CHLOROPLAST DNA DIVERSITY IN *PACKERA* (ASTERACEAE)

A Phylogeographic Study of *Packera contermina* and Three Related Species from Southwestern Alberta based on Chloroplast DNA Variation

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Abstract

Members of the genus *Packera* (Asteraceae) are widespread in North America, but most are found in western regions of the continent where extensive morphological intergradation is common. Previous molecular systematic studies found that four species in southwestern Alberta, a region proposed to be at the interface of the Cordilleran and Laurentide ice sheets during the last advance of Pleistocene glaciation, showed unusually high levels of inter- and intrapopulational chloroplast DNA variation. The present study analyzed chloroplast haplotype phylogeny, frequency variation, and geographic distribution patterns in *Packera contermina* and closely related species *P. pseudaurea*, *P. cana*, and *P. cymbalarioides* from southwestern Alberta, northern Montana, and northwestern Wyoming. Restriction site analyses of chloroplast DNA from 730 individuals across 34 populations of the four species revealed fifteen haplotypes, of which seven are commonly found in other North American *Packera* species. Three haplotypes were detected in *P. cymbalarioides*, seven in *P. cana*, eight in *P. pseudaurea*, and twelve in *P. contermina*. The level of haplotype frequency variation among populations was high in *P. cymbalarioides* ($\theta = 1$) moderate to low in *P. contermina* ($\theta = 0.333$) and *P. cana* ($\theta = 0.261$), and very low in *P. pseudaurea* ($\theta = 0.085$), possibly reflecting differences in the species' history. Phylogenetic analyses revealed 2 groups of haplotypes, one of which is found mainly in populations from the Great Basin of North America and the second in populations of more coastal and northern regions. The presence of haplotypes from both groups of *Packera* species suggests that the cpDNA diversity in southern Alberta has arisen through hybridization/introgression events that have involved a number of species from outside of the region.

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Native plants have always been intriguing to me. My early attempts at field identification were limited to color photos in pocket field guides. The names I found for plants were usually forgotten as soon as I flipped the page. When field botany was offered as a summer course at the University of Lethbridge, I decided it was a good opportunity to learn the local flora. The course marked a change in direction for me. My career path as an occasional student was replaced with that of biology major, and ultimately focussed on the research presented in this thesis.

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INTRODUCTION

The role played by geohistorical events associated with glaciation in the evolution of the North American flora and fauna has long been and remains a subject of great interest to evolutionary biologists (Avise 1994, Myers and Giller 1988, Riddle 1996, Stebbins 1950). Efforts to identify the genetic consequences of historical influences on the diversity and distribution patterns commonly center around the events associated with Quaternary climate changes, in particular Pleistocene glaciation events (Comes and Abbott 1998, Soltis et al. 1997). The response of species to climate changes involves many factors including local adaptation or migration to more suitable habitat; therefore, by comparing patterns of intraspecific genetic structure, a more complete picture of the biogeographic history of a species can be achieved.

Changes in geographic distribution due to climate changes may be evident in macro-evolutionary changes (phylogenetic factors) such as lineage sorting and hybridization or in micro-evolutionary changes such as genetic drift, migration, mutation and natural selection (Avise et al. 1987). In the past, studies of such changes were frequently divided into either phylogenetic or population genetic studies, and evolutionary studies of genetic diversity seldom integrated the two approaches. More recent investigations emphasize the importance of historical factors in explaining present-day spatial distribution patterns of genotypes (Demesure et al. 1996, Sewell et al. 1996, Soltis et al. 1997, Strand et al. 1996, Zink and Dittmann 1993). The term "phylogeography" was coined to describe this approach of relating gene genealogies to geographic distribution in a historical context (Avise et al. 1987).

Although traditional methods of studying inter- and intraspecific genetic variation such as morphological and cytological analyses were important in establishing early theories of phylogeny, more recent studies use molecular methods including isozyme characterization and DNA analyses using RAPDs, restriction fragment and sequence data. The importance of molecular data is under-scored by the increasing number of studies using these methods to examine a wide range of questions such as the evolutionary history of related species and intraspecific relationships including population structure and gene flow (Avise 1991, Comes and Abbott 1998, Gottlieb 1977, Soltis et al. 1997). While both isozyme studies and DNA analyses provide the opportunity to assess patterns and levels of polymorphism and heterozygosity in distinct genetic loci, only DNA analyses enable the researcher to select genetic markers with different modes of inheritance and potentially different rates of evolution (Avise 1994, Moritz and Hillis 1996). This is an advantage in phylogeographic studies because specific target loci may be chosen according to predicted rates of accumulated variation and subsequently related to the timing of specific geohistorical events.

Phylogeographic studies have made particular use of DNA from cytoplasmic organelles, especially mitochondria and chloroplasts, as sources of genetic markers (Birky et al. 1989, Dong and Wagner 1994, Tomaru et al. 1998). Characteristics of cytoplasmic organelles such as uniparental inheritance and the lack of genetic recombination, commonly allow mtDNA and cpDNA polymorphisms to be treated as alleles at single haploid loci. In population genetic studies, this has the effect of reducing the effective population size, and increasing geographic subdivision of genetic diversity when compared with data from nuclear genomes. These characteristics not only enable

the lineage of a species to be traced through a single parental line, but also reflect the evolutionary divergence in the gene itself, information which is extremely valuable in studies of vicariance events and timing of speciation events.

In zoological studies, variation in mtDNA (Avise 1991) has been used to explore speciation and vicariance events brought about by Pleistocene glaciation. Strange and Burr (1997) examined variation in freshwater fish in eastern North America, Byun et al. (1997) documented patterns in bears dispersing from proposed glacial refugia on the west coast, and Klicka and Zink (1997) related diversity to proposed patterns of songbird speciation across the continent. In the American midwest, Zamudio et al. (1997) determined the phylogeny of mitochondrial genomes in lineages of short-horned lizards and related the regional patterns to historic climate changes. The phylogeographic patterns apparent in these studies all utilize mtDNA variation as population genetic markers and relate the apparent divergence to geohistorical factors.

In botanical studies, cpDNA genomes are more commonly used for phylogeographic studies because there are evolutionary differences between mitochondrial genomes in plants and animals that make cpDNA a more attractive marker. In plants, the size of the mitochondrial genome and the gene order is highly variable, while at the same time, gene and spacer sequences are very conserved (Palmer 1987). By contrast, cpDNA is so highly conserved in size, gene arrangement and nucleotide substitution that early predictions were that the genetic profile of a single individual would characterize the entire species (Palmer et al. 1988). Subsequent studies show that levels of variation are higher than expected in some species and that cpDNA data are increasingly useful at both inter- and intraspecific levels (Harris and Ingram 1991, Soltis

et al. 1992). Interspecific cpDNA variation has been used to investigate the relationship of sympatric oak species (Whittemore and Schaal 1991) and to detail the evolution of natural hybrid zones from patterns of DNA variation in irises (Arnold 1994, Cruzan et al. 1993). More recently, high levels of intraspecific variation have been observed in many species and used for population level studies where interpopulational variation is high but intrapopulational levels are low (McCauley 1994, Strauss et al. 1993). Chloroplast DNA markers have been used to detail the post-glacial dispersal of beech trees (*Fagus sylvatica* L.) from refugia in Europe (Demesure et al. 1996) and to trace the diversity within species of Saxifragaceae on the west coast of North America (Soltis et al. 1997). Both of these phylogeographic studies show that cpDNA diversity is closely related to historical factors.

In a continent-wide survey of cpDNA variation in *Packera*, Bain and Jansen (1996) used restriction site analyses to examine the taxonomic relationships of three proposed subgroups in North America, where a previous study of nuclear ribosomal DNA sequence analyses showed extremely low levels of variation (Bain and Jansen 1995). The authors found that high levels of cpDNA polymorphisms in the genus persisted even to the population level. The detection of this high variation was consistent with theories that hybridization occurred frequently in the evolution of *Packera* species (Bain and Jansen 1996, Barkley 1968, Kowal 1975).

Species of *Packera* are found in several geographic and climatic regions in North America where they may co-occur with one or more other *Packera* species. Some, such as *P. cana* (Hooker) Weber and Löve, *P. paupercula* (Michaux) Weber and Löve and *P. streptanthifolia* (Greene) Weber and Löve are widespread species that are

morphologically variable across the range and intergrade in areas of overlap, whereas others like *P. quarens* (Greene) Weber and Löve and *P. quebradensis* (Greene) Weber and Löve are narrow endemics that resemble other widespread and geographically disjunct species (Barkley 1988). Most studies of evolution of the *Packera* complex have suggested that geohistorical events such as Pleistocene glaciation have played a major role in establishing these patterns. Additional evidence comes from detailed morphological studies such as those examining related *Packera* species in eastern North America (Kowal 1975) or the arctic-alpine disjunct *Packera cymbalaria* (Pursh) Weber and Löve (Packer 1972, Whitton and Bain 1992). Both studies suggest that high amounts of phenotypic variation are common and that often species boundaries are blurred either through intergradation or as a result of disjunction.

The additional data from the restriction site analysis of cpDNA in bulk collections from *Packera* populations showed that while many species were characterized by the same chloroplast types, others had several different chloroplast types both within and among populations (Bain and Jansen 1996). In addition, most populations in coastal regions were characterized by a single haplotype whereas highly polymorphic populations were identified in southwestern North America, in southwestern Alberta and in the Alaska/Yukon region. The latter two are regions at the boundaries of continental and Cordilleran ice sheets during the last advance of Pleistocene glaciation (Rutter 1984) suggesting that these regions may be glacial refugia where several species were sympatrically associated.

Besides these two glacial boundaries, there are other regions in western North America that are thought to have been refugia during the Pleistocene. Geological records

of the Quaternary period suggest that most of Canada experienced at least one major glaciation event, with as many as four in some regions (Alley 1973). At the onset of glaciation, Cordilleran and Laurentide icesheets originated and advanced from mountainous regions in the west and Hudson Bay region in the east. At the interface of the two converging icesheets, in southwestern Alberta and adjacent Montana, the unglaciated areas may have served as refugia for regional species. Glacial refugia that are proposed to have existed along the coast of western North America, are thought to have sustained life during the maximum advance of glaciation (Pielou 1989). Evidence from patterns of colonizing species supports the notion that several coastal refugia may have existed, and it is from these regions that major recolonization and post-glacial expansion of recolonizing species would have originated (Rogers et al. 1991, Soltis et al 1997).

In southwestern Alberta, the most recent glacial advance reached its peak around 18000 years before present. However, the timing of the maximum of the Cordilleran advance was not necessarily coordinated with that of the Laurentide and at any one time, open areas along the eastern edge of the continental divide may have existed between the two ice masses (Alley 1973). At the edge of these ice sheets, small high elevation regions called nunataks were proposed to have remained ice-free (Packer and Vitt 1974). Like larger glacial refugia, these nunataks are hypothesized to have harbored life during periods of glaciation. Biological evidence for such areas has been deduced from patterns of post-glacial recolonization and the presence of rare disjunct species (Cwynar and MacDonald 1986, Packer and Vitt 1974). Because of their proximity to newly deglaciated habitat, nunatak areas, like larger scale glacial refugia, may have been important sources of colonizing species (Bennett 1985, Bird and Marsh 1973, Mulligan

1970, Packer and Vitt 1974, Ritchie and MacDonald 1986). If this dispersal pattern exists, then intraspecific patterns of genetic variation associated with these events may be detectable (Packer, 1980).

The influence of historical events on existing patterns in populations may be evident in bottlenecks causing reduced genetic diversity, historical patterns of gene flow by migration (Wright 1951), and fragmentation and isolation events (Templeton et al. 1990). Wright's island model and the stepping stone model of migration (Wright 1951) were designed to describe two different cases of colonization. The former describes the establishment of a sink population (the island) by the migration of individuals from a large adjacent panmictic source. Therefore, due to founder events, the island population is a subset of the total variation in the source population. The stepping stone model describes a directional reduction in genetic diversity as the variation in each advancing population becomes a subset of that in the previous one. This model is tested by correlating the number of migrants between populations with the distance between them with the prediction that adjacent populations would exchange a higher number of migrants and more distant populations would exchange fewer migrants.

Bottleneck events are those in which the effective population size is reduced by selection or drift. In such cases, the total amount of diversity remaining in a species or population is reduced when compared with pre-event levels (Avise 1994). Fragmentation and isolation of small populations may result of a spatial or temporal series of small bottlenecks where each population is characterized by a different subset of the original diversity. The severity and timing of the bottleneck would determine the proportion of original diversity that was maintained in each population. After the bottleneck is

removed, these fragmented populations may become the source of migrants for unoccupied regions. This type of historical structuring would be expected to show evidence of founder effects once the bottleneck is lifted, such as reduced genetic diversity in sink populations relative to source populations. An alternate pattern of diversity might be expected if geneflow is not restored between adjacent populations after a bottleneck event. The metapopulations model as described by McCauley (1995) suggests that bottlenecks in maternally inherited genes result in high interpopulational variation across a range with corresponding low levels of intrapopulational diversity. Additional data from nuclear genes would be necessary to distinguish between the two cases.

Interspecific patterns of genetic variation may also have been influenced by glacial events through range expansion and contraction. However, other factors may also be evident. For example, a species that is forced from its native range into a sympatric association with close relatives may show evidence of gene flow through hybridization when reproductive barriers are poorly established. In his geohistorical discussion of aureoid Senecios (=*Packera*), Barkley (1988) suggested that high levels of variation in the complex are the result of hybridization facilitated by glacial forces. Support for this idea may be evident in the high incidence of polymorphic cpDNA in *Packera* species in the Alaska/Yukon territory and in southwestern Alberta (Bain and Jansen 1996), but has been largely uninvestigated.

In the present study, four species of *Packera* from southwestern Alberta, northern Montana, and northwestern Wyoming are compared for chloroplast haplotype diversity. *Packera contermina* (Greenm.) Bain, *P. cana*, *P. pseudaurea* (Rydb.) Weber and Löve, and *P. cymbalarioides* Buek. are four closely related species found in southwestern

Alberta in prairie and montane or alpine areas directly affected by Pleistocene glaciation. While all four species were shown exhibit high levels of cpDNA variation, three of the four, *P. contermina, P. cana, P. pseudaurea,* were also polymorphic at the intrapopulational level (Bain and Jansen 1996).

Within the study region, the species fall into two ecologically distinct groups, high elevation alpine types and lower elevation montane or prairie types. The ecological variation suggests that each species has been affected by a different set of geohistorical forces and that each has a distinct history. For example, the potential for a mid-elevation understory species such as *P. pseudaurea* to survive in open exposed high elevation glacial nunataks is low compared with that of alpine taxa such as *P. contermina* and *P. cymbalarioides.* Furthermore, populations of *P. contermina* and *P. cymbalarioides* are generally small (fewer than 2000 individuals) and geographically isolated from adjacent populations by deep, heavily treed valleys, whereas physical barriers between populations of *P. cana* and *P. pseudaurea* are not as pronounced because of their lower elevation habitat.

Packera cana is a wide spread prairie species of the Great Plains, but within the southwestern Alberta region, small populations can be found at high alpine elevations along the continental divide as well as on open plains and exposed hillsides at mid- to low elevations and drier habitats. During the maximum extent of glaciation, the Laurentide ice sheet covered many native prairie sites where this species is found (Alley 1973).

Packera pseudaurea is found along stream banks and ponds at mid-elevations as an understory species in coniferous forests in southern Alberta. Although the species'

overall range is from New Mexico in the south to Minnesota in the east and from the Cascades of Washington in the west to southern Alberta in the north, the taxon is commonly segregated into three varieties whose ranges are distinct. The typical variety *P. pseudaurea* var. *pseudaurea* is the only one in southwestern Alberta.

Packera cymbalarioides overlaps with *P. cana* and *P. pseudaurea* in southwestern Alberta where it is at the northernmost limit of its range (Moss 1983). It is a high alpine species of wet meadows and seepage zones from the mountains of northern Wyoming and California to Washington, Idaho and western Montana, an area south of the proposed maximum extent of Cordilleran glaciation.

Packera contermina is of particular interest in this study since its range is restricted to southwestern Alberta and adjacent Montana. Populations inhabit a very narrow range from the Highwood Pass to northern Montana (Moss 1983). The populations are confined to high, dry, windy alpine ridges and calcareous outcroppings above 2000 m. Most are in highly erosional scree slopes or open disturbed areas commonly associated with alpine species of *Erigeron aureus* Greene, *Dryas octopetala* L., *Crepis nana* Rich., and *Erigeron lanatus* Hook.

Taxonomic treatments of *P. contermina* are varied. As outlined by Packer (1972), past treatments have considered these populations to be disjunct members of the arctic species *P. hyperborealis* (Greenm.) Löve and Löve, or *P. resedifolia* (Less.) (currently *P. cymbalaria*). Studies using flower and leaf morphology, and cytology and pollen morphology concluded that *P. contermina* is a distinct species (Whitton and Bain 1992, Packer 1972). Recent floristic treatments have adopted this view (Douglas 1982, Moss 1983).

