other defining characteristics of the genus are the plants are rhizomatous, sometimes tap rooted; heads are borne singularly or in corymbiform arrays; leaves are entire, dentate or pinnatifid; and disk corolla throats usually tubular, sometimes strongly inflated-indurate. (Nesom 2006).

E. lemmonii has a taproot, growing into and out of crevices and ledges on cliff faces (Figure 2.5). The basal portion of the stems may be “subnaked and rhizomatous in appearance, but apparently not subterranean.” Stems are decumbent-ascending to prostrate; greenish, hirsute to hispidopilose and minutely glandular. Leaves are basal and cauline, greenish with proximal blades oblanceolate to ovate, 5-12 x 1-3 mm, margins with 1-2 pairs of teeth or shallow lobes, or entire, faces piloso-hirsute, minutely glandular (Nesom 2006). Flowering heads observed and counted, can range from 1-3 on small plants to 1- 47 on larger plants. Involucres are 2.5 x 5-10 mm.; phyllaries in 2-3 (-4) series (erect, apices appressed), sparingly piloso-hirsute, minutely glandular (Nesom 2006). The disc corolla is 1.8–2.4 mm. in diameter, and yellow in color (Figure 2.6). There are 42-60 white ray florets (Figure 2.7), without an abaxial midstripe, and the laminae do not coil or reflex (Nesom 2006). The cypselae are 1-1.2 mm, two-nerved, with faces that are sparsely strigose; pappi: outer of setae, 7-10 bristles (Nesom 2006).

Figure 2.5. *Erigeron lemmonii* in May 2011 at the peak of its flowering season. Photo by P. Bailey

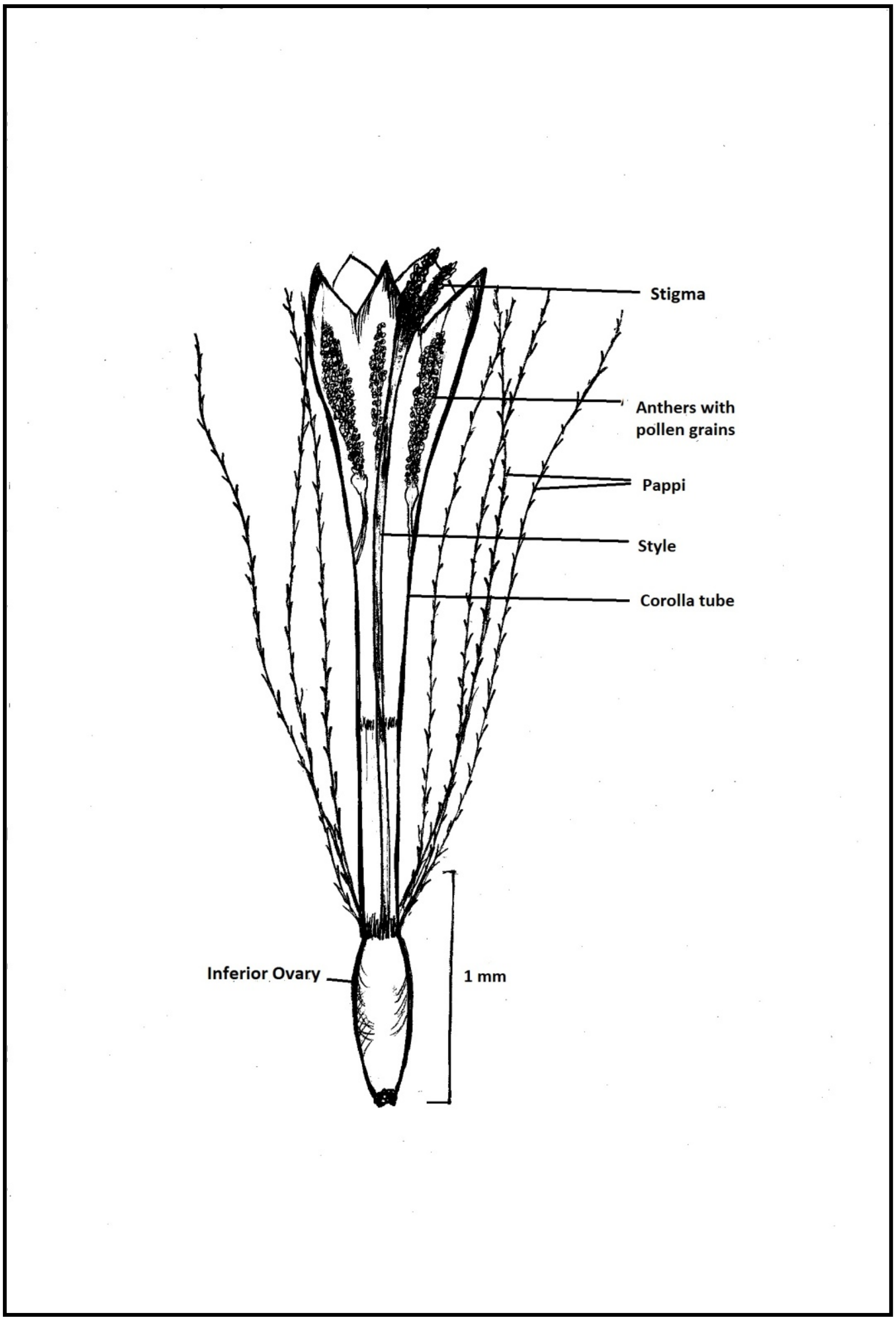

Figure 2.6. *Erigeron lemmonii* disk floret with an inferior ovary at base. The style is in the center of the corolla tube, the stigma is located at the apex of the style, and seven barbed pappi bristles are attached at the top of the ovary. Typically shorter pappi within a secondary whorl are also attached at the top of the ovary and this is represented by the dark band at the top of the ovary as seen under the microscope. Drawing by P. Bailey.

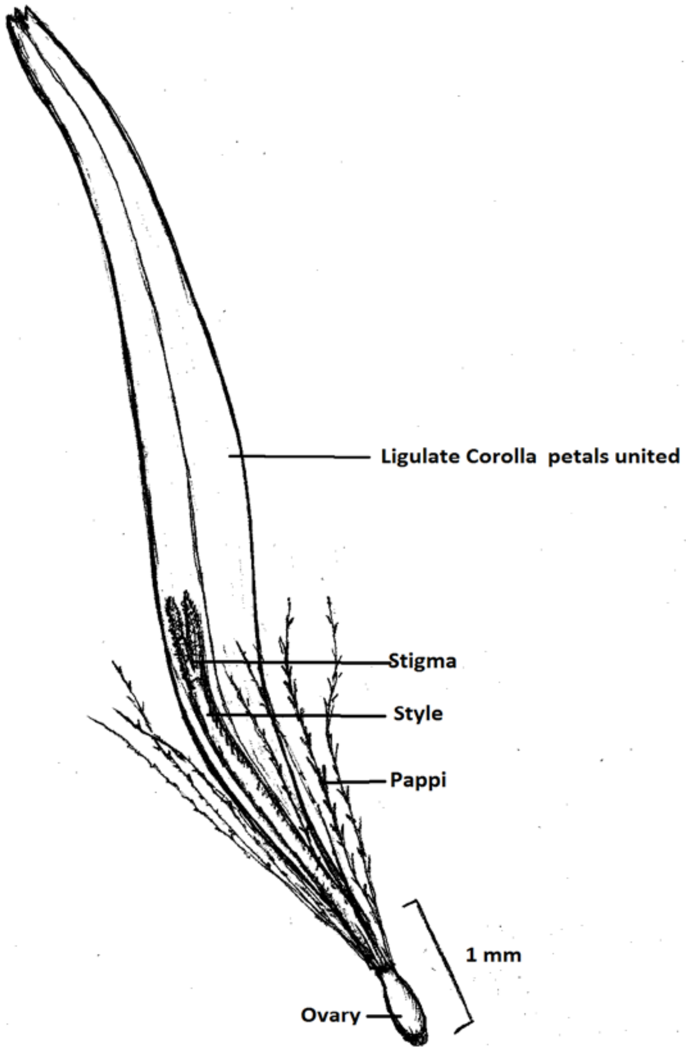

Figure 2.7. Ray floret of *Erigeron lemmonii* as seen under the microscope**.** The bi-lobed stigma opens as it becomes receptive. Seven pappi, approximately 2.6-2.8 mm in length, are attached to the top of the inferior ovary and the secondary pappi reduced but present in the dark band at the top of the ovary. Drawing by P.Bailey.

Reproduction.

Mating system

There is a diversity of mating systems, and self-incompatibility (SI) and self-compatibility (SC) are documented within the family Asteraceae (Proctor et al. 1996; Horsburgh et al. 2011). The Asteraceae conform to a general rule that sporophytic self-incompatibility is correlated with the dry papillate stigma type and the trinucleate condition of the pollen grain (Heslop-Harrison and Shivanna 1977). Within the tribe Astereae there are many species that are 1) obligate outcrossers (xenogamy), with self pollination mechanisms by either autogamy (selfed within the same flower) or geitonogamy (selfed from a different flower of the same plant) producing a low seed set (Brouillet 1981; Jones 1987; Horsburgh et al. 2011).

This genus is characterized by conspicuous rays and relatively numerous disc florets, features consistent with outcrossing mating system (Noyes 2000b) and has been confirmed with molecular techniques (Noyes *et al*. 1995). Self incompatibility (SI) is also important in promoting species within the genus of *Erigeron* (Noyes 2000b). However, Knuth (1906-1908) reported that automatic self-pollination is possible in *Erigeron* during later stages by contraction of the stylar branches, which are brought into contact with pollen of the same flower.

Data suggest that agamospermy (the ability of a plant to produce seeds without fertilization, also known as apomixis) has arisen a minimum of three times in the genus of *Erigeron* (Noyes 2000a) and has been reported for *Erigeron strigosus* (Noyes 2006a, 2006b) *E. compositus* (Noyes *et al*. 1995), and *E. mucronatus* (Spongberg 1971). Seven species have odd ploidal chromosome counts, which is a strong correlate of agamospermy (Noyes 2000a). Documented presence of polyploidy and agamospermy in an increasing number of species of *Erigeron*, points to hybridization and polyploidy affiliated with genetic drift, or gradual geographic isolation, or both, as evolutionary mechanisms within the genus (Spongberg 1971). For *Erigeron* species under greater environmental stress, the chromosome number is increased and reproduction becomes vegetative and /or agamospermic (Spongberg 1971). Triploidy is rare in the Asteraceae and the Astereae. Triploids probably survive because they are apomicts where meiosis is absent, and thus eliminates the problem of triploidy in sexually reproducing plants (Semple Pers. Comm. 2013).

Vegetative reproduction

Field observation and root scan analysis (Bailey et al, in preparation) show that *Erigeron lemmonii* is capable of reproduction by ramets, producing a tangled system of rhizomes that root at the nodes and produce vegetative flowering mats. Where rich organic soil builds up in the cracks and crevices of cliffs, *Erigeron lemmonii* is found growing, and a large plant can split into several small plants. These plants are capable of producing roots at nodes along roots and stems (15 new plants have been produced from small stem sections under greenhouse conditions). Spongberg (1971) reported the same reproductive strategy for two species of *Erigeron; E. vagus and E. lanatus* also growing in rocky substrate where the original plants increase in size, and eventually split into several smaller plants which grow in pockets of suitable soil. Vegetative reproduction would not significantly increase the population size quickly or add genetic diversity; however, it could maintain the population for a long time.

Breeding system

In *Erigeron*, the heads are generally heteromorphic; the peripheral ray florets are pistillate, surrounding fertile, inner tubular disk florets which are pistillate or perfect (Mani and Saravanan 1999). If the stigmas and anthers of the same flower are not simultaneously mature the flower is referred to as dichogamy. If this is well-marked and the stigmas are dried when the anthers spring up or vice-versa, only cross-pollination is possible. However, if it is not well-marked, self-pollination is possible later on (Knuth 1906-1908).

In general, *Erigeron* is protandrous, with earlier anther dehiscence preceding stigma receptivity within a flower (Spongberg 1971). As anthesis progresses centripetally, modifications also spread to the immediate inner series of tubular disk florets from the periphery. In the tightly packed peripheral florets, corolla development becomes slender, with the inhibition of the stamen development (gynomonoecy). As more disk florets open, the head becomes more protogynous with the stigma receptivity preceding anther dehiscence (Mani and Saravanan 1999), and with this change, the majority of pollination shifts to butterflies. However, automatic self-pollination may be possible in *Erigeron* during later stages of flowering by contraction of the stylar branches, which are brought into contact with pollen of the same flower, as reported in Knuth (1906-1908).

Chromosome number

The chromosome number for *Erigeron lemmonii* has not been previously reported in the literature (Noyes and Bailey, in preparation). The ancestral number in Astereae is x = 9 (Semple and Watanabe 2009). The majority of *Erigeron* species are diploid (2n=18) and exhibit normal chromosome associations and divisions when undergoing meiosis (Spongberg 1971).

METHODS

Staining and microscopy

Erigeron lemmonii flowers from 26 individual plants from the Scheelite Canyon were collected (May 2011), and the flowers were dissected, examined, and photographed at the Monsanto Center, the herbarium at the Missouri Botanical Garden (June and December 2011 and April 2012 (Missouri Botanical Garden 2011)). Staining slides for the dissecting microscope, light microscope at 4x, 10x and 40x scales, and the scanning electron microscope (SEM) were prepared and viewed to understand the plants' morphology and pollen grain structure. Sketches were made to study the ray and disc floret morphology of *Erigeron lemmonii.* Photographs were taken of style and stigma development and the pollen presentation mechanism in relation to its morphology, and of the pollen grains.

Calberla's Stain was used in slide preparation of *Erigeron lemmonii* for the dissecting and compound microscopes (Figures 2.4, 2.5, and 2.6). Calberla's Stain is translucent pink and is used to identify pollen grains. In Asteraceae most genera have very similar pollen (size, shape and spinescent surface). Calberla solution, composed of 5 mls of glycerin, 10 mls of 95% ethanol, and 15 mls of distilled water, is placed onto a specimen slide. Basic fuchsin solution is added drop by drop to a slide, until a translucent pink color is obtained. The prepared slides were viewed under a compound or dissecting microscope (Dafni et al. 2005) and the floral structures were photographed.

Pollen investigation using scanning electron microscope (SEM).

Slides were prepared for the SEM, from a dry pollen sample using 2, 2–dimethoxypropane (DMP). The following process was used: the preserved *Erigeron lemmonii* samples were rehydrated in deionized water. The anthers were placed into acidified DMP for 20-30 minutes, the samples were then transferred to pure acetone for a few minutes, and then critical point dried in CO2 (using acetone; Erdtman 1969). The dried pollen grains were mounted on stubs using double-stick tape and placed in a Denton-Desk V Sputter Coater. Stubs were sputter coated with gold, and observed in a Nikon –JEOL Neoscope JCM-500 Scanning Electron Microscope.

Blooming data. This section includes flowering progression for a single capitulum based on observation, and seasonal flowering period data based on statistical analysis. Fifty-five individual *E. lemmonii* plants (approximately. 0.05% of the population) growing on ten distinct cliff faces were surveyed once a month from April to September 2012. For each cliff face, the numbers of individual plants were counted and each individual plant was mapped and observed. Flowering data specific to each plant were also recorded over the 6-month survey period. Records included the number of formed buds, actively blooming flowers, and, lastly, the number of spent flowers (peduncles with fluffy seed or no seeds) for each month of observation.

Statistical analysis using an analysis of variance (ANOVA) was done with SAS (2008). Changes over time in the botanical variables (e.g., number of buds, number of flowers, and number of spent flowers) were analyzed using the blocking design of plants and month as main effects. Data represented count data, which follow the Poisson distribution. Hence, prior to any analysis, the standard transformation of log(y+1) was used to help stabilize the variance. The model is $\log(y+1) = \mu + \mathrm{Plant}_j + \mathrm{Month}_k + \epsilon_{jk}$. The results section presents the statistical data for three plant stages of buds, flowers, and spent flowers, and totals were summarized for each. Graph analyses of these data were also summarized using Origin8.6® (Origin Lab Corporation 2012).

Mating system experiment.

Seed set

The purpose of this experiment was to determine if *Erigeron lemmonii* is outcrossing, selfing, or agamospermous. These possible combinations have different ramifications for the population: if the plant is an outcrosser and self incompatible it would rely on insect pollinators; if the plant is selfing (autogamous or geitonogamy) it would be self-compatible and individual plants would be inbreeding; or if it were able to produce seed without being fertilized (apomixis) it could be self-sustaining. The population may also exhibit a combination of reproductive strategies. The mating system experiment was conducted over a three week period during May 2012 in the Scheelite Canyon at Fort Huachuca Army Base, Arizona. Three sites, on three separate cliff faces were chosen. For each site, five treatments were set up within a group of five or more *Erigeron lemmonii* plants, as described by Dafni, et al. (2005).

To prepare the five treatments, all open flowers on the plants were removed before the plant was bagged with a mesh bag to exclude pollinators (except for the open-pollinated plants) and labeled. The five treatments were: 1) Control, consisting of two plants bagged with no manipulations; 2) Outcrossed, (consisting of three plants bagged; 3) Self-within-same-flower, consisting of three plants bagged; 4) Self-within-same-plant, consisting of three plants bagged; and 5) Open-Pollination, consisting of three plants, unbagged and untreated manually. At 24 and 48 hours, when buds opened to produce flowers from the bagged plants, the treatments were applied as described below, and they were rebagged to prevent insects from getting to the plants. The control treatment was untouched, and bagged, leaving the bag in place until the flowers became dry, when the flowering heads were collected. The outcrossed treatments were manually manipulated by brushing one plant's flowers (on the capitulum–flowering head consisting of ray and disk florets) with another plant's florets (from one capitulum), and bagging them. For the self-within-same flower treatment, florets were dusted with their own pollen, but this was hard to discern because of the size of the individual florets. The open flowering heads have florets at various stages, so some of the florets dusted would be receptive to fertilization. Open-pollinated treatments consisted of plants with flowering heads that were not bagged and available to visiting insects (including an unknown subset of pollinators). After the crossings were conducted, plants were re-visited during every 24-hour period, until the flowering heads became dry and/or fluffy (spent flower stage). Dried flowering heads were picked prior to seed dispersal, and placed in separate labeled envelopes for each plant/treatment.

Slides were prepared from the harvested seed heads (one flowering head per slide), and stained with Aniline Blue stain (Dafni et al. 2005). In Asteraceae, each ovary can produce only one seed, or the seed is aborted (shriveled). Total florets from each flowering head were counted within each treatment type (combining disk and ray florets). The cypselae: floret ratio was determined by analyzing heads with a Swift dissecting microscope (at 40 X), and deriving the percentage by dividing the number of seeds by the number of ovules for each head collected for the different treatment types. These data were analyzed using SAS and treatment types were compared.

RESULTS

Microscopy. *Erigeron lemmonii* flowers from 26 individual plants were examined (ranging from 1-3 flowers on small plants to 1- 47 flowers on larger plants). Involucres ranged from 2.5 x 5-10 mm in diameter as reported by Nesom (2006). Within the genus of *Erigeron,* the flower heads are dichromatic, which is associated with dimorphic capitulum of disc and ray florets. Attraction mechanisms are visual (color and pattern in both visible and UV light (Baagøe 1978)); chemical (scent); and nutritive (pollen and nectar). On an individual capitulum, there were a mean of 56 yellow disk florets (28 minimum and 73 maximum disk florets per capitulum). There were a mean of 45 white ray florets per head (35 minimum and 60 maximum ray florets). The rays are without an abaxial midstripe, and the laminae do not coil or reflex. The average seed set in the natural setting consisted of 62 cypselae per capitulum from both disk and ray florets, as observed under the microscope.

Floral parts were measured. The corolla tube is 3.2-3.4 mm in length from measurements taken. Filament length is approximately 1 mm, and the filament is inserted in the upper third of the corolla tube and highly appressed. Anther length is 0.08 mm to 1mm. Two distinct whorls of bristles are attached at the top of the inferior ovary; one whorl of 7 pappi have the pappus length of 3.0 -3.2 mm. The second whorl has very short bristles as seen as the dark band above the ovary (in Figure 2.6, 2.7 and 2.8). Style length in the disk floret is 3 mm. As the style emerges from the corolla tube, and the two stigmatic lobes separate, but do not reflex back (as reported for the genus by Knuth in 1908). The stigmas are dry and papillate (with unicellular papillae) or epapillate (Heslop-Harrison and Shirvanna 1977) and once the stigmatic head desiccates, the style shrivels back into the corolla tube.

The ray florets are 8 mm in length and 1 mm wide (at the widest point). The florets have an inferior ovary 1 mm in length. The style length is also 3 mm. The seven to ten pappi are 2.6-2.8 mm in length and attached at the top of the outside of the ovary. In the pistillate ray florets, the stigmatic lobes open and reflex back; however, in the disk florets they do not reflex back (Figure 2.8).

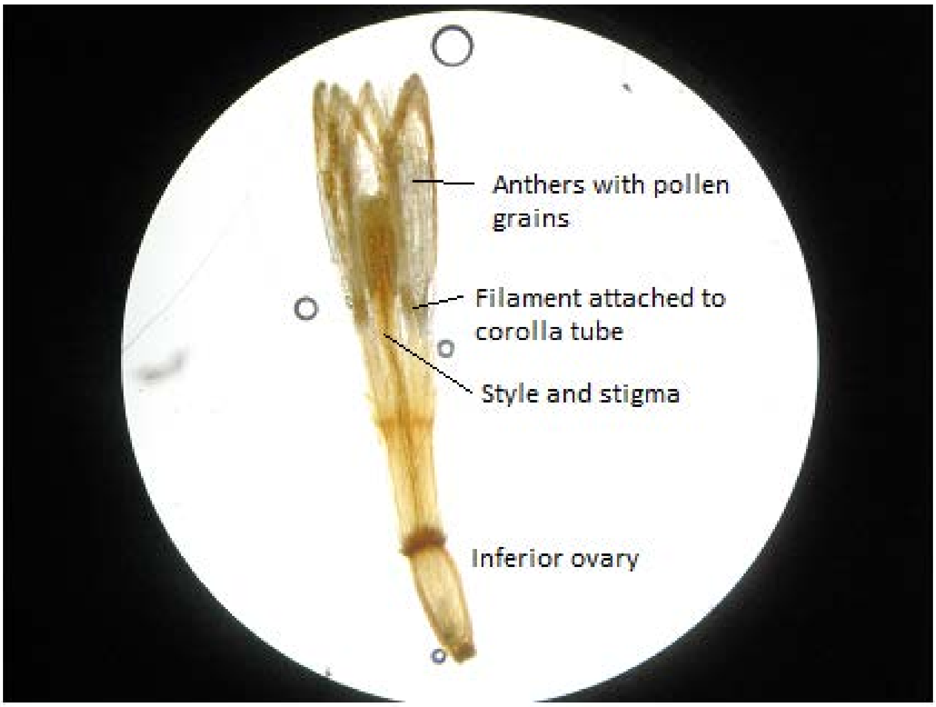

Figure 2.8. Disk floret with inferior ovary, the style is in the center of the corolla tube, and the two-lobed stigma at the top of the style is in the process of retracting (4x). Photo by P. Bailey, 2011.

Pollen presentation and pollen morphology. In Asteraceae, there are three common types of pollen presentation (Yeo 1993): 1) on a stylar brush projecting beyond the anther-cylinder; 2) by the stylar piston mechanism causing pollen to appear on the apex of the anther-cylinder; and 3) via a combination of these two arrangements, extrusion first, followed by emergence of style branches with the remainder of the pollen. The correct position of the distal part of the style immediately before dehiscence of the anthers is essential for this system. This depends on the floral parts involved and the type of pollen presentation.

In Asteraceae, the ray florets can protect the pollen from moisture in some genera (Knuth 1908) and the pollen is enclosed in an anther-tube, from which it is discharged by a shortening of the filaments when they are disturbed by insects. Many members of the family have stamen filaments that are irritable when being touched. In *Erigeron lemmonii* the anthers are attached to the inner wall, at the upper third of the corolla tube (Figure 2.9). It is difficult to tell when the anthers dehisce (Yeo 1993). According to Thiele (1988), they dehisce at the beginning of anthesis and are reported to dehisce around noon. The pappus may also aid in removing pollen from visiting pollinators.

Asteraceae pollen grains are characterized by a thick, elaborate, multi-layer exine (Blackmore et al. 2009). In Astereae, pollen are generally small, oblate or speheroidal, round, or in polar view, triangular. The tectorum is microperforate, covering the whole surface and spines are average to many, evenly distributed, and conical pointed 1.5-5.0 µm (Blackmore et al. 2009). Pollen grains in Asteraceae are trinucleate, as far as is known (Brewbaker 1967).

Figure 2.9. Pollen grains visible on anthers attached to the wall within the corolla tube (10x). Photo by P. Bailey, 2011.

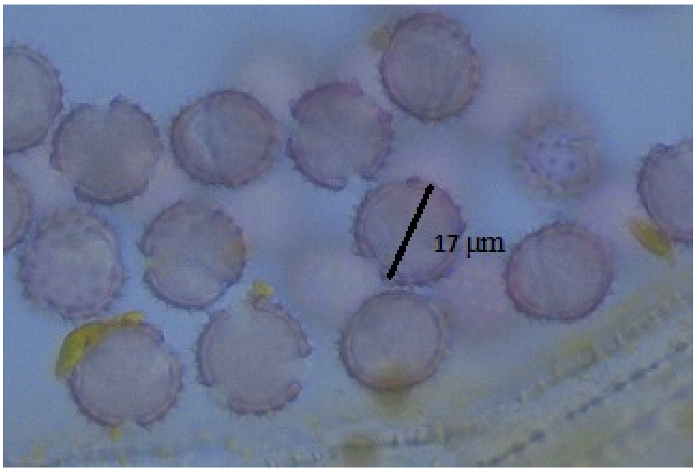

Figure 2.10. Trinucleate spherical pollen grains of *Erigeron lemmonii* with a spinescent exine (100 x). Photo by P. Bailey, 2011.

Photos taken of the *Erigeron lemmonii* pollen grains confirmed the trinucleate spherical morphology (Figures 2.10 and 2.11). Pollen samples measured from exine to exine were 17µm in diameter, on average. The pollen surface was spinescent exine with 2µm spikes. The spines increase the surface area for adhesion, to aid in attachment of pollen grains to animal pollinators (Bolick 1986). Pollen viability was estimated to be 85.8% by Noyes (Noyes and Bailey, in preparation).

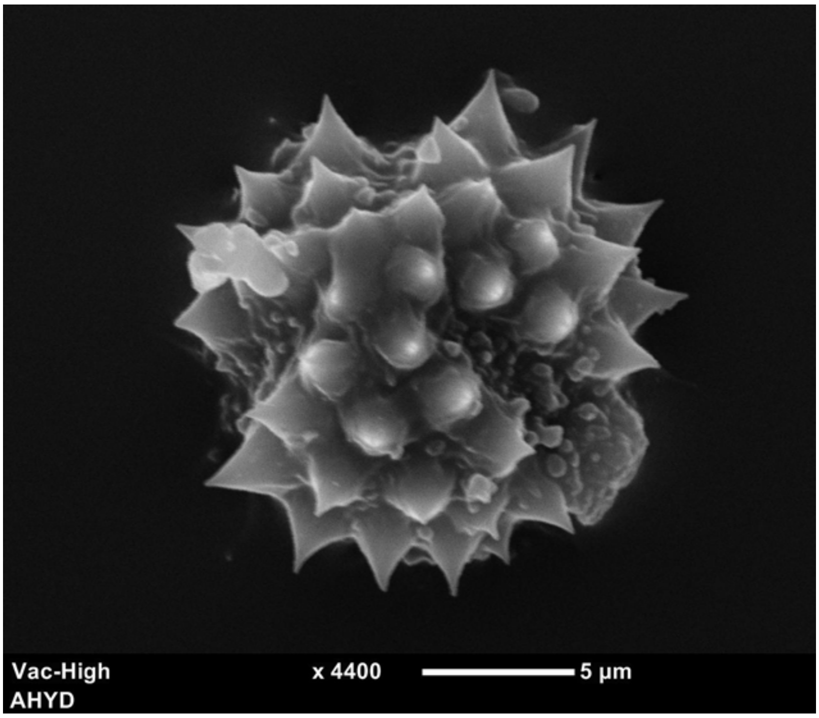

Figure 2.11. *Erigeron lemmonii* pollen grain has a spinescent exine surface with 2µm spikes. SEM Photo by David Bogler, 2011.

Bloom data.

Flowering Progression

Erigeron lemmonii's progression of flowering was observed under natural conditions, once a month during evenly spaced trips from April–September, 2012 with the exception of May. During May 2012 a longer trip was made to conduct the mating system experiment and observe the plant over an extended period. Over the winter *Erigeron lemmonii* plants are a dormant with much dead leaf material visible on the top of the mat. As the weather warms, the brittle, dead leaves are broken off and replaced with new growth from the live stem tips. As the plant become green and vibrant, small green tight buds start to appear and these grow to larger buds in approximately 6-7 days. The large buds with white ray florets (petals) just appearing at the top of the capitulum take approximately 24-30 hours to open, with the ray florets fully extended around the disk florets. At this time, the ray's stigmas open and two stigmatic lobes are presented. The disk florets start to open centripedinally from the outside towards the middle in about 6–12 hours with the stigmas extending to the apex of the corolla tube. The disk florets can take several days to open. However, the disk floret stigmas of *Erigeron lemmonii* are presented at the apex, and do not appear to open fully in florets observed under the microscope. The florets are persistent (during the day and night) remaining open for another 6-9 days, before becoming spent. The rays will turn from white to light pink to lavender as they age, and the center disk florets will often turn brown and not open fully. The entire process depends on weather conditions; primarily temperature and moisture. The flowering progression for a single capitulum is approximately three weeks as observed in natural conditions. The seeds may remain on the capitulum, or easily shatter and are dispersed by natural forces such as wind or animals, after the three-week flowering period.

The number of buds on a plant ranged from a minimum of 3 to a maximum of 122. Not all buds opened during the season. The total number of flowers was 828 for the 55 plants sampled and ranged from 0 to 90 flowering heads on individual plants (five plants had 0 flowers). The average number of flowering heads per plant was 15.

Flowering season

Results from this analysis are shown below (Tables 2.1.; 2.2; and 2.3. and Figure 2.12.).

Table 2.1. Analysis of Variance for *Erigeron lemmonii* buds, flowers and spent flower stages for 6 months in 2012.

Source/model	DF	Sum of Sqs.	Mean Sq.	F Ratio	Prob >F
Bud	59	288.61	4.89	15.09	<.0001
Error	270	87.52	0.32		
C: Total	329	376.13			
Flower	58	158.96	2.74	6.66	<.0001
Error	216	88.81	0.41		
C. Total	274	247.78			
Spent flower	58	351.28	6.05	18.40	<.0001
Error	216	71.07	0.32		
C. Total	274	422.36			

Note: >0.05 not significant

Life stage: Buds

Of the 330 observations, (66 plants over five measuring periods), the data indicated that the least squares mean for the number of buds per plant was 1.03 with an error mean square of 0.32. The model as defined above explained 76.7% of the total variance, which indicates a relatively good fit. The ANOVA further indicated that the overall model was highly significant (F = 15.09, p-value < 0.0001). Table 2.2 partitions the total variance into all known sources, which are differences among plants and differences among sampling months. As is readily observed from Table 2, both main effects are significant. That is, for differences among plants, the F-value is 4.40 with an associated p-value of < 0.0001 and for differences among sampling months, the F-value is 130.48 with an associated p-value of <0.0001. Ancillary analyses using Tukey’s hsd test (Table 2.3) further indicate that the largest number of buds/plant occurred during the month of May and the least occurred in July. Furthermore, the average number of buds/plant in April was not significantly different from those observed in July. The months of August, September, and June each exhibited average values which were different from each other, but smaller than that observed in May and larger than those observed in April and July.

Life stage: Flower

The flowers per plant data set contained 275 observations, as no readings were recorded for the month of April (bud stage in April only). With these data, the model explained 64.14% of the total variance and exhibited an overall mean of 0.77 with a root mean square of 0.64. The ANOVA indicated an overall significant model effect (F= 6.66, p-value < 0.0001).

As with the life stage parameter of number of buds/plant, the data that represent the number of flowers/plant likewise exhibited significant main effects. With this parameter, the F- and p-values were F = 3.92, p < 0.0001 for plant, and F = 43.60, p < 0.0001 for sampling month, respectively. The largest number of flowers/plant occurred in May, with the least number occurring in July. The months of June, August, and September exhibited similar means that were all smaller than the mean observed in May, yet larger than that observed in July (Table 2.3).

Life stage: Spent flowers

The life stage parameter of spent flowers/plant also contained 275 readings. The model explained 83.17 % of the total variance and exhibited a least squares mean of 1.72 with a root mean square of 0.57. The ANOVA indicated an overall significant model effect (F= 18.40, p-value < 0.0001). As with the other two parameters, the main effects were also highly significant, F=5.78 (p < 0.0001) and F = 43.60 (p < 0.0001), respectively, for differences among plants and differences among sample months. The largest number of spent flowers/plant occurred in June and July, whereas the least amount of spent flowers/plant occurred in May. The average numbers of spent flowers/plant for August and September were similar, but different from those observed during the other sampling months (see Table 2.3).

