Taxonomic investigation of Erigeron lackschewitzii
by Tulli Ann Kerstetter

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
This study investigates the taxonomic status of Erigeron lackschewitzii Nesom and Weber (Asteraceae), a narrow Montana endemic restricted to windy, exposed ridgelines and calcareous substrates in the Bob Marshall and Scapegoat Wildernesses of northwestern Montana. By means of morphological and molecular techniques, the relationships of E. lackschewitzii to four other Erigeron (E. grandiflorus, E. simplex, E. ochroleucus var. scribneri, E. radicatus) were investigated in order to ascertain parentage and determine whether populations of this taxon were sufficiently distinct to warrant specific status. A total of 307 individuals comprising the five species were examined. In addition, six other species were included as outgroups in genetic analyses in order to estimate the direction of evolution of genetic markers. Lack of success in obtaining chromosome counts from root tips prompted an analysis of the number of stomates per unit area and estimates of mean stomate sizes of populations of each of the five species. Results suggest that plants belonging to E. lackschewitzii are tetraploid. Chloroplast DNA (cpDNA) restriction site analysis with ten restriction endonucleases revealed two mutations unique to E. lackschewitzii and further supported a relationship of E. lackschewitzii to E. ochroleucus var. scribneri. A phenetic analysis of Random Amplified Polymorphic DNA (RAPD) markers likewise revealed that E. lackschewitzii is distinct from other Erigeron and most closely related to E. ochroleucus var. scribneri. Individuals from populations of E. lackschewitzii were found to be genetically uniform, suggesting recency of origin from a single progenitor individual or population. Results from genetic studies corroborate findings from geographic and population phenetic analyses of morphological data, suggesting that populations of E. lackschewitzii, although closely related to E. ochroleucus var. scribneri, are sufficiently distinct to warrant species status. The persistent lack of pollen in individual E. lackschewitzii examined, coupled with the presence of fully formed achenes at a very early state of floret development, suggest some form of apomixis. Populations of E. lackschewitzii most likely resulted from selection for traits often found in other polyploid derivatives that colonize areas left barren after glaciation, with maintenance of the adaptive genotype through apomixis.

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Date November 28, 1994

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ABSTRACT

This study investigates the taxonomic status of *Erigeron lackschewitzii* Nesom and Weber (Asteraceae), a narrow Montana endemic restricted to windy, exposed ridgelines and calcareous substrates in the Bob Marshall and Scapegoat Wildernesses of northwestern Montana. By means of morphological and molecular techniques, the relationships of *E. lackschewitzii* to four other *Erigeron* (*E. grandiflorus*, *E. simplex*, *E. ochroleucus* var. *scribneri*, *E. radicatus*) were investigated in order to ascertain parentage and determine whether populations of this taxon were sufficiently distinct to warrant specific status. A total of 307 individuals comprising the five species were examined. In addition, six other species were included as outgroups in genetic analyses in order to estimate the direction of evolution of genetic markers. Lack of success in obtaining chromosome counts from root tips prompted an analysis of the number of stomates per unit area and estimates of mean stomate sizes of populations of each of the five species. Results suggest that plants belonging to *E. lackschewitzii* are tetraploid. Chloroplast DNA (cpDNA) restriction site analysis with ten restriction endonucleases revealed two mutations unique to *E. lackschewitzii* and further supported a relationship of *E. lackschewitzii* to *E. ochroleucus* var. *scribneri*. A phenetic analysis of Random Amplified Polymorphic DNA (RAPD) markers likewise revealed that *E. lackschewitzii* is distinct from other *Erigeron* and most closely related to *E. ochroleucus* var. *scribneri*. Individuals from populations of *E. lackschewitzii* were found to be genetically uniform, suggesting recency of origin from a single progenitor individual or population. Results from genetic studies corroborate findings from geographic and population phenetic analyses of morphological data, suggesting that populations of *E. lackschewitzii*, although closely related to *E. ochroleucus* var. *scribneri*, are sufficiently distinct to warrant species status. The persistent lack of pollen in individual *E. lackschewitzii* examined, coupled with the presence of fully formed achenes at a very early state of floret development, suggest some form of apomixis. Populations of *E. lackschewitzii* most likely resulted from selection for traits often found in other polyploid derivatives that colonize areas left barren after glaciation, with maintenance of the adaptive genotype through apomixis.

CHAPTER 1

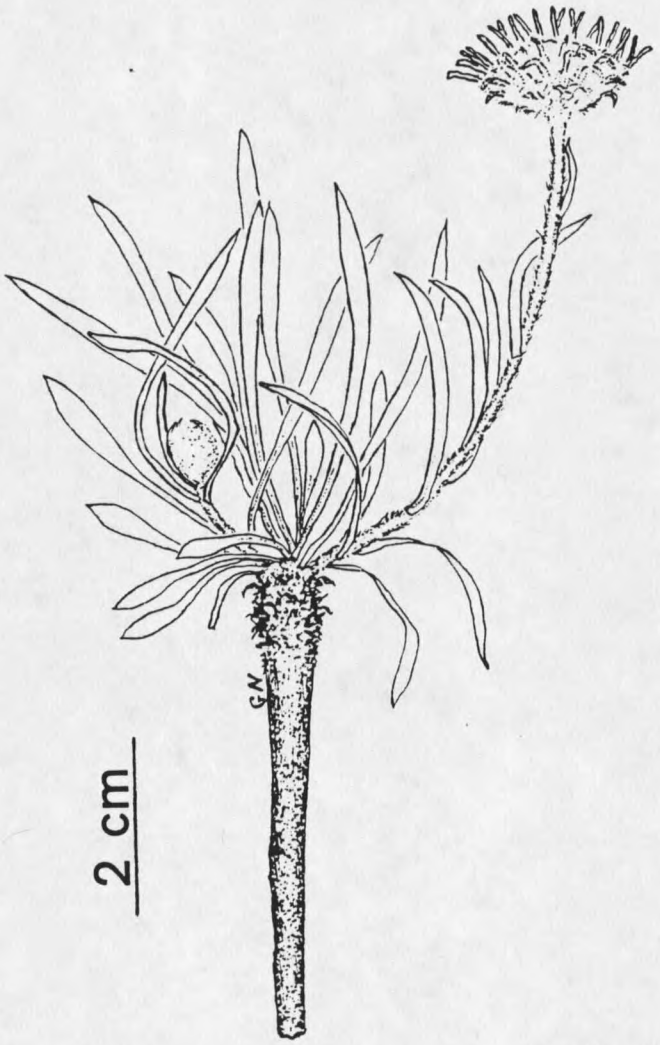
INTRODUCTION

Erigeron lackschewitzii Nesom and Weber (Figure 1) is described as a narrow endemic from the Bob Marshall and Scapegoat Wildernesses of northwestern Montana (Nesom and Weber, 1983). The species description is based on 18 individual plants in two collections from the Flathead Range and named for its collector, Klaus Lackschewitz, of Missoula. Judging from the very few and highly abortive pollen grains produced by plants of this taxon, the species is believed by its authors to be apomictic and probably polyploid.

Erigeron lackschewitzii is currently included in Category 2 of the U.S. Fish and Wildlife Service Notice of Review under consideration for federal listing as a threatened or endangered plant species and has been tracked by the Lewis and Clark National Forest as a species of concern (Heidel, 1993). It is currently ranked as "threatened throughout its range, but having taxonomic questions associated with it." The Montana Natural Heritage Program lists *E. lackschewitzii* as "imperiled" at both the global and state level, and the taxon was previously listed as "rare in Montana" by the Montana Rare Plant Project, according to Heidel.

Erigeron lackschewitzii is one of the few endemics in Montana (Heidel, 1993). It is found in exposed alpine settings on limestone substrates of the Madison group and Devonian formations containing dolomite. The species is known from 12 sites in four

Fig. 1. *Erigeron lackschewitzii* Nesom and Weber. From Nesom and Weber, 1983.



counties (Teton, Flathead, Pondera, Lewis and Clark) within a north-south 45-mile perimeter along the Front Range. *Erigeron lackschewitzii* grows in association with intermittent mats of *Dryas octopetala* as well as *Arctostaphylos uva-ursi,* with *Carex rupestris* as the dominant sedge between mats, according to Heidel. The mats of *Dryas*

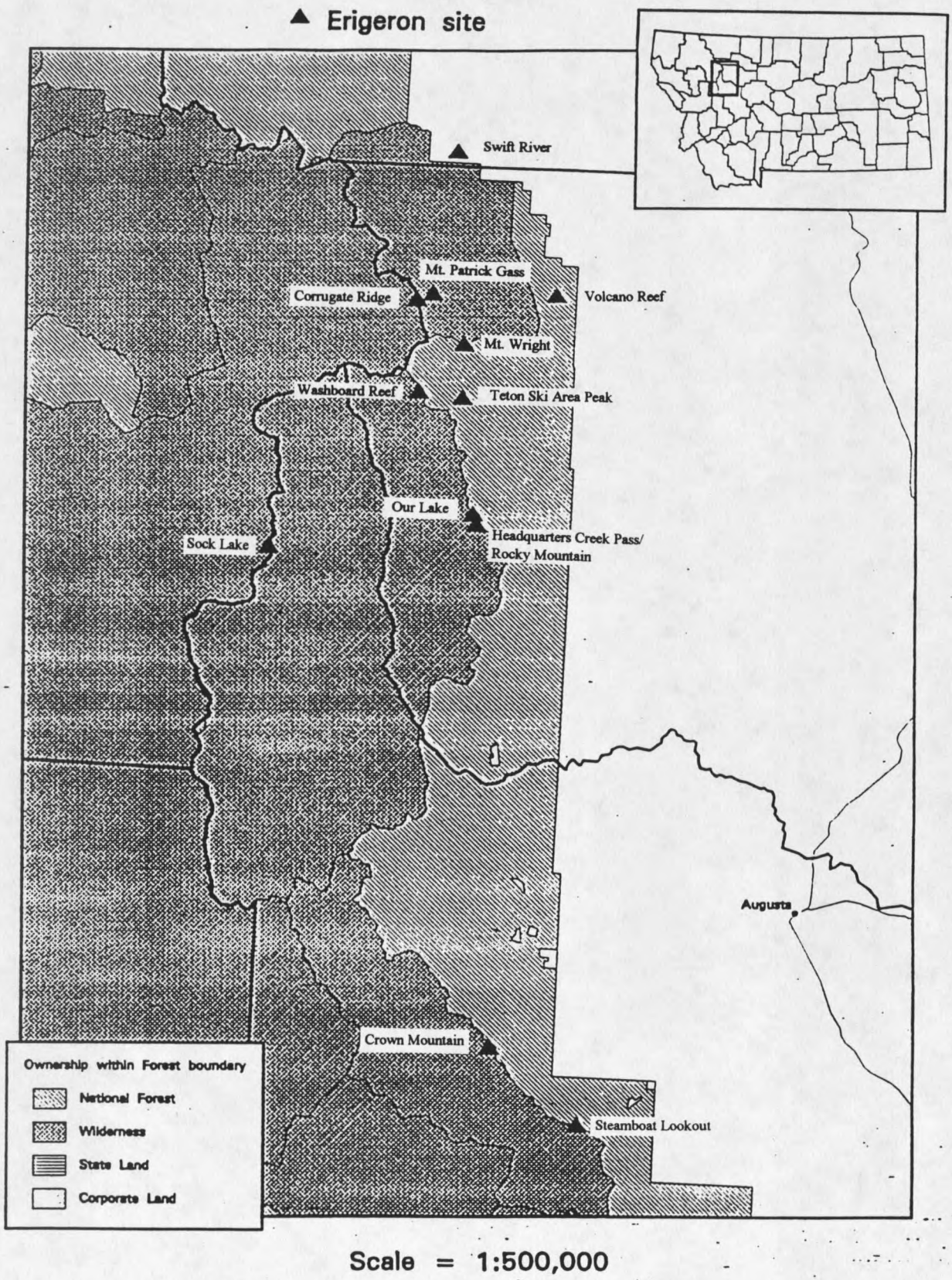
octopetala occupy exposed, snow-free sites on calcareous parent material where the soil is thin, rocky and unstable, acting as stable microsites in which other species of plants are able to exist (Bamberg, 1964).

The species is most frequently found on exposed alpine slopes with a southwest aspect, with smaller alpine and subalpine populations facing northwest (Heidel, 1993). Elevation ranges from 1951m to 2500m. *Erigeron lackschewitzii* is not a dominant community type, and populations range from 5 to 500 individuals in about 10 acres for denser populations to a mile for more scattered groups, according to Heidel. The largest populations (over 200 individuals) are found at Headquarters Creek Pass, Sock Lake, Crown Mountain, and Our Lake (Figure 2).

According to Nesom and Weber (1983), *E. lackschewitzii* is most closely related to *E. simplex* Greene and the southern alpine race of *E. grandiflorus* Hooker *sensu* Spongberg (1971). This relationship is inferred by the shared occurrence of monocephalous stems with entire leaves, involucral bracts with lanate-villous indumentum, blue-rayed outer florets, and an outer pappus of conspicuous squamellae. Additionally, cross walls of involucre trichomes are often pigmented red-purple, particularly near the base, in all three taxa. *Erigeron lackschewitzii* differs from both *E. simplex* and the southern alpine race of *E. grandiflorus* in the following features, as quoted from Nesom and Weber (1983):

1. Basal leaves linear-oblanceolate to narrowly oblanceolate without a well-demarcated blade versus spatulate in *E. simplex* and *E. grandiflorus*.
2. Ray flowers equal 30-68 versus 50-125 in *E. simplex* and 56-123 in *E. grandiflorus*.
3. Stem pubescence woolly-villous with glandularity lacking or not evident versus spreading but not villous or woolly, with erect, stipitate-glandular

Fig. 2. Locations of *Erigeron lackschewitzii* in Montana. After Heidel, 1993



trichomes usually conspicuous in *E. simplex* and *E. grandiflorus*.

4. Presence of a long thick taproot versus a short length of thick unbranched root with a fibrous system below (*E. grandiflorus)* or fibrous roots from a slender branching caudex (*E. simplex*).
5. More pappus bristles (15-24) than *E. grandiflorus* (9-14) but comparable to *E. simplex*.

Subsequent to describing *E. lackschewitzii*, Nesom (1989 and personal communication) synonymized it with another species, *E. ochroleucus* var. *scribneri.* Dorn (1984 and personal communication) concurs, delimiting *E. lackschewitzii* as a variation within the more widespread *E. ochroleucus* var. *scribneri.* Lesica (personal communication) suggests *E. lackschewitzii* and *E. ochroleucus* var. *scribneri* are quite close, proposing that the former may be a subset of the latter.

Erigeron lackschewitzii ("Front Range fleabane") is one of approximately 200 species of cosmopolitan *Erigeron* L. (Asteraceae/Compositae). The genus is composed of mostly low perennial herbs with narrow or chiefly basal leaves and commonly naked peduncles (Cronquist, 1947). The flowering heads are subtended by relatively long and narrow phyllaries usually subequal or sometimes regularly imbricate. Ray florets range in color from white to pink and purple and are commonly narrow and numerous, although occasionally broad and few. Style appendages are short, commonly obtuse or acute. In most species achenes are two-nerved and often surmounted by a double pappus of bristles and setae/squamellae. The name is an ancient one, used by Theophrastus for a plant with early developing, grayish-white hairs or pappus (Polunin, 1959). *Erigeron* usually bloom in spring and early summer, and nearly all species are of temperate or boreal regions or tropical mountainous areas. In South America, however, Section *Leptostelma* has

diverged into humid swampy openings of tropical and subtropical forests, and other species of the genus have spread to the oceanic islands of Galapagos, Juan Fernandez, and the Falklands (Solbrig, 1962).

The largest number and least specialized species of *Erigeron* are concentrated in the cordillera of western North America (Cronquist, 1947). In most groups of *Erigeron*, species tend to be fairly well defined with relatively limited ranges, and numerous endemics occur. More than 40 of the 133 North American species (ca. 30%) occupy ranges either entirely confined to a single state or not more than 250 miles long, according to Cronquist. Flowering heads and vegetative plant bodies of *Erigeron* are almost monotonously uniform, which may have prompted Cronquist to conclude in his revision that indumentum was the single most important taxonomic criterion (Spongberg, 1971). Geographical and ecological isolation of montane populations, comparable to insular isolation, promote genetic drift and result in populations with slight morphological differences, according to Spongberg. With many variations on a few themes, a large degree of morphological parallelism may occur in the genus and require molecular techniques to discover patterns of common ancestry (Nesom, 1989).

According to Cronquist (1947) *Erigeron* is closely related to *Aster* and *Conyza*, and derives as an early offshoot of the *Aster* lineage. Embryological data (Harling, 1951) and chloroplast DNA restriction site studies (Jansen et al., 1992) support a close relationship between *Erigeron* and *Aster*. Other workers (e.g., Bremer, 1994) have found a closer relationship of *Erigeron* to *Vittadinia* — a relatively small genus of perennial

herbs and shrubs from Australia, New Zealand, and New Guinea — based on cladistic analysis of 26 binary morphological characters of the tribe Astereae.

Most species of *Erigeron* are diploid, although many triploids, tetraploids, a few hexaploids, and several apomicts have been reported (McDonald 1927; Harling 1951; Spongberg, 1971; Nesom and Weber, 1983). Very few hybrids have been well-circumscribed, probably due to similarity of morphology between parent species (Spongberg, 1971). Hybridization, polyploidy and apomixis, combined with genetic drift due to geographic isolation, may serve as speciation mechanisms in the genus, according to Spongberg.

CHAPTER 2

MATERIALS AND METHODS

This study addresses the taxonomic status of *Erigeron lackschewitzii.* By means of morphological and molecular techniques, the relationships of *E. lackschewitzii* to four other *Erigeron* were investigated. Three analyses based on morphology (phenetic cluster analysis, canonical variates analysis, unpaired t-tests), and three molecular studies (chloroplast DNA, ribosomal DNA, Random Amplified Polymorphic DNA) were performed on the five species of interest. In addition, mitotic chromosome counts from root tips were attempted for *E. lackschewitzii*. The four species investigated as possible progenitors of *E. lackschewitzii* include the southern alpine race of *E. grandiflorus* Hooker (hereinafter *E. grandiflorus*), *E. simplex* Greene, *E. ochroleucus* var. *scribneri* (Canby) Cronquist, and *E. radicatus* Hooker. Although *E. radicatus* was not originally perceived as a putative relative, it was included in reponse to preliminary findings that suggested a very close relationship to *E. ochroleucus* var. *scribneri*. Also, proper identification of taxa sympatric with *E. lackschewitzii* prompted inclusion of *E. radicatus*.

Examined were 79 individuals from herbarium specimens including type specimens for all five species, and 228 plants field-collected from Montana and Wyoming during the summers of 1992-1994. Thirty-one quantitative and 51 qualitative characters were scored for 307 individuals as follows: *E. grandiflorus,* 63 specimens; *E. lackschewitzii*, 56

specimens; *E. ochroleucus* var *scribneri,* 78 specimens; *E. radicatus,* 56 specimens; and *E. simplex,* 54 specimens.

Discussion of Characters

Characters were selected in response to preliminary visual examination of specimens and review of diagnostic keys delimiting species (e.g., Cronquist, 1947; Hitchcock, et al., 1955; Spongberg, 1971; Nesom and Weber, 1983; Dorn, 1984; Dorn, 1992). Characters were also chosen in accordance with phenetic analysis techniques of Sokal and Sneath (1963) and Sneath and Sokal (1973), that is, from all parts of the plant and those which varied within the groups studied, not merely conventional diagnostic characters. The 31 quantitative and 51 qualitative characters were grouped according to root system, flowering stem, cauline leaves, basal leaves, involucre, ray florets, disc florets, and achenes.