Although the *P. contermina* life history is largely unknown, individuals are clonal and assumed to be outcrossing like most *Packera* species (Barkley, 1988). Chromosome counts of 2n=160+ were reported by Packer (1972), and because the base chromosome number of *Packera* is x=23, the count is expected to be closer to the octoploid 2n=184.

While the habitat in which individuals are found is relatively uniform, considerable intrapopulational morphological variation exists within the species across the range. For example, a single population may include several variations in vegetative and floral morphology. Individuals with tomentum limited to leaf axils and basal leaf rosettes can be found along with others exhibiting a thick covering of hair over upper leaf surfaces. Two forms of inflorescences, radiate and discoid, can also be found within a single population.

Molecular studies using sequence data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA suggest that *P. contermina* is closely related to a southern alpine species, *P. cymbalarioides* (Bain and Jansen 1995), but low levels of intrageneric sequence divergence do not eliminate phylogenetic links to other *Packera* species. Similarly, data from a chloroplast DNA restriction site analysis uncovered high levels of variation and cpDNA polymorphisms which were shared by many species in the complex (Bain and Jansen 1996), giving no indication of the relationship of *P. contermina* to other taxa.

For ecological and historical reasons, genetic and geographical variation would be predicted to be different for each of the *Packera* species in southern Alberta. By examining the type and magnitude of those differences among species and within

Packera contermina in greater detail, a more complete picture of the phylogeography of *Packera* may be possible.

This study was initiated to address several issues in *Packera* species: 1) to explore the haplotype diversity within and among populations of *P. contermina* for evidence of migration, founder effects and bottlenecks associated with effects of Pleistocene glaciation, 2) to compare haplotype diversity and distribution patterns in related species in the southern Alberta region to identify concordant patterns of glaciation effects, and 3) to analyze the phylogenetic relationships of known haplotypes within the genus and apply this information to an analysis of their present-day distribution in southern Alberta taxa.

Chloroplast DNA restriction site analysis of the variation among individuals in four closely related species is an ideal system for examining these questions. Evaluation of haplotype variation at three levels, 1) type of haplotype present in populations and species, 2) frequency of haplotype among and within populations and, 3) the number of mutational differences among haplotypes provides the data necessary to describe phylogeographic effects. Each provides evolutionary information necessary to explain diversity in *Packera* species in southwestern Alberta.

Patterns of population differentiation and structure are most simply evaluated using unbiased estimates of haplotype diversity and effective number (Kimura and Crow 1964, Nei 1987), where high values usually characterize highly variable populations that are not distinct from one another. More detailed comparisons are made through calculations of population subdivision, *Fst* (or θ in the case of haploid data), based on haplotype frequencies (Raymond and Rousett 1995). Estimates of gene flow (*Nm*),

expressed as the average effective number of migrants exchanged between populations, and the geographical distance between populations are examined for correlations that support an isolation by distance model (Goudet 1995).

Based on theories of hybridization (Barkley 1988) and molecular evidence, Bain and Jansen (1996) suggested that the whole *Packera* complex was behaving like a single biological species. Therefore, one final point addressed by this study concerns the discrimination of *P. contermina*, *P. cana*, *P. pseudaurea* and *P. cymbalarioides* based on differences in haplotypes and haplotype frequencies.

The proposed analyses of patterns of genetic variation will not only provide the opportunity to assess the importance of geohistorical forces such as glaciation for facilitating hybridization and/or introgression in the evolution of *Packera* species, but to identify the potential forces that have shaped population structure and diversity.

MATERIALS AND METHODS

Plant Materials and Sampling Procedures

Populations of *Packera contermina* and three related species were sampled in southern Alberta, northern Montana, and northern Wyoming. The species were chosen because they were known to exhibit intrapopulational cpDNA variation in this region (Bain and Jansen 1996). Potential collection sites for *P. contermina*, *P. cana*, *P. pseudaurea* and *P. cymbalarioides* were identified by a survey of herbarium specimens at University of Lethbridge (LEA), University of Calgary (UAC), and University of Alberta (ALTA) and by investigating areas with similar habitat to known sites for the presence of each species. The data for all collections are summarized in Table 1. The abbreviations listed for each population will be used throughout the paper. A total of 350 individuals in 14 populations of *Packera contermina*, 122 individuals in 8 populations of *P. cana*, 161 individuals in 7 populations of *P. pseudaurea*, and 94 individuals in 5 populations of *P. cymbalarioides* were sampled throughout the study range.

In all collections, 2-3 leaves were removed from individual plants, packaged, and frozen at –80°C. within 48 hours of harvesting. Samples were taken from plants at least 1 m apart to reduce the chance of re-sampling clonal individuals. At the time of sampling, approximate population sizes of *P. contermina* were estimated by counting the number of individuals in a 1 m^2 quadrat and averaging 4-6 random counts across the area of a population. Estimations of the dimensions of the population were made visually. Both flowering and non-flowering individuals were counted and obvious offshoots and clones were ignored.

One population (SMC) was intensively collected (100 individuals) and the position of each plant mapped on a grid by triangulation from known coordinates relative to adjacent collections. As before, specimens were collected 1 m apart. For each individual, distance measurements were recorded from a specimen plant to each of two posts set 20 m apart. Triangulation calculations were performed to determine the coordinates of each plant in the population and the locations were graphed on to a grid using a computer spreadsheet program.

Collections of *Packera contermina* included peripheral populations from the northern (HWP) and southern limits (SMC, FL, CR) of the species distribution. Alpine populations (YCR, SC, SPR, PVT) of the wide-ranging species *P. cana* were collected inside the same geographic area as *P. contermina*. In addition, populations of *P. cana* from one prairie location (CS), one montane site, (RM), and one southern alpine area (BTP) in northern Wyoming were also collected. Restriction site data from the study by Bain and Jansen (1996) for *P. cana* population #75 (CD), a second prairie site, were also appended to the data set.

A total of seven populations of *P. pseudaurea* was sampled, including five (RR, CL, BR, VCR, BML) from the same geographical region as *P. contermina* collections. Five populations of *P. cymbalarioides* were sampled, three from the southern Alberta Rocky mountains, the northernmost extent of the species range, and two populations from northern Wyoming. Voucher specimens for all collections are deposited in the herbarium at the University of Lethbridge (LEA).

DNA Extraction and Isolation

Total genomic DNA was isolated from leaf tissue by the method of Doyle and Doyle (1987) using 2X CTAB extraction buffer, chloroform:octanol partitioning solvent, and isopropanol precipitation. DNA purification was carried out by ultracentrifugation on a CsCl gradient or by digestion with a caylase cocktail according to the methods of Okada et al. (1997). Modification of the caylase digestion included precipitating the DNA from the aqueous phase with 100% isopropanol, washing the pellet once with a 76% ethanol, 10 mM ammonium acetate solution, with a final wash of 70% ethanol. The DNA pellet was resuspended in sterile nanopure water and stored at 4°C.

Restriction Site Analysis

Aliquots of purified total DNA suspensions were digested with restriction enzymes known to generate polymorphic profiles as documented by Bain and Jansen (1996). In that study, a total of four restriction enzymes combined with seven lettuce chloroplast probes revealed nine polymorphic sites in species of *P. contermina*, *P. pseudaurea*, *P. cana* and *P. cymbalarioides*. To avoid re-examination of non-informative restriction sites, only those enzyme-probe combinations showing polymorphic sites were chosen for this study (Table 2). Resulting fragments of digested DNA were separated by electrophoresis in 1.3% - 1.5% agarose gels in 1% TAE buffer. Gels were stained with ethidium bromide, and trimmed of excess agarose. DNA fragments embedded in the gel were transferred to nitrocellulose membrane by southern blotting for 12 - 48 hours and fixed to the filters by heating to 80°C for 2 hours according to the methods of Jansen and Palmer (1987).

Probes were cloned from the 14.7kb, 18.8kb, 6.3kb, 6.9kb, 5.4kb, 6.7kb and 3.8kb fragments of the lettuce chloroplast genome (Jansen and Palmer 1987), and radio-labeled with ^{32}P-dATP by nick-translation. These probes were hybridized to fixed *Packera* cpDNA fragments at 65°C for 16 – 36 hours according to the methods of Palmer (1986) and Jansen and Palmer (1987). Hybridized filters were exposed to autoradiography film supplemented by intensifying screens, and developed after 4 - 72 hours at -80°C, depending on the strength of radioactive signal before exposure. Restriction fragments were interpreted and sites scored as present or absent, and the resulting binary characters were used to describe the haplotypes. Haplotypes were identified in individuals of *P. contermina*, *P. pseudaurea*, *P. cana* and *P. cymbalarioides*. Haplotypes that could be unambiguously characterized with the same 10 restriction sites in other *Packera* species across North America (Bain and Jansen 1996) were identified and added to the present data set.

Phylogenetic analysis of haplotypes

The evolutionary relationship of all composite haplotypes was analyzed by phylogenetic analysis and strict consensus trees were generated. Two parsimony analyses were performed using the computer program, PAUP ver. 4.0b2 (Swofford 1996). A Wagner parsimony analysis was conducted by a heuristic search for the most parsimonious trees with TBR swapping and MULPARS option in effect. Wagner parsimony assumptions dictate that all character state changes are equally likely but the nature of restriction site data suggests that losses are more common than gains (Albert et al. 1992). Therefore, a second parsimony analysis was performed using step-matrix asymmetric character weighting of restriction site gain to loss ratio of 1.3:1 as

recommended by Albert et al. (1992) and Olmstead and Palmer (1994). Ancestral character states were assigned as those in the most common haplotype, H1. Strict consensus trees of all most-parsimonious trees were computed for both analyses.

The locations of individual haplotypes outside of southwestern Alberta were plotted on a map of North America and the geographical distribution was related to the phylogenetic distribution on the step-matrix tree described above.

Discrimination of species

A discriminant analysis of cpDNA diversity in the four *Packera* species in this study was employed to test the utility of haplotype diversity in differentiating each of the species. The variation in total number of haplotypes and the frequency detected in each species was used to derive all discriminant functions and to judge their significance in separating the four groups. The frequency data were arcsine transformed (Sokal and Rohlf 1995) prior to analysis using the statistical package SPSS (Release 4.0 for Macintosh). The results were plotted in two dimensions using the first and second discriminant function as the x- and y- axis. A table of classification results for each species was produced.

Statistical Analysis of Haplotype Variation

Interspecific haplotype variation in four *Packera* species

For populations of *Packera contermina*, *P. cana*, *P. pseudaurea* and *P. cymbalarioides*, haplotype frequency was tabulated and the distribution plotted on regional maps. Genetic variation within all four *Packera* species was described by treating the chloroplast genome as a single locus and the haplotypes as alleles at that locus (Nei and Tajima 1981).

Genetic variability was estimated by the number of alleles at a locus where a high number indicates high levels of polymorphism (Nei 1987). The most basic estimate of diversity is the effective number of haplotypes, n_e. These values were calculated by the formula (8.17) in Nei (1987) such that $n_e = 1/\sum x_i^2$. In this formula, x_i is the frequency of the i^{th} haplotype for each population. For each species, sample haplotype diversities across all populations were calculated as $h = 1 - \sum x_i^2$ according to formula 8.3 of Nei (1987). Unbiased estimates of haplotype diversity for each population were calculated as $\hat{h} = 2n(1 - \sum x_i^2)/(2n-1)$ where x_i is the estimate of allele frequency and n is the number of individuals sampled (Nei 1987, Nei and Roychoudhury 1974) and the variance calculated according to the methods of Nei and Roychoudhury (1974). This formula allows for comparisons of diversity between collections of unequal sample sizes. The calculated values are reported as $\hat{h}$ and n_e for each population.

For each species, an estimator of population structure, θ, (the equivalent of Wright's *Fst*) (Weir and Cockerham 1984) was calculated using cpDNA haplotype variation subdivided among populations. The computer program, GENEPOP (vers. 3.1b, Raymond and Rousset 1995) was used to compute the values that are a measure of the total genetic variation due to among population variation. The standard deviation values were determined by jackknifing over populations using the computer program FSTAT (vers. 1.2, Goudet 1995). The effective number of migrants, that is, the estimate of gene flow due to seed dispersal between all populations was calculated by Wright's formula relating N_m to (θ) as outlined in Strand et al., (1996): $N_m = 1/2\ ((1/\theta) - 1)$, and is reported for each species.

Intraspecific variation in *Packera contermina*

1) Intrapopulational Spatial Structure of Genetic Variation

One population of *P. contermina* (SMC) was intensively sampled and examined for an intrapopulational pattern of haplotype distribution. The distribution was evaluated by a Mantel test for significance between genetic identity and geographic distance using the R Package: Multidimensional Analysis, Spatial Analysis computer program (Macintosh version). The correlation matrix for pairwise geographic distances between individuals was calculated using the SIMIL option in the R Package. The matrix for pairwise genetic distances between haplotypes was calculated from character state changes using the restriction site distance algorithm of Nei and Li in the computer program PAUP ver. 4.0b2 for Macintosh (Swofford 1996).

2) Among Population Spatial Structure of Genetic Variation

Latitudinal and longitudinal coordinates for all populations were recorded from 1:50,000 scale topographic maps. Pairwise geographic distances of populations of *P. contermina* were calculated using the GEODIST option in the R Package: Multidimensional Analysis, Spatial Analysis computer program (Legendre and Vaudor 1991). The distances are reported in linear kilometers.

Potential gene flow between populations was examined by calculating pairwise estimates of θ, the probability that populations share the same haplotypes and haplotype frequencies, using the ISOLDE program in GENEPOP (ver. 3.1b, Raymond and Rousset 1995). The values were converted to estimates of the number of migrants exchanged between populations (*Nm*) (Slatkin and Barton 1989) and natural log transformed.

Pairwise estimates of gene flow (*Nm*) between populations were plotted against natural log transformed distance measures. The significance of relationships between interpopulational distances and pairwise estimates of θ was evaluated by Mantel tests using the computer program R Package: Multidimensional Analysis, Spatial Analysis (Legendre and Vaudor 1991). In this calculation matrices of θ values were examined for correlation with pairwise distances and a Mantel relation determined. The test resampled the data by randomizing columns of the triangular matrix output from pairwise θ values and pairwise geographic distance values to obtain 1000 multiple randomized matrices, then calculated the correlation coefficient and the rejection zone (*P*-values) of the test.

3) Interpopulational genetic distance relationships

Relationships between populations were examined using genetic identity measures. Pairwise genetic distances between populations were calculated from haplotype frequency data using two methods, Nei and Li, and Cavalli-Sforza chord measure in the program PHYLIP (Felsenstein 1993). The former assumes that neutral mutations accumulate in a genetic drift model, while the latter is a pure genetic drift model (Felsenstein 1993). Fitch cluster analyses were performed on the distance matrices and plotted as unrooted trees. These analyses reflect genetic distances and the trees are constructed by global optimization of the data set before constructing an unrooted tree.

RESULTS

Population sizes of *P. contermina* were variable across the range. The smallest were HSL, HWP, SFT, CR, VR, PM, and AR populations with an estimated 50 to 200 individuals and a density of approximately 1 plant/ m^2. The largest populations were estimated at between 3000 and 5000 individuals in sites at LFT, SFL, MDP and SMC with densities of between 5 and 10 plants/m^2.

Restriction Site Characterization

The character states of 10 cpDNA restriction sites using the enzyme-probe combinations are shown in Table 2. Nine of the restriction sites were anticipated from the data of Bain and Jansen (1996), but Character 6 (Table 2) is a new restriction site polymorphism that was previously undetected with the Dra I - 6.7+3.8 enzyme-probe combination. This mutation describes an autapomorphy that characterizes H14 in two *P. cana* (SM, PVT) and two *P. contermina* (HSL, VR) populations (Table 3). Haplotypes differ by as few as one mutation and as many as eight restriction site changes. A total of 15 haplotypes was compiled from the polymorphic sites as shown in Table 3. Using only the restriction site characters employed in this study, three additional unambiguous haplotypes (H16, H17, H18) were identified from the study by Bain and Jansen (1996).

Phylogeny and Geographic Distribution of Haplotypes

The phylogenetic analyses of all 18 haplotypes are described in Table 3 and illustrated in Fig. 1 and show strict consensus trees with two clades of haplotypes. In both the Wagner parsimony (Fig. 1a.) and the step-matrix parsimony analysis (Fig. 1b) haplotypes H1, H2, H6, H8, H12 and H5, H14, H18 formed a group (clade A) at one end of the unrooted tree, and haplotypes H3, H4, H7, H9, H10, H11, H13, H15, H16, H17

(clade B) at the other. In the Wagner parsimony analysis, two steps separated the two groups of haplotypes while all other branches were single steps. A bootstrap value for the branch separating the two clades was 53%. A total of 540 most parsimonious trees of 20 steps were found with a homoplasy index of 0.500, consistency index of 0.500, and retention index of 0.750.