Table 2.2. Effect Tests: number of buds, flowers and spent flowers per *Erigeron lemmonii* plant and sampling month, 2012

Source	Nparm	DF	Sum of Sqs.	F Ratio	Prob > F
Buds					
Plant	54	54	77.10	4.40	<.0001
Sampling Month	5	5	211.50	130.48	<.0001
Flower					
Plant	54	54	87.24	3.92	<.0001
Sampling Month	4	4	71.72	43.60	<.0001
Spent flower					
Plant	54	54	102.78	5.78	<.0001
Sampling Month	4	4	248.50	43.60	<.0001

Note: >0.05 not significant

Table 2.3. Least square means for bud, flower, and spent flower stages by sampling month for *Erigeron lemmonii*, 2012.

Level	Least Sq Mean	Untransformed Mean
Buds		
4: April	0.22 E	0.25
5: May	2.51 A	11.30
6: June	0.71 D	1.04
7: July	0.20 E	0.22
8: Aug	1.51 B	3.54
9: Sept	1.04 C	1.84
Flowers		
5: May	1.68 A	11.30
6: June	0.58 B	0.80
7: July	0.12 C	0.13
8: Aug	0.62 B	0.87
9: Sept	0.83 B	1.30
Spent Flowers		
5: May	0.16 C	0.13
6: June	2.68 A	1.30
7: July	2.74 A	4.36
8: Aug	1.41 B	0.80
9: Sept	1.58 B	0.87

Note: Means followed by the same letter are not significantly different at P > 0.05.

Ancillary analyses using Tukey's hsd test (Table 2.3) further indicate that the largest number of buds occurred during the month of May and the least occurred in July. Furthermore, the average number of buds per plant in April was not different than that observed in July. The months of August, September, and June each exhibited average values that were different from each other, but smaller than that the values observed in May and larger than those observed in April and July. The largest number of flowers per plant occurred in May, whereas the least amount of flowers per plant occurred in July. The average numbers of flowers per plant for June, August, and September were similar, but different from the values observed in May and July. The largest numbers of spent flowers/per plant occurred in June and July, whereas the least amount of spent flowers/plant occurred in May. The average numbers of spent flowers/per plant occurred in August and September, but were different from the numbers observed during the other sampling months.

Figure 2.12 shows the mean for buds, flowers, and spent flowers in each month sampled.

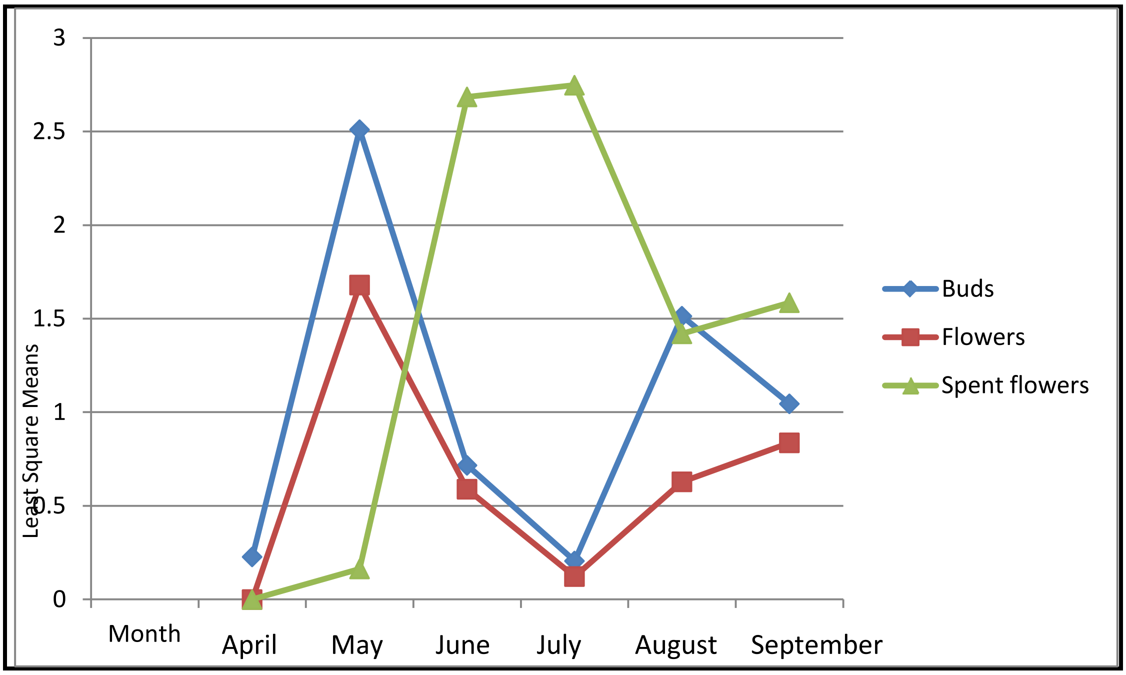

Figure 2.12. Least square means for *E. lemmonii* buds, flowers, and spent flowers showing the mean for each sampling month, in 2012.

Analysis and interpretation of the mating system.

This research consisted of analysis to determine which plant mating system *Erigeron lemmonii* has under natural field conditions. This analysis applied five treatments to three groups of *E. lemmonii* and measured the number of cypselae (stained) produced and the number of total florets (both disk florets and ray florets combined) per flowering head. For some plants there was more than one flowering head (capitulum). The research consisted of recording outcomes for the following treatments that manually hand-pollinated crosses (replicating the different types of mating systems). This resulted in five treatments; open, outcrossed, selfed, gietonogamy, and the control. Table 2.4 summarizes the number of flowers and seed/ovule percentages for plants in each treatment type. One of the exclusion nets for the control was removed (by an animal or bird) and found on the ground near the plant, so data were based on only two plants. The data collected were analyzed with SAS software (SAS 2008) as a completely randomized design (CRD) to determine seed set, indicating which type of mating system occurs in *E. lemmonii*.

Table 2.4. Seed development for the five mating treatment types for *E.lemmonii, 2*012.

Treatment type	No. of plants sampled	No. of heads sampled	No. florets per head	% filled cypselae per head
Open	3	7	109.2	18.7%
Outcrossed	3	7	95.8	12.1%
Selfed	3	6	84.1	1.1%
Gietonogamy	3	6	87.1	1.8%
Control	2	2	108.0	0%

Response: Ratio of the number of seeds to the number of florets.

The ratio of the number of cypselae/floret indicated that the CRD model explained 87.81% of the total variance. Additionally, the overall mean for these responses was 0.34 with a root mean square of 0.10. Prior to the analysis, the Levene's test was used to check the assumption of equal variances among the five groups. This evaluation did not find enough evidence to suggest that the variances were not equal (Levene's F = 1.16, p-value = 0.35). Hence, the cypselae/floret data did not require a variance stabilizing transformation and they were analyzed accordingly.

The ANOVA (Table 2.5) strongly indicates that differences in cypselae/floret are present (F = 41.43, p-value < 0.0001). Tukey's hsd test (see Table 2.6) further shows that two major groups were present among the treatments.

Table 2.5. Effects Tests for five mating treatments applied to *E. lemmonii*, 2012.

Source	**Nparm**	**DF**	**Sum of Sqs.**	**F Ratio**	**Prob>F**
Treatment	4	4	1.81	41.43	<.0001

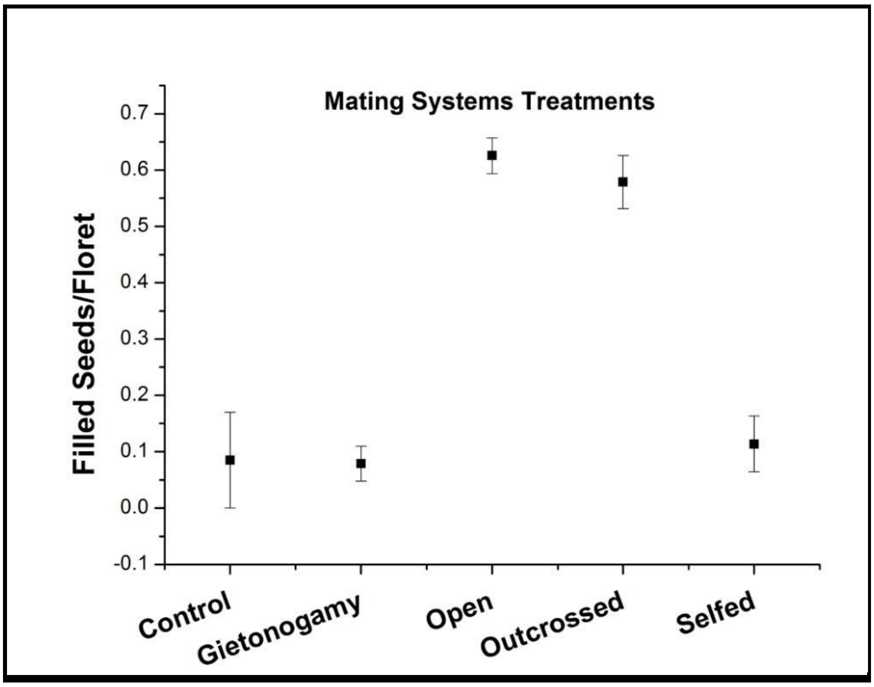

Figure 2.13. Least square means of cypselae/floret ratios for tested mating system treatments for *E.lemmonii*, 2012.

Table 2.6. Significant differences in Least Square Means for five mating treatments applied to *E. lemmonii*.

Treatment	Least Sq Mean
Open	0.62 A
Outcrossed	0.57 A
Selfed	0.11 B
Control	0.08 B
Gietonogamy	0.07 B

Note: Means not connected by the same letter are significantly different at P > 0.05.

The Open and Outcrossed treatment groups each displayed similar means, whereas the treatments of Selfed, Gietonogamy, and Control exhibited mean characteristics that were similar to each other. The open pollinated plants in field conditions had the most seed set, whereas pollinator exclusion treatments yielded the least seed set. Therefore, *Erigeron lemmonii* does not appear to set seed with its own pollen, and thus is xenogamous, dependent on insect pollinators. The low seed set within the selfed, gietonogamy, and control treatments indicates *E. lemmonii* has a self-incompatibility system.

DISCUSSION AND CONCLUSION

Pollen presentation. The small size of the pollen grain (consistently 17µm in diameter), and its circular shape and spinescent exine surface with 2-µm spikes is characteristic of the *Erigeron* genus. This pollen morphology aids in attachment of pollen grains to animal pollinators with hairs, such as flies, native bees, wasps, beetles, and butterflies. A large quantity of pollen is produced that is 85% viable (unpublished data, Noyes). Sexual plants produce pollen of uniform size and high viability (Noyes et al. 2006), whereas apomictic plants produce pollen of low quality and high size variation. *Erigeron lemmonii* has uniform-sized pollen that is of high quality, consistent with an outcrossing mating system. However, many plant species include both sexually and apomictically reproducing populations (Gustafsson 1946-1947, Grant 1982, Asker and Jerling 1992). The sizes of pollen grains are also important; pollen grains of apomictic plants generally measure 17 µm (length) or greater compared to pollen grains produced by sexual plants, which are usually between 12 and –15 µm and lack micrograins (Noyes et al. 2006).

The nectar of the Asteraceae family is known to be hexose rich (Baker and Baker 1983, Lane 1994) and is the most sought after resource (Kevan and Baker 1983), whereas the pollen is eaten by a diversity of insects, and can provide a balanced diet compared to plant tissues or nectar (Willmer 2011). Many plants reliant on xenogamy have longer-lived pollen, so the pollinators can spread the pollen as they forage, and thus fertilize the plant with viable pollen (Willmer 2011). Additionally outcrossing plants also produce a higher amount of pollen (can range from 1,000, to 10,000 grains); of which only a small amount is necessary for maximum seed set (Willmer 2011). *Erigeron lemmonii* produces a large amount of viable pollen within the multiple florets.

Flowering progression. *Erigeron lemmonii's* progression of flowering within a single capitulum was observed under natural conditions, once a month from April–September 2012. Small buds grow to larger buds in approximately 6-7 days, and the large buds start to open in approximately 24-30 hours, with ray florets fully open. At this point, the ray's stigmas open and two stigmatic lobes are presented. The disk florets start to open centripedinally approximately 6–12 hours after the rays are fully open. In the disk florets the stigmas extend to the apex of the corolla tube, and separate into two lobes, but do not curl backwards. The florets are persistent (during the day and night), remaining open for approximately six to nine more days, before becoming spent. The flowering process is long, about 3 weeks total from the tight bud formation to the spent flower stage of the dried flowering head. This long flowering progression for a single capitulum can accommodate a wide range of insects including flies, native bees and wasps, beetles, butterflies, and nocturnal moths.

Flowering period. In winter the plant is dormant for approximately 4 months (temperature dependent). Over the spring much of the dead leaf material falls off as new growth occurs from the crown and vigorous stems. For the 55 plants sampled (approximately 5% of the population) and the flowering data summed, May was the month with the most flower buds (802) and flowers (513) for the sampled plants (Figure 2.14). The other period of significant bud formation is August (321 buds), after the monsoonal rains occur in July and August. Flowering occurs throughout September, the second-most important flowering month (136 flowers). Although data collection ended in September, buds were still being produced to a lesser degree in September, suggesting a sustained but small flowering period into October and possibly November depending on the temperatures during any given year. June and July have significantly higher counts for spent flowers than any other months (1061 and 1113, respectively). July's high spent flower count resulted from many dried flower heads being counted again, plus an additional amount from the past month's flowers.

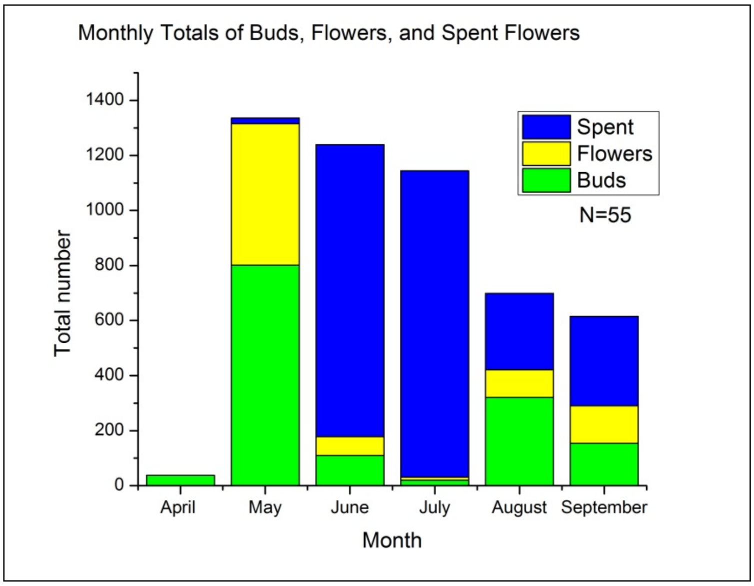

Figure 2.14. Flowering data including monthly totals of bud, flower, and spent flower data for *Erigeron lemmonii*, 2012.

There were a total of 1445 buds and 828 flowers, with an overall flowering ratio of 57%. The proportion of buds, flowers, and spent flowers for *E. lemmonii* during the 6-month flowering period was analyzed (Figure 2.15). This analysis confirms May as the primary flowering period and September as the secondary flowering period after the monsoon rains. The flowering season for *Erigeron lemmonii* is much longer than previously reported in the literature, starting in April and continuing through October, and quite possibly extending into November.

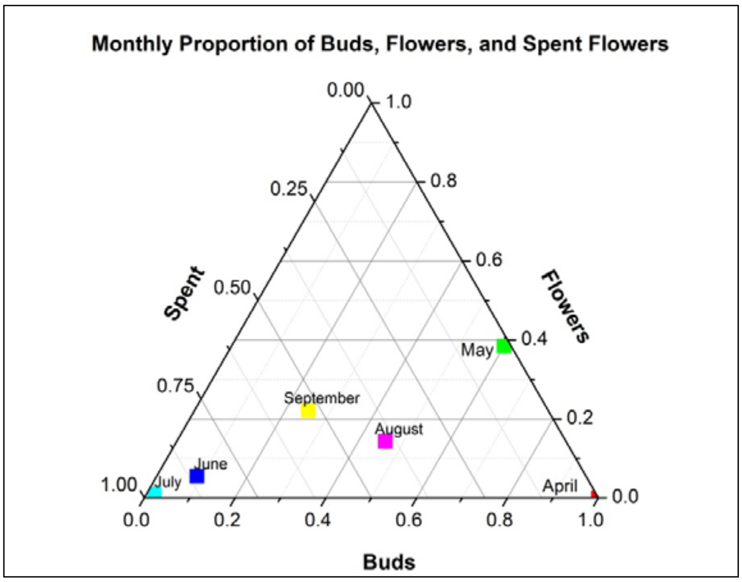

Figure 2.15. The mean proportion of buds, flowers and spent flowers for *Erigeron lemmonii*. Each point represents an individual month, April through September, 2012.

Erigeron lemmonii takes advantage of the right environmental conditions to flower and grow throughout an extended 6-month period; blooming profusely in May, after spring rains, and again in September, after monsoon rains, and persisting into October (and perhaps November and December if weather conditions remain warm). This bimodal flowering season allows the plant to take advantage of two different rainfall seasons, thereby ensuring survival in the arid Southwest. The continuity of flowering allows for the maximum visitation, and for a different suite of pollinators throughout its long flowering period. The long duration of the flowering season and floral progression would suggest that a variety of pollinators are able to fertilize the flowers located at different elevations on the cliffs. Flower visitation by flies, wasps, beetles, and butterflies was observed during the day (Bailey, Higgins and Cobb, in preparation), and also by moths at dusk. Pleasants (1983) reported that *Erigeron* has diurnal separation of pollinators and visitation based on dehiscence at midday for the genus, which suggests this temporal division actually supports a greater number of insect visitors continually until senescence.

Mating system investigation. Results indicate that the open pollinated treatment (0.625) and the outcrossed by manual treatment (0.578) were not distinctly different and had the highest values for all five treatment types. This indicates that *Erigeron lemmonii* is dependent on insect pollinators to outcross with other individuals within its population. Conspicuous rays and numerous disk florets are consistent with an outcrossing mating system (Noyes 2000b) and xenogamy occurs in other members of the genus of *Erigeron. Erigeron lemmonii* flowering heads examined under the microscope showed greater seed set for open pollinated plants yielded approximately 18.7% developed cypselae (cypselae /floret ratio). Pollinator exclusion significantly reduced seed set for *E. lemmonii*. Geitonogamous, Selfed and the Control (<1.8%, <1.1% and 0 respectively), showing the species is not capable of making seed autonomously either by selfing or apomixes. The 1.8% is considered “leaky” for the geitonogamous treatment, meaning that within the second generation the seeds would not be fertile (Noyes, pers. comm.). This evidence is consistent with the hypothesis that *E. lemmonii* possesses sporophytic incompatibility (Noyes and Bailey, in preparation), as has been described for other Asteraceae (Gerstel 1950; Nettancourt 1977).

Similarly, *Erigeron kachinensis*, another endemic population with a similar life-history found growing on cliffs in the arid Southwest (Allphin and Harper 1994, 1997) is an obligate outcrosser, with a self-incompatible system (Allphin et al. 2002). It is further surmised (Allphin et al. 2002) that *E. kachinensis* appears to exhibit a homomorphic sporophytic type of self-incompatibility system, as found in other composites. Self-incompatibility is believed to have evolved to prevent inbreeding in plants (Nettancourt 1977). In small, isolated populations, a self-incompatible plant species could exhibit reduced reproductive success from the inability to cross with compatible plants (Byers and Meagher 1992) and experience limited seed set from expression of lethal genes during embryo development (Wein et al. 1987, 1989; Allphin et al 2002).

Erigeron lemmonii is also capable of vegetative reproduction, as it reproduces by rooting easily from nodes on its stems and roots (Noyes and Bailey, in preparation, Bailey et al. in preparation). This has implications for the population; it may continue to increase in numbers as older plants find new pockets of suitable soil and plantlets divide by vegetative means from the parent plant. This reproductive method produces a clone of the adult parent, so the genetics are preserved within the population and limits inbreeding. However, this method precludes the introduction of new genetic combinations. This has also been observed in

Erigeron mucronatus DC. (Mehra and Gill 1972), which has a similar rock habitat and long flowering period, but grows on rock substrates in the western Himalayan Mountains.

Xenogamy can create new genetic combinations to keep the population from becoming inbred. An initial genetic investigation was done to obtain microsatellite markers (Lindsay et al. 2012). Eight loci were developed for *E. lemmonii* exhibiting a range of observed heterozygosity, indicating some genetic diversity within the population. Genetic viability in individual plants has been correlated with increased survivorship (Schaal and Levin 1976, Hamrick 1979). Allphin and Windham (2002) found a positive correlation between heterozygosity and longevity in *Erigeron kachinensis,* and they suggest this is the reason why this cliff-growing perennial is a long-lived species. Many cliff-growing species are long-lived perennials and once established, the population can remain relatively stable in the environment without significantly changing (Larson et al. 2000). Although individuals may grow at different rates depending on microhabitat differences, *Erigeron lemmonii* appears to be a stable population and the population may be slowly increasing as observed by monitoring efforts (Stone 2012 Personal Communication).

Erigeron lemmonii has recently been removed from the Candidate Species List (USFWS 2012) because human disturbance and wildfire do not appear to be significant threats to this cliff-adapted species. *Erigeron lemmonii* is well adapted to its environment, and has many traits that allow the population to be successful. These traits include its multiple floret morphology, which produces large amounts of viable pollen. *Erigeron lemmonii* has a xenogamous mating system with a long flower progression, and extended bimodal flowering season, which allows for outcrossing by a range of insects (Bailey, Higgins and Cobb, in review). For the open pollinated flowers examined (n=14), there was a cypselae/floret ratio of approximately 18.7%. Total flowering was 57% (n= 55), for 5% of the population over the six month flowering season during 2011. However, further genetic study of the population would be useful to determine more about the genetic structure within the *Erigeron lemmonii* population and to detect patterns leading to a better understanding of the genetic consequences of isolation for this plant species. Phylogenetic analysis would also be valuable in determining *Erigeron lemmonii's* close relatives, and its lineage and evolution.

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CHAPTER 3

Habitat Soil Properties and Root Characteristics of a Rare Cliff-dwelling *Erigeron* Species Compared to Two Other *Erigeron* Species”

ABSTRACT:

Background: This study focused on three species of *Erigeron,* Asteraceae, that co-occur in the Huachuca Range, which is part of the Sky Island Region, in Arizona, USA. The three species of *Erigeron* have very different life histories. *E. arisolius,* Dry Sun Fleabane; and *E. neomexicanus,* New Mexico Fleabane; are habitat generalists and grow in a broad range of environmental conditions. *Erigeron lemmonii,* a rare endemic species that is a habitat specialist, grows only on cliffs in one canyon. The objectives of this research were to develop an understanding of the relationship between soil nutrient properties and the three species of Erigeron using standard soil chemical testing procedures, and to investigate the root architecture of the three species in order to understand how the species are adapted to their soil and environment. One plant from each *Erigeron* species was scanned and analyzed using Win Rhizo, to determine the relationships between rooting structure and soil attributes, and to understand more about the environmental conditions and the various habitats of each of the three species (Regent Instruments Inc. 2009). Key findings included that *Erigeron lemmonii* soils had larger concentrations of soil nitrogen, potassium, and organic matter, whereas *E. arisolius* had midrange levels of nitrogen and potassium, and had the highest concentration of phosphorous. *Erigeron neomexicanus* soils had the least amount of all three nutrients. Root architecture analysis showed that *E.lemmonii* had the greatest root-to-shoot ratio (4.12), compared to *E. arisolius* (3.80) or *E. neomexicanus* (1.04). *Erigeron lemmonii* has four times the amount of root material supporting its top stems and leaves. *Erigeron lemmonii*, as a long-lived perennial, is easy to root from stem nodes or root pieces, and can easily reproduce by vegetative means, if habitat conditions are right. *Erigeron neomexicanus* which had the most extensive root system is a perennial, found growing in the most diverse conditions of the three *Erigeron* species. *Erigeron arisolius* is an annual and has the smallest root system.

Keywords: *Erigeron lemmonii, Erigeron arisolius, Erigeron neomexicanus,* cliff endemic, root system architecture.

INTRODUCTION:

Region. Little is known about the rare, endemic plant species *Erigeron lemmonii* A. Gray, Lemmon’s Fleabane, Asteraceae family. This species has a limited distribution, growing in the rugged Scheelite Canyon at Fort Huachuca, Arizona, USA. This canyon is within the Huachuca Range, which is part of the Madrean Sky Island Archipelago. The term 'sky island' was first used by Weldon Heald in 1967 to describe mountain ranges that are separated from each other by valleys of hot, dry grassland or desert. The Madrean Archipelago is unique from a planetary perspective (Warshall 1994): it exists between two cordilleras (the Sierra Madre Occidental and the Rocky Mountains); the archipelago contains 40 sky islands within this complex (Figure 3.1); the complex has a north- south orientation but contains an east-west valley bridge through the Deming Gap of the Rocky Mountain Cordillera; the islands have a mixed geological composition unique in the world and a high relief (1,524 m).This sky island complex is the only one known to straddle two major floristic and two major faunal realms, as well as three major climatic zones (tropical, subtropical, and temperate). The 181,300-square-kilometer (70,000-square mile) Madrean Sky Islands region of southeastern Arizona, southwestern New Mexico, and northwestern Mexico is of particular interest to botanists because of its striking diversity of plant species and habitats, with more than 3,000 species of plants recorded (Austin 2010). This topography has created an area of high endemism within the flora and fauna inhabiting the various sky island mountain ranges. In particular the Huachuca Mountain Range is exceptionally rich in plant species (Bowers and McLaughlin 1994) because it has a complex topography, has a wide elevation range, and a diversity of geographical substrates and aquatic habitats, and includes a large Apachian floristic district element.

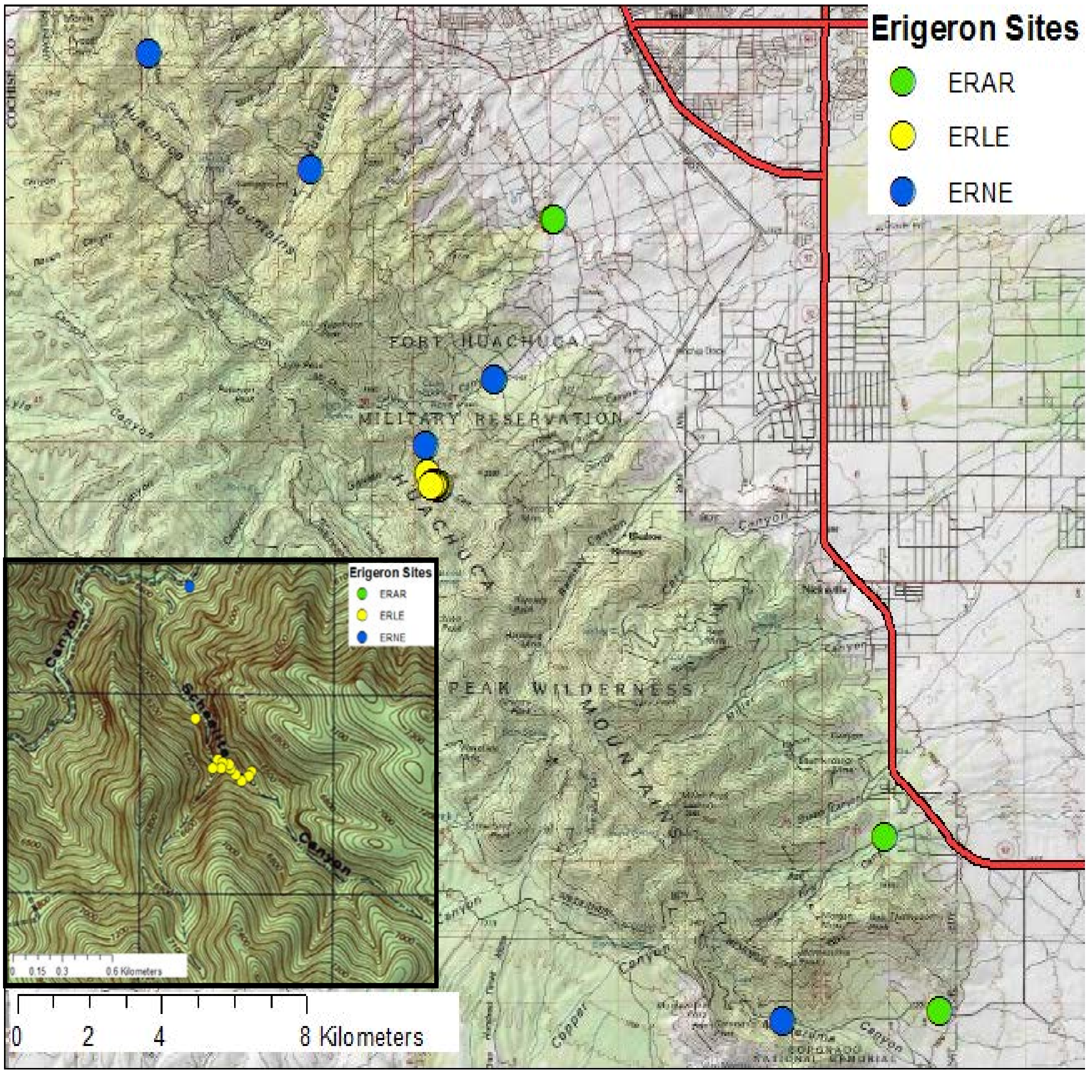


Figure 3.1. Map of Huachuca Range showing the distribution of *Erigeron* sampling sites for *E. arisolius* (ERAR), *E. lemmonii* (ERLE), and *E. neomexicanus* (ERNE). Insert is the distribution of *E. lemmonii* sampled within the Scheelite Canyon (map by Jacob Higgins, 2012).

Geology. The geology of the Huachuca Range is composed of Bolsa Quartzite, the basal sedimentary unit, on Precambrian granite (Bowers and McLaughlin 1994). On the eastern slope, Palezoic sedimentary rocks, mainly limestone but also some siltstone and shales, top the quartzite (Bowers and McLaughlin 1994). On the western slope, sedimentary rocks of the Cretaceous age, including conglomerates and shales are interspersed with volcanic and sedimentary rocks (Keith and Wilt 1978). *Erigeron lemmonii* grows on north- south- and west- facing cliff walls (Warren et al. 1991), at an elevation between 1900 and 2000 m (6300 and 6600 feet) (Nesom 2006). It is a prostrate, perennial plant growing in dense clumps, spreading 10-20 cm (and occasionally up to 60 cm), and is found in crevices and on ledges of vertical cliffs composed of Escabrosa Limestone (Mississipian age).

Climate. A weather station is located at Fort Huachuca at the northern end of the range. Annual precipitation at Fort Huachuca is 37.1 cm (14.6 inches); about half falls as high-intensity “monsoonal” rains in July and August that originate as scattered thunderstorms. Winters are rather dry, the wettest months being December and January, with an average of 4.5 cm precipitation (1.78 inches). Summers and winters at Fort Huachuca are mild. The average temperature in January is 7.9°C (46° F) with average daily maximum and minimum temperatures of 14.7°C and 1.2°C (58.4° and 34° F). Summer temperatures are moderated by afternoon cloud cover. The average July temperature is 25.3°C (77.5°F), with average daily maximum and minimum temperatures of 31.4°C and 19.1°C (88.6° and 66.4° F), respectively.

The study focused on the soil and root properties of three species of *Erigeron* (Fleabanes); one endemic plant adapted to a naturally sparse cliff wall habitat (*E. lemmonii,* Lemmon’s Fleabane) and two others (*E. arisolius,* Dry-sun Fleabane *and E. neomexicanus,* New Mexico Fleabane) adapted to a more diverse habitat. The soil component provides important baseline information to better understand conditions controlling *E. lemmonii* establishment and growth, and to make comparisons between the three *Erigeron* species. This analysis compares the root morphology of the three *Erigeron* species and provides an understanding of how they have adapted their root structure to the soil and their habitats.

Habitat characteristics for the three *Erigeron* species. The three species have very different habitats: *Erigeron lemmonii* is a cliff-growing species, *E. arisolius* is a grassland species, and *E. neomexicanus* occurs in “disturbance” habitats. *Erigeron lemmonii* is an endemic species found at only one location in the rugged Scheelite Canyon, Huachuca Mountains (Cochise County), on Fort Huachuca Army Base, Arizona. It is adapted to cliffs in a naturally fragmented canyon landscape. *Erigeron lemmonii* is a prostrate, perennial plant growing in dense clumps spreading 10-25 cm (occasionally up to 60 cm) growing on shady south-, north-, and west-facing cliff walls and vertical faces of large boulders along the canyon bottom, at an elevation between 1,900 and 2,200 m (6,300 and 7,300 feet) (Nesom 2006). At these locations it is found in crevices and on ledges growing in highly organic soil, on Escabrosa limestone substrate (Warren et al., 1991). There are approximately 954 individuals (Malusa 2006) in the population.

