THE EVOLUTIONARY ORIGINS OF *ERIGERON TRIFIDUS*, A RARE PLANT IN ALBERTA

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Abstract

The present study analyzed the evolutionary history of *Erigeron trifidus* Hook. by addressing two main questions: 1) Is the current hypothesis of the origin of the species by hybridization between *E. compositus* and *E. lanatus* supported by molecular data? and 2) Is the species monophyletic?

An analysis of uni-and-biparentally inherited molecular markers from three species throughout the range of *E. trifidus* yielded data that supports the hybridization hypothesis. First, a restriction site analysis of cpDNA revealed 4 haplotypes. In most cases, cpDNA haplotypes were the same as in *E. lanatus*, suggesting *E. lanatus* as the maternal parent. Sequencing and cloning the nuclear ETS region revealed the presence of multiple repeat types in most individuals sampled. This further supports the hybrid origin hypothesis in that *E. trifidus* contained only repeat types present in one or the other of the putative parents. In addition, *E. trifidus* displayed the highest percentage of intraindividual repeat type polymorphism, a common trait of hybrid species. *Erigeron trifidus* populations collected in the northern region appear to be monophyletic as they all exhibited a particular pattern of repeat type variation, a pattern absent in Ram Mountain and Waterton Lakes National Park populations. As *E. lanatus* has never been recorded from Ram Mountain, it is likely that the populations identified as *E. trifidus* are instead a different agamospermous variant of *E. compositus*. In Waterton Lakes National Park, populations of *E. trifidus* are thought to be the product of local hybridization but the identity of the putative parents remains uncertain. Therefore, *E. trifidus* is concluded to be polyphyletic.

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INTRODUCTION

The role of hybridization in the evolution of plant populations is uncertain. Two viewpoints exist. One states that hybrid species are fit enough to persist through time (Arnold, 1997; 1996; Rieseberg et al., 2003). The increased variation resulting from hybridization then promotes the development of novel adaptations. Hybridization is thus considered to be an important evolutionary force that creates opportunities for speciation. The contrasting position accords hybrids little evolutionary importance. Here, hybridization is considered as a transient process that leads to organisms with lowered fitness. It is therefore viewed as a local phenomenon with temporary effects (Rieseberg et al. 2003; Linder and Rieseberg, 2004). Definitive support for either viewpoint is lacking.

In hybrid speciation, two otherwise independent lineages recombine sexually to create a new species (Rieseberg and Carney, 1998). Hybrid speciation in plants occurs in at least two ways: allopolyploid speciation and diploid (homoploid) speciation. An allopolyploid (a new species that has the complete diploid chromosome complement of both its parents) is generally reproductively cut off from its parents as any backcrossing produces a high proportion of unviable or sterile triploid offspring. The occurrence of diploid hybrid speciation is thought to be much lower as the partially fertile hybrids are often able to backcross to the parents. Therefore, in order for the new hybrid species to be maintained over time, reproductive isolation from parental species is required. The present study considers both types of hybrid speciation. Asexually reproducing *Erigeron compositus* populations are known allopolyploids while the origins of *Erigeron trifidus* may involve homoploid speciation.

The extent of plant hybridization in nature is unclear. Measurements generally represent a substantial underestimate, as many hybrids have gone undetected due to inadequate systematic attention (Arnold, 1992; Rieseberg, 1997). Current reviews identify a worldwide frequency of hybridization at 11%. When considering angiosperms, this value rises to between 50-60% (Rieseberg, 1997). Natural hybrids were also found to be taxonomically unevenly distributed within angiosperms, with certain families exhibiting a high incidence of hybridization while in other families, hybrid species have yet to be detected (Ellstrand and Elam, 1993).

The process of hybridization is frequently correlated with asexual reproduction (apomixis) in plants (Marshall and Brown, 1981; van Dijk, 2003). Hybrids often exhibit odd ploidy levels resulting from a combination of divergent genomes; sexual reproduction is thus prohibited. Apomixis is one strategy by which a new allopolyploid lineage can be maintained over time. Extant apomicts always have closely related sexual taxa and can often be crossed with these, the apomicts acting as pollen donors (Stebbins, 1950). This influx of genetic variation from occasional sexual reproduction is thought to be important as the persistence of a species over time is dependent on availability of genetic diversity. Initial genetic variability in apomicts is a consequence of their hybrid and/or polyploid origins. Although fixed as a result of asexual reproduction, this variability cannot be maintained indefinitely without sexual recombination; it will continuously be eroded via selection. Therefore, obligate apomixis is rare. Facultative or partial apomixis (with some sexual seed set) is much more common (Richards, 1973).

Apomixis is reported from at least 30 families of flowering plants (Holsinger, 2000). Agamospermy ("seeds without sex") is a specific type of apomixis that has some

advantages over vegetative apomixis through seed dispersal, although most of the genetic and evolutionary consequences of asexuality do not differ whatever the form (Richards, 2003). Agamic complexes are often conspicuous components of North Temperate floras and exhibit puzzling patterns of variation that pose innumerable problems for systematists (Grant, 1981). When agamospermous species have arisen many times, as in *Crepis* (Babcock and Stebbins, 1938) and *Rubus* (Gustafsson, 1934), or have uncertain origins, as in *Boechera*; (Schranz et al., 2005), the patterns of variation make it difficult to identify distinct lineages that can be called species. Populations resulting from different hybridization and/or polyploid events and that exhibit agamospermy are often referred to as 'microspecies' (Grant, 1981).

Allozyme and DNA marker studies have demonstrated that microspecies can harbour values of genetic diversity similar to those of sexually reproducing populations (Van der Hulst et al., 2000; Van Dijk, 2003). Four theories have been put forth to explain this observation (Richards, 2003): (1) mutation, (2) backcrossing of apomicts with sexual relatives, (3) facultative meiotic recombination and cross-fertilization within apomictic populations, and (4) multiple hybrid origins of agamosperms from genetically divergent sexual ancestors (Richards, 2003). A study of the *Antennaria* agamic complex in North America led Bayer (1991) to conclude that the variability displayed in a variety of taxa was best explained by polyphyletic origins from extant sexual progenitors. In the case of *A. media*, some populations were found to contain a mixture of obligate and facultative agamosperms and sexuals (Bayer et al., 1990), making both multiple polyploid and hybridization events as well as occasional sexual crosses likely. In comparison, Menken et al. (1995) explained the high levels of genotypic diversity within and among clonal

populations of *Taraxacum* section *Ruderalia* by the extensive, recurrent exchange of genetic material between sexual diploids and agamospermous triploids. Finally, variation resulting from mutation has been identified as an important in other asexual lineages of *Taraxacum* (King and Schaal, 1990).

Microspecies demonstrate an astonishing diversity of morphological traits and geographic distribution in addition to variety in genetic composition (Grant, 1981; Gornall, 1999). Characterizing this variety and divergence and understanding the mechanisms by which they arise is one goal of both population genetics and systematics. Avise et al. (1987) coined the term phylogeography to describe work carried out to further our understanding of these processes. Phylogeography builds upon work by De Candolle (1820), who was the first to propose that the current geographical distribution of living organisms depends upon both ecological and historical parameters. Today, phylogeography characterizes population subdivision by recognizing geographical patterns of genealogical structure across the range of a species (Taberlet et al., 1998). Phylogeography has been applied to many aspects of plant populations as it has long been recognized that genetic variation at this level is determined not only by the contemporary forces of genetic exchange but also by historical patterns of relationships (Schaal et al., 1998; Tribsch and Schonswetter, 2003).

Patterns of glacial recolonization of European tree species were among the first phylogeographic studies of plant species. The majority of these studies relied solely on chloroplast DNA. The chloroplast genome is nonrecombining and uniparentally inherited, which allows polymorphisms to be treated as alleles at a single, haploid locus. This characteristic makes cpDNA useful for tracking haplotype lineages and distinguishing maternal from paternal parents. For example, Demesure et al. (1996) discovered a surprising

level of homogeneity in chloroplast DNA amongst populations of *Fagus sylvatica* (common beech) from northern Europe compared to those in Italy and concluded that these patterns resulted from recolonization from two distinct refugia. Phylogeographic studies have also focused on herbaceous species. Chloroplast DNA variation among circumpolar populations of *Saxifraga oppositifolia* identified historic migration routes from a relict population in western Beringia during the late Tertiary (Abbott et al., 2000; Holderegger and Abbot, 2003; Abbott and Comes, 2004).

One focus of recent phylogeographic studies is the question of monophyly of polyploid taxa. Due to their reticulate past, naturally occurring polyploids have historically been treated in one of two ways in phylogenetic analyses: they are excluded, or their origins have been inferred from intermediacy in morphology (Rieseberg and Ellstrand, 1993) and/or additivity patterns of fragment analyses (Brochmann et al., 1992; Koch et al., 2003). A limitation with such analyses is that evolutionary events such as selection, mutation or drift, which may have taken place after the hybridization event in the hybrid species as well as in the parental lineages, may obscure original patterns of intermediacy or additivity. The use of multiple molecular approaches in phylogeny reconstruction, such as analysis of more than one gene region, may overcome such limitations. A familiar example of an evolutionarily successful and geographically widespread agamic complex is the genus *Taraxacum* (Asteraceae). Morphological, ecological, and geographic variation within asexual *Taraxacum* polyploids has led to the description of nearly 2000 microspecies (Richards, 1973). King (1993), utilizing traditional cpDNA as well as nuclear DNA, demonstrated a polyphyletic origin of several asexual *Taraxacum* taxa. In addition, the data show that

multiple hybridization events are a more important source of genotypic variation than mutation for these asexual polyploids.

Finally, phylogeographic studies have focused on identifying specific lineages involved in allopolyploid events. *Draba lacteal* (Brassicaceae) is an allopolyploid that, along with its close allies, forms a taxonomically intricate arctic-alpine complex including sympatric diploids, tetraploids and hexaploids (Brochmann et al., 1992). A study by Grundt et al. (2004) utilized a multiple molecular approach involving sequence analysis as well as DNA fingerprinting methods, and concluded that, although *D. lacteal* originated from a single diploid lineage, it has most likely originated several times in the Beringian area and is thus not monophyletic.

The role of hybridization and agamospermy in the evolution of microspecies is particularly uncertain in speciose, widespread plant groups where they may yield phylogeographic patterns that defy ready interpretation. *Erigeron* L. (Asteraceae: Asteraceae), with over 400 species, provides a useful system in which to study this phenomenon. The majority of *Erigeron* species occur in North America, although taxa are also numerous in temperate Europe, Asia and South America (Nesom, 1989). A typical *Erigeron* species occurs in exposed subalpine and alpine habitats and exhibits specific morphological traits; namely, a monocephalous head, 'caudex'-branching, narrow white to pinkish rays, yellow discs and oblanceolate leaves. Despite these similarities, variation in ray to disc floret ratio, breeding system, annual versus perennial habit and leaf dissection exists within the genus (Nesom, 1989). In addition to its complex geographical distribution and paucity of reliable morphological characters, *Erigeron* is further notable because of the incidence of agamospermy in several of its species (Noyes, 2000a).

Erigeron trifidus (three forked fleabane) is a small alpine daisy originally described in the late 1800's. In recent history, it has been treated as a synonym of *Erigeron compositus* Pursh var. *discoideus* A. Gray. This treatment follows a monograph by Cronquist (1947) in which var. *discoideus* is distinguished from the other varieties by the "mostly once ternate leaves" compared to leaves "mostly 2-4 times ternate". Recently, Packer (1983a) recognized a component of *E. compositus* var. *discoideus* as the distinct species *Erigeron trifidus* Hook. An analysis of the two type specimens reveals a number of morphological differences, with *E. trifidus* exhibiting invariably three-lobed leaves, wider leaf lobes, dilated petioles and larger heads with more villous involucres. Packer (1983a) also separated *E. trifidus* on the basis of cytology. The chromosome number for *E. compositus* in Alberta is normally 2n=54 (Love and Love, 1975) while *E. trifidus* is 2n=45 (Packer, 1983a). Based on the above evidence, Packer proposed that *E. trifidus* arose via hybridization between *E. compositus* Pursh and *E. lanatus* Hook (2n=36).