The topology of the tree produced by step-matrix parsimony analysis (Fig. 1b) was similar to the tree produced by Wagner parsimony in that the haplotypes split into two groups H1, H2, H5, H6, H8, H12, H14, H18, and H3, H4, H7, H9, H10, H11, H13, H15, H16, H17, but bootstrap support for the two clades was low (47%). The consensus tree was calculated from 8 most parsimonious trees requiring 212 steps (CI, RI, HI undefined because of asymmetric character weighting).

From the data reported by Bain and Jansen, (1996), the collection sites of *Packera* species characertized by H1 and H2 were most commonly found in the Great Basin region of western North America as shown in Table 4. Outside this region, H1 was found in Quebec and Newfoundland populations of *P. cymbalaria*, and H2 in a population of *P. paupercula* from the Yukon Territories, as well as in all four species in southwestern Alberta. The haplotypes not found in Alberta (H16, H17, and H18) were unique to populations of *P. ogotorukensis* (Packer) Löve and Löve (#496, #498), *P. bolanderi* (A. Gray) Weber and Löve (#470), and *P. bernardina* (Greene) Weber and Löve (#465), respectively. Haplotypes H4, H7, H10, and H13 were found in species from the coastal regions such as Vancouver Island, and northern regions such as the Yukon. They were also detected in polymorphic species in southwestern Alberta and in monomorphic

populations of *P. pauciflora* (Pursh) Löve and Löve (#77) and *P. cymbalaria* (#259) in Quebec.

Eleven of the fifteen haplotypes detected in individuals of *P. contermina*, *P. cana*, *P. pseudaurea*, and *P. cymbalarioides* (Table 5) in this study were found in more than one species. Two haplotypes (H1, H2) were found in all four species. Twelve haplotypes were found in *P. contermina*, seven in *P. cana*, nine in *P. pseudaurea*, and three in *P. cymbalarioides*. The three haplotypes detected in *P. cymbalarioides* were also found in *P. pseudaurea* (H1, H2, H3), and all seven haplotypes in *P. cana* were also found in *P. contermina* (H1, H2, H7, H10, H11, H12, H14). *Packera pseudaurea* and *P. contermina* shared six haplotypes (H1, H2, H6, H7, H8, H9).

H1 accounted for a major portion of the diversity in *P. contermina*, *P. cana*, *P. pseudaurea*, and *P. cymbalarioides* as shown by the frequency values for individual haplotypes in Table 6, 7, 8, and 9 respectively. It was found in varying proportions in all populations of the four species, with the exception of *P. cymbalarioides*. In this species, all populations were characterized by a single haplotype and three different haplotypes (H1, H2, H3) were detected amongst the five monomorphic populations. The population frequencies of H1 range from 10.5% to 100% in *P. contermina*, 11.1% to 83.3% in *P. cana*, and 37.5% to 100% in *P. pseudaurea*.

Discriminant analysis of four species of *Packera*

The plot of discriminant scores of populations (Fig. 3) showed that *P. pseudaurea* populations may be slightly differentiated, but the canonical discriminant analysis failed to derive any statistically significant functions at the $p < 0.05$ level. The predicted group memberships of populations of *Packera* were correctly identified in over 70% of the

cases, indicating that there was a moderate amount of overlap among the groups (Table 10).

Survey of Haplotype Frequencies and Distribution

Distribution of Intraspecific Variation

Within-population frequencies of haplotypes other than H1 were highly variable among the four species. In *P. contermina* (Table 6, Fig.4) H8 and H11 were the most frequent haplotypes (between 4% and 5% of the total), but H2 and H10 were more evenly distributed among the populations (more than 5). Although H11 accounted for approximately 84% of the diversity in the most northern population, HWP, it was found in only 3 other individuals, all from southern populations (SMC, and SFL). By contrast, in *P. cana* (Table 7, Fig. 5), aside from H1, haplotypes H2 and H7 were the most common haplotypes with H7 detected in more northern populations (RM, CS, SPR), and H2 in more southeastern regions (BTP, YCR, CD). In *P. pseudaurea* (Table 8, Fig. 6), H7 was the most common after H1, and it was detected in all populations except the low elevation population, FHL. All *P. cymbalarioides* populations (Table 9, Fig. 7) were monomorphic for H1 or H2 in southwestern Alberta, whereas the northwestern Wyoming populations were monomorphic for H2 and H3. The new combination of restrictions sites which describe H14 (Table 3) was found in two populations each of *P. contermina* (HSL, VR) and *P. cana* (SC, PVT).

The effective number of haplotypes (n_e) and the haplotype diversity indices ($\hat{h}$) were moderate to high in *P. cana* populations where they ranged from n_e = 1.8 to 3.92,

and $\hat{h} = 0.485$ to 0.775. The overall haplotype diversity across all populations of *P. cana* was $h = 0.756$.

Packera pseudaurea populations exhibited highly variable levels of haplotype diversity ($\hat{h} = 0$ to $\hat{h} = 0.742$) with overall values of $h = 0.452$ and effective number of haplotypes (n_e=1-3.556). The most differentiated populations were the two southernmost populations where extreme values in FHL ($\hat{h} = 0$, $n_e = 1$) and HWM ($\hat{h} = 0.742$, n_e=3.556) were recorded.

Packera cymbalarioides populations were all monomorphic for a single haplotype giving population haplotype diversity values of $\hat{h} = 0$. The overall haplotype diversity for all populations was $h = 0.587$.

Population differentiation as indicated by estimates of θ (Table 11) ranged from extremely high values in *P. cymbalarioides* ($\theta = 1.0$) to extremely low values in *P. pseudaurea* ($\theta = 0.0855$). Estimates in *P. cana* and *P. contermina* were $\theta = 0.2619$, and $\theta = 0.3331$, respectively, indicating a moderate level of differentiation. Calculations of estimated numbers of migrants exchanged between populations were $N_m = 0.00$ in *P. cymbalarioides*, $N_m = 1.00$ in *P. contermina*, $N_m = 1.41$ in *P. cana*, and $N_m = 5.35$ in *P. pseudaurea*.

Frequencies and distribution in *P. contermina*

Across populations of *P. contermina*, the haplotype diversity was $h = 0.436$. Within population haplotype diversity values ranged from $\hat{h} = 0$ (CR) to $\hat{h} = 0.788$ (SLR), with corresponding effective number of haplotypes of $n_e = 1$ and $n_e = 4.122$. Over 42% of the populations exhibited two or more effective haplotypes. Populations SFL and

SLR in the central to southern regions of the collection range showed the highest diversity $\hat{h} = 0.717$ and $\hat{h} = 0.788$, respectively).

In the most northerly population of *P. contermina* (HWP), H11 made up 84.2% of the variation and appeared in only three other individuals from more southerly populations (2 in SFL and 1 in SMC). H1 made up less than 40% of the variation in five *P. contermina* populations (HWP, PM, SFT, SFL, SLR), and 63.6% or more in the remaining populations. One haplotype was unique to *P. contermina* (H15) and appeared only once in SFL. Other less common haplotypes (H6, H8, H9, H10) were scattered in populations throughout the region. Haplotype H12 was found in only 4 individuals across the range, one in HWP, one in SLR, and two in the southern population of SMC. Haplotype H14 was found in a single individual at HSL, and in 6 individuals at VR. This unusual mutation was also identified in 2 populations of *P. cana* (PVT, SC). Haplotype H13 was found in only one southern Alberta species (*P. contermina*, SMC), but identical characters made up the haplotypes in one population of *P. bolanderi* (A. Gray) Weber and Löve, from Oregon and two populations of *P. ogotorukensis* (Packer) Löve and Löve from Alaska (Table 4). Haplotype H15 was not identified in species outside of the region.

Spatial structure of haplotype diversity in *Packera contermina*

Intrapopulational haplotype distribution

Although seven of the *P. contermina* haplotypes were identified in the intensively collected population (SMC), over 88% of the haplotypes in the population were H1 (Table 6). Patterns of spatial structure (Fig. 8) within the population, tested by a Mantel

test for correlation between geographic location and genetic identity revealed a very low positive r-value ($r = 0.0103$) which was not significant at the $\alpha = 5\%$ level ($p_{1000\ permutations} = 0.363$, $p_{approximation} = 0.410$). These values support the lack of visible spatial patterning in this population possibly due to the predominance of H1.

Interpopulational haplotype distribution

Packera contermina populations were tested for effects of isolation by distance by computing the Mantel relation between pairwise geographic distances and pairwise estimates of genetic identity computed as θ (Table 12). The Mantel relation ($r = 0.349$) was positive and significant at the $\alpha = 5\%$ level ($p_{1000\ permutations} = 0.012$, $p_{approximation} = 0.002$), indicating that near-by populations are more genetically alike than distant populations as predicted by an isolation by distance model. Eleven data points were omitted from the plot of 80 pairwise comparisons (Fig. 9) because natural log transformation of negative or 0 values are undefined. Seven pairwise data points appeared in this plot above ln (M) values of 2 and ln(distance) values of 3 suggesting high amounts of geneflow exist between geographically distant populations.

Interpopulational genetic distance relationships

The tree topology of analyses of interpopulational relationships based on pairwise genetic distances (Table 13) showed that both Cavalli-Sforza (Fig. 10) and Nei algorithms (Fig. 11) produced similar trees. Each analysis reflected the significant distance of HWP from all other populations and the clustering of PM and HSL populations. This relationship generally reflects the geographic location of these populations relative to all others. SFT and SLR formed a distant association that is

consistent in both tree topologies. The tree generated from Cavalli-Sforza distances provided a more divergent view of genetically close populations such as LFT, CR, MDP, FL, HFT, and AR than the tree derived from Nei's genetic identities.

DISCUSSION

The cpDNA data presented provide persuasive evidence that evolution within *Packera* has been influenced by geohistorical events and that hybridization and introgression have probably played a major role in shaping the variation in patterns within the genus. An understanding of the interplay among the numerous factors that have contributed to the variation pattern can best be achieved by concentrating the discussion on two main points. The first aspect involves the diversity and phylogeny of the cpDNA haplotypes and the geographical and taxonomic distribution of these haplotypes within the genus. The recurrence of the same divergent haplotypes in all four species in southwestern Alberta suggests that historic geneflow has influenced the diversity at some level. The second point focuses on the geographical and taxonomic distribution of haplotypes within species and populations of the southwestern Alberta study area with reference to historical factors that may have influenced those patterns. These factors are necessary to assess the potential impact of Pleistocene glaciation on present-day intraspecific geneflow

cpDNA haplotype phylogeny and geographic variation in *Packera*

The phylogenetic relationships of *Packera* cpDNA haplotypes indicate that most branches of the trees are only one step in length. This clearly shows that the haplotypes are closely related. Preliminary surveys of haplotypes from other members of the tribe Senecioneae (discussed in Bain and Jansen 1996), including Senecioid and Tussilaginoid taxa thought to be closely related to *Packera*, indicate that *Packera* haplotypes are very distinct. Therefore, the overall low levels of haplotype divergence within the group

suggest that the haplotypes have probably arisen in the complex and may form a monophyletic group, quite distinct from other taxa in the tribe. This does not support the idea presented by Bain et al. (1997) that *Packera* is of hybrid origin. In such a case, more divergent chloroplast types would be expected if both parental types were maintained in the genus.

The high number of haplotypes that differ from each other by only one step is consistent with the observations of haplotype evolution made by Crandall and Templeton (1996). They predict that because there are multiple copies of an organellar genome within individuals, mutations are not likely to result in the extinction of the parental type, but rather the accumulation of new types in populations along with the ancestral forms. In addition, the coalescence theory of genetic relationships supports the notion that ancestral haplotypes are the most widespread and common because they persist along with newly evolved forms (Watterson and Guess 1977, reviewed in Crandall and Templeton 1996). Therefore, these data suggest that clade A haplotypes are ancestral and, within that clade, H1 would be predicted as the most ancestral haplotype because it is both common and widespread in the group. The coexistence of ancestral and derived haplotypes also reduces the resolution of the phylogeny (Crandall and Templeton 1996) so it is not surprising that bootstrap support for the phylogeny is low.

The distribution of *Packera* haplotypes in North America (Table 4, Fig. 2) shows that the clades exhibit some regional affiliations. Clade A haplotypes are widespread across continental North America but probably originated in the American midwest because all populations in Wyoming, Utah, Arizona, and New Mexico are characterized by ancestral haplotypes H1 and H2 (Table 4). These haplotypes are found in multiple

collections of wide-ranging species such as *P. multilobata* (T. & G. ex Gray) Weber and Löve and *P. streptanthifolia*, as well as those with limited distribution such as *P. quarens* (#458) and *P. eurycephala* (T. & G. ex Gray) Weber and Löve (#468). For the most part, the distribution of the other clade A haplotypes, is consistent with this Great Basin concentration, for example, H12 is found with H2 in a population of *P. streptanthifolia* (# 451) in Utah.

Haplotype H18 is the only clade A haplotype not found in southwestern Alberta. It was detected in a monomorphic population of the southern California endemic, *P. bernardina* Weber and Löve (#465), and may be also be a haplotype of the polymorphic taxa, *P. multilobata* (#451) in New Mexico based on restriction site characters identified in Bain and Jansen (1996). Because H18 has a restricted distribution in a geographically distant region, it is not surprising that it was absent from southwestern Alberta.

The presence of clade A haplotypes in the Yukon is not unexpected. As shown in Table 4, these haplotypes are found in *P. paupercula* (#305), and *P. pauciflora* (Pursh) Löve and Löve (#500). *Packera paupercula* is one of the most wide-ranging *Packera* species across North America (Barkley 1978), and *P. pauciflora* is a widespread alpine species. Both species are found predominantly, but not exclusively in regions east of the continental divide. Assuming that clade A haplotypes arose in the American midwest (Great Basin region), it is likely that *P. paupercula* and *P. pauciflora* individuals bearing H2 migrated to northern regions during an episode of range expansion. However, the presence of clade A haplotypes in *P. cymbalaria* (#52, #53) on the east coast of North America (Table 4) is harder to explain. It is thought to be an arctic-alpine species with disjunct distribution (Whitton and Bain 1992) between northwestern North America and

the east coast. Bain and Jansen (1996) detected a highly polymorphic *P. cymbalaria* population (#489) in the Yukon with haplotypes from both clades (likely H1 from clade A, H10 from clade B based on polymorphic restriction sites at AvaI – 6.3 and AvaII – 5.4). On the east coast, *P. cymbalaria* populations (#52, #53, and #259) were apparently monomorphic for either H1 or H10, and may represent bottlenecked populations of a more wide-ranging distribution in a polymorphic taxon. However, this pattern may also be an artifact of limited sampling of the total cpDNA diversity in eastern populations. Sampling of individuals of *P. cymbalaria* in eastern Canada and the Yukon and a similar analysis to that in the present study is warranted to determine the distribution of cpDNA polymorphisms in the species. It may be that the highly polymorphic population of *P. cymbalaria* detected in the Yukon is ancestral, and that the eastern populations were bottlenecked as the Laurentide ice sheet advanced during the last glacial episode. In this case, the polymorphism in *P. cymbalaria* must have been established before the most recent onset of glaciation.

In contrast to the continental distribution of clade A haplotypes, clade B haplotypes are distributed through the northern and coastal areas. Based on the notion that the flora of the eastern coastal areas of Quebec and Newfoundland are part of the arctic-alpine complex (Morrisett 1971), *Packera* species in this region would be expected to exhibit clade B haplotypes. The presence of clade B haplotypes H7 and H10 in *P. pauciflora* (#77) and *P. cymbalaria* (#259) in Quebec as well as western North America is consistent with the geographic affiliation of this clade with northern and coastal species.

A separate explanation is necessary for the present-day distribution of only clade

B haplotypes on the west coast of North America. Bain and Jansen (1996) showed that populations are generally monomorphic for or at least exhibit lower levels of intrapopulational variation of clade B haplotypes. All haplotypes except H16 (*P. ogotorukensis*) and H17 (*P. bolanderi*) were found in more than one species within the complex and can be found in populations scattered throughout the range as indicated in Table 4. This suggests that clade B haplotypes were established early after the split from clade A, and that species with northern and coastal haplotypes were displaced to coastal regions where they continued to differentiate.

This present study determined that clade A and clade B haplotypes are not only present in southwestern Alberta, but that all four *Packera* species are polymorphic for these haplotypes. This suggests that the geographical proximity of the coastal regions and the Great Basin (< 1500 km.) to southwestern Alberta, and a biogeographic history that includes floristic range expansion during interglacial periods of the Quaternary era and contraction due to Pleistocene events are two important factors in establishing this pattern. In support of this observation, Bain and Jansen (1996) show that highly polymorphic populations also exist in Yukon/Alaska (for example, *P. cymbalaria*), the west coast (*P. macounii* Weber and Löve), and southwestern North America (*P. neomexicana* Weber and Löve). The former regions are adjacent to the maximum limits of Pleistocene glaciation and the polymorphisms may be due to the same process of past interspecific gene flow as that predicted in southwestern Alberta.