Quantitative Characters

Root System. Nesom and Weber (1983) cite the long thick taproot of *E. lackschewitzii* as distinctive of the species. Preliminary examination of specimens belonging to *E. ochroleucus* var. *scribneri* disclosed a possible distinction between this species and *E. lackschewitzii* in that the taproot of *E. lackschewitzii* seemed to be consistently thicker and more robust. *Erigeron radicatus* is distinguished from *E. ochroleucus* var. *scribneri* in diagnostic keys by presence of a well-developed and branched caudex as opposed to the slightly branched caudex of *E. ochroleucus* var.

scribneri (Cronquist, 1947). Root system characters include: (1) caudex width at ground level, (2) width below caudex branches, and (3) width below caudex.

Length of the perennating organ was initially included in the analysis but eliminated when it became obvious that many taproots were cut during collection.

Flowering Stem. *Erigeron lackschewitzii* is described as having a woolly-villous stem indumentum (Nesom and Weber, 1983). Plants belonging to *E. simplex* possess smaller trichomes which are more variable in density, and the glandular trichomes in particular may be less abundant than in *E. grandiflorus,* according to Nesom and Weber. The authors state that, unlike *E. lackschewitzii*, neither *E. grandiflorus* nor *E. simplex* possess trichomes so long and dense as to obscure completely the other types of vestiture. In contrast, Spongberg (1971) found differences in stem trichomes to be slight between monocephalous arctic and alpine species. For example, Spongberg found that variation in trichome density of plants belonging to *E. simplex* ranged from nearly glabrous to densely woolly-villous.

Information regarding stem trichome density of *E. radicatus* and *E. ochroleucus* var. *scribneri* is lacking, although preliminary examination revealed that a combination of density and type may distinguish these species from each other and from *E. lackschewitzii*. Because trichome type and length can effect the appearance of density, data were collected on type (see under qualitative characters) as well as number per unit area and length.

Within broad limits, stem height is often useful, although any of the larger species may have occasional depauperate low forms Cronquist (1947). Since longer stems tend to

correlate with lower altitude, exposure at a particular habitat and/or light intensity in arctic and alpine species (Spongberg, 1971), consistency in stem length within species may indicate habitat preferences and, by extension, ecological isolation.

Flowering stem characters include: (4) number of stems, (5) height of tallest stem, (6) width 5 mm below involucre, (7) number of trichomes per unit, and (8) trichome length.

Cauline Leaf Number. Diagnostic keys state that plants belonging to *E. lackschewitzii* have five to ten cauline leaves (Nesom and Weber, 1983). *Erigeron ochroleucus* var. *scribneri* display "a few" leaves on the stem, and *E. radicatus* typically have two or three or none (Cronquist, 1947). Although cauline leaf numbers in arctic/alpine *Erigeron* species appear to correlate with stem length, number of leaves were "disproportionately greater" in *E. grandiflorus* (Spongberg, 1971).

Basal Leaves. Cronquist (1947) states that the size and shape of the leaves and their comparative development from base to top of stem are more important diagnostic tools than stem height, although in most species of the genus there is considerable variation in absolute size of the leaves. Basal leaf sizes reported for *E. lackschewitzii* and *E. radicatus* are a subset of sizes reported for *E. ochroleucus* var. *scribneri*, while leaf dimensions for plants belonging to *E. grandiflorus* and *E. simplex* are several-fold wider. Therefore, basal leaf dimensions are diagnostic in delimiting the latter two taxa from the former three. Basal leaf characters include: (10) length of longest leaf, (11) width of longest leaf, (12) length of widest leaf, and (13) width of widest leaf.

Involucre. Characters of the involucre are diagnostically important, but there are many slight and overlapping differences between species (Cronquist, 1947). Although involucre sizes for the five taxa studied do overlap, relatively consistent size ranges were noted for each species (cf. Cronquist, 1947; Hitchcock, et al., 1955; Spongberg, 1971). Involucre characters include: (14) width, (15) height, (16) number of phyllary rows, (17) outer phyllary width, (18) outer phyllary height, (19) number of trichomes/mm, and (20) trichome length.

Ray Florets. The taxonomically important variations in the rays are in color, length, width, and number, all being occasionally useful within broad limits (Cronquist, 1947). Length and width of rays are variable in monocephalous arctic/alpine species (Spongberg, 1971). Polyploids possess the longest rays and diploids the shortest, except at higher altitudes, according to Spongberg. Additionally, increased disc diameter correlates with narrower more numerous florets, with smaller discs having fewer but wider rays. Ray floret numbers overlap in *E. lackschewitzii*, *E. ochroleucus* var. *scribneri*, and *E. radicatus,* but are well below the range found in plants belonging to *E. grandiflorus* and *E. simplex*. Ray floret characters include: (21) length of rays, (22) width of rays, (23) number, and (24) number of apex notches.

Number of apex notches was added based on preliminary examination of *E. lackschewitzii* specimens. Plants belonging to *E. lackschewitzii* possess consistently prominent two- and three-notched apices which differ from the more variable tip types of the other taxa.

Disc Florets. The disc florets furnish few taxonomic characters, although occasionally gross differences in length facilitate differentiation of related species (Cronquist, 1947). Length of the style appendages are frequently helpful as secondary characters, although some species show a surprising amount of variation, according to Cronquist. Within arctic/alpine monocephalous taxa, length of style branches is relatively consistent (Spongberg, 1971). Disc floret characters include: (25) length of corolla, (26) width of corolla, and (27) style branch length.

Achenes. Important variations in the pappus are in number, texture, and occasionally length and color of the bristles, with number of bristles helpful within broad limits (Cronquist, 1947). Spongberg (1971) disagrees in part, maintaining that one of the most overused characters is number of pappus bristles, which can vary between plants of the same species, between heads on the same plant, and are frequently broken off of herbarium specimens. Pappus height, rather than number of bristles, remains consistent within arctic/alpine monocephalous *Erigeron* taxa, according to Spongberg.

Curiously, achene sizes are rarely found in the literature. The only important variation in achenes, according to Cronquist (1947), is nervation. Achene nervation was originally included as a character but abandoned before final analysis due to lack of consistent variation among the five taxa studied. Achene size, however, was included, based on preliminary examination of the five species which suggested achene sizes may be species specific and suggestive of ploidy level. Achene characters include: (28) pappus height, (29) number of pappus bristles, (30) length, and (31) width.

Qualitative Characters:

Root System: Taproot. Despite evidence of some environmental modifications, *Erigeron* species comprising the arctic/alpine complex can be separated into loosely defined groups on the basis of similar morphology and parallel variation in perennating organs (Spongberg, 1971). *Erigeron simplex* plants produce fibrous roots, often with branched caudices, while *E. grandiflorus* individuals possess well-developed vertical caudices surmounting definite taproots. Other workers (Cronquist, 1947; Dorn, personal communication) assert that individual species of long-lived perennials sometimes display more than one type of root system, i.e., a stout rhizome with fibrous roots, a taproot and well-developed branching caudex, or merely a taproot and crown. Plants belonging to *E. lackschewitzii*, *E. ochroleucus* var. *scribneri*, and *E. radicatus* uniformly possess a vertical taproot surmounting a caudex. Taproot (character 32) is coded as present (1) or absent (0).

Root System: Secondary Roots. In their species description of *E. lackschewitzii*, Nesom and Weber (1983) indicate that probably the most conspicuous unique feature is a long, thick taproot lacking any obvious secondary branches. The absence of numerous secondary/fibrous roots would be of diagnostic value if it were a consistent and persistent feature of plants belonging to *E. lackschewitzi*. Presence of secondary roots (character 33) is classified as less than 10 secondary/fibrous roots (1), or more than 10 secondary/fibrous roots (2).

Root System: Caudex Branching Pattern. One of the distinguishing features separating *E. radicatus* from *E. ochroleucus* var. *scribneri* is the caudex, being only

slightly branched in *E. ochroleucus* var. *scribneri,* and well developed, with primary branches usually again branched, in *E. radicatus* (Cronquist, 1947). Hooker (1834) includes with his species description of *E. radicatus* a drawing of a highly branched caudex which undoubtedly prompted the specific epithet "radicatus." Spongberg (1971) maintains that caudex size is a function of age, along with soil conditions (moisture, soil depth, etc.). Since *E. radicatus* has been rarely collected (Hitchcock, et al., 1955), this feature is of interest as a possible diagnostic trait, inasmuch as the separation of *E. radicatus* from *E. ochroleucus* var. *scribneri* has been problematic. Caudex branching pattern (character 34) is classified as primary (1), secondary (2), or tertiary (3).

Root System: Caudex Habit. A perpendicular caudex is present in *E. grandiflorus*, as opposed to the decumbent, thinner caudex of *E. simplex*, (Spongberg, 1971). Since *E. lackschewitzii*, *E. ochroleucus* var. *scribneri* and *E. radicatus* possess erect caudices surmounting definite taproots, this character was included specifically for separation of *E. simplex* from *E. grandiflorus*. Habit of the caudex (character 35) is classified as decumbent (0) or erect (1).

Flowering Stem: Monocephalous. Hitchcock et al. (1955) delimit *E. ochroleucus* var. *scribneri* with solitary or a few heads per stem, while solitary heads are found in plants belonging to *E. lackschewitzii* (Nesom and Weber, 1983) and *E. radicatus* (Hooker, 1834). It is relevant here that more than one head per flowering stem in the *Erigeron andicola* complex of South America tended to be more frequent if the plants were over 10 cm tall (Solbrig, 1962). Clonal plants of *E. grandiflorus* grown under alpine conditions in a growth chamber produced branched flowering stems when moved to a

greenhouse (Spongberg, 1971). Spongberg suggests that one of the parents of this triploid may have been branched, and/or environmental change resulting from a warmer temperature regime and different photoperiod may have induced branching. This character (36) is coded according to whether all stems are monocephalous (0) or possess more than one head (1).

Flowering Stem: Growth Pattern. This character and the following two characters are included because they are commonly found in species descriptions and diagnostic keys. Growth pattern of the stem (character 37) is classified as decumbent (1), ascending (2), or strict (3).

Flowering Stem: Base Color. See above. Stem-base color (character 38) is classified as red/purple (1) or green/white (2).

Flowering Stem: Stem Color. See above. Color of the stem as a whole differs in many cases from the color of the stem base. Stem color (character 39) is classified as red/purple (1), partially red/purple (2) or wholly green (3).

Flowering Stem: Trichomes. Stem pubescence in *E. lackschewitzii* consists of lanate-villous, loosely ascending to appressed flattened and twisted trichomes, (Nesom and Weber, 1983). In contrast, *E. ochroleucus* var. *scribneri* trichomes are described as short, white, appressed hairs (Canby, 1890); not at all hirsute (Rydberg, 1900); closely hirsute-strigose, often loosely spreading (Cronquist, 1947), or appressed and ascending (Dorn, 1984). Stem indumentum in *E. radicatus* consists of finely hirsute or villous spreading or appressed hairs (Cronquist, 1947). According to Spongberg (1971), stem indumentum differences are slight in monocephalous arctic/alpine *Erigeron*, although *E.*

grandiflorus and *E. simplex* are easily separated from the above-described taxa by the presence of conspicuous glandular and tiny appressed Type A trichomes *sensu* Nesom (1976). Trichome characters of the flowering stem include: (40) presence of villous trichomes (1) or not (0), (41) presence of strigose trichomes (1) or not (0), (42) presence of glandular trichomes (1) or not (0), and (43) presence of other types of trichomes (1) or not (0).

Cauline Leaves: Position on Stem. According to Nesom and Weber (1983), plants belonging to *E. lackschewitzii* possess leaves which extend halfway up the stem. If leaf placement were consistently and persistently halfway up the stem in individuals belonging to *E. lackschewitzii*, this character would be diagnostic of the species. Leaf position on the stem (character 44) is classified as extending greater than halfway up the stem (1) or less than halfway (0).

Cauline Leaves: Margin Undulation. Spongberg (1971) found a tendency of leaf margins to undulate in plants belonging to *E. simplex*. Leaf margin undulation (character 45) is coded according to whether leaf margins undulate (1) or not (0).

Cauline Leaves: Leaf Types. More important than stem height are the size and shape of leaves and their comparative development from base to top of stem, that is, reduced or unreduced in size as they are positioned from stem base to flowering head (Cronquist, 1947). Because cauline leaves were reduced upward in all five species studied, comparative development was omitted from the analysis. Several plants of all species displayed leaf dimorphism which correlated with location of insertion on the stem. Basal-like leaf shapes were observed near stem bases, with linear to lanceolate shapes

(depending on the species) near flowering heads. Cauline leaf characters include: (46) presence of basal-like leaves (1) or not (0), (47) presence of linear leaves (1) or not (0), and (48) presence of lanceolate leaves (1) or not (0).

Cauline Leaves: Trichomes. Indumentum types are noted in all species descriptions. Trichome characters of cauline leaves include: (49) presence of villous trichomes (1) or not (0), (50) presence of strigose trichomes (1) or not (0), and (51) presence of glandular trichomes (1) or not (0).

Basal Leaves: Leaf Types. In his study of the *Erigeron andicola* complex of South America, Solbrig (1962) found leaf types and shapes to be highly variable and independent, with no correlation to other characters. Preliminary examination of plants belonging to *E. lackschewitzii* and *E. ochroleucus* var. *scribneri* indicated striking similiarity in leaf shapes, although basal leaves of *E. ochroleucus* var. *scribneri* appeared to be consistently narrower. A search of the literature has substantiated personal observation that *E. radicatus* individuals produce narrowly spatulate to linear-spatulate basal leaves in addition to the narrowly oblanceolate leaf shape of *E. lackschewitzii* and *E. ochroleucus* var. *scribneri*. Plants belonging to *E. grandiflorus* display lanceolate and broadly lanceolate to elliptical or subovate basal leaves, in contrast to the narrowly to broadly spatulate shape generally found in *E. simplex* (Spongberg, 1971). Dimorphic leaf types were noted for several specimens of all species.

Basal leaf characters include: (52) presence of linear leaves (1) or not (0), (53) presence of narrowly oblanceolate leaves (1) or not (2), (54) presence of

oblanceolate/obovate leaves (1) or not (0), and (55) presence of spatulate leaves (1) or not (0).

Basal Leaves: Longest Leaf Tip Type. Because immature leaves are extremely variable, and mature aspects of leaf apices and shapes are often disguised in juvenile forms (Spongberg, 1971), tip types of the longest and widest leaves were included as characters. Spatulate leaves usually have obtuse or rounded apices, whereas lanceolate leaves most often terminate in acute apices (Harrington and Durrell, 1957; Spongberg, 1971). Longest leaf tip type (character 56) is classified as acute (1), obtuse (2), or other (3).

Basal Leaves: Longest Leaf Trichomes. Leaf indumentum is included in all diagnostic keys found in the literature. Trichome characters of the longest leaf include: (57) presence of ciliate trichomes (1) or not (0), (58) presence of laminar trichomes (1) or not (0), and (59) presence of glandular trichomes (1) or not (0).

Basal Leaves: Widest Leaf Tip Type. This character (60) is discussed and coded according to that found under "Basal Leaves: Longest Leaf Tip Type."

Basal Leaves: Widest Leaf Trichomes. These characters (61-63) are discussed and coded according to those found under "Basal Leaves: Longest Leaf Trichomes."

Involucre: Shape. A hemispherical involucre is characteristic of *E. grandiflorus* and *E. simplex* (Spongberg, 1971), with plants of *E. lackschewitzii* described as shallowly hemispheric (Nesom and Weber, 1983). The turbinate shape is described only for *E. radicatus* (Hitchcock et al., 1955), although preliminary examination of field specimens failed to corroborate this attribute. The presence of turbinate involucres was noted on a few *E. lackschewitzii* specimens. No descriptions of involucre type are noted for *E.*

ochroleucus var. *scribneri* in the literature. Involucre shape (character 64) is classified as turbinate (1) or hemispheric (0).

Involucre: Tip Reflexion. Reflexion of phyllary tips was enhanced in clones of *E. grandiflorus* placed in a growth chamber simulating arctic conditions (24-hour photoperiod) over those in alpine growth conditions with a 14-hour photoperiod (Spongberg, 1971). Although no plants of the five species collected were grown under arctic conditions, differences in reflexion of phyllary tips were noted. Tip reflexion (character 65) is classified as reflexed (1) or not (0).

Involucre: Tip Color. The character of the involucre is highly significant, but there are so many slight and overlapping differences between various species that no broad clearcut divisions are possible (Cronquist, 1947). Pigmentation in trichomes was found to be highly variable in *E. grandiflorus* and *E. simplex* (Spongberg, 1971), although preliminary examination of field-collected *E. grandiflorus* specimens from Montana and Wyoming suggests that these plants may be more extensively pigmented than *E. simplex* individuals. Because pigment concentration in trichome cells can effect the appearance of color in the involucre, this character only pertains to the pigmentation of epidermal cells of the phyllaries. Tip color (character 66) is classified as herbaceous (0), slightly red/purple (1), or mostly red/purple (2).

Involucre: Trichomes. Possibly because of invariance in flowering heads and vegetative plant bodies, indumentum has assumed some importance in floral keys and species descriptions (Spongberg, 1971). Identification of trichomes is inexact and at times obtuse, leading to difficulty in objectifying what is visualized. For example, the involucre

of *E. ochroleucus* var. *scribneri* has been variously described as pubescent with short white appressed hairs becoming more copious and spreading on the involucre (Canby, 1890), not at all hirsute (Rydberg, 1900), more or less densely hirsute-villous with slender, usually loose hairs, often also somewhat viscid (Cronquist, 1947), and woolly-villous with tangled glassy or blackish multicellular hairs (Dorn, 1984). Involucre trichome characters include: (67) presence of woolly appearance (1) or not (0), (68) presence of villous trichomes (1) or not (0), (69) presence of strigose trichomes (1) or not (0), and (70) presence of glandular trichomes (1) or not (0).

A woolly appearance is defined as covered with dense, long, soft, entangled, curled hairs, not appressed close to the surface (Harrington and Durrell, 1957). If floral bracts (phyllaries) looked white to the naked eye, indicating that trichomes were either very dense, very long and tangled, or both, the involucre was designated as woolly.

Ray Florets: Angle to Involucre. Studies of *E. grandiflorus* clones in arctic and alpine growth chambers suggest that the angle of ray florets to the involucre is environmentally influenced (Spongberg, 1971). Personal observation in the field when comparing sympatric species did not completely corroborate this finding. Nesom and Weber (1983) describe the ray flowers of *E. lackschewitzii* as somewhat erect, an observation similar to that noted by Spongberg for the southern alpine race of *E. grandiflorus*. Species descriptions of the other three taxa of interest fail to mention this character. Angle to involucre (character 71) is classified as oblique (1) or not (0).

Ray Florets: Trichomes. It is unknown whether trichome lengths on ray florets correlate with trichome lengths elsewhere on the plant because ray floret trichomes have

only been described in the literature for *E. lackschewitzii* (Nesom and Weber, 1983). The most evident correlation in the *Erigeron andicola* complex of South America is that of pubescence of leaves, scapes, and involucre; no plant with a glabrous scape had pubescent phyllaries or leaves (Solbrig, 1962). Ray floret trichomes (character 72) are classified as short (1), medium (2), or long (3).

Ray Florets: Color Dried. Herbarium specimens of *E. grandiflorus* are often described as having a deep cobalt blue color when in actuality the true fresh color is lavender to pink. Nesom and Weber (1983) describe ray floret color of *E. lackschewitzii* as light lilac to purple when dried, while field collections indicate that fresh ray floret color is a consistent pink which dries to a deep blue. Because colors are not coded from a color chart, ray color other than white can be subjective and, consequently, confusing. Due to the possibility of more than one color on individual ray florets, each color was coded as a separate character. Characters include: (73) color dried blue/lavender (1) or not (0), (74) color dried pink (1) or not (0), and (75) color dried white (1) or not (0).

Ray Florets: Color Fresh. This suite of characters was determined after rays were immersed in hot deionized water. Ray floret color after immersion was compared, where possible, to label descriptions and found to consistently match the natural fresh color. Due to the possibility of more than one color on individual ray florets, each color was coded as a separate character. Characters include: (76) color fresh blue/lavender (1) or not (0), (77) color fresh pink (1) or not (0), and (78) color fresh white (1) or not (0).

