Systematics of North American *Petasites* (Asteraceae: Senecioneae). II. Isozyme analysis and population genetic structure

Donna M. Cherniawsky and Randall J. Bayer

Abstract: *Petasites* Mill. (Asteraceae: Senecioneae) is a genus of diploid, perennial, clonal herbs with a widespread distribution across North America. Because of the low variability in floral morphology and high diversity in leaf morphology, considerable taxonomic confusion surrounds the genus. *Petasites* is generally recognized in North America by five ill-defined native taxa and two introduced species. Enzyme electrophoresis was conducted to assess the genetic variation in *Petasites* and obtain an understanding of the evolutionary relationships within the genus. Results show divergence at isozyme loci is not associated with morphological divergence. The mean number of alleles per locus and proportion of polymorphic loci are comparable with plants with similar life-history traits; however, levels of heterozygosity ranged substantially. Although there is a weak, negative association between geographical distance and genetic identity, this is not concordant with morphological differences. Typical of clonal species, genetic identities of *Petasites* are more comparable with selfing plants than with other diploid, outcrossing perennials. *Petasites* also maintains a level of genetic diversity that is similar to other clonal plants and exhibits high levels of differentiation among its populations. This study provides the first contribution to the population genetic structure of *Petasites*. The relatively high values of genetic identities between the different taxa of *Petasites* and the similarity in isozymes and chromosome number indicates a rapid and recent origin in North America. These data, in accordance with the close morphological associations of the four taxa in *Petasites*, suggest the recognition of one species only, *Petasites frigidus*.

Key words: *Petasites*, Asteraceae, North America, clonal, systematics, genetic variation.

Résumé : *Petasites* Mill. (Asteraceae: Senecioneae) est un genre d'herbacées diploïdes et pérennes largement distribuées en Amérique du Nord. La faible variabilité de la morphologie florale et la forte diversité des morphologies foliaires ont entrainé une confusion considérable autour de ce genre. En Amérique du Nord, on reconnait généralement cinq taxons indigènes mal définis de *Petasites* et deux espèces introduites. Les auteurs ont utilisé l'électrophorèse des enzymes pour évaluer la variation génétique chez les *Petasites* et ils ont obtenu une meilleure compréhension des relations évolutives dans ce genre. Les résultats montrent que la divergence aux lieux isozymiques n'est pas associée avec la divergence morphologique. Le nombre moyen d'alléles par lieu et la proportion des lieux polymorphes sont comparables avec des plantes ayant les mêmes caractères des cycles vitaux; cependant, les niveaux d'hétérozygocité varient substantiellement. Bien qu'il y ait une faible association négative entre les distances géographiques et l'identité génétique, ceci ne concorde pas avec les différences morphologiques. Typiques des espèces clonales, les identités génétiques des *Petasites* sont plus comparables aux plantes autofertiles qu'aux autres plantes pérennes diploïdes hétérofertiles. Les *Petasites* maintiennent également un niveau de diversité génétique qui est similaire à d'autres plantes clonales et montrent de hauts niveaux de différenciation dans leurs populations. Cette étude constitue la première contribution sur la structure génétique des populations de *Petasites*. Les valeurs relativement élevées des identités génétiques entre les différents taxons de *Petasites* et la similitude des isozymes et du nombre de chromosomes indiquent une origine rapide et récente en Amérique du Nord. Ces données, en concordance avec les associations morphologiques étroites des quatre taxons de *Petasites*, suggèrent la reconnaissance d'une seule espèce, le *Petasites frigidus*.

Mots clés : *Petasites*, Asteraceae, Amérique du Nord, clonal, systématique, variation génétique.

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Introduction

Petasites Mill. (Asteraceae: Senecioneae) represents a morphologically diverse group of diploid, perennial, clonal herbs that commonly occur in disturbed habitats. Populations tend to exhibit a patchy distribution and range in size from only one or two to several hundred individuals. *Petasites* has a widespread geographical distribution across Canada, Alaska, and the northern contiguous United States. All North American taxa have sympatric ranges in northwestern North America.