The presence of a large number of divergent haplotypes within a single species is unusual, but to have four different polymorphic taxa in a single region is remarkable. The number of shared polymorphisms indicates that high levels of gene exchange

characterize the *Packera* complex, an observation that is supported by the high frequency of morphological intergradation (Bain 1988, Barkley 1988, Kowal 1975) and low divergence values for ITS sequence data (Bain and Jansen, 1995). Mechanisms that may account for high levels of intraspecific cpDNA diversity include hybridization and/or introgression, lineage sorting, or divergence within the taxon itself (Raamsdonk et al. 1997, Rieseberg & Brunsfeld 1992, Whittemore & Schaal 1991). Variations that arise within a species may be the result of secondary contact and geneflow between divergent populations that have had enough time to accumulate mutational differences. If one considers only the modern distribution, this is a possible explanation for the patterns in *P. cana*, *P. cymbalarioides* and *P. pseudaurea*. However, the present day distribution of *P. contermina* is so limited that this mechanism alone is unlikely to have infused the high number of haplotypes that characterize the species. Combined with the observation that other *Packera* species share many of the same haplotypes, the high levels of cpDNA diversity in *P. contermina* have likely arisen through other mechanisms.

High levels of intraspecific variation in the *Packera* complex might also be explained by lineage sorting of a highly polymorphic ancestor from which each species receives a slightly different subset of polymorphic haplotypes. Support for this is not warranted because the geographic distribution of haplotypes and polymorphic populations of *Packera* detected by Bain and Jansen (1996) shows that polymorphisms are widely distributed. By contrast, a pattern of lineage sorting would be expected to show a polymorphic source surrounded by monomorphic populations, or at least populations with relatively reduced variability. It is more likely that the haplotypes have evolved within the *Packera* complex, and have been widely distributed through frequent

hybridization and/or introgression events, an idea discussed by Bain and Jansen (1996) and supported by their observation of low levels of divergence in nrDNA ITS sequence data (Bain and Jansen 1995).

However, a third explanation of recurrent restriction site mutations that characterize haplotypes in *Packera* is that they have arisen in each species by convergence. If convergence is the mechanism by which these recurring haplotypes appear, a random pattern of haplotype distribution might be expected in the complex. This is because constituent restriction site mutations would be independent of species and geographic regions, a pattern contradicted by the distribution shown in Fig. 2 where clade B haplotypes have a coastal and northern affiliation, and clade A haplotypes are more continental. On a more local scale, convergent evolution of the eleven shared polymorphisms in *Packera contermina*, *P. cana*, *P. pseudaurea* and *P. cymbalarioides* (Table 5) in southwestern Alberta would be unprecedented and unlikely. Such a high number of convergent haplotypes is improbable because the mutation rate in the chloroplast genome is low; therefore, each haplotype is more likely to have arisen only once, and been re-distributed in the complex through other mechanisms.

The best explanation of shared diversity is that introgressive hybridization of species outside of the region and from both sides of the continental divide has enabled frequent chloroplast capture events. The presence of haplotypes found in southwestern Alberta in species in adjacent regions, and the observation of widespread intergradation in the complex supports this interpretation. However, none of these models is mutually exclusive. Mechanisms such as chloroplast capture which may have influenced *Packera* in this region would benefit from research designed to detect introgressive hybridization

in species with high levels of polymorphisms, and to compare the apparent absence of chloroplast capture in those species which are demonstrably monomorphic.

Discriminant Analysis of *Packera* species

While the four *Packera* species exhibit morphological and ecological differences that allow their identification in the field (Moss 1983), the discriminant analysis of cpDNA haplotype frequency data from populations in southwestern Alberta region failed to detect any significant differences among them (Fig. 3). This is congruent with ITS sequence data that show low levels of sequence divergence within the genus (Bain and Jansen 1995). In the case of widespread *P. cana* and *P. pseudaurea*, population samples may reflect only a small portion of the total chloroplast diversity in these taxa because they were drawn from a very small portion of the range. However, increased sampling is unlikely to provide any additional support for discriminant functions given the existing trends for shared haplotypes (approximately 98% of the individuals exhibit shared haplotypes (Table 5, and Tables 6-9) (Sokal and Rohlf 1995)).

All three *P. cymbalarioides* haplotypes are found in *P. pseudaurea* and all seven *P. cana* haplotypes in *P. contermina*. From a geographical and ecological perspective, this trend does not make sense in all cases. *Packera cana* and *P. pseudaurea* are both widespread with presumed increased potential for diversity, whereas *P. cymbalarioides* and *P. contermina* are restricted to alpine regions. From a population genetic perspective, the alpine populations are potentially restricted by bottlenecking and may have survived glaciation *in situ*, factors that would be expected to contribute to reduced diversity. This may be the case when comparing *P. cymbalarioides* and *P. pseudaurea*, but it is not a good explanation of the apparent subset of haplotypes that widespread *P. cana* shares

with the narrow endemic, *P. contermina*. This apparent contradiction can be re-examined by considering the haplotype diversity indices for *P. contermina* ($h = 0.436$) and *P. cana* ($h = 0.756$) (Table 11). They indicate that even though fewer haplotypes were detected, *P. cana* is more diverse. The significance of this is discussed below.

Geographic structuring of cpDNA Haplotypes within *Packera*

The geographic structure of diversity in each species is shaped by historic effects relating to migration events, bottleneck effects, founder events and hybridization and/or introgression. The overall values of population subdivision (θ) for each of the four species revealed that geographic structuring of cpDNA diversity is highly variable among *P. cana, P. cymbalarioides, P. pseudaurea* and *P. contermina*. High levels of intraspecific diversity were detected in all four ($h > 0.436$), but interspecific differences in population structure as measured by θ suggest that in the southwestern Alberta region each species was influenced by different historical forces.

The species that shows the least amount of cpDNA diversity subdivision is *P. pseudaurea* ($\theta = 0.0855$) even though levels of within population haplotype diversity are moderate ($h = 0.452$). In *P. pseudaurea* (Fig. 6), there is a directional trend of decreasing diversity from north to south with adjacent populations tending to share similar haplotypes. This trend suggests that the distribution is the result of a migration event from the north. It is supported by a high estimated number of migrants between populations ($Nm = 5.35$) (Table 11). Not surprisingly, the exception to this north to south gene flow trend is HWM, a montane population in central Montana, geographically isolated from other populations by a wide buffer of prairie. It has a high haplotype

diversity index ($\hat{h}$ = 0.742) which suggests that it was not established by a founder event from adjacent populations, but that it probably survived the most frequent glaciation event in or near that location.

Packera cana populations show the highest level of haplotype diversity (h = 0.756) of the four species studied, but the diversity is only moderately subdivided among the populations (θ = 0.2619). Like *P. pseudaurea, P. cana* is a widely distributed species, but unlike *P. pseudaurea*, the range includes diverse habitat from prairies across the Great Plains up to high alpine regions in the Rocky mountains. Although a heterogeneous environment such as this would provide the opportunity for isolated populations to differentiate within the species, this has not occurred. Two possible explanations are 1) the versatility of *P. cana* to survive in diverse niches has ensured that gene flow is not restricted to a narrow ecological zone, and 2) the effective number of migrants is sufficient to prevent differentiation of populations (Nm = 1.41). The haplotype profile of *P. cana* is not dominated by H1 as it is in *P. contermina* or *P. pseudaurea* in which H1 makes up more than 75% of the diversity overall. This suggests that *P. cana* arose through a different sequence of phylogenetic events. A possible explanation of this high diversity is that *P. cana* is a polyploid species that has arisen from divergent parental types within the complex then acquired additional haplotypes by chloroplast capture. This suggestion is supported by chromosome counts in *P. cana* that show as many as three ploidy levels (Moss 1983).

At the extreme northern limit of the range in southwestern Alberta, *Packera cymbalarioides* populations are limited to very high elevation wet alpine meadows. Only

three haplotypes characterize the species and all populations are monomorphic. Two patterns of historic forces that would fit such a distribution are: i) a bottlenecking event in which a large polymorphic ancestral population has been reduced to subsets of monomorphic populations, and ii) a recent range expansion event in which the leading edge populations are the products of founder events from a polymorphic source population. Without evidence from other molecular markers or wide geographic sampling, it is impossible to distinguish between these events. From a geohistorical perspective, bottlenecking events are likely to have occurred during the last advance of Pleistocene glaciation (Packer and Vitt 1974). Polymorphic populations likely exist near the Alberta collection sites because adjacent populations are characterized by different haplotypes, i.e. YCL includes only H1, and AVR only H2. Evidence of a polymorphic population may be found with more extensive sampling. The high alpine reaches where *P. cymbalarioides* resides may have remained ice-free allowing the existing populations to survive the last advance of Pleistocene glaciation very close to existing locations.

Packera contermina, a highly polymorphic species, has a limited distribution in southwestern Alberta and is restricted to geographically isolated populations in high alpine areas. This study shows that the spatial structuring generally evident in interpopulational comparisons within *P. cana*, *P. pseudaurea*, and *P. cymbalarioides* does not follow the same pattern in *P. contermina*. Because the range of the species has been sampled more broadly and intensively than those of the other three species, the results can be discussed in more detail.

Phylogeographic patterns in *Packera contermina*

Spatial Distribution of Intrapopulational cpDNA Haplotypes

The only population (SMC) that was analyzed for intrapopulational distribution of haplotypes showed no evidence of significant spatial structure (Mantel $r = 0.0103$, $P = 0.362$) in the distribution of chloroplast haplotypes. Visual inspection of the distribution of chloroplast haplotypes in SMC (Fig. 8) shows that although the non-H1 haplotypes are not evenly distributed in the population, they are not clustered together based on their phylogenetic identity. However, there was a high frequency of H1 haplotypes (~88%) in the collection; only two other haplotypes were found more than once (H12 in 2 individuals, H7 in 5 individuals). Thus, the Mantel relation for correlation of haplotype with location was heavily dependent on a small amount of variation. This low level of diversity may be a factor of a sampling strategy that was designed to minimize the chance of re-sampling clones, but may have failed to identify true neighbors. Other potential factors contributing to the lack of detectable spatial patterning relate to the distribution of cpDNA haplotypes through seed dispersal. The seeds of *Packera contermina* are wind dispersed and the populations inhabit highly exposed alpine regions where there are few barriers to dispersal. It is possible that the dimensions of the collection site were significantly smaller than dispersal distances and that within-population structure existed but on a much larger scale than was sampled. This may be the case in the SMC collection because the mapped individuals were only part of a population that extended over an area approximately 2500 m^2 on the floor of a high alpine valley. Thus, a larger spatial sampling scheme than was employed here may be warranted for investigating intrapopulational structure in this species.

Interpopulational Variation

Among the 14 populations of *Packera contermina* analyzed there is a wide range of chloroplast haplotype diversity values displayed over the limited geographic range. The overall value of θ (0.333) indicates moderate levels of haplotype subdivision among populations compared to within populations. The range of intrapopulational haplotype diversity was highly variable ($\hat{h} = 0$ (CR) to $\hat{h} = 0.788$ (SLR)) indicating that different forces are shaping the diversity in each population. An examination of the frequency pie diagrams (Fig. 4) gives no indication that there is a regional trend of diversity such as that seen in *P. pseudaurea*. However, a Mantel relation for correlating geographic distance with genetic distance was positive ($r = 0.349$, $P = 0.012$) indicating that a significant part of the distribution can be explained by migration events. Evidence for other effects, such as bottlenecks, can be found in the cpDNA haplotype profiles but cannot be statistically verified without evidence from additional molecular markers. One example of a possible bottleneck is HWP, a population that is not only geographically distant from its southern counterparts, but also shows a lower haplotype diversity index ($\hat{h} = 0.285$) than many of the southern populations; furthermore, it is dominated by a different haplotype (H11) than is common in the south (H1). Other possible bottlenecks and founder effects are also shown in the PM, SFT, HSL, HFT region where presence of H2, a low frequency haplotype is found in adjacent populations and not in other areas. The CR population may be the result of a founder event because it is monomorphic for the predominant haplotype in its nearest neighbors at FL and SMC.

The data also suggest range fragmentation, as would have occurred through

spatial and temporal inter-digitation of Cordilleran and Laurentide ice sheets leaving some unglaciated nunatak areas. Evidence of such an occurrence is visible in the high degree of differentiation between HWP and all other populations, and to a lesser degree, among populations such as PM, HSL, SFT, SLR, VR, and SFL. There is a marked increase in the effective number of haplotypes ($\hat{h} > 0.500$) and a lack of continuity of haplotype frequencies with adjacent populations in estimates of pairwise values of Nei's genetic distances (most values are greater than approximately 0.400). A further line of evidence that supports a hypothesis of range fragmentation is that adjacent populations do not share the same complement of haplotypes. For example, HWP, SLR, and SMC are the only populations with H12, yet they are widely separated geographically as well as by other populations without H12. Similar disjunct patterns are evident in H8, H10, H11, and H14. Although HWP and SFL are both characterized by H11, the frequency of this haplotype is quite different between populations (84.2% and 10.5% respectively). There are also six intermediate sample sites between these populations with no trace of the haplotype. The presence of H11 in both populations suggests that either this haplotype was once widespread in the region and that it survived a severe bottlenecking event in the two southern populations, or that long-distance dispersal of individuals bearing the haplotype is responsible for the disjunct distribution. These hypotheses are difficult to distinguish without evidence from additional molecular markers, but long-distance dispersal seems an unlikely explanation since other low frequency haplotypes such as H6 and H7 also exhibit apparent disjunctions, but for different populations. If long-distance dispersal events were so common and undirected, lower levels of population diversity

might be expected. This suggests that many of these haplotypes may have been widespread across a pre-glaciated region. It is likely that climatic and geological changes associated with Pleistocene glaciation resulted in fragmentation and bottlenecking and that these factors account for most of the differences. The lack of clear evidence in many cases may be the result of early post-glacial migration and colonization events that have broken up ancient patterns of these events.

Many populations of *P. contermina* are linked by apparent gene flow because the estimated number of migrants exchanged among populations is $Nm = 1.00$, a value thought to be high enough to prevent differentiation of populations (Slatkin and Barton 1989). However, as shown above, apparent links by seed dispersal have been insufficient to homogenize the present-day diversity pattern. Therefore, the heterogeneous pattern of cpDNA polymorphisms detected in *P. contermina* may be due to the lack of equilibrium between drift and migration brought about by frequent local extinctions and recolonizations as described by metapopulation models (McCauley, 1995). This cannot be discounted because the environment in which these populations are found consists of highly erosional rocky ridges at high elevations where it might be expected to be less stable than lower elevation montane regions. However, because the apparent level of gene flow was measured indirectly by cpDNA haplotype analysis rather than by direct movement of individuals between existing populations (Slatkin 1987), the genetic similarity is a measure of identity in state rather than an identity by descent. Therefore, the possibility exists that the overall high frequency of H1 in the species is biasing the estimates of gene flow upward and the actual number of migrants is lower.

The relationship of pairwise geographic distances with estimated number of

migrants (Fig. 9) shows that at least one geographically distant population pair is linked by apparently high seed dispersal, and that over half of the comparisons had homogenizing levels of gene flow (values of ln(M) above 0). This pattern could be explained by long distance dispersal in the case of ongoing gene flow or by fragmentation of an ancient polymorphic population in the absence of gene flow. These events may be distinguished by examining the sequence divergence in intraspecific markers such as microsatellites to determine the relative timing of population structuring, but there is no evidence of such details in the data from this study. The present day distribution is likely the result of a complex history involving bottlenecks, founder events, and migration events.

Dendrograms of the genetic relationships among populations of *P. contermina* both consistently differentiate one population, HWP, the most northern and geographically separated of all the samples (Fig. 10 and Fig. 11). The grouping of SLR with SFT, and HSL with PM is consistent with their close geographical proximity and suggests that these populations probably diverged from a local gene pool. This line of evidence supports the survival of *P. contermina* in small nunatak areas in southwestern Alberta during the maximum advance of Pleistocene glaciation.