Erigeron lemmonii has recently been delisted as a Category 2 Candidate Species within the U.S. Fish and Wildlife System. In October 2012, the U.S. Fish and Wildlife Service (USFWS) published a proposed rule change in the Federal Register: “The Service also announced that the Lemmon fleabane, a candidate for listing under the Act since 1996, is being removed from the list of candidate species because the main threat (wildfire) does not rise to a level of significance such that the species is in danger of extinction or likely to become so in the foreseeable future.” (USFWS 2012).

Dry Sun Fleabane (*E. arisolius*), a state-sensitive species (Arizona Revised Statutes, 1999), has a wider distribution that includes Arizona and Mexico. *E. arisolius* is reported to be an annual or short –lived perennial (Nesom 2006), is 30-70 cm tall, taprooted, with erect stems consisting of 20-50 flowering heads in loose corymibiform arrays. It grows in grassland habitats at an elevation range of 1300 to 1700 m (4,260 to 5,570 feet), and is reported to bloom in (May-)June - August (-November). There is an abundance of this species at the Fort Huachuca Army Base.

New Mexico fleabane (*E. neomexicanus*) grows in various natural habitats, including ridges, and has the largest range: Arizona, New Mexico, and Mexico (Nesom 2006). *Erigeron neomexicanus* is a 30- to 70-cm tall perennial, with a tap root having cudices; woody, erect stems; and basal leaves with pinnatifid margins and (1-)5-15(-30) floweringheads in loose corymibiform arrays. New Mexico fleabane grows at an elevation range of 1500 to 2700 m (4,920-8,850 feet) on rocky substrates. It appears to be a ruderal species in that it is able to grow in a wide range of conditions including road cuts and stream beds. It is reported to bloom between July or August and October or as late as December.

Cliff habitat characteristics. Environmental factors such as direct sunlight, rapidly moving air, fluctuating temperatures, and a lack of well-developed soil characterize cliff plant habitats (Larson et al. 2000). Within cliff ecology, the relationships between soil chemistry and plant species composition, richness, and growth have not been well-established (Larson et al. 2000). Hora (1947) reports that few cliff species show clear site selection based on soil pH and other soil chemical properties. Soil distribution on cliffs is patchy, but where soils do occur, concentrations of phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) may be even higher than in soils on level ground forest at cliff edges (Young 1996).

The availability of water necessary for plant growth on cliffs has been investigated (Larson et al. 2000). Whereas rocks have little water storage capacity compared to soils, rocks hold water at low tension levels, making water readily available to plants, and, as such, rock substrates are not as unfavorable for plants as they might otherwise appear. If the rock cliff is hydrated over a long period of time by such means as rainfall, dew, or humidity, a small but steady source of water is available to the plant (Larson et al. 2000). The amount of water required to hydrate rock is much less than that needed for soil, and as a result, under dry conditions rock can be a more favorable growing substrate than soil. The advantage that results from cliffs being wetter than the soil at ground level is offset by the fact that cliffs retain less precipitation than a level soil surface.

McAuliffe and Burgess (1994) present an overview of the geomorphology and soil characteristics that affect water dynamics, and the responses of plants to various soil water conditions found in the piedmont grassland landscapes at the base of the sky islands.
A recent article (Poot et al. 2012) examines specialized root architecture and endemism on granite outcrops. Endemic species have similar root characteristics that related congeners growing in deeper soils do not have; the endemic species have a larger biomass of roots overall, they can root faster, and have lower specific root length. Poot et al. (2012) conclude that these endemics have undergone selective pressure to adapt the species to the granite outcrop habitat, and their root growth and root morphology traits are maladapted for these species to grow on the ground, in deeper soils, suggesting this may explain the narrow endemism that many endemic plants exhibit (Poot et al. 2012). They also pose an interesting question: are these narrow endemic species “canaries” for sensing climate change or warming or cooling trends? Would a cliff-growing endemic plant species be able to adapt to an increase in temperature, resulting in drier conditions within its environment?

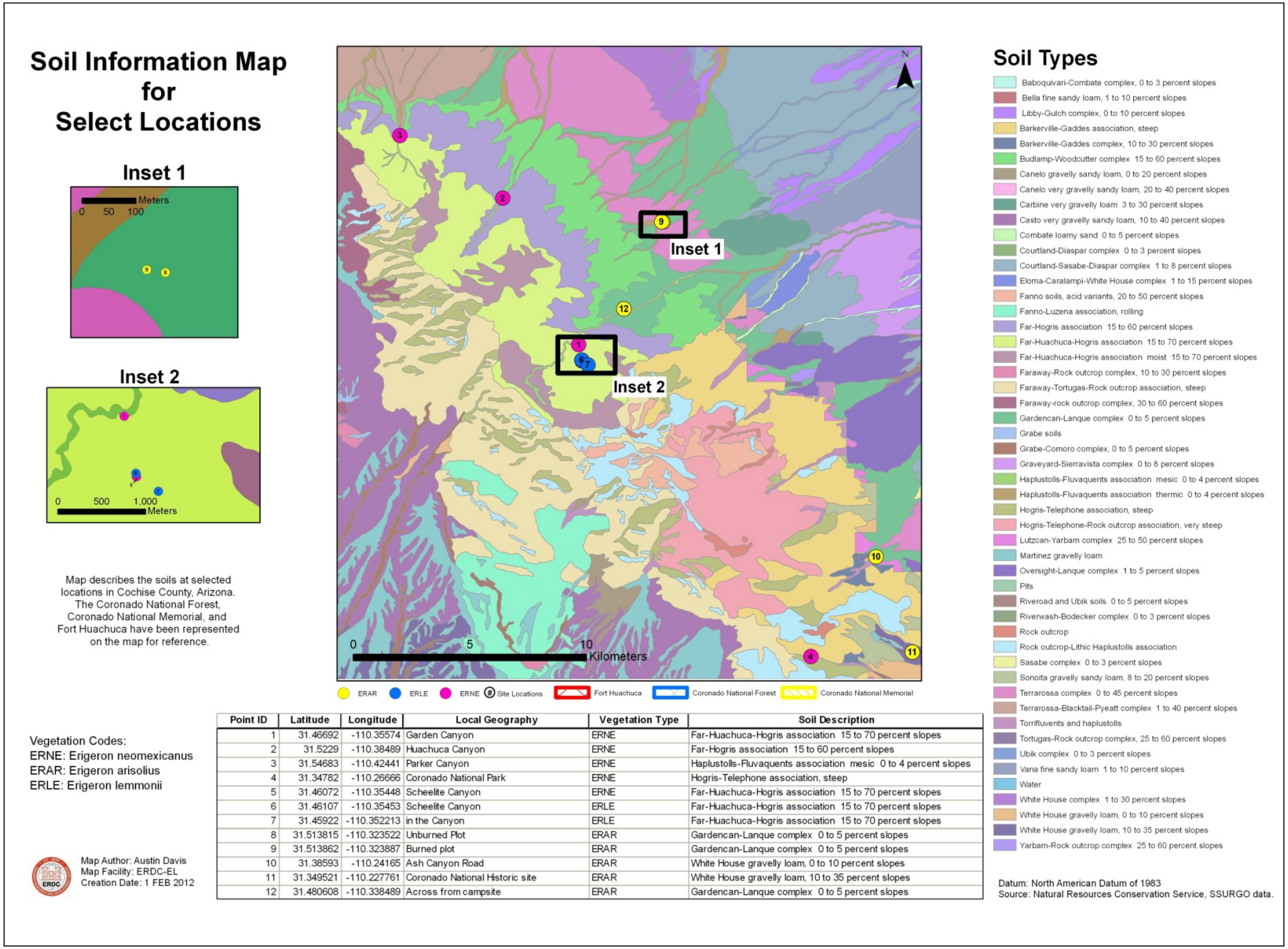
Soil types

The soil types and soil map data were taken from the US Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS) database (2008, 2010). Table 3.1 lists the plots. The point ID and four-letter plant species name (ERNE (*Erigeron neomexicanus*), ERLE (*Erigeron lemmonii*) and ERAR (*Erigeron arisolius*)) are listed first, followed by the GPS coordinates, where they are located, and the soil type at each site. For soil descriptions, see Appendix 3. II.

Table 3.1. Soil type and coordinates for the experimental plots located in the Huachuca Range, 2011

Point ID	Latitude	Longitude	Nominal	TEXT
ERNE 1	31.46692	-110.356	Garden Canyon	Far-Huachuca-Hogris association 15 to 70 % slopes
ERNE 2	31.5229	-110.385	Huachuca Canyon	Far-Hogris association 15 to 60 % slopes
ERNE 3	31.54683	-110.424	Parker Canyon	Haplustolls-Fluvaquents association mesic 0 to 4 % slopes
ERNE 4	31.34782	-110.267	Coronado National Park	Hogris-Telephone association, steep slopes
ERNE 5	31.46072	-110.354	Scheelite Canyon	Far-Huachuca-Hogris association 15 to 70 % slopes
ERLE 6	31.46107	-110.355	Scheelite Canyon	Far-Huachuca-Hogris association 15 to 70 % slopes
ERLE 7	31.45922	-110.352	in the Canyon	Far-Huachuca-Hogris association 15 to 70 % slopes
ERAR 8	31.51382	-110.324	Unburned Plot	Gardencan-Lanque complex 0 to 5 % slopes
ERAR 9	31.51386	-110.324	Burned plot	Gardencan-Lanque complex 0 to 5 % slopes
ERAR 10	31.38593	-110.242	Ash Canyon Road	White House gravelly loam, 0 to 10 % slopes
ERAR 11	31.34952	-110.228	Coronado National Historic site	White House gravelly loam, 10 to 35 % slopes
ERAR 12	31.48061	-110.338	Across from campsite	Gardencan-Lanque complex 0 to 5 % slopes

Soils map of Erigeron plots. The locations of all plots for *Erigeron arisolius* (yellow dots), *E. lemmonii* (blue dots), and *E. neomexicanus* (pink dots) within the Huachuca Range are recorded in the ARC GIS map (Figure 3.2). This map was generated from the Natural Resource Conservation Service, SSURGO data; North American Datum of 1983.



Map describes the soils at selected locations in Cochise County, Arizona. The Coronado National Forest, Coronado National Memorial, and Fort Huachuca have been represented on the map for reference.

Vegetation Codes:
ERNE: Erigeron neomexicanus
ERAR: Erigeron arisolius
ERLE: Erigeron lemmonii

Point ID	Latitude	Longitude	Local Geography	Vegetation Type	Soil Description
1	31.46692	-110.35574	Garden Canyon	ERNE	Far-Huachuca-Hogris association 15 to 70 percent slopes
2	31.5229	-110.38489	Huachuca Canyon	ERNE	Far-Hogris association 15 to 60 percent slopes
3	31.54683	-110.42441	Parker Canyon	ERNE	Haplustolls-Fluvaquents association mesic 0 to 4 percent slopes
4	31.34782	-110.26666	Coronado National Park	ERNE	Hogris-Telephone association, steep
5	31.46072	-110.35448	Scheelite Canyon	ERNE	Far-Huachuca-Hogris association 15 to 70 percent slopes
6	31.46107	-110.35453	Scheelite Canyon	ERLE	Far-Huachuca-Hogris association 15 to 70 percent slopes
7	31.45922	-110.352213	in the Canyon	ERLE	Far-Huachuca-Hogris association 15 to 70 percent slopes
8	31.513815	-110.323522	Unburned Plot	ERAR	Gardencan-Lanque complex 0 to 5 percent slopes
9	31.513862	-110.323887	Burned plot	ERAR	Gardencan-Lanque complex 0 to 5 percent slopes
10	31.38593	-110.24165	Ash Canyon Road	ERAR	White House gravelly loam, 0 to 10 percent slopes
11	31.349521	-110.227761	Coronado National Historic site	ERAR	White House gravelly loam, 10 to 35 percent slopes
12	31.480608	-110.338489	Across from campsite	ERAR	Gardencan-Lanque complex 0 to 5 percent slopes

Map Author: Austin Davis
Map Facility: ERDC-EL
Creation Date: 1 FEB 2012

Datum: North American Datum of 1983
Source: Natural Resources Conservation Service, SSURGO data.

Figure 3.2. Soils map of all *Erigeron* plots (by Austin Davis, 2012).

OBJECTIVES:

The scientific objectives for this study are: 1) to determine and compare the composition of soil nutrients of the habitats where each of the three species of Erigeron occur; and 2) to investigate the root architecture of the three species, in order to understand how the species are adapted to their specific soil and environment.

Soil characteristics. Cation-exchange capacity (CEC) is defined as the degree to which a soil can absorb and exchange cations. Cations (Daniels and Orndorff 2007) are positively charged ions (Ca^{2+}, Mg^{2+}, K^+, NH_4^+, etc.), and anions are negatively charged ions (NO_3^-, PO_4^{2}, etc.). Soil particles and organic matter have negative charges on their surfaces. The positive mineral cations can bond with the negative surface charges on the soil particle surfaces, so the cation is not easily lost when the soil is leached. This provides the plant with a nutrient reserve that is readily available to plant roots. These nutrients can then be replaced or exchanged by other cations. CEC is dependent on soil texture and organic matter content. Generally, the higher the level of clay and organic matter in the soil, the higher the CEC.

Two factors determine the relative proportions of the different cations absorbed by clay: 1) the cations are not held equally tight by the soil colloids, and the order of strength of absorption is $Al^{3+} > Ca^{2+} > Mg^{2+} > K^+ = NH_4^+ > Na^+$. Second, the concentration of the cations in the soil helps determine the degree of adsorption. Very acidic soils will have high concentrations of H+ and Al^{3+}; and in neutral to alkaline soils, Mg^{2+} and Ca^{2+} dominate (Daniels and Orndorff 2007).

Soil pH is a measure of the acidity or alkalinity of the soil**.** Macronutrients tend to be less available in soils with low pH, whereas micronutrients tend to be less available in soils with high pH. A pH of 1-6 is in the acidic range, 7 is neutral, and 8-14 is in the alkaline range. The desired range of pH is 6.0-6.5 where nutrients are more available to plants. In general, for most soils CEC will increase with an increase in pH. The proportion of CEC satisfied by basic cations (Ca, Mg, K and Na) is termed percentage base saturation (BS%), and as the BS% increases, so does the pH. As the nutrient cations Ca, Mg, K, and Na to plants increase, the BS% will increase (Daniels and Orndorff 2007).

Moisture, light, and temperature drive plant growth. There is a correlation between soil texture and water retention within a soil. Clay particles will hold and swell with water, having small spaces between the small-sized clay particles, and are the least porous. They can either become saturated holding too much water for a long time so that plants will drown, or as they dry, water can become bound so tightly that it is not available to plants. Sand has the largest particle size and is also the most porous, resulting in large voids and low water-holding capacity, so that is dries out quickly. Loam is a combination of sand, clay, and organic material. Organic matter within loams can result in large aggregate soil particles, and loams containing organic matter can hold water and supply water to plants more evenly over a longer duration of time. These characteristics were included in the current analysis to understand critical differences in the three Erigeron species.

Root morphology. Root characteristics and soil types were compared for the three species of *Erigeron.* Win Rhizo V3-9 software (Regent Instruments 2009) was used to obtain root growth measurements. Soil nutrient data were correlated to obtain a better understanding of how the three species of *Erigeron* have adapted to their habitats.

METHODS:

The overall project study design involves five distinct plots for each *E. arisolius* and *E. neomexicanus* (10m x 25m) at different locations, and for *E. lemmonii* at its one known location.

Soil nutrient analysis. A standard soil sample was collected at each site, using the Extension Service protocol (Soil, Water, and Air Testing Lab 2010). This protocol collects equal amounts of soil from across the plot and then composites them so as to obtain one soil sample for each plot. However, to minimize the disturbance of habitat of *E. lemmonii,* only one entire plant was withdrawn for examination and measurement. For soil analysis, soil was taken from 5-15 individual plants, by digging out a small amount (200 grams) of soil around the plant, within the crevices on cliff walls. Care was taken in the soil collection to minimize the amount of material taken for analysis, leaving sufficient soil for the plants to continue growing. In the case of *E. lemmonii,* the soil samples collected from each individual plant were mixed so that all nutrient analysis could be completed with one soil sample, for each pre and post-monsoon season. Each sample was uniquely numbered and an accurate location was recorded using GPS. Soil tests were taken pre-monsoon, on 16-17 May 2011. A second collection was taken post-monsoon, 16-19 August 2011.

Soil analysis for May and August samples was conducted at the Soil, Water, and Agricultural Testing Lab at New Mexico State University. The soil analysis followed standard test procedures used by the Soil, Water, and Agricultural Testing Lab, which are listed in Appendix 3.I and referenced in the literature review. For each nutrient, the following standard procedures were used: Nitrate- Nitrogen (NO3) was measured in a cadmium reduction column; Potassium (K) was measured by ICP, 1:5 soil: Phosphorous (NaHCO3) followed the Olsen procedure; and organic matter was measured by the Walkley-Black procedure.

Soil statistical analysis. SAS (Windows Version 9.2, SAS Institute, 2008) was used to conduct soil nutrient analysis, important in understanding the differences between the three species of *Erigeron.* Major soil nutrients and physical properties including (Nitrogen (N), Phosphate (P2O5), Potassium (K2O), and Organic matter (%) were analyzed and compared. Soil data for each species represent data for one variable with split plot (pre-monsoon and post monsoon) in time treated as a repeated measure in PROC MIXED to accommodate probable covariance between seasonal measurements within a plot. Variance analysis was accomplished with either PROC GLM or PROC MIXED, which gave identical results for key tests of interest; however, variance was easier to identify with the PROC MIXED output.

Prior to any analysis, the assumption of equal variances was evaluated using Levene’s test. This analysis indicated that the equal variances assumption was not valid for each of the soil chemistry variables in the study. The logarithmic transformation was applied to these data and the assumption retested. In the transformed state, Levene’s test indicated that the assumption of equal variances is supported. Thus, prior to any analysis, the data were transformed and the repeated measures model was applied. For these particular data, effects of interest were all fixed effects and the split plot effect was not randomized. The interaction of the monsoon with the soil for each species was the split plot effect. The type I error rate was set at 0.05.

Table 2 displays the logarithmic least squares means for each of these variables classified by the two main effects of Season and Species. Ancillary to this analysis, the Bonferroni LSD procedure was used to delineate any and all pairwise comparisons. This analysis is represented by the letters A, B, and C; and as indicated, means with the same letters are not different, whereas means with different letters are different. The Bonferroni LSD technique was chosen over other procedures, as it controls the individual error rate to restrict the experiment-wise error rate α. This is achieved by setting the individual alpha as α/m, where m is the number of comparisons to be made.

Root analysis with Win Rhizo. This study measured one medium-sized individual from each of the three *Erigeron* species. Measurements were analyzed with Win Rhizo software V3-9 software (Regent Instruments 2009). To obtain measurements, individual plants were placed in a tray with de-ionized water, then onto the root scanner. As plants were scanned, measurements were taken, including root length, root diameter, total root surface and root-to-shoot ratios. At the time of sampling (2011), *E. lemmonii* was considered as a candidate species by the USFWS (1975, 1980, 1993, and 1996) because of its rarity. Permission to remove one individual from its population was granted by the Resource Manager at Fort Huachuca. Statistical analysis was not completed for the root analysis because only one specimen was removed and measured.

The Win Rhizo program automatically calculates different root characteristics from the root scans. For the analysis of root morphology, the "Tennent" method was used to calculate root diameter interpolation. This analysis includes: 1) Root length measurement, a direct comparison to the length of the roots (in centimeters); 2) Root diameter measurement, (i.e., fine, medium, and large root classes and a direct comparison to the root diameter classes); 3) Surface area measurement of the root, a measure of biomass; and 4) Stem length to root length ratio, a way of indexing the plant (the length of stem divided by the length of roots). This measure can show the amount of root growth needed to support the plant growth (stems within the plant above ground). Soil volume was not calculated. Measurements for the three *Erigeron* species were taken and their root structure was compared. These root growth measurements were correlated to the soil nutrient data to obtain a better understanding of how the plants have adapted to their habitats.

Greenhouse-grown plants from stem segments of *Erigeron lemmonii*. In spring 2012, a small plant was taken for a chromosome number analysis using fresh root tip growth, for use in a related study, conducted at the University of Central Arkansas (Noyes and Bailey, in review). This plant was transplanted in potting soil, so fresh root tips could be harvested. Six other plants were started from 6- to10-cm stem pieces cut from the specimen, treated with Root Tone, a rooting hormone, placed in potting soil mix, and cared for with routine watering by greenhouse staff. Additional 10-cm-long stem pieces were cut from six other large individuals in the field, wrapped in damp paper, and shipped to the greenhouse, where they were started by the same method, yielding eight new plants. These 15 plants have been grown in the greenhouse since spring 2012 (for further genetic research in another study). This adventitious rooting characteristic of *Erigeron lemmonii* will be discussed in this paper.

RESULTS:

Soil nutrient analysis. The field layout of this research consisted of 11 soil samples taken from the three habitat areas that supported the species used in this study.   Five of these soil samples were taken from each of the habitats dominated by the *E. arisolius* and *E. neomexicanus* species and one sample was taken from the habitat dominated by the *E. lemmonii* species. Samples were taken pre-monsoon and post-monsoon, for a total of 22 samples.

The experimental design for this study is represented by the following multi-factor repeated measures model, where the total variance is partitioned as either between plots or within plots. The factors of interest are species, plots, and season. The between-plots factor is species, whereas the within-plots factors are season and species-by-season interaction, as represented in Table 2.2.

Table 3.2. Anova comparison of habitat soil nutrients for three species of *Erigeron*, plot, pre- and post-monsoon season, and species/season interrelationships (2011).

Variable	df	SS	F	Pr>F
Organic matter(%)				
Species	2	8.29	41.74	0.0001***
Plot (Species)	8	5.29	6.65	0.0073**
Season	1	0.04	0.50	0.4991
Species*season	2	0.50	2.54	0.1397
Nitrogen (ppm)				
Species	2	10.43	5.61	0.0299*
Plot(Species)	8	6.54	0.88	0.5691
Season	1	8.16	8.79	0.0180*
Species*season	2	0.23	0.12	0.8835
Phosphorous (ppm)				
Species	2	5.25	7.79	0.0132*
Plot(Species)	8	3.33	1.23	0.3866
Season	1	0.09	0.27	0.6115
Species*season	2	0.18	0.27	0.7635
Potassium (ppm)				
Species	2	1.23	5.20	0.0357*
Plot(Species)	8	2.36	2.47	0.1103
Season	1	0.49	4.16	0.0756
Species*season	2	0.54	2.27	0.1649

Note: Pr>F refers to the main effect describing species:* $P < 0.05$; ** $p < 0.01$; ***$p < 0.001$

Table 3.2 indicates that the “Species” variable exhibited differences in soil chemistry characteristics/nutrients among the three species. The p-values of 0.0001, 0.0299, 0.0132, and 0.0357 were noted for each characteristic in the study, respectively. However, differences among seasons (pre- and post-monsoon) were only observed for the soil characteristic of nitrogen (0.0180). Organic matter was significantly different for each species and within the plots (by species) comparison.

Table 3.3 showed that the factor season was significant only for the soil characteristic of nitrogen. Hence, the data indicate that the nitrogen concentrations in the soil were significantly greater in the post-monsoon season when compared to those concentrations pre-monsoon. The nitrogen concentration in the post-monsoon season was approximately 2.5 times larger than that observed pre-monsoon in the soil sampled. The mean concentrations of the other soil characteristics of percent organic matter, phosphorous, and potassium were not significantly different.

Table 3.3. Mean comparison of total soil nutrients (log transformed) for pre- and post-monsoon season (pre-mon and post-mon) and the three *Erigeron* species, 2011.

Season	%Organic matter		Nitrate (ppm)		Phosphorous (mg/Kg)		Potassium (ppm)	
Pre-mon	0.83	A	1.13	A	2.71	A	3.59	A
Post-mon	0.95	A	2.79	B	2.66	A	3.46	A
Species								
E. lemmonii	2.72	A	4.02	A	2.87	AB	4.19	A
E. arisolius	0.49	C	1.99	AB	3.18	A	3.58	AB
E. neomexicanus	0.92	B	1.52	B	2.16	B	3.34	B

Significant at $P < 0.05$
Note: Means with the same letter are not significantly different.

With regards to species, the soil characteristics of nitrogen and potassium exhibited similar findings. The soil in which *E. lemmonii* was growing had significantly larger concentrations than those found for *E. neomexicanus,* and the concentrations observed for *E. arisolius* soil were not different from either *E. lemmonii* or *E. neomexicanus*. The mean concentrations exhibited for phosphorous showed similar characteristics to those observed for nitrogen and potassium; however, the order of the means changed. The largest mean concentration of 3.182 was observed for *E. arisolius* soil, whereas the smallest was observed for *E. neomexicanus* soil (2.165). The mean concentration for *E. lemmonii* soil (2.877) was not different for either of these two species. Soil- organic matter content differed among the three sites. The larger mean concentrations were found for the species site of *E. lemmonii* (2.722) and the smallest at the *E. arisolius* site (0.494). Both of these sites were also different from the mean concentration found at the *E. neomexicanus* site (0.928).

Root morphology analysis. The scans for each Erigeron were taken (Figure 3.3) from one medium–sized individual for each Erigeron species, and the analysis was done using only Win Rhizo (Tables 3.4, 3.5, and 3.6). The plant top refers to the above-ground portion of the plant. For *E. lemmonii* and *E. arisolius,* the entire plant with root is shown in the scan. For *E. neomexicanus,* roots are scanned separately from the plant top because the size of the plant exceeded the scanner; so only its roots are shown. *Erigeron lemmonii* has an underground rhizome with smaller roots protruding from it. All underground portions of all *Erigeron* plants were treated and referred to as “roots” in the analyses.

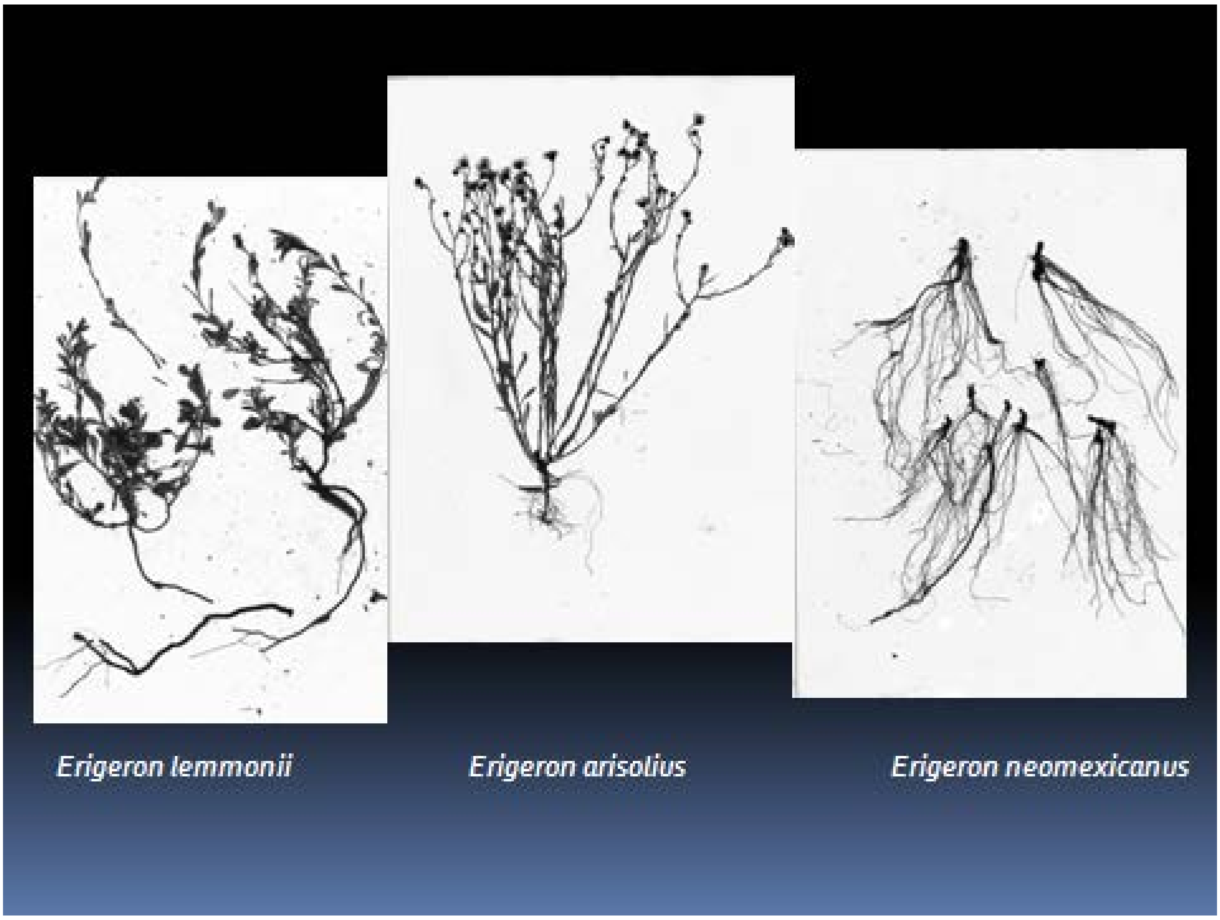


Figure 3.3. Win Rhizo® root scans for the three species of *Erigeron*, 2011.

Table 3.4. Total root and plant top characteristics for each *Erigeron* species (Win Rhizo data, 2011).

Roots	Length (cm)	Surface Area(cm)	Avg. Dia.(mm)	Length per Vol.(cm/m^3)	Tips(#)
E. arisolius	150.85	21.18	0.44	150.85	113
E. lemmonii	86.25	29.68	1.09	86.25	108
E. neomexicanus	1676.39	268.67	0.51	1676.39	1414
Tops					
E. arisolius	575.09	170.67	0.94	575.09	661
E. lemmonii	355.15	170.59	1.52	355.15	570
E. neomexicanus	1923.15	653.34	1.07	1923.15	2399

Erigeron lemmonii is the smallest sized plant of the three species, followed by *E. arisolius*, whereas *E. neomexicanus* is a much larger, more robust sized plant. Root characteristics from the Win Rhizo data indicate *E.lemmonii* has the largest average diameter of roots and top stems (1.09, 1.52) compared to *E. arisolius* (0.44, 0.94) and *E. neomexicanus* (0.51, 1.07), respectively. By contrast *E. neomexicanus* has much longer roots, with greater surface area than the other two species. *E. neomexicanus* has significantly more fine, medium and large roots than the other two species (Table 3.5), and many more growing tips. The leafy top data in Table 3.4 also indicate that *E. neomexicanus* has the longest stems, largest surface area of stem and leaves, and more growing tips than the other two species.

Table 3.5. Total root length within the three classes of root diameter (fine, medium, and large) for each *Erigeron* species (Win Rhizo data). 2011).

Species	Fine (0.25-2.0 mm)	Medium (2.0- 4.0mm)	Large (4.0-4.75mm)
E. arisolius	72.47	2.34	0.09
E. lemmonii	81.74	3.16	1.33
E. neomexicanus	965.09	21.17	6.09

Erigeron neomexicanus has the most roots overall including roots within the three root diameter classes, whereas *Erigeron arisolius* has the least amount of roots including roots within the three root classes.

Table 3.6. Stem length/root length ratio for each *Erigeron* Species (Win Rhizo data, 2011).

Species	# of shoots	# of roots	Ratio
E. arisolius	575	150	3.80
E. lemmonii	350	86	4.12
E. neomexicanus	1923	1676	1.14

Table 3.6 data indicate *E. lemmonii* has the greatest stem length to root length ratio (4.12) compared to *E. arisolius* (3.80) or *E. neomexicanus* (1.04). *E. lemmonii* has four times the amount of root supporting its top stems and leaves. As demonstrated by the greenhouse specimens, *Erigeron lemmonii* is easy to root from stem, rhizome or root pieces. Rootlets grow from the nodes of both stem and underground rhizome if conditions are right (15 plants have been started in the green house by rooting 5- to 10-cm pieces of stem in potting soil). This stem length/ root length ratio supports findings of the adventitious rooting capability of *Erigeron lemmonii.*

DISCUSSION AND CONCLUSION:

Table 3.7 summarizes soil, root, and plant attributes comparing and contrasting differences between the three Erigeron species. These factors are discussed below.

Table 3.7. Soil, root, and plant attributes for *Erigeron lemmonii, E. arisolius,* and *E. neomexicanus,* 2012.