The utilization of both asexual and sexual modes of reproduction has contributed to the extensive geographical distribution of *Petasites*. The possession of an extensive, bracteate rhizome promotes the invasion and colonization of various habitats through vegetative spreading and often results in the development of large clones. The capacity for long-distance seed dispersal is a factor that has also facilitated *Petasites* in achieving its widespread occupation in North America. Although its polygamodioecious habit may inhibit sexual reproduction to some degree, this is considered minimal in *Petasites* because all populations observed in the field were comprised of both functionally staminate and functionally pistillate plants.

Petasites is a taxonomically difficult genus as a result of highly conservative floral structures and highly diverse leaf forms. Between several extreme leaf forms are numerous intergrading ones, all of which have been reported to be interfertile (Bogle 1961). It is largely the absence of marked discontinuities between these intergrading leaf forms that has generated confusion surrounding the taxonomic delineation of the genus.

Traditionally, taxonomists have constructed classification systems of *Petasites* on the basis of slight differences in leaf morphology. The precise relationships among the various taxa in *Petasites* have not been explicitly defined; therefore, it has not been clear how much polymorphism in the leaves is inherent within each taxon, and how much of it might be attributed to environmental influence or hybridization.

The uncertainty of the relationships in *Petasites* is reflected in the inconsistences of the various circumscriptions of the species. Rydberg (1927) recognized as many as ten species native to North America, whereas Cronquist (1953) and Bogle (1968) recognized only two: *P. frigidus* with three varieties (*frigidus*, *nivalis*, *palmatus*) and *P. sagittatus*. Additionally, Bogle (1968) recognized one hybrid taxon, *P.* ×*vitifolius* (Greene) Bogle, which he showed to be a diverse assemblage of plants which originated from hybridization between *P. sagittatus* and *P. frigidus* var. *palmatus*. Although *P. sagittatus* tends to exhibit the most cohesive leaf morphology of all the taxa of *Petasites* in North American, its apparent intergradation with other taxa in the genus has raised questions about its taxonomic status as a species. Hooker (1833) and Bogle (1968) speculated that *P. sagittatus* might be conspecific with *P. frigidus*. Another point of contention in *Petasites* has been the status of *P. frigidus* var. *frigidus* and var. *nivalis* (sensu Cronquist (1978) and Bogle (1968)) and the forms intermediate in morphology between these taxa. Some authors, including Hultén, recognized these entities as species. Hultén (1950) and Bogle (1961) have regarded the intermediates as hybrids between the *frigidus* and *nivalis* taxa.

Our working classification for *Petasites* is based on our interpretation of the various authors' keys, descriptions, type specimens, and annotations on herbarium specimens. It most closely follows Bogle (1968) and recognizes six groups. For simplicity, we use specific or subspecific epithets only. Our concept of *frigidus* and *nivalis* is in accordance with Bogle's (1968) for *P. frigidus* var. *frigidus* and var. *nivalis*, respectively. These two taxa and the forms intermediate in morphology between them are collectively referred to as the *frigidus* complex. The intermediate forms are denoted as "intermediates." However, because of the difficulty in discriminating between the various entities of the *frigidus* complex in our field collections, this group is regarded as the *frigidus* complex only. The taxa *palmatus*, *sagittatus*, and *vitifolius* are recognized *sensu* Bogle's *P. frigidus* var. *palmatus*, *P. sagittatus*, and *P.* ×*vitifolius*, respectively. For a more detailed description of each taxon and the different taxonomic treatments, refer to Cherniawsky and Bayer (1998).