Ray Florets: Apex Notches. Preliminary examination of plants belonging to *E. lackschewitzii* revealed that apex notches were relatively invariable and might be

diagnostic of the taxon. Although most *Erigeron* possess rays which are bi- or trifurcated, the degree of cleft formation is more varied in the other four species. Apex notches (character 79) are classified as microscopic (1) or macroscopic (2) under 15X power.

Disc Florets: Style Branch Shape. This character was included in order to ascertain whether style branch shape, like style branch length (character 27), is relatively consistent within plants of a given taxon. Style branch shape (character 80) is classified as deltoid (1), deltoid-lanceolate (2), or lanceolate (3).

Disc Florets: Pollen. The consistent and persistent absence of pollen in *E. lackschewitzii* is a diagnostic feature of the taxon and may indicate reproductive isolation resulting from obligate apomixis. *Erigeron grandiflorus* is a facultative apomict, while style presentation in several plants belonging to *E. simplex* may be indicative of self-compatibility (Spongberg, 1971). Preliminary examination of several *E. radicatus* specimens revealed a high degree of variability in pollen production and style presentation at anthesis when compared to sexual taxa. Disc floret pollen (character 81) is coded as present (1) or absent (0).

Achenes: Trichome Density. Density of trichomes in arctic/alpine monocephalous *Erigeron* proved too variable to be of diagnostic value except in combination with other characters (Spongberg, 1971). Cronquist (1947) maintains that occasionally indumentum is an important variable, while Solbrig (1962) states that achene trichome density is a character completely independent of indumentum as a whole in the South American *Erigeron andicola* complex. A review of the literature and personal observation indicate that trichome density of the achenes might be diagnostic in delimiting *E. lackschewitzii*

from *E. ochroleucus* var. *scribneri* and *E. radicatus*. Achene trichome density (character 82) is classified as dense (1) or sparse (2).

Species Examined

Originally described as occurring only in northern Montana and Canada, *E. grandiflorus* has been separated by Spongberg (1971) into three widely disjunct northern alpine, southern alpine, and arctic races. The southern alpine race of *E. grandiflorus* Hooker (Figure 3) is a triploid facultative apomict with a discontinuous distribution in the western United States. It is found in exposed, dry areas and unstable locations (e.g., along roadcuts, mine dumps, tourist overlooks, trails) from the Beartooth Plateau south into New Mexico and west into the Great Basin. Where sympatric with *E. simplex*, it occurs in drier, more exposed sites. Other workers (Dorn, 1984, and personal communication; Hartman, personal communication) synonymize the southern alpine race of *E. grandiflorus* with *E. simplex* Greene, maintaining that *E. grandiflorus* does not occur in the contiguous 48 states outside of northwestern Montana. Synonomy of the southern alpine race of *E. grandiflorus* with *E. simplex* in Montana and Wyoming is discussed in the Results chapter of this study. No races of *E. grandiflorus* are sympatric with *E. lackschewitzii*.

In contrast, *E. simplex* Greene (Figure 4) is a widespread diploid species of alpine and subalpine mesic sites, preferring a *Geum*-meadow habitat in the southern part of its range, and rarely found on exposed ridge tops (Spongberg, 1971). Above 12,000 feet in the southern Rockies, *E. simplex* is found in more sheltered patches near boulder bases

and on south- and east-facing slopes. It occurs in the northern Rockies west to Olympic Mountains, east to the Medicine Bow Range of Wyoming, and south to Colorado's Sangre de Cristo Range. The species is less common in the northern part of its range and is not found north of continental glaciation, according to Spongberg. *Erigeron simplex* is sympatric with *E. lackschewitzii*.

Erigeron ochroleucus var. *scribneri* (Canby) Cronquist is a diploid herb not known to occur west of the continental divide (Cronquist, 1947). It is a relatively low and slender plant, with few small cauline leaves. The variety is found from southern Alberta to southeastern Wyoming, occasionally as far east as Cypress Hills, Saskatchewan, the Black Hills of South Dakota, and northwestern Nebraska (Cronquist, 1947; Hitchcock, et al., 1955). Originally described as *Erigeron scribneri* by Canby (1890) from Scribner's 1883 collection of the Northern Transcontinental Survey of Montana's Little Belt Mountains, it assumed variety status in Cronquist's (1947) revision of North American *Erigeron*. *Erigeron ochroleucus* var. *scribneri* is known to occur in dry plains and barren places in the mountains of Montana (Dorn, 1984). In Wyoming, where it appears in greatest abundance and variation (personal observation), the taxon inhabits rocky places in the north, central, south-central, and eastern part of the state (Dorn, 1992). *Erigeron ochroleucus* var. *ochroleucus* (Figure 5) differs from var. *scribneri* only in its larger size, presence of moderately well-developed cauline leaves, and habitat preference for hills, slopes and meadows at lower elevations (Cronquist, 1947; Dorn, 1992; personal observation). Results of this study indicate that, with one or two exceptions, *E. ochroleucus* var. *scribneri* is not sympatric with *E. lackschewitzii*.

Fig. 3. *Erigeron grandiflorus* Hooker.
From Hitchcock et al., 1955.

Fig. 4. *Erigeron simplex* Greene.
From Hitchcock et al., 1955.

Erigeron radicatus Hooker (Figure 6) is a tetraploid perennial species with a taproot and branching caudex, with primary branches usually again branched (Cronquist, 1947). Reported as rarely collected, it is found in the Rocky Mountains of southern Alberta and the Cypress Hills of Saskatchewan (Hitchcock, et al., 1955). It is also known to occur in the highest mountains of the Central Rockies (Coulter and Nelson, 1909) as well as on ridges and slopes of high mountains in western and south-central Montana (Dorn, 1984). *Erigeron radicatus* is sympatric with *E. lackschewitzii*.

Geographic Variation

Phenetic cluster analysis was performed in order that samples of individual variation across a wide geographical area could be classified using similarity coefficients in triangular matrix form (Sokal and Sneath, 1963). The analysis generated a dendrogram of phenetic distance for all pair-wise combinations of 147 individuals, each circumscribed by the 82 standardized characters discussed previously (see "Discussion of Characters"). Table 1 lists specimens included in the analysis.

Fig. 5. *Erigeron ochroleucus* Nuttall.
From Hitchcock et al., 1955.

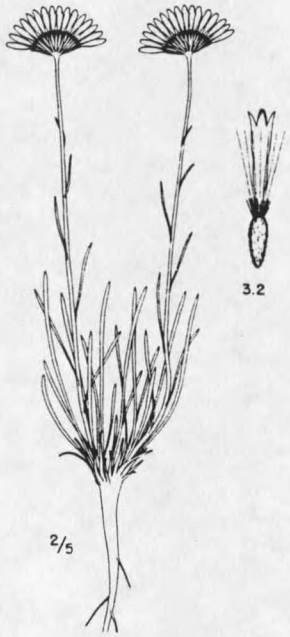
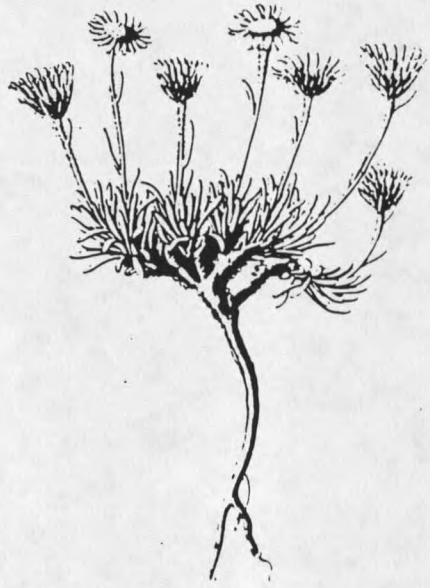


Fig. 6. *Erigeron radicatus* Hooker.
From Hooker, 1834.



Population Variation

Canonical variates analysis (CVA) was performed on a limited set of populations of the five species of interest to assess the within-population variability versus that between populations. CVA establishes population distinctions based on relationships between groups explored in multidimensional space (Sokal and Sneath, 1963). The object of CVA is to determine functions whose application to original data maximizes observed differences between groups. Each entity is assigned a score which is the summation of each attribute score. Summation scores are then weighted by a correlation matrix established a priori. The procedure has been used for studying infraspecific hybridization and incipient speciation in various taxa, according to Sokal and Sneath.

For the first of two CVA analyses, data from ±20 individuals representing the most northern and ±20 individuals representing the most southern populations collected of *E. ochroleucus* var. *scribneri*, *E. radicatus*, *E. simplex*, and *E. grandiflorus* were used to establish population parameters. Accessions underlined in Table 1 identify these populations. Twenty individuals each from the most northern and most southern populations collected of *E. lackschewitzii* (underlined in Table 1) were added as a test group of "unknowns" in order to determine which of the aforementioned species this taxon most resembled.

The most northern and most southern populations of *E. lackschewitzii* were included in the second CVA to test species identification. One hundred and twelve of the 147 individuals included in phenetic cluster analysis of geographic variation were placed in a test group of "unknowns." The test group individuals include representatives of all five

species and are indicated by asterisks in Table 1. CVA placement of an individual into a particular group was based on the generalized distance of each specimen from each population's group mean (Rohlf, 1993). Results from this CVA were compared with the phenetic cluster analysis of geographic variation in order to cross-verify species identification. Individuals with missing data could not be included in the analysis (Rohlf, 1993).

Table 1. *Erigeron* specimens utilized for geographic and population analyses. Labels refer to those shown in Fig. 9. Numbers in parentheses indicate individuals sampled per accession. Underlined accessions = CVA populations used to establish species identities. Starred accessions = CVA "test group" specimens.

Taxa	Label	Locality
E. grandiflorus	EGtype	Summit of Rocky Mountains, *Drummond s.n.* (1)
	EGm924*	Montana: Teton Co., *Lackschewitz 10085* (1)
	EGm085*	Montana: Flathead Co., *Lackschewitz 10604* (1)
	EGw090*	Wyoming: Albany Co., *Osterhout 1805* (1)
	EGw046*	Utah: Uintah Mountains, *Godding 1385* (3)
	EGw565*	Colorado:above Beaver Creek, *Crandall 2819* (1)
	EGw637*	Colorado: Larimer Co., *Osterhout 2860* (1)
	EGw462*	Wyoming: Johnson Co., *Porter 7092* (1)
	EGw033*	Wyoming: Albany Co., *Beaman and Stone 1394* (1)
	EGw035*	Utah: Uintah Co., *Beaman and Stone 1439* (4)
	EGw664*	Colorado: Park Co., *Neely and O'Kane 3139* (2)
	EGw615*	Colorado: Summit Co., *Gierisch 2192* (1)
	EGw461*	Wyoming: Albany Co., *Spongberg 69-54* (1)
	EGw879	Colorado: Grand Co., *Tweedy 5796* (1)
	EGw805	Wyoming: Albany Co., *Nelson 138805* (1)
	EG2800	Wyoming: Johnson Co., *Kerstetter EG2800* (20)
	EG3000*	Montana: Carbon Co., *Kerstetter EG3000* (2)
	EG3200*	Montana: Carbon Co., *Kerstetter EG3200* (2)
	EG3400	Montana: Carbon Co., *Kerstetter EG3400* (20)
E. lackschewitzii	ELtype*	Montana: Flathead Co., *Lackschewitz 9191*
	ELm887*	Montana: Teton Co., *Schassberger 339* (1)
	ELm490*	Montana: Lewis and Clark Co., *Lesica 4044* (1)
	ELm845*	Montana: Teton Co., *Lackschewitz 10552* (1)
	EL200*	Montana: Teton Co., *Kerstetter EL200* (2)

	EL700*	Montana: Teton Co., *Kerstetter EL700* (2)
	EL1000	Montana: Teton Co., *Kerstetter EL1000* (20)
	EL1100*	Montana: Teton Co., *Kerstetter EL1100* (2)
	EL1200	Montana: Teton Co., *Kerstetter EL1200* (10)
	EL1300	Montana: Teton Co., *Kerstetter EL1300* (10)
	EL1400*	Montana: Teton Co., *Kerstetter EL1400* (2)
	EL14a02*	Montana: Teton Co., *Kerstetter EL14a02* (1)
	EL15a01*	Montana: Teton Co., *Kerstetter EL15a01* (1)
E. ochroleucus	EOtype	Montana: Little Belt Mountains, *Scribner 77*
var. *scribneri*	EOc840	Wyoming: Sweetwater Co., *Dorn 3114* (1)
	EOc905	Wyoming: Albany Co., *Goodding 224* (1)
	EOg112	Montana: Glacier Co., *DeSanto s.n.* (1)
	EOg104	Montana: Glacier Co., *DeSanto s.n.* (1)
	EOm378*	Montana: Teton Co., *Lackschewitz 10574* (2)
	EOm164*	Montana:Broadwater Co., *Lesica 756* (1)
	EOm188*	Montana: Lewis and Clark Co., *Craighead 28* (2)
	EOm093*	Montana: Teton Co., *Lackschewitz 10539* (2)
	EOm441*	Montana: Beaverhead Co., *Lackschewitz11307* (2)
	EOm972*	Montana: Carbon Co., *Lackschewitz 7852* (1)
	EOw322*	Wyoming, Big Horn Co., *Williams & Williams 3221* (1)
	EOw681*	Wyoming, Big Horn Co., *Williams 1940* (1)
	EOw274*	Wyoming: Bighorn NF, *Hurd 2008* (1)
	EOw610*	Wyoming, Big Horn Co., *Dorn 3206* (1)
	EOw005*	Wyoming: Bighorn NF, *Johnston 1310* (1)
	EOw527*	Wyoming: Johnson Co., *Hardman 9555* (1)
	EOw528*	Wyoming: Sheridan Co., *Hartman & Odasz 9274* (1)
	EOw094*	Wyoming: Fremont Co., *Hartman & Haines 20250* (1)
	EOw463	Wyoming: Johnson Co., *Porter 7091* (1)
	EOw967	Wyoming: Big Horn Co., *Lichvar 1662* (1)
	EOw778*	Montana: Meagher Co., *Hitchcock & Muhlick 12233* (2)
	EO100*	Montana: Gallatin Co., *Kerstetter EO100* (2)
	EO1400N*	Montana: Teton Co., *Kerstetter EO1400* (5)
	EO1900*	Wyoming: Sheridan Co., *Kerstetter EO1900* (2)
	EO2100*	Wyoming: Sheridan Co., *Kerstetter EO2100* (2)
	EO2300*	Wyoming: Big Horn Co., *Kerstetter EO2300* (2)
	EO2500*	Wyoming: Johnson Co., *Kerstetter EO2500* (2)
	EO2800	Wyoming: Johnson Co., *Kerstetter EO2800* (19)
	EO3400	Montana: Carbon Co., *Kerstetter EO3400* (19)
	EO3700*	Montana: Lewis & Clark Co., *Kerstetter EO3700* (3)

E. radicatus	ERtype	Summit of Rocky Mountains, *Hooker s.n.*
	ERc642	Montana: Anaconda-Pintler Range, *Lackschewitz 5397* (1)
	ERc789*	Montana: Fergus Co., *Bamberg 224* (1)
	ERm252*	Montana: Flathead/Lewis & Clark Co., *Lackschewitz 9102* (3)
	ERm480	Montana: Deerlodge Co., *Lackschewitz 3770* (1)
	ERm908	Montana: Carbon Co., *Lesica 2860* (1)
	ERny991	Canada: Saskatchewan, Cypress Hills, *Macoun s.n.* (1)
	ERw477*	Idaho: Lemhi Co., *Moseley 363* (1)
	ERw157*	Montana: Madison Co., *Lackschewitz 8973* (5)
	ERw160*	Montana: Flathead/Lewis & Clark Co., *Lackschewitz 9102* (3)
	ERw697	Idaho: Fremont Co., *Moseley 835* (2)
	ER1000	Montana: Teton Co., *Kerstetter ER1000* (12)
	ER1403*	Montana: Teton Co., *Kerstetter ER1403* (1)
	ER3500	Montana: Madison Co., *Kerstetter ER3500* (17)
E. simplex	EStype	Colorado, *Greene, s.n.*
	ESw637*	Colorado: Larimer Co., *Osterhout 2860* (1)
	ESws1293*	Colorado: Chaffee Co., *Goodall s.n.* (1)
	ESws1298*	Colorado: Boulder Co., *Arnold 190A* (1)
	ESws2320*	Colorado: Gunnison Co., *Gierisch 2845* (1)
	ESws4869	Colorado: Pitkin Co., *Santos and Weber 213* (1)
	ES200*	Montana: Teton Co., *Kerstetter ES200* (4)
	ES1000*	Montana: Teton Co., *Kerstetter ES1000* (4)
	ES1100	Montana: Teton Co., *Kerstetter ES1100* (10)
	ES1300	Montana: Teton Co., *Kerstetter ES1300* (10)
	ES1500*	Montana: Teton Co., *Kerstetter ES1500* (1)
	ES2400	Wyoming: Big Horn Co., *Kerstetter ES2400* (15)
	ES2700*	Wyoming: Johnson Co., *Kerstetter ES2700* (2)
	ES2800*	Wyoming: Johnson Co., *Kerstetter ES2800* (2)

Variation between *E. lackschewitzii* and *E. ochroleucus* var. *scribneri*

Unpaired t-tests were performed a posteriori on the identical 31 quantitative variables used to determine CVA population identities in order to identify which

characters differed significantly between the two species. Computations were performed using the computer program E-Z Stat (Towner, 1990).

Uniparental Molecular Variation

Specimens for cpDNA restriction site analysis of the five species of interest were obtained from 27 sites in Wyoming, Montana, and Oregon (Table 2). Whole plants were either pressed and dried or placed in plastic bags on ice and later stored in a -80°C freezer prior to DNA isolation. Leaves were ground in CTAB DNA extraction buffer following Doyle and Doyle (1987). For a preliminary report to the Montana Natural Heritage Program, nine specimens representing six species were digested with 12 restriction endonucleases having six-base nucleotide recognition sequences. These included *Ase*I, *Bam*HI, *Bcl*I, *Dra*I, *Eco*RI, *Eco*RV, *Nsi*I, *Sac*I, *Sca*I, *Stu*I, *Xba*I, and *Xho*I. Fragments were separated on 0.9% agarose/TBE gels.

For the final analysis, total DNA from 43 specimens representing 11 species was digested with ten restriction endonucleases having six-base nucleotide recognition sequences: *Ase*I, *Bam*HI, *Bcl*I, *Bst*BI, *Dra*I, *Eco*RI, *Eco*RV, *Eco*0109I, *Hind*III, and *Stu*I. Restriction endonucleases *Nsi*I, *Sac*I, *Sca*I, *Xba*I and *Xho*I were discontinued after completion of the preliminary report due to lack of informative sites. In order to make a tentative assessment of the direction of evolution, the five study taxa were compared against *Erigeron caespitosus* Nutt., *E. filifolius* Nutt., *E. formosissimus* Greene, *E. pumilus* Nutt., *E. speciosus* (Lindl) DC, and *E. ursinus* DC. Restriction site analysis, including Southern blotting, nick-translation, hybridization to radioactive probes, and

autoradiography essentially followed Sytsma and Gottlieb (1986). Cloned fragments from the large single copy region of the cpDNA library of *Petunia* (provided by J. Palmer) were used in the hybridization experiments. Figure 7 illustrates location of cpDNA probe regions.

Table 2. Specimens of *Erigeron* species utilized for molecular variation studies. Labels refer to those shown in Figs. 12, 13, and Tables 4-7. Vouchers are deposited at MONT.