	Erigeron lemmonii	*Erigeron arisolius*	*Erigeron. neomexicanus*
Soil Attributes			
Soil chemistry	Highest N, and K	Highest P	Lowest N, P, K
Soil structure	Organic soil/compost	Sand	Sand
Root Attributes			
Below ground (surface area)	Medium	Least	Largest
Above ground	Least	Medium	Largest
Stem length to root length ratio	Largest ratio	Medium ratio	Smallest ratio
Plant attributes			
Habitat	Crevices in cliff	Open grassland	Seral and disturbed areas
Life cycle	Long-lived perennial	Annual	Perennial
Flowering period	May-Oct. (Dec.)	June to Aug.(Sept.)	July-Aug. (Oct.)
Clonal capacity	Highly clonal	None	Possible root division

Soil nutrients and organic matter. Although the cliff environment appears to be dry and harsh, the soil analysis for *E.lemmonii* indicates the cliff soils are not as harsh as they appear; they have a higher percentage of organic matter in the soil and higher levels of nitrogen and potassium, compared to the two other more widely spread *Erigeron* species, *E. arisolius* and *E. neomexicanus*. Organic matter in the soil was distinctly different for each of the three *Erigeron* species and significantly higher in the soil of *E. lemmonii*.

Nitrogen, an important nutrient, was significantly higher in soils for *E. lemmonii*. Nitrogen in the soil was found to increase approximately 2.5 times after the monsoon (July-August); nitrogen moves through the soil profile after the monsoon rains as the season progresses. As plant-available nutrients and nitrogen from organic material are taken up by microbes, they release ammonium N, which is then nitrified and causes a flush of plant growth. The released nitrogen provides for continual growth throughout the extended growing season of *E. lemmonii* (Bailey, Whitley and Kevan, in preparation). Nitrogen and phosphorous enrichment of the cliff soils can occur from mammal and bird activity such as rock perches in localized areas (Derry et al. 1998), and decaying plant matter caught in the crevices can contribute to the build-up of organic soil and microbial nitrification. Nitrogen is well-known to be mobile in soil moisture (Derry et al. 1999). Potassium was also higher for *E. lemmonii* than the other two species. Potassium is necessary for the synthesis of protein, for photosynthesis, and to produce flowers, seed, and fruit. Potassium also helps plants absorb and hold water, and increases their defense against diseases. Phosphorous levels were higher for *E. arisolius,* closely followed by *E. lemmonii,* but were lower for *E. neomexicanus. E. neomexicanus* grows in the least fertile soil, having the lowest levels of nitrogen, phosphorous, and potassium.

Organic matter varies between the three *Erigeron* species. The soil that *Erigeron lemmonii* is growing in is composed of rich organic debris washed down the rock walls and caught in crevices. It is highly organic and has a large aggregate texture, which holds water. After rain showers, this water can be held and stored for plant use for a long time, which helps to buffer plant growth from extremes of hot/cold temperature and wet/dry soil conditions. *Erigeron neomexicanus* can survive in a large variety of conditions and unlike the other two *Erigeron*, its soil is the most variable; in some places it has little organic content, while in other locations it has more organic matter. Soils can range from dusty, sandy soils near disturbances such as road cuts, shoulders, and stream edges in open sun, to richer soils in semi-shaded woodlands, and on the ground below rock cliffs. However, these areas can provide the plants with more water, such as sheet flow across asphalt roadways and the roughened edges along the upper banks of streams. *Erigeron arisolius* grows in sandy soils in less disturbed areas within the semi-arid grasslands.

Root Analysis. Table 6 shows there are approximately four times the number of stem length to root length ratio for a typical medium-sized *Erigeron lemmonii* plant (4.12). The ratio for

Erigeron arisolius is almost the same (3.80), compared to *E. neomexicanus* (1.14) which has the lowest ratio (1:1). The roots of *E. lemmonii* have a larger average root diameter (1.09) compared to the congeners *E. arisolius* (0.44) and *E. neomexicanus* (0.51*). E. lemmonii* produces the least root length per root mass compared to the congeners. These findings are consistent with the conclusions of other outcrop endemic plant research; i.e., the outcrop species sends out roots faster to explore new space and transport water with a high investment in a few larger diameter laterals and a strong apical dominance (Poot et al. 2012). Larger diameter roots have been associated with greater and faster growth (Eissenstat 1991), increased longevity of the plant (Eissenstat 1992), and greater hydraulic conductance (Passioura 1988). All of these characteristics are necessary to allow a long-lived outcrop plant to access and obtain permanent and fast water supply through fissures within the rock. Plant roots have been shown to easily change direction to grow towards areas of less resistance (Clark et al. 2003, Saoirse et al. 2012), or divert resources to increase growth of other laterals or initiate primordia (Mirra and Gibbons 1996, Falik et al. 2005). Zohary (1973) reports that in desert conditions, cliffs have conditions more favorable for plants than other habitat types because most of the rainwater that falls, collects in crevices, and is protected from fast evaporative loss. As rainwater also drips down the face of the cliff, it is collected in shallow soil pockets creating mesic refuges (Poot et al. 2012). The ability of this species to produce roots at stem nodes when conditions are right allows it to grow quickly in a new suitable habitat, with limited competition from other cliff-growing plant species. Available water is held by the organic soils of *E. lemmonii* for a longer duration than other soil types, which allows the plant to have a steady supply even during hot, dry times. Under water-limiting conditions, *E. lemmonii* leaves start to dry out and die back, thereby reducing its mass so it has less above-ground biomass to support. When the spring and monsoon rains come, this plant quickly responds with root and stem growth to take advantage of these environmental conditions.

Unlike *E. lemmonii*, the other two *Erigeron* species bloom only after the monsoon rains. *Erigeron arisolius* blooms from June to August/September and is reported to be an annual or short-lived perennial (Nesom 2006). It has the smallest above-ground biomass of the three species. *Erigeron arisolius* grows in the hot dry grasslands, and the high stem length -to-root length ratio would indicate that the plant is better adapted to take up water and nutrients with greater root mass, helping this annual plant to grow quickly after the monsoon rains to fulfill its lifecycle and to produce fertile seed.

Erigeron neomexicanus blooms from July/August to October and is a perennial. *Erigeron neomexicanus* has a stem length to root length ratio of 1, indicating the root and upper plant growth are about the same; it has the greatest number of stems, more leaf growth, and more ability of the plant to photosynthesize. This is a large, vigorous plant compared to the other two species, so its extended root system supports an equal amount of above-ground growth. This plant grows in the widest range of conditions, and is often found on the edges of roadways, along the eroded edges of streams and bank cuts, and grows in the harshest soils as indicated by the soil results. *E. neomexicanus* is adapted to growing quickly in disturbed and eroded areas that contain gravel and rocks. Rock fragments or large stones in soil can improve water availability and play a significant role in the water budgets of plants in semi-arid regions. In these situations, water readily infiltrates, and is held between the rocks, which act as mulch and reduce evaporative water losses (Hanson and Blevins 1979, Flint and Childs 1984).

There are distinct differences between the soil characteristics for the three species and each displays a particular root development to take advantage of soil characteristics. Each species has uniquely adapted to its habitat. The cliff habitat is the least harsh environment, offering highly organic soil rich in nitrogen and potassium, which supports a long growing and flowering season for the rare endemic, *Erigeron lemmonii*. It does not appear to be experiencing nutrient limitations and this plant benefits from early spring and monsoon rains.

Results indicate that *E. lemmonii* is well adapted to the cliff environs it grows in. *Erigeron lemmonii* soils have high levels of nitrogen, potassium, and organic material, and sufficient level of phosphorous, which has a low solubility and slow movement through the soil (Stevenson 1986). It grows in the richest organic soil of the three species and is able to take advantage of the organic soils deposited in cracks within the cliff face, grow many roots to stabilize the plant, and allow for greater uptake of water and nutrients, particularly nitrogen, as shown by the data. The plant also produces adventitious roots easily from nodes on the stems, which can aid in plantlet development if a stem is broken off the parent plant. Adventitious roots can help maintain a large established individual, as the plant seeks new soil to grow in along the cracks, allowing for it to adapt to changes that may occur within the cliff environment. These plantlets can separate from the initial plant and grow as additional individuals, expanding the population by vegetative reproduction. Clones produced by vegetative reproduction (asexual), which are the same genetically as the parent plant, will ensure that the population will maintain its present genetic diversity. The findings of this study suggest that the root system *of E. lemmonii* may have been shaped by selection forces increasing the chance to access fissures in the rock strata. *E. lemmonii* grows in the richest organic soil, which stabilizes water fluctuations, and it grows in a cliff habitat, which stabilizes temperature fluctuations, offering greater stability than the other two *Erigeron* species habitats, while providing less competition from other plant species. Compared to *E. lemmonii's* environment, the other two *Erigeron* species grow in deeper soil habitats, which are generally less open, supporting more standing biomass and higher plant densities. Under these conditions, *E. lemmonii* roots may be maladapted to growing on the ground (Poot et al. 2012). Early root formation would cost aboveground competitiveness, which may be necessary for plants growing in deeper soils with more plant competition (Lavergne et al. 2004).

At present *Erigeron lemmonii* appears to be stable as observed from monitoring efforts at Fort Huachuca (Stone, 2012 Personal Communication) and, like many cliff species (Larson et al. 2000), it is a long-lived species that is well adapted to its environment. However, climate change is a conservation concern; it can potentially cause future drying of the environment in this region. Although the cliff environment provides some buffering capability of temperature and available water, if this occurs, *Erigeron lemmonii* may be the most likely to be impacted; increased temperatures would cause the rock substrate to be warmer which, in turn, could cause the organic soils to start drying. As an annual, *Erigeron arisolius'* seeds will take advantage of the conditions suitable for its germination. However, competition dynamics among the other grassland species may cause extreme competition for plant-available water, which may have a serious impact on this species. *Erigeron neomexicanus* is the most adaptable species, and least likely to be impacted because presently it takes advantage of a wide range of environmental conditions.

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APPENDIX 3.I

References for Soil Analysis, Soil, Water, and Air Testing Lab, New Mexico State University, Las Cruces, NM.

REFERENCES FOR SOIL ANALYSIS		
Test	**Procedure**	**Reference**
Standard Soil Test		
Organic Matter	Walkley - Black	6
pH	Saturated Paste	2
Salts	Solution conductivity	2
Phosphorus	Olsen	3 & 7
ESP	SAR Estimation	2
Texture	By Feel	10
Potassium (K)	ICP, 1:5 H20 Extract	1
Nitrate - Nitrogen (NO3)	Cadmium Reduction Column	3 & 4
Fertitility Tests		
Phosphorus	Olsen	3 & 7
Nitrate	Cd reduction, 1:5 H2O Extract	4
Potassium	1:5 NH4OAc Extract	3
Nitrogen	Kjeldahl	5
Fe, Zn, Cu, and Mn	DPTA Extract	3
Organic Matter	Walkley - Black	5
Boron	Hot Water Soluble	5
Soil Characterization Tests		
pH	Choice of procedure	8
E.C. of saturated extract	Solution conductivity	2
% H2O at saturation	Drying at 110 +/- 5 degrees C	12
CaCO3 (equivalent)	Rapid titration	5
Gypsum	Acetone precipitation	8
Extractable Cations	NH4OAc at pH 7 or 9	8
Exchangeable Cations	NH4OAc extractable minus soluble	8
Cation Exchange Capacity	Na saturated then NH4OAc extracted	5
Extractable Hydrogen	BaCl2 - TEA Extraction	8
Extractable NH4	2N KCl (colorimetric)	5

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APPENDIX 3.II

Map Unit Soil Description (Brief, Generated)
Santa Cruz and Parts of Cochise and Pima Counties, Arizona
[Minor map unit components are excluded from this report]

Map unit: HoF - Hogris- Telephone association, steep
Component: Hogris (50%)
The Hogris component makes up 50 percent of the map unit. Slopes are 35 to 45 percent. This component is on mountains. The parent material consists of mixed colluvium and/or mixed residuum. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 2 percent. This component is in the F041XA124AZ Quercus Arizonica-Quercus Emoryi/bouteloua ecological site. Nonirrigated land capability classification is 6e. This soil does not meet hydric criteria.

Component: Telephone (20%)
The Telephone component makes up 20 percent of the map unit. Slopes are 35 to 45 percent. This component is on mountains. The parent material consists of mixed alluvium. Depth to a root restrictive layer, bedrock, lithic, is 5 to 20 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is moderately high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 2 percent. This component is in the F041XA120AZ Quercus Emoryi-Quercus Arizonica/juniperus Deppeana/muhlenbergia Emersleyi ecological site. Nonirrigated land capability classification is 6e. This soil does not meet hydric criteria.

Map unit: WgC - White House gravelly loam, 0 to 10 percent slopes
Component: White House (80%)
The White House component makes up 80 percent of the map unit. Slopes are 0 to 10 percent. This component is on fan piedmonts. The parent material consists of mixed old alluvium. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is moderately high. Available water to a depth of 60 inches is high. Shrink-swell potential is moderate. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 2 percent. This component is in the R041XC313AZ Loamy Upland 12-16" P.z. ecological site. Nonirrigated land capability classification is 6e. This soil does not meet hydric criteria. The calcium carbonate equivalent within 40 inches, typically, does not exceed 6 percent.

Map unit: WgE - White House gravelly loam, 10 to 35 percent slopes
Component: White House (80%)
The White House component makes up 80 percent of the map unit. Slopes are 10 to 35 percent. This component is on fan piedmonts. The parent material consists of mixed old alluvium. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is moderately high. Available water to a depth of 60 inches is high. Shrink-swell potential is moderate. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 2 percent. This component is in the R041XC314AZ Loamy Slopes 12-16" P.z. ecological site. Nonirrigated land capability classification is 6e. This soil does not meet hydric criteria. The calcium carbonate equivalent within 40 inches, typically, does not exceed 6 percent.

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Survey Area Version: 6, Survey Area Version Date: 02/24/2010
USDA Natural Resource Conservation Service

Map Unit Description (Brief, Generated)
Cochise County, Arizona, Douglas-Tombstone Part
[Minor map unit components are excluded from this report]

Map unit: 62 - Far- Hogris association, 15 to 60 percent slopes
Component: Far (55%)
The Far component makes up 55 percent of the map unit. Slopes are 25 to 60 percent. This component is on mountains, hills. The parent material consists of mixed slope alluvium. Depth to a root restrictive layer, bedrock, lithic, is 5 to 20 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 3 percent. This component is in the F041XA120AZ Quercus Emoryi-Quercus Arizonica/juniperus Deppeana/muhlenbergia Emersleyi ecological site. Nonirrigated land capability classification is 6c. This soil does not meet hydric criteria.

Component: Hogris (25%)
The Hogris component makes up 25 percent of the map unit. Slopes are 15 to 50 percent. This component is on hills, mountains. The parent material consists of mixed slope alluvium. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is somewhat excessively drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 1 percent. This component is in the F041XA124AZ Quercus Arizonica- Quercus Emoryi/bouteloua ecological site. Nonirrigated land capability classification is 6c. This soil does not meet hydric criteria.

Map unit: 63 - Far-Huachuca-Hogris association, 15 to 70 percent slopes
Component: Far (35%)
The Far component makes up 35 percent of the map unit. Slopes are 25 to 60 percent. This component is on Hills, mountains. The parent material consists of mixed slope alluvium. Depth to a root restrictive layer, bedrock, lithic, is 5 to 20 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 3 percent. This component is in the F041XA120AZ Quercus Emoryi-Quercus Arizonica/juniperus Deppeana/muhlenbergia Emersleyi ecological site. Nonirrigated land capability classification is 6c. This soil does not meet hydric criteria.

Component: Huachuca (35%)
The Huachuca component makes up 35 percent of the map unit. Slopes are 25 to 70 percent. This component is on hills, mountains. The parent material consists of slope alluvium derived from limestone. Depth to a root restrictive layer, bedrock, lithic, is 5 to 20 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is moderately high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 2 percent. This component is in the F041XA121AZ Pinus Discolor-Juniperus Deppeana/cercocarpus Montanus Var. Paucidentatus ecological site. Nonirrigated land capability classification is 6c. This soil does not meet hydric criteria. The calcium carbonate equivalent within 40 inches, typically, does not exceed 10 percent.

Map Unit Description (Brief, Generated)
Cochise County, Arizona, Douglas-Tombstone Part

Map unit: 63 - Far-Huachuca-Hogris association, 15 to 70 percent slopes
Component: Hogris (20%)
The Hogris component makes up 20 percent of the map unit. Slopes are 15 to 50 percent. This component is on hills, mountains. The parent material consists of mixed slope alluvium. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is somewhat excessively drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 1 percent. This component is in the F041XA124AZ Quercus Arizonica-Quercus Emoryi/bouteloua ecological site. Nonirrigated land capability classification is 6c. This soil does notmeet hydric criteria.

Map unit: 71 - Gardencan-Lanque complex, 0 to 5 percent slopes
Component: Gardencan (45%)
The Gardencan component makes up 45 percent of the map unit. Slopes are 0 to 5 percent. This component is on fan terraces. The parent material consists of fan alluvium derived from granite and gneiss. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is moderately high. Available water to a depth of 60 inches is moderate. Shrink-swell potential is moderate. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 1 percent. This component is in the R041XA110AZ Sandy Loam Upland 16-20" P.z. ecological site. Nonirrigated land capability classification is 6c.This soil does not meet hydric criteria.

Component: Lanque (35%)
The Lanque component makes up 35 percent of the map unit. Slopes are 0 to 5 percent. This component is on stream terraces, alluvial fans. The parent material consists of fan and stream alluvium derived from granite and gneiss. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is well drained. Water movement in the most restrictive layer is moderately high. Available water to a depth of 60 inches is moderate. Shrink-swell potential is low. This soil is not flooded. It is not ponded. There is no zone of water saturation within a depth of 72 inches. Organic matter content in the surface horizon is about 2 percent. This component is in the R041XA110AZ Sandy Loam Upland 16-20" P.z. ecological site. Nonirrigated land capability classification is 6c. This soil does not meet hydric criteria.

Map unit: 86 - Haplustolls-Fluvaquents association, mesic, 0 to 4 percent slopes
Component: Haplustolls, mesic (70%)
The Haplustolls, mesic component makes up 70 percent of the map unit. Slopes are 0 to 4 percent. This component is on flood plains. The parent material consists of mixed stream alluvium. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is somewhat poorly drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is occasionally flooded. It is not ponded. A seasonal zone of water saturation is at 90 inches during January, February, March, April, August, September, October, November, December. Organic matter content in the surface horizon is about 2 percent. This component is in the F041XA125AZ Platanus Wrightii-Juglans Major/muhlenbergia Rigens ecological site. Nonirrigated land capability classification is 6c. This soil does not meet hydric criteria.

Map Unit Description (Brief, Generated)
Cochise County, Arizona, Douglas-Tombstone Part

Map unit: mesic, 86 - Haplustolls-Fluvaquents association, 0 to 4 percent slopes
Component: Fluvaquents, mesic (20%)
The Fluvaquents, mesic component makes up 20 percent of the map unit. Slopes are 0 to 4 percent. This component is on flood plains. The parent material consists of mixed stream alluvium. Depth to a root restrictive layer is greater than 60 inches. The natural drainage class is somewhat poorly drained. Water movement in the most restrictive layer is high. Available water to a depth of 60 inches is very low. Shrink-swell potential is low. This soil is frequently flooded. It is not ponded. A seasonal zone of water saturation is at 18 inches during January, February, March, April, May, August, September, October, November, December. Organic matter content in the surface horizon is about 2 percent. This component is in the F041XA125AZ Platanus Wrightii-Juglans Major/muhlenbergia Rigens ecological site. Nonirrigated land capability classification is 6c. This soil meets hydric criteria.

CHAPTER 4

Network Analysis of Floral Insect Visitors of the Rare *Erigeron lemmonii* Compared to More Widespread Species, *Erigeron arisolius* and *Erigeron neomexicanus* (Asteraceae), in Arizona, U.S.A.

ABSTRACT

We examined flower-visiting insect community associated with three species of *Erigeron* (Daisy Fleabanes). We were specifically interested in determining if insect assemblages on the one rare *Erigeron* species were shared by the other two more common *Erigeron* species, and the degree to which they shared flower-visitors with other flowering plants in each of their respective habitats. *Erigeron lemmonii* is a rare endemic plant adapted to sparse cliff wall habitats in one canyon fragmented by topography. *Erigeron arisolius* and *E. neomexicanus* are more widespread species adapted to more diverse habitat conditions. *Erigeron lemmonii* has a many insect visitors, but is not linked to other plants within its habitat through its insect visitors. Our findings indicate *Erigeron lemmonii* has a unique assembly of flower-visiting insects, compared to either *E. arisolius* or *E. neomexicanus.* Its network architecture indicates it has no redundant relationships with other plant species in its habitat to support its insect visitors, unlike the networks of *E. arisolius* and *E. neomexicanus,* which have more core clusters resulting in robust networks. Their network architecture indicates the latter two species would be less impacted by fragmentation than would *E. lemmonii. Erigeron lemmonii* has a fragile insect visitation network structure because, if *E. lemmonii* at the population level disappears, it may cause a local collapse of its dependent insect visitors. The insect visitor community contains the pollinators of *E. lemmonii,* without which outcrossing would not occur. This has serious implications for the conservation of *E. lemmonii* and its associated insect visitors.

Keywords: *Erigeron*, endemic plant**,** insect visitation networks, Huachuca Range

INTRODUCTION

Pollination networks have been identified as critical to the survival of many native ecosystems (Kearns et al. 1998). In pollination networks, pollinators are more sensitive and quicker to respond than plants to habitat loss, however, the loss of pollinators is proposed to lead to eventual plant extinctions (Taki & Kevan 2007). In small populations, pollinator abundance and diversity decrease with decreasing plant population size, and may drop to a point at which pollinator service deteriorates (Fritz & Nelson 1994). Plant in-breeding depression can lead to eventual plant and pollinator extinction (Fritz & Nelson 1994). Gene flow between habitat fragments can be expected to decrease by total or partial loss of pollinators or the inability of the pollinator to carry pollen over large distances. This in turn may lead to increased rates of genetic drift. Inbreeding depression is the loss of fitness of sexually reproducing individuals caused by mating of close relatives. Genetic drift causes the loss of genetic variation (allelic richness) in populations. As a population becomes smaller, its gene pool will become smaller within which there is a higher chance of a stochastic event where deleterious alleles become fixed (genetic drift, also known as inbreeding depression). If genetic diversity is lost, phenotypes that may be more adapted to particular environmental conditions and essential for adaption could be lost and therefore make the population more prone to local extirpation. That set of processes can set in motion an interrelated cascade of extinction events of populations on a regional level (Richards 2000), or extinction vortices linked with plant/pollination losses within a pollination network (Pauw 2007).

Military bases and conservation lands may become genetic islands with increased development occurring outside many military bases and conservation areas. The loss of species and genetic diversity from restricted genetic pools that result from fragmentation is of crucial concern to military and conservation resource managers across the country. With increased fragmentation there are other associated impacts to the natural resources which challenge management and operations. Land managers responsible for rare plant conservation must also consider the resource requirements of pollinators (Tepedino et al. 1997; Kearns et al.1998; Tepedino et al. 2012) because many rare plant species are also commonly outcrossed and dependent on pollinators (Weller 1994).

The role of insect pollinators can vary with season, locality, and other environmental factors. Asteraceae flowers are habitually visited by a varied group of insects; including pollinating insects gathering pollen and nectar, and other insects that may secondarily spread pollen among plants (Mani & Saravanan1999). The role of flower-visiting insect species may vary depending on the season, locality and interactions with other species within the insect community of the plant. True pollinators are easier to recognize because of the specialized adaptations for nectar feeding, and gathering and transporting pollen grains. Bees are the most important pollinators of Asteraceae species and are highly specialized for the collection of pollen and nectar (Mani & Saravanan 1999). Johnson and Midgley (1997) reported the most important pollination vectors of Asteraceae to be solitary bees and flies. Flies (Diptera) are partly specialized and can be very effective pollinators of Composite heads and are liquid feeders, sucking the plant or other insects. Hoverflies (Syrphids) suck juices of the flower heads and parasitize aphids, and are also considered important pollinators. Butterflies and moths (Lepidoptera) have mouthparts consisting of a long proboscis and are important pollinators of the Composites, including *Erigeron.* Butterflies are known to migrate in swarms; they migrate up from Mexico into the United States through the Sky Island Region, which influences flower pollination patterns (Felger and Wilson 1994). Butterflies are diurnal and depend on nectar for carbohydrates and amino acids. Moths are crepuscular or nocturnal, often visiting white, strongly scented flowers that have long, narrow corolla tubes that cannot be reached by bees. Lycaenidae, mostly small bluish or copper colored butterflies are known pollinators of several species of *Erigeron*. In another study by Spongburg (1971) muscoid flies were very active in morning hours for *Erigeron simplex and E. grandiflorus.* However, other insects visiting the flowers were sulphur butterflies, mosquitos, and a large presence of thrips in the disks.

The genus *Erigeron* is in the largest plant family, Asteraceae and shows a wide range of distribution patterns from species that have extensive high-density populations to rare low-density populations. Our study focused on three species of *Erigeron* (Fleabanes), a rare endemic plant adapted to a naturally sparse cliff wall habitat in one canyon fragmented by topography (*E. lemmonii*), and two others (*E. arisolius and E. neomexicanus*) adapted to more diverse habitat conditions over a larger range (Figure 4.1). This research was conducted in the Huachuca Range, on the Fort Huachuca Military Reserve, U.S. Forest Service, and National Park Service lands. We applied network approaches to understanding how plant species subjected to fragmented and sparsely vegetated habitat have adapted insect visitation network strategies compared to more widespread congeners. We compared and contrast the insect visitation networks of three species of *Erigeron* by recording insect visitor/ plant interactions. We focused on variation in such variables as plant and insect visitor richness and abundance, network structure, and the degree of plant and insect visitor specialization. We have also used non-metric multidimensional scaling (McCune & Grace, 2002) to further define the insect visitor interactions and communities for each of the three *Erigeron* species. Our study addresses the role of preserving the entire plant - insect visitor network that includes *E. lemmonii*, and is relevant to the future management conservation priorities for this rare plant.

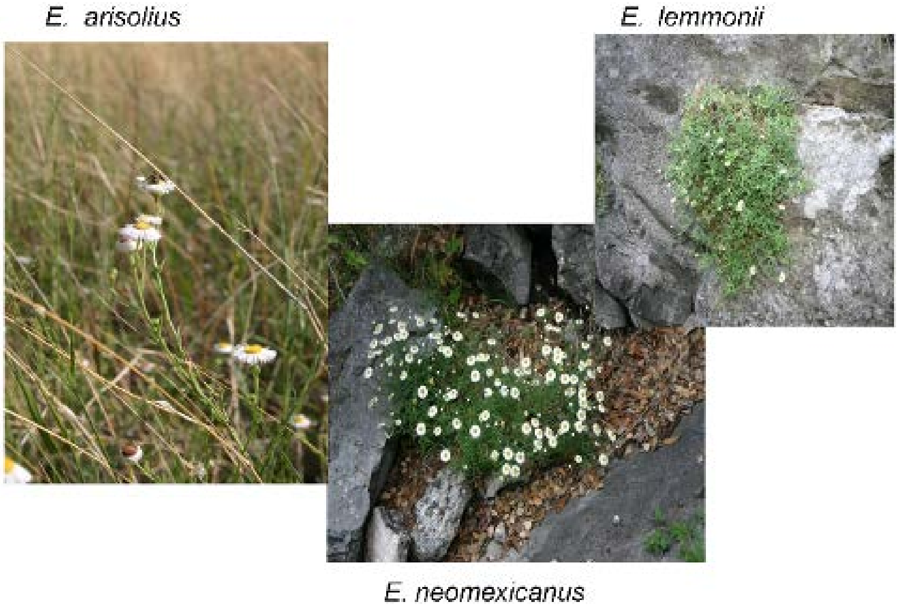

Figure 4.1. *Erigeron arisolius* (left), *Erigeron lemmonii* (upper center and right), and *Erigeron neomexicanus* (bottom right), Fort Huachuca, Arizona, USA.

Floral visitor networks. It has been demonstrated by several studies that species richness and abundance increases with increased patch area (Rosenzweig 1995; Connor et al. 2000; Taki & Kevan 2007). Species loss in small habitat fragments is generally much greater than for most other groups in terrestrial habitats (Rosenzweig 1995). Taki and Kevan (2007) showed the number of plant species increased with habitat area suggesting a higher resource diversity supporting a greater number of insect visitors. Secondly, as the percent coverage of flowering plants increases with area, they found a higher density of bees.

Network science has expanded the field of ecology, by investigating relationships within the ecosystem to more fully understand how all the species function within these systems. Initially network science was applied to food webs in ecology, and currently many other applications are being explored including pollination networks. Ecological networks contain nodes (species) and links are the interactions between them. Plant pollinator networks typically have sparse matrices which describe their topology, because most interactions are not observed, and only a fraction of the total interactions actually occur (Jordano et al. 2006). A node is characterized by its degree of distribution, which is defined as the number of links to other nodes (Bascompte & Jordano 2007), and the more connected a node is, the more stable it will be within the network. The generalized patterns have implications for the stability and resilience to disturbance of the network (Barabasi & Albert 1999). Insect visitation networks share a general pattern of a dense core of species which interact with each other, surrounded by many species with few interactions connected with those in the core. Regardless of the size of the network, plant-pollinator mutualisms analyzed, generally center around a core of generalist and specialist species (Jordano et al. 2006).

Hypotheses

We made the following predictions based on *Erigeron lemmonii* having evolved in a highly specific habitat (i.e., cliff faces) under naturally fragmented, conditions, as opposed to the two more wide spread and habitat generalists species of *Erigeron.*

H1: *Erigeron lemmonii* will have weakly linked nodes supporting a small number of insect visitors within its network.

H2: *Erigeron lemmonii* will share insect visitors with relatively common plants that are insect visitor generalists (and are found in a core within the insect visitation network structure).

H3: *Erigeron arisolius* and *E. neomexicanus* will be supported by more redundant, robust network relationships than *E. lemmonii*.

METHODS

Study locale. We conducted our survey in the Huachuca Range on federal agency owned property, located in Cochise County of southeastern Arizona (Figure 4.2). Plots were established at Fort Huachuca Army Military Reserve, U.S. Forest Service land in Ash Canyon, and the Coronado National Historic Site, National Park Service (see Supplementary Material for GPS locations). Seven plots were located at Fort Huachuca; a 33,000 ha (127.4

Sq. mi) military installation that has well characterized vegetative communities (Schlichting 2008; Nature serve 2012), with high diversity of plants. Two plots, one each for *E. arisolius* and *E. neomexicanus* were located at Coronado National Historic Site, and one *E. arisolius* plot was located in Ash Canyon. Surveys and sampling were conducted over two years, 2010 and 2011.

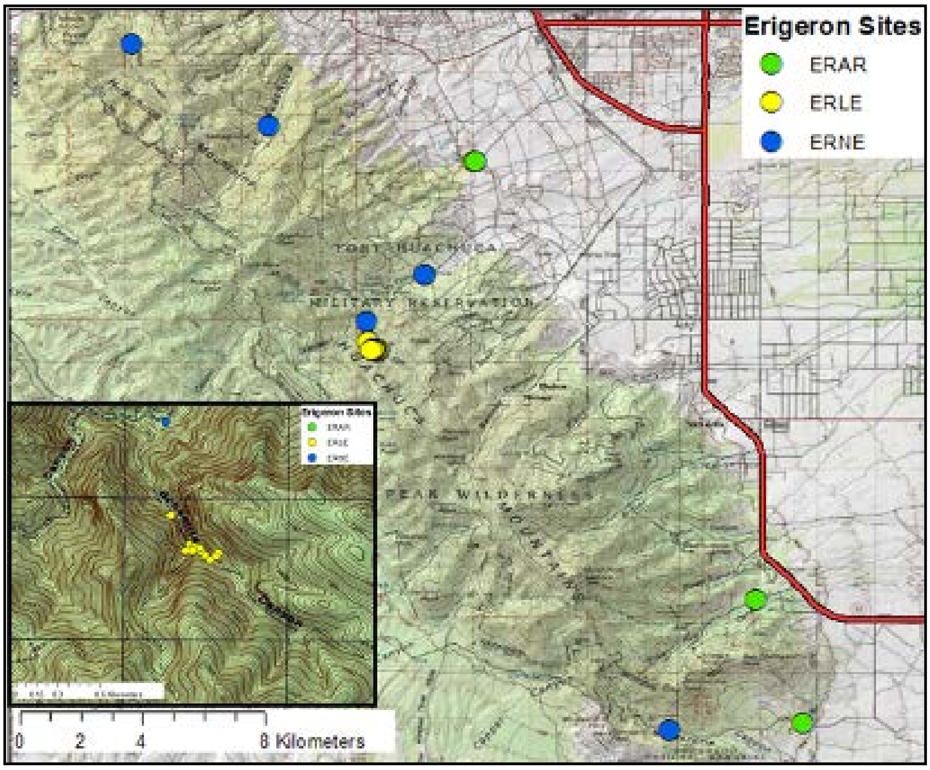

Figure 4.2. Map of Huachuca Range showing the distribution of *Erigeron* sampling sites for *E. arisolius* (ERAR), *E. lemmonii* (ERLE), and *E. neomexicanus* (ERNE). Insert is the distribution of *E. lemmonii* sampled within the Scheelite Canyon.