It is necessary to understand the phenetic and genetic relationships among the various taxa to resolve the taxonomic problems in North American *Petasites*. Multivariate morphometric analyses were conducted to assess the phenetic relationships in the genus (Cherniawsky and Bayer 1998). Results from the morphometric analyses showed that North American *Petasites* was comprised of four polymorphic entities. Morphological variation was continuous for most reproductive characters, and although there was a high degree of overlap, leaf characters provided the most discrimination. The morphological differentiation alone was not considered sufficient to warrant the recognition of separate species. It was recommended that one polymorphic species of *Petasites* be recognized (*P. frigidus* with three varieties) in addition to one hybrid taxon originating from crosses between two of the varieties.

The primary objective of this study is to elucidate the genetic relationships within *Petasites* through the use of starch gel electrophoresis of enzymes. From the synthesis of this information and results from the morphometric analyses, our ultimate goal is to propose a classification system of *Petasites* that better expresses evolutionary relationships among the taxa. The genetic diversity results also provide information about the genetic structure of populations of *Petasites* as compared with other clonal plants, which in turn, contributes to our understanding of the relationships among the various taxa.

Materials and methods

Population sampling

Prior to population sampling, same gel electrophoresis of rhizomes (fresh and frozen) and leaves (young and mature, fresh and frozen) revealed that tissue types, excluding frozen leaves, showed similar activity and resolution. Since rhizomes were hardier than leaves, they were chosen as the tissue to be used from distant populations. Leaves were used from populations near Edmonton.

Plants were collected from 47 populations in the summers of 1992 and 1993 from Alberta, British Columbia, the Yukon Territory, the Northwest Territories, and Alaska (Table 1; Cherniawsky 1994). Depending on the population size, 10–60 individuals per population were randomly sampled. Because of the rhizomatous habit of *Petasites*, plants were collected a few metres apart to

Table 1. Locality data (latitude and longitude) and sample sizes for 47 populations of *Petasites* used in enzyme electrophoresis.

Taxon	Population designation*	Sample size	Locality
The *frigidus* complex	AK-92026	29	64°30′N, 141°15′W
	AK-92027	30	64°38′N, 141°20′W
	AK-92028	29	64°05′N, 141°50′W
	AK-92029	27	63°25′N, 142°20′W
	AK-92031	38	65°32′N, 145°30′W
	AK-92032	31	68°25′N, 149°25′W
	AK-92033	28	69°06′N, 149°32′W
	AK-92034	33	63°15′N, 145°40′W
	NW-92021	29	67°10′N, 136°00′W
	NW-92022	28	67°12′N, 135°40′W
	YT-92015	24	63°30′N, 136°20′W
	YT-920161	29	63°55′N, 135°18′W
	YT-920162	39	63°56′N, 135°18′W
	YT-92017	23	63°55′N, 135°17′W
	YT-92018	31	64°23′N, 138°21′W
	YT-92019	32	65°05′N, 138°21′W
	YT-92020	28	66°36′N, 136°19′W
	YT-92023	27	63°33′N, 136°20′W
	YT-92024	29	61°19′N, 135°37′W
	YT-92025	36	64°03′N, 140°43′W
	YT-92036	34	62°11′N, 131°48′W
	YT-92037	30	63°15′N, 131°48′W
palmatus	AB-92001	34	55°33′N, 113°28′W
	AB-92002	34	55°33′N, 113°28′W
	AB-92003	34	55°33′N, 113°28′W
	AB-92004	31	55°33′N, 113°28′W
	AB-92008	10	54°00′N, 114°05′W
	BC-92010	19	55°44′N, 120°08′W
	BC-92012	30	57°27′N, 122°53′W
	NW-92021	34	67°10′N, 136°00′W
	NW-92022	29	67°12′N, 135°40′W
	YT-92014	34	60°10′N, 129°47′W
	YT-92023	35	66°33′N, 136°20′W
sagittatus	AB-92005	32	53°00′N, 111°32′W
	AB-92006	30	53°22′N, 112°40′W
	AB-92007	14	54°00′N, 114°05′W
	AB-92009	60	55°33′N, 113°28′W
	AK-92030	35	63°20′N, 143°00′W
	BC-92011	18	57°13′N, 121°42′W
	BC-92013	28	58°50′N, 125°00′W
	AK-92035	26	60°49′N, 137°27′W
vitifolius	AB-93038	35	54°00′N, 113°59′W
	AB-93039	35	53°58′N, 114°06′W
	AB-93040	36	53°25′N, 114°50′W
	AB-93041	36	53°10′N, 114°50′W
	AB-93042	36	53°10′N, 114°30′W
	AB-93043	36	53°10′N, 113°50′W