Species	Label	Procedures	Locality
E. caespitosus	EC	cpDNA, rDNA	Montana: Gallatin County
E. filifolius	EF	cpDNA, rDNA	Oregon: Deschutes County
E. formosissimus	EFO	cpDNA, rDNA	Oregon: Crook County
E. grandiflorus	EG28	cpDNA, rDNA, RAPD	Wyoming: Johnson County
	EG30	cpDNA, rDNA	Montana: Carbon County
	EG32	cpDNA, rDNA, RAPD	Montana: Carbon County
	EG34	cpDNA, rDNA, RAPD	Montana: Carbon County
E. lackschewitzii	EL1	cpDNA, rDNA	Montana: Flathead County
	EL2	RAPD	Montana: Flathead County
	EL7	cpDNA, rDNA, RAPD	Montana: Teton County
	EL10	cpDNA, rDNA, RAPD	Montana: Teton County
	EL11	cpDNA, rDNA, RAPD	Montana: Teton County
	EL12	cpDNA, rDNA	Montana: Flathead County
	EL13	cpDNA, rDNA, RAPD	Montana: Flathead County
	EL14	cpDNA, rDNA	Montana: Teton County
E. ochroleucus	EOs1a	cpDNA, rDNA	Montana: Gallatin County
var. *scribneri*	EO14N	cpDNA, rDNA, RAPD	Montana: Teton County
	EO20	cpDNA, rDNA, RAPD	Wyoming: Sheridan County
	EO21	cpDNA, rDNA	Wyoming: Sheridan County
	EO23	cpDNA, rDNA	Wyoming: Big Horn County
	EO25	cpDNA, rDNA, RAPD	Wyoming: Johnson County
	EO28	cpDNA, rDNA	Wyoming: Johnson County
	EO34	cpDNA, rDNA, RAPD	Montana: Carbon County
	EO37	RAPD	Montana: Lewis & Clark Co.
E. ochroleucus var. *ochroleucus*	EO17	cpDNA, rDNA	Montana: Sweetgrass County
E. pumilus	EP	cpDNA, rDNA	Montana: Gallatin County
E. radicatus	ER10	cpDNA, rDNA, RAPD	Montana: Teton County
	ER35	cpDNA, rDNA, RAPD	Montana: Madison County
E. simplex	ES2	RAPD	Montana: Teton County

	ES10	cpDNA, rDNA	Montana: Teton County
	ES11	cpDNA, rDNA, RAPD	Montana: Flathead County
	ES13	cpDNA, rDNA, RAPD	Montana: Flathead County
	ES24	RAPD	Wyoming: Big Horn County
	ES27	cpDNA, rDNA, RAPD	Wyoming: Johnson County
	ES28	cpDNA, rDNA	Wyoming: Johnson County
E. speciosus	ESP	cpDNA, rDNA	Montana: Gallatin County
E. ursinus	EU1	cpDNA, rDNA	Montana: Gallatin County
	EU26	cpDNA, rDNA, RAPD	Wyoming: Johnson County
	EU33	cpDNA, rDNA	Wyoming: Park County

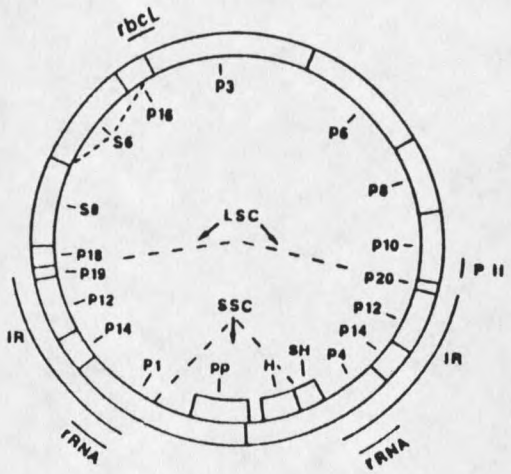
Biparental Molecular Variation

Biparental molecular variation was assessed for nuclear ribosomal DNA (rDNA) and Random Amplified Polymorphic DNA (RAPD). For rDNA single digests were performed with the same endonucleases and DNAs used for cpDNA restriction site analysis. Cloned fragments from the nuclear ribosomal repeat of *Glycine max* (PGMR1, provided by L. Zimmer) were used in the hybridization experiments.

RAPDs were used in place of allozymes due to recent studies of alpine *Erigeron* in Europe which suggested that interspecific allozyme variation was low and did not exceed intraspecific variation, due possibly to recent speciation during glaciation epochs and retention of ancestral polymorphisms (Huber and Leuchtmann, 1992). RAPDs have also been found to be of value where previous allozymic studies have failed to reveal any variation (Brauner, Crawford and Stuessy, 1992; Heidel, personal communication). Additionally, a genetic study using RAPDs has been used to determine the relationship of an endangered endemic species of *Ranunculus* (Ranunculaceae) to its close congeners and

to provide repeatable quantitative data with which to evaluate prior taxonomic classifications (Van Buren et al., 1994).

Fig. 7. Locations of cpDNA clones used against DNA of *Erigeron* species. From Sytsma and Gottlieb, 1986.



A total of 21 populations representing six *Erigeron* species (Table 2) served as sources of DNA for RAPDs. DNA extracted for cpDNA restriction site analysis was used, along with DNA isolated using a "shortcut" procedure developed at Montana State University (See Appendix C for the "quick" DNA extraction protocol). All DNA was purified over sepharose columns and concentration in ng/µl determined by DNA fluorometer (Model TKO 100, Hoefer Scientific Instruments, San Francisco). Amplifications were performed in 25-µl reaction volumes containing 85mM Tris-HCl (pH 9.0), 25mM $(NH_4)_2SO_4$, 3.5mM $MgCl_2$, 100mM each dATP, dCTP, dGTP, and dTTP,

400mM primer, 2.5U Taq DNA polymerase (Gibco BRL, Life Technologies, Inc., or Perkin Elmer, Roche Molecular Systems, Inc.), and 150 ng DNA from dried specimens. Primers consisted of random ten-base sequences from the 'A' kit obtained from Operon Technologies (A-01, A-02, A-03, A-04, A-05, A-07, A-09, A-11, and A-13). Each amplification was performed using a single primer with two replicates where possible within time constraints. Amplifications were carried out in either a Temp.Tronic thermocycler (Barnstead/Thermolyne Corp.) or PTC-100 Programmable Thermal Controller (MJ Research, Inc.) programmed for 40 cycles of 1 minute at 94°C, 1 minute at 36°C, and 2 minutes at 72°C, following Williams et al. (1990), with a postdwell extension time of 5 minutes at 72°C. Amplification products were separated on 1.5% agarose/TBE gels and photographed on a UV illuminator. Data were analyzed using phenetic procedures on fragments scored by simple presence or absence. Jaccard's coefficient of similarity was utilized to obtain estimates of genetic similarity for phenetic analysis.

Determination of Ploidy Level

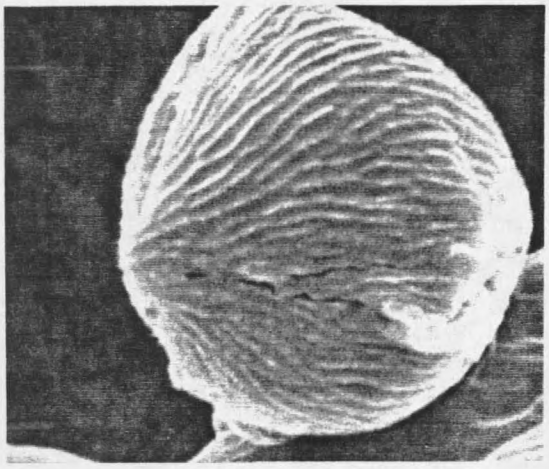
Immature capitula of *Erigeron lackschewitzii* gathered in order to obtain meiotic chromosome counts were found to lack pollen. Several specimens of *E. lackschewitzii, E. simplex* and *E. ochroleucus* var. *scribneri* were sent to Western State College in Gunnison, Colorado, for scanning electron microscopy. A single pollen grain of *E. lackschewitzii* was photomicrographed by Wayne Warnken and found to be abnormal (Figure 8a). Pollen grains of *E. simplex* (Figure 8b) and *E. ochroleucus* var. *scribneri*

(Figure 8c) were typical of the Astereae tribe of Asteraceae (cf. Skvarla, et al., 1977). Pollen of *E. radicatus* and *E. grandiflorus* were not available for SEM.

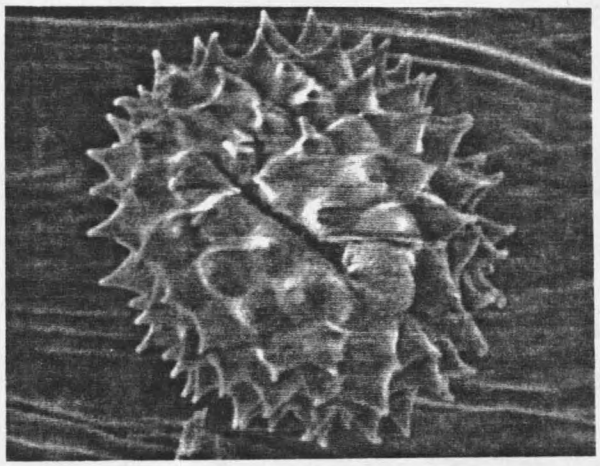
Due to absence of pollen in plants belonging to *E. lackschewitzii*, seed germinations for mitotic counts were attempted using root tips. The procedure involved surface sterilizing disc floret achenes in a mixture of double-deionized water (840 µl/ml), dilute clorox (150 µl/ml) and 20% SDS (10 µl/ml) followed by a wash of Captan fungicide. The achenes were then placed in Petri dishes on filter paper for germination. Each filter paper was moistened with double-deionized water, to which was added 10^{-6}M gibberellic acid to stimulate germination. The Petri dishes were sealed with Parafilm and placed under GTE/Sylvania Gro-Lux fluorescent lights with 24-hour illumination until germination. Germlings were transferred into 8-hydroxyquinoline for two to four hours, then killed and fixed in Farmer's Fluid (3:1 95% EtOH:glacial acetic acid) 6-24 hours, then stored in 70% EtOH.

Because of reported correlations between polyploidy and increased cell size (Babcock and Stebbins, 1938; Cronquist, 1988), counts of the number of stomates filling the viewing area under 200X magnification and measurements of stomate size under 100X magnification were undertaken. Counts and measurements were taken from three to five populations of each of the five species of interest. Pairwise comparisons of diploid *E. simplex* and *E. ochroleucus* var. *scribneri* with triploid *E. grandiflorus* and tetraploid *E. radicatus* were subjected to unpaired t-tests in order to ascertain whether stomate counts and sizes differed significantly among the different ploidy levels and to estimate ploidy level of *E. lackschewitzii* individuals. Calculations were performed using the computer

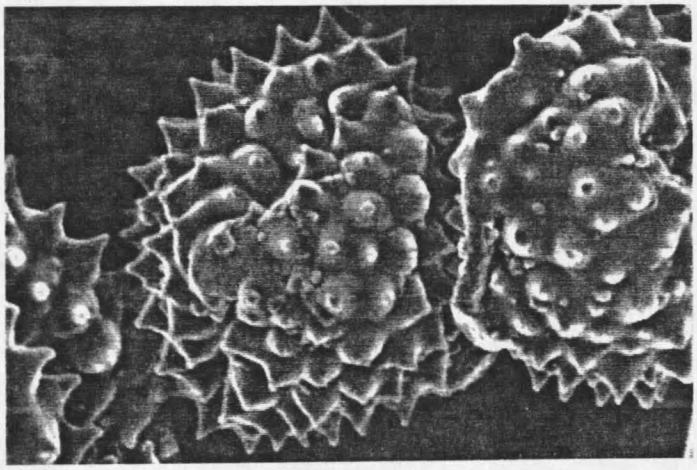
Fig. 8. Scanning electron micrographs of pollen grains from three species of *Erigeron*. (a) *Erigeron lackschewitzii* x 3500. (b) *Erigeron ochroleucus* var. *scribneri* x 3500. (c) *Erigeron simplex* x 3500. SEM photos by Wayne Warnken, Western State College, Gunnison, Colorado.



(a)



(b)



(c)

program E-Z Stat (Towner, 1990). Stomate studies were undertaken in response to lack of success in obtaining chromosome counts.

CHAPTER THREE

RESULTS

Geographical Variation

Cluster analysis of phenetic distances calculated between all pairwise comparisons of individuals belonging to *E. grandiflorus*, *E. simplex*, *E. lackschewitzii*, *E. ochroleucus* var. *scribneri*, and *E. radicatus* revealed a distinction between a group containing *E. grandiflorus* and *E. simplex* and a group with *E. lackschewitzii*, *E. ochroleucus* var. *scribneri*, and *E. radicatus* (Figure 9). Herbarium specimens EGm085UR and EGm924LR from the University of Montana clustered with *E. lackschewitzii*, reflecting their clear resemblance to *E. lackschewitzii* and misidentification as *E. grandiflorus*.

The majority of plants confirmed as *E. ochroleucus* var. *scribneri* through visual examination ("a" on Figure 9) clustered near *E. lackschewitzii* ("b" on Figure 9). All of these *E. ochroleucus* var. *scribneri* specimens are from Montana and Wyoming. A smaller group of *E. ochroleucus* var. *scribneri* ("c" on Figure 9) that clustered with *E. radicatus* are from Wyoming, likewise a third group of *E. ochroleucus* var. *scribneri* clustering near *E. lackschewitzii* ("d" on Figure 9). Specimens EO3702 and EO3703 ("e" on Figure 9), clustering near *E. lackschewitzii*, are sympatric with *E. lackschewitzii*.

Fig. 9. Cluster analysis of phenetic distance of all pairwise combinations of *Erigeron* taxa. See Table 1 for label identification. Starred accessions are type specimens. See text for discussion of specimens marked a-f.

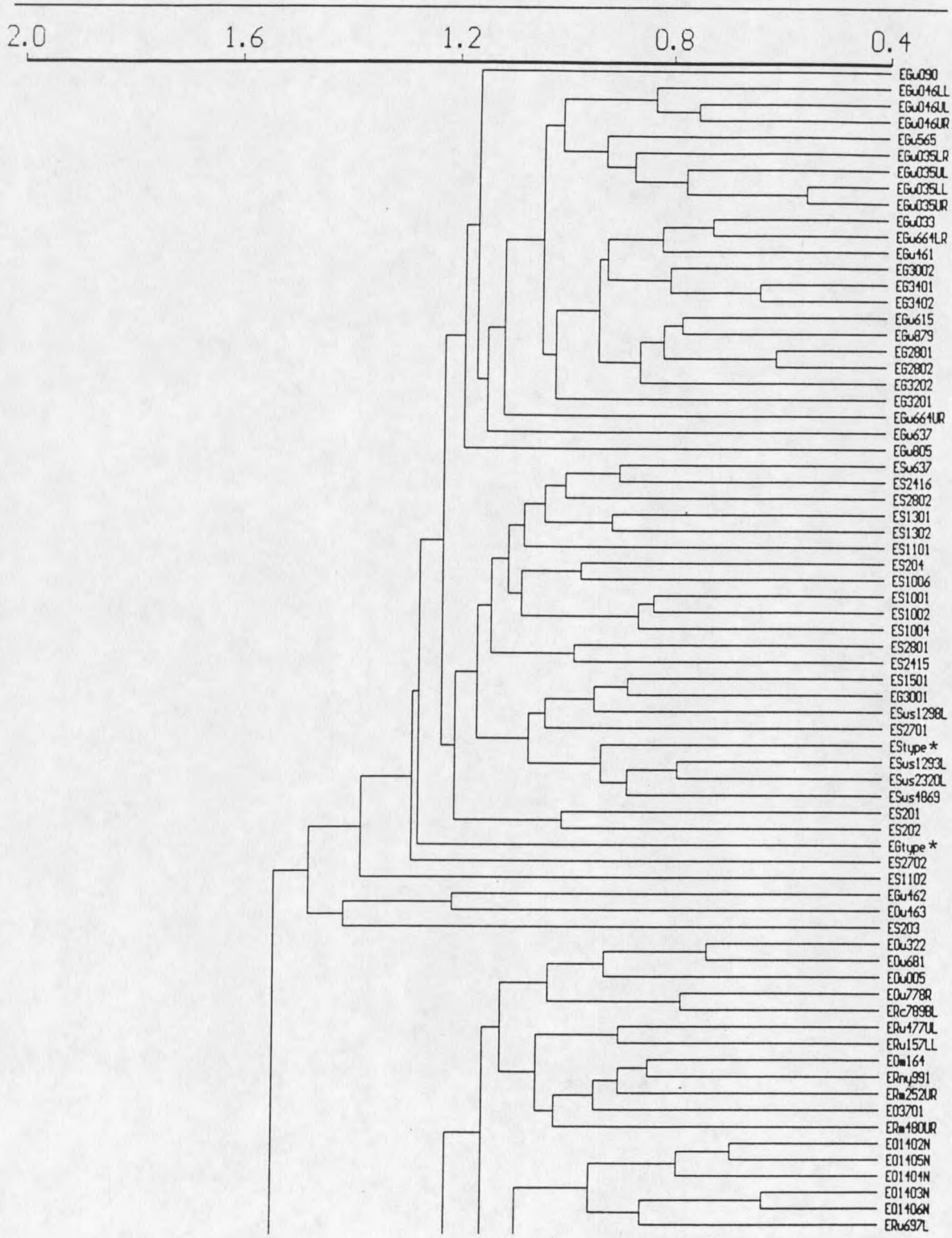


Fig. 9 — continued

Erigeron ochroleucus var. *scribneri* is morphologically more distinct from *E. lackschewitzii* than it is from *E. radicatus*. Eighteen specimens labeled as *E. ochroleucus* var. *scribneri* clustered with *E. radicatus* (Figure 9). Among these were several specimens from the Bob Marshall and Scapegoat Wilderness areas ("f" on Figure 9). These results confirm the confusion surrounding identity of specimens labeled as *E. ochroleucus* var. *scribneri* in northwestern Montana.

Visual examination of many specimens correctly identified as *E. radicatus* revealed gross abnormalities in floret morphology. Abnormalities of disc florets included wide variation in shape and size, highly irregular pappus of twisted uneven bristles, lack of discrete corolla limbs, and tiny anthers. Also observed were a wide range of achene trichome densities and occasional fusing of achenes. Ray florets displayed wide variation in shape, from flat and wide to inrolled and narrow. These abnormalities were especially evident from sites in the Gravelly Range of southwestern Montana.

Erigeron radicatus ray florets were occasionally tubular and filiform, unlike most species of *Erigeron*. This variation in ray florets was observed in *E. radicatus* plants from Sock Lake in the Bob Marshall Wilderness and also in individuals tentatively identified as *E. ochroleucus* var. *scribneri* from Crown Mountain in the Scapegoat Wilderness (See Figure 2 for site locations). Tubular ray florets are known to occur in *E. acris*, a diploid biennial or perennial of rocky places in the mountains of Montana. Since plants belonging to *E. radicatus* are tetraploids, the species may have been formed by hybridization between *E. acris* and *E. ochroleucus* var. *scribneri* and/or an as-yet unknown taxon.

Molecular analysis of *E. acris* and other possible progenitors may reveal whether *E. radicatus* arose through one or more hybridization events.

Specimens labeled as *E. ochroleucus* var. *scribneri* from Crown Mountain (EO3701-EO3703) require special mention since Crown Mountain is one of only two sites where *E. lackschewitzii* may be sympatric with *E. ochroleucus* var. *scribneri*. Two other sites (Mount Wright and Mount Patrick Gass on Figure 2) where sympatric populations of *E. ochroleucus* var. *scribneri* have been reported are locations more accurately described as containing *E. radicatus*. Crown Mountain, however, is unusual in that the specimens identified as *E. ochroleucus* var. *scribneri* occasionally possess the tubular filiform ray florets observed in some *E. radicatus*. The number of ray florets is highly variable, from 42 in one specimen to 78 in another and 102 in a third, with the number of pappus bristles at 6, 7 and 9 respectively. Pappus bristles for these specimens are closer in range to that found for *E. radicatus* (5-13) than *E. ochroleucus* var. *scribneri* (10-17), although the number of ray florets is higher than observed in *E. radicatus* (12-50) and more consistent with *E. ochroleucus* var. *scribneri* (30-88).

In addition to the presence of tubular ray florets in Crown Mountain plants, there are abnormalities in floret morphology and gross differences in achene sizes similar to those found in *E. radicatus*. For example, specimen EO3702 possesses two types of ray florets: "normal" laminar rays about 8 mm long and 2 mm wide, and long narrow tubular rays about 10.2 mm long and 0.7 mm wide. The tubular ray florets displayed long narrow achenes (unmeasured) while the shorter "normal" ray florets displayed large achenes more

characteristic of *E. lackschewitzii* and *E. radicatus* than *E. ochroleucus* var. *scribneri*. In addition, disc florets contained very little pollen.