Plant and flower-visitor sampling. In 2010 we sampled herbaceous plants for plant diversity data within the *E. lemmonii* plots, and we sampled for flower-visiting insects for two years. The following year we did not sample the flowering co-blooming, plants on the ground, with the exception of the two *Erigeron* species (*E. neomexicanus* and one individual *E. platyphyllus)* which remains in the 2011 plant data. We sampled the adjacent *Erigeron* species to examine if there were any shared insect visitors between them and *E. lemmonii*. The *E. lemmonii* plot data is separate from the *E. neomexicanus* plot data used to create their respective networks. We showed 2010 and 2011 networks for *E. lemmonii* and only the 2011 data was shown for *E. arisolius* and *E. neomexicanus* because the similarity in the network architecture between years.

Within each plot, a complete list of plant species present (Supplementary material) and percent cover for all species within the plot was recorded. The percent coverage of all herbaceous plants within each plot was taken using the National Vegetation Classification System methodology (Nature Conservancy 1998), and averaged together to determine the total percent cover within plots for each of the three *Erigeron* species. The identification of plant species was made using the following references: *Flora of North America* (Nesom 2006); Arizona Flora (Kearney and Peebles 1960); A Field Guide to the plants of Arizona (Epple & Epple 1995); and the Botanical Survey at Fort Huachuca (Schlichting 2008).

We sampled flower-visiting insects from each of the three *Erigeron* species as well as from all other plants in flower in the plots. We sampled insect visitors from *E. lemmonii* during the peak flowering season (May- October) on 10 different rock faces (approximately 5m x 5m areas). The 10 rock faces constituted 1 plot for this endemic plant which only occurs in the Scheelite Canyon at Fort Huachuca, In this study, 5 plots (50 x 10m) each were established for *Erigeron arisolius* and *E. neomexicanus*. All *Erigeron* plots were sampled while the *Erigeron* species were in bloom. Foraging activity is usually low in the early morning when temperatures are cool or when it rains (Kevan and Baker 1983). Plots were sampled in the morning at 800 to 1400 in the afternoon, until it got extremely hot or thundershower activity started, as was the case in monsoon season. All *Erigeron* plots were systematically sampled for 30 consecutive minutes with battery powered handheld vacuum cleaners (Ryobi 18v hand vacuum) modified for insect collection (Figure 4.3).

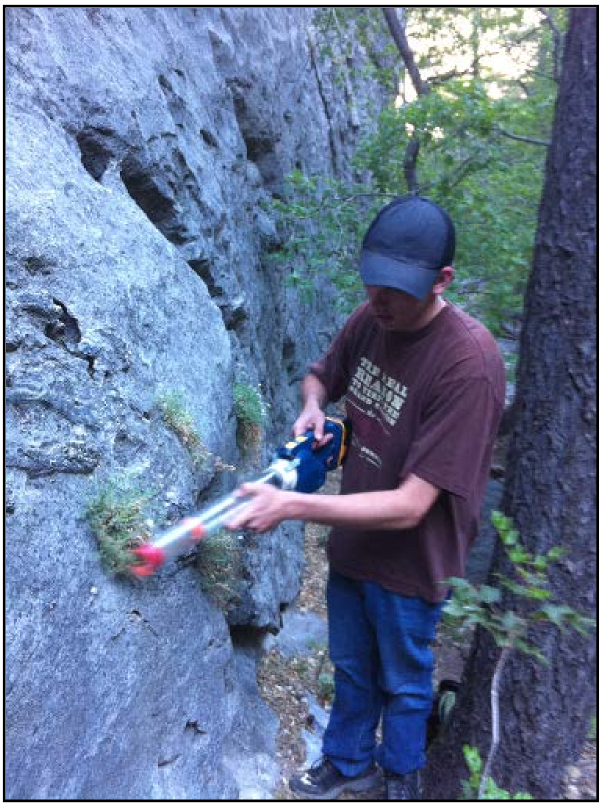

Figure 4.3. Collecting insect visitors on *Erigeron lemmonii* with vacuum cleaner adapted for this purpose, in Scheelite Canyon, AZ. during sampling year 2010.

We also collected insect visitors from two other more widespread species, *E. arisolius* (August- September), and *E. neomexicanus (*September- October*).* When more than one species of *Erigeron* was blooming, all plots at Fort Huachuca (n=8) were sampled on two days, and the NPS and USFS plots (n=3) were sampled on another day during each monthly sampling trip, due to the distance between the different ownership lands where plots were located. The visitation sequence of plots sampling were varied each day by using a random number table.

A synoptic collection insect species collected in this study are curated at the Colorado Plateau Museum of Arthropod Biodiversity. We developed an image library of exemplars for many insect species using a Canon EOS 5D Mark II attached to a Visionary Digital BK Plus Lab System, Zerene Stacker for montaging, and final editing in Adobe Photoshop CS5. Images of specimens were submitted to experts for identification confirmation.

Network analysis. Insect visitors and their host plants were used to create adjacency matrices and corresponding bipartite (or 2-mode) networks: for each *Erigeron* species the data set for

2011 were chosen. Plant (P) and insect visitor (A) are defined within an adjacency matrix and shown in a bipartite graph**.** In these matrices, plant species composed the row categories and insect visitors the column categories, with the number of individuals of an insect species captured on a plant represented in the corresponding matrix cell. In the bipartite network, each plant and insect visitor corresponds to a node, and the number of insect visitors captured on a plant provide a weighting for the edges (= links) between plant and insect visitor nodes. Another measure is centrality. Eigenvector centrality is a measure of the degree to which a node is a component of overall connectedness in the network (Borgatti & Everett 1997-1999). For example, a species with a higher eigenvector centrality value could be a generalist interacting with other generalists in a more central location of the network (a core cluster of the network).

In order to visualize and analyze the structure of plant-plant interactions (= shared insect visitors), the adjacency matrices and corresponding bipartite networks were created and visualized using Netdraw 2.085 in UCINET (Borgatti 2002). In the adjacency matrices, the constituent plants comprise both column and row categories and the matrix cells correspond to the number of insect visitor species shared by pairs of different plant species. All matrix processing and network analyses were done by UCINET 6.0 (Borgatti et al. 1999). Network parameters (Börner et al. 2007) related to network topology, are the number of nodes or size of the network (N), number of edges or links (E), and several measures of network connectedness, including mean number of edges per node ($\overline{k}$) or mean *degree centrality* ($\overline{C_D}$), and mean *eigenvector centrality* ($\overline{C_E}$). For each of the three *Erigeron* species, the insect visitation networks analyses included species richness and species abundance, and examined specialist/generalist relationships within the networks. We examined the networks for clustering and centrality properties and derived eigenvector centrality values, important in understanding if the networks are highly connected with multiple cores.

Initially we ran network analysis software and the Non-metric Multidimensional Scaling (NMS) software with the complete flower-visiting insect community. We reran the networks constructed from a subset the component communities as described below; the resulting network patterns were similar for each of these applications. We also combined years together but the networks were so dense, we chose to show data for one year within each network.

Non-metric Multidimensional Scaling. Non-metric multidimensional scaling was used to compare insect community composition. The Primer6 software looks at an insect community as a group and places the group by distance and direction between two points (not numerical). Ordinations were made first with a fourth root transformation (standard transformation for dissimilarity comparison tests) of the data (Mantel 1967), and then a resemblance function was run on the data (PRIMER6 2007). Our requirement for adequate differentiation was a stress level of no greater than 0.25 and a minimum stress of 0.01, indicating a sufficient degree of separation of points to draw general conclusions about dissimilarity within the data. NMS also provides a graphical output that allowed us to make assessments of our differentiating factors such as collection date (both yearly and seasonal), flower species, etc.

After NMS analyses were conducted, we also ran Multiple Response Permutation Procedures (MRPPs) on the same dataset as above, with no adjustments or transformations. This was a way to confirm our observed groupings in the NMS analyses and examine the driving factors behind the NMS point differentiations. We selected post-hoc analyses to distinguish between our factors but otherwise used the default settings for MRPP available in the PC-ORD 5 data analysis package.

Four NMS were run using Primer6 software; the first using the complete insect visitor communities from the three species of *Erigeron* hosts while the other three used subsets of this community. The subset NMS were of the fly, bee, and beetle visitors only. All three NMS had points distinguished for each host *Erigeron* species from which samples were collected. The data for each NMS was modified prior to running by the removal of singleton (single occurrence) species to eliminate outliers in this test. Any outliers were further reduced with the MDS Subset tool in Primer6.

Insect Indicator species. We quantified both species that were only found on one of the three *Erigeron* species and more common species that may have visited more than one *Erigeron* species but were statistical “indicators”. An indicator species is one that is significantly more abundant (defined by simple quantity) and frequent (defined by multiple collection dates) on one plant host than any other. We used PC-ORD 5 software to determine our indicators through their Group Indicator Analysis tool. This tool tests indicator values based on the abundance of species of insects visiting flower hosts as well as the frequency of capture of these species and ranks them based on these to provide an overall Indicator Value.

A Monte Carlo test of similarity is run on the overall indicator value of each species to which it assigns a p-value and a ‘Max Group’ value. An insect species with a significant p-value is assigned as an Indicator Species to the flower host species that is listed as its Max Group.

It is possible that there were additional indicator taxa that we did not recognize. Many insect taxa are poorly known taxonomically and there might be some taxa that we designated as a single morphospecies but are actually multiple species. It is much easier to distinguish taxa that show obvious morphological differences. For example, if two taxa in the same family are known to be in different genera, we know that they are different species even though we were not able to identify them to species. Thus, most species were easy to distinguish because they occurred in different genera than the next closest taxa.

RESULTS

Plant diversity and percent cover. For each *Erigeron* species the plot data, including associated percent cover and total plant species were tallied by year (Figure 4.4). *Erigeron lemmonii* had the lowest associated plant species diversity growing adjacent to it, with 28 species in 2010 and 22 species in 2011. *Erigeron arisolius* had the most plant species diversity within the combined plots, with 63 species in 2010, and 34 species in 2011. In 2011 there were early spring wild fire which burnt two of the *Erigeron arisolius* plots, and this explains the reduction in the diversity of plants between the two years. The plant diversity within the *Erigeron neomexicanus* combined plots were 32 plant species for each year.

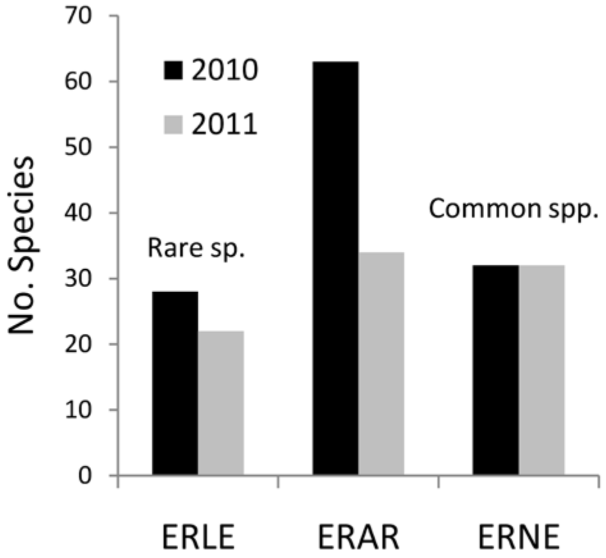

Figure 4.4. Total number of plant species represented in plots for *Erigeron arisolius* (ERAR); *E. lemmonii* (ERLE); and *E. neomexicanus* (ERNE) for two sampling years (2010 and 2011).

The percent coverage of all herbaceous plants within each plot was taken and averaged together to determine the total percent cover within plots for each of the three *Erigeron* species. Only herbaceous plant cover is reported. Although grasses were abundant in many of the *E. arisolius* plots, grasses are mostly wind pollinated and no insect visitors were collected on them. There was a small percent of tree cover present in two plots; however this percentage is not reported here because no collections of pollinating insects were made from trees in either plot. Mean percent cover for *Erigeron lemmonii* was 15 percent on the cliff faces. In contrast the mean percent cover for the five *E. arisolius* plots was 33 percent and the mean percent cover for the five *E. neomexicanus* plots was 32 percent. For *E. lemmonii* habitat, more plant species are present growing adjacent to *E. lemmonii* on the rock faces, however the herbaceous plant cover is much less consistent on the rock cliffs (Figure 4.5), with plants scattered across the rocks where there are pockets of soil built up in crevices. This is in contrast to the close correlation between the species diversity within the plots and the total percent cover within the plots for *E. arisolius* and *E. neomexicanus.*

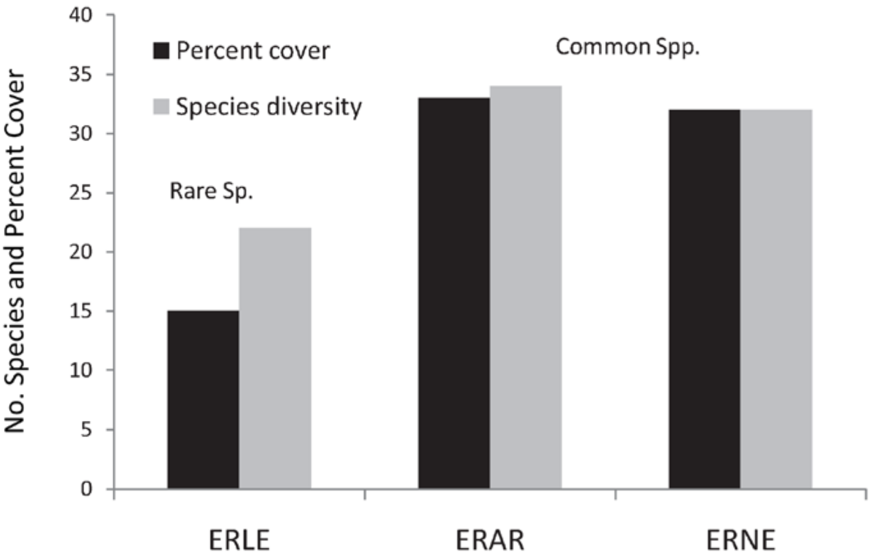

Figure 4.5. Percent cover compared to the total number of herbaceous plant species represented within plots for *E. lemmonii* (ERLE), *Erigeron arisolius* (ERAR), *and E. neomexicanus* (ERNE) in 2011.

Insect visitor data. The findings from our insect collection describe the number of insect visitors, within their order, for the three *Erigeron* networks, for each year (Table 4.1). We ran NMS, MRPP, and network analysis, including insect species from taxa having the capacity to carry pollen (i.e. hairs on legs and/or body, and mouthparts). The component community consisted of insect visitor orders including: Diptera (Flies) were the most diverse insect visitor order for the three species of *Erigeron* (26%), followed by the Hymenoptera (Bees, Wasps and Ants) (23%). Coleoptera (Beetles) also formed a very diverse group of insect visitors (22%), followed by Lepidoptera (Butterflies and Moths) (8%). We excluded the following orders of insect floral visitors (21%) because they are dystropous insects, flower visitors not adapted to pollination; Hemiptera and Orthoptera, and the known predators, Araneae and Phasmida. These visitors were not represented in the plant/ insect visitor networks or the NMS analysis.

Table 4.1. Species richness and abundance of flower-visting insects from each insect order for each Erigeron species for 2010 and 2011 collection years.

Order	ERAR2010	ERAR2011	ERLE2010	ERLE2011	ERNE2010	ERNE2011
Diptera	12	4	5	22	17	8
Hymenoptera	10	6	1	7	8	6
Coleoptera	17	13	4	3	13	13
Lepitodtera	0	4	2	3	0	3

Note: ERAR = *E. arisolius* for 2010, and 2011; ERLE = *E. lemmonii* for 2010 and 2011; ERNE= E. neomexicanus for 2010 and 2011.

Erigeron lemmonii were most importantly visited by Diptera, followed by Coleoptera and Lepidoptera (specifically micro-moths). For *E. arisolius*, Coleoptera was the most diverse group of insect visitors, followed by the Diptera and Hymenoptera. For *E. neomexicanus* both the Diptera and Coleoptera were the most diverse insect visitors, followed by Hymenoptera.

Bipartite networks for each species of Erigeron. Networks were created for each year for all three species of *Erigeron.* Although some of the insects and plants varied within the networks from year to year, the basic architecture of the network for *Erigeron arisolius* and *E. neomexicanus* remained similar between years. However, for *E. lemmonii* the pattern was different between years. The 2010 and 2011 networks are included for *E. lemmonii* because of the differences between years are important. However, only 2011 networks are shown for the other two *Erigeron* species. In 2010 (Figure 4.6) the *E. lemmonii* network has 12 species of insects with 2 insect visitors (Aclypterate 002, a small fly, and *Melyridae Trichochrous* 001), connected to Utah Fendlerbush (*Fendlerella utahensis).* The 2011 network for *Erigeron lemmonii* (Figure 4.7) is portrayed as a star structure with no connection to other plants through insect visitors, and therefore no redundant network structure is provided for its insect visitors. This network, composed of 35 links (all insects), demonstrates *E. lemmonii* had a greater number of insect visitors linked to it than either *E. arisolius* or *E. neomexicanus* for this year, but it had no connection to other plants during 2011 sampling. Overall *Erigeron lemmonii* has only two insect visitor connections with one other plant, in one of two years sampled. It is very weakly connected to other plant species growing adjacent to it on the cliff. By contrast the networks for *E. arisolius* and *E. neomexicanus* were dense networks having multiple core clusters providing some redundant structure within their respective networks

(Figures 4.8 and 4.9). The network for *Erigeron arisolius* contained 184 links composed of 161 insect species and 24 plant species. Of this number there are 15 insect species and 6 number of plant species directly connected to *E. arisolius* as a core cluster within its network. The network for *Erigeron neomexicanus* contained 218 links composed of 197 insect species and 22 plant species. Of this number there are 20 insect species and 19 plant species directly connected to *E. neomexicanus* as a core cluster.

An important measure of networks is centrality. Examination of the eigenvector centrality values can reveal which species in the network is more important by having more interactions with other generalist species, and have a more central location of the network (as a core cluster within the network). Eigenvector centrality values are provided (Table 4.2) for the three species of *Erigeron* and some of the other associated plant species dominantly present in the plots in 2011. A central insect visitor species is more central in the network by being a generalist, interacting with other generalist plants, but it can also interact with specialized species.

Erigeron lemmonii has the highest eigenvector centrality value (0.167) in its network, and is not linked to any other plant species growing on the surrounding cliffs in 2011. There are many co-blooming plant species growing adjacent to the *E. lemmonii* plants on the cliffs (see Supplementary Materials). The *E. lemmonii* networks demonstrate an asymmetric pattern, and its insect visitors are not connected to other plant species.

Table 4.2. Eigenvector values for 2011 networks for each species of *Erigeron*, and the most highly connected core plants within their networks.

	E.arisolius	*E.lemmonii*	*E. neomexicanus*
E. arisolius	0.219	-	-
E. lemmonii	-	0.167	-
E. neomexicanus	0.114	-	0.165
Dalea albiflora	0.105	-	0.026
Eriogonum polycladum	1.143	-	0.043
Viguiera cordifolia	0.155	-	0.265

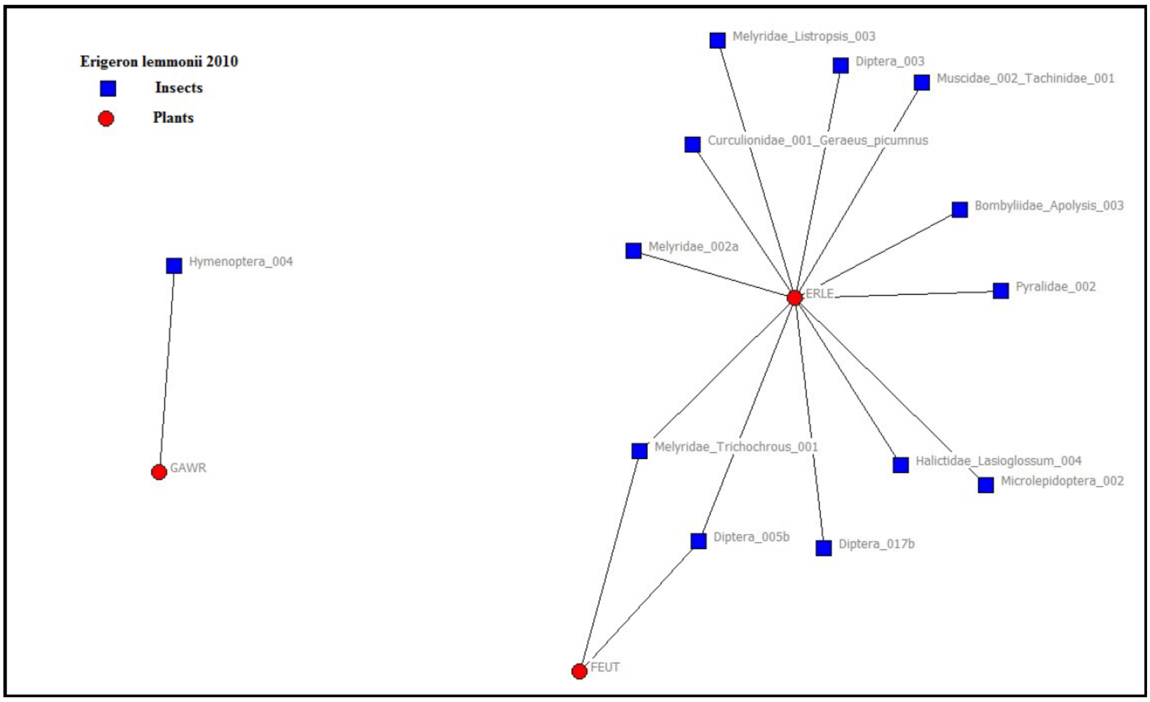

Figure 4.6. *Erigeron lemmonii* plant/insect visitor bipartite network with 12 links within 2010 network; *Erigeron lemmonii* connected to 1 plant by Diptera 005b (Aclypterate 002 b) and Melyridae Trichochrous 001.

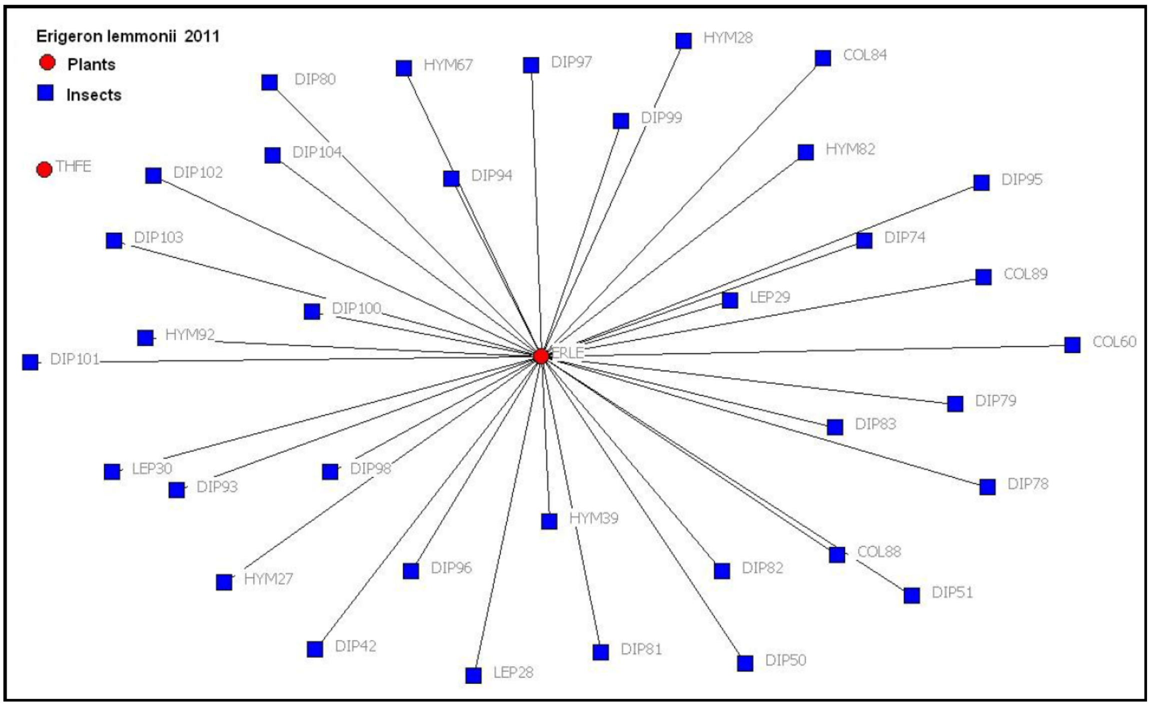

Figure 4.7. *Erigeron lemmonii* plant/insect visitor bipartite network with 35 links (all insects) within 2011 network.

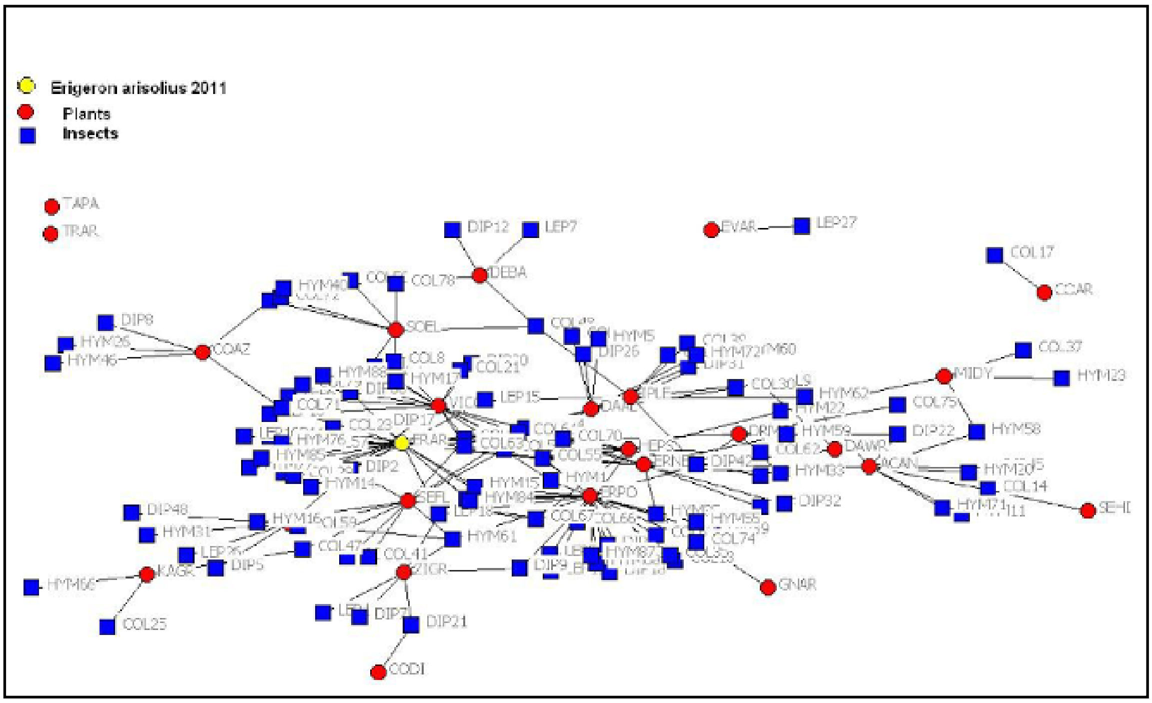

Figure 4.8. *Erigeron arisolius* plant/ insect visitor bipartite network with 184 links (161 Insect species and 24 Plant species) for 2011 network.

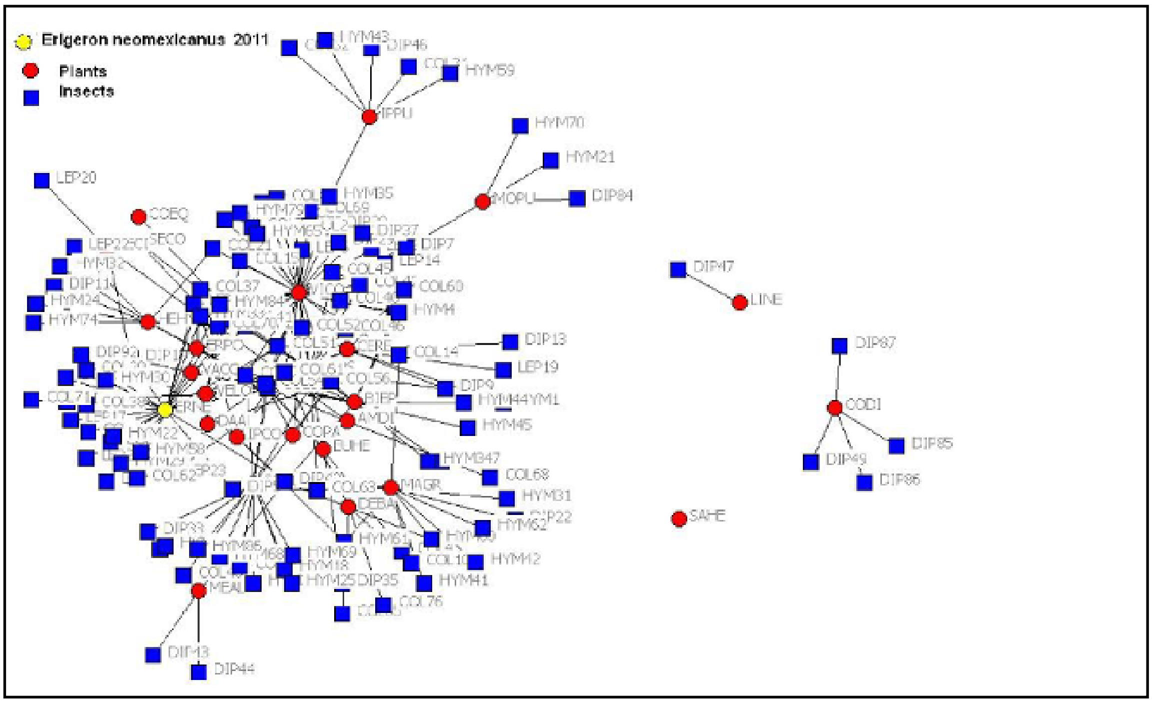

Figure 4.9. *Erigeron neomexicanus* plant/ insect visitor bipartite network with 218 links (197 Insect species and 22 Plant species) for 2011 network.

In 2011 the eigenvector values were compared for the three *Erigeron* species. *E. arisolius* has the highest eigenvector value (0.219) in its network, and therefore is a central core cluster within the network. In contrast the networks of *E. arisolius* and *E. neomexicanus* exhibit centrality with a number of core clusters around several plant species, providing pollen, nectar, shelter or other products for flower visitors. For example *Viguiera cordifolia* Gray (Asteraceae) is another prevalent plant which has a core cluster centrality within the *E. arisolius* and *E. neomexicanus* networks with eigenvector centrality values of 0.155 and 0.265 respectively. In fact within the *E. neomexicanus* network, *Viguiera cordifolia* had the highest Eigenvector centrality value meaning more plants and insect flower visitors were connected to it than any other plant including *E. neomexicanus*, which had the second highest value (0.165) in its network. There were other plants that also had high Eigenvector centrality values, which indicates that there were central core clusters around other plant species, important because this pattern creates redundancy within the network structure. With redundant patterns built into the network architecture, they can be expected to be more resilient to disturbance such as drought, fire, and other and catastrophic circumstances.

Non-metric Multidimensional Scaling (NMS). Multiresponse Permutations Procedures (MRPPs) were run for each of the NMS iterations to provide a statistical understanding of observed groupings. The NMS associated MRPP (Table 4.3) show a significant distinction between the insect visitor component communities at each of the sites, with *E. lemmonii* very distinct from the other *Erigeron* species. Of these, ERLE samples were found to be the most variable among the same plant type.

Figure 4.10 was run using only samples that came from *Erigeron* hosts to distinguish the insect visitors belonging to each species. The NMS and MRPP (Table 4.3) show a significant distinction between ERLE insect visitors and the insect visitors of ERAR and ERNE, though the latter two are not significantly distinct at this level of analysis. This suggests that the nature of ERLE habitat and the unique conditions of ERLE growth lend themselves to differing flower visitors than the other species which grown in more generalized and open habitats. The lack of distinction between ERAR and ERNE in the *Erigeron*-only analysis compared with the significant distinction of the associated flowering species suggests that these *Erigeron* are being visited by similar insect visitor species despite the difference in surrounding habitat.

Table 4.3. Multiresponse Permutation Procedure results showing the differentiation between the flower-visitor communities of each *Erigeron* species*: E.arisolius* (ERAR), *E.lemmonii* (ERLE), *E.neomexicanus* (ERNE).