The morphological differences that are intermediate in *E. trifidus* compared to its putative parents include leaf shape, petiole width and degree of involucre pubescence. *Erigeron compositus* exhibits ternately lobed or dissected leaves with linear petioles. Involucres show highly variable pubescence, ranging from glandular to densely villous. The leaves of *E. lanatus* are much wider (up to 5 cm), entire or 3-toothed at the apex, with undifferentiated petioles (Packer, 1983b). The specific epithet '*lanatus*' refers to the copiously woolly-villous or lanate hairs of the involucre. *Erigeron trifidus* individuals have three-lobed leaves with petioles that are dilated compared to *E. compositus* but narrower than those of *E. lanatus*. The involucres of *E. trifidus* are consistently more densely villous than

those of *E. compositus* but exhibit a lower degree of pubescence compared to *E. lanatus* (Packer, 1983a).

Erigeron trifidus shares the habitat preference of *E. compositus* and *E. lanatus* where their distributions overlap: high elevation scree slopes. Despite this, the overall distribution for the three species differs dramatically. *Erigeron compositus* occurs in the western United States and Canada, the Gaspe Peninsula of Quebec, Newfoundland and Greenland where it can be found growing at both low and high elevations in alpine regions as well as across prairie landscapes. *Erigeron lanatus* populations are much more restricted. They are identified from scattered locations along the Rocky Mountains from Willmore Wilderness Area in northern Alberta to the southern alpine regions of Idaho in the United States (Packer, 1983b). In comparison, *E. trifidus* exists only in Alberta, where it is considered rare, although Packer (1983a) writes that "its occurrence in adjacent British Columbia seems quite probable". The known distribution for *E. trifidus* includes Jasper National Park, Mountain Park, Willmore Wilderness Area, Ram Mountain and Waterton Lakes National Park. Examining each species' range enables the identification of three geographical scenarios: locations where all three *Erigeron* species are present (Mountain Park, Jasper National Park, Willmore Wilderness area and Waterton Lakes National Park), locations where one of the putative parents is absent (Ram Mountain) and locations where *E. compositus* and *E. lanatus* have been found but the putative hybrid, *E. trifidus*, has not (Banff National Park and Kananaskis).

Traditionally, *E. compositus* has been separated into three highly variable varieties (Cronquist, 1947). Beaman (1977) demonstrated that this traditional concept is unnatural as *E. compositus* is an agamospermous species complex comprising both sexually and asexually

reproducing populations. Based on an extensive survey of herbarium specimens, Beaman informally proposed that the sexual component of the complex is made up of five subspecies. These subspecies are morphologically distinct and geographically restricted, occupying diverse habitats primarily in the western United States. The sexual entities have been characterized cytologically and genetically (Noyes et al., 1995) and were shown to be diploid (2n=18) and outcrossing. In comparison, agamospermous *E. compositus* is widespread in Alberta and is extremely variable in both vegetative and floral morphology (Beaman, unpubl. data) with triploid (3X) through septaploid (7X) chromosome counts reported. Both cytology and geographic distribution indicate that putative hybridization events resulting in the evolution of *E. trifidus* involved agamospermous rather than sexual populations of *E. compositus* (Packer, 1983a).

Different geographical distributions of sexuals and parthenogens are common in animals and plants (Bell 1982; Bierzychudek, 1987). This phenomenon is known as geographical parthenogenesis. In Europe, *Taraxacum* shows a classic north-polyploid, south-sexual contrast of geographical parthenogenesis. Several hypotheses have been put forward to explain the tendency for asexuals to occur farther to the north in the Northern Hemisphere, the most widely accepted stating that asexuals are confined to environments with limited biotic interactions (Levin, 1975; Bell, 1982). In these regions, apomicts could replace sexuals owing to their intrinsic reproductive advantage. However, in areas with complex communities, sexuals would dominate because they are better at filling narrow and specialized niches (van Dijk 2003).

The breeding system of *Erigeron lanatus* is unknown. Based on large amounts of pollen that is relatively uniform in size (personal observation), sexual reproduction is a

possibility (personal observation). Conversely, *E. trifidus* produces little viable pollen, yet is highly fertile producing abundant, viable seed that germinates to form large (if geographically restricted) populations. These observations, in conjunction with an odd chromosome number (2n=45) suggest it is agamospermous (Packer, 1983a).

As indicated, much is known of the morphology, cytology, geographical distribution and breeding system of *Erigeron trifidus* and its putative parental species, *Erigeron compositus* and *Erigeron lanatus*. Despite this, molecular analyses have not yet been undertaken. To that end, this study assessed the evolutionary history of *E. trifidus* by addressing two questions: 1) Is the current hypothesis of the hybrid origin of the species supported by molecular data? and 2) Is the species monophyletic or has it had multiple origins?

The detection of reticulate evolution requires an analysis of molecular characters in addition to morphological and cytological data (Rieseberg and Ellestrand, 1993). Recent advances in molecular methods have enabled an examination of genetic markers with different modes of inheritance and corresponding different evolutionary rates. This has translated into the recognized distinction between gene and species trees, which has emphasized the need for more than one gene for phylogenetic analyses (Hewitt, 2001). A combination of chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) has been successfully used in phylogeographic studies to differentiate reticulate from divergent relationships (Soltis et al., 1992; Hughes et al., 2002).

Although the use of cpDNA is well documented, nuclear ribosomal DNA has historically been given a limited role in the detection of hybridization, in part because it was thought that concerted evolution acted to promote sequence homogeneity at the individual,

population and species level despite biparental inheritance and high copy number (Volkov et al., 1999; Wendel et al., 1995). Thus, additivity of expected parental sequences in hybrids was not expected. A false inference of homogeneity may also result from a failure to detect the infrequent repeat type in direct sequencing due to unequal ratios of parental nrDNA repeats in hybrids (Rauscher et al., 2002). Conversely, when intra-individual polymorphism in nrDNA is observed, hybrid origins cannot necessarily be assumed. This is because repeat diversity within an individual may arise from the evolution of nonfunctional nrDNA repeats (pseudogenes) or to the intragenomic evolution of functional, but divergent, paralogous gene copies (Bailey et al., 2003; Buckler et al., 1997). However, despite these limitations, nrDNA additivity has been successfully used in some instances to document hybridization at both diploid (Fuertes Aguilar and Feliner, 2003; Sang et al., 1995) and polyploid (Rauscher et al., 2004; Andreasan and Baldwin, 2003) levels.

Rauscher et al. (2004) utilized the ITS region of nrDNA to analyze multiple origins within the *Glycine tomentella* (Leguminosae) allopolyploid complex. It is known that the species *G. tomentella* is a large polyphyletic complex of diploid and tetraploid cytotypes. The diploid 'races' have been described genetically and represent unique groups with separate phylogenetic origins. Allopolyploid lineages have arisen by crosses among these diploid races and with other species. Currently, six major tetraploid races are recognized and their diploid progenitors have been identified. From direct sequencing, it was determined that in most allopolyploid accessions, both homeologous nrDNA repeats were present. In addition, phylogenetic analyses of these accessions provided evidence for multiple origins in several of the polyploid races. This study was one of the first to provide clear evidence for long term maintenance of nrDNA repeat types in allopolyploids.

The ETS region has also proven useful for resolving historical patterns of hybridization (Linder et al. 2000; Markos and Baldwin, 2002). Recent molecular investigations of *Sidalcea* (Malvaceae) discovered extensive polymorphism in both the ITS and ETS for a large number of individuals (Andreasen and Baldwin, 2003). These polymorphic DNAs were cloned and sequenced and included in phylogenetic analyses together with direct sequences of non-polymorphic samples. The positions of the cloned ITS and ETS sequences suggested that three *Sidalcea* lineages were the product of hybridization. Further, putative parental lineages were identified.

A major strength of phylogeography is its ability to test for explicit evolutionary relationships between phylogenetically related taxa occupying different geographical areas (Felsenstein, 1982; Dobes et al., 2004). This advantage is of particular importance at the intra-and interspecific level, where closely related lineages may occur sympatrically. Thus, the monophyly of *E. trifidus* can be examined by looking for phylogeographical correlations within the spatial distribution of the three *Erigeron* species. If regional variation patterns within *E. trifidus* populations can be attributed to similar regional differences in the putative parental species of the same region, this will be strong evidence that *E. trifidus* has originated numerous times from different hybridization events in each region and is thus not monophyletic. Conversely, if the patterns for each region fail to show significant differences despite variation in the putative parental species, this would suggest that *E. trifidus* originated through one hybridization event and colonized each region post-origin.

MATERIALS AND METHODS

Plant Materials and Sampling Procedures

Erigeron trifidus is known from restricted alpine locations throughout the province of Alberta. Potential collection sites were identified by a survey of herbarium specimens at the University of Lethbridge (LEA), the University of Alberta (ALTA) and the University of British Columbia (UBC) and through personal communications with Joyce Gould (senior botanist, Alberta Natural Heritage Information Centre), Peter Achuff (Species Assessment Biologist, Ecological Integrity Branch, Parks Canada), and Peter Lesica (affiliate faculty member, Division of Biological Sciences, University of Montana). Areas with similar elevation and habitats to known sites were also investigated. *Erigeron compositus* and *E. lanatus* populations were collected at the same sites as *E. trifidus*, as well as from a small number of additional locations. Population size was estimated visually at each collection site. The data for all collections are summarized in Appendix 1. Sampling for each species was as follows: for *E. trifidus*, a total of 142 individuals in 18 collections (representing 10 populations), for *E. compositus* a total of 148 individuals in 17 collections (representing 13 populaitons, and for *E. lanatus*, a total of 92 individuals in 14 collections (representing 9 populations). At the time of sampling, individuals were taken from throughout the visible range of the local population. As certain sites were visited more than once, collections made within 300 m of one another but in different years were combined (see Table 1) for each species. Voucher specimens for all collections are deposited in the herbarium at the University of Lethbridge (LEA).

DNA Isolation and Amplification

Two to five leaves from individual plants were collected from each area, maintained in liquid nitrogen for a maximum of 48 hours and stored at –80°C. In addition, live plants from each population were maintained in the greenhouse or, in a few cases, were grown from seed collected in the field.

Total genomic DNA was obtained from each individual following the CTAB procedure of Doyle and Doyle (1987). Some modifications were applied, involving grinding only 50-75 mg of frozen leaf tissue and washing the DNA pellet with 76% ethanol/0.2M sodium acetate. DNA was dissolved in 1.0 mL of sterile nanopure water for long-term storage.

Twenty -five-microlitre polymerase chain reactions (PCR) were performed in a master mix containing 1 x PCR buffer (10 mM Tris-HCl/50 mM KCl buffer, pH 8.0), 3mM $MgCl_2$, 0.4 uM of each primer, 0.2 mM of each dNTP, 1 unit Taq DNA polymerase (NEB) and approximately 1 ng of template DNA using a GeneAmp 9600 (Perkin Elmer) thermal cycler. PCR product was checked for length and concentration on 1.5% agarose gels and purified using the QIAquick PCR purification Kit (Qiagen, Valencia, CA). The purified PCR product was then sequenced using both forward and reverse primers for verification. All sequences were exported to the program Se-Al v2.0a11 (RAMBAUT 1995), manually aligned, and inspected for point mutations incorporating restriction enzyme cut sites.

Sequence data were generated from all three genomes. Initially, a wide range of regions found to be phylogenetically useful at the interspecific level in other studies were screened (Sang et al., 1997; Shaw et al., 2005; Taberlet et al., 1991; Demesure et al., 1995; Dumolin-Lapeque et al., 1997; Markos and Baldwin, 2001) (refer to Table 2). Sequence

variation was discovered in two chloroplast regions, the *psb*A-*trn*H intron and the *trn*C-*psb*M intron as well as in the external transcribed spacer (ETS) region of nuclear ribosomal DNA (nrDNA). The *psb*A-*trn*H intron was amplified using primers *psb*A (forward 5' to 3': GTT ATG CAT GAA CGT AAT GCT C) and *trn*H (reverse 5' to 3': CGC GCA TGG TGG ATT CAC AAA TC) from Sang et al. (1997). Thermal cycling started with a denaturation step at 95°C, an annealing temperature of 55°C, a 72°C extension for 45 seconds (increasing 3 seconds per cycle for 30 cycles) and a final extension of 7 minutes (Sang et al. 1997). The *trn*C-*psb*M intron was amplified as described by Shaw et al. (2005) (80°C denaturing, 50°C annealing and 72°C extension for 30 cycles, final extension of 5 min.) using primers *trn*C (forward 5' to 3': CCA GTT CRA ATC TGG GTG) and *psb*M (reverse: ATG GAA GTA AAT ATT CTY GCA TTT ATT GCT). The ETS region was amplified using primers AST-8 (forward 5' to 3': TTC TCT TCT TCG TAT CGT GCG GT) from Markos and Baldwin (2001) and 18S-ETS (reverse 5' to 3': ACT TAC ACA TGC ATG GCT TAA TCT) and the following cycling conditions: 94°C denaturing; 55°C annealing; 72°C extension for 1 min, for 39 cycles; final extension of 7 min.(Baldwin and Markos,1998).