The Pleistocene period is marked by fluctuations in the timing and extent of the Cordilleran and Laurentide ice sheets (Stalker and Harrison 1977), so that the number of habitable areas during this time may have been in constant flux. There is strong support for survival of a population in the most northern region (at HWP) or for founding from a separate refugium due to the high frequency of H11 that is almost absent from regions to the south. It is possible that the more southern populations are less differentiated than

HWP because their proximity to each other has increased the potential for ameliorating gene flow during post-glaciation times. However, relictual populations may have survived in the southern part of the species range also. Haplotype profiles such as those that characterize PM, SLR, SFT, and SFL show a lower frequency of H1. This suggests that these areas are possible bottlenecks of a widespread and diverse ancestral population in which H1 was common, but not predominant, and are the source of individuals for post-glacial colonization. Because H1 is present in all source populations, the likelihood that it becomes established early in the founding of a new population is high. These data suggest such a pattern because intervening populations (for example, HSL, LFT, MDP, AR) have high levels of H1 and may be the recipients of haplotypes from more than one adjacent source. However, without congruence among many of the rarer haplotypes across the species range, it is difficult to determine refugial species and centers of diversity that have become source populations for recolonization. Other studies show that refugial populations are important sources of dispersal and are usually characterized by higher levels of genetic diversity relative to nearby founder populations, as was shown in the Carpathian mountains in Europe in the dispersal of *Fagus sylvatica* (Demesure et al. 1996) and on the west coast of North America in disjunct cpDNA haplotypes in *Tolmiea menziesii* (Soltis et al. 1997). The geographic scale of these studies is broader than this study, but the generalizations apply equally well to the relationships between *Packera contermina* populations when suggesting nunatak survivorship.

The complexities revealed in *Packera contermina* are also reflected in the genus as a whole. Levels of cpDNA variation in the four species are unusually high, but are consistent with hypotheses of other researchers that hybridization/introgression has been

frequent and contributes to intergradation (Bain 1988, Bain and Jansen 1996, Barkley 1962, 1988, Kowal 1975). In southwestern Alberta, it is likely that Pleistocene glaciation events have facilitated contact between diverging lineages and that this contact has resulted in introgression of non-local chloroplast haplotypes as well as exchange of local types. The lack of discrimination between species based on cpDNA variation suggests that these events have occurred frequently in this area.

There is no evidence of contemporary interspecific gene flow between any of the four species. The same lack of spatial structure generally evident in *P. contermina* populations is apparent between adjacent populations of different species. Populations of *P. cymbalarioides* (SMQ), *P. cana* (SC) and *P. contermina* (SMC) were collected at Sofa Mountain Cirque. Each collection at this site is characterized by a different set of haplotype frequencies that suggests that interspecific gene flow is unlikely.

CONCLUSIONS

This study provided evidence of two groups of diverging lineages in the cpDNA genome of *Packera*. The most common and widespread haplotype in southwestern Alberta (H1) appears to be part of an ancestral group in the genus that is found in widely dispersed species across North America. The second group of haplotypes (group B) appears to have diverged later and survived in more coastal and northern species. The presence of haplotypes from both groups in southwestern Alberta suggests that hybridization between species from these two groups is responsible. Because the advance of Pleistocene glaciation would restrict species to glacial refugia and boundary regions, the pre-glacial distribution of haplotypes from the coastal and northern groups either may have been more widespread or extensive migration may have taken place during the advance and retreat of glaciers. Subsequent fragmentation and bottlenecking by glacial events may have restricted the distribution of species to ice-free areas near southwestern Alberta and to refugia in the Pacific northwest coastal region, a displacement which may have facilitated hybridization/ introgression. Comparative studies of other species provide evidence of such a region in southwestern Alberta and/or northern Montana (Wilson and Hebert 1998, Zamudio et al. 1997).

This study also provides evidence that the species' histories of *P. cana*, *P. pseudaurea*, *P. cymbalarioides*, and *P. contermina* are affected by different forces in spite of a close phylogenetic and geographic relationship. Differences in population subdivision and estimated levels of gene flow exist even though levels of haplotype diversity and effective numbers of haplotypes were similar among the four species.

These insights demonstrate the need for more extensive comparative analyses of

species such as *P. contermina*, *P. cana*, and *P. pseudaurea* with those from other regions of high haplotype diversity such as the Great Basin of southwestern North America, and the Alaska-Yukon region. Not only would such studies increase our knowledge of phylogeographic patterns in the genus, they would provide valuable insights into the mechanisms responsible for generating high amounts of intra-specific variation.

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Table 1. Population name, abbreviation, location, elevation and collection numbers for 34 populations of *Packera contermina, P. cana, P. pseudaurea, and P. cymbalarioides* in southwestern Alberta, northern Montana, and northwestern Wyoming.

Taxon	Coll. no.	Abbrev.	Location	Latitude/Longitude	Elevation (m. above sea level)
P. contermina					
	Golden 264	AR	Avion Ridge, AB.	49° 10' N., 114° 06' W.	2260
	Golden 269	CR	Carthew Ridge, AB.	49° 01' N., 114° 01' W.	2500
	Golden 250	FL	Forum Lake, B.C.	49° 00' N., 114° 04' W.	2010
	Golden 245	HFT	Hailstone Fire Tower, AB.	50° 12' N., 114° 27' W.	2360
	Bain 535	HSL	Hailstone Lake, AB.	50° 11' N., 114° 26' W.	2150
	Bain 357	HWP	Highwood Pass, AB.	50° 36' N., 114° 58' W.	2600
	Golden 258	LFT	Livingstone Fire Tower, AB.	49° 55' N., 114° 20' W.	2100
	Golden 271	MDP	MacDonald Pass, AB.	49° 22' N., 114° 31' W.	1900
	Golden 267	PM	Plateau Mountain, AB.	50° 13' N., 114° 32' W.	2430
	Bain 536	SFL	Southfork Lakes, AB.	49° 20' N., 114° 20' W.	2025
	Golden 252	SFT	Sugarloaf Fire Tower. AB.	49° 56' N., 114° 32' W.	2500
	Golden 272	SLR	S. Livingstone Ridge, AB.	49° 40' N., 114° 20' W.	2190
	Golden 262	SMC	Sofa Mountain Cirque, AB.	49° 02' N., 113° 47' W.	1990
	Golden 251	VR	Victoria Ridge, AB.	49° 17' N., 114° 09' W.	2150
P. cana					
	Golden 229	BTP	Beartooth Pass, Mon.	44° 57' N., 109° 27' W.	3108
	Lowry 75	CD	Cardston, AB.	49° 11' N., 113° 18' W.	1190
	Golden 246	CS	Carstairs, AB.	51° 35' N., 114° 04' W.	1030
	Golden 216	PVT	Prairie View Trail, AB.	51° 03' N., 115° 03' W.	1560
	Golden 207	RM	Ram Mountain, AB.	52° 20' N., 115° 47' W.	2040
	Golden 248	SC	Sofa Mountain Cirque, AB.	49° 02' N., 113° 47' W.	1990
	Golden 257	SPR	Spionkop Ridge, AB.	49° 12' N., 114° 07' W.	2160
	Golden 259	YCR	Yarrow Canyon Ridge, AB.	49° 11' N., 114° 06' W.	2130
P. pseudaurea					
	Yates 3	BML	Beaver Mines Lake, AB.	49° 26' N., 114° 35' W.	1450
	Yates & Golden 1	BR	Belly River, AB.	49° 01' N., 113° 42' W.	1342
	Golden 247	CL	Chinook Lake, AB.	49° 40' N., 114° 35' W.	1460
	Bain & Schultz 572	FHL	Flathead Lake, Mon.	47° 38' N., 114° 17' W	960
	Golden 189	HWM	Highwood Mountains, Mon.	47° 10' N., 110° 37' W.	2320
	Yates & Golden 8	RR	Raspberry Ridge	50° 18' N., 114° 37' W.	1940
	Yates 5	VCR	Victoria Ridge, AB.	49° 19' N., 114° 03' W.	1750

Table 1. ....cont.

Taxon	Coll. no.	Abbrev.	Location	Latitude/Longitude	Elevation (m. above sea level)
P. cymbalarioides					
	Golden 263	AVR	Avion Ridge, AB.	49° 10' N., 114° 06' W.	1980
	Golden 240	BHM	Bighorn Mountains, WYO.	44° 45' N., 107° 39' W.	3095
	Golden 234	BTM	Beartooth Mountains, WYO.	44° 56' N., 109° 33' W.	3334
	Golden 270	SCQ	Sofa Mountain Cirque, AB.	49° 02' N., 113° 47' W.	1990
	Golden 260	YCL	Yarrow Canyon Lake, AB.	49° 11' N., 114° 05' W.	1980

Table 2. Chloroplast DNA restriction site mutations detected in four species of *Packera* from southwestern Alberta, northern Montana, and northwestern Wyoming. Probes are cloned *Latuca* cpDNA fragments.

			Mutation (fragment size in kb)*	
Site Number	Probe length (kb)	Restriction endonuclease	Absent	Present
1	6.3	Ava I	1.6	1.1 + 0.5
2	6.9	Ava I	10.0	6.7 + 3.3
3	14.7	Ava I	6.7	5.3 + 1.4
4	18.8	Ava I	12.3	7.2 + 5.1
5	5.4	Ava II	6.7	6.2 + (0.5)
6	6.7+3.8	Dra I	4.9	3.5 + (1.4)
7	6.7+3.8	Dra I	1.9	0.8 + 1.1
8	14.7	Dra I	8.6	4.4 + 4.2
9	14.7	Dra I	8.6	6.1 + 2.5
10	14.7	Hae III	1.9	1.7 + (0.2)

* Inferred fragments in brackets

Table 3. Polymorphisms in 15 chloroplast DNA haplotypes detected in four *Packera* species from southwestern Alberta, northwestern Wyoming, and northern Montana.

Haplotype	Restriction site characters (Table 2.)										Populations where haplotypes detected.
	1	2	3	4	5	6	7	8	9	10	
H1	1	1	1	1	1	1	1	1	1	0	HFT, SFT, FL, VR, LFT, SMC, AR, PM, CR, MDP, SLR, HWP, HSL, SFL, PVT, BTP, CS, SC, SPR, YCR, RM, CD, RR, CL, BR, VCR, BML, FHL, YCL, SCQ
H2	1	1	1	1	1	1	0	1	1	0	HFT, SFT, LFT, SMC, PM, HSL, BTP, SPR, CD, CL, HWM, BHM, AVR
H3	0	1	0	0	0	1	0	0	0	1	RR, CL, BTM
H4	0	1	1	0	0	1	0	0	0	0	CL
H5	1	1	1	1	1	1	0	1	0	1	RR, CL, BR, BML, HWM
H6	1	1	1	1	1	1	1	1	1	1	HFT, VR, AR, SFL, BR, BML
H7	0	1	1	0	0	1	0	0	0	1	SMC, PM, HSL, CS, YCR, RM, RR, CL, BR, VCR, BML, HWM
H8	1	1	1	1	1	1	1	0	1	0	SFT, VR, MDP, SLR, VCR
H9	0	1	1	0	0	1	1	0	0	1	MDP, SLR, RR
H10	0	0	1	1	0	1	0	1	0	1	HFT, FL, SMC, SLR, SFL, PVT, CS, SC, YCR,
H11	0	0	1	1	0	1	0	0	0	1	SMC, HWP, SFL, SC, CD
H12	1	1	1	1	1	1	0	0	1	0	SMC, SLR, HWP, BTP, SC
H13	0	1	1	1	0	1	0	1	0	1	SMC
H14	1	1	1	1	1	0	0	0	1	1	VR, HSL, PVT, SC
H15	0	1	1	1	0	1	1	0	1	1	SFL
Haplotypes derived from data in Bain & Jansen (1996).											
H16	0	1	1	1	0	1	0	0	0	1	
H17	1	1	1	1	0	1	0	1	0	0	
H18	1	1	1	1	1	1	0	0	0	1	

Table 4. Haplotypes identified in *Packera* populations across North America (Bain & Jansen 1996), compiled using character states of restriction site mutations1-5, and 7-10 from Table 3.

Taxon (coll. #)	Location	H 1	H 2	H 4	H 7	H 10	H 12	H 13	H 16	H 17	H 18
P. bernardina (#465)	San Bernardino Co., CA										X
P. bolanderi (#470)	Curry Co., OR							X		X	
P. cymbalaria (#52)	Forillon, PQ.	X									
P. cymbalaria (#53)	Port as Port, NF	X									
P. cymbalaria (#259)	Mt. Griscom, PQ					X					
P. eurycephala (#468)	Mendocino Co., CA		X								
P. ganderi (#464)	San Diego Co., CA				X						
P. hyperborealis (#491)	Dempster Hwy., YT				X						
P. indecora (#300)	Takini, YT			X							
P. laynae (#467)	Eldorado Co., CA		X								
P. moresbiensis (#271)	Vancouver Is., BC			X							
P. moresbiensis (#480)	Queen Charlotte Is., BC				X						
P. multilobata (#449)	Cache Co., UT		X								
P. multilobata (#461)	Coconino Co., NM		X								
P. multilobata (#474)	Lincoln Co., WY		X								
P. neomexicanus (#463)	Gila Co., AZ		X								
P. ogotorukensis (#494)	Tok, AK							X	X		
P. ogotorukensis (#496)	Sheep Mtn., YT								X		
P. ogotorukensis (#498)	Rock Glacier, YT					X		X			
P. pauciflora (#77)	Blanc Sablon, PQ				X						
P. pauciflora (#500)	S. Canol Rd. YT		X								
P. paupercula (#305)	Km 1814, Alcan Hwy., YT		X								
P. pseudaurea (#99)	Gunnison Co., CO	X									
P. quarens (#458)	Catron Co., NM.		X								
P. quercetorum (#462)	Gila Co., AZ		X								
P. streptanthifolia (#152)	Crowsnest Mtn., AB	X									
P. streptanthifolia (#446)	Cache Co., UT	X	X								
P. streptanthifolia (#450)	Duchesne Co., UT		X								
P. streptanthifolia (#451)	Dushesne Co., UT		X				X				

Table 5. Haplotypes identified in individuals from 4 species of *Packera* from southwestern Alberta, northwestern Montana, and Northwestern Wyoming.

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15
P. contermina	X	X				X	X	X	X	X	X	X	X	X	X
P. cana	X	X					X			X	X	X		X	
P. cymbalarioides	X	X	X												
P. pseudaurea	X	X	X	X	X	X	X	X	X						

Table 6. Sample size (N), haplotype frequency expressed percent of detected haplotypes, haplotype diversity ($\hat{h}$) with standard errors in parentheses, and effective number of haplotypes (n_e) in 14 *Packera contermina* populations.

Population	N=	H1	H2	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	$\hat{h}$	n_e
HFT	21	0.810	0.048	0.095	0	0	0	0.047	0	0	0	0	0	0.339 (0.001)	1.495
SFT	6	0.167	0.167	0	0	0.667	0	0	0	0	0	0	0	0.546 (0.015)	2.000
FL	33	0.939	0	0	0	0	0	0.061	0	0	0	0	0	0.116 (0.000)	1.128
VR	22	0.636	0	0.045	0	0.046	0	0	0	0	0	0.273	0	0.529 (0.001)	2.068
LFT	21	0.952	0.048	0	0	0	0	0	0	0	0	0	0	0.093 (0.001)	1.100
SMC	95	0.884	0.011	0	0.053	0	0	0.011	0.011	0.021	0.011	0	0	0.216 (0.000)	1.273
AR	14	0.929	0	0.071	0	0	0	0	0	0	0	0	0	0.138 (0.003)	1.153
PM	15	0.400	0.200	0	0.400	0	0	0	0	0	0	0	0	0.662 (0.002)	2.778
CR	19	1.000	0	0	0	0	0	0	0	0	0	0	0	0	1.000
MDP	31	0.935	0	0	0	0.032	0.032	0	0	0	0	0	0	0.125 (0.000)	1.114
SLR	13	0.308	0	0	0	0.154	0.308	0.154	0	0.077	0	0	0	0.788 (0.003)	4.122
HWP	19	0.105	0	0	0	0	0	0	0.842	0.053	0	0	0	0.285 (0.001)	1.383
HSL	22	0.682	0.091	0	0.182	0	0	0	0	0	0	0.046	0	0.503 (0.001)	1.967
SFL	19	0.316	0	0.421	0	0	0	0.105	0.105	0	0	0	0.053	0.717 (0.001)	3.312

Table 7. Sample size (N), haplotype frequency, haplotype diversity ($\hat{h}$) with standard errors in parentheses, and effective number of haplotypes (n_e) in 8 *Packera cana* populations.

Population	N=	H1	H2	H7	H10	H11	H12	H14	$\hat{h}$	n_e
PVT	16	0.375	0	0	0.188	0	0	0.438	0.653 (0.002)	2.723
BTP	25	0.56	0.240	0	0	0	0.200	0	0.601 (0.001)	2.432
CS	14	0.714	0	0.143	0.143	0	0	0	0.466 (0.003)	1.815
SC	13	0.385	0	0	0.154	0.077	0.154	0.231	0.775 (0.003)	3.922
SPR	6	0.667	0.333	0	0	0	0	0	0.485 (0.015)	1.800
YCR	9	0.111	0	0.778	0.111	0	0	0	0.392 (0.006)	1.588
RM	6	0.833	0	0.167	0	0	0	0	0.303 (0.015)	1.385
CD	33	0.152	0.606	0	0	0.242	0	0	0.559 (0.000)	2.227

Table 8. Sample size (N), haplotype frequency, haplotype diversity ($\hat{h}$) with standard errors in parentheses, and effective number of haplotypes (n_e) in 7 *Packera pseudaurea* populations.