A					B			
All Visitors		Test Statistic (T)	Heterogeneity Value (A)	p-value	**Flies**	Test Statistic (T)	Heterogeneity Value (A)	p-value
ERAR	ERNE	-0.2252	0.0063	0.3343		0.6817	-0.0230	0.7408
ERAR	ERLE	-5.7515	0.1379	0.0008*		-1.9941	0.0256	0.0399*
ERNE	ERLE	-6.6932	0.1809	0.0001*		-5.4076	0.0476	0.0002*

C					D			
Beetles		Test Statistic (T)	Heterogeneity Value (A)	p-value	**Bees**	Test Statistic (T)	Heterogeneity Value (A)	p-value
ERAR	ERNE	-0.0066	0.0002	0.4004		1.3548	-0.0335	0.9402
ERAR	ERLE	-5.7953	0.1665	0.0007*		-1.0400	0.0258	0.1350
ERNE	ERLE	-6.4877	0.2004	0.0002*		0.3662	-0.0095	0.5631

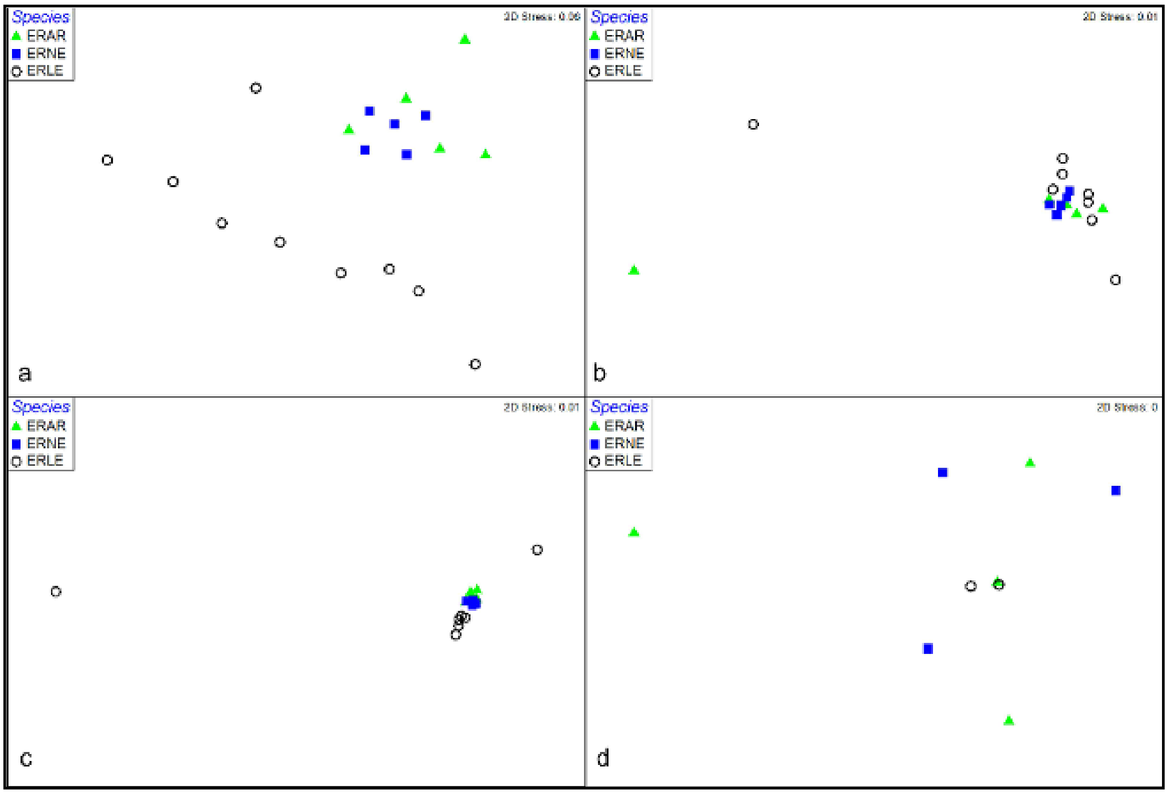

Figure 4.10. Non-metric Multidimensional Scaling graphics showing distinction between all insect visitors (a) on flowers at each of the *Erigeron* sites. Each point represents one sample from one flower (any species) at one of the study sites. Color is distinguished by the variety of *Erigeron* present at the site. This figure also presents NMS that used only Fly (b), Beetle (c), and Bee (d) visitors to look for visual patterns to help identify the driving community for differences observed in NMS Indicator species.

Indicator species. Of the 94 flower visitor species identified among *Erigeron* species, eight were found to be Indicator Species of one of the three *Erigeron* hosts, two for *Erigeron lemmonii*, one for *Erigeron arisolius*, and five for *Erigeron neomexicanus*. All Indicator species were accepted at a p-value of 0.05 or less. Of these, only three species - *Allograpta obliqua, Chauliognathus werneri, and Chauliognathus opacus* - were found to have any overlap between the species and, in all three instances, were found to be greatly more abundant on *E. arisolius*. Within the *E. lemmonii* network Aclypterate 002 and *Trichochrous* sp.1 were connected to other plant species. However, our findings indicate that a species of microlepidoptera (Microlep 002) and *Trichochrous* sp.1 are indicator species for *Erigeron lemmonii* and potentially important pollinators.

DISCUSSION

Plant richness and abundance. There were a greater number of plant species and increased percent cover within the plots for *E. arisolius* and *E. neomexicanus* compared to *E. lemonii*. Both species also had larger habitat areas than *E. lemmonii* and supported more insect visitors. Bascompte and Jordano (2007) found species degree (diversity in a network) to be correlated with its geographic distribution. Our findings demonstrated a positive correlation for plant species diversity; percent cover within the plots (Figure 4.3 and 4.4), and size of the greater habitat area on a landscape scale. This trend within our study was similar to other studies (Rosenzweig 1995, Connor et al 2000, Taki & Kevan 2007). *Erigeron lemmonii* is a habitat specialist and is well connected to its insect visiting community, however it was very weakly linked (with only two occurrences) to one other plant species by its two insect visitor species. It has a 6 month-long flowering season that can support a large number of insect visitors.

Insect visitor richness and abundance. There was also a positive correlation between plant richness and abundance, to insect visitor richness and abundance. Secondly, as the percent coverage of flowering plants increased with area, we found a higher density of insect visitors. Taki & Kevan (2007) showed the number of plant species increased with habitat area suggesting a higher resource diversity supporting a greater number of insect visitors. Our results indicate flies, beetles and the hymenoptera (bees, wasps and ants) were the most important groups of flowering visitors.

Network structure. Nestedness is a network measure, defined as the distribution among species which reveals nested patterns whenever species with fewer interactions appear to be included within those with more generalized species. A high level of nestedness implies the network is cohesive, regardless of the type of mutualism the community represents (Bascompte & Jordano 2007). The interactions of more specialized species are a subset, nested within the interactions of generalized species. Nested patterns typically yield a core of species usually having a higher eigenvector centrality in the network, and they act as a pivot cluster for other peripheral species (Borgatti et al.1999). Nestedness has two important features; the first being that highly asymmetric interactions are generated at the level by specialist species interacting with the most generalist species (Varquez & Aizen, 2006). The second, is the intrinsic implication that there is a core of taxa with a higher density and symmetry at the level of generalist which is understood to be a redundant structure (Memmot et al. 2004). Nestedness can be seen from the points of view as community assembly (Jordano et al. 2006) and would provide alternative links for system response after disturbance.

Networks were created for each year for all three species of *Erigeron*, and although some of the insects and plants varied within the networks from year to year, the basic architecture of the network for *Erigeron arisolius* and *E. neomexicanus* remained similar between years. The pattern was different between years for *E. lemmonii* (Figure 4.5 and 4.6). In our examination between years, the 2010 *Erigeron lemmonii* network has 12 species of insects with 2 insects connecting to one other plant species; Aclypterate 002 and *Trichochrous* 001 are connected to Utah Fendlerbush, *Fendlerella utahensis.* However, this is the only insect interactions recorded for the *Fendlerella utahensis*; it has no other redundant connections to any other plants by its insect visitors. In the 2011 network, *Erigeron lemmonii* has 35 links to insects only and none to other plants creating a simple star pattern with *E. lemmonii* being a hub plant species. Although in 2010 *E. lemmonii* was connected to one other plant, the network architecture can still be described as a simple star because the other plant does not have any other connections. In general for both years, *Erigeron lemmonii* can be described as a generalist, with an asymmetric relationship to its insect visitors and appears to have specialized insect visitors dependent on this plant. Predominately its insect visitors are not linked to other plant species, with two exceptions; Aclypterate 002 and *Trichochrous 001,* both connected to *Fendlerella utahensis.* There were many other co-occurring blooming plant species growing on the surrounding cliffs through-out the extended six-month flowering period of *E. lemmonii* (Supplementary materials). In contrast, the networks for *E. arisolius* and *E. neomexicanus* were very dense, with multiple core clusters nested within the network architecture. In each case *E. arisolius* and *E. neomexicanus* have insect visitors which used other host plants within the network. For *E. arisolius* 184 links were recorded with 160 being insect visitors and 24 plant species. For *E. neomexicanus* 218 links were recorded with 196 being insect visitors and 22 plant species.

For 2011 the eigenvector centrality values for *E. arisolius* (0.219), *E. lemmonii* (0.169) and *E. neomexicanus* (0.114) indicate each is an important node within their respective networks. *Erigeron lemmonii* is the only plant node supporting a number of insect visitors. For *E. arisolius* and *E. neomexicanus* there were other plants having a high Eigenvector centrality value, which indicates multiple core clusters which were central within their respective networks. This pattern creates redundancy within the network structure and allows the network more flexibility and more resilience to disturbance such as drought, fire, and other and catastrophic circumstances.

There are three important points that indicate *Erigeron lemmonii* has a fragile network. First, although the *E. lemmonii* networks between years reflect some differences, it is not connected to other plants by insect visitors (with only one exception in 2010, *Fendlerella utahensis,* which is not further connected to any other plant species), therefore the network architecture has no other core clusters to support its insect visitors. Secondly, while there are a number of insect visitors connected to *E. lemmonii,* which creates the network structure of a simple star structure, there are no redundant structures within the networks to support the insect visitors during environmental disturbances. Third, the majority of insect visitors (with one exception) are specific to *E. lemmonii* and not to any other plants as shown by these networks. The only exception is a small weevil, *Geraeus picumnus*, the only insect visitor found within all three *Erigeron* species webs.

NMS and MRPP data. The NMS and MRPP data demonstrates that *E. lemmonii* flower-visiting insect community is much more distinct than either *E. arisolius* or *E. neomexicanus.* Coleoptera was the most important group of insect visitors for *E. arisolius,* followed by the Diptera and Hymenoptera. For *E. neomexicanus* both the Diptera and Coleoptera were the most prominent insect visitors, followed by Hymenoptera. By contrast, *Erigeron lemmonii* were primarily visited by the Diptera, followed by Coleoptera and Lepidoptera (i.e., moths), and the insect species composition within these orders were distinctly different from the other two *Erigeron* species.

The NMS and MRPP analyses confirm the uniqueness of the *Erigeron lemmonii* community and show the amount of variability. Due to the low capture rate of insects on *E. lemmonii*, the patterns of dissimilarity within those captures can be seen in the NMS, showing that individual communities tend to be varied and unique in comparison to the other two *Erigeron* which tend to be more similar within groups and between species.

Indicator species. Two patterns emerged when we examined both the indicator species that showed a significant statistical affinity for one of the *Erigeron* species, and species that were only found on one of the *Erigeron* species but not were too rare in samples to show statistical affinity. NMS patterns (Figure 4.10) and MRPP analysis (Table 4.3) suggests that these orders are the driving species-groups behind the overall dissimilarity, as bee visitors were not found to be significantly different in MRPP. There were only eight indicator species (Supplementary Material): for *E. lemmonii* there were two, a melyrid beetle (*Trichochrous sp.*) and Microlepidoptera sp.2. There was one indicator for *E. arisolius* (*Chauliognathus omissus*). There were five indicators for *E. neomexicanus,* buprestid and cantharid beetles (*Acmaeodora opacula*, *Chauliognathus opacus*, and *Chauliognathus werneri*), a bombyliid fly (*Apolysis sp.*), and a syrphid fly (*Allograpta obliqua*). Of these, members of family Melyridae, Buprestidae, Syrphidae, and Bombyliidae are known pollinators of flowers and members of Cantharidae may be incidental or minor pollinators as they are known to supplement their diet with nectar and pollen. None of the eight Indicator Species overlap in presence among the *Erigeron* hosts (Supplementary Material). However, there was one insect visitor found on all three *Erigeron* species, a small weevil, *Geraeus picumnus,* a non-indicator that may potentially be a vector for cross pollination. *Erigeron arisolus* and *E. neomexicanus* had 47 and 52 species of flower visitors respectively, sharing 27 of these between them, whereas *E. lemmonii* had a total of 23 flower visitor species and shared only one, *Geraeus picumnus*, with the other two *Erigeron*. Within this identified group of insect visitors is a likely subset of key *E. lemmonii* pollinators. We did not do the pollen washes of all insects captured over the course of the collection to identify the pollinators, so we do not have this definitive proof. However, we do have clearly significant indicator species for each *Erigeron* host species, and the single species found in common to all three *Erigeron* species. Photographs with identifications are included in Appendix 4.III.

CONCLUSIONS

Our first hypothesis was that plants that have evolved under naturally fragmented, highly specific habitat conditions (*Erigeron lemmonii)* have weakly linked nodes supporting a small number of insect visitors in the insect visitation network. Our findings indicate this hypothesis was false as shown from our network examination; *Erigeron lemmonii* has a large number of insect visitors in its network over a long six month flowering season that are directly linked to it. It has been reported there is a diurnal separation of pollinators including visitation based on dehiscence at midday for the genus *Erigeron* (Pleasants 1983), which suggests this temporal division actually supports a greater number of insect visitors.

Our second hypothesis was that plants that have evolved under naturally fragmented cliff habitat share insect visitors with relatively common plants that are insect visitor generalists (and are found in a core within the network). We found this to be false because our findings indicate *E. lemmonii* shared two insect visitors with only one other plant species that was not connected to anything else in 2010, and had no connections to any other plant species in 2011. *Erigeron lemmonii* has one species of flower visitor in common with the other two *Erigeron* species; *Garaeus picumnus,* a weevil, is found on all three *Erigeron* host species. *Garaeus picumnus* are not present in great numbers, and therefore is not an indicator species. Other than this one insect visitor, no others were shared with either of the other two *Erigeron* species, and *E. lemmonii* insect visitors are specific to it. *Erigeron lemmonii's* star-like network does not exhibit multiple cores within the network architecture, which indicates it has no redundant relationships to support its insect visitors.

The third hypothesis stated that plants that have evolved as habitat generalists are supported by more redundant, robust network relationships, and are relatively less impacted by fragmentation, than plants that have evolved as habitat specialists. We found this to be true. The findings indicate *E. arisolius* and *E. neomexicanus,* which are habitat generalists, are supported by more redundant core clusters interconnected together through-out the web resulting in robust network relationships. Their network architectures indicate they will be relatively less impacted by environmental changes or instability. Conversely *Erigeron lemmonii* only shares two insect visitors with one other plant (2010), and none with any other *Erigeron* species studied. *Erigeron lemmonii* has a much more fragile star-like network structure, without any redundant core clusters. This network will have a low resiliency to environmental disturbances.

Assuming a close one to one relationship between a single insect pollinator species and plant species should result in the extinction of the remaining mutualistic partner in case of extinction of either the pollinator or plant species (Steffan-Dewenter 2006). *Erigeron lemmonii* exhibits an unbalanced relationship where a number of insect visitors rely on this one plant species. This indicates a high extinction risk for the insect visitors, including the subgroup of pollinators of *Erigeron lemmonii*. Whereas in the networks for *E. arisolius* and *E. neomexicanus*, both generalist species, the network architecture reflects more core groups providing redundancy within the network, and resiliency to environmental changes. The more redundancy in the network, the more stable the network architecture is, which provides protection from loss of plant and insect visitor species.

Conservation factors for Erigeron lemmonii. *Erigeron lemmonii* is reliant on its pollinators because it is an outcrosser, with a xenogamous mating system (Bailey et al., in preparation). As a habitat specialist, *E. lemmonii* has only one other plant that shares insect visitors within its network, and has a very unique insect community specific to it compared with the other two *Erigeron* species. The NMS and MRPP data demonstrates that *E. lemmonii* insect visitors are much more distinct than either *E. arisolius* or *E. neomexicanus* are to each other due to the distinction of the flower visitor community (Figure 4.10). If *E. lemmonii* at the population level disappears, it may cause a collapse of its dependent insect visitors, because they have so few links to other plants within the network with no demonstrated redundant connections within the network structure. Redundancy within the network provides resiliency from disturbances. This has serious implications for the conservation of *E. lemmonii* and its associated insect visitor community and pollinators.

The integrity of *E. lemmonii* population is not being eroded through hybridization. Our results indicate hybridization of *E. lemmonii* with *E. neomexicanus* is not possible because we did not capture insects on *E. lemmonii* that were directly connected through its network, shared by *E. neomexicanus. Erigeron lemmonii* is found growing within 2-4 meters from *Erigeron neomexicanus,* which grows on the ground below the cliffs. *E. lemmonii* does not share insect visitors, or the subset of pollinators, with *E. neomexicanus.* The likelihood of pollen movement between these two *Erigeron* species with no shared pollinators is nonexistent. This is significant because there is distinct habitat separation of the two *Erigeron*, even though they grow in close proximity to one another.

Recently in October, 2012, the U.S. Fish and Wildlife System (USFWS) published a proposed rule change in the Federal Register: Lemmon fleabane, a candidate for listing under the Act since 1996, is being removed from the list of candidate species because the main threat (wildfire) does not rise to a level of significance such that the species is in danger of extinction or likely to become so in the foreseeable future. In addition to fire, recreational repelling which is not allowed in the canyon, might pose as a minor threat, but would not threaten the entire population if it were to occur. Additionally in 2011, the Desert Botanical Garden collected hundreds of viable seeds for long term storage. This and future planned seed collections by the Desert Botanical Garden may offset any impacts to the species in the event of fire or other catastrophic events.

Currently the Army Resource Manager monitors the population and is drafting a Cooperative Agreement with the U.S. Fish and Wildlife Service, as a result of the proposed change. Fort Huachuca Army Base maintains permanent photo- monitoring plots using a tripod and specific coordinates for future re-measurement of plots in an effort to survey for new plants within the population, and has monitored the *Erigeron. lemmonii* population since 2008 (Stone, pers. comm.2010). This monitoring indicates the population has been stable during the time it has been monitored and may even be increasing slowly (Stone, pers. comm. 2012). The cooperative agreement will ensure continued monitoring, 1) will promote adaptive management of this species, 2) continue to restrict recreational activities in its habitat, and 3) encourage further research into the life history and population biology. Our network and NMS analysis indicates this species warrants monitoring because of its fragile ecological relationships with its insect visitors.

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APPENDICES

Additional supporting information may be found in the online version of this article

Appendix 4.I. Associated flowering herbaceous plants growing within collection plots for the three *Erigeron* species.

Appendix 4.II. Indicator Values of flower visitors of the three *Erigeron* species.

Appendix 4.III. Photographs of the significant indicator species of flower visitors to each of the *Erigeron* hosts and the flower visitor common to all three *Erigeron* species.

Appendix 4.IV. GPS coordinates for *Erigeron* study plots.

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SUPPLEMENTARY MATERIAL

Appendix 4.I. Associated flowering herbaceous plants growing within collection plots for the three *Erigeron* species.

Grasses, and trees were eliminated because we did not collect insect visitors from these plants if found within plots. Grasses were eliminated because they are mainly wind pollinated and tree species were eliminated because they were not flowering or prevalent within the plots.

Erigeron arisolius G.L. Nesom

Family	Scientific Name	Common Name
Fabaceae	*Acacia angustissima* (Mill) Kuntze	Whiteball acacia
Liliaceae	*Agave palmeri* Engelm.	Palmer's Agave
Asteraceae	*Ambrosia acanthicarpa* Hook	Annual Bursage
Papaveraceae	*Argemone pleiacantha* Greene	Bluestem Prickle Poppy
Asteraceae	*Artemisia dracunculoides* Pursh.	Wild Tarragon
Asteraceae	*Baccharis pteroniodes* DC.	Yerba de pasmo
Nyctaginaceae	*Boerhaavia purpurascens* Gray	Purple Spiderling
Fabaceae	*Calliandra eriophylla* Benth.	Fairy duster
Euphorbiaceae	*Chamaesyce albamarginata* Torr. & Gray	Rattlesnake plant
Chenopodiaceae	*Chenopodia alba* L.	Pigweed
Commelinaceae	*Commelina dianthifolia* Delile.	Birdbill Dayflower
Convolvulaceae	*Convolvulus arvensis* L.	Field Bindweed
Convolvulaceae	*Convolvulus equitans* Benth	Pink Bindweed
Asteraceae	*Conyza canadensis* (L.) Conquist	Horseweed
Cactaceae	*Cylindropuntia spinosior* Engelm. and Bigel.	Cane Cholla
Fabaceae	*Dalea albiflora* Gray	Scruffy Prairie Clover
Solanaceae	*Datura wrightii* Regel	Jimsonweed
Fabaceae	*Desmodium batocaulon* Gray	Bushy Tickclover
Asteraceae	*Dieteria canescens var. glabra* (Pursh) Nutt.	Purple Aster
Asteraceae	*Dyssodia pentachaeta* (DC) Robins	Five needle Fetid Marigold
Polygonaceae	*Eriogonum wrightii* Torr.	Buckwheat
Convolvulaceae	*Evolvulus arizonicus* Gray	AZ Blue eyes
Convolvulaceae	*Evolvulus sericeus* Swartz.	Silver Morning Glory
Asteraceae	*Gnaphalum arizonicum* Gray	Pearly Everlasting
Onegraceae	*Guara coccinea* Nutt.	Scarlet Guara
Asteraceae	*Helianthus annus* L.	Annual Sunflower
Asteraceae	*Heterotheca subaxillaris ssp latifolia* Semple	Camphorweed
Convolvulaceae	*Ipomoea coccinea* L.	Scarlet Creeper
Euphorbiaceae	*Jatropha macrorhiza* Benth. McNary	Ragged-leaf Spurge
Zygophyllaceae	*Kallstoemia grandiflora* Torr.	AZ. Caltrop Poppy
Brassicaceae	*Lepidium thurberi* Wooton	Thurber's peppergrass
Linaceae	*Linum lewesii* Pursh.	Blue Flax
Linaceae	*Linum neomexicanum* Greene	Yellow Mexican Flax
Linaceae	*Linum puberlum* Engelm.	Plains flax
Asteraceae	*Xanthisma gracile* (Nuttall) Morgan & Hartman	Spiny yellow daisy
Liliaceae	*Millia biflora* Cav.	Mexican Star lily
Nyctaginaceae	*Mirabilis longiflora* L.	Nicotiana
Cactaceae	*Opuntia englemannii* Salm-Dyck	Prickly Pear
Portulacaceae	*Phemeranthus aurantiacus* (Engelm.) Kiger	Fameflower
Portulacaceae	*Portulaca umbraticola* L.	Chinese Hat
Chenopodiaceae	*Salsola kali* L.	Tumbleweed
Asteraceae	*Senecio flaccidus* Less	Threadleaf Groundsel
Asteraceae	*Senecio multicapitatus* Greenm.	Grounsel
Fabaceae	*Senna hirsuta* (L.) Inwin & Barneby	Wild Senna
Solanaceae	*Solanum elaegnifolia* Cav.	Silver Nightshade
Malvaceae	*Sphraeralcea parvifolia* A Nels.	Little-leaf globemallow
Portulacaceae	*Talinum paniculatum* (Jacq.) Gaertn.	Jewel of Opar
Asteraceae	*Thelesperma megapotamicum* (Spreng.)	Hopi Tea

	Kuntze	
Lamiaceae	*Trichostema arizonicum* Gray	AZ Bluecurls
Verbenaceae	*Verbena hastata* L.	Verbena
Scrophulariaceae	*Veronica peregrina* L.	Wandering Speedwell
Asteraceae	*Viguiera stenoloba* Blake	Skeletonleaf Goldeneye
Asteraceae	*Zinnea grandiflora*	Zinnea

Erigeron lemmonii Gray

Family	Scientific Name	Common Name
Liliaceae	*Allium cernuum* Roth	Wild onion
Rubiaceae	*Bouvardia glaberrima* Engelm	Smooth Bouvardia
Scrophulariaceae	*Castilleja patriotica* Fern	Huachuca Mtn. Paintbrush
Fumariaceae	*Corydalis aurea* Willd.	Golden Cordalis
Asteraceae	*Erigeron neomexicanus* Gray	New mexico fleabane (on ground)
Asteraceae	*Erigeron platyphyllus* Greene	Tall Erigeron (on ground)
Saxifragaceae	*Fendlerella utahensis* (Wats.) Heller	Utah Fendlerbush
Asteraceae	*Galinsoga parviflora* Cav.	Raceweed
Rubiaceae	*Galium aparine* L.	Bedstraw
Rubiaceae	*Galium wrightii* Gray	Wright's Galium
Geraniaceae	*Geranium richardsonii* Fisch. & Trautv.	Richardson's Geranium
Saxifragaceae	*Heuchera sanguinea* Engelm.	Coralbells
Convolvulaceae	*Ipomoea purpurea* (L.)Roth	Purple Morning glory
Scrophulariaceae	*Maurandella antirrhiniflora* Humb. & Bonpl.	Little Snapdragon Vine
Oxalidaceae	*Oxalis amplifolia* (Trel.) Knuth	Oxalis/ Shamrocks
Fabaceae	*Phaseolus angustissimus* Gray	Slim-leaf Lima Bean
Solanaceae	*Physallis lobata* Torr.	Purple nightshade
Asteraceae	*Stephanomeria thurberi* Gray	Thurber's Stephanomeria
Ranunculaceae	*Thalictrum fendleri* Engelm.	Fendler's Meadowrue

Erigeron neomexicanus Gray

Family	Scientific Name	Common Name
Fabaceae	*Acacia greggii* Gray	Cat claw Acacia
Asteraceae	*Amauriopsis dissecta* (A. Gray) Rydberg	Bahia/ Yellow Ragweed
Ericaceae	*Arctostaphylos pungens* Parry	Point-leaf Manzanita
Asteraceae	*Artemisia dracuncularus* Pursh	Wild Tarragon
Asteraceae	*Bahia dissecta* (Gray) Britton	Wild Chrysanthemum
Asteraceae	*Bidens leptocephala* Sherff	Few flowered Beggar Ticks
Asteraceae	*Carduus nutans* L.	Musk Thistle
Scrophulariaceae	*Castilleja patriotica* Fern	Huachuca Mtn. Paintbrush
Chenopodiaceae	*Chenopodium capitatum* (L.). Asch.	Strawberry Blight
Santalaceae	*Commandra pallida* A. DC.	Bastard Toadflax
Commelinaceae	*Commelina dianthifolia* Delile	Birdbill Dayflower
Convolvulaceae	*Convolvulus equitans* Gray	Pink morning glory
Asteraceae	*Cosmos parviflorus* (Jacq.) H.B.K.	Wild Cosmos
Fabaceae	*Dalea albaflora* Gray	Scruffy Prairie Clover
Fabaceae	*Dalea leporina* (Ait) Kerney & Peebles	Fox tail Dalea
Ranunculaceae	*Delphinium nuttallianum* Pritz ex Walp	Nuttall's Larkspur
Fabaceae	*Desmodium batacaulon* Gray	Bushy Tickclover
Polygonaceae	*Eriogonum polyclaon* Benth.	Buckwheat
Asteraceae	*Eupatorium herbaceum* (Gray) Greene	Ageratina
Convolvulaceae	*Evolvulus arizonicus* Gray	AZ. Blue eyes
Geraniaceae	*Geranium carolinianum* L.	Wild Geranium
Onegraceae	*Guara coccinea* Nutt.	Scarlet Guara
Lamiaceae	*Hedeoma hyssopifolium* Gray	Sweet Scent
Asteraceae	*Helianthus annua* L.	Annual Sunflower

Asteraceae	*Heliomeris multiflora* Nutt	Nevada Showy False Goldeneye
Asteraceae	*Heterotheca subaxillaris ssp latifolia* Semple	Camphorweed
Convolvulaceae	*Ipomoea coccinea* L.	Scarlet bindweed
Convolvulaceae	*Ipomoea purpurea* (L.) Roth	Purple morninglory
Linaceae	*Linum lewesii* Pursh.	Blue flax
Linaceae	*Linum neomexicanum* Greene	New Mexico yellow flax
Fabaceae	*Macroptilium gibbosifolium* (Ortega) A. Del.	Orange stringbean
Fabaceae	*Melilotis alba* Desr.	Sweet White Clover
Lamiaceae	*Monarda punctata* L.	Spotted Monarda
Scrophulariaceae	*Penstamon barbatus* (Cav) Roth	Goldenbeard Penstamon
Lamiaceae	*Salvia henryi* Gray	Red Sage
Asteraceae	*Schkuhria wislizeni var. frustrata* Gray	Threadleaf
Portulacaceae	*Sedum cockerellii* Britton	Stonecrop
Asteraceae	*Solidago lepida* D.C. *var. salebrosa* (Piper) Semple.	Rocky Mountains Canada Goldenrod
Asteraceae	*Solidago wrightii* Gray	Wright's goldenrod
Malvaceae	*Sphaeralcea parvifolia* A. Nels.	Globemallow
Fabaceae	*Trifolium agrarium* L.	Yellow Hop Clover
Verbenaceae	*Verbena ciliata* Benth	Mexican Verbena
Asteraceae	*Verbesina longifolia* Gray	Long leaf Crownbeard
Asteraceae	*Viguiera cordifolia* Gray	Heartleaf Goldeneye
Asteraceae	*Viguiera longifolia* (Robins & Greem.)Blake	Goldeneye
Asteraceae	*Viguiera multiflora* (Nutt.) Blake	Many-flowered Goldeneye

Appendix 4. II. Indicator Values of flower visitors of the three *Erigeron* species.

Singletons, non-insects, and insects from non-pollinating orders were removed prior to the indicator analysis being run. Indicator analysis was performed using PCORD 5.0. Specimens were placed into morphospecies when a full identification was not possible. Each morphospecies was identified to the extent possible by the staff at the Colorado Plateau Museum of Arthropod Biodiversity.