Restriction Site Analysis

Purified PCR product (4-5 uL) was digested overnight using between 1 and 2 units of each 4-bp cutter restriction enzyme (in separate digestions). The resulting fragments were separated using 2.0% agarose gels run for 3-4 hours at between 60 and 75 volts. The gels were stained using ethidium bromide, visualized under ultraviolet (UV) light and photographed. Restriction fragments were interpreted and sites scored as present or absent. The resulting binary characters were used to describe the haplotypes (for the chloroplast regions) (Table 3) as well as the repeat profiles (for the ETS region) (Table 4).

Cloning of PCR product

Initial screening of the ETS region revealed digest enzyme profiles that summed to greater than 700 bp (the length of a single ETS repeat unit), thereby indicating the presence of more than one repeat type per individual. Therefore, a subset of individuals that together included all of the variation detected was cloned in order to recover each repeat type. Incorporation of PCR product into plasmid vector and transformation was performed using the pGEM®-T Easy Vector Systems (Promega Corp, Madison, WI, USA). Positive colonies were grown in overnight cultures and plasmids were isolated using the QIAprep Spin Miniprep Kit (#27106; QIAGEN, Inc., Valencia, CA, USA). Cycle sequencing of plasmid inserts was accomplished with the above ETS primers and conditions, using approximately 350 ng of plasmid template. All clones were sequenced and the sequences matched with initial digest banding patterns in order to exclude the possibility of PCR artifact.

Analysis of ETS repeat profiles

PCR product from the ETS region was digested with two restriction enzymes: Tsp5091 and HpyCH4IV. For Tsp5091, a single mutation was identified at the 300 base pair (bp) site. If this restriction site was absent, the repeat type was uncut resulting in a 700 bp fragment. If the restriction site was present, the repeat type was cut yielding 300 and 400 bp fragments. Two mutations corresponding to HpyCH4IV restriction cut sites were identified; one at the 350 bp mark and a second at the 500 bp mark. Additional mutations were present near the 3' end of the region but these resulted in fragments too small to be resolved on an agarose gel and were therefore not used for coding purposes. Sequence analysis indicated that the mutation at the 500 bp mark was present and invariant for all accessions, so coding was based on the variation at the 350 bp site. If this restriction site was present, a 350 bp

fragment resulted while its absence yielded a 500 bp fragment. Combining the information from the two restriction enzymes yielded 4 repeat types.

Statistical Analysis of Haplotype Variation and Repeat Type Variation

Analysis of molecular variance (AMOVA) computes molecular variance components at different hierarchical levels (Excoffier et al. 1992). Conventional AMOVAs were performed on both cpDNA and ETS data sets and were based on pairwise squared Euclidean distance between haplotype frequencies for cpDNA and repeat type frequencies for the ETS region. Total genetic variance for conventional AMOVAs was partitioned into two hierarchical levels: among populations and within populations.

While the assignment of individuals to populations remained the same throughout all analyses (Table 1), morphology and ETS repeat type variation for *E. trifidus* populations suggested two geographical groupings: a northern region (including populations from Mountain Park, Willmore Wilderness Area and Jasper National Park) and a southern region (including populations from Waterton Lakes National Parks). The affiliation of centrally located Ram Mountain population (T-RM1) to the northern vs. the southern region was tested by including it in both regions and observing which gave the highest discrimination among regions. These regional analyses added a third hierarchical level of variation: among populations within groups. To quantify measurements of population subdivision, F_{ST} values for the chloroplast and ETS markers using corresponding variation components from AMOVA were measured. In all cases, 9999 permutations were run. AMOVAs were carried out with ARLEQUIN Version 2.000 (Schneider et al. 2000).

Mantel tests were performed to determine whether genetic divergence among populations was correlated with the geographical distances separating them. Mantel analyses

compute a Mantel statistic value, or R_M. The R_M statistic, which can range in value from –1.0 to +1.0, indicates the overall linear relationship between genetic and geographic distance. If the R_M value is negative, the genetic distance between populations is larger than would be predicted based on geographic distance: the populations are genetically distinct. If the R_M value is positive, there is a positive correlation between genetic and geographic distance: populations that are closer together are genetically more similar. Finally, R_M values approximating 0 indicate that there is no relationship between genetic relatedness and geographic distance. Mantel tests were applied in two different ways. First was the 'classical' use of a Mantel test (Mantel 1967; referred to as the 'overall' Mantel test in the following text), which was carried out for *E. trifidus*, *E. compositus* and *E. lanatus* populations using both cpDNA and nrDNA. Here, pairwise geographical distances were calculated for each population within each species group. The resulting geographic distance matrix was compared to the genetic Euclidean distance between each pair of populations within each species group. All R_M values for the overall Mantel test were calculated using R Package 4.0 (Casgrain and Legendre, 2001) and tested for significance with 9999 permutations.

The second application of the Mantel test incorporated distance classes. As in the overall Mantel test, a Mantel test with distance classes compares geographic and genetic distances matrices. However, the distance class method enables examination of directional variation over the area of study (Oden and Sokal, 1986). This allowed differences in genetic distance for populations within and between the southern and northern regions to be quantified. In order to create the distance classes, a matrix summarizing the geographic distance between each pair of populations was constructed for each species. Based on this

matrix, classes of geographic distances were created so that each class contained similar numbers of pairwise comparisons (Oden and Sokal, 1986; Gabrielson et al., 1997). A geographical matrix, or model matrix, was then constructed for each distance class. For example, to assess the relationship of populations in distance class one, inter-populational comparisons with a chosen geographical limit above 0 and below 100 km were given a value of 0, all others had a value of 1. The resulting model matrix was then compared to the genetic distance matrix (based on Euclidean distance). For distance class two, the model matrix was constructed by giving inter-populational comparisons with geographical limits between 100 km and 160 km a value of 0 and all others a value of 1. The relationship between this model matrix and the genetic distance matrix was then evaluated. This process was repeated for all distance classes for each species. Corresponding R_M values were computed individually and plotted against distance classes. Four distance classes were generated for *E. trifidus*: (1) $0 \leq d < 100$ km; (2) $100 \leq d < 160$ km); (3) $160 \leq d < 250$ km; (4) $250 \leq d < 600$ km. Distances classes for *E. compositus* and *E. lanatus* were as follows: (1) $0 \leq d < 100$ km; (2) $100 \leq d < 160$ km; (3) $160 \leq d < 250$ km; (3) $250 \leq d < 400$ km; (4) $400 \leq d < 600$ km. All R_M values were calculated and Bonferroni-corrected using R Package 4.0 (Casgrain and Legendre, 2001) and tested for significance with 9999 permutations. The results of the overall Mantel test for cpDNA did not indicate a significant correlation between geographic and genetic distance. Therefore, the Mantel test with distance classes was performed on ETS repeat types only.

Chromosome Counts

Mitotic squashes were prepared from root tips using modifications of Soltis (1980) and Noyes (personal communication). Individual plants were repotted and allowed to

generate new roots for approximately four months. Root tips were collected in early morning and pretreated in 0.002 M 8-hydroxyquinoline in uncapped vials for 3 hours at 16°C in the dark. The root tips were then fixed in 3:1 acetocarmine to acetic acid in foiled-wrapped, capped vials for one to four weeks at 16°C. In preparation for squashing, tips were placed in water for 3 minutes, transferred to 15% HCl (aq) for 26 minutes and returned to the water bath for 1 min. Squashing followed Soltis (1980).

Morphological Analysis

The specific morphological characters utilized in this study represent characters identified by Packer (1983a) and J.H. Beaman (unpublished data) as being useful in distinguishing *E. trifidus* from *E. compositus*.

Petiole width and degree of phyllary pubescence were measured for each voucher specimen for a total of 79 individuals. Petiole width was measured at the midpoint between the leaf axil and blade. Measurements were made for between 5 and 18 individual petioles per voucher individual. Mean petiole width for each individual was analyzed by one-way ANOVA.

To measure degree of pubescence, a digital photograph was taken at the base of the phyllaries. The photographs were then imported into Photoshop ver. 8.0 (Adobe Systems Incorporated, CA, USA) and a 2.5 cm x 2.5 cm detail cropped from the same position from each photograph. All hairs within the detail were transformed to white pixels while everything else was transformed to black pixels. A ratio of white:black was calculated and the mean ratios for each species analyzed by one-way ANOVA. For both characters, residuals were tested for normality and homogeneity.

For both morphological analyses, individuals falling outside of the 95% confidence interval for their group mean were identified.

RESULTS

Population sizes of *E. compositus*, *E. trifidus* and *E. lanatus* varied considerably over their ranges. *Erigeron compositus* populations were the largest: between 40-70 individuals for each site except Willmore Wilderness Park, where between 10 and 30 individuals were counted. The size of *E. trifidus* populations varied between 20-80 individuals in Willmore Wilderness Park and between 10-50 individuals in all other areas. *Erigeron lanatus* populations were the smallest, with sizes ranging from 5 individuals to no more than 20.

Restriction site Characterization

The character states of two cpDNA restriction sites and the 4 resulting cpDNA haplotypes are shown in Table 3. Haplotypes differed by either one or two mutations. Table 4 summarizes the four ETS repeat type profiles, which also differed by one or two mutations. Based on the four repeat types described, five combined repeat types are possible. In four out of five cases, the repeat types constituting the combined repeat types were unambiguous. However, in the case of combined repeat type 1+2+3+4, there were numerous possibilities. Repeat types 1 and 3 as well as combined repeat type 1+3 were not recovered on their own in any of the individuals sampled (Table 4).

Geographic Distribution of cpDNA haplotypes and ETS repeat types

cpDNA Haplotypes

The distribution of the four cp haplotypes is described in Table 5 and illustrated in Fig.1. The within-population frequencies illustrated in Fig. 1 are reported in Table 6. The majority of *Erigeron lanatus* populations were monomorphic for haplotype 1 although haplotype 3 was present in a single polymorphic population in Willmore Wilderness Area. Haplotypes 1, 3 and 4 were recovered from *E. trifidus* individuals, although the majority of

the populations were monomorphic for haplotype 1. Three *E. trifidus* populations exhibited polymorphism (T-WW2, T-WL2, and T-RM1). All four haplotypes were found in *Erigeron compositus* populations, almost half of which (6 out of 13) were polymorphic.

ETS repeat types

The distribution of ETS repeat types and combined repeat types is described in Table 5 and illustrated in Fig.2. The within-population frequencies illustrated in Fig. 2 are reported in Table 6. The ETS data from the three *Erigeron* species can be described by the presence or absence of multiple repeat types within individuals (intra-individual repeat type polymorphism). Polymorphism was also detected at the population level with individuals exhibiting varying combinations of repeat types and/or combined repeat types. *Erigeron trifidus* showed the highest percentage of intra-individual repeat type polymorphism, at 83.7% while 71.2% of *E. trifidus* populations were polymorphic. In *Erigeron compositus* populations, 69% exhibited polymorphism, with 70% of *E. compositus* individuals showing intra-individual repeat type polymorphism. The percentages in both categories were lowest for *E. lanatus*, at 55% for intra-individual repeat type polymorphism and 67% for polymorphic populations.

Figure 2 and Tables 5 and 6 also show that the HpyCH4IV restriction site at the 350 bp mark was absent for all *Erigeron trifidus* populations in the southern region.

Hierarchical structuring of genetic variance

cp DNA haplotypes

The AMOVAs on cpDNA haplotype data for *E. trifidus* populations led to similar partitioning of genetic variance components. i.e. grouping populations into two regions had little effect on among-population variation (29.96% when Ram Mountain population T-RM1

was included in the northern region and 30.44% when T-RM1 was included in the southern region). Comparing these to the value obtained without grouping (32.47% of variation among populations) indicates that there is little genetic differentiation for cpDNA across the range of *E. trifidus*, an observation that is further supported by the extremely low 'among group' percentages for both the southern (3.01%) and northern (4.04%) placements of T-RM1. Finally, the F_{ST} values for the various regional groupings of *E. trifidus* populations are not significantly different from one another, providing further support for the lack of population subdivision. Table 7 summarizes these results.