Solbrig (1962) encountered a similar type of situation in achenes of the southern South American *Erigeron andicola* complex. In some plants achenes were shrunken and aborted, while in others fully formed achenes were present at a very early stage of floret development, suggesting some sort of precocious fertility. If staining is taken as a measure of pollen fertility, Solbrig's results suggested that all degrees from complete fertility to almost complete sterility were present. Solbrig concluded that since apomixis is not rare in *Erigeron*, its presence may have caused the observed patterns of variation in achene development. By extension, hybridization followed by some degree of apomixis may be present in populations of *E. ochroleucus* var. *scribneri* and *E. radicatus* in Montana, confounding species boundaries. Genetic analyses beyond the scope of this study may be required to delimit the two species.

Population Variation

The initial CVA (with *E. grandiflorus*, *E. simplex*, *E. ochroleucus* var. *scribneri* and *E. radicatus* populations only) resulted in all *E. lackschewitzii* "unknowns" grouping with *E. ochroleucus* var. *scribneri*, corroborating the obvious morphological resemblance of the two species. In the second CVA (with north and south populations of *E. lackschewitzii* included) all but one *E. lackschewitzii* specimen (ELm490) were assigned to *E. lackschewitzii*. ELm490 has shorter stem and involucre trichomes and shorter outer phyllaries than other *E. lackschewitzii* plants examined, with a considerably greater

number of narrow rays. These attributes could more closely align this specimen with *E. ochroleucus* var. *scribneri* in a computer analysis. However, phenetic clustering placed ELm490 close to the type specimen for *E. lackschewitzii*, verifying its strong resemblance to *E. lackschewitzii*.

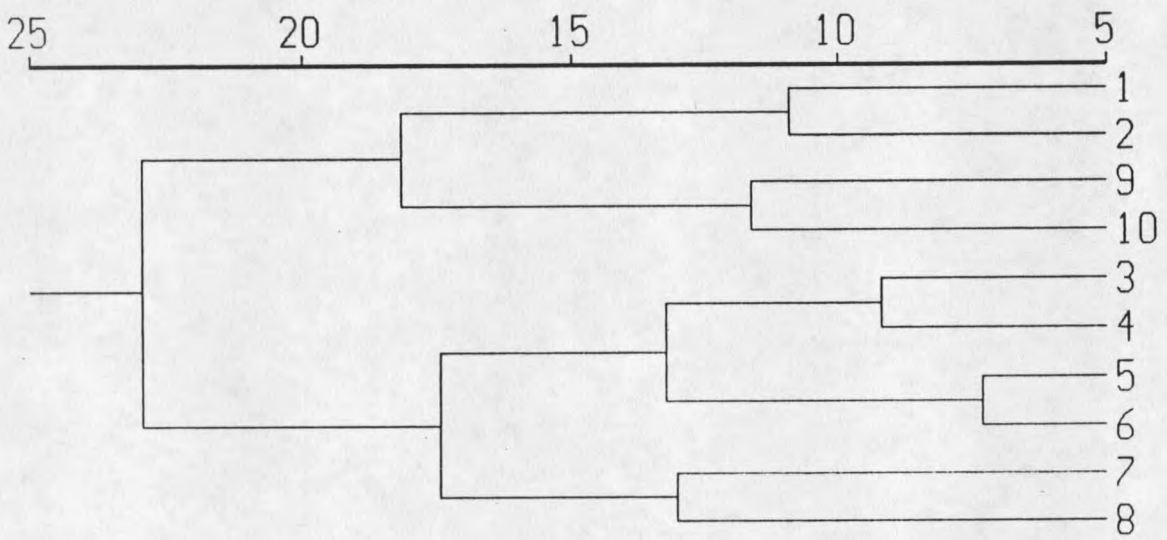
A cluster analysis of CVA-generated generalized distances of the ten populations mirrored results from geographic analysis, i.e., a cluster containing *E. grandiflorus* and *E. simplex* and a cluster with *E. lackschewitzii*, *E. ochroleucus* var. *scribneri*, and *E. radicatus* (Figure 10). Within these two major groups were smaller clusters separating each species into its two populations.

Using the ten population samples described above to establish population parameters for each of the five species, a CVA "classification test" was performed on a selected set of specimens. Of 21 specimens assigned to *E. grandiflorus*, CVA placed two with *E. lackschewitzii* and three with *E. simplex*. Twelve of 39 specimens identified as *E. ochroleucus* var. *scribneri* were consigned to *E. radicatus*, while seven of eight specimens identified as *E. radicatus* were retained with *E. radicatus*. All 22 specimens identified as *E. simplex* remained within *E. simplex* in the CVA classification test.

Comparison of the CVA classification test results with geographic cluster analysis results in conflicting placement of some of the above-listed specimens. Visual re-examination of the specimens in question confirms placements as follows. The three specimens identified as *E. grandiflorus* and placed with *E. simplex* should be retained within *E. grandiflorus*. *E. grandiflorus* specimens placed with *E. lackschewitzii* (previously discussed) were simply misidentified and properly belong with *E.*

lackschewitzii. With one exception, all of the 12 individuals originally labeled as *E. ochroleucus* var. *scribneri* but classified as *E. radicatus* by the CVA test belong with *E. radicatus*. The lone exception displays a morphology intermediate between *E. ochroleucus* var. *scribneri* and *E. radicatus* and cannot be correctly placed without DNA analysis, although habitat information would tend to place it among *E. ochroleucus* var. *scribneri*. The *E. radicatus* individual placed by the CVA test with *E. ochroleucus* var. *scribneri* should be retained within *E. radicatus*.

Fig. 10. UPGMA dendrogram reflecting generalized distances derived from canonical variates analysis (CVA) of ten populations from five *Erigeron* taxa. *E. grandiflorus* = populations 1, 2; *E. simplex* = populations 9, 10; *E. lackschewitzii* = populations 3, 4; *E. ochroleucus* var. *scribneri* = populations 5, 6; *E. radicatus* = populations 7, 8.



Variation between *E. lackschewitzii* and *E. ochroleucus* var. *scribneri*

Twenty-four of the quantitative characters varied significantly ($p < 0.05$) in unpaired t-tests between the two populations of *E. lackschewitzii* and the two of *E.*

ochroleucus var. *scribneri* (Table 3). Eight of the significant variables are illustrated in graph form in Figure 11. The two populations of *E. ochroleucus* var. *scribneri* were determined a priori to be accurate representatives of the species and are not confused with *E. radicatus*.

Table 3. Quantitative characters found to differ significantly ($p < 0.05$) between populations of *E. lackschewitzii* (EL) and *E. ochroleucus* var. *scribneri* (EO).

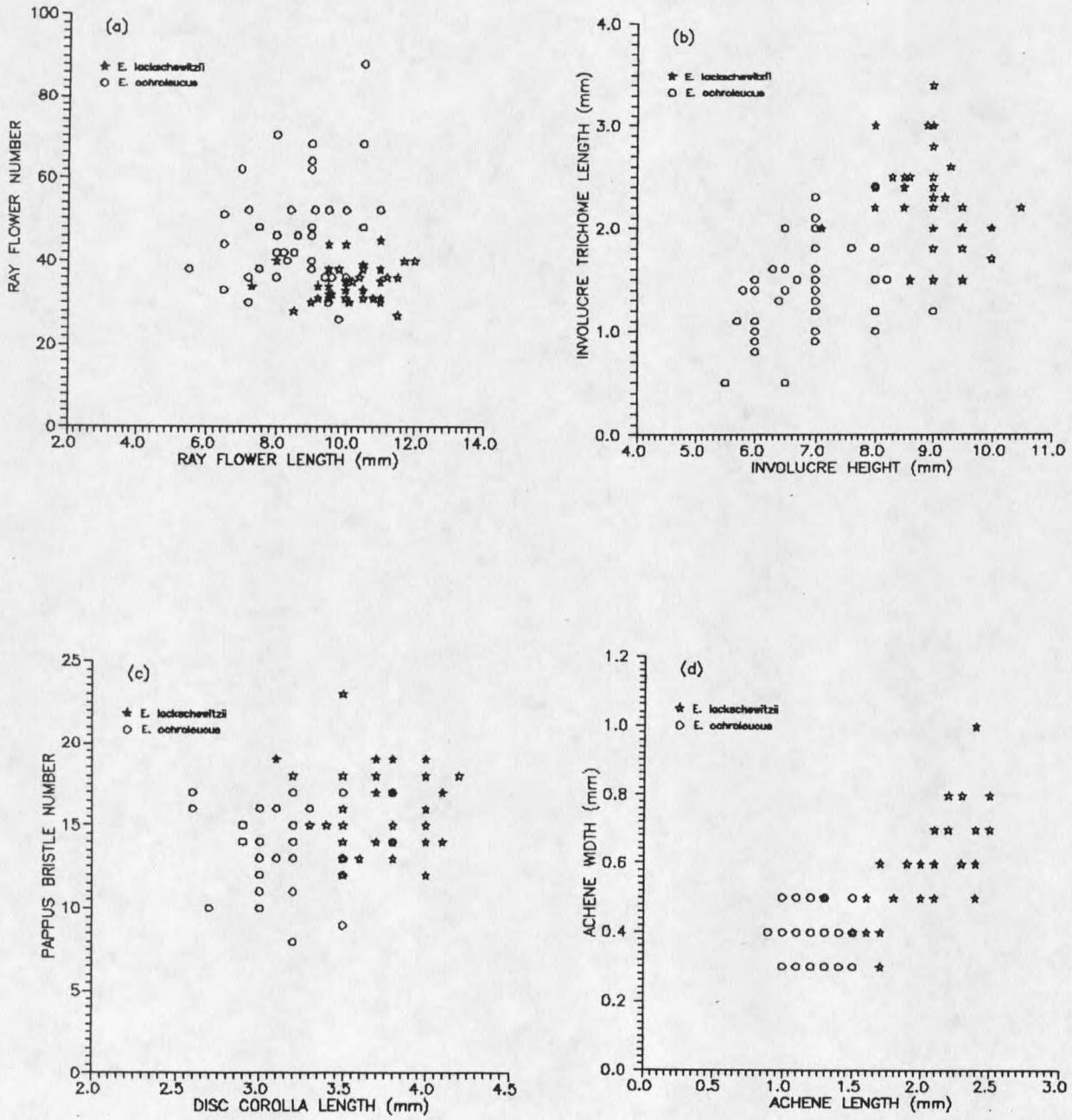
Character Number	Character Description	Mean EL	Mean EO	Variance EL	Variance EO
	Habit/Root System:				
2	Width below branches	0.579	0.465	0.010	0.012
3	Width below caudex	0.435	0.313	0.020	0.010
	Flowering Stem:				
6	Width below head	1.170	0.988	0.051	0.011
8	Trichome length	1.550	0.861	0.165	0.078
	Cauline Leaves:				
9	Number of leaves	5.375	8.175	6.343	2.712
	Basal Leaves:				
10	Length of longest leaf	33.325	45.250	117.071	120.192
11	Width of longest leaf	1.872	1.515	0.256	0.112
12	Length of widest leaf	30.100	38.475	117.746	63.025
	Involucre:				
14	Width	14.038	12.425	2.428	2.353
15	Height	8.913	6.900	0.372	0.626
18	Outer phyllary height	6.762	5.253	0.298	0.508
19	No. trichomes/mm	9.750	13.550	2.962	4.715
20	Trichome length	2.267	1.425	0.209	0.187
	Ray Florets:				
21	Length	10.113	8.645	0.960	1.692
22	Width	1.933	1.327	0.104	0.112
23	Number of rays	34.975	47.275	19.102	160.769
24	No. apex notches	1.975	1.450	0.076	0.459
	Disc Florets:				
25	Corolla length	3.662	3.115	0.078	0.073
26	Corolla width	0.850	0.725	0.014	0.011
27	Style branch length	0.863	0.740	0.015	0.014

	Achenes:				
28	Pappus height	3.305	2.563	0.067	0.071
29	No. of pappus bristles	16.275	13.550	7.128	5.023
30	Length	2.060	1.180	0.096	0.034
31	Width	0.595	0.380	0.019	0.005

Further observations regarding quantitative characters are as follows. First, although the average involucre on *E. lackschewitzii* is about as large as those found in *E. simplex* and *E. grandiflorus* (14.04 mm versus 13.19 mm and 14.57 mm, respectively), the number of ray florets averaged about half as many (34.98 versus 61.49 and 80.30 respectively). Therefore, the correlation of increased involucre diameter with more and narrower ray florets found in monocephalous arctic/alpine *Erigeron* discussed previously fails to hold for *E. lackschewitzii*. Secondly, Spongberg's (1971) contention that involucre height is correlated with stem length was less pronounced in *E. lackschewitzii* and *E. ochroleucus* var. *scribneri*. The southern population of *E. lackschewitzii* is composed of low individuals with stems averaging 2.67 cm versus 6.46 cm in the northern population, yet involucre heights for both populations were not significantly different ($p = 0.156$). Likewise, stem lengths of plants belonging to *E. ochroleucus* var. *scribneri* differed significantly between populations ($p < 0.001$) yet involucre heights were practically identical ($p = 0.814$). Visual observation of population habitats suggests that stem length for both *E. ochroleucus* var. *scribneri* and *E. lackschewitzii* may be in part inversely correlated with windy conditions.

Concerning qualitative characters, the following nonstatistical observations between *E. lackschewitzii* and *E. ochroleucus* var. *scribneri* were noted. Plants belonging

Fig. 11. Graphs depicting eight variables found to vary significantly between most northern and most southern populations of *E. lackschewitzii* and *E. ochroleucus* var. *scribneri* collected during 1992 and 1993. Stars = *E. lackschewitzii*, circles = *E. ochroleucus* var. *scribneri*.



to *E. lackschewitzii* exhibit less tendency toward multiple flower stems, more often possess turbinate involucres, and display fewer strigose trichomes on phyllaries than *E.ochroleucus* var. *scribneri*. Additionally, *E. lackschewitzii* ray florets are usually held at an angle oblique to the involucre (versus at right angles), are consistently pink in color (versus white to pink and lavender), and are relatively deeply notched at the apex (versus varied). Finally, *E. lackschewitzii* individuals were universally lacking normal pollen and were consistently and persistently sparse in achene pubescence when compared with plants belonging to *E. ochroleucus* var. *scribneri*.

Uniparental Molecular Variation

A total of approximately 305 cpDNA restriction sites were surveyed, of which 19 sites were informative (Table 4). Probe regions P6 and P3 of the *Petunia* chloroplast genome displayed the greatest variation, each with seven restriction site mutations. Probe regions S6 and S8 contained two site mutations each, while P8/10 contained a single site. Smaller fragments associated with the loss of the larger molecular weight bands may have gone undetected, either because they were too small or they lie outside the probe regions (e.g., Doebley, et al, 1992; Fenster and Ritland, 1992), but were inferred to be present due to a lack of length mutations (e.g., Soltis, et al., 1988).

Unique cpDNA mutations were found for four of the five species of interest: *E. lackschewitzii* (2), *E. radicatus* (1), *E. simplex* (2), and *E. grandiflorus* (1). All restriction sites noted for specimen EO17 of *E. ochroleucus* var. *ochroleucus* were consistent with those found in *E. ochroleucus* var. *scribneri*. Specimen EO14N of *E. ochroleucus* var.

scribneri shared restriction sites with *E. radicatus*, confirming morphological analyses which grouped all specimens from this site (Mount Wright, Figure 2) with *E. radicatus*. Polymorphisms were observed only among populations of *E. simplex*, possibly reflecting the wider geographical range and considerable morphological variation known to exist in this species (Spongberg, 1971).

Table 4. Restriction site mutations of cpDNA in ten species of *Erigeron*. The five species of interest and unique sequences are listed first under "Mutation." Labels with no number designations indicate species. See Table 2 for label identification.

Enzyme	Region	Mutation (kb)	Individuals/taxa with mutated DNAs
*Ase*I	S8	2.8 + 0.6[a] = 3.4	EL
*Ase*I	P6	1.4 + 1.4 = 2.8	EL
*Ase*I	P6	4.4 = 3.8 + 0.6	EG, ES
*Bam*HI	P6	3.7 = 2.0 + 1.7	EO14N, ER
*Bam*HI	P6	2.2 + 1.2 = 3.4	EL, EO, ER, EF, EP
*Bam*HI	P6	3.1 + 0.3[a] = 3.4	ES27, ES28
*Bcl*I	S6	9.2 = 5.2 + 4.0	EL, EO, ER
*Bcl*I	S8	5.8 + 1.6 = 7.4	EO14N, ER, EP
*Bst*BI	P3	1.55 + 0.65 = 2.2[b]	ES28, EFO, ESP, EU
*Bst*BI	S6	3.8 = 2.0 + 1.8	ES
*Bst*BI	P8/10	4.4 = 3.5 + 0.9	EL, EO (excluding EO14N)
*Dra*I	P3	6.7 + 1.4 = 8.1[b]	EF, EFO, EU
*Dra*I	P6	3.4 + 0.5[a] = 3.9	EG
*Eco*0109I	P3	5.4 = 4.2 + 0.8 + 0.4[ab]	ES
*Eco*0109I	P3	4.6 + 0.8 = 4.2 + 0.8 + 0.4[ab]	EG, EL, EO (excluding EO14N), EF
*Eco*0109I	P3	1.4 + 0.4[a] = 1.8	ES, EG
*Eco*0109I	P3	2.6 + 2.0 = 4.6	EO14N, ER, EP
*Eco*RI	P6	4.1 = 3.8 + 0.3[a]	EL, EO14N*, ER
*Hind*III	P3	3.9 + 0.2[a] = 4.1	ES, EG

[a]Fragments were not seen either because they were too small to detect or they lie outside the probe regions. [b]Direction of evolution unable to determine due to split in outgroup (see text for discussion). *Unable to determine fragment pattern for other populations of *E. ochroleucus* var. *scribneri*.

The presence of a cpDNA mutation unique to *E. grandiflorus* and two mutations found in *E. simplex* but not shared with *E. grandiflorus* confirms geographic and population cluster analyses based on morphology. These results support Spongberg's (1971) delimitation of *E. grandiflorus* and *E. simplex* as distinct species in Wyoming and southern Montana. Morphological and genetic similarity of the two species suggests some sort of hybrid origin of triploid *E. grandiflorus* from diploid *E. simplex*. Genetic and morphological analyses of other possible progenitors may reveal whether *E. grandiflorus* originated from hybridization events involving *E. simplex* and another species or from fertilization of unreduced egg cells by reduced pollen within *E. simplex* populations.

A genetic distance matrix (Table 5) incorporating restriction site mutations listed in Table 4 was computed according to Nei (1987); computations were performed using the computer program RESTSITE (Miller, 1990). Interspecific divergence values in *Erigeron* species examined varied from 0.0004 to 0.0136 nucleotide substitutions per site, a range comparable to values reported for other congeneric species. For example, values of 0.0000-0.0070 were found for *Lycopersicon* (Palmer and Zamir, 1982), and 0.0000-0.0260 for *Pisum* (Palmer et al., 1985). A dendrogram resulting from cluster analysis of the genetic distance matrix (Figure 12) was generated using the computer program RESTSITE (Miller, 1990).

Variation in fragment patterns of the five outgroup species *E. speciosus*, *E. filifolius*, *E. formosissimus*, *E. pumilus*, and *E. ursinus* confounded attempts to assess the direction of evolution in several cases. However, because this study was essentially an effort to delimit and circumscribe *E. lackschewitzii* and all potentially confusing species,

no phylogenetic analysis was undertaken. Nei's genetic distances and cluster analysis were performed in essence to delimit groups and only by indirect inference suggest relationships.