Insect	Max Group	Observed Indicator Value	Mean Indicator Value	Standard Deviation	p-value	
Braconidae_001	ERAR	3	4.9	2.8	0.7734	
Braconidae_002	ERAR	4.5	4.5	0.25	0.6457	
Braconidae_004	ERLE	4.2	4.5	0.26	1	
Braconidae_005	ERAR	4.5	4.5	0.25	0.6469	
Ichneumonide_002	ERNE	4.8	4.5	0.26	0.3075	
Vespidae_Polistes_004	ERAR	4.5	4.5	0.25	0.6457	
Vespidae_Pterocheilus_arizonicus	ERAR	4.5	4.5	0.26	0.6451	
Nymphalidae_Chlosyne_zacinia	ERNE	4.8	4.5	0.25	0.3159	
Noctuidae_Schinia_001	ERAR	9.1	5.4	2.48	0.2006	
Noctuidae_002	ERAR	4.5	4.5	0.25	0.6457	
Pyralidae_001	ERAR	4.5	4.5	0.25	0.6555	
Pyralidae_002	ERLE	4.2	4.5	0.26	1	
Pyralidae_003	ERNE	4.8	4.5	0.26	0.3045	
Microlepidoptera_001	ERNE	3.2	4.9	2.78	0.5421	
Microlepidoptera_002	ERLE	16.7	6.6	3.32	0.0326	*
Lepidoptera Immature 001	ERLE	8.3	4.5	3.04	0.3219	
Bombyliidae_Apolysis_002	ERNE	14.3	6.8	3.03	0.024	*
Bombyliidae_Apolysis_003	ERLE	4.2	4.5	0.26	1	
Bombyliidae_Bombylius_barbatus	ERAR	4.5	4.5	0.25	0.6457	
Bombyliidae_Geron_albarius	ERAR	4.5	4.5	0.25	0.6469	
Bombyliidae_Geron_Empidigeron_sp1	ERLE	8.3	4.7	2.94	0.3291	
Bombyliidae_Geron 006	ERNE	9.5	4.5	3.03	0.0922	
Bombyliidae_Phthiria_001	ERNE	14.4	10.7	4.83	0.2188	
Bombyliidae 002	ERAR	3	4.9	2.8	0.7668	
Bombyliidae 003	ERLE	8.3	4.6	3.07	0.3313	
Dolichopodidae_Mesorhaga_sp1	ERLE	12.5	5.8	3.12	0.1	
Dolichopodidae_002	ERNE	4.8	4.5	0.26	0.3075	
Syrphidae_Allograpta_obliqua	ERNE	17	7.8	3.79	0.0296	*
Syrphidae_Ocyptamus_001	ERNE	9.5	4.6	3.07	0.0982	
Tachinidae_001	ERLE	4.2	4.5	0.26	1	
Tachinidae_002	ERNE	4.8	4.5	0.26	0.3097	
Tachinidae_003	ERAR	4.5	4.5	0.26	0.6357	
Tephritidae_Urophora_001	ERAR	9.1	4.6	3.06	0.2016	
Tephritidae_001	ERAR	4.5	4.5	0.26	0.6485	
Acalypterate_001	ERNE	8.2	6.3	3.24	0.2384	
Acalypterate_002	ERLE	4.2	4.5	0.26	1	
Nematocera_001	ERLE	4.2	4.5	0.26	1	
Diptera_003	ERLE	4.2	4.5	0.26	1	
Diptera_005	ERNE	4.8	4.5	0.26	0.3197	
Diptera_006	ERAR	4.5	4.5	0.26	0.6485	
Diptera_007	ERAR	4.5	4.5	0.26	0.6427	
Diptera_014	ERNE	2.4	4.5	3.03	0.7566	
Diptera_017a	ERAR	6	5.8	3.21	0.5291	
Diptera_017b	ERLE	4.2	4.5	0.26	1	
Diptera_017c	ERNE	4.8	4.5	0.25	0.3207	
Diptera_017d	ERNE	4.8	4.5	0.26	0.3169	
Diptera_021	ERAR	4.5	4.5	0.25	0.6469	
Diptera_024	ERAR	4.5	4.5	0.26	0.6485	
Diptera_028	ERLE	8.3	4.6	3.07	0.3319	
Diptera_033	ERLE	4.2	4.5	0.25	1	
Bruchidae_001	ERNE	4.8	4.5	0.26	0.3097	
Bruchidae_Acanthoscelides_001	ERNE	4.8	4.5	0.25	0.3207	

Insect	Max Group	Observed Indicator Value	Mean Indicator Value	Standard Deviation	p-value	
Buprestidae_Acmaeodora_opacula	ERNE	42.9	10.1	3.97	0.0002	*
Buprestidae_Acmaeodora_001	ERNE	4.8	4.5	0.25	0.2965	
Buprestidae_Acmaeodora_002	ERNE	4.9	6.5	3.2	0.5995	
Buprestidae_Acmaeodora_003	ERAR	5.4	5.7	3.11	0.6059	
Cantharidae_Chauliognathus_opacus	ERNE	21.7	12.3	4.38	0.0414	*
Cantharidae_Chauliognathus_omissus	ERAR	31.8	9.5	4.39	0.0004	*
Cantharidae_Chauliognathus_lewisi_vittatus	ERNE	17	13.1	4.55	0.1798	
Cantharidae_Chauliognathus_misellus	ERAR	10.4	10.1	3.88	0.3811	
Cantharidae_Chauliognathus_nr._werneri	ERNE	37.1	19.1	6.09	0.0092	*
Chrysomelidae_Cryptocephalinae_001	ERLE	4.2	4.5	0.26	1	
Coccinellidae_Hippodamia_convergens	ERAR	12.8	7.3	3.45	0.092	
Coccinellidae_Scymus sp	ERNE	9.5	5	2.82	0.0894	
Curculionidae_001_Geraeus_picumnus	ERNE	1.7	5.7	3.13	1	
Curculionidae_Apion 001	ERNE	4.8	4.5	0.26	0.3045	
Melyridae_Listropsis_001	ERNE	9.4	8.6	3.96	0.3691	
Melyridae_Listropsis_002	ERAR	10.6	7.9	3.84	0.2396	
Melyridae_Listropsis_003	ERLE	4.2	4.5	0.26	1	
Melyridae_Rhadalus_001	ERAR	13.6	6	3.24	0.0684	
Melyridae_Trichochrous_001	ERLE	25	8.2	3.76	0.003	*
Melyridae_Trichochrous_003	ERNE	7.7	5.8	3.12	0.1718	
Melyridae_001	ERAR	13.6	7.1	3.03	0.0614	
Melyridae_002a	ERLE	4.2	4.5	0.26	1	
Melyridae_002b	ERAR	4.5	4.5	0.26	0.6451	
Mordellidae_001	ERNE	7.3	8.4	3.95	0.5315	
Mordellidae_002	ERAR	2.8	4.8	2.88	0.7612	
Phlacridae_001	ERNE	2.4	4.5	3.02	0.7596	
Scraptiidae_Anaspis_001	ERLE	4.2	4.5	0.26	1	
Tenebrionidae_Lobometopon_001	ERNE	2.4	4.5	3.02	0.7528	
Coleoptera_001	ERNE	4.8	4.5	0.26	0.3097	
Coleoptera_008	ERNE	4.8	4.5	0.25	0.3045	
Apidae_Melissodes_001	ERAR	3	4.9	2.79	0.757	
Apidae_003	ERNE	4.8	4.5	0.25	0.3045	
Halictidae_Lasioglossum_001	ERNE	2.4	4.5	3.05	0.7487	
Halictidae_Lasioglossum_002	ERAR	4.5	4.5	0.25	0.6469	
Halictidae_Lasioglossum_003a	ERNE	4.8	4.5	0.26	0.3197	
Halictidae_Lasioglossum_003b	ERNE	4.8	4.5	0.26	0.3197	
Halictidae_Lasioglossum_003c	ERNE	4.8	4.5	0.26	0.3197	
Halictidae_Lasioglossum_004	ERLE	8.3	4.5	3.03	0.3187	
Halictidae_Lasioglossum_005	ERLE	4.2	4.5	0.26	1	
Halictidae_Perdita_001	ERAR	4.5	4.5	0.26	0.6345	
Halictidae_Dialictus_prunisiformes	ERAR	3.8	5.4	2.43	0.7776	
Halictidae_Dialictus 006	ERAR	4.5	4.5	0.26	0.6427	

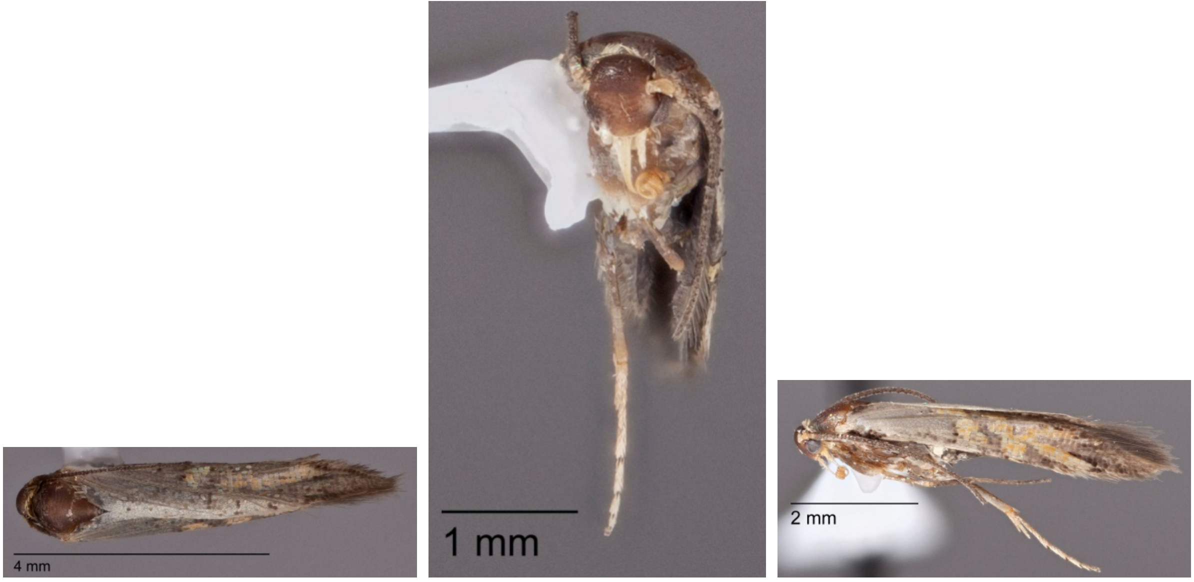
Appendix 4. III. Photographs of the significant indicator species of flower visitors to each of the *Erigeron* hosts and the flower visitor common to all three *Erigeron* species.

These figures display CPMAB's images of the eight species found to be indicator species of *Erigeron*. Also, the single species found to be common among Erigeron hosts is displayed at the end.

Indicators for *Erigeron lemmonii*

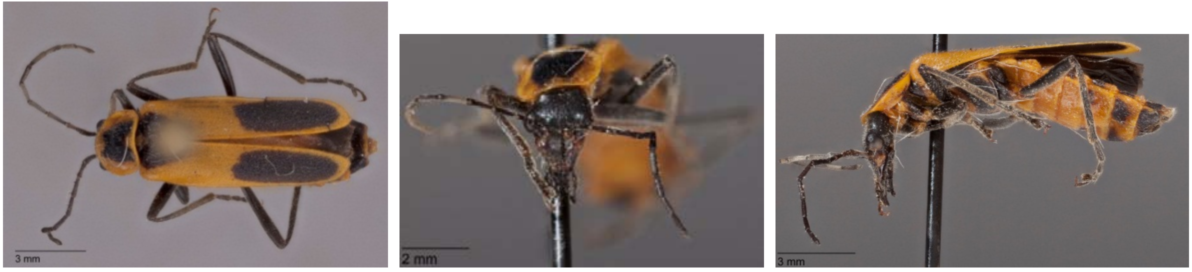
Trichochrous sp.1

Microlepidoptera sp.2



Indicator for *Erigeron arisolius*

Chauliognathus omissus



Indicators for *Erigeron neomexicanus*

Acmaeodora opacula

Chauliognathus opacus

Chauliognathus werneri

Apolysis sp.2

Allograpta obliqua

Insect common to all three *Erigeron* species. Note: This species is NOT an indicator for any of the *Erigeron* plant hosts

Curculionidae *Geraeus picumnus*

Websites to view insect collections.
(http://bugguide.net/bgimage/user/28808,
http://bugs.nau.edu/BIO322/Fort_Huachuca_ERLE_Curc001.html,
http://www.bugs.nau.edu/Bio322/Fort_Huachuca_ERLE_HLasio.html,
http://www.bugs.nau.edu/Bio322/Fort_Huachuca_ERLE_Geron.html).

Appendix 4.IV. GPS Coordinates for *Erigeron* plots.

GPS coordinates taken with a Garmin Etrex Venture HC, datum WGS84.

Erigeron lemmonii	31.46104884, -110.35493150

Erigeron arisolius

1. Site across road from Picnic area (Fort H)	31.48060760, -110.33848864
2. Woodcutter’s Pond Road (Non-burned plot)	31.51381544, -110.32358604
3. Woodcutter’s Pond Road (Burned plot)	31.51386238, -110.32388653
4. Ash Canyon (USFS)	31.38589636, -110.24126041
5. Coronado National Historic Site (NPS) Field to right of entrance	31.34952108, -110.22776110

Erigeron neomexicanus

1. Huachuca Canyon	31.52844136, -110.38096318
2. Parker canyon	31.54819985, -110.42423878
3. Scheelite Canyon trail	31.45917205, -110.35379894
4. Scheelite Canyon parking area	31.46701567, -110.35525949
5. Coronado National Historic Site (NPS) On road shoulder	31.34657803, -110.26177579

CHAPTER 5

Isolation and characterization of microsatellite loci for a hyper-rare cliff endemic, *Erigeron lemmonii*, and a more widespread congener, *Erigeron arisolius* (Asteraceae).

ABSTRACT

We identified eight novel polymorphic microsatellite loci in *Erigeron lemmonii* and nine novel polymorphic microsatellite loci in *Erigeron arisolius*. Of these, three loci developed for *E. arisolius* cross-amplified *E. lemmonii* DNA and four loci developed for *E. lemmonii* cross-amplified *E. arisolius* DNA. This set of 17 loci will be used to analyze patterns of genetic structure in populations of these two *Erigeron* species in southeastern Arizona with the ultimate goal of comparing patterns of genetic structure within a hyper-rare cliff endemic (*E. lemmonii*) to those of a more widespread congener (*E. arisolius*).

INTRODUCTION

The Lemmon's Fleabane, *Erigeron lemmonii* A. Gray, is an endemic plant occurring solely in Scheelite Canyon, on the Fort Huachuca Army installation, in the Madrean Sky-Island Region of southeastern Arizona. This plant was removed from candidate species status under the endangered species act in October 2012 (USFWS 1996: USFWS 2012). *Erigeron lemmonii* consists of a single population of less than 1000 individuals (Malusa 2006). It grows on shaded limestone cliffs with western, southern, and northern aspects at elevations of 1900-2200 meters (Nesom 2006). *E. lemmonii* has been determined to be diploid via chromosome counts of 20 different cells (Noyes and Bailey, in review). The Dry-sun Fleabane, *Erigeron arisolius* G.L.Nesom, is more widespread, growing in grassland habitats at elevations of 1300-1700 meters in Arizona and northern Mexico (Nesom 2006), and is also diploid (Sundberg 1990). The New Mexico Fleabane, *Erigeron neomexicanus* A. Gray, grows in various natural habitats and has the largest range: Arizona, New Mexico, and Mexico (Nesom 2006). It grows at an elevation range of 1500 to 2700 m in rocky substrates. We developed a series of microsatellite loci for each species and tested them for cross-amplification to investigate patterns of genetic diversity and population structure in both *Erigeron* species in a future study.

METHODS

Collection of 110 genetic samples of *E. lemmonii* from the accessible areas of the cliffs (approximately 10 % of the total population), and 30 samples for *E. arisolius* and *E. neomexicanus* at 5 different plot locations for each were made in the Fall, 2009 and Spring 2010. Fresh plant material was stored in RNA lader and shipped to the lab for further processing. All *Erigeron* samples were extracted using an initial disruption step with Lysing Matrix D tubes containing 1.4 mm ceramic spheres (MP Biomedicals LLC, Solon, Ohio) followed by extraction with the DNeasy Plant Mini Kit (Qiagen, Valencia, California). DNA from 10 individuals of *Erigeron lemmonii* (31.46136, -110.35474, Voucher: P. Bailey 301 (MO)), 10 individuals of *Erigeron arisolius* (31.52544 , -110.32944, Voucher: P. Bailey 307 (MO)), and 10 individuals of *Erigeron neomexicanus* (31.46701, -110.35525, Voucher : P. Bailey 303 (MO)) was pooled and concentrated, separately (providing a total of 25 µg for each species), and sent to Genetic Identification Services (GIS, Chatsworth, California) for the development of microsatellite libraries.

The *Erigeron* genomic DNA was enriched for four different microsatellite motifs (AAC, ATG, CATC, and TAGA). Positive colonies containing uninterrupted tri- and tetra-nucleotide repeats were amplified with universal M13 primers, and clones with sequence lengths of 300-700 bp were sequenced. Of the 141 clones examined, 81 yielded unique sequences with repeat motifs for *E. lemmonii* and 133 clones yielded 60 unique sequences with repeat motifs for *E. arisolius*. Primers were designed for 51 loci for *E. lemmonii* and 45 loci for *E. arisolius* using the web-based program DESIGNERPCR 1.03 (Research Genetics, Inc.) with the following primer selection parameters: 1) primer length between 18 and 22 base pairs, 2) primer melting temperature (*Tm*) or this tween 53-60°C, with a variance of ±1°C between forward and reverse primers, and 3) primer GC content between 35 and 65%. All primers were tested for amplification and polymorphism in seven individuals of each species, as well as the genomic DNA used to construct the libraries. From this initial screening, nine polymorphic loci were identified for *E. lemmonii* (Elem) and 15 polymorphic loci were identified for *E. arisolius* (Eari). Initially GIS used similar methods for *Erigeron neomexicanus,* however they had trouble developing these markers. GIS had many failed PCRs, and extreme size polymorphism of gel bands, and tried multiple attempts at cloning and sequencing to produce the contracted 15 markers.

Forward primers were labeled with 6FAM or HEX (Eurofins MWG Operon, Huntsville, Alabama) and further screened, optimized, and characterized by genotyping 60 individuals from *E. lemmonii* (ERLE-SC; 31.46136, -110.35474, Voucher: P. Bailey, 301 (MO)) and 30 individuals each from two populations of *E. arisolius* (ERAR-WP; 31.513862 , -110.323887, Voucher: P. Bailey, 304 (MO) and ERAR-CG; 31.480608 , -110.338489, Voucher: P. Bailey, 306 MO)). PCR amplifications for *E. arisolius* were performed in 10 μL reactions containing 1 U of Platinum *Taq* DNA polymerase (Invitrogen, Grand Island, New York), 1× PCR Buffer -$MgCl_2$ (Invitrogen), 2 mM $MgCl_2$, 1 μM each primer, 0.2 mM each dNTP, and 5-10 ng genomic DNA. PCR amplifications for *E. lemmonii* were run with the same conditions, except that the amount of Platinum *Taq* DNA polymerase was doubled. PCR temperature cycling conditions consisted of: 1) an initial denaturation cycle of 94°C for 5 min, 2) 34 cycles of 94°C for 1 min, annealing temperature for 1 min., and elongation at 72°C for 30 sec, and 3) a final extension of 72°C for 10 min. Labeled PCR products were run on an ABI 3100 Genetic analyzer (Applied Biosystems, Foster City, California) by loading 2 μL of PCR product, 0.5 μL of Genescan ROX 500 size standard (ABI), and 10 μL of HIDI formamide

(ABI). Resulting electropherograms were analyzed using GeneMapper software (ABI) and scored manually.

RESULTS

Erigeron lemmonii and *Erigeron arisolius*

Of the original nine Elem and 15 Eari loci, one Elem locus and six Eari loci displayed inconsistent or confounding nontarget amplification. A total of 17 primer pairs (eight Elem and nine Eari) amplified a scorable microsatellite in at least one of the two species, with four Elem primers cross-amplifying *E. arisolius* DNA and three Eari primers cross-amplifying *E. lemmonii* DNA (Table 5.1 and 5.2). For each *Erigeron* species, one locus cross-amplified DNA from the other species even though it did not amplify a scoreable microsatellite in the intended species. Descriptive statistics for each locus in each population, including number of alleles per locus (A), observed heterozygosity (H_O), and expected heterozyosity (H_E) were calculated using Microsatellite Analyser 4.05 (Dieringer and Schlötterer 2003). The number of alleles present in the *E. lemmonii* population ranged from 2 to 5 for Elem primers and 7 to 12 for the cross-amplified Eari primers.

Table 5. 1. Results of primer screening in the single known population of *E. lemmonii* in Scheelite Canyon, Fort Huachuca, AZ (ERLE-SC) and two populations of *E. arisolius* on Fort Huachuca, AZ (ERAR-WP, Woodcutter's Pond and ERAR-CG, Campground), reporting number of individuals that amplified (*n*; out of a subset of 60 random samples from the *E. lemmonii* population and 30 samples for each population of *E. arisolius*), number of alleles (*A*), and average expected (H_E) and observed heterozygosity (H_O) for each of the 17 loci (i.e. the eight loci developed for *E. lemmonii* = Elem and the nine loci developed for *E. arisolius* = Eari).

	ERLE-SC				**ERAR-WP**				**ERAR-CG**			
Locus	***n***	***A***	H_E	H_O	***n***	***A***	H_E	H_O	***n***	***A***	H_E	H_O
Elem3-1	56	3	0.351	0.339	-	-	-	-	-	-	-	-
Elem3-2	46	4	0.492	0.457	25	11	0.605	0.640	23	14	0.697	0.391
Elem3-3	-	-	-	-	25	3	0.607	0.280	22	5	0.608	0.500
Elem4-1	50	4	0.427	0.240	16	6	0.518	0.313	17	6	0.494	0.176
Elem4-2	58	2	0.466	0.448	-	-	-	-	-	-	-	-
Elem4-3	58	2	0.503	0.500	30	8	0.824	0.733	26	7	0.808	0.692
Elem4-4	58	2	0.276	0.259	-	-	-	-	-	-	-	-
Elem4-5	28	5	0.471	0.357	-	-	-	-	-	-	-	-
Eari3-3	56	10	0.765	0.446	-	-	-	-	-	-	-	-
Eari4-1	59	7	0.627	0.542	29	7	0.789	0.759	30	8	0.749	0.633
Eari4-4	-	-	-	-	30	12	0.850	0.767	24	9	0.858	0.792
Eari4-5	42	12	0.827	0.500	28	12	0.841	0.536	26	9	0.847	0.692
Eari4-6	-	-	-	-	29	15	0.915	0.828	25	14	0.913	0.920
Eari4-7	-	-	-	-	6	8	0.924	0.500	15	14	0.929	0.733
Eari4-8	-	-	-	-	23	5	0.761	0.261	28	6	0.764	0.393
Eari4-10	-	-	-	-	19	12	0.892	0.316	14	13	0.907	0.643
Eari4-12	-	-	-	-	27	11	0.830	0.704	24	13	0.889	0.708

Table 5.2. Primer sequences (forward and reverse) and characteristics of 17 novel microsatellite loci developed for *Erigeron*, including repeat motif, annealing temperature (T_A), allelic size range (as determined in 60 individuals from the single known population of *E. lemmonii* and two populations of 30 individuals each of *E. arisolius*; see Table 2), and GenBank Accession Number. The eight loci developed for *E. lemmonii* = Elem and the nine loci developed for *E. arisolius* = Eari.

			E. lemmonii		***E. arisolius***		
Locus	**Primer Sequences (5’-3’)**	**Repeat Motif**	**T_A (°C)**	**Range (bp)**	**T_A (°C)**	**Range (bp)**	**Accession No.**
Elem3-1	F: GACGAACACAGATTAGTAGCAG R: GCCTCTCTACCTTTGAGTAGG	AAC$_{10}$	57	145-151	-	-	JQ868414
Elem3-2	F: CCCGCAAGTGTGTAGTAGTC R: ATGGTGGATTGGTTATGCT	AAC$_{7}$	55	256-265	50	145-293	JQ868415
Elem3-3	F: TTCTGGATGTGTATTGTTCGT R: GAGTGGTCTGCTGTTGTCA	CAT$_{14}$	-	-	55	228-243	JQ868416
Elem4-1	F: TAGGGTTTCGGATTTTAGGAG R: GAGACCACTTGTTTCCACTTG	CATC$_{7}$	50	203-219	53	120-272	JQ868417
Elem4-2	F: AAACCATTTCTCGCATCAC R: CAAAGCCAGGTGTTGTTG	CATC$_{6}$	50	96-104	-	-	JQ868418
Elem4-3	F: AACCACGACCACACCATC R: TGGAGGCAGAGAGTGATTG	ATCT$_{6}$	57	214-222	57	182-246	JQ868419
Elem4-4	F: AGGCCGGGATACTGATAAAT R: GATCGCCAGAGTAGCAGATT	TAGA$_{7}$	57	221-229	-	-	JQ868420
Elem4-5	F: TCGTGATTATTGGACGTAGATC R: CCTTCAATCTGTCATGTTTTTC	TAGA$_{8}$	55	228-248	-	-	JQ868421
Eari3-3	F: ACATTGGTCCTTTGCTATTTAG R: GCTCTGATTCTHATTTGAGTTC	CAC$_{10}$	55	263-302	-	-	JQ868422
Eari4-1	F: CCAACACTGCTAACCACATG R: TTAGGGATGGTCGGATTG	CATC$_{5}$	50	213-249	57	183-235	JQ868423
Eari4-4	F: CTCCAAACAAGATTAAATGTGC R: ACCCACTTATTGTGTGATACGA	GATG$_{8}$	-	-	57	232-300	JQ868424
Eari4-5	F: ATGATGGTGGTGATGAGAAGTC R: TGGGTTTCAATGGATTCAAAG	ATCT$_{9}$	55	212-362	59	262-334	JQ868425
Eari4-6	F: GCGGTTTGTGTAGAAGTCC R: ATCTCACTGGTGAATTTCAGAG	TATC$_{23}$	-	-	57	207-295	JQ868426
Eari4-7	F: AGTTGAGGTCCGGGATATG R: TTGATTGTGAGCTTGGTTTAAG	TAGA$_{19}$	-	-	55	212-336	JQ868427
Eari4-8	F: CTGCTTCTGGCTCATAACC R: ATTCCTCCATACCGATTCC	TAGA$_{5}$	-	-	55	113-137	JQ868428
Eari4-10	F: AATTCCATTCACCAAGTTCC R: ATTCGCTGTCTAACCAATGA	ATCT$_{9}$	-	-	55	278-366	JQ868429
Eari4-12	F: TGCTCACTGCTTTCTTCTTAG R: AGGAGGTGGTTATCTTCAATG	TAGA$_{16}$	-	-	55	221-293	JQ868430

***Erigeron neomexicanus*.**

The development of microsatellite markers for the species *Erigeron neomexicanus* did not go as we expected/hoped, and thus we have been unable to determine microsatellite genotypes for the 197 *E. neomexicanus* samples. The following is a list of issues the geneticists had while working with *E. neomexicanus* and what we did to try to overcome those issues: 1) Extracted DNA contained inhibitors (common inplants) delayed msDNA development by GIS; 2) Inhibitors in the DNA also caused delays and issues with primer optimization process took much longer than expected at ERDC; and 3) The genetic samples may have included some polyploid individuals, which made scoring difficult, since some samples showed a diploid genotype while others showed a tetraploid genotype. The amplified samples generally gave an electropheragram, showing a greater number of peaks than what would be expected, even for a tetraploid individual. It would have been unethical to score the peaks, since there was no way to tell which peaks were real, which were due to “junk”, and which were stutter, etc. (Fig. 5.1) in the opinion of the geneticists. They tested a variety of PCR enhancers, to reduce primer dimers and stutter, but nothing worked to remove the extra peaks (Fig 5.2).

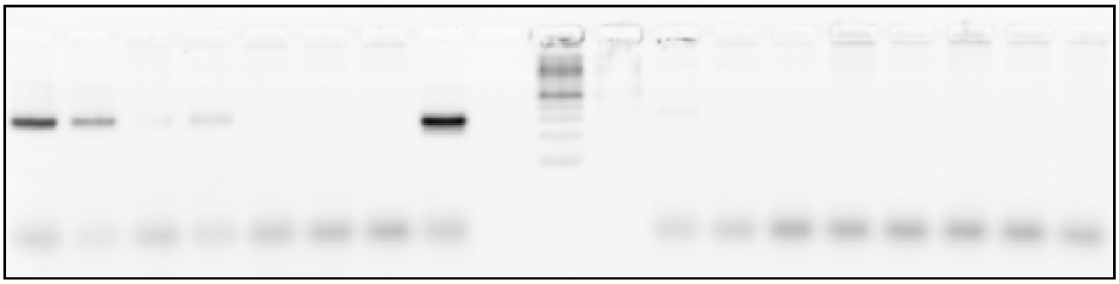
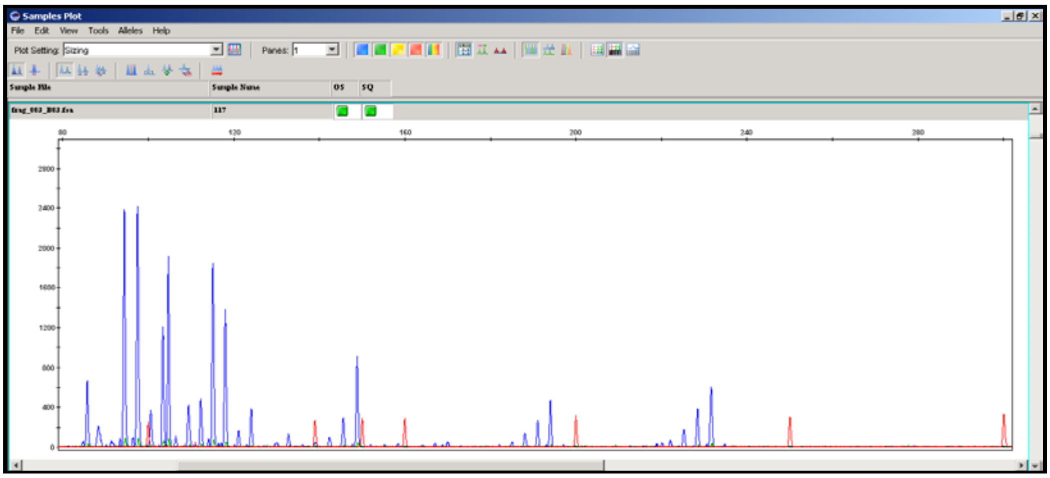


Figure 5.1. Example of ERNE primer optimization gel, showing dimers and nonamplification (blank lanes).



Plot Setting: Sizing

Panes: 1

Sample File	Sample Name
frag_003_B03.fsa	117

Figure 5.2. An example of an electropheragram showing too many peaks to determine the microsatellite markers for *E. neomexicanus*.

DISCUSSION AND CONCLUSION

The levels of polymorphism exhibited in these microsatellite loci suggest that they will be useful for detecting patterns of population structure in their respective *Erigeron* populations. This data will be used to evaluate patterns of genetic structure in rare and widespread species, and determine whether a narrow endemic (*E. lemmonii*) has adapted strategies to ensure adequate gene flow in comparison to a widespread species (*E. arisolius*). In the future, this should lead to a better understanding of the genetic consequences of isolation within a rare endemic plant species. The Microsatellite Markers (Appendix 5.I) appears in the order as listed on-line and unchanged from the database GenBank® entry. There were 8 microsatellite markers developed for *Erigeron lemmonii,* and 9 microsatellite markers for *Erigeron arisolius*. Files were downloaded from GenBank®, accessed on Jan 8, 2013.

E. neomexicanus is primarily reported as a diploid (2n= 18) in the majority of the accounts (DeJong and Longpre 1963; Schaack and Windham 1983; Sundberg 1983; Ward 1984, Semple and Chmielewski, 2 counts 1987; Carr et.al.1999). However, it is also reprted as a tetraploid (2n= 36) in two accounts (Harms 1969; Pinkava and Keil 1977). Individuals of differing ploidy levels may possibly have been collected originally. Although initially GIS and the ERDC geneticist used similar methods in the microsatellite development for *E. neomexicanus* as the other two species, it did not yield results, and held up the microsatellite development. These initial problems became magnified at every step of the process. Interpreting the data was not possible because the electropheragram, showed a greater number of peaks than what would be expected, and the geneticists were not able to definitively determine the microsatellites for *E.neomexicanus.* I had to make the decision to halt its development due to time and budget constraints.

However, despite difficulties, the microsatellite markers for *E. lemmonii* and *E. arisolius* were produced for the first time for the genus of *Erigeron.* Initial analysis using the program "STRUCTURE" (University of Chicago 2003) showed that there are no distinct subpopulations within either of the populations of *E. lemmonii,* or *E. arisolius* (Edwards, unpublished data); however this needs further examination. The insect pollinators and seed dispersers are promoting gene flow among individuals in *E. lemmonii* and among populations of *E. arisolius,* which can be measured by the mean observed heterozygosity. The observed mean heterozygosity (based on the total number of genetic samples per population) was 0.4

for *E. lemmonii* (n=110) and 0.6 for *E. arisolius* (n=147). The *Erigeron lemmonii* population had mean number of alleles of 7.0 from a possible range of 16. The *Erigeron arisolius* population had mean number of alleles of 16 from a possible range of 18. Results indicate the overall genetic diversity of *E. lemmonii* is significantly less than the genetic diversity of *E. arisolius* (Edwards, pers. comm., 2013).

Two future uses of these markers include: 1) further analysis to examine genetic structure within each population and gene flow for each *Erigeron* population; and 2) analyze genetic differences between *Erigeron* species in order to determine the phylogeny as future research.

ACKNOWLEDGEMENTS

The authors thank C. G. Cox, B. L. Escalon, A. J. Hollingsworth, R. D. Noyes, and S. Stone for assistance with specimen collection and lab work. The study described and the resulting data presented herein were obtained from research conducted under the U.S. Army Environmental Quality Technology Program by the U.S. Army Engineer Research and Development Center.