Variation among populations accounted for the majority of the genetic variation for *E. compositus* (76.44% compared to 23.56% for within population variation; F_{ST} value of 0.76). For *E. lanatus*, most of the genetic variation was among individuals within populations (88.62%) rather than between populations (11.38%). An F_{ST} value of 0.14 also indicates low levels of variation at the population level. (Table 7).

ETS Repeat Types

The assignment of the *E. trifidus* population from Ram Mountain (T-RM1) to different regions had a major influence on the variance component percentages. The 'among population variation' for *E. trifidus* when populations were not divided was 54.21%. When populations were separated into regions and population T-RM1 was included in the southern region, the variation among populations decreased to 15.30%. This decrease wasn't as pronounced when the Ram Mountain population was included in the northern region (28.98%) (Table 8). The inclusion of T-RM1 in the southern group also resulted in the highest percentage of variation among groups (56.21%).

The F_{ST} value of ungrouped *E. trifidus* was 0.54. Dividing *E. trifidus* populations into regions increased the F_{ST} values (0.69 when T-RM1 was included in the northern region and 0.76 when T-RM1 was included in the southern region), indicating a corresponding increase in the level of genetic differentiation among populations (Table 8).

For both *E. compositus* and *E. lanatus*, most of the total variation was accounted for by 'among population variation' (69.44% and 80.82%, respectively). High levels of population differentiation were also reflected in the high F_{ST} values (0.69 for *E. compositus* and 0.81 for *E. lanatus*) (Table 8).

Mantel Analyses

Classical Mantel Tests

The overall R_M values calculated with genetic and geographic distance matrices for cpDNA were as follows: -0.033 (p=0.458) for *E. compositus*, -0.068 (p=0.638) for *E. trifidus* and –0.023 (p=0.564) for *E. lanatus*. These values indicate that geographic distance had little effect on inter-populational genetic distance, an unsurprising result given the relatively low levels of cpDNA haplotpe variation for the three *Erigeron* species.

For ETS repeat types and combined repeat types, an overall R_M value of 0.206 (p=0.013) for *E. trifidus* indicates that inter-populational genetic distance moderately increased with increasing geographical distance. The R_M value for *E. compositus* was not significant (R_M = -0.114, p=0.171). *Erigeron lanatus* showed the highest R_M value at 0.670 (p=0.003), indicating a strong relationship between genetic and geographic distance.

Mantel Test with Distance Classes

Mantel tests calculated with distance classes showed a decrease from a significantly positive value (up to 100 km) to a significantly negative value (above 250 km, Fig. 3) for *E.*

trifidus populations (Fig 3a). The highest R_M value was found in distance class 1 (R_M = 0.445, p=0.0180) and the lowest in distance class 4 (R_M = -0.414, p=0.0100). The distance class correlogram for *E. compositus* showed no significant correlations (Fig. 3b): R_M values were slightly positive in distance classes one (up to 100 km) and five (from 400 to 600 km) and slightly negative in classes 2-4 (from 100 to 400 km). For populations of *E. lanatus*, distance class 1 (up to 100 km) exhibited a significantly positive R_M value of 0.553 (p=0.017) while a significantly negative R_M value (–0.337, p=0.024) was found in distance class 4 (from 250 to 400 km). Although not significant, slightly positive values were exhibited by distance classes 2 and 3 (from 100 to 250 km) while distance class 5 (from 400 to 600 km) showed a slightly negative R_M value (Fig.3c).

Chromosome Counts

A count of 2n=54 was recorded for *E. compositus* (population108). *Erigeron trifidus* (population 104) had a count of 2n=45.

Morphological Analysis

Petiole width was significantly different for each *Erigeron* species (Fig.4a, one way ANOVA: $F_{2,721} = 1872.4$, $p = 0.002$, $r^2 = 0.84$). Individuals from populations 156 and 171 are above the *E. lanatus* mean, indicating that the petiole width for these individuals was higher than the average for the species. *Erigeron trifidus* individuals from populations 157 and 170 had wider petioles compared to the species average while narrower petiole widths were recorded for individuals from populations 133 and 173. All *E. compositus* individuals fell within the 95% confidence interval for its respective group mean.

The degree of phyllary pubescence showed similar results to those reported for petiole widths, with each species exhibiting a significantly different mean (Fig. 4b, one way

ANOVA: $F_{2,394} = 3008.3$, $p < 0.05$, $r^2 = 0.89$). However, only *E. trifidus* populations contained individuals that exhibited measurements placing them outside of the 95% confidence interval for its group mean. A high degree of phyllary pubescence was found for individuals from populations 157 and 170, whereas a low degree of phyllary pubescence was measured for individuals from population 133.

DISCUSSION

As *E. trifidus* is morphologically very similar to *E. compositus*, our initial goal was to determine whether the populations identified as *E. trifidus* exhibited molecular patterns distinct from populations identified as *E. compositus*. Thus, examining the observed variation for *E. compositus* creates a base from which to analyze *E. trifidus* populations.

Hybridization in conjunction with polyploidy has played a major role in the origin and maintenance of inter- and-intraspecific polymorphism within the agamospermous *E. compositus* complex (Noyes et al., 1995; Noyes 2000b). In addition to the sexual diploid populations described by Beaman (1977), Noyes et al. (1995) discovered a number of tetraploid individuals that were considered to be the result of crosses between sexual diploids. Northern agamospermous populations are also derived from sexual progenitors. The predominantly hexaploid condition of agamic *E. compositus* allows for additivity of the divergent ancestral genomes. Thus, higher levels of heterozygosity are exhibited by agamic *Erigeron* populations compared to their sexual progenitors (Noyes and Soltis, 1996).

The present study supports the mosaic nature of *Erigeron compositus* populations. First, high levels of inter-and-intra-populational variation were uncovered for chloroplast DNA (Fig. 1). The different cpDNA haplotypes could have originated in a number of ways. Noyes et al. (1995) analyzed genetic variation for the five informally proposed subspecies of sexual *E. compositus* and found that the genetic similarity between them was only slightly lower than that recorded between populations of two different *Erigeron* species. The taxonomic categorization of the taxa as recognized by Beaman (1977) was thus supported. The possibility therefore exists that the sexual subspecies harbour different chloroplast

genomes. Hybridization events involving various subspecies could then result in offspring with differing chloroplast genomes, depending on which subspecies was the maternal parent.

The accumulation of mutations within a cpDNA lineage could also produce this pattern. There are regions of the cpDNA molecule that are more susceptible than others to recurring mutational events (Graham et al., 2000). In addition, Avise et al. (1987) cite several studies indicating that particular restriction sites "blink" on and off during evolution. Because gain and loss of restriction sites is influenced by nucleotide changes at several positions, independent events could lead to the same apparent character state. Thus, the distribution of some restriction sites among the different *Erigeron compositus* haplotypes, which contribute to cpDNA haplotype diversity, may be due to the repeated loss and gain of particular sites. Finally, the four chloroplast haplotypes recovered in this study are separated by a small number of mutations; only two steps are required to move between those most distantly related (H1 and H4 or H2 and H3) (Table 3). A minimal number of mutation events would therefore result in the inter-populational chloroplast variation observed. Secondary contact between divergent populations that have had enough time to accumulate mutational differences could then explain polymorphism within populations.

For the external transcribed spacer region, combined repeat types were recovered from *E. compositus* populations in each geographic region sampled. Therefore, results of this study parallel those of Linder et al. (2000) and Andreasen and Baldwin (2003) in reporting multiple ETS repeat types within a single individual. As the repeat types present in sexual *E. compositus* have not yet been described it is difficult to relate these patterns to hybridization, but given the established importance of hybridization in the complex it is likely that repeat types recovered from hexaploid agamospermous *E. compositus* are also

present in their diploid sexual relatives. Mutation could also result in the observed variation in nrDNA. Because of the presumed rarity of recombination in agamic taxa, nuclear mutations may become automatically fixed in clonal lineages and accumulate rapidly (Maynard Smith, 1978; Richards, 1986). However, Noyes and Soltis (1996) determined that allozyme variation in agamospermous *E. compositus* is very similar to that of sexual diploid *E. compositus,* suggesting that most of the genetic variation in agamospermous populations is ultimately derived from diploid populations rather than mutation.

The maintenance of intra-populational genetic variation within agamic populations is difficult to explain. One common hypothesis invokes occasional sexual reproduction either among facultative agamic clones or between agamic clones and sexual plants (Asker and Jerling, 1992). In *E. compositus*, agamic and sexual populations have a mainly allopatric distribution. Recent crossing between agamic and sexual plants is therefore an unlikely source of genetic variation within agamic populations. Sexual reproduction between agamospermous individuals with different genotypes is considered more likely (Noyes and Soltis, 1996). The occurrence of occasional sexual reproduction could also act to maintain intraindividual variation as the homogenizing effects of concerted evolution are diminished in allopolyploids (Doyle et al., 2002). This is because disomic inheritance limits interaction between the divergent repeats during meiosis (Noyes and Rieseberg, 2000). Agamospermous *E. compositus* individuals therefore have the potential to exhibit higher levels of nrDNA polymorphism than diploid populations at both the population and individual level.

Although the evolution of the nrDNA family is extremely complex and the mechanisms of concerted evolution are not well understood (Yoong Lim et al., 2000; Rauscher et al., 2002), hexaploid agamospermous *E. compositus* has the potential to help

clarify these mechanisms due to its hybrid genome. This is because, at least in the first generation, the newly formed genome contains a minimum of two unique, homeologous ribosomal DNA loci. The fate of these loci following the polyploid event is unclear, although evidence from this study, as well as others, points to the maintenance of multiple repeat types over time (Buckler et al., 1997; Rauscher et al., 2002; Singh et al. 2001).

Statistical analyses of chloroplast and nrDNA for *Erigeron compositus* reveal patterns consistent with an agamospermous breeding system. First, the AMOVA revealed that the percentage of variation for *E. compositus* was higher among populations (76% for cpDNA and 69% for the ETS region; Tables 7 and 8) than within populations. A high level of population differentiation was further supported by F_{ST} values of 0.76 for cpDNA haplotypes and 0.69 for ETS repeat types. Although most of the variation was accounted for at the 'among population' level, more than one repeat type was recovered in the majority of populations (Fig. 2). Noyes and Soltis (1996) described similar population structure for agamospermous *E. compositus* (variation was identified both within and between populations), as have other researchers examining clonal taxa (Mogie, 1992; van Dijk and Bakx-Schotman, 2004). That is, clonal species tend to be multiclonal both within and among populations (Ellstrand and Roose, 1987). Multiclonal populations also show intermediate levels of diversity and evenness indicating that most populations are neither composed of numerous genotypes in equal frequencies nor dominated by a single genotype. Instead, the typical population genetic structure is complex (Ellstrand and Roose, 1987).

In comparison, results of the AMOVA for *Erigeron lanatus* attributed the highest percentage of the total variation for cpDNA to 'within population variation' (88.62%). The variation between populations was therefore low (11.38%) (Table 7). This is perhaps due to a

combination of low levels of polymorphism in ancestral populations and a short divergence time. Descendant populations would then exhibit a similar level of variation. *Erigeron lanatus* populations are geographically isolated so cytoplasmic gene flow among genetically diverse populations is likely unimportant . Over time, it is expected that different haplotypes within each population will move to fixation, so the observed variation within populations suggests drift is incomplete. The different pattern exhibited by *E. compositus* thus likely relates to higher levels of cpDNA polymorphism in ancestral populations, an unsurprising observation given the recognized hybrid origins of agamospermous *E. compositus*.

Patterns of nrDNA for *E. lanatus* do not correspond to those of cpDNA. The ETS region exhibited polymorphism at both the individual and population level. In addition, the AMOVA for the ETS region (Table 8) reveals that 'among population' variation was the higher of the two variation percentages for *E. lanatus*. This observation could be explained by higher levels of nrDNA polymorphism in ancestral populations compared to those observed for chloroplast DNA.

Both the overall Mantel test and the Mantel test with distance classes failed to link the observed genetic variation within and among *Erigeron compositus* populations to geographic distance. Such a continuous range of variation has been detected in agamic grasses (Kellogg 1987, 1990). There are numerous explanations for this observation. The first relates to the high elevation, alpine habitat of *E. compositus*. This environment is less stable than those at lower elevations. Thus, frequent local extinctions and recolonizations could result in a lack of equilibrium between drift and migration, as described by metapopulation models (McCauley, 1995).