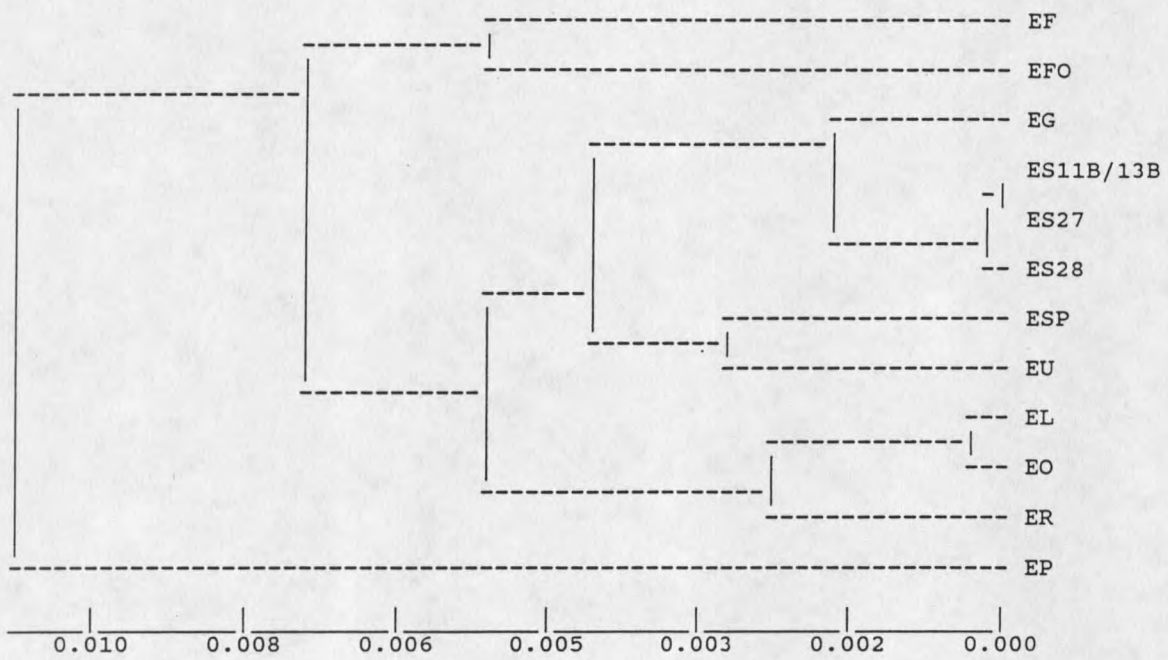
Table 5. Matrix of genetic distances estimated from restriction site data for all pairwise comparisons of cpDNA genomes. See Table 2 for label identification. Labels with no numbers indicate species. EO14N is included in ER, reflecting the proper identification of this population as *E. radicatus*.

	EF	EFO	EG	EL	EO	EP	ER
EF	1.000000						
EFO	0.005606	1.000000					
EG	0.009681	0.008753	1.000000				
EL	0.007134	0.009163	0.005211	1.000000			
EO	0.005194	0.006545	0.004405	0.000429	1.000000		
EP	0.008489	0.011461	0.012992	0.011386	0.009873	1.000000	
ER	0.007667	0.007780	0.006137	0.003017	0.002186	0.009588	1.000000
ES11B/13B	0.009535	0.007708	0.001036	0.005975	0.005164	0.013501	0.006448
ES27	0.009450	0.008192	0.001867	0.005928	0.005123	0.013368	0.006397
ES28	0.010234	0.007329	0.002497	0.006607	0.005809	0.013615	0.007081
ESP	0.007780	0.004476	0.005206	0.006275	0.003952	0.010437	0.006275
EU	0.008364	0.005241	0.003591	0.005002	0.004192	0.011523	0.005463

	ES11B/13B	ES27	ES28	ESP	EU
ES11B/13B	1.000000				
ES27	0.000000	1.000000			
ES28	0.000619	0.000000	1.000000		
ESP	0.004550	0.005468	0.004731	1.000000	
EU	0.003855	0.004718	0.004032	0.003114	1.000000

Chloroplast DNA variation among and within *Erigeron* species studied herein reflect variation patterns found with other Asteraceae genera. Some groups exhibit few, if any, polymorphisms (e.g., *Antennaria*, *Coreopsis*, *Marshallia*) whereas others (e.g., *Krigia*) have some of the highest levels of intraspecific variation documented so far in angiosperms (Jansen, et al., 1992).

Fig. 12. Dendrogram reflecting genetic distances derived from the number of nucleotide substitutions per site. See Table 2 for label identification. Labels with no numbers indicate species. EO14N is included in ER, reflecting the proper identification of this population as *E. radicatus*.



Biparental Molecular Variation

Nuclear genes that code for rDNA are repeated thousands of times within the typical plant genome (Schaal and Learn, 1988). Ribosomal DNA is arranged in tandem repeats, each repeat unit consisting of a transcribed region separated from the next region by an intergenic spacer (Hamby and Zimmer, 1992). The transcribed region consists of an 18S gene, a 5.8S gene and a 26S gene, along with external and internal transcribed spacers, all of which total approximately 6 kb. Intergenic spacers (IGS) separate the tandem repeat units and are themselves variable in length, causing repeat units to range in

size from about 8 to 15 kb (Hamby and Zimmer, 1992). Restriction enzymes which cut repeat units in one or two places produce bands on a Southern blot, allowing size analysis of one unit.

The sizes of the tandem repeats in the *Erigeron* taxa studied are about 14 kb and 10.6 kb (Table 6). Monomorphic fragment patterns were detected by two restriction endonucleases, *Bst*BI and *Stu*I. Although inconclusive, results paralleled those found in cpDNA for *E. lackschewitzii* and *E. ochroleucus* var. *scribneri*, i.e., rDNA band patterns were similar for the two species, with one exception. *Erigeron ochroleucus* var. *ochroleucus* displayed two bands of 6.0 kb and 4.6 kb with *Hind*III while a single *E. lackschewitzii* specimen displayed a band of 10.6 kb, which may ultimately prove to denote a polymorphism within the *E. lackschewitzii/E. ochroleucus* complex. Lack of resolution with other populations of *E. lackschewitzii* and *E. ochroleucus* var. *scribneri* did not allow for determination of consistency with respect to this mutation. As with cpDNA restriction sites, no patterns emerged among the outgroup species so as to estimate the direction of evolution.

The use of randomly amplified primers in RAPD analysis can detect polymorphisms in the absence of specific nucleotide sequence information, and the polymorphisms can function as genetic markers (Williams et al., 1990). Nearly all RAPD markers are dominant, although it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous or homozygous for a RAPD marker. It is also unknown whether RAPDs represent a random sample from throughout the genome (Brauner, et al., 1992).

Table 6. Taxa grouped by rDNA repeat units. See Table 2 for label identification. EO14N = ER, reflecting the proper identification of this population as *E.* radicatus. Labels with no numbers indicate species.

Enzyme	Fragment (kb)	Taxa
*Bam*HI	6.7 + 3.0 + 1.3	EL, ER, ES, EU, EC, ESP
*Bcl*I	14.0	EL, EO, EG, EF, EU, EFO
	7.0 + 7.0	ER, ES, EC, ESP
*Bst*BI	14.0	All species
*Dra*I	9.0 + 1.6	EL, EO, EU, EP, ESP
	6.0 + 3.0 + 1.6	ES, EC
*Eco*0109I	11.0 + 1.6 + 1.4	EL, EO, ER, EG, EU, ESP, EP
*Eco*RI	6.7 + 3.8 + 3.4	EL, EO28, ES, ESP
*Hind*III	10.6	EL13B, EF
	6.0 + 4.6	EO17
*Stu*I	14.0	All species

Nine decanucleotide RAPD primers of the 'A' kit produced 122 bands that could be repeatably scored. Only fragments with molecular sizes between 200-1000 base pairs were scored due to inconsistency in resolution above and below this range. Application of the UPGMA clustering algorithm of the SAHN and TREE options of NTSYS-pc (Rohlf, 1993) to a matrix of Jaccard similarity coefficients derived from the presence/absence of RAPD fragments (Table 7) produced the dendrogram illustrated in Figure 13.

RAPD markers revealed no genetic diversity among individuals from three populations of *E. lackschewitzii*. The lack of variation in RAPDs among *E. lackschewitzii* populations probably reflects recent ancestry from a single or a few ancestral populations of its presumed progenitor (e.g., Fenster and Ritland, 1992), and maintenance of the genotype through apomictic reproduction.

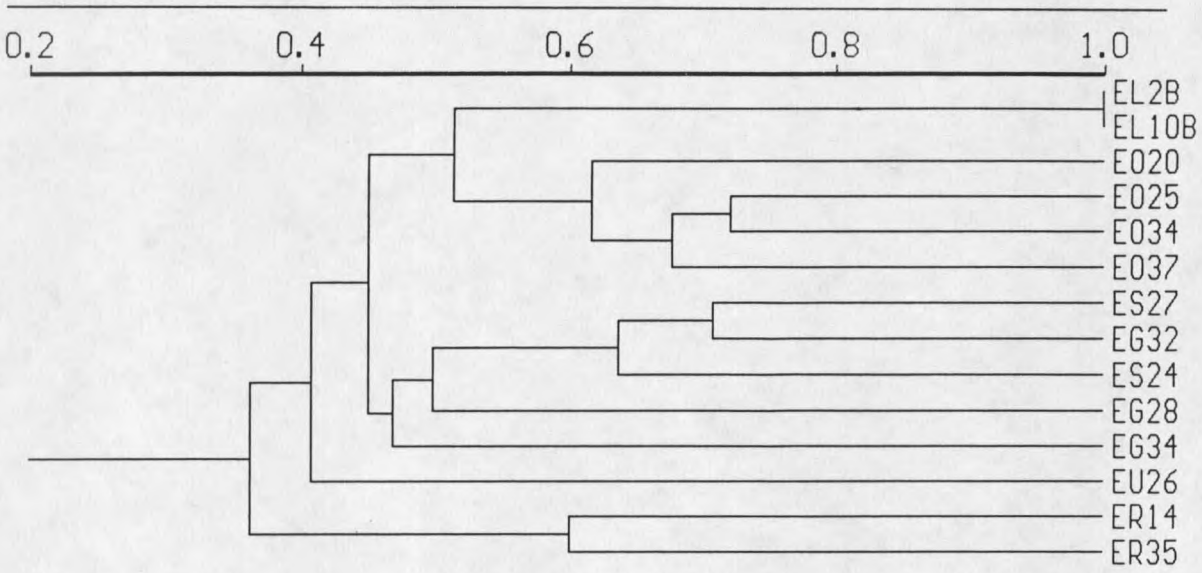
Table 7. Matrix of Jaccard similarity coefficients based on the presence/absence of fragments for all pairwise comparisons of RAPD genomes. See Table 2 for identification. ER14 = EO14N in Table 2, reflecting the proper identification of this population as *E. radicatus*.

	EL2B	EL10B	EO20	EO25	EO34	ES27	EG28	EG32
EL2B	1.000000							
EL10B	1.000000	1.000000						
EO20	0.564103	0.540230	1.000000					
EO25	0.550000	0.516854	0.658537	1.000000				
EO34	0.523810	0.472222	0.585714	0.721311	1.000000			
ES27	0.444444	0.437500	0.426829	0.475000	0.484375	1.000000		
EG28	0.440000	0.433735	0.456790	0.455696	0.491803	0.492537	1.000000	
EG32	0.509804	0.440678	0.483333	0.527273	0.520000	0.707317	0.528302	1.000000
ER14	0.440000	0.409639	0.432099	0.525641	0.451613	0.434783	0.328947	0.444444
EU26	0.411765	0.394737	0.413333	0.397436	0.440678	0.400000	0.359375	0.437500
EO37	0.470588	0.470588	0.606061	0.709677	0.645161	0.500000	0.392857	0.583333
ES24	0.390625	0.380952	0.442623	0.426229	0.478261	0.642857	0.473684	0.631579
EG34	0.500000	0.500000	0.363636	0.300000	0.333333	0.433333	0.379310	0.578947
ER35	0.360000	0.333333	0.434783	0.450000	0.450000	0.320000	0.380952	0.217391

	ER14	EU26	EO37	ES24	EG34	ER35
ER14	1.000000					
EU26	0.352113	1.000000				
EO37	0.424242	0.470588	1.000000			
ES24	0.298246	0.425532	0.545454	1.000000		
EG34	0.280000	0.333333	0.333333	0.480000	1.000000	
ER35	0.600000	0.200000	0.307692	0.227273	0.210526	1.000000

Few reports on intra- and interpopulational variation in RAPDs are available for evaluating the level of variation in *E. lackschewitzii* as compared to other endemic species. However, studies by Brauner, Crawford and Stuessy (1992) on the rare family Lactoridaceae have found low variation among populations of *Lactoris fernandeziana* endemic to the island of Masatierra in the Juan Fernandez Archipelago. The level of variation in RAPDs was low relative to other endemic Juan Fernandez species surveyed, and nearly all variants were restricted to single populations, suggesting to the authors minor genetic differences among populations. A previous study of allozymes at 22 loci in 83 plants failed to reveal variation.

Figure 13. Dendrogram reflecting generalized distances derived from the presence or absence of RAPD fragments. See Table 2 for label identification. ER14 = EO14N in Table 2, reflecting the proper identification of this population as *E. radicatus*.



In contrast to *E. lackschewitzii*, the other five taxa displayed considerable individual variation. The levels of RAPD variation in the other *Erigeron* species are high enough in some cases so as to confound species relationships. However, this is most likely due to inadequate sampling, especially of *E. simplex* and *E. grandiflorus*. Of interest is the clustering of *E. ochroleucus* var. *scribneri* individuals in relation to *E. lackschewitzii*, i.e., accessions of *E. ochroleucus* var. *scribneri* are distinct and most similar to *E. lackschewitzii*. RAPD results support cpDNA restriction site variation and morphological analyses with respect to these two species.

The low coefficient of similarity among accessions of *E. grandiflorus* may indicate a hybrid origin from several populations of *E. simplex* with an as-yet unknown species.

Spongberg (1971) notes that each population of *E. grandiflorus sensu lato* is unique and more similar to sympatric populations of other species than to other races of *E. grandiflorus*. Within a given race, population individuality might be interpreted as evidence of a unique origin, and each population may constitute a separate biotype, according to Spongberg.

Determination of Ploidy Level

Results from stomate counts reveal a significant difference ($p < 0.001$) between diploids and polyploids with reference to number of stomates contained within the viewing field under 200X magnification. A significant difference also exists between diploid and triploid populations and between triploid and tetraploid populations. No significant difference ($p = 0.054$) was noted between populations of the two diploid species. Tetraploid *E. radicatus* and triploid *E. grandiflorus* had fewer stomates per unit area ($\bar{x}$ = 19.5 and 18.3 respectively) than diploids *E. ochroleucus* var. *scribneri* ($\bar{x} = 26.2$) and *E. simplex* ($\bar{x} = 24.4$). Mean number of stomates for *E. lackschewitzii* was 19.7. Unpaired t-tests of sets of stomate counts revealed a significant difference ($p < 0.001$) between *E. lackschewitzii* and both diploids. Although no significant difference ($p = 0.139$) was noted between *E. lackschewitzii* populations and triploid *E. grandiflorus* populations, stomate counts were essentially identical ($p = 0.873$) when *E. lackschewitzii* and tetraploid *E. radicatus* populations were compared. These results suggest that populations of *E. lackschewitzii* are more likely to be tetraploid than triploid.

The presence of numerous trichomes on leaf surfaces in several specimens of *E. grandiflorus* and *E. simplex* may have been instrumental in reducing the number of stomates per unit area in these two species.

Estimates of the relative sizes of stomates likewise revealed a significant difference ($p < 0.001$) between polyploids and diploids and between ploidy levels. Polyploids *E. radicatus* and *E. grandiflorus* had larger stomates ($\bar{x} = 46.5$ µm and 40.5 µm respectively) than diploids *E. ochroleucus* var. *scribneri* and *E. simplex* ($\bar{x} = 33$ µm and 30.5 µm respectively). Mean stomate size for *E. lackschewitzii* was 47.5 µm. No significant differences were noted between diploid *E. ochroleucus* var. *scribneri* and diploid *E. simplex* ($p = 0.431$) or between tetraploid *E. radicatus* and *E. lackschewitzii* ($p = 0.533$), but significant differences were observed between triploid *E. grandiflorus* and tetraploid *E. radicatus* ($p < 0.001$) and between *E. grandiflorus* and *E. lackschewitzii* ($p < 0.001$).

Stomate sizes for these *Erigeron* species correlate with those found for ploidy levels in *Crepis* (Asteraceae). Although ploidy levels above tetraploid were not investigated, pentaploid *Crepis* populations were found to have stomates whose average sizes ranged from 48.7µm to 51.3µm (Babcock and Stebbins, 1938). Whereas no pentaploids have been noted for any *Erigeron*, and stomate sizes for *E. lackschewitzii* are below the population averages found in pentaploid *Crepis*, results of stomate size measurements suggest that *E. lackschewitzii* populations are most likely tetraploid.

Effects of polyploidy have long been known, and the most immediate effect is an increase in cell size (Stebbins, 1971). Although enlarged cells do not always increase plant size, *gigas* effects are commonly found, particularly in organs having a highly determinate

growth pattern, such as flowers and seeds, according to Stebbins. Leaves and petals of polyploids are usually thicker and firmer than those of their diploid progenitors, and they usually flower later due to retardation of the mitotic cycle. These general characteristics of polyploids were found in *E. lackschewitzii* individuals when compared with plants belonging to *E. ochroleucus* var. *scribneri*.

CHAPTER FOUR

DISCUSSION

The data herein presented suggest retention of specific status for *Erigeron lackschewitzii*. Morphologic and genetic studies concur and clearly show that *E. lackschewitzii*, although similar to *E. ochroleucus* var. *scribneri*, is distinct. Phenograms displaying geographic and population variation, coupled with genetic divergence values and nuclear genome comparisons, illustrate the taxon's relationship to, as well as separation from, *E. ochroleucus* var. *scribneri*. None of the other three putative close relatives of *E. lackschewitzii* (*E. grandiflorus*, *E. simplex*, *E. radicatus*) are as closely related.

Based on the results of this study, the morphologic and genetic similarities of *E. ochroleucus* var. *scribneri* and *E. lackschewitzii* suggest a progenitor-derivative relationship of quite recent and probably rapid origin. It has been predicted that recently derived species exhibit high genetic similarity with a progenitor taxon due to inadequate time for new mutations to accumulate (Gottlieb, 1976). Changes in breeding system (commonly from outcrossing to selfing) may allow rapid fixation of new chromosomal and/or genetic variants, resulting in "quantum" speciation (Grant, 1981). High genetic identities found in isozyme loci by Purdy, Bayer, and Macdonald (1994) suggest a recent

origin for the narrow sand dune endemic *Stellaria arenicola* (Caryophyllaceae) where morphological divergence had occurred despite a lack of genetic divergence. *Stellaria arenicola* populations were also found to have significantly higher rates of selfing relative to progenitor populations. Low morphological and DNA divergence is not unusual and has been found in several species pairs (e.g., Sytsma and Smith, 1988; Crawford, 1990; Fenster and Ritland, 1992).

The lack of genetic variation among populations of *E. lackschewitzii* may also be viewed in light of limited genetic variation of ancestral populations and founding individuals (Gottlieb, 1976). For example, reduced cpDNA and isozyme variation in highly selfing *Mimulus micranthus* (Scrophulariaceae) when compared to its mixed-mating congener *M. guttatus* suggests a recent origin of *M. micranthus* from a limited number of *M. guttatus* populations (Fenster and Ritland, 1992). Genetic variation in this case may have been reduced due to a bottleneck effect since only one cpDNA mutation was unique to all populations of *M. micranthus.* The relative levels of species-wide isozyme diversity were similar to patterns found in cpDNA variation and may reflect isozyme sensitivity to population bottlenecks, according to the authors.