Note: Population designations consist of province, territory, or state; year; and collection number. Voucher specimens are deposited at ALTA.

*AB, Alberta; AK, Alaska; BC, British Columbia; NW, Northwest Territories; YT, Yukon Territory.

avoid sampling from the same vegetative clone. The rhizomes were packed in moist *Sphagnum* and stored in refrigeration to keep them fresh. Several plants from each population were pressed as voucher specimens and are deposited in ALTA.

Extraction and electrophoresis of enzymes

Standard methods for horizontal starch gel electrophoresis (Soltis et al. 1983) were employed in this study to assess genetic variation in *Petasites*. When the population was large enough, 30 individuals per population were used in the analysis because it has been estimated that there is a 95% probability of detecting all alleles in a population when 30 diploid individuals are examined (Crawford 1990). Small pieces of rhizomes or 1-cm^2 pieces of fresh leaf tissue were ground in ice-cold extraction buffer (Bayer 1991). Two drops of β-mercaptoethanol per 10 mL were added to the buffer prior to use. Supernatant was absorbed onto filter paper wicks and stored at –70°C. Wicks were thawed and then loaded onto 12.5% starch gels.

The resolution of 14 enzymes was accomplished using three different gel/electrode buffer systems. The following system was used to resolve alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), and isocitrate dehydrogenase (IDH): gel buffer of one part 0.038 M lithium hydroxide monohydrate – 0.188 M boric acid (pH 8.2) and nine parts 0.045 M Tris – 0.007 M citric acid (pH 8.4); the electrode buffer consisted of only the lithium borate component (Soltis et al. 1983). Acid phosphatase (ACP), glucose-6-phosphate dehydrogenase (G6PDH), glutamate dehydrogenase (GDH), 6-phosphogluconic dehydrogenase (6-PGD), and diaphorase (DIA) were resolved with a gel buffer of 0.02 M histidine hydrochloride (1 M NaOH to pH 7.0) and an electrode buffer of 0.40 M citric acid trisodium salt, (1 M HCl to pH 7.0) (Soltis et al. 1983). Shikimic acid dehydrogenase (SKD), phosphoglucomutase (PGM), menadione reductase (MNR), malic enzyme (ME), and aldolase (ALDO) were resolved with the following system: electrode buffer of 0.065 M L-histidine (free base) and 0.007 M citric acid monohydrate (pH 6.5) and a gel buffer consisting of a 1:3 ratio of electrode buffer and distilled water (modified from Cardy et al. 1981).

Electrophoresis for the lithium borate system was conducted at 50 mA for the first hour then 60 mA for the following 4 h. The histidine-HCl system was run at 100 mA for the first hour and 70 mA for the next 4 h. The histidine citrate system was run at 40 mA for the first hour and 30 mA for the next 4 h. All gels were run at 4°C, and this temperature was maintained with crushed ice on the gel.

Most of the staining protocols followed Soltis et al. (1983) with the modification of a 0.05 M Tris-HCl buffer. The protocol for ADH followed that for GDH (Soltis et al. 1983) with the addition of 5 mL of ethanol. The agar (2%) overlay method was used for PGI only. Staining protocols for DIA and MNR followed methods 1 and 2, respectively, of Wendel and Weeden (1989), with the omission of NADH from DIA.