A second explanation for populations of *E. compositus* failing to exhibit spatial structure is also based on habitat. Agamospermous *E. compositus* populations that are sympatric with *E. trifidus* and *E. lanatus* inhabit exposed alpine slopes. As such, few barriers exist to the dispersal of their seeds via wind. Geographic patterns resulting from this dispersal mechanism may be present, but at a scale that wasn't detectable with the sampling strategy used for this study. Thus, collecting and analyzing a greater number of individuals over a more continuous range may be required to identify correlations between genetic and geographic distance.

Third, the variation present in and between *E. compositus* populations could be due to mutation. *Erigeron compositus* is geographically widespread. Some authors suggest that in large areas that are absolutely dominated by apomicts, the maintenance of genotypic diversity through sexual recruitment will be limited if not absent (Ellstrand and Roose, 1987; Richards, 2003). As a result, genotypic differentiation will mostly depend on accumulation of mutations in apomicts. Noyes and Soltis (1996) detected unique alleles in agamospermous *E. compositus* based on electrophoretic data and identified mutation as a possible source. Variation resulting from mutation would also lack widespread geographic structure. Further, the possibility exists that the sites analyzed in this study represent mutational 'hotspots' within chloroplast and/or nrDNA regions. However, unique repeat types were not identified from *E. compositus* populations in this study. Unless this is a direct result of sampling error, mutation is an unlikely source of inter-population variation.

Our results indicate that in one of the regions analyzed, *Erigeron trifidus* populations represent a distinct hybrid species rather than variants within the *Erigeron compositus* microspecies complex. However, the situation in the other *E. trifidus* regions differs. An

understanding of the various evolutionary forces at work can best be achieved by focusing on patterns within each geographic region.

In the northern region, where *Erigeron trifidus* is the most common, populations exhibited characteristics that support their hybrid origin and identify *E. compositus* and *E. lanatus* as the likely parental species. First, an intermediate chromosome number of 2n=45 was confirmed for an *E. trifidus* individual in the northern region. Morphology also supports the hybrid origin hypothesis (Fig. 4). A primary criterion of hybridity is morphological intermediacy in several characters (Gottlieb, 1972). This criterion is one on which Packer (1983a) based his hybrid origin hypothesis. Individuals of *E. trifidus* from northern populations showed very similar petiole widths and degrees of phyllary pubescence whose average measurements were both statistically different from those reported for *E. compositus* and *E. lanatus* as well as intermediate to them. Outliers were present for *E. trifidus* for both characters, but these populations were not located in the northern region (Figures 4a and 4b).

The chloroplast genome is usually nonrecombining and uniparentally inherited, making it useful for tracking haplotype lineages and distinguishing maternal from paternal parents (Soltis et al., 1992). *Erigeron trifidus* populations across the northern region invariably show the same cpDNA haplotypes as *E. lanatus* from this same area (Fig 1). *Erigeron lanatus* could therefore be the maternal parent in putative crosses. Polyploid *E. compositus* is facultatively agamospermous (Noyes and Soltis, 1996) and it is well documented that when agamosperms are involved in sexual reproduction, they often act as the pollen donor (Gustafsson, 1946-1947; Nordal and Iverson, 1993). Even plants that are obligate apomicts in female function may produce reduced, functional pollen (Gornall, 1999; Richards, 2003). However, northern *E. compositus* populations also exhibit haplotypes 1 and

3, like *E. trifidus*, thus allowing the possibility of *E. compositus* being the maternal parent. Data from Noyes and Soltis (1996) have shown that crossing between genetically distinct facultative agamosperms accounts for most of the variation that exists in polyploid *E. compositus* populations. This observation indicates that certain individuals within these populations have either retained or secondarily evolved the ability to act as a maternal parent in sexual reproduction. Thus, *Erigeron trifidus* could also have inherited its chloroplast genome directly from *E. compositus*. Populations within an agamospermous microspecies complex may exhibit diverse capacities for pollen production, with corresponding differences in female reproductive function (Van Baarlen et al., 1999). Therefore, an additional possibility is that both *E. compositus* and *E. lanatus* have acted as the maternal parent in sexual crosses. However, cytonuclear interactions have a strong effect on the relative fitness of hybrid species. Thus, crosses often occur in only one direction (Asker and Jerling, 1992). Additional molecular studies are required to distinguish between these hypotheses.

The existence of cpDNA haplotypes 1 and 3 in both *E. compositus* and *E. lanatus* populations could be due to a number of factors. First, lineage sorting could produce the observed variation patterns. In this case, each species would receive a different subset of haplotypes from a polymorphic ancestor (Avise, 1994). Lineage sorting therefore suggests that haplotypes 1 and 3 evolved within the *Erigeron* complex via ancient hybridization events and were then distributed randomly. Further, haplotype 1 is present in *E. trifidus, E. compositus* and *E. lanatus* populations across their sampled ranges. The coalescence theory of genetic relationships states that ancestral haplotypes are widespread and common because they persist along with newer forms (Watterson and Guess, 1977). The existence of haplotype 1 in an historical ancestor is thus supported. A second explanation is that the

shared haplotypes arose via convergence. If convergence is the means by which haplotypes 1 and 3 appear, a random distribution should result as the mutations creating the characteristic restriction sites would be independent of both taxonomic classification and geographic location. This is the case for cpDNA haplotypes for all three *Erigeron* species, as revealed by the insignificant R_M values for the overall Mantel test. However, haplotypes 1 and 3 are very widely distributed in both *E. compositus* and *E. lanatus* populations. Multiple origins of similar chloroplast haplotypes across a wide geographic range is unparsimonious and therefore relatively unlikely.

The level of repeat type variation uncovered in the ETS region in this study was surprising. Multiple repeat types were recovered from all three *Erigeron* species (Fig. 2, Tables 5 and 6) so the expectation of strictly monomorphic parents and a hybrid which exhibited both parental repeat types was not realized. However, *E. trifidus* exhibited only repeat types and combined repeat types present in one or both of the putative parents across its geographic range (Fig. 2, Tables 5 and 6). Additivity of parental repeat types in nrDNA is a recognized hybrid trait (Andreasen, 2003;Rauscher et al., 2004) so the pattern shown by *E. trifidus* is consistent with a hybrid origin. In addition, *E. trifidus* showed the highest levels of polymorphism at the individual and population level, a characteristic directly attributable to genome combination (Rieseberg, 1997).

The variation in *E. trifidus* repeat type patterns from populations in the northern region was not directly attributable to variation in the putative parental populations. This provides some support for monophyly of northern *E. trifidus* populations. Despite this hypothesis, differences in ETS variation patterns do exist within these populations. For example, combined repeat type 1+2 is present in Mountain Park and Jasper National Park but

is absent in Willmore Wlderness area. Further, combined repeat type 2+4 is present in Jasper National Park only (Fig. 2). Gene flow is the principal factor acting to reduce genetic differentiation between populations (Slatkin, 1987). The fact that populations of *E. trifidus* exhibit a degree of genetic differentiation is evidence against rampant sexual reproduction. This observation is in line with other lines of evidence pointing toward an apomictic breeding system for *E. trifidus* (sterile pollen and large population size in northern regions) (Packer, 1983). Therefore, the variation in ETS repeat types in each northern region could be explained by origin and maintenance of variation through agamospermy, similarly to *E. compositus*.

Patterns in the ETS region separate northern *Erigeron trifidus* populations from *E. trifidus* populations further south. The HpyCH4IV restriction site at the 350 bp mark was absent in all northern populations but present at Ram Mountain and in the southern region (Fig.2). This north/south geographic split including the Ram Mountain population in the south was supported by statistical analyses. First, the AMOVA for ETS repeat type data revealed that the percentage of variation among groups was higher when northern populations were considered in isolation (56.21%) compared to the value when T-RM1 was included in the northern region (39.99%) (Table 8). In addition, the 'among population' variation percentage was reduced from 54.2% for ungrouped *E. trifidus* populations to 15.3% when the northern region consisted solely of populations from Mountain Park, Willmore Wilderness Area and Jasper National Park (Table 8). Further, according to the Mantel test with distance classes, populations separated by distances of up to 100 km (which included only northern populations) were significantly positively correlated whereas all other distance classes failed to show a significantly positive correlation (Fig. 3a). Finally, Figure 3a

illustrates a significantly negative R_M value for distance class 4, which consists of *E. trifidus* populations in the northern region and those from Waterton Lakes National Park. Regional variation is uncommon in arctic-alpine species of restricted distributions (Gabrielson et al., 1997; Tollesfrud et al., 1998; Bauert et al., 1998) and is likely a result of multiple origins for *E. trifidus*.

Erigeron trifidus populations in the southern region at Waterton Lakes National Park (157 and 170) exhibited wider petioles and a greater degree of phyllary pubescence compared to the average for the species. Local variation for *E. trifidus* thus parallels that observed for *E. lanatus* (Figures 4a and 4b), supporting the hypothesis of local hybridization. However, *Erigeron trifidus* population T-WL2 is polymorphic for chloroplast haplotypes 1 and 3 whereas *E. lanatus* populations from the same region are all monomorphic for H1 (Fig.1). Assuming mutation is unimportant, possible explanations for this observation include a restricted sample size for *E. lanatus*. If more individuals had been sampled, haplotype 3 may have been recovered as it was in Willmore Wilderness Area (L-WW2). Second, *E. lanatus* could have been the hybrid pollen donor. If local *E. compositus* populations comprise other parental species, this scenario would require *E. compositus* filling the role of the maternal parent, a distinct possibility given the presence of haplotype 3 in population C-WL2 (Fig. 1) and the ability of *E. compositus* to undergo occasional sexual reproduction (Noyes and Soltis, 1996). Both of these hypotheses indicate that, despite the morphological differences observed for *E. lanatus* populations at Waterton Lakes National Park, collections made in this region were of *E. lanatus* individuals rather than of a different species.

A third explanation for the presence of haplotype 3 in southern *Erigeron trifidus* populations is that *E. lanatus* was not involved in the putative hybridization event as either a

maternal or a paternal parent. *Erigeron lanatus* from Waterton Lakes National Park was genetically isolated, as comparisons of *E. lanatus* populations from this region to populations occurring further north failed to show any significantly positive R_M values in the Mantel test with distance classes (distance classes 4 and 5; Fig. 3c). This observation could identify an isolated population of *E. lanatus* or it could point to populations L-WL1 and L-WL2 being comprised of a different species. *Erigeron pallens* occurs in Waterton Lakes National Park and is morphologically similar to *E. lanatus* in exhibiting solitary, large, villous heads and oblanceolate to spatulate leaves that are 3-lobed at the apex (Cronquist, 1947; Packer, 1983b). In addition, *E. pallens* is diploid (2n=18) (Packer, 1983b), indicating that a sexual breeding system is likely. *Erigeron pallens* is thus capable of acting as a paternal parent in a putative cross with local *E. compositus*. Chromosome counts for all Waterton Lakes National Park collections would assist in differentiating between *E. lanatus* and *E. pallens*.

Our results suggest that the identity of *Erigeron trifidus* populations from Ram Mountain is different from both the northern and the Waterton Lakes National Park populations. First, one putative ancestor, *E. lanatus*, was not collected at Ram Mountain. Nor has it ever been identified from this area (Packer, 1983b). Second, *E. trifidus* populations from Ram Mountain (133 and 173) exhibited narrower petioles and a lower degree of phyllary pubescence compared to *E. trifidus* individuals from other regions. Values for these two measurements were therefore close to that reported for the *E. compositus* species average. Third, chloroplast haplotypes 1 and 2 were recovered from *E. trifidus* population T-RM1 (populations 133 and 173; Table 1) although they were absent in *E. compositus* populations from Ram Mountain. The only other locations exhibiting haplotype 2 were *E. compositus* populations from Willmore Wilderness Area and Jasper

National Park. Overall, the data indicate that populations of *E. trifidus* from Ram Mountain (populations 133 and 173) consist of another group in the *E. compositus* microspecies complex rather than hybrid *E. trifidus*.