Population	N=	H1	H2	H3	H4	H5	H6	H7	H8	H9	$\hat{h}$	n_e
RR	26	0.539	0	0.039	0	0.077	0	0.308	0	0.039	0.618 (0.001)	2.541
CL	27	0.778	0.074	0.037	0.037	0.037	0	0.037	0	0	0.391 (0.001)	1.624
BR	27	0.852	0	0	0	0.074	0.037	0.037	0	0	0.271 (0.001)	1.363
VCR	25	0.800	0	0	0	0	0	0.120	0.080	0	0.346 (0.001)	1.513
BML	26	0.731	0	0	0	0.159	0.039	0.077	0	0	0.443 (0.001)	1.770
FHL	14	1.00	0	0	0	0	0	0	0	0	0	1
HWM	16	0.375	0.125	0	0	0.250	0	0.250	0	0	0.742 (0.002)	3.556

Table 9. Sample size (N), haplotype frequency, haplotype diversity ($\hat{h}$), and effective number of haplotypes (n_e) in 7 *Packera cymbalarioides* populations.

Population	N=	H1	H2	H3	$\hat{h}$	n_e
BTM	12	0	0	1	0	1
BHM	31	0	1	0	0	1
YCL	4	1	0	0	0	1
AVR	16	0	1	0	0	1
SCQ	23	1	0	0	0	1

Table 10. Predicted group membership of populations reported as actual number and percent of total in parentheses based on discriminant functions derived from cpDNA haplotypes detected in species of *Packera*. Overall percent of groups correctly classified: 73.53% as calculated by SPSS for the Macintosh® ver 4.0.

Actual taxon identity	# of populations	Predicted taxon identity			
		P. contermina	*P. cana*	*P. pseudaurea*	*P. cymbalarioides*
P. contermina	14	12 (85.7%)	2 (14.3%)	0	0
P. cana	8	2 (25.0%)	5 (62.5%)	0	1 (12.5%)
P. pseudaurea	7	2 (28.6%)	0	5 (71.4%)	0
P. cymbalarioides	5	2 (40.0%)	0	0	3 (60.0%)

Table 11. Estimates of Wright's Fst, θ , with standard deviations in parentheses (Weir and Cockerham, 1984), overall haplotype diversity (h), and estimates of number of migrants (N_m) exchanged among all populations of each species based on cpDNA haplotypes. Calculations for haplotype diversity and estimates of number of migrants explained in text.

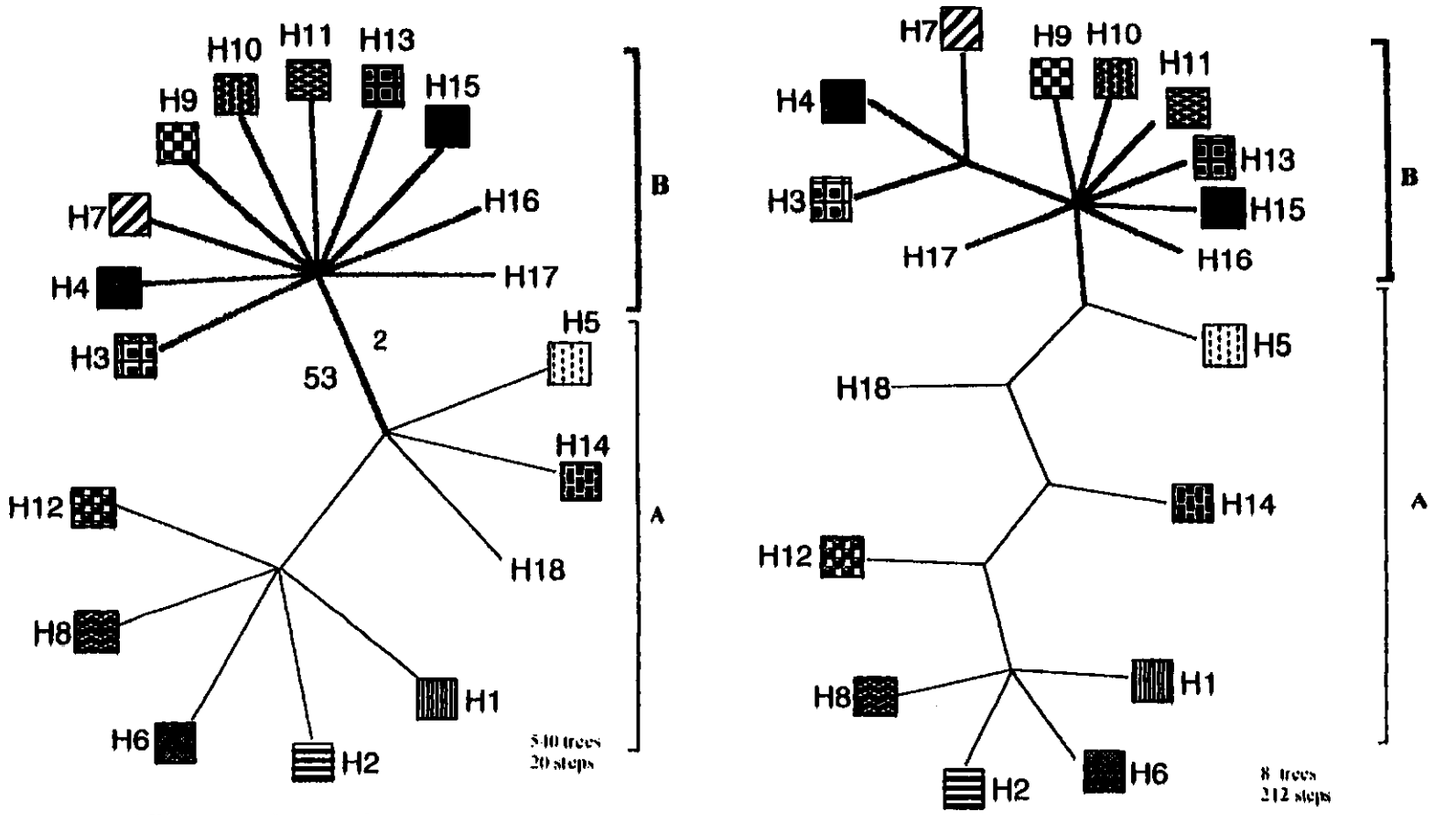
Taxon	θ	h	N_m
P. contermina	0.3331 (+/-0.11)	0.436	1.00
P. cana	0.2619 (+/-0.10)	0.756	1.41
P. pseudaurea	0.0855	0.452	5.35
P. cymbalarioides	1.00	0.587	0.00

Table 12. Pairwise comparisons between 14 populations of *Packera contermina*: geographical distances calculated from latitude and longitude coordinates reported in linear kilometres (below diagonal); and probability of identity in state as calculated by GENEPOP Vers. 3.1b (above diagonal).

	HFT	SFT	FL	VR	LFT	SMC	AR	PM	CR	MDP	SLR	HWP	HSL	SFL
HFT		0.5005	0.0290	0.0744	0.0235	0.0142	-0.024	.02395	0.0794	0.0323	0.2410	0.6491	0.0273	0.2290
SFT	29.43		0.7395	0.3495	0.7249	0.6669	0.6480	0.2759	0.8135	0.7166	0.1423	0.6069	0.3751	0.2769
FL	136.33	109.86		0.2108	-0.011	0.0033	-0.016	0.4454	0.0074	-0.008	0.4465	0.7947	0.1441	0.4485
VR	104.77	78.535	31.57		0.1887	0.1884	0.1308	0.1824	0.2381	0.1949	0.1583	0.5473	0.0462	0.1832
LFT	33.572	14.917	102.83	71.27		-0.005	-0.025	0.3992	-0.005	-0.020	0.4146	0.7893	0.1158	0.4165
SMC	137.94	114.03	21.324	38.031	104.58		-0.015	0.4002	0.0151	0.0001	0.4433	0.7377	0.0871	0.4570
AR	118.57	92.323	17.732	13.735	85.041	26.94		0.3341	0.0226	-0.025	0.3338	0.7484	0.0811	0.3215
PM	5.819	29.834	138.58	107.05	36.473	141.02	120.87		0.4663	0.4295	0.1427	0.5039	0.0608	0.1849
CR	136.04	109.90	3.110	31.414	102.50	18.27	17.595	138.43		-0.003	0.4711	0.8366	0.1747	0.4615
MDP	92.95	63.934	52.648	28.677	62.014	65.282	38.474	93.727	53.87		0.4242	0.7859	0.1324	0.4397
SLR	60.753	34.215	75.872	44.371	27.820	79.969	58.232	62.751	75.776	35.26		0.4121	0.1690	0.1158
HWP	57.227	79.453	189.17	157.96	89.015	193.19	171.74	52.923	189.31	140.77	113.69		0.5595	0.4140
HSL	3.110	27.116	133.24	101.70	30.476	134.71	115.47	8.518	132.93	90.283	57.774	60.51		0.2053
SFL	97.447	69.308	42.648	16.894	64.985	53.784	27.205	98.876	43.490	11.844	37.130	147.82	94.58	

Table 13. Pairwise genetic distances for 14 populations of *P. contermina*: Cavalli-Sforza distances reported below the diagonal, and distances calculated by Nei methods above. All values computed by PHYLIP vers. 3.57.

	HFT	SFT	FL	VR	LFT	SMC	AR	PM	CR	MDP	SLR	HWP	HSL	SFL
HFT		1.383	0.01	0.083	0.009	0.012	0.004	0.386	0.011	0.012	0.452	2.054	0.047	0.391
SFT	0.153		1.434	1.264	1.382	1.424	1.435	1.432	1.432	1.315	0.83	3.475	1.356	1.985
FL	0.022	0.172		0.084	0.003	0.004	0.005	0.408	0.002	0.003	0.437	2.045	0.047	0.525
VR	0.06	0.139	0.064		0.083	0.079	0.079	0.487	0.082	0.08	0.51	2.125	0.097	0.484
LFT	0.021	0.145	0.016	0.063		0.003	0.004	0.381	0.001	0.002	0.467	2.045	0.039	0.546
SMC	0.032	0.163	0.019	0.057	0.018		0.005	0.347	0.002	0.003	0.456	1.953	0.029	0.538
AR	0.014	0.172	0.019	0.048	0.017	0.027		0.408	0.003	0.004	0.468	2.046	0.047	0.454
PM	0.095	0.159	0.111	0.141	0.081	0.063	0.112		0.406	0.407	0.871	2.449	0.164	0.951
CR	0.029	0.168	0.009	0.057	0.007	0.018	0.01	0.105		0.001	0.465	2.043	0.045	0.545
MDP	0.036	0.131	0.017	0.053	0.016	0.026	0.019	0.111	0.009		0.42	2.044	0.046	0.546
SLR	0.12	0.13	0.104	0.134	0.131	0.114	0.132	0.185	0.127	0.085		2.397	0.51	0.856
HWP	0.2	0.247	0.194	0.21	0.193	0.162	0.194	0.226	0.191	0.194	0.215		2.088	1.3
HSL	0.055	0.153	0.057	0.065	0.037	0.023	0.059	0.022	0.05	0.057	0.155	0.208		0.58
SFL	.0630	0.219	0.106	0.086	0.128	0.109	0.081	0.184	0.124	0.129	0.159	0.145	0.14	



a) Wagner parsimony strict consensus tree

b) Step-matrix strict consensus tree

Fig. 1. Phylogenetic relationships of cpDNA haplotypes in *Packera* species across North America as described in Table 3. In both trees, Clade A haplotypes are H1, H2, H5, H6, H8, H12, H14,and H18, denoted by the narrow lines. Clade B haplotypes are H3, H4, H7, H9, H10, H11, H13, H15, H16, and H17, denoted by the bold line. On the Wagner parsimony tree, branch lengths greater than one are indicated by the number to the right of the line and bootstrap values (>50%) to the left.

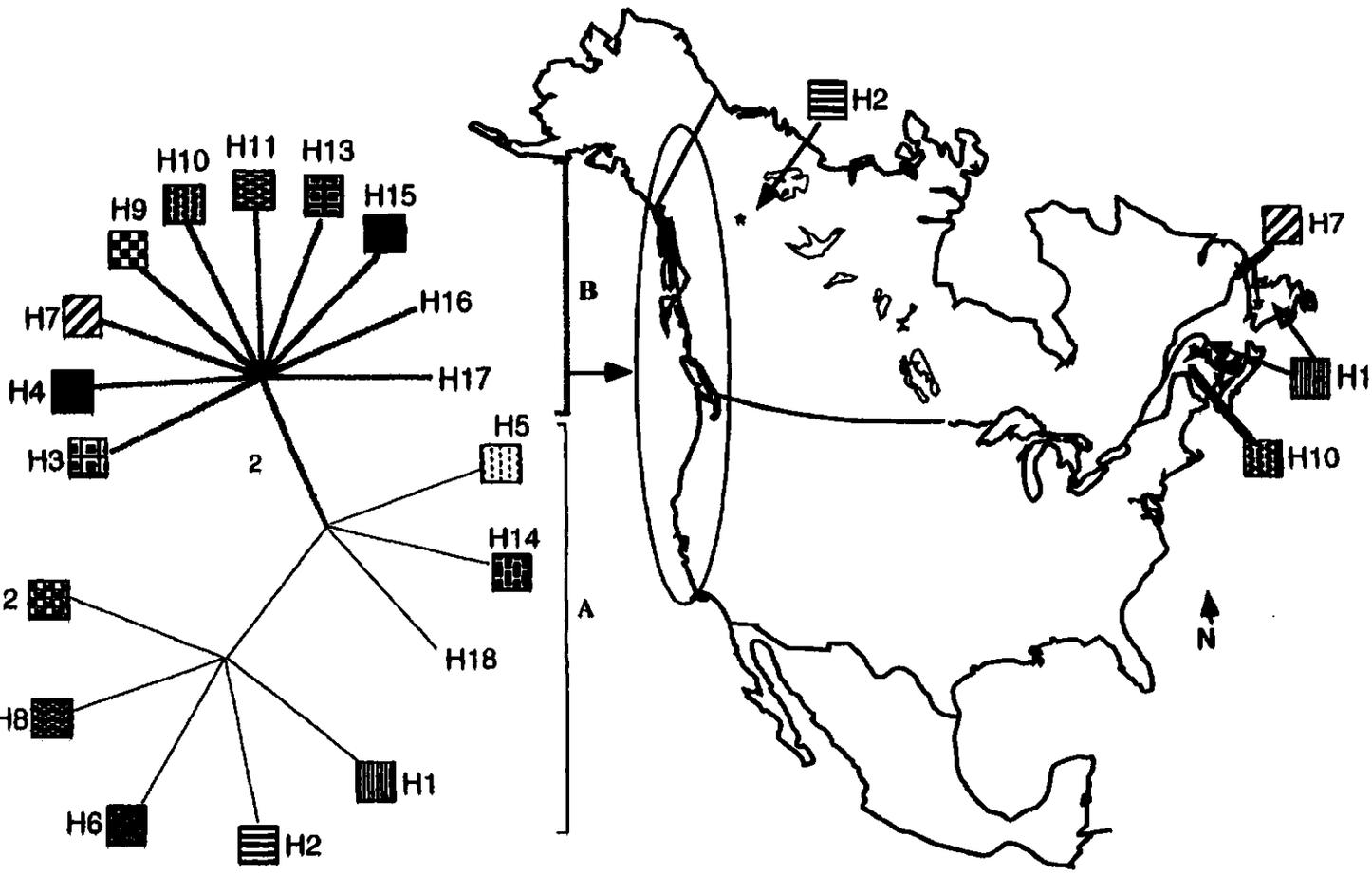


Fig. 2. Phylogeographic relationships and regional affiliations of 18 cpDNA haplotypes of *Packera* from North America as described in Table 3. Clade A haplotypes (shown with narrow branch lines) most common in Great Basin populations and widespread across North America. Clade B haplotypes (denoted by heavy branch lines) most common in coastal and northern regions. All haplotypes except H16, H17, and H18 are found in southwestern Alberta.