Genetic interpretation of isozymes and allozymes was based on the segregation of bands among individuals in the populations sampled, in association with information on the typical subunit structure of enzymes in other plant species (Gottlieb 1981; Kephart 1990), subcellular compartmentalization, and expected number of isozymes which are highly conserved among diploid plants (Gottlieb 1982; Kephart 1990). The locus that migrated the most anodally was designated as 1, the next as 2, and so forth. Similarly, the most anodal allozyme was designated as a, then b, and so on.

Analysis of genetic variation

Genetic variation was evaluated using mean number of alleles per locus (A), including monomorphic loci, proportion of polymorphic loci (P), observed and expected mean number of heterozygotes (H_{obs} and H_{exp}, respectively), and the mean fixation index (F_{T}) per population. A locus was considered to be polymorphic if the most common allele was present at a frequency of 0.99 or less (Gottlieb 1981). Chi-square tests were used to determine whether observed values deviated from those expected under Hardy–Weinberg equilibrium, at 5 and 1% significance levels. The mean fixation index provides an indication of the degree of inbreeding in populations (Hartl 1980). Calculations of all statistics are outlined in Hartl (1980).

Allelic frequencies were determined for all populations of *Petasites*. They were used to calculate standard genetic distances (*D*) and genetic identities (*I*) for all pairwise comparisons of populations for each taxon using the methods of Nei (1972, 1973). These statistics provide an estimate of genetic differentiation within and among populations. Allelic frequencies were also used to calculate gene diversity statistics which are described by Nei (1973) with the following equations:

$$[1] \qquad H_T = H_S + D_{ST}$$

and

$$[2] \qquad G_{ST} = D_{ST}/H_{HT}$$

Total gene diversity, (H_T), is partitioned into diversity within populations (H_S) and among populations (D_{ST}). G_{ST}, the coefficient of gene differentiation, is the ratio of the among population diversity to the total gene diversity. Nei's statistics were calculated using the GENESTAT program, version 2.1 (Whitkus 1988).

A dissimilarity matrix using Lotus 1-2-3, version 3.1 (Lotus Development Corporation 1989), was made from the genetic distance data. This matrix was used to generate an average linkage cluster diagram using SYSTAT, version 5.03 (Wilkinson 1990). Bootstrap values (Felsenstein 1985) were calculated to assess the strength of relationships in the tree. This was accomplished by creating 100 random data sets, running them individually through GENESTAT to create 100 distance matrices, then creating 100 UPGMA (unweighted pair group method) trees through PAUP (Swofford 1991), and finally a 50% majority rule consensus tree.

Results

Eleven enzyme systems assayed are interpreted as being encoded by 22 putative loci. Three enzyme systems, DIA, ALDO, and ME were excluded from the analysis because they were not interpretable. Additionally, as a result of inadequate resolution, MNR-1, PGM-1, and IDH-2 were also omitted. Thus, the 19 isozymes (and their allozymes) used in the analysis include GDH-1 (A), 6PGD-1 (A), 6PGD-2 (A–D), G6PDH-1 (A and B), G6PDH-2 (A–D), ACP-1 (A), ACP-2 (A), LAP-1 (A and B), LAP-2 (A–C), ADH-1 (A–F), ADH-2 (A–F), IDH-1 (A), PGI-1 (A), PGI-2 (A–F), SKD-1 (A–C), SKD-2 (A–C), PGM-2 (A–C), PGM-3 (A and B), and MNR-2 (A). The number of isozymes in *Petasites* is in close agreement with reports for other diploid plants (Gottlieb 1982; Weeden and Wendel 1989; Bayer 1992; Kephart 1990).