We are left to explain the absence of *Erigeron trifidus* in Banff National Park in areas where both *E. compositus* and *E. lanatus* exist. The Rocky Mountains of Alberta represent the boundary of Laurentide and Cordilleran ice sheets during the last advance of Pleistocene glaciation (Rutter, 1984). Based on geomorphological evidence and high levels of species diversity, *in situ* glacial survival in the Mountain Park area is considered plausible for flowering plants during the Wisconsin period (Packer and Vitt, 1974). The possibility of long-term survival on nunataks (an Inuit word meaning an ice-free mountain prominently emerging above the surface of a glacier) within glaciated areas has been supported for many arctic-alpine species (Abbott et al., 1995; Demesure et al., 1996; Soltis et al., 1997). Therefore, the possibility exists that *E. trifidus* persisted through the last glacial advance on nunataks within the Mountain Park area and spread to the other northern regions after the retreat of the ice. Nunataks have not been identified in Banff National Park. Perhaps *E. trifidus* existed in this area prior to Pleistocene glaciation, during which the lack of favourable environments caused the species to become locally extinct. Or, perhaps the initial hybridization event between *E. compositus* and *E. lanatus* occurred on nunataks within Mountain Park and subsequent migration to Banff National Park never occurred.

CONCLUSIONS

This study provided evidence for polyphyletic hybrid origins of *Erigeron trifidus* and identified *E. compositus* and *E. lanatus* as the parental species in the northern region of Alberta. Morphological characters of northern *E. trifidus* populations were found to be intermediate to those measured in *E. compositus* and *E. lanatus*. Intermediacy was also present in the chromosome number of *E. trifidus*. Chloroplast DNA haplotype patterns indicate that *E. lanatus* is likely the maternal parent in the majority of *E. trifidus* populations. Finally, a surprising level of ETS repeat type variation was recovered from populations of *E. compositus* and, to a lesser degree, *E. lanatus*. Additivity of this repeat type variation, as well as the highest levels of intraindividual repeat type polymorphism, were observed in hybrid *E. trifidus* populations.

Populations of *E. trifidus* at Waterton Lakes National Park are also hypothesized to be the product of local hybridization. However, it was not possible to identify maternal or paternal parents here due to discrepancies in chloroplast DNA haplotype patterns between *E. trifidus*, *E. compositus* and *E. lanatus*. *Erigeron trifidus* has also been identified from Ram Mountain. However, the absence of *E. lanatus* from Ram Mountain combined with chloroplast DNA haplotype patterns and morphology of individuals from this area, point to populations being comprised of another group in the *Erigeron compositus* microspecies complex rather than individuals of hybrid origin.

An odd chromosome number of 2n=45 is the proposed mechanism by which *Erigeron trifidus* is reproductively isolated from its sympatric parents. This count is based on very few individuals and needs to be verified with a larger sample size. Cytology also supports an agamospermous breeding system for *E. trifidus*, although the identification of

agamospermy will require additional analyses in the form of in-depth breeding system studies.

Overall, this study identifies the process of hybridization as important in the evolution of plant populations. *Erigeron trifidus*, with its multiple hybrid origins, represents a novel genotype that is well adapted to its environment (based on observed population size). The study also supports the usefulness of multigene families like the ETS region in the detection of hybridization events. Future investigations examining numerous aspects of the structure of *E. trifidus* populations would increase our knowledge of the long term evolutionary potential for hybrid species, and agamospermous hybrid species in particular.

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Table 1. Location and population components for combined populations of *Erigeron trifidus*, *E. compositus* and *E. lanatus*

Species/combined Pop. Abbreviation	Location	Collection numbers included in combined populations
E. trifidus		
T-CD1	Cardinal Divide	105, 108, 136
T-CD2	Cardinal Divide	103, 139
T-CD3	Cardinal Divide	140, 175
T-JNP1	Jasper Nat'l. Park	110, 113
T-JNP2	Jasper Nat'l. Park	143, 153
T-RM1	Ram Mt.	133, 173
T-WW1	Wilmore Wilderness Pk.	163, 167
T-WW2	Wilmore Wilderness Pk.	160
T-WLNP1	Waterton Lakes Nat.'l Pk.	157
T-WLNP2	Waterton Lakes Nat.'l Pk.	170
E. compositus		
C-CD1	Cardinal Divide	104, 109
C-CD2	Cardinal Divide	106, 135
C-CD3	Cardinal Divide	174
C-JNP1	Jasper Nat'l. Pk.	112, 145
C-JNP2	Jasper Nat'l. Pk.	154
C-RM1	Ram Mt.	134, 172
C-BNP1	Banff Nat'l. Pk.	116, 148
C-WW1	Wilmore Wilderness Pk.	161
C-WW2	Wilmore Wilderness Pk.	164
C-WLNP1	Waterton Lakes Nat'l. Pk.	158
C-WLNP2	Waterton Lakes Nat'l. Pk.	169
C-AR1	Kananaskis	102
C-AR2	Kananaskis	159

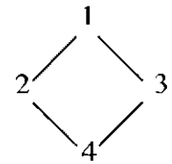
Table 1....cont.

Species/combined	Location	Collection numbers included
E. lanatus		
L-CD1	Cardinal Divide	107, 138
L-JNP1	Jasper Nat'l. Pk.	111, 114, 142
L-BNP1	Banff Nat'l. Pk.	115
L-BNP2	Banff Nat'l. Pk.	146
L-WW1	Wilmore Wilderness Pk.	162
L-WW2	Wilmore Wilderness Pk.	165, 168
L-WLNP1	Waterton Lakes Nat'l. Pk.	156
L-WLNP2	Waterton Lakes Nat'l. Pk.	171
L-AR1	Kananaskis	129, 101

Table 2. Genome regions analyzed for *Erigeron trifidus*, *E. compositus* and *E. lanatus*.

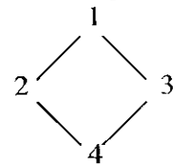
Chloroplast region	Reference	Genome	Variation present (+) Or absent (-)
*trn*K intron	Demesure et al., 1995	chloroplast	-
trnC-*trn*D intergenic spacer	Demesure et al., 1995	chloroplast	-
*trn*D-*trn*T intergenic spacer	Demesure et al., 1996	chloroplast	-
*trn*L intron	Taberlet et al., 1991	chloroplast	-
*trn*T-*trn*L intergenic spacer	Taberlet et al., 1991	chloroplast	-
*trn*L-*trn*F intergenic spacer	Taberlet et al., 1991	chloroplast	-
*trn*F-*trn*V intergenic spacer	Dumolin-Lapegue et al., 1997	chloroplast	-
nad1 intron	Demesure et al., 1995	mitochondrial	-
*psb*A-*trn*H intergenic spacer	Sang et al., 1997	chloroplast	+
*trn*C-*psb*M intergenic spacer	Shaw et al., 2005	chloroplast	+
ETS	Markos and Baldwin, 2001	nuclear	+

Table 3 . Haplotype profiles and fragment size for 4 cpDNA haplotypes detected in *Erigeron trifidus*, *E. compositus* and *E. lanatus* using two enzyme-genome combinations

Haplotype	Restriction site		Fragment size(s) for *Rsa*I site (bp)	Fragment size(s) for *Mun*I site (bp)	cpDNAhaplotypes (lines = single mutations)
	I. *Rsa*I	II. *Mun*I			
1	1	1	210 + 110	500 + 600	
2	1	0	210 + 110	1100	
3	0	1	320	500 + 600	
4	0	0	320	1100	

I. *Rsa*I site located within the *psb*A-*trn*H intron
II. *Mun*I site located within the *trn*C-*psb*M intron
For Restriction sites: 1 = restriction cut site present
0 = restriction cut site absent

Table 4. Repeat type profiles of 4 ETS repeat types and summary of combined repeat types detected in *Erigeron trifidus*, *E. compositus* and *E. lanatus*. For restriction enzyme HpyCH4IV, the uninformative mutation at the 500 bp mark is not shown. (⊕) indicate inferred small fragments. Asterisks indicate repeat types and combined repeat types possible but not recovered in any individual sampled.

Repeat Type	Restriction site from Tsp5091	Restriction site from HpyCH4Iv	Fragment size(s) for Tsp5091	Fragment size(s) for HpyCH4IV	Combined Repeat Types	Relationships of ETS repeat types (lines=single mutations)
					1+2	
1*	0	1	700	350 ⊕	1+3*	
2	0	0	700	500 ⊕	2+4	
3*	1	1	300 + 400	350 ⊕	3+4	
4	1	0	300+400	500 ⊕	1+2+3+4	

For Restriction sites: 1 = restriction cut site present
0 = restriction cut site absent

Table 5. Distribution of chloroplast DNA haplotypes from Table 2 and ETS repeat types and combined repeat types from Table 3.

Taxon/ Combined Population	Location	cp DNA haplotypes				ETS repeat types & combined repeat types					
		H1	H2	H3	H4	1+2	1+2+3+4	2	1+2	4	3+4
E. trifidus											
T-CD1	Cardinal Divide	X					X	X	X		
T-CD2	Cardinal Divide	X					X				
T-CD3	Cardinal Divide		X						X		
T-JNP1	Jasper Nat'l. Pk.	X					X	X	X		
T-JNP2	Jasper Nat'l. Pk.	X				X		X	X		
T-RM1	Ram Mt.	X	X			X					
T-WW1	Willmore Wilderness Pk.	X					X	X		X	X
T-WW2	Willmore Wilderness Pk.	X		X			X			X	
T-WLNP1	Waterton Lakes Nat'l. Pk.	X				X	X				
T-WLNP2	Waterton Lakes Nat'l. Pk.	X		X		X	X				
E. compositus											
C-CD1	Cardinal Divide				X				X		
C-CD2	Cardinal Divide		X			X	X	X			
C-CD3	Cardinal Divide		X						X		
C-NP1	Jasper Nat'l. Pk.	X	X			X		X			
C-JNP2	Jasper Nat'l. Pk			X		X		X			
C-RM1	Ram Mt.			X	X		X		X		
C-BNP1	Banff Nat'l. Pk.	X			X		X	X		X	
C-WLNP1	Waterton Lakes Nat'l. Pk.			X		X		X	X		
C-WLNP2	Waterton Lakes Nat'l. Pk.	X		X			X	X	X		
C-WW1	Willmore Wilderness Pk.		X		X		X	X	X		
C-WW2	Willmore Wilderness Pk				X				X		
C-AR1	Kananaskis					X			X		
C-AR2	Kananaskis				X	X	X			X	
E. lanatus											
L-CD1	Cardinal Divide		X							X	
L-JNP1	Jasper Nat'l. Pk.	X								X	X
L-BNP1	Banff Nat'l. Pk.	X									X
L-BNP2	Banff Nat'l. Pk.	X								X	
L-WLNP1	Waterton Lakes Nat'l. Pk.	X				X					
L-WLNP2	Waterton Lakes Nat'l. Pk.	X				X	X	X			
L-WW1	Willmore Wilderness Pk.	X								X	
L-WW2	Willmore Wilderness Pk.	X		X		X			X	X	
L-AR1	Kananaskis		X			X					

Table 6. Sample Size (*N*), cpDNA haplotype frequency and ETS repeat type and combined repeat type frequency

		cp DNA haplotypes				ETS repeat types/combined repeat types					
Species/Population	*N*	H1	H2	H3	H4	2+4	1+2+3+4	2	1+2	4	3+4
E. trifidus											
T-CD1	39	1.000	0	0	0	0	0.564	0.077	0.359	0	0
T-CD2	10	1.000	0	0	0	0	1.000	0	0	0	0
T-CD3	10	1.000	0	0	0	0	0	0	1.000	0	0
T-JNP1	22	1.000	0	0	0	0	0.773	0.045	0.182	0	0
T-JNP2	15	1.000	0	0	0	0.067	0	0.200	0.733	0	0
T-RM1	11	0.818	0.182	0	0	1.000	0	0	0	0	0
T-WW1	12	1.000	0	0	0	0	0.750	0.083	0	0.083	0.083
T-WW2	10	0.875	0	0.125	0	0	0.900	0	0	0.100	0
T-WLNP1	8	1.000	0	0	0	0.700	0.300	0	0	0	0
T-WLNP2	5	0.500	0	0.500	0	0.800	0.200	0	0	0	0
E. compositus											
C-CD1	5	0	0	1.000	0	0	0	0	1	0	0
C-CD2	24	0.833	0	0	0.167	0.912	0.042	0.042	0	0	0
C-CD3	8	1.000	0	0	0	0	0	0	1	0	0
C-JNP1	14	0.857	0.143	0	0	0.857	0	0.143	0	0	0
C-JNP2	15	0	0	1.000	0	0.800	0	0.200	0	0	0
C-RM1	16	0	0	0.267	0.733	0	0.750	0	0.250	0	0
C-BNP1	13	0.846	0	0	0.154	0	0.154	0.077	0	0.769	9
C-WW1	9	0	0.375	0	0.625	0	0.375	0.125	0.500	0	0
C-WW2	8	0	0	0	1.000	0	0	0	1.000	0	0
C-WLNP1	8	0	0	1.000	0	0.222	0	0.667	0.111	0	0
C-WLNP2	7	0.500	0	0.500	0	0	0.200	0.200	0.600	0	0
C-AR1	10	0	0	0	1.00	0	0	0	1.00	0	0
C-AR2	14	0	0	1.00	0	0.214	0.714	0	0	0.0714	0
E. lanatus											
L-CD1	11	1.000	0	0	0	0	0	0	0	1.000	0
L-JNP1	11	1.000	0	0	0	0	0	0	0	0.933	0.067
L-BNP1	12	1.000	0	0	0	0	0	0	0	0	1.000
L-BNP2	8	1.000	0	0	0	0	0	0	0	1.000	0
L-WW1	8	1.000	0	0	0	0	0	0	0	1.000	0
L-WW2	5	1.000	0	0	0	0.077	0	0	0.077	0.846	0
L-WLNP1	12	1.000	0	0	0	1.000	0	0	0	0	0
L-WLNP2	13	0.800	0	0.200	0	0.600	0.200	0.200	0	0	0
A-AR1	8	1.000	0	0	0	1.000	0	0	0	0	0

Table 7. Results of the analyses of molecular variance (AMOVA) of chloroplast restriction site data from *Erigeron trifidus*, *E. compositus and E. lanatus*.