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GenBank ®. 2012. *Erigeron lemmonii* Accession numbers JQ868414- JQ868421. www.ncbi.nlm.nih.gov

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APPENDIX 5.I

GenBank ® Microsatellite Markers

1) Erigeron lemmonii microsatellite Elem4-4 sequence

GenBank: JQ868420.1

FASTA Graphics

Go to:

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2012
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  ORGANISM  Erigeron lemmonii
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Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 536)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 536)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
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      121 taacgagaaa ttaattataa aattgaaaaa cgatacgata gatagataga tagatagata
      181 gatagatata caaggtaacc ctaataaccc taaaaaaatc aatcgctagc tcagcaagca
      241 atcgggggga agtggatatt gagttcacgc ccgagggtat accgttatta atctgctact
      301 ctggcgatcc ttgggacatc tctgatgaag aggcagacag ccagcgccgt ctgttgcagg
      361 aattaggtat attatatatc atcgatcatt acattccctt ccttaattcc cttcttctta
      421 atatatatat acaaagttag gatttttttt tttgacaatc atgggcaata atataacttt
      481 ttttatatat aacaactttg caatgtttat tttacatcgt aaagttcatg aactag
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2) Erigeron lemmonii microsatellite Elem4-2 sequence

GenBank: JQ868418.1

FASTA Graphics

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2012
DEFINITION  Erigeron lemmonii microsatellite Elem4-2 sequence.
ACCESSION   JQ868418
VERSION     JQ868418.1  GI:387912628
KEYWORDS    .
SOURCE      Erigeron lemmonii
  ORGANISM  Erigeron lemmonii
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 279)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
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            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
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            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 279)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
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      121 agtacaataa tggcaaacat caaccgcttt catacaacaa aaccatttct cgcatcactt
      181 gctagcattg tctctccatc catccatcca tccatccatc ctccatggcc caacttcaaa
      241 aaaccatcca acaacacctg gctttgcata tcctactag
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3) Erigeron lemmonii microsatellite Elem3-3 sequence

GenBank: JQ868416.1

FASTA Graphics

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2012
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VERSION     JQ868416.1  GI:387912626
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  ORGANISM  Erigeron lemmonii
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 502)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
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            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 502)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
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            Research and Development Center, 3909 Halls Ferry Road,
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4) Erigeron lemmonii microsatellite Elem3-1 sequence

GenBank: JQ868414.1

FASTA Graphics

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2012
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Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 502)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
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  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 502)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
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5) Erigeron lemmonii microsatellite Elem4-5 sequence

GenBank: JQ868421.1

FASTA Graphics

Go to:

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            Astereae; North American clade; Erigeron.
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REFERENCE   1  (bases 1 to 720)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 720)
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and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
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      601 gagtctcctt ttatacgtat gaattacaag aatgaatgtt acggcccgtt cggtccggga
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6) Erigeron lemmonii microsatellite Elem4-3 sequence

GenBank: JQ868419.1

FASTA Graphics

Go to:

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2012
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            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 527)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 527)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1189047"
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                     aaccacgaccacaccatc, rev_name: Elem4-3R, rev_seq:
                     tggaggcagagagtgattg"
     repeat_region   283..306
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                     /satellite="microsatellite:Elem4-3"
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      121 accatttcca aaagtctttc ccactcccca taaatatcat ttccctctcc tgaccggcat
      181 tgacttaaca accacgacca caccatcaac cctccactgg agtcaagaaa ctggcgattt
      241 aatttttgat ttccgatcaa cttcaacaat attctcactt gcatctatct atctatctat
      301 ctatctatat atataatgca acaatgatct ccacactctt cctcctcctc atttctgcca
      361 cttccgccgc cgccgccgac atccgcccac cacaatcact ctctgcctcc aactcatcac
      421 aaagatggtc ttcacccaac aacaccttca ttttcacctt catttccacc tcacccaaca
      481 cttactacgc cgccatcact tataacaaca tccccatctg gaaagcc
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7) Erigeron lemmonii microsatellite Elem4-1 sequence

GenBank: JQ868417.1

FASTA Graphics

Go to:

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LOCUS       JQ868417                 781 bp    DNA     linear   PLN 22-OCT-
2012
DEFINITION  Erigeron lemmonii microsatellite Elem4-1 sequence.
ACCESSION   JQ868417
VERSION     JQ868417.1  GI:387912627
KEYWORDS    .
SOURCE      Erigeron lemmonii
  ORGANISM  Erigeron lemmonii
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 781)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 781)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1189047"
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                     gagaccacttgtttccacttg"
     repeat_region   501..528
                     /rpt_type=tandem
                     /satellite="microsatellite:Elem4-1"
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       61 ttccataatt tttcaagatt ttcaggggtg caggttctcc cccccttgtt cagcttcctc
      121 tcaccatcca ccttataata tcggttcctg ttataattat catacttgtc gataatacga
      181 ccagcctcct ccttggtgac tttcctacca tctacgtctc tcccgtcgtt ttcaagcgta
      241 tcataaaagg ttccctcttt catctccccc agtgaaaccc cattttccgt catccaacac
      301 tcggtgcgta ataaaatacg tgattcaaca gtcttaagat ccctatcagt acccggtttc
      361 agtttgttga gattgatccc acccttaatc agattatcaa ccataaaatc cctcattccc
      421 atctcaccgt aaatttcatc ctccatatct tacaagataa ttttttcga tctatagggt
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      481 ttcggatttt aggagggatc catccatcca tccatccatc catccatcgt atcgtattta
      541 tttagttata tttactttta cttaccgtat ataatatatg tattacactc aatgtccccc
      601 tactttgccg ggatttcaat atctaaccct gtacccaacg ccttttactg tactaaccca
      661 taatctacaa gtggaaacaa gtggtctctt ttcgggaaat aatcccatgc tancntttgg
      721 aaacaatgct aacaataagt cctctactaa caatattaac atttttgggg gttcataacc
      781 a
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8) Erigeron lemmonii microsatellite Elem3-2 sequence

GenBank: JQ868415.1

FASTA Graphics

Go to:

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LOCUS       JQ868415                 700 bp    DNA     linear   PLN 22-OCT-
2012
DEFINITION  Erigeron lemmonii microsatellite Elem3-2 sequence.
ACCESSION   JQ868415
VERSION     JQ868415.1  GI:387912625
KEYWORDS    .
SOURCE      Erigeron lemmonii
  ORGANISM  Erigeron lemmonii
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 700)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 700)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:1189047"
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      121 aaccataacc acaatgttct caagtatgaa atacttggga gtacgtcgta cgaaagcacg
      181 ttctcaagtg tatgacactt gggagtacgt agtacaaatc ttcaagtgtg aatacttggg
      241 agtacatcga accaatcatc aagtacaagc ataaccaatc caccataaca taagcacata
      301 cacttcctaa gactctacac tcaaggaatc ctagatgctt atatataggg ttacccgcag
      361 ccttcccacc acatttaaaa ctctttgaga gcgttgtcgt attccatgta tgcactacta
      421 tcctaaacaa tgcataataa tcacagacga aagcataata gccaaaccat aagtatatcc
      481 gacaatcaaa ccaaacaatc aaacaaacaa gtacttgtac acttttcagc ccgaatctca
      541 aacgagtagg gagctacgga ctcaccttga ttggcaaaag atgttaagat tgatataaga
      601 tgaggttata agtgattaat gtgctccaag cgtccacaac ctaataatgt atacaataca
      661      cattatta accaaagacg attctttaac tagattttag
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1) Erigeron arisolius microsatellite Eari4-12 sequence

GenBank: JQ868430.1

FASTA Graphics

Go to:

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LOCUS       JQ868430                 614 bp    DNA     linear   PLN 22-OCT-
2012
DEFINITION  Erigeron arisolius microsatellite Eari4-12 sequence.
ACCESSION   JQ868430
VERSION     JQ868430.1  GI:387912640
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 614)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 614)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
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  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1189046"
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                     tgctcactgctttcttcttag, rev_name: Eari4-12R, rev_seq:
                     aggaggtggttatcttcaatg"
     repeat_region   445..508
                     /rpt_type=tandem
                     /satellite="microsatellite:Eari4-12"
ORIGIN
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       61 acccatccat cggctgaaaa tgacttgatc tcaccagcga ttttctgaaa tgaagaatgg
      121 aaataagacg agtaacaacc ccaacaatag tatagtaatg tcaagaaaag gaaggaggag
      181 ccgtacagtg ggaaactgat cacagttgaa agcctcaata tgagtaatgc cactaacacc
      241 ttgaagcaga ttgtcgtaaa aaacatcagc agcatgacca acgggagata ccacaccaag
      301 ccccgtaacc acaactcttc ttttcttgct cactgctttc ttcttagttg tggcttcttt
      361 tgtaggctgc acggcaattg ccatggtttt gcctaatata tttatattat tatttattat
      421 atcaaatcaa agaaaagagt attatagata gatagataga tagatagata gatagataga
      481 tagatagata gatagataga tagatagata ctgcagtaat tagtaataac actccagcct
      541 ccggggctat gtatgtttaa tacgttatta catacattga agataaccac ctcctatata
      601      ttacggat tgat
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2) Erigeron arisolius microsatellite Eari4-8 sequence

GenBank: JQ868428.1

FASTA Graphics

Go to:

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LOCUS       JQ868428                 430 bp    DNA     linear   PLN 22-OCT-
2012
DEFINITION  Erigeron arisolius microsatellite Eari4-8 sequence.
ACCESSION   JQ868428
VERSION     JQ868428.1  GI:387912638
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 430)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
```

```
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 430)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:1189046"
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                     attcctccataccgattcc"
     repeat_region   242..261
                     /rpt_type=tandem
                     /satellite="microsatellite:Eari4-8"
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       61 catcgccggt gccggaaccg tcgccggagg tctgccgcgc cgcatatttg atgttttaaa
      121 cctaattaca gatcgaagaa atggaagctg taacttacaa tctcgatctg cttctggctc
      181 ataacctcat gtttcaattg atagatataa gatatataga tatagatata atagatacag
      241 atagatagat agatagatag atatatagtg agttaggaat cggtatggag gaatcctaac
      301 tcgtctcgat ccccattttc tttctcccct ttttttctgt tgttcatcgt tcccaattac
      361 tttaataaat tgttttttta tttttctatg ggaaatgatc gaggtggttt acgtagtaaa
      421      actaaagt
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3) Erigeron arisolius microsatellite Eari4-6 sequence

GenBank: JQ868426.1

FASTA Graphics

Go to:

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LOCUS       JQ868426                 518 bp    DNA     linear   PLN 22-OCT-
2012
DEFINITION  Erigeron arisolius microsatellite Eari4-6 sequence.
ACCESSION   JQ868426
VERSION     JQ868426.1  GI:387912636
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
```

```
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 518)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 518)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     atctcactggtgaatttcagag"
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      121 ccaagacttg tagacgtagt atttccccag caaacgaaac aaattttccc gcggtttgtg
      181 tagaagtcca aaggttgaat ttcaatacca acagtggctg tttgcaatgc agcaacaacc
      241 agacatgtcc aattaattat agcaaatata tacatagtat acctaaatat tgtatctatc
      301 tatctatcta tctatctatc tatctatcta tctatctatc tatctatcta tctatctatc
      361 tatctatcta tctatctatc tatctatgtt gctaaataag gctggacata gagaataact
      421 tacgttcata tctgctctga aattcaccag tgagatgcct ttttagaaag gtagtattcc
      481      gtgtgaca caaaagagga tatatatata tgatcagt
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4) Erigeron arisolius microsatellite Eari4-4 sequence

GenBank: JQ868424.1

FASTA Graphics

Go to:

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LOCUS       JQ868424                 796 bp    DNA     linear   PLN 22-OCT-
2012
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DEFINITION  Erigeron arisolius microsatellite Eari4-4 sequence.
ACCESSION   JQ868424
VERSION     JQ868424.1  GI:387912634
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 796)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 796)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     ctccaaacaagattaaatgtgc, rev_name: Eari4-4R, rev_seq:
                     acccacttattgtgtgatacga"
     repeat_region   514..545
                     /rpt_type=tandem
                     /satellite="microsatellite:Eari4-4"
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      121 atcaaatcca tgttcaaatc ctttaaaaac taaaccaaac cctaacaaaa ccctgaatcg
      181 acccaaaatg cgttttgact caattcatgc cccgaaaaca ctttgttata aatagaaaca
      241 agtttataag tataaaatga aggttatgag atagaattta cttaccaaga gttccacgaa
      301 ccaccaaatc cgaaattgac ctaaatgtgg agatttattg tgtcgtggaa cccaaatttt
      361 tgatatgatg agtagatgat gataatgatg attatcctaa cttaaatctt gctccaaaca
      421 agattaaatg tgcaagaatt tagagagagg gagagagaag agtgtgtcta attagagaga
      481 gaaagagtgg gtggatgaat gaatgaatga atggatggat ggatggatgg atggatggat
      541 ggatggagaa tgggataggg ttgggtcttg ggtcatggtt agacccatgg gttgacccgt
      601 ctatattttc tagtttaatt cacatatcgt tcagtatcgt atcacacaat aagtgggtaa
      661 accgacccgt taggcctatc gcccgtcatt tagtctcaaa gtgatgtcaa ttatatcatt
      721 aattgcatta actaatcaaa ctagatcaat acccgggcgt tgaccgggtt aaaacaatat
      781      atatagccta tatata
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5) Erigeron arisolius microsatellite Eari3-3 sequence

GenBank: JQ868422.1

FASTA Graphics

Go to:

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LOCUS       JQ868422                 456 bp    DNA     linear   PLN 22-OCT-
2012
DEFINITION  Erigeron arisolius microsatellite Eari3-3 sequence.
ACCESSION   JQ868422
VERSION     JQ868422.1  GI:387912632
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 456)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 456)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:1189046"
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                     gctctgattcthatttgagttc"
     repeat_region   181..210
                     /rpt_type=tandem
                     /satellite="microsatellite:Eari3-3"
ORIGIN
        1 cggtgatcat acgacaacat tggtcctttg ctatttagaa acggatcact ccaataataa
       61 ttagtctcct caggtcccat tggtatctct cttttaatca aatacggtca ttcatatcca
      121 tctgctcttg tggcttgtta gacaacgaca atcccggctg tggtttatgt tcaaaacaac
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181 caccaccacc accaccacca ccaccaccac aaccactacc accaccatca tcatcatcat
241 catcatcatc atgtatatga atatgaccat tattgtcatg gccatgaaaa ttggaactca
301 aatcagaatc agagccagat ggcggccgta gagcatcatc atcatcaggt gacgaagaag
361 gcggtatatc aagctgttga tccacaaatc ttgaaagggt gtgacccata tctttaagtg
421      tgaaaata agctacatgt gaattcgata actcgt
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6) Erigeron arisolius microsatellite Eari4-10 sequence

GenBank: JQ868429.1

FASTA Graphics

Go to:

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LOCUS       JQ868429                 614 bp    DNA     linear   PLN 22-OCT-
2012
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ACCESSION   JQ868429
VERSION     JQ868429.1  GI:387912639
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 614)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 614)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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7) Erigeron arisolius microsatellite Eari4-7 sequence

GenBank: JQ868427.1

FASTA Graphics

Go to:

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VERSION     JQ868427.1  GI:387912637
KEYWORDS    .
SOURCE      Erigeron arisolius
  ORGANISM  Erigeron arisolius
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Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 578)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 578)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
FEATURES             Location/Qualifiers
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8) Erigeron arisolius microsatellite Eari4-5 sequence

GenBank: JQ868425.1

FASTA Graphics

Go to:

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2012
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Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 634)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 634)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
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            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
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9) Erigeron arisolius microsatellite Eari4-1 sequence

GenBank: JQ868423.1

FASTA Graphics

Go to:

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2012
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            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
            asterids; campanulids; Asterales; Asteraceae; Asteroideae;
            Astereae; North American clade; Erigeron.
REFERENCE   1  (bases 1 to 570)
  AUTHORS   Lindsay,D.L., Bailey,P., Anderson,J.L., Jung,M.G., Edwards,C.E.
and
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            Lance,R.F.
  TITLE     Isolation and characterization of microsatellite loci for a
            hyper-rare cliff endemic, Erigeron lemmonii, and a more
widespread
            congener, Erigeron arisolius (Asteraceae)
  JOURNAL   Conserv Genet Resour 4 (4), 849-852 (2012)
REFERENCE   2  (bases 1 to 570)
  AUTHORS   Lindsay,D.L., Anderson,J.L., Jung,M.G., Bailey,P., Edwards,C.E.
and
            Lance,R.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-MAR-2012) Environmental Laboratory, US Army
Engineer
            Research and Development Center, 3909 Halls Ferry Road,
Vicksburg,
            MS 39180, USA
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//
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CHAPTER 6

CONCLUSIONS

SUMMARY

This study focused on three species of *Erigeron,* Asteraceae, that co-occur in the Huachuca Range which is part of the Sky Island Region, in Arizona, U.S.A. The three species of *Erigeron* have very different life histories. *Erigeron arisolius* (Dry Sun Fleabane) and *E. neomexicanus* (New Mexico Fleabane) are habitat generalists that grow in a wide range of environments whereas *E. lemmonii* (Lemmon's Fleabane) is a rare endemic species and a habitat specialist that grows only on cliffs in one canyon.

There was greater research focus on *Erigeron lemmonii*, because I discovered that little was known about this endemic species. New findings of my research included an improved description of the morphology and pollen characteristics of *E. lemmonii* relevant to its pollination biology, flowering period for the population, and duration of flowering for a single flowering head, and it's mating system. The addition of the soil component tied the nutrient composition of the soils for each *Erigeron* species to its rooting characteristics, in better defining habitat specific information. The floral insect visitor networks showed the floral insect visitors in relationship to their connected plants for each of the three *Erigeron* species. The addition of the MNS analysis and indicator species added significant data to the study of the insect communities for each of the three *Erigeron species*. The first microsatellites were developed for the genus of *Erigeron*.

Erigeron lemmonii was found to have an extended flower progression of approximately 3 weeks for an individual capitulum from bud to senescence. The population takes advantage of environmental conditions over an extended six month flowering period, with peak flowering occurring in May. *Erigeron lemmonii* exhibits xenogamy, reliant on insect cross pollination. It appears to have a self incompatibility system, preventing fertilization by autogamy, or geitonogamy. It does not appear to reproduce by apomixes, but can reproduce itself by vegetative means; plant rhizomes and stems produce roots easily from nodes when conditions are favorable.

Soils where *Erigeron lemmonii* grew had higher concentrations of nitrogen, potassium and organic matter, whereas those found with *E. arisolius* had midrange levels of nitrogen and potassium, and had the highest concentration of phosphorous. *Erigeron neomexicanus* soils had the least amount of all three nutrients. The root architecture analysis showed *E.lemmonii* (n=1) with the greatest stem length to root length ratio (4.12) compared to *E. arisolius* (3.80) or *E. neomexicanus* (1.04). *Erigeron lemmonii* has four times the amount of root supporting its top stems and leaves, which gives it the ability to take up available water to sustain itself in its cliff environment, and to carry out rapid exploration of suitable soil contained in crevices supporting expansion of the plant as conditions become favorable. *Erigeron lemmonii* as a long-lived perennial is easy to root from stem nodes or root pieces, and can easily reproduce from vegetative means under favorable habitat conditions. *Erigeron neomexicanus*, a perennial, had the most extensive root system and was found growing in the most diverse conditions of the three *Erigeron* species. *Erigeron arisolius* is an annual or tender perennial and has the smallest root system.

The most important orders of flower visiting insects for the three *Erigeron* species were recorded. For *E. arisolius* the order of Coleoptera was the most diverse group of insect visitors, followed by the Diptera and Hymenoptera. *Erigeron lemmonii* were most importantly visited by the Diptera, followed by Coleoptera and Lepidoptera (specifically micro-moths). For *E. neomexicanus*, both the Diptera and Coleoptera had the most diverse insect visitors, followed by Hymenoptera.

Of the 94 flower visitor species identified (not including singleton species) among *Erigeron* species, eight were found to be Indicator Species of one of the three *Erigeron* hosts, two for *Erigeron lemmonii*, one for *Erigeron arisolius*, and five for *Erigeron neomexicanus*. Of these only three species - *Allograpta obliqua, Chauliognathus werneri, and Chauliognathus opacus* - were found to have any overlap between the species and, in all three instances, were found to be greatly more abundant on *E. arisolius*. Within the *E. lemmonii* network, Diptera 005b and *Trichochrous* sp.1 were connected to one other plant species. However, the findings indicate that a species of microlepidoptera (Microlep 002) and *Trichochrous* (sp.1) are indicator species for *Erigeron lemmonii* and are potentially important pollinators.

Erigeron lemmonii has a unique assembly of flower-visiting insects, compared to either *E. arisolius* or *E. neomexicanus.* Examination of the network architecture of *E. lemmonii* shows no redundant core cluster relationships with other plant species in its habitat to support its insect visitors. This is unlike the networks of *E. arisolius* and *E. neomexicanus*, which have more core clusters that result in robust networks. The network architecture of the latter two species suggests that they would be less impacted by fragmentation than would *E. lemmonii. Erigeron lemmonii* has a fragile insect visitation network structure. *Erigeron lemmonii* is a keystone species within its cliff habitat. If *E. lemmonii* at the population level disappears, a collapse of its dependent insect visitors may also occur.

A genetic note on the microsatellites developed for *Erigeron lemmonii* and *Erigeron arisolius* was published in Conservation Genetic Resources, April 2012 and available on line at Springer. Additionally, the GenBank ® record for the microsatellite markers (Accession numbers JQ868414- JQ868430) are available in the appendix 5.I in Chapter 5. Our microsatellite data for *Erigeron lemmonii* and *E. arisolius* show all individuals have a range of genetic variability. Eight microsatellites were developed for *E. lemmonii,* and nine microsatellites were developed for *E. arisolius* (for all amplified and cross amplified primers). The *Erigeron lemmonii* population had mean number of alleles of 7.0 from a possible range of 16 and the mean observed heterozygosity was 0.4 (n=110). The *Erigeron arisolius* population had mean number of alleles of 16 from a possible range of 18, and the mean observed heterozygosity was 0.6 (n=147). The proportion of total alleles occurring in each population may be more important to conservation biologist (Hamrick et al 1991) than the heterozygosity diversity. Results indicate the overall genetic diversity of *E. lemmonii* is significantly less than the genetic diversity of *E. arisolius*.

Erigeron neomexicanus is mostly diploid as reported in the majority of the accounts (DeJong and Longpre 1963; Schaack and Windham 1983; Sundberg 1983; Ward 1984, Semple and Chmielewski 1987; Carr et.al.1999). However, it is also reported as a tetraploid in two accounts (Harms 1969; Pinkava and Keil 1977). This may have contributed to the difficulties with developing the microsatellites for this species, possibly individuals of differing ploidy levels may have been collected originally. Although initially GIS and the ERDC geneticist used similar methods in the microsatellite development for *E. neomexicanus,* it did not yield a number of reliable microsatellites. I had to make the decision to halt its development due to time and budget constraints. However, despite difficulties, the microsatellite markers for *E. lemmonii* and *E. arisolius* were produced for the first time for the genus of *Erigeron.*

Gene flow potential is correlated with pollen and seed dispersal mechanisms, as well as with longevity of the plant species and its size (Hamrick et al 1991). *Erigeron lemmonii,* a long-lived perennial, has 85% viable pollen (Noyes and Bailey, in preparation), and wind is the primary seed dispersal mechanism. Based on these findings, and the fact that E. lemmonii is an outcrosser, and the insect pollinators and seed dispersment are promoting gene flow among individuals in the *E. lemmonii* population.

My research results indicate *Erigeron lemmonii* is well adapted to its environment, and that it has many traits which allow the population to be successful. These traits include a multiple floret morphology which produces large amounts of viable pollen, a xenogamous mating system with a long flower progression, and an extended bimodal flowering season which allows for outcrossing by a range of insects (Bailey, Higgins and Cobb, in preparation). In the open pollinated flowers examined (n=14) there was an 18.7% cypselae/floret ratio. This seedset percentage may be low as a result of environmental conditions (such as weather) to which the plant was responding. Total flowering was 57% (n= 55), for 5% of the population during the 2011 flowering season. Seed is easily dispersed up and down the cliff face on the wind by updrafts and gravity.

The carrying capacity of its habitat depends on the slow accumulation of organic debris in the cracks of the rock and ledges. The habitability of the site is of long duration because there is very slow successional displacement within the stable, cliff plant community. The rock substrate modifies the ambient temperature by retaining heat to provide a more constant temperature. The vertical habitat offers protection from wildfire, grazing, floods and human disturbance; however droughts, insects and disease are possible threats. Climate change may also be a threat to the population.

Specimens of the three *Erigeron* species have been vouchered and deposited in the Missouri Botanical Garden's Herbarium, in St. Louis, MO. (Bailey, P. #301-#312, MO). Website for the herbarium is available at Tropicos.org. Missouri Botanical Garden. <http://www.tropicos.org>. A synoptic collection insect species collected in this study are curated at the Colorado Plateau Museum of Arthropod Biodiversity, located at Northern Arizona University, Flagstaff, AZ. Additionally they have developed an image library of exemplars for many insect species. Websites to view insect collections are available at http://bugguide.net/bgimage/user/28808, http://bugs.nau.edu/BIO322/Fort_Huachuca_ERLE_Curc001.html, http://www.bugs.nau.edu/Bio322/Fort_Huachuca_ERLE_HLasio.html.

CONSERVATION EFFORTS

Recently in October, 2012, the U.S. Fish and Wildlife Service (USFWS) published a proposed rule change in the Federal Register: "Lemmon fleabane, a candidate for listing under the Act since 1996, is being removed from the list of candidate species because the main threat (wildfire) does not rise to a level of significance such that the species is in danger of extinction or likely to become so in the foreseeable future. In addition to fire, recreational repelling which is not allowed in the canyon, might pose as a minor threat, but would not threaten the entire population if it were to occur." Additionally in 2011, the Desert Botanical Garden collected hundreds of viable seeds for long term storage. This and future planned seed collections by the Desert Botanical Garden may offset any impacts to the species in the event of fire or other catastrophic event.

Currently the Army Resource Manager monitors the population and is drafting a Cooperative Agreement with the U.S. Fish and Wildlife Service, as a result of the proposed change. Fort Huachuca Army Base maintains permanent photo- monitoring plots using a tripod and specific coordinates for future re-measurement of plots in an effort to survey for new plants within the population, and has monitored the *Erigeron lemmonii* population since 2008 (Stone, pers. comm.2010). This monitoring indicates the population has been stable during the time it has been monitored (Stone, pers. comm.2012). The cooperative agreement 1) ensures continued monitoring, 2) promotes adaptive management of this species, 3) continues to restrict recreational activities in its habitat, and 4) encourages further research into the life history and population biology. The network and NMS analysis indicate that this species warrants monitoring because of its fragile ecological relationships with its insect visitors. Though *E. lemmonii* has recently been removed from the USFWS Candidate Species List however, it is still listed as a G1 plant species by Nature Serve (2012).

Erigeron lemmonii is reliant on its pollinators because it is an outcrosser, with a xenogamous mating system. As a habitat specialist, *E. lemmonii* has only one other plant species that shares insect visitors within its network, and *E. lemmonii* has a unique insect community specific to itself compared with the other two *Erigeron* species. The NMS and MRPP data demonstrate that *E. lemmonii* insect visitors are much more distinct than either *E. arisolius* or *E. neomexicanus* are to each other due to the distinction of the flower visitor community. If *E. lemmonii* at the population level disappears, it may cause a collapse of its dependent insect visitors, because they have so few links to other plants within the network with no demonstrated redundant connections within the network structure. Redundancy within the network provides resiliency from disturbances. The insect visitor community contains the pollinators of *E. lemmonii*, without which outcrossing would not occur. This has serious implications for the conservation of *E. lemmonii* and its associated insect visitors. An effective approach to protect the entire insect visitor community may be essential in protecting rare insect species, and *E. lemmonii*.

The integrity of *E. lemmonii* population is not being eroded through hybridization. Hybridization of *E. lemmonii* with *E. neomexicanus* is low or absent because we did not capture insects on *E. lemmonii* that were directly connected through its network, shared by *E. neomexicanus.* *Erigeron lemmonii* is found growing within 2-4 meters from *Erigeron neomexicanus,* which grows on the ground below the cliffs. *Erigeron lemmonii* does not share insect visitors, or the subset of pollinators, within its network with *E. neomexicanus.* The likelihood of pollen movement between these two *Erigeron* species with no shared pollinators is highly unlikely. This is significant because there is distinct habitat separation of the two *Erigeron,* even though they grow in close proximity to one another. *Erigeron arisolius* does not grow in close proximity to *E. lemmonii* and therefore do not share insect visitors/ pollinators either. However, one weevil species was collected separately on all three *Erigeron* species, but not as an interconnected network component between all three species of *Erigeron.* This generalist insect species apparently has a wide habitat range.

One goal of conservation biology is to preserve the evolutionary potential of a species. Plant species exhibit a continuum of evolutionary potential; sexual reproduction has the greatest potential, followed by apomixes, and clonal reproduction has the least potential (Grant 1973, Hamrick et al. 1991). However, plants that reproduce vegetatively can reproduce for thousands of years without any apparent decline in vigor (Grant 1971). My findings show that *Erigeron lemmonii* can reproduce by at least two means; sexually by xenogamy with some type of self-incompatibility mechanism present, and asexually by clonal, vegetative division. In the review of plant allozyme literature (Hamrick et al. 1991), it has been consistently shown that the plant mating systems significantly influence genetic diversity within and among populations. The mating system should influence how genetic variation is linked with other traits. For example, outcrossers should experience faster increases in direct gene expression owing to increased heterozygosity, compared to selfers (Hamrick et al 1991). Gene flow is occurring in both *Erigeron* populations as seen by their observed heterozygosity. However, genetic findings indicate that *Erigeron lemmonii* has significantly less genetic diversity than *E. arisolius*. Future genetic work to thoroughly analyze gene flow and the genetic structure will be completed for *E. lemmonii* and *E. arisolius* by Edwards. Results will provide a baseline for population genetics, and in the future *E. lemmonii* could be monitored to see if there is any genetic decline. Additionally the entire population should be monitored by counting the number of individual plants annually to ensure the population is stable, and not in decline.

This information is new for the species. DoD Resource managers and the USFWS managers can input this information to their respective resource management plans in the future.

FURTHER RESEARCH DIRECTIONS

Microsatellites for all three *Erigeron* species were initially developed through a contract I had with Genetic Information Services, Inc. to develop genetic libraries for the three *Erigeron* species, and select likely microsatellite marker sequences. Dr. Richard Noyes agreed to assist me in obtaining the chromosome number of *E. lemmonii*, so that other genetic research could proceed. We will publish the chromosome number and another aspect of *E. lemmonii's* reproduction in the near future. With the genetic library data and chromosome number for *Erigeron lemmonii*, geneticists at the Engineer Research and Development Center (ERDC) confirmed and published the microsatellite markers for *E. lemmonii* and *E. arisolius*, the first for the genus of *Erigeron*. However, further genetic work is necessary to confirm and publish the microsatellites for *Erigeron neomexicanus*.

Future genetic work to understand the phylogeny of all three species of *Erigeron* would advance understanding of these species and their evolutionary relationships, particularly for *Erigeron lemmonii.* Dr. Noyes has grown fifteen plants from cuttings taken from the original specimen of *E. lemmonii* used to determine the chromosome number. These plants now reside in the greenhouse at the Central University of Arkansas under his care, and will be used for further research.

Cliffs remain as one of the few ecosystem types that have largely escaped landscape conversion (Larson et al. 2000), and provide refugia and migration pathways for unusual communities of plant and animal species (Kuntz and Larson 2006). Very limited insight exists on how gene flow is influenced by pollinator movement, landscape context, and the spatial arrangement of plant populations (Steffan-Dewenter et al. 2006). Future genetic research of these *Erigeron* species and inter-related aspects would further this knowledge.

Rarity can occur for different reasons, which has important implications for the genetic structure within a population (Wiens et al 1989, Barret and Kohn 1991). The four evolutionary forces; mutation, natural selection, migration and genetic drift, interact with an organism's recombinant system, and this can have different genetic consequences. In small populations, genetic drift will have a dominant influence on the population structure (Barret and Kohn 1991). In populations where drift predominates, they are more prone to extinction. Increased inbreeding can occur in small populations; however, inbreeding effects on plants are also variable because of the diverse reproductive systems (Barret and Kohn 1991). Inbreeding depression results from an increase of deleterious recessive alleles (Karron 1991). However, species that occur naturally in small populations may possess genetic systems adjusted to close inbreeding, as well as adaptations that offset the disadvantage of rarity (Stebbins and Major 1965; Wiens et al 1989). Outbreeding depression is fitness decline resulting from hybridization, however this is not occurring between *E. lemmonii* and *E. neomexicanus, or E. arisolius*. The genetic structure and fitness for the *E. lemmonii* population and to what extent geneflow is occurring will be the subject of a future paper by Edwards and Bailey.

Pollen washes from insect visitors captured would definitively confirm the subset of pollinators for each of the three species of *Erigeron*. This was not possible in the network study, due to time and money constraints. Although there does not appear to be pollinator limitation, knowing more about which of the insect visitors are pollinators of *E. lemmonii* may be critical to prevent a collapse of the pollination network. However, a more effective approach to protect the entire insect visitor community may prove to be essential in protecting rare insect species, and *E. lemmonii*.

The possibility of hybridization between *E. lemmonii* and *E. neomexicanus* is low or absent, as shown by the lack of connections between these species in their insect visitor networks. Currently, a growing area of network research assigns traits to allow advanced ecological and evolutionary analysis of relationships. It is believed in the Southwest, *Erigeron* specie(s) had a larger range. For whatever reason the range decreased in size, leaving isolated pockets of *Erigeron* in specific cliff habitats, and these evolved into separate, relic species over time (Nesom 1990). The evolutionary relationships are not known for *Erigeron lemmonii* or the *Erigeron pringlei* group. Fleabanes like *E. lemmonii*, are only known from one or a few mountain ranges in Coconino, Gila, Graham and Santa Cruz Counties, whose members, *E. pringlei*, *E. heliographis, E. anchana* and *E. saxatilis,* also live on vertical rock ledges and boulders (Nesom 1990). *Erigeron scopulinus* described by Nesom and Roth (1981) is also thought to be closely related to the *E. pringlei* group, and grows on two isolated mountain ranges in the Southwest. Future phylogenetic and network analysis of the *pringlei* group would be a useful subject for future research to elucidate their evolutionary relationships. It would also be interesting to examine their pollination networks to see if similar patterns are found among these endemic *Erigeron* species. Soil, moisture, rooting and other morphological characteristics, pollinator data, and genetic data could be assigned as traits to these species in yielding new network information.

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