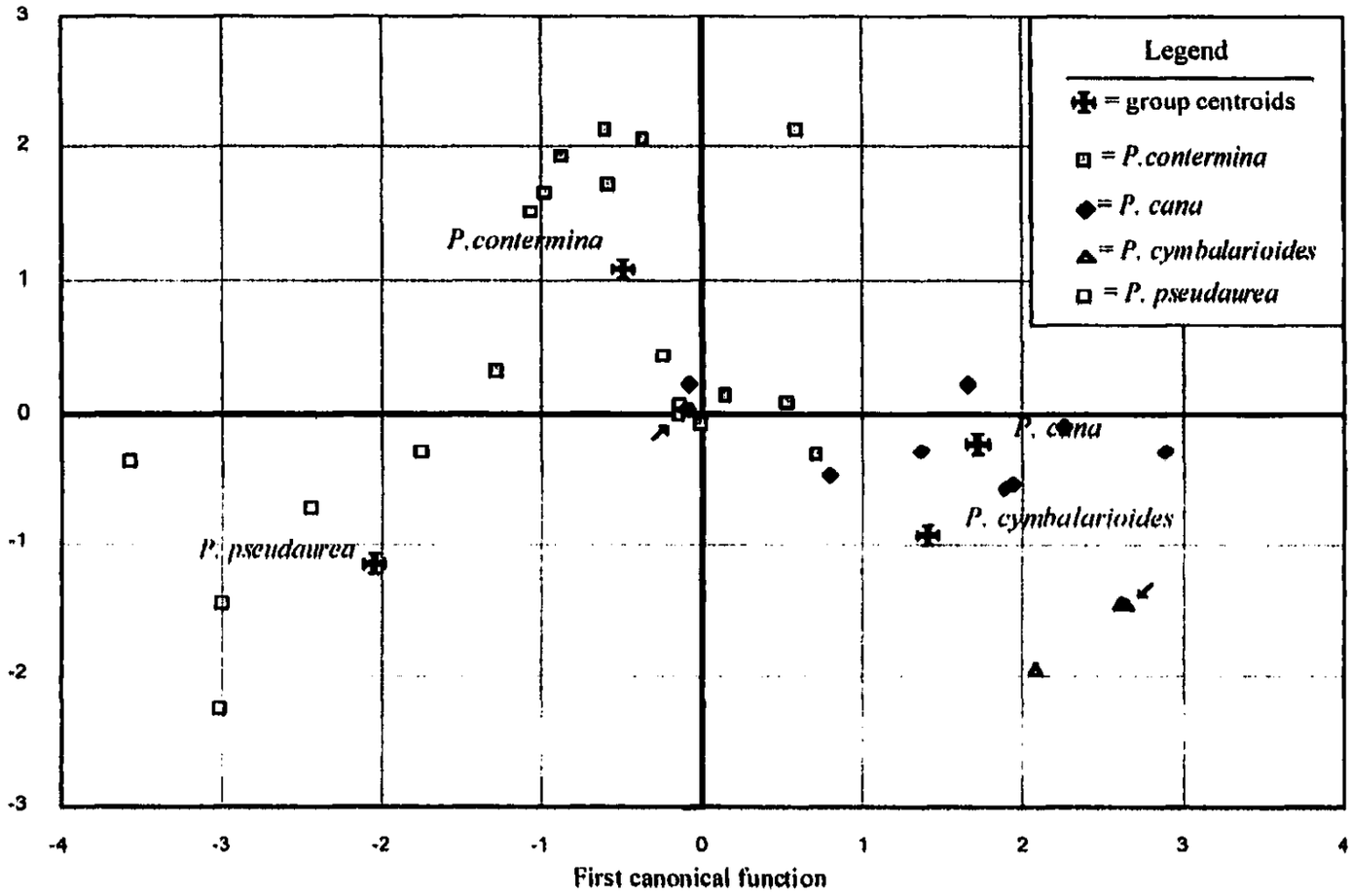


Fig. 3. Relationships of 34 *Packera* populations based on discriminant functions derived from cpDNA haplotypes and haplotype frequency. Group centroids are labelled with species name. Overlap of more than one population and/or species indicated by arrows.

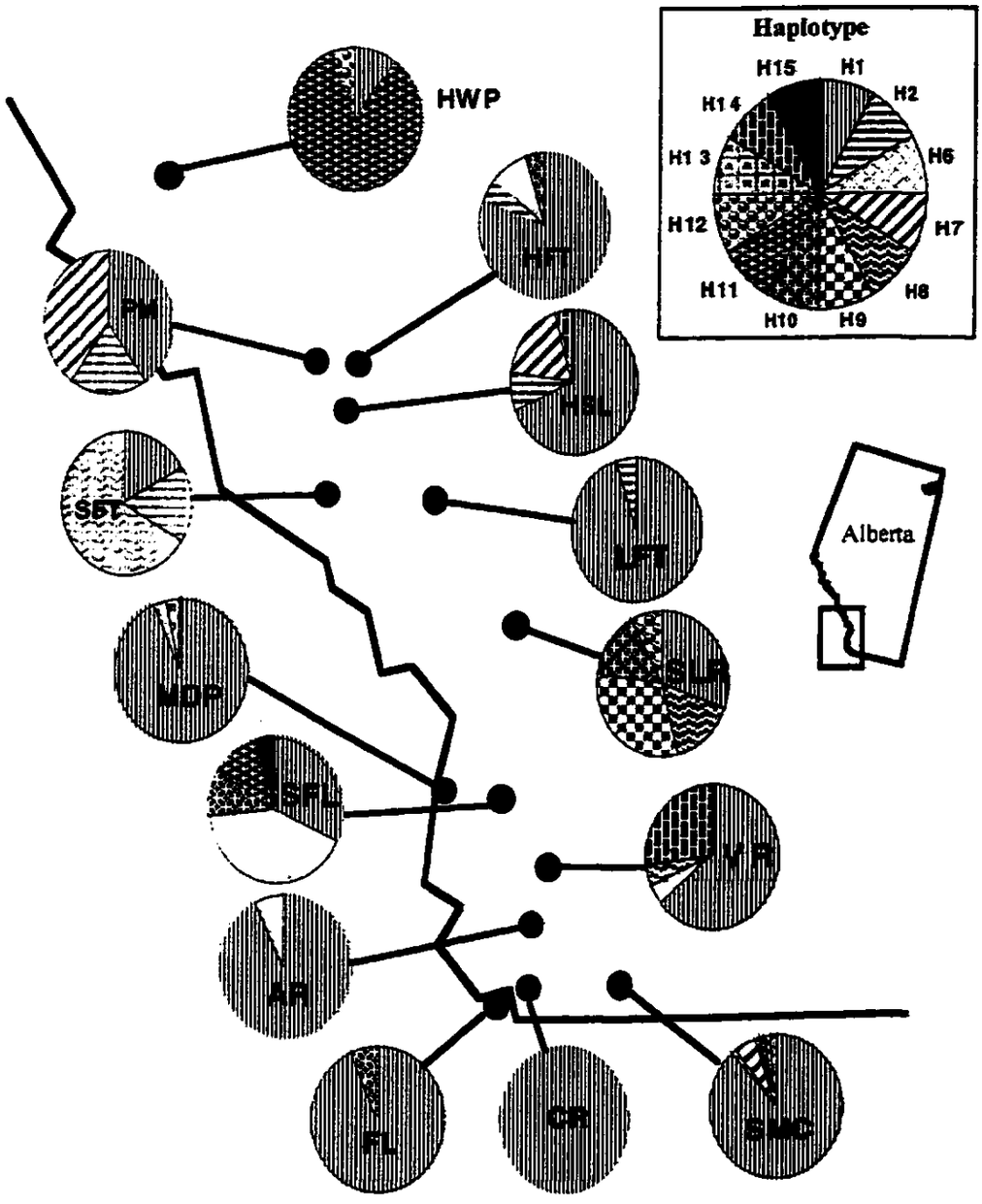


Fig. 4. Haplotype frequencies in *Packera contermina* populations in Alberta and southeastern British Columbia. Lettering in pie-diagrams refers to population abbreviations in Table 1. Collection sizes reported in Table 6.

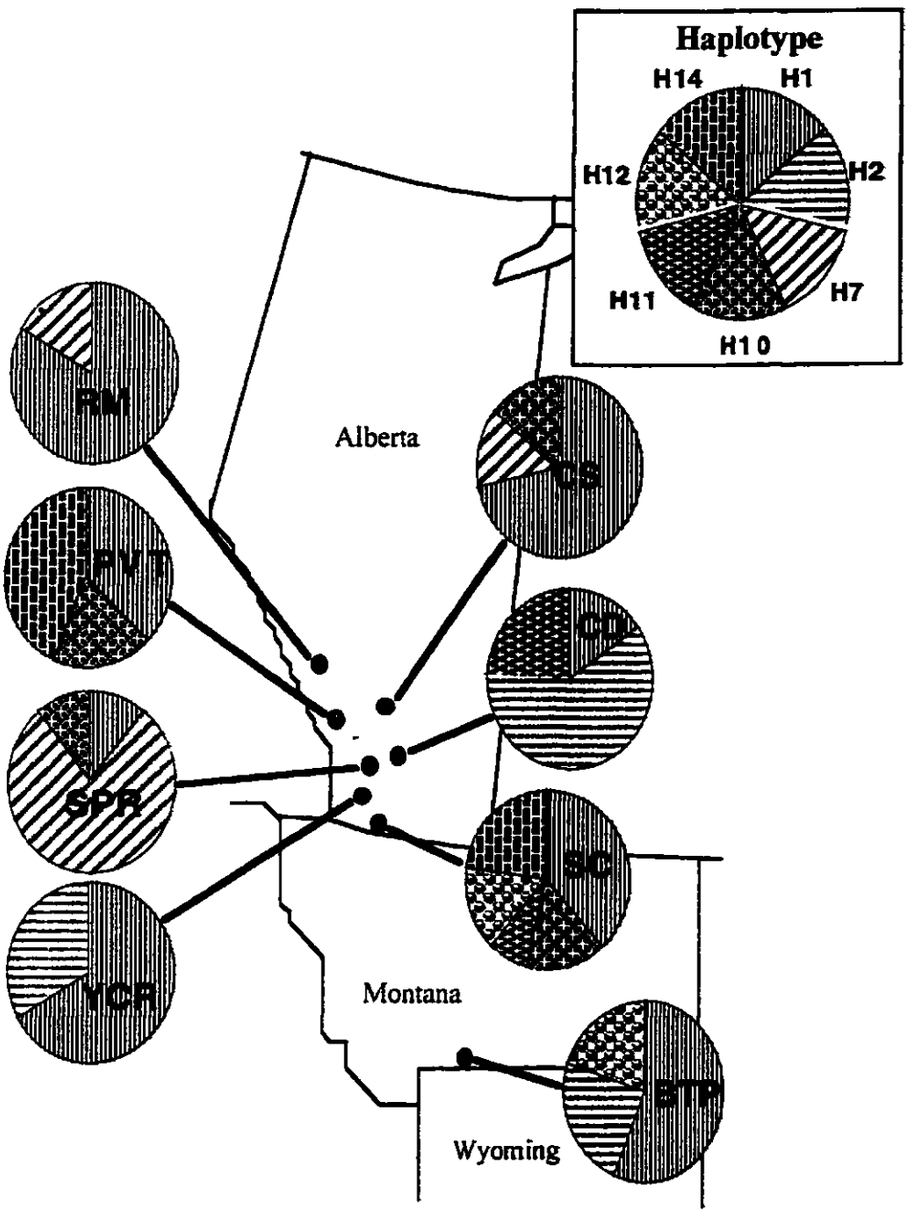


Fig. 5. Haplotype frequencies in populations of *Packera cana* in Alberta and Montana. Lettering in pie diagrams refers to population abbreviations as reported in Table 1. Collection sizes reported in Table 7.

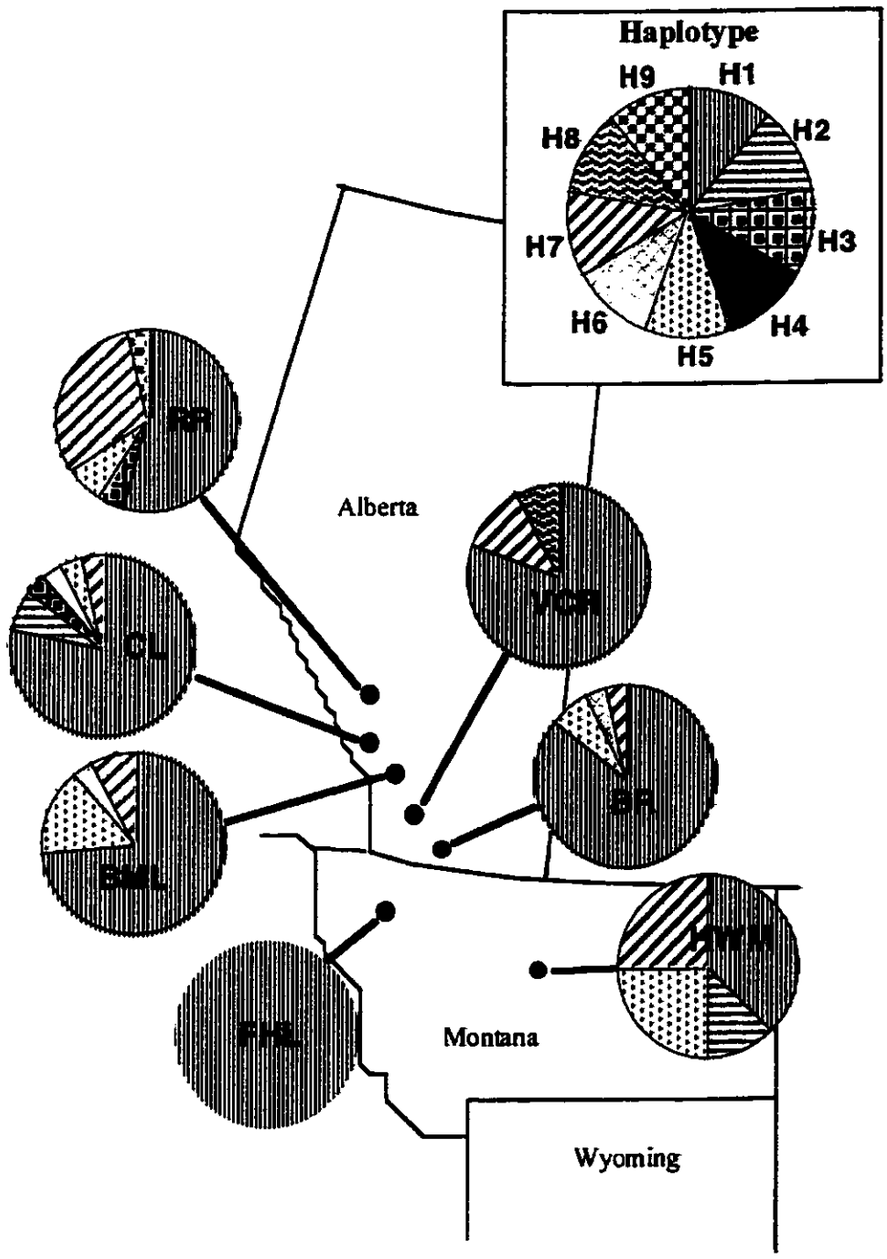


Fig. 6. Haplotype frequencies in populations of *Packera pseudaurea* in Alberta and Montana. Population abbreviations described in Table 1. Sample collection sizes shown in Table 8.

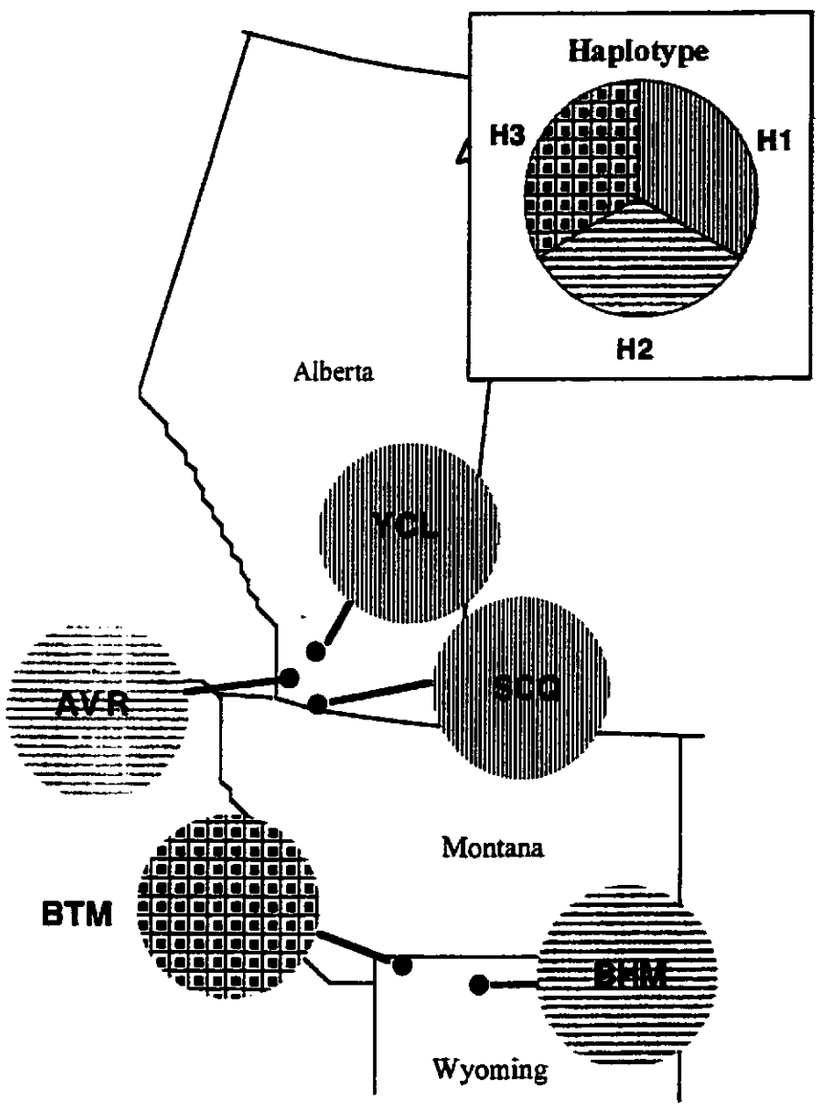


Fig. 7. Haplotype frequencies in *Packera cymbalarioides* populations in Alberta and Wyoming. Population abbreviations described in Table 1. Population sizes shown in Table 9.