The emergence of a new species such as *E. lackschewitzii* by way of a change in ploidy level and fixation of the genotype through apomixis initially involves a few individuals which form subpopulations reproductively isolated from diploid progenitors (Harlan and deWet, 1975). Previously hidden variation may arise from the breakdown of developmental canalization following inbreeding and strong directional selection, while changes in the genetic background may lead to changes in the expression of coordinated

sets of genes, which in turn yield novel phenotypes subject to selection (Levin, 1983). Due to reduced recombination because of a reduction in sexual reproduction in the more obligate of apomicts, little rearrangment of genetic material occurs, and the novel phenotype would be perpetuated asexually.

Polyploidy can arise through union of gametes, one or both of which are unreduced (Harlan and deWet, 1975). Hybridization need not be involved since such events occur in selfed progenies as well as among interbreeding populations, species and/or genera. A change in the number of chromosomes, even without complications such as apomixis, introduces an immediate barrier to hybridization with the ancestral diploid (Cronquist, 1988).

The contention that polyploidy, and specifically autopolyploidy, can be a common and successful speciation process in some groups of plants was demonstrated by Wolf, Soltis and Soltis (1990). Chloroplast DNA and allozymic variation in diploid and tetraploid *Heuchera grossulariifolia* (Saxifragaceae) suggested multiple origins of autotetraploids. Morphological differentiation of the two cytotypes was restricted to leaf size differences which were more pronounced in natural habitats than under common garden conditions, suggesting that such differences were partly a function of habitat. The two cytotypes were largely reproductively isolated and exhibited slight differences in substrate specificity. Autotetraploid populations tended to occupy drier, south-facing slopes and metamorphic substrates. Diploids were more variable, occurring on granite and basalt in addition to metamorphic substrates. Electrophoretic data coupled with geographic distribution of populations and a cpDNA-based phylogeny suggested that

autotetraploid populations evolved several times as migration of diploids occurred down river systems. The authors conclude that, despite their morphological similarity, diploid and autotetraploid *H. grossulariifolia* could be considered separate species.

Features found in polyploid derivatives of diploid progenitors include increased gene activity and enzyme diversity, lower transpiration rates but higher photosynthetic rates, novel flavonoid production, lower germination rates accompanied by greater dormancy, later flowering extended over a longer period, greater longevity and increased pathogen resistance, lower growth rates, and higher tolerance to mineral and nutrient stress (Levin, 1983). Any combination of these traits could result in greater ecological amplitude of the polyploid independent of a broad base of genetic diversity, according to Levin. Additionally, later flowering in polyploids as a result of retardation of the mitotic cycle could result in temporal divergence which might prove significant in preventing or reducing gene flow between diploid progenitor and polyploid derivative (Lewis, 1980). Speciation may then proceed in some instances to designation of populations as sibling species if significant and readily detectable morphological, physiological, or ecological changes accompany the change in ploidy level (Stebbins, 1971). The evolutionary process of polyploidy occurring in species populations is a generalized phenomenon that is a primary mechanism of speciation in plants (Lewis, 1980).

In rapidly evolving families, such as Asteraceae, polyploidy is absent from the least specialized and present in the more specialized species groups (Stebbins, 1980). *Erigeron* species considered "advanced" by Cronquist were later found to be exclusively or predominantly tetrasporic, that is, all four megaspore nuclei are involved in formation of

the embryo sac (gametophyte). Less "advanced" taxa display bisporic or mixed monosporic and bisporic conditions (Harling, 1951). Variation and lability in embryo sac development in *Erigeron* is in striking contrast to the uniform and invariable conditions found in other genera of the Astereae tribe of Asteraceae, most of which produce normal monosporic embryo sacs, (Harling, 1951). Two *Erigeron* species examined by Harling that display tetrasporic embryo sac development are *E. ochroleucus* (variety unknown) and *E. simplex*, in addition to three apomicts: *E. divergens, E. annuus, and E. mucronatus*.

Harling (1951) suggests that different types of embryo sac development are found in some *Erigeron* species due to variable wall formation during meiotic divisions. In studies of unrelated apomictic *Elymus rectisetus* (Poaceae) and a close sexual relative, significantly thicker cell walls were found in the sexual relative (Carman, et al., 1991). Absence of callose in and around unreduced megaspore mother cells in the apomict appeared to be the major reason for the difference in cell wall thickness, according to the authors. In certain types of apomicts studied by Bell (1992), unreduced egg cells were found in embryo sacs lacking a callosed boundary. Bell suggests that thinner cell walls may allow an inflow of nutrients which could stimulate parthenogenetic development of the egg. Given the propensity of *E. ochroleucus* to form tetrasporic embryo sacs (Harling, 1951; variety unknown), it may not be unusual to find a tendency towards polyploidy and apomixis in this *Erigeron* species.

Plants with closed reproductive systems such as apomixis, which emphasize constancy in reproduction, may be found in open and pioneering habitats that include post-

glacial areas (Grant, 1981). Since most apomicts are polyploids, the advantages conferred by a change to polyploidy may greatly alter cytological, biochemical, genetic, physiological and developmental character, providing new polyploids with unique transgressive tolerances and developmental patterns which could suit them to conditions beyond the limit of diploid progenitors (Levin, 1993). The ability of polyploid apomicts to survive and expand appears to be higher in areas where succession has not reached a mature stage (Ehrendorfer, 1980).

Speciation by selfing can form new local races or narrowly endemic species arising on the periphery of ancestral species through fixation of variation (Grant, 1981). Endemics which are narrowly defined ecologically may be relictual and ancient, moderately old, or young (Stebbins, 1971). Based on its localized condition in specialized habitats of western North America, with close relatives of more generalized distribution in surrounding vegetation, *E. lackschewitzii* could be classified as a young endemic *sensu* Stebbins. Specifically, *E. lackschewitzii* occupies windy ridgeline habitats at the edge of continental glaciation in northern Montana, at the northwestern boundary of the range of its progenitor. The species grows exclusively on a calcareous limestone or dolomite substrate, which has been shown to be higher in calcium, magnesium and pH, with less phosphorus, than sandstone soils (Mooney, 1966). Plants belonging to *E. lackschewitzii* commonly bloom later in July and early August than sympatric *Erigeron* (*E. simplex, E. radicatus, E. compositus*) and commonly remain in flower later into August (personal observation). Given the subtle physiological differences mentioned previously that can be induced by polyploidy which may affect the optimum conditions for growth (Cronquist,

1988), it is not surprising that this polyploid occupies a different habitat than its progenitor, or that it is found parapatrically in a different geographic range.

Although the expected population structure of apomictic plants is morphological uniformity (Grant, 1981), apomicts may achieve levels of heterozygosity comparable to sexuals over time due to occasional sexuality (Campbell and Dickinson, 1990). Since studies by several workers suggest that very few apomicts are obligate (Asker and Jerling, 1992), a knowledge of time since origin plus amount of sexuality within populations may reveal the amount of genetic variation likely to be attained in certain apomictic groups. Further studies involving *E. lackschewitzii* may disclose the extent to which sexual reproduction occurs and may lead to an assessment of the amount of genetic variation attainable by this new species over time.

Apomicts may not be able to rapidly adapt to environmental changes due to lower rates of recombination and extreme genetic constancy brought about by asexual reproduction (Stebbins, 1971), yet they may survive and reproduce for millions of years, evolving slowly, as long as their habitat remains unchanged (Cole, 1980). Persistence of agamic complexes throughout long periods of time is suggested for *Rubus* subgenus *Eubatus* and many tropical grasses, which include *Panicum, Setaria*, and *Pennisetum* (Sebbins, 1971). In other genera (*Hieracium*, *Alchemilla*, *Sorbus*, *Crepis*, *Taraxacum*, and *Crataegus)*, the number of agamospecies far exceeds the number of sexual species (Richards, 1973).

CHAPTER FIVE

CONCLUSION

There are several concepts under which recently-derived polyploid apomict populations such as constitute *E. lackschewitzii* would merit specific status. Under a phenetic species concept such as espoused by Cronquist (1947, 1988), a species is defined as a self-perpetuating natural population distinguished from related populations by reasonably constant morphological and physiological characters and partial or complete barriers to interbreeding. Physiologic differences between species can be inferred from differences in distribution, habitat preference, flowering season, and the like. Partial or complete barriers to interbreeding may depend on such factors as number and size and shape of chromosomes, secondary metabolites, pollinators, anatomical and micromorphological characters, geography, and sterility barriers, according to Cronquist. Any or all of the above may contribute to reproductive isolation. Farrar (1990) states that species recognition is appropriate when taxa, in addition to meeting other species criteria, have attained a degree of predictability in their occurrence in a particular habitat.

Under the phylogenetic species concept, a species is a cohesive group with common descent (Mishler and Brandon, 1987; Templeton, 1989; Nixon and Wheeler, 1990). A variety of cohesive mechanisms exist and are not limited to gene flow. Singly or in combination, these include common history, developmental constraints, and stabilizing

selection. The apparent coherence of morphologically recognizable asexual species, despite the lack of gene flow, may indicate that processes responsible for cohesion of species may be other than gene flow, even in sexual groups (Mishler, 1990).

The nature of species in asexual groups may be confused with a different question concerning the adaptive significance of sexual versus asexual reproduction (Mishler and Budd, 1990). Thus, because asexual reproduction is seen as a poor adaptive strategy, it is reasoned that species status in asexuals is of little importance. Mishler and Budd counter this philosophy by suggesting that, first, it is not a settled question of whether or in what cases sexual reproduction is selectively advantageous, and secondly, whether or not asexuals are less adaptive does not preclude the importance of species formation as a phylogenetic pattern in these groups. Under the phylogenetic species concept, according to the authors, speciation is the origin of distinctive new lineages by a breakdown in any of a number of integrative and/or cohesive mechanisms in parent lineages. These mechanisms can include a key regulatory mutation which produces a new morphology or ecological preference, or a release of selective constraints in some geographically or ecologically marginal population.

Most asexually reproducing species may be short-lived branches from sexually reproducing ancestral stocks and lack major phylogenetic significance in terms of further diversification (Budd and Mishler, 1990). This statement, among other reasons, may have prompted workers (e.g., Beaman, 1957; Babcock and Stebbins, 1938) who have studied asexual populations to deny species status to these groups. However, according to

Kellogg (1990), there is no biological reason why a group that fails to diverge and subsequently diversify cannot constitute a species.

Based upon the above discussion regarding species concepts, together with conclusive data supporting the distinction of *E. lackschewitzii* populations from those of *E. ochroleucus* var. *scribneri*, *E. lackschewitzii* qualifies as a species. *Erigeron lackschewitzii* individuals are reproductively isolated from their progenitor, are morphologically discrete, are ecologically and geographically distinct from progenitor populations, and result from one of the commonly accepted modes of speciation in plants, namely, through a change in ploidy level. The species probably arose in response to environmental selection for polyploids with tolerances to nutrient and habitat stresses, enabling colonization of open habitats left barren by glaciation. Individuals belonging to *E. lackschewitzii* are all descended from a unique common ancestor and display a distinctive genetic profile. These characteristics of plants belonging to *E. lackschewitzii* would be sufficient for sexual populations to be considered as separate species and should therefore be sufficient to denote specific status for this Montana alpine endemic.

REFERENCES CITED

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

Babcock, E.B., and G.L. Stebbins. 1938. The American species of *Crepis*: their interrelation and distribution as affected by polyploidy and apomixis. Publ. Carn. Inst. Wash. 504: 1-199.

Bamberg, S.A. 1964. Ecology of the vegetation and soils associated with calcareous parent material in the alpine region of Montana. PhD dissertation, University of California, Davis.

Beaman, J.H. 1957. The systematics and evolution of *Townsendia* (Compositae). Contr. Gray Herb. 183: 1-151.

Bell, P.R. 1992. Apospory and apogamy: implications for understanding the plant life cycle. Int. J. Plant Sci. 153(3): S123-S136.

Brauner, S., D.J. Crawford, and T.F. Stuessy. 1992. Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. Amer. J. Bot. 79: 1436-1439.

Bremer, K. 1994. Asteraceae: cladistics and classification. Portland: Timber Press, Inc.

Budd, A.F., and B.D. Mishler. 1990. Species and evolution in clonal organisms — summary and discussion. Syst. Bot. 15: 167-171.

Campbell, C.S., and T.A. Dickinson. 1990. Apomixis, patterns in morphological variation, and species concepts in subfamily Maloideae (Rosaceae). Syst. Bot. 15: 124-135.

Canby, W.M. 1890. *Erigeron* —No. 77. Bot. Gaz. 15: 150.

Carman, J.G., C.F. Crane, and O. Riera-Lizarazu. 1991. Comparative histology of cell walls during meiotic-apomeiotic megasporogenesis in two hexaploid Australasian *Elymus* species. Crop Science 31: 1527-1532.

Cole, C.J. 1980. Newly attained polyploidy and/or clonal reproduction in animals: the brink of extinction, or threshold to new frontiers? *In* Lewis, W.H., ed., Polyploidy: biological relevance. New York: Plenum Press.

Coulter, J.M., and A. Nelson. 1909. New manual of botany of the central Rocky Mountains (Vascular plants). New York: American Book Company.

Crawford, D.J. 1990. Plant molecular systematics. New York: John Wiley & Sons.

Cronquist, A. 1947. Revision of the north American species of *Erigeron*, north of Mexico. Brittonia 6:121-302.

_____. 1988. Species and infraspecific taxa. *In*: The evolution and classification of flowering plants. Bronx: New York Botanical Garden.

Doebley, J., R. von Bothmer, and S. Larson. 1992. Chloroplast DNA variation and the phylogeny of *Hordeum* (Poaceae). Amer. J. Bot. 79: 576-584.

Dorn, R.D. 1984. Vascular plants of Montana. Cheyenne: Mountain West Publishing.

_____ 1992. Vascular plants of Wyoming. Cheyenne: Mountain West Publishing.

Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

Ehrendorfer, F. 1980. Polyploidy and distribution. *In*: Lewis, W.H., ed., Polyploidy: biological relevance. New York: Plenum Press.

Farrar, D.R. 1990. Species and evolution in asexually reproducing independent fern gametophytes. Syst. Bot. 15: 98-111.

Fenster, C.B., and K. Ritland. 1992. Chloroplast DNA and isozyme diversity in two *Mimulus* species (Scrophulariaceae) with contrasting mating systems. Amer. J. Bot. 79: 1440-1447.

Gottlieb, L.D. 1976. Biochemical consequences of speciation in plants. *In*: Ayala, F.J., ed., Molecular evolution. Sunderland, Mass.: Sinauer Associates.

Grant, V. 1981. Plant speciation. New York: Columbia University Press.

Hamby, R.K., and E.A. Zimmer. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. *In*: Soltis, P.S., D.E. Soltis, and J.J. Doyle, eds., Molecular systematics of plants. New York: Chapman and Hall.

Harlan, J.R., and J.M.J. deWet. 1975. On Ö. Winge and a prayer: the origins of polyploidy. Bot. Rev. 41: 361-384.

Harling, G. 1951. Embryological studies in the Compositae. Part III.: Astereae. Acta Hort. Berg. 16: 73-120.

Harrington, H.D., and L.W. Durrell. 1979. How to identify plants. Chicago: Swallow Press.

Heidel, B.L. 1993. Report on the conservation status of *Erigeron lackschewitzii*, a candidate threatened species. Helena: Montana Natural Heritage Program.

Hitchcock, C.L., A. Cronquist, M. Ownbey, and J.W. Thompson. 1955. Part 5: Compositae. *In* Vascular plants of the pacific northwest. Seattle: University of Washington Press.

Hooker, W.J. 1834. Flora boreali americana, vol. II. London: Henry G. Bohn.

Huber, W., and A. Leuchtmann. 1992. Genetic differentiation of the *Erigeron* species (Asteraceae) in the Alps: a case of unusual allozymic uniformity. Pl. Syst. Evol. 183: 1-16.

Jansen, R.K., H.J. Michaels, R.S. Wallace, K. Kim, S.C. Keeley, L.E. Watson, and J.D. Palmer. 1992. Chloroplast DNA variation in the Asteraceae: phylogenetic and evolutionary implications. *In*: Soltis, P.S., D.E. Soltis, and J.J. Doyle, eds., Molecular systematics of plants. New York: Chapman and Hall.

Kellogg, E.A. 1990. Variation and species limits in agamospermous grasses. Syst. Bot. 15: 112-123.

Levin, D.A. 1993. Local speciation in plants: the rule not the exception. Syst. Bot. 18: 197-208.

_____ 1983. Polyploidy and novelty in flowering plants. Am. Nat. 122: 1-25.

Lewis, W.H. 1980. Polyploidy in species populations. *In*: Lewis, W.H., ed., Polyploidy: biological relevance. New York: Plenum Press.

McDonald, C.C. 1927. A study of seed development in three species of *Erigeron* with special reference to apogamy. Bull. Torrey Bot. Club 54: 479-497.

Miller, J.C. 1990. RESTSITE. Cambridge: Whitehead Institute.

Mishler, B.D. 1990. Reproductive biology and species distinctions in the moss genus *Tortula*, as represented in Mexico. Syst. Bot. 15: 86-97.

_____, and R.N. Brandon. 1987. Individuality, pluralism, and the phylogenetic species concept. Biol. Phil. 2: 397-414.

_____, and A.F. Budd. 1990. Species and evolution in clonal organisms — introduction. Syst. Bot. 15: 79-85.

Mooney, H.A. 1966. Influence of soil type on the distribution of two closely related species of *Erigeron*. Ecology 47: 950-958.

Nei, M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Nesom, G.L. 1989. Infrageneric taxonomy of new world *Erigeron* (Compositae: Astereae). Phytologia 67: 67-93.

_____, and W.A. Weber. 1983. A new woolly-headed, monocephalous *Erigeron* (Asteraceae) from Montana. Madroño 30: 245-249.

_____. 1976. A new species of *Erigeron* (Asteraceae) and its relatives in southwestern Utah. Brittonia 28:263-272.

Nixon, K.C., and Q.D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6: 211-223.

Palmer, J.D., R.A. Jorgensen, and W.F. Thompson. 1985. Chloroplast DNA variation and evolution in *Pisum*: patterns of change and phylogenetic analysis. Genetics 109: 195-213.

Palmer, J.D., and D. Zamir. 1982. Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc. Natl. Acad. Sci. USA 79: 5006-5010.

Polunin, N. 1959. Circumpolar arctic flora. London: Oxford University Press.

Purdy, B.G., R.J. Bayer, and S.E. Macdonald. 1994. Genetic variation, breeding system evolution, and conservation of the narrow sand dune endemic *Stellaria arenicola* and the widespread *S. longipes* (Caryophyllaceae). Amer. J. Bot. 81(7): 904-911.

Raeder, U., and P. Broda. 1985. Rapid preparation of DNA from filamentous fungi. Lett. Appl. Microbiol. 1:17-20.

Richards, A.J. 1973. The origin of *Taraxacum* agamospecies. Bot. J. Linn. Soc. 66: 189-211.

Rohlf, F.J. 1993. NTSYS-pc, Version 1.80. Applied Biostatistics, Inc., distributed by Exeter Software, Setauket, New York.

Rydberg, P.A. 1900. Catalogue of the flora of Montana and the Yellowstone national park. Mem. N.Y. Bot. Gard. 1:399-408.

Schaal, B.A., and G.H. Learn. 1988. Ribosomal DNA variation within and among plant populations. Ann. Missouri Bot. Gard. 75: 1207-1216.

Skvarla, J.J., B.L. Turner, V.C. Patel, and A.S. Tomb. 1977. Pollen morphology in the Compositae and in morphologically related families. *In*: Heywood, V.H., H.B. Harborne, and B.L. Turner, eds., The biology and chemistry of the Compositae. London: Academic Press.

Sneath, P.H.A., and R.R. Sokal. 1973. Numerical taxonomy. San Francisco: W.H. Freeman and Company.

Sokal, R.R., and P.H.A. Sneath. 1963. Principles of numerical taxonomy. San Francisco: W.H. Freeman and Company.

Solbrig, O.T. 1962. The South American species of *Erigeron*. Contr. Gray Herb., 191: 3-79.

Soltis, D.E., P.S. Soltis, T.A. Ranker, and B.D. Ness. 1988. Chloroplast DNA variation in a wild plant, *Tolmiea menziesii*. Genetics 121: 819-826.