Grouping	Source of variation	d.f.	SS	Variance components	% total variation	F_{ST}*
E. trifidus						
Ungrouped	Among populations	9	2.31	0.01668	32.47	0.325
	Within populations	130	4.51	0.03470	67.53	
North vs. South	Among groups	1	0.35	0.00212	4.04	0.340
(T-RM1 in northern region)	Among pop.'s within groups	8	1.97	0.01575	29.96	
	Within populations	130	4.51	0.03470	66.00	
North vs. South	Among groups	1	0.37	0.00157	3.01	0.335
(T-RM1 in southern region)	Among pop.'s within groups	8	1.95	0.01587	30.44	
	Within populations	130	4.51	0.03470	66.55	
E. compositus						
Ungrouped	Among populations	12	49.16	0.35485	76.44	0.764
	Within populations	135	14.77	0.10937	23.56	
E. lanatus						
Ungrouped	Among populations	8	0.189	0.00141	11.38	0.114
	Within populations	73	0.80	0.01096	88.62	

*All p-values were < 0.05

Table 8 Results of the analyses of molecular variance (AMOVA) of ETS repeat type data from *Erigeron trifidus*, *E. compositus* and *E. lanatus*

Grouping	Source of variation	d.f.	SS	Variance components	% total variation	F_{ST}*
E. trifidus						
Ungrouped	Among populations	9	32.35	0.25017	54.21	0.542
	Within populations	132	27.89	0.21132	45.79	
North vs. South	Among groups	1	8.26	0.27232	39.99	0.690
(T-RM1 in north.region)	Among pop.'s within groups	8	24.08	0.19732	28.98	
	Within populations	132	27.89	0.21132	31.03	
North vs. South	Among groups	1	18.10	0.41697	56.21	0.755
(T-RM1 in south.region)	Among pop.'s within groups	8	14.24	0.11350	15.30	
	Within populations	132	27.89	0.21132	28.49	
E. compositus						
Ungrouped	Among populations	12	57.64	0.41288	69.44	0.694
	Within populations	135	24.53	0.18173	30.56	
E. lanatus						
Ungrouped	Among populations	8	25.69	0.31026	80.82	0.809
	Within populations	83	6.07	0.07315	19.08	

*All p-values were < 0.05

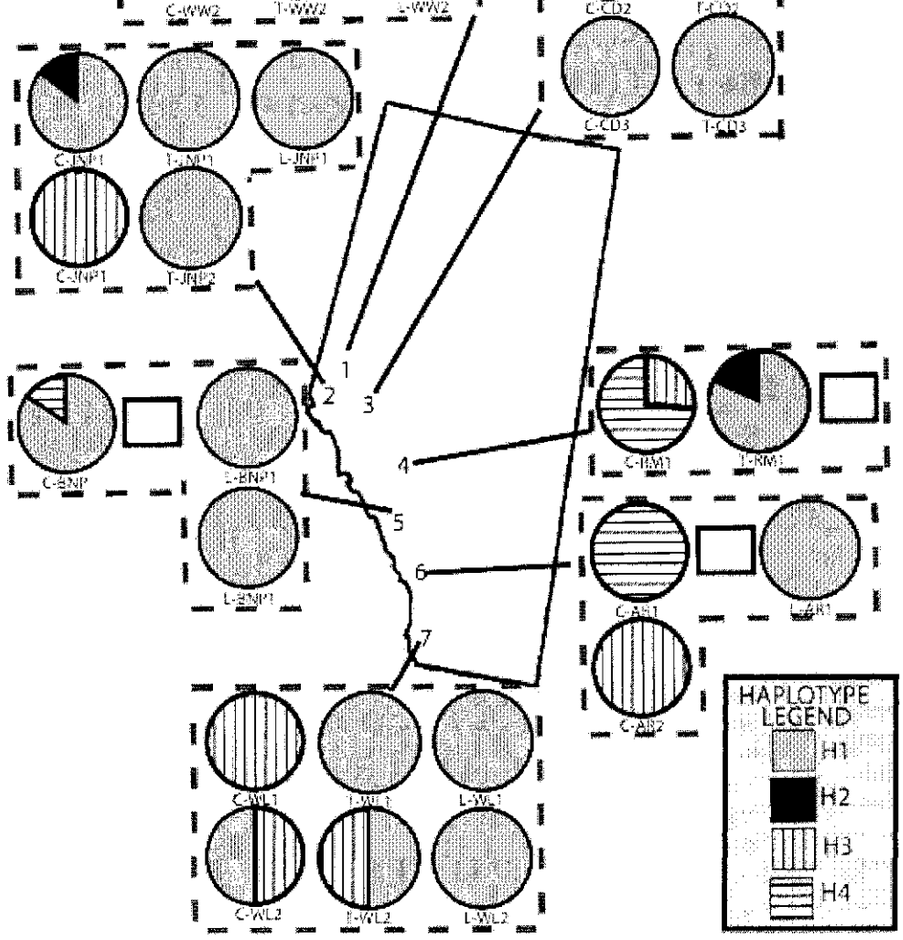


Fig. 1. Chloroplast DNA haplotype map for *Erigeron trifidus* (middle column of pie diagrams in each outlined region), *E. compositus* (first column of pie diagrams in each identified region) and *E. lanatus* (third column of pie diagrams in each identified region). Location numbers are as follows: 1) Willmore Wilderness Area; 2) Jasper National Park; 3) Mountain Park; 4) Ram Mountain; 5) Banff National Park; 6) Waterton Lakes National Park. Squares indicate the absence of a species in that region. Population abbreviations as in Table 1.

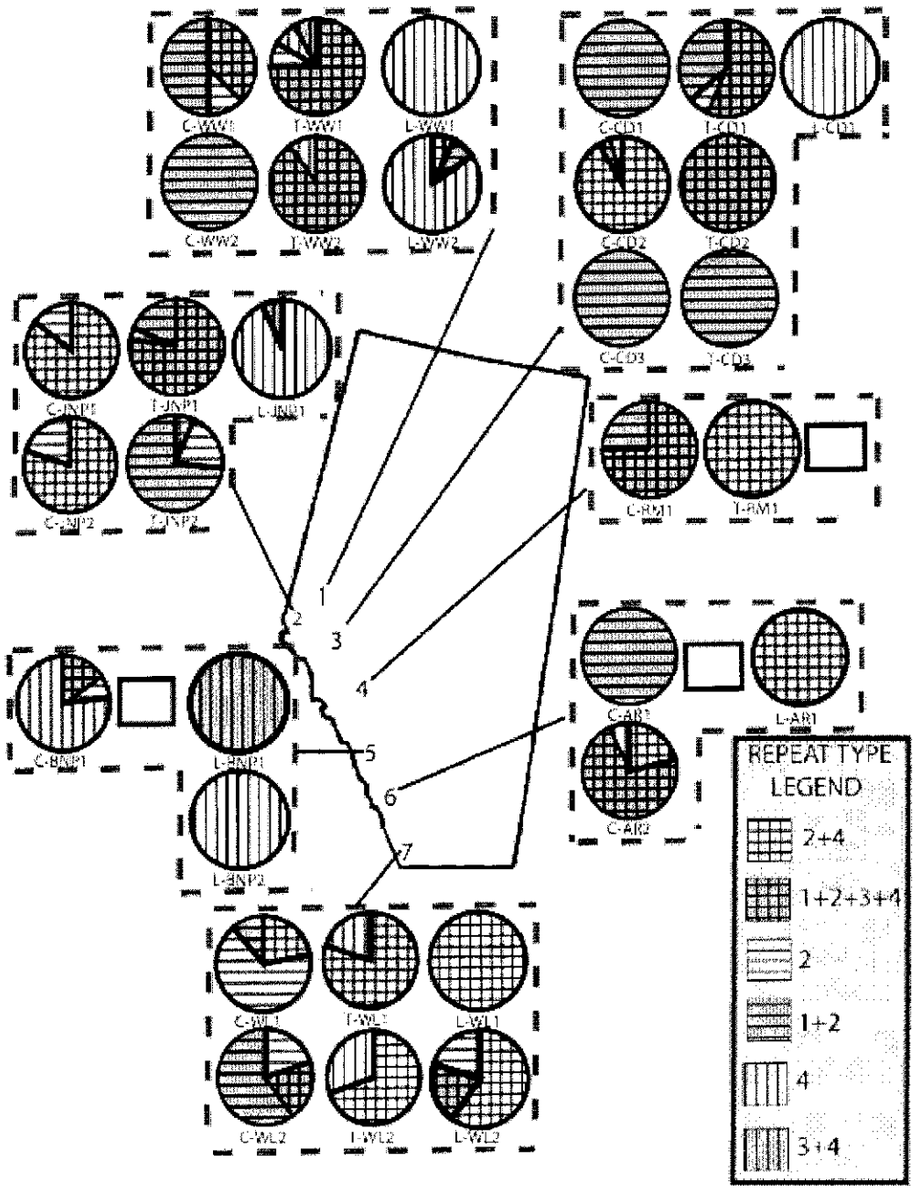


Fig. 2. ETS repeat type map for *Erigeron trifidus* (middle column of pie diagrams in each outlined region), *E. compositus* (first column of pie diagrams in each identified region) and *E. lanatus* (third colulmn of pie diagrams in each identified region). Location numbers are as follows: 1) Willmore Wilderness Area; 2) Jasper National Park; 3) Mountain Park; 4) Ram Mountain; 5) Banff National Park; 6) Waterton Lakes National Park. Squares indicate the absence of a species in that region. Population abbreviations as in Table 1