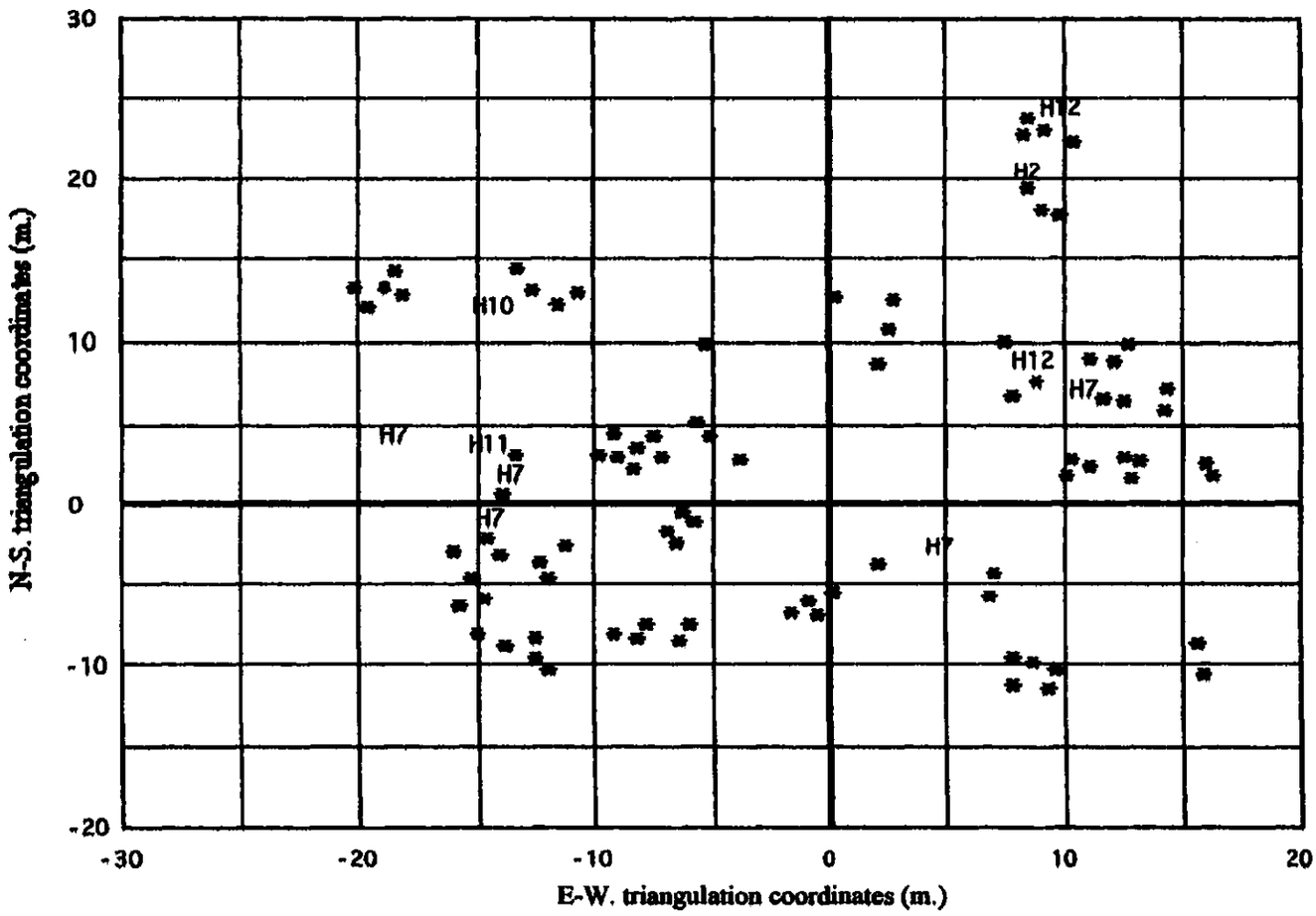


Fig. 8. Distribution of cpDNA haplotypes of 100 individuals of *P. contermina* in SMC population. Haplotype 1 is designated by (*) and H2, H7, H10, H11, H12 are individually labelled. Grid positions are calculated from triangulation coordinates relative to horizontal baseline at 0 m.

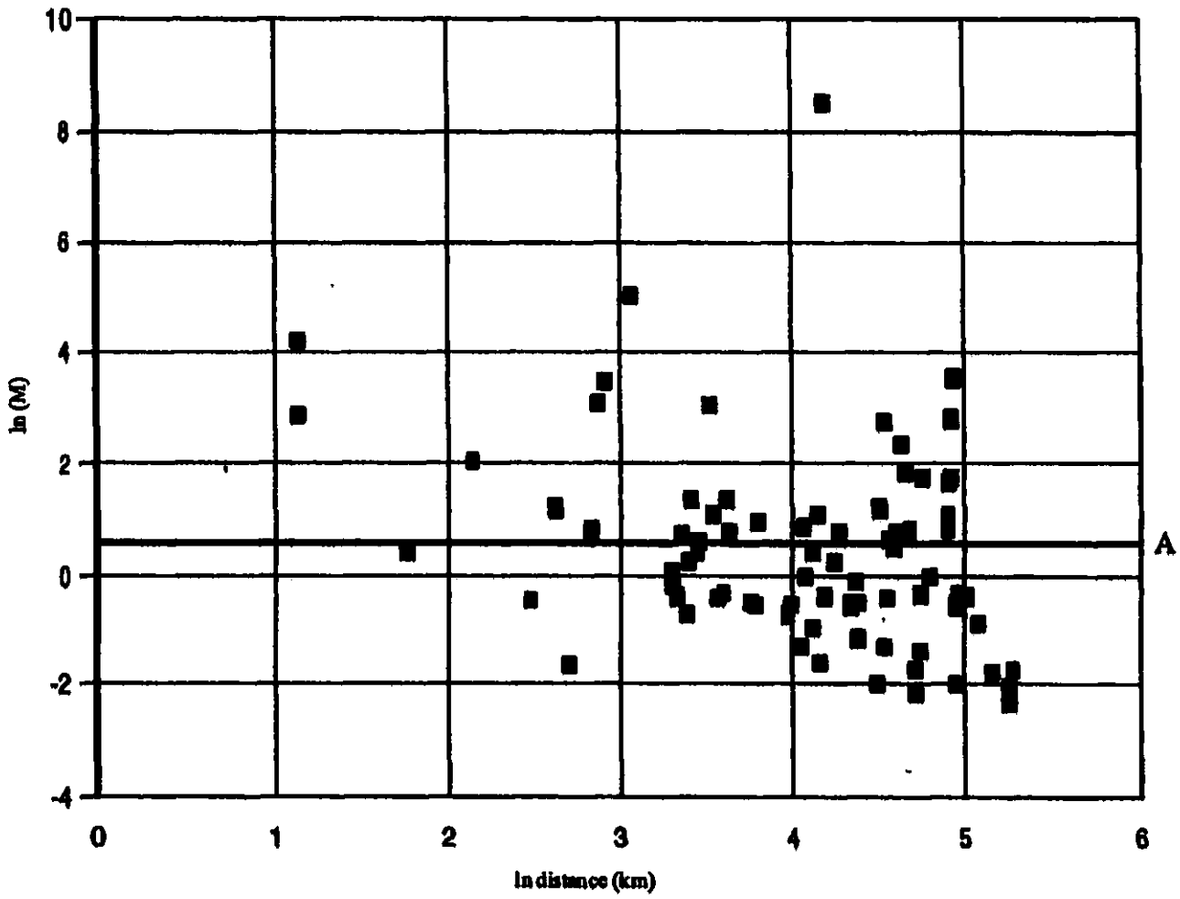


Fig. 9. Relationship of calculated pairwise estimates of migrants (M) exchanged between populations with geographic distance for 14 *Packera contermina* populations. Line A indicates the mean for all comparisons. Mantel relation for all comparisons ($r = 0.349$, $p = 0.012$).

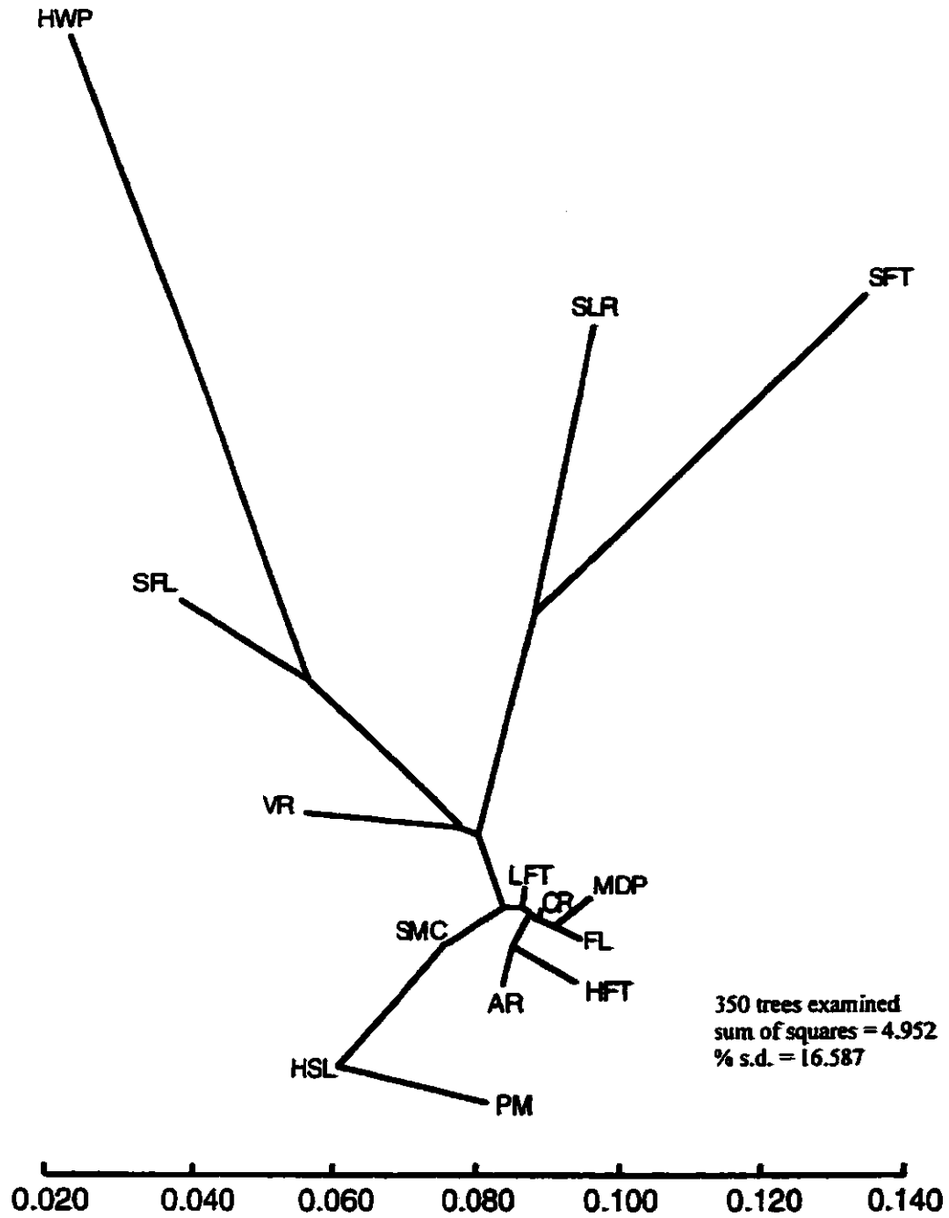


Fig. 10. Fitch-Margoliash cluster analysis of 14 *Packera contermina* popu.ations, based on Cavalli-Sforza distances. Pairwise distances reported in Table 12.

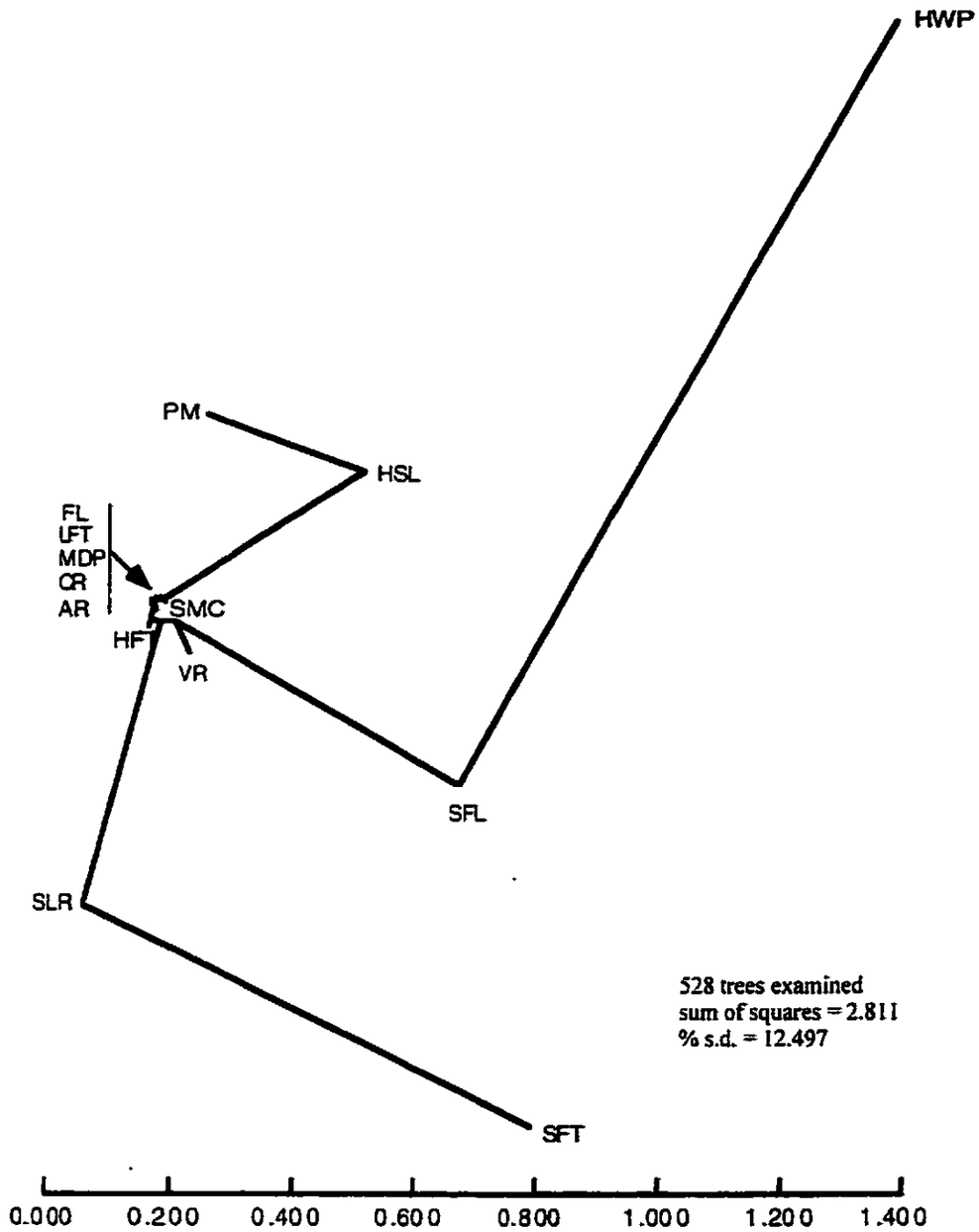


Fig. 11. Fitch-Margoliash cluster analysis of 14 *Packera contermina* populations, based on Nei's genetic identities. Pairwise distances reported in Table 12.