Spongberg, S.A. 1971. A systematic and evolutionary study of North American arctic and alpine monocephalous species of *Erigeron* (Compositae). PhD dissertation, University of North Carolina at Chapel Hill.

Stebbins, G.L. 1971. Chromosomal evolution in higher plants. Menlo Park: Addison-Wesley Publishing Company.

Stebbins, G.L. 1980. Polyploidy in plants: unsolved problems and prospects. *In*: Lewis, W.H., ed., Polyploidy: biological relevance. New York: Plenum Press.

Sytsma, K.J., and L.D. Gottlieb. 1986. Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (Onagraceae). Evolution 40: 1248-1261.

_____, and J.F. Smith. 1988. DNA and morphology: comparisons in the Onagraceae. Ann. Missouri Bot. Gard. 75: 1217-1237.

Templeton, A.R. 1989. The meaning of species and speciation: a genetic perspective. *In*: Otte, D., and J.A. Endler, eds., Speciation and its consequences. Sunderland, Mass.: Sinauer Associates, Inc.

Towner, H.F. 1990. E-Z Stat, Version 2.0. Trinity Software, Campton, New Hampshire.

Van Buren, R., K.T. Harper, W.R. Andersen, D.J. Stanton, S. Seyoum, and J.L. England. 1994. Evaluating the relationship of autumn buttercup (*Ranunculus acriformis* var. *aestivalis*) to some close congeners using random amplified polymorphic DNA. Amer. J. Bot. 8: 514-519.

Williams, J.G.K., A.R. Kubelik, J.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Wolf, P.G., D.E. Soltis, and P.S. Soltis. 1990. Chloroplast-DNA and allozymic variation in diploid and autotetraploid *Heuchera grossulariifolia* (Saxifragaceae). Amer. J. Bot. 77(2): 232-244.

APPENDICES

APPENDIX A

FLORAL KEY TO THE

FIVE SPECIES

(Floral key to the five species of interest occurring in Montana.)

1. Involucre villous to densely woolly-villous with tangled, sometimes glassy or red to purplish-black multicellular hairs; usually 1 head per stem
   2. Rays blue-purple or pink, rarely white
      3. Lower leaves all linear or narrowly oblanceolate, rarely over 3 mm wide, with narrowly acute tips; stem and involucre with minute glandular trichomes or not
         4. Disc corollas (3.0)3.5-4.1 mm long; ray florets (27)30-40(52); achenes usually 1.5-2.5 mm long; alpine endemic, windy slopes near ridge lines on calcareous soil; nw

            E. LACKSCHEWITZII Nesom and Weber
         4. Disc corollas (2.5)3.0-3.5(3.8) mm long; ray florets (30)40-70(88); achenes usually 1.1-1.5 mm long; dry exposed areas, montane to rarely alpine; w, c

            E. OCHROLEUCUS var. SCRIBNERI (Canby) Cronq.
      3. Lower leaves, or some of them, oblanceolate to obovate or spatulate and rounded or obtuse at tip, usually over 3 mm wide; stem and involucre with conspicuous glandular trichomes
         5. Disc corollas (2.7)3.0-3.5(4.0) mm long; pappus bristles 13-22; ray florets 42-90; protected areas alpine to subalpine; w

            E. SIMPLEX Greene
         5. Disc corollas (3.1)3.5-4.1 mm long; pappus bristles 8-13(16); ray florets (48)58-112(132); alpine, windy, exposed, disturbed areas; Carbon county

            southern alpine race of E. GRANDIFLORUS Hook.
   2. Rays white, rarely pale purple
      6. Lower leaves, at least some of them, spatulate-linear or wider, with broadly acute tips; rocky outcrops and exposed locations high in the mountains; w

         E. RADICATUS Hook.
      6. Lower leaves linear or narrowly oblanceolate, usually with narrowly acute tips
         7. Caudex with primary branches commonly twice or thrice branched; disc corollas (2.0)2.5-3.0(3.5) mm long, sometimes lacking pollen at anthesis; pappus bristles (5)8-11(13) sometimes kinky and irregular; alpine, subalpine, in rocky outcrops and exposed locations; w

            E. RADICATUS Hook.
         7. Caudex usually with unbranched primary branches; disc corollas (2.5)3.0-3.5(3.8) mm long containing pollen at anthesis; pappus bristles 10-17 and regular; dry exposed areas, montane to rarely alpine; w, c

            E. OCHROLEUCUS var. SCRIBNERI (Canby) Cronq.
1. Involucre moderately to densely strigose-villous, sometimes with more than one head per stem
   8. Rays blue-purple, pink, or white; phyllary tips red/purple and somewhat reflexed; dry exposed areas, montane to rarely alpine; w, c

      E. OCHROLEUCUS var. SCRIBNERI (Canby) Cronq.

8. Rays white; phyllary tips various
 9. Caudex with primary branches commonly twice or thrice branched; no. of cauline leaves 1-6; alpine, subalpine, in rocky outcrops and exposed locations; w
 E. RADICATUS Hook.
 9. Caudex usually with unbranched primary branches; no. of cauline leaves usually (5)7-11; dry exposed areas, montane to rarely alpine; w, c
 E. OCHROLEUCUS var. SCRIBNERI (Canby) Cronq.

APPENDIX B

SPECIMENS EXAMINED

Erigeron caespitosus Nutt. Trans. Am. Phil. Soc. II. 7:307. 1840.

SPECIMEN EXAMINED. U.S.A. Montana: Gallatin Co., City of Bozeman, Pete's Hill on Sourdough Ridge, in sagebrush-grass habitat, at 5000 ft., *Lavin s.n.* (MONT).

Erigeron filifolius Nutt. Trans. Am. Phil. Soc. II. 7:308. 1840.

SPECIMEN EXAMINED. U.S.A. Oregon: Deschutes Co., ca. 6 miles east of Sisters, Oregon, along Highway 126 in dry volcanic soil, at 4300 ft., *Kerstetter s.n.* (MONT).

Erigeron formosissimus Greene, Bull. Torrey Club 25:121. 1898.

SPECIMEN EXAMINED. U.S.A. Oregon: Crook Co., along Ochoco Creek and Highway 26, in wet, peaty meadow, at 4000 ft., *Kerstetter s.n.* (MONT).

Erigeron grandiflorus Hook. Fl. Bor. Am. 2:18. 1834. TYPE: Summits of the Rocky Mountains, *Drummond s.n.* (holotype: GH!)

REPRESENTATIVE SPECIMENS EXAMINED. U.S.A. Colorado: above Beaver Creek, at 11-12,000 ft. *Crandall 2819* (RM); Larimer Co., mountains of Estes Park, *Osterhout 2860* (RM); Grand Co., no locality, *Tweedy 5796* (RM); Park Co., east-facing slope of Leadville limestone, at 12,000 ft., *Neely and O'Kane 3139* (RM). Montana: Carbon Co., Absaroka-Beartooth Wilderness, near summit of Glacier Lake Trail, at 10,004 ft., *Kerstetter EG3000* (MONT); rim of Beartooth Mountains overlooking Quad Creek, at 10,000 ft., *Kerstetter EG3400* (MONT); gravel pit off Highway 212 at Montana-Wyoming border, at 10,005 ft., *Kerstetter EG3200*; Flathead Co., Corrugate Ridge, along Continental Divide above W. side of headwaters of Bruce Creek, at 8000 ft., *Lackschewitz 10604* (MONTU); Teton Co., Bob Marshall Wilderness, east flank of Mt. Wright, north of trail in saddle, at 7500 ft., *Lackschewitz 10,085* (MONTU). Utah: Uintah Mountains, Fish Lake, *Godding 1385* (RM); Uintah Co., Leidy Peak, at 11-11,500 ft., *Beaman and Stone 1439* (RM). Wyoming: Albany Co., LaPlata Mines, *Osterhout 1805* (RM); Medicine Bow Mountains, Libby Flats, at 11,000 ft., Spongberg 69-54 (RM); Medicine Bow Mountains, at 11,000 ft., *Nelson 138805* (RM); near Medicine Bow Peak, at 11-11,800 ft., *Beaman and Stone 1394* (RM); Johnson Co., summit of Sheep Mountain, at 9600 ft., *Porter 7092* (RM); north of Powder River Pass, at 9677 ft., *Kerstetter EG2800* (MONT).

Erigeron lackschewitzii Nesom and Weber, Madroño 30:245. 1983. TYPE: U.S.A. Montana: Flathead Co., Continental Divide "N" wall, summit of mtn S. above Sock L., at 8200 ft., *Lackschewitz 9101* (holotype MONTU!).

REPRESENTATIVE SPECIMENS EXAMINED. U.S.A. Montana: Lewis and Clark Co., Scapegoat Wilderness, south-facing slopes of Crown Mountain west of the pass, across from pack trail, at 7200-7700 ft., *Lesica 4044* (MONTU); Teton Co., Bob Marshall Wilderness, north flank of NW-SE ridge south of Mt. Patrick Gass between Bruce Creek and Crazy Creek, at 7840 ft., *Kerstetter EL1200* (MONT); Bob Marshall Wilderness, south flank of Mt. Patrick Gass, on divide between Bruce Creek and Crazy

Creek, at 7700 ft., *Kerstetter EL1300* (MONT); Bob Marshall Wilderness, Front Range Mountains, ca. 25 miles west of Choteau, above Our Lake, at 8400 ft., *Kerstetter EL200* (MONT); Bob Marshall Wilderness, Headquarters Creek Pass, at 7743 ft., *Kerstetter EL700* (MONT); ca. 34 miles west of Choteau, on west side of peak above Teton Pass Ski area, at 8040 ft., *Schassberger 339* (MONTU); Bob Marshall Wilderness, south flanks of Mt. Patrick Gass, on divide betweeen Bruce Creek and Crazy Creek, at 7800 ft., *Kerstetter EL1100* (MONT); east flank of Mt. Wright, East Front Range, south of trail in saddle, at 7500 ft., *Kerstetter EL1400* (MONT); Lewis and Clark National Forest, Rocky Mountain District, T28N R10W S14, at 7800 ft., *Lackschewitz 10552* (MONTU).

Erigeron ochroleucus var. *scribneri* (Canby) Cronq. Brittonia 6:189. 1947. TYPE: U.S.A. Montana: Little Belt Mountains, *Scribner 77* (holotype: NY!)

REPRESENTATIVE SPECIMENS EXAMINED. U.S.A. Montana: Beaverhead Co., Tendoy Mountains, above Muddy Creek Road, *Lackschewitz 11307* (MONTU); Broadwater Co., Belt Mountains, west side of Mt. Edith, at 9200 ft., *Lesica 756* (MONTU); Carbon Co., rim of Beartooth Mountains overlooking Quad Creek, at 10,000 ft., *Kerstetter EO3400* (MONT); Beartooth Mountains, near old gravel pit, at 9700 ft., *Lackschewitz 7852* (MONTU); Gallatin Co., Fairy Lake Trail, at 8300-8900 ft., *Kerstetter EO100* (MONT); Glacier Co., Glacier National Park, Chief Mountain, at 6400 ft., *DeSanto s.n.* (Glacier National Park herbarium); Glacier National Park, Chief Mountain, at 6750 ft., *DeSanto s.n.* (Glacier National Park herbarium); Lewis and Clark Co., Scapegoat Mountain, at 8200 ft., *Craighead 28* (MONTU); Scapegoat Wilderness, Front Range Mountains, south-facing slopes of Crown Mountain east and west of the pass along pack trail, at 7200-7700 ft., *Kerstetter EO3700* (MONT); Meagher Co., western edge of Little Belt Mountains, *Hitchcock and Muhlick 12233* (RM); Teton Co., Bob Marshall Wilderness, East Front Mountains, on ridge of argellite and metamorphic rock, at 7900 ft., *Lackschewitz 10574* (MONTU); Bob Marshall Wilderness, above saddle east of Mt. Wright, at and above location of *E. lackschewitzii*, at about 7500 ft., *Kerstetter EO1400* (MONT). Wyoming: Bighorn-Sheridan boundary, low rocky limestone ridge, at 9700 ft., *Johnston 1310* (RM); community along roadside, Buffalo district, Rabbit park, muddy C&H Allot., at 8000 ft., *Hurd 208* (RM); Albany Co., Laramie Hills, *Goodding 224* (COLO); Big Horn Co., 4 miles SE of Hyattsville, at 5200 ft., *Lichvar 1662* (RM); growing in clump of *Petrophytum*, at 5200 ft., *Dorn 3206* (RM); summit of Medicine Mountain, at 9500-10,000 ft., *Williams and Williams 3221* (RM); summit of Medicine Mountain, at 9500-10,000 ft., *Williams 1940* (RM); south ridge of Medicine Mountain along road to Medicine Wheel, at 9600 ft., *Kerstetter EO2300* (MONT); Fremont Co., near Beaver Rim, ca. 14 airmiles SW of Sweetwater Station, at 6800 ft., *Hartman and Haines 20250* (RM); Johnson Co., summit of Sheep Mountain, west of Buffalo, at 9600 ft., *Porter 7091* (RM); ca. 1 air mile west of Dullknife Reservoir Spillway, at 8400 ft., *Hartman 9555* (RM); off National Forest Road 29 east of Powder River Pass, at 9500 ft., *Kerstetter EO2500* (MONT); north of Powder River Pass, at 9800 ft., *Kerstetter EO2800* (MONT); Big Horn Mountains, Ice Creek, ca. 8.8 air miles west of Burgess Junction, at 9000 ft., *Hartman and Odasz 9274* (RM); Sheridan Co., west of Fallen City overlook near mile marker 67 north of Highway 14, at 7800 ft., *Kerstetter EO1900* (MONT); along

National Forest Road 185 on windy open ridge at 8000 ft., *Kerstetter EO2000* (MONT); edge of limestone formation west of National Forest Road 15, at 7600 ft., *Kerstetter EO2100* (MONT); Sweetwater Co., Little Mountain, at 8400 ft., *Dorn 3114* (COLO).

Erigeron ochroleucus Nutt. var. *ochroleucus*, Trans. Am. Phil. Soc. II. 7:311. 1840.

SPECIMEN EXAMINED. U.S.A. Montana: Sweetgrass Co., along Big Timber Canyon Road, 5 miles west of Highway 191 north of Big Timber, at 4072 ft., *Kerstetter EO1700* (MONT).

Erigeron radicatus Hook. Fl. Bor. Am. 2:17. 1834. TYPE: Canada. Alberta: Mountains near Jasper's Lake, Rocky Mountains, *Hooker s.n.* (isotype: NY!)

REPRESENTATIVE SPECIMENS EXAMINED. Canada. Saskatchewan: Cypress Hills, *Macoun s.n.* (NY). U.S.A. Idaho: Fremont Co., proposed Targhee RNA, NE-facing slope of saddle above lake, at 9900 ft., *Moseley 835* (RM); Lemhi Co., Beaverhead Mountains, continental divide 1 mile north of Peak 11860, ca. 20 miles SE of Leadore, at 9900 ft., *Moseley 363* (RM). Montana: Anaconda-Pintler Range, below summit of E. Pintler Peak, at 9400 ft., *Lackschewitz 5397* (COLO); Carbon Co., Beartooth Mountains, on ridge east of Rosebud Plateau, at 10,800 ft., *Lesica 2860* (MONTU); Deerlodge Co., Pintler Range, Little Rainbow Mountain, SW above Storm Lake Basin, at 9900 ft., *Lackschewitz 3770* (MONTU); Fergus Co., Big Snowy Range, at 8300-8700 ft., *Bamberg 224* (COLO); Flathead Co., above Sock Lake, continental divide "N" wall, at 8200 ft., *Lackschewitz 9102* (MONTU); Bob Marshall Wilderness, summit above Sock Lake, at 8200 ft., *Lackschewitz 9102* (RM); Madison Co., crest of steep limestone ridge off Gravelly Range Road, at 9250 ft., *Lackschewitz 8973* (RM); on hillside east of Gravelly Range Road, at 9250 ft., *Kerstetter ER3500* (MONT); Teton Co., Front Range Mountains, west slope of Rocky Mountain, at 8380 ft., *Kerstetter ER1000* (MONT).

Erigeron simplex Greene, Fl. Fran. 387. 1897. TYPE: U.S.A. Colorado: no locality, *Greene s.n.* (lectotype: ND!).

REPRESENTATIVE SPECIMENS EXAMINED. U.S.A. Colorado: mountains of Estes Park, *Osterhout 2860* (RM); Cottonwood Pass on mountainside, at about 12,000 ft., *Goodall s.n.* (Western State College herbarium); Boulder Co., 8 miles NW of Nederland, at 11,600 ft., *A.L. Arnold 190A* (Western State College herbarium); Gunnison Co., Cumberland Pass, alpine grassland, at 12,100 ft., *Gierisch 2845* (Western State College herbarium); summit of Independence Pass, alpine tundra, rocky soil, *Santos and Weber 213* (Western State College herbarium). Montana: Flathead Co., south flank of Mt. Patrick Gass, south of divide between Bruce Creek and Crazy Creek, at 7400 ft., *Kerstetter ES1300* (MONT); Bob Marshall Wilderness, in boulder field south of Mt. Patrick Gass, at 7300 ft., *Kerstetter ES1100* (MONT); Teton Co., Bob Marshall Wilderness, below ridgeline of saddle west of Our Lake, at 8400 ft., *Kerstetter ES200* (MONT); Bob Marshall Wilderness, below saddle west of the summit of Rocky Mountain, at 8000 ft., *Kerstetter ES1000* (MONT); Bob Marshall Wilderness, in saddle on north side of trail to summit of Mt. Wright, at 7500 ft., *Kerstetter ES1500* (MONT). Wyoming: Big

Horn Co., on ridge above road to Medicine Wheel, at 9600 ft., *Kerstetter ES2400*; Johnson Co., off National Forest Road 29 east of Powder River Pass, at 9500 ft., *Kerstetter ES2700* (MONT); north of Powder River Pass, at 9800 ft., *Kerstetter ES2800* (MONT).

Erigeron speciosus (Lindl) DC. Prodr. 5:284. 1836.

SPECIMEN EXAMINED. U.S.A. Montana: Gallatin Co., Bridger Range, Bridger Bowl Ski area, in lodgepole pine-Douglas fir habitat, at 8000 ft., *Lavin s.n.* (MONT).

Erigeron ursinus DC. Eat. Bot. King Exp. 148. 1871.

REPRESENTATIVE SPECIMENS EXAMINED. U.S.A. Montana: Gallatin Co., summit of Fairy Lake Trail, at 8900 ft., *Kerstetter EU100* (MONT). Wyoming: Johnson Co., off National Forest Road 29 east of Powder River Pass, at 9500 ft., *Kerstetter EU2600* (MONT); Park Co., west of National Forest Road 142 to Clay Butte Lookout Station, at 9000 ft., *Kerstetter EU3300* (MONT).

APPENDIX C

PROTOCOL FOR

QUICK DNA EXTRACTION

(Protocol obtained from B. FitzGerald, unpublished, Montana State University.)

1. Grind one or two leaves in 300 µl extraction buffer consisting of 200 mM Tris-HCl (pH 8.5), 250 mM NaCl, 25 mM EDTA, and 0.5% SDS (from Raeder and Broda, 1985).
2. Spin at 14,000 rpm for 5 minutes. Dump supernatant into a clean tube.
3. Centrifuge at 10,000 for 15 minutes. Dump supernatant into another clean tube.
4. Precipitate DNA by adding 20 µl of 3M sodium acetate (pH 5.2) and 300 µl cold isopropanol. Invert to mix, and place in -20°C for 20-30 minutes.
5. Spin at 14,000 rpm for 4 minutes to pelletize. Discard supernatant.
6. Wash pellet with 70% EtOH and air dry.
7. Resuspend pellet in 100 µl sterile water.
8. Add 10 µl RNAse and incubate at 37°C for 1 hour.
9. Freeze for 15-20 minutes at -20°C. Thaw without disturbing.
10. Centrifuge at 14,000 rpm for 1 minute.
11. Place supernatant containing DNA in a clean tube and label.