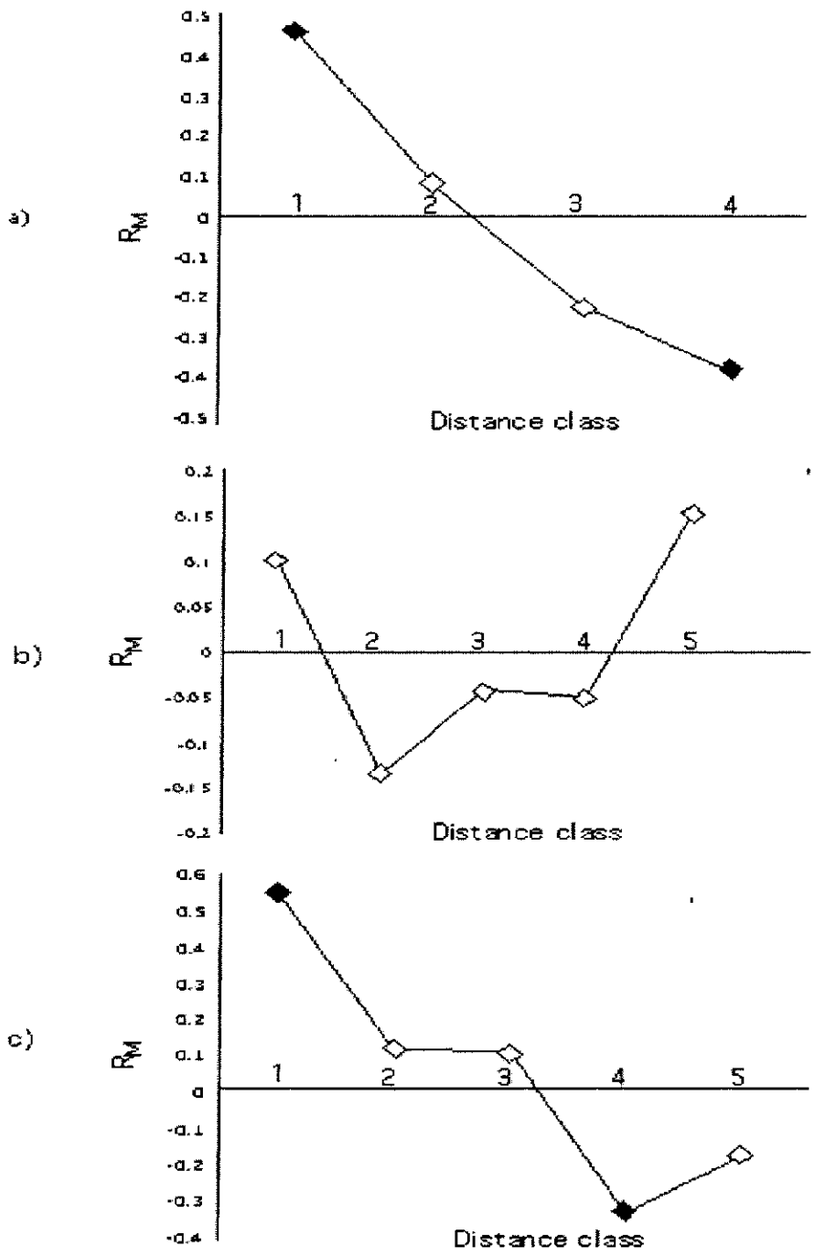


Fig. 3. Correlograms of Mantel R_M per distance class for the ETS region. a) *Erigeron trifidus*; b) *E. compositus*; c) *E. lanatus*. Distance classes for *E. trifidus* as follows: 1) 0 km $\leq$ d < 100 km; 2) 100 km $\leq$ d < 160 km; 3) 160 km $\leq$ d < 250 km; 4) 250 km $\leq$ d < 600 km. Distance classes for *E. compositus* and *E. lanatus* as follows: 1) 0 km $\leq$ d < 100 km; 2) 100 km $\leq$ d < 160 km; 3) 160 km $\leq$ d < 250 km; 4) 400 km $\leq$ d < 600 km; 5) 400 km $\leq$ d < 600 km. Filled diamonds indicate Bonferroni-corrected R_M values that are significantly different from zero at p = 0.05.

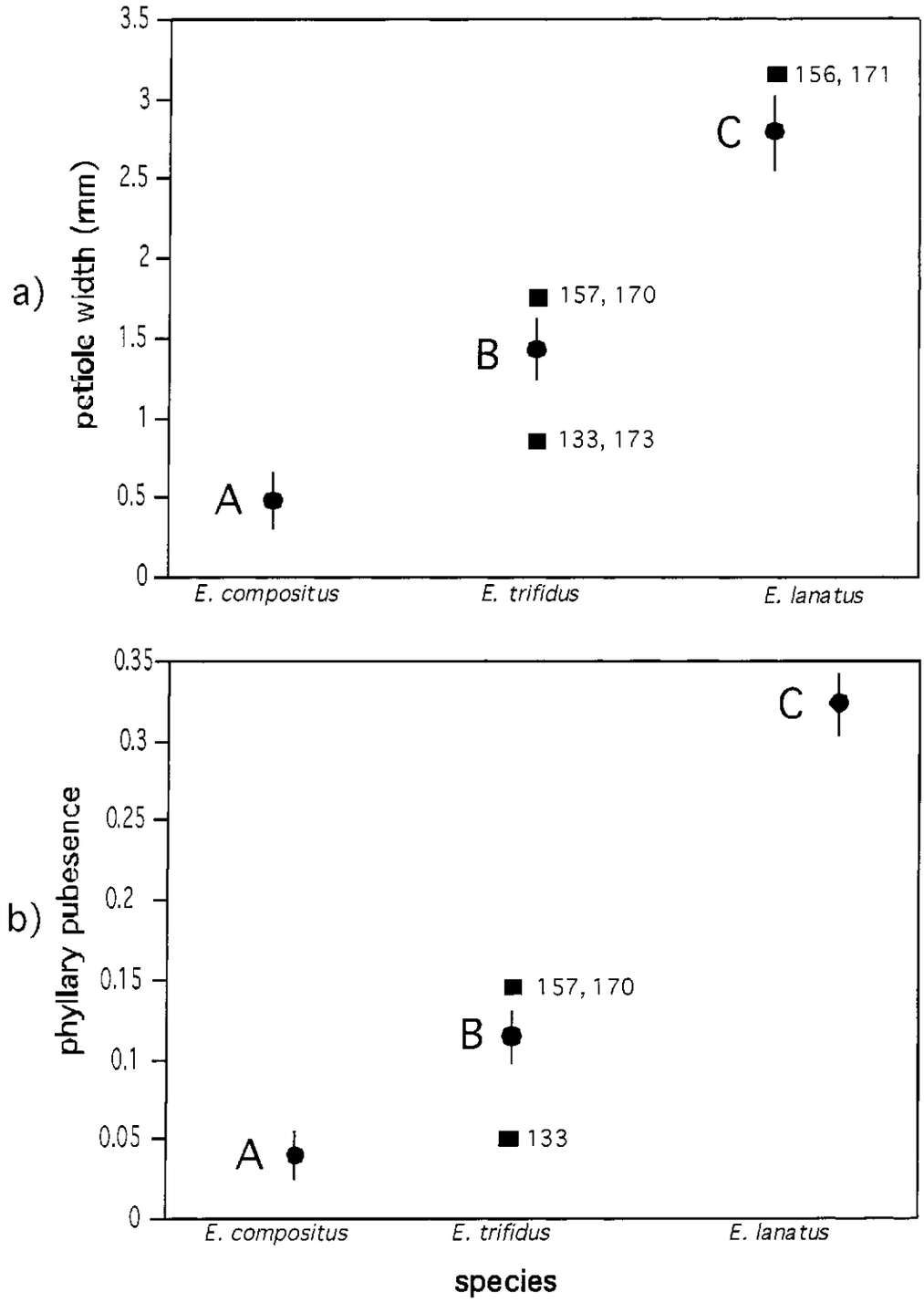


Fig. 4. Mean (± 1 SE) for: a) petiole width; b) phyllary pubescence for *Erigeron trifidus* (*n*=42), *E. compositus* (*n*=56) and *E. lanatus* (*n*=35). Letters represent statistically different means (Tukey-Kramer test, $p < 0.05$) for a one-way ANOVA analysis. Numbers identify specific populations. Population numbers as in Appendix 1.

Appendix 1. Collection numbers, location and elevation for populations of *Erigeron trifidus*, *E. compositus* and *E. lanatus*.

Taxon/ Coll. No.	Location	Lat./Long.	Elevation (m Above sea level)
E. trifidus			
103	Cardinal Divide	52°55'44.4" N., 117°22'40.4" W.	2093
105	Cardinal Divide	52°55'20.2" N., 117°22'40.4" W.	2335
108	Cardinal Divide	52°55'20.4" N., 117°22'40.2" W.	2349
110	Jasper Nat'l. Pk.	52°49'08.4" N., 118°08'52.5" W.	2452
113	Jasper Nat'l. Pk.	52°49'08.5" N., 118°09'52.4" W.	2451
133	Ram Mt.	52°21'24.5" N., 115°47'12.5" W.	2159
136	Cardinal Divide	52°55'20.4" N., 117°22'40.2" W.	2364
139	Cardinal Divide	52°55'44.6" N., 117°22'40.8" W.	2189
140	Cardinal Divide	52°55'26.2" N., 117°22'47.4" W.	2135
143	Jasper Nat'l. Pk.	52°49'01.2" N., 118°09'07.2" W.	2448
153	Jasper Nat'l. Pk.	52°49'01.3" N., 118°08'07.5" W.	2030
157	Waterton Lakes Nat'l. Pk.	49°09'23.4" N., 114°06'24.2" W.	2423
160	Willmore Wilderness Pk.	53°33'12.4" N., 118°30'04.5" W.	2340
163	Willmore Wilderness Pk.	53°29'19.3" N., 118°30'10.3" W.	2181
167	Willmore Wilderness Pk.	53°29'19.4" N., 118°30'10.6" W.	2204
170	Waterton Lakes Nat'l. Pk.	49°08'19.4" N., 113°56'12.9" W.	2238
173	Ram Mt.	52°21'24.4" N., 115°47'12.3" W.	2173
175	Cardinal Divide	52°55'26.3" N., 117°22'47.6" W.	2044
E. compositus			
102	Kananaskis	52°36'02.9" N., 114°58'25.3" W.	2676
104	Cardinal Divide	52°55'44.4" N., 117°22'28.1" W.	2093
106	Cardinal Divide	52°55'20.2" N., 117°22'40.4" W	2335
109	Cardinal Divide	52°55'44.2" N., 117°22'28.3" W.	2440
116	Banff Nat'l. Pk.	52°10'46.9" N., 117°07'42.8" W.	2441
112	Jasper Nat'l. Pk.	52°49'03.4" N., 118°09'02.8" W.	2451
134	Ram Mt.	52°21'26.7" N., 117°07'10.5" W.	2264
135	Cardinal Divide	52°55'20.2" N., 117°22'40.4" W.	2264
145	Jasper Nat'l. Pk.	52°49'03.2" N., 118°09'02.9" W.	2448
148	Banff Nat'l. Pk.	52°10'46.6" N., 117°07'42.5" W.	2490
154	Jasper Nat'l. Pk.	52°46'08.5" N., 118°08'08.2" W.	2150
158	Waterton Lakes Nat'l.Pk.	49°09'23.6" N., 114°06'30.1" W.	2237
159	Kananaskis	50°36'09.1" N., 114°59'20.4" W.	2570
161	Willmore Wilderness Pk.	53°33'17.6" N., 118°30'11.2" W.	2349
164	Willmore Wilderness Pk.	53°29'11.8" N., 118°30'09.3" W.	2159
169	Waterton Lakes Nat'l. Pk.	49°08'18.3" N., 113°56'11.3" W.	2238
172	Ram Mt.	52°21'26.9" N., 117°07'10.3" W.	2173
174	Cardinal Divide	52°54'39.5" N., 117°14'20.5" W.	2154
E. lanatus			
101	Kananaskis	50°36'02.9" N., 114°58'25.3" W.	2676
107	Cardinal Divide	52°55'20.5" N., 117°22'38.5" W.	2087
111	Jasper Nat'l. Pk.	52°49'11.2" N., 118°09'15.4" W.	2460
114	Jasper Nat'l. Pk.	52°49'11.0" N., 118°09'15.2" W.	2451
115	Banff Nat'l. Pk.	52°10'46.5" N., 117°07'45.6" W.	2441
129	Kanasaskis	50°36'02.6" N., 114°58'25.8" W.	2679
138	Cardinal Divide	52°55'20.4" N., 117°22'38.7" W.	2182
142	Jasper Nat'l. Pk.	52°49'11.4" N., 118°09'15.3" W.	2446

Appendix 1....cont.

Taxon/ Coll. No.	Location	Lat./Long.	Elevation (m Above sea level)
E. lanatus			
146	Banff Nat'l. Pk.	52°11'40.1" N., 117°09'30.4" W.	2320
156	Waterton Lakes Nat'l. Pk.	49°09'10.3" N., 114°06'17.5" W.	2423
162	Willmore Wilderness Pk.	53°33'10.7" N., 118°30'40.3" W.	2401
165	Willmore Wilderness Pk.	53°29'09.4" N., 118°30'21.4" W	2204
168	Willmore Wilderness Pk.	53°29'10.2" N., 118°30'21.6" W.	2210
171	Waterton Lakes Nat'l. Pk.	49°08'09.4" N., 113°56'21.4" W.	2238