The subunit composition of the enzymes used in this study is in agreement with results from Gottlieb (1981), Weeden and Wendel (1989), and Kephart (1990). Monomeric enzymes include ACP, SKD (Weeden and Wendel 1989; Kephart 1990), PGM (Gottlieb 1981; Weeden and Wendel 1989; Kephart 1990; Hashemi and Estali 1992), and LAP (Gottlieb 1981; Kephart 1990). Dimeric enzymes include 6PGD (Gottlieb 1981; Kephart 1990), ADH, PGI (Gottlieb 1981; Weeden and Wendel 1989; Kephart 1990), G6PDH, and IDH (Weeden and Wendel 1989; Kephart 1990). MNR is considered to be a tetramer (Hashemi and Estilai 1992). Weeden and Wendel (1989) and Kephart (1990) consider GDH to be a hexamer.

A table of allelic frequencies for *Petasites* is provided in Cherniawsky (1994). Seven isozymes were monomorphic for identical alleles in all 47 populations: GDH-1, 6PGD-1, ACP-1, ACP-2, IDH-1, PGI-1, and MNR-2. All populations of *vitifolius* were monomorphic for 6PGD-2 D and ADH-1 C. All taxa shared the same high frequency allele at 5 of 12 polymorphic loci (G6PDH-1 A, LAP-1 A, LAP-2 A, PGM-2 B, and PGM-3 A). Two unique alleles were observed in the *frigidus* complex, allele *6Pgd-2*^{a} was present in moderately high frequencies (0.18–0.50) in six populations of the *frigidus* complex, while allele *Adh-2*^{e} was present in very low frequencies (0.02 and 0.03) in only two populations. Several alleles were shared by only two taxa: *Adh-1*^{a} and *Adh-1*^{e}, *sagittatus* and the *frigidus* complex; *Adh-2*^{b}, the *frigidus* complex and *palmatus*; *Adh-2*^{f}, the *frigidus* complex and *vitifolius*; and *Skd-1*^{a}, *vitifolius* and *palmatus*.

Genetic variation in 47 populations of *Petasites* is presented in Table 2. The following values are those for all taxa. The values of *A* range from 1.00 (all loci monomorphic) to 1.94 with a mean of 1.54. The values for the range and mean of *P* are 0.00–0.63 and 0.38, respectively. The observed and expected heterozygosities were not significantly different from Hardy–Weinberg expectations in just over half of the cases as indicated from the chi-square analyses. Values of H_{obs} ranges from 0.00 to 0.29 (mean 0.15), and those of H_{exp} range from 0.00 to 0.26 (mean 0.14). Values for F_T range from –1.00 (indicating complete outcrossing) to 0.66 with a mean of –0.03.

Gene diversity statistics for individual and pooled taxa of *Petasites* are presented in Table 3. PGI-2, G6PDH-2, and 6PGD-2 have the highest average levels of H_T for individual taxa (not shown) and for pooled taxa. The mean value of H_T for all taxa over all loci is 0.33. The lowest level of H_T occurred in *vitifolius* with a value of 0.25, whereas *palmatus* has the highest level of H_T with a value of 0.35.

The average value of G_{ST} for the *frigidus* complex and *vitifolius* is 0.42 and 0.41, respectively. In contrast, the average value of G_{ST} for *palmatus* and *sagittatus* is 0.57 and 0.57, respectively. The value of G_{ST} for all taxa (0.52) follows this trend.

Presented in Table 4 are genetic identities (*I*) and distances (*D*) for all pairwise comparisons of populations within four taxa of *Petasites*. The highest value of *I* (0.96) and lowest value of *D* (0.04) occurs between *palmatus* and *sagittatus*. The lowest value of *I* (0.87) occurs between *vitifolius* and *sagittatus* and highest value of *D* (0.14) occurs between *vitifolius* and *palmatus*.

The mean and range of genetic identities and genetic distances for pairwise comparisons of populations within each taxon of *Petasites* is presented in Table 5. The mean genetic identity values (and genetic distance values) in all taxa range from 0.74 to 0.88 (*D* = 0.32–0.13). The widest range of genetic identities, 0.49–0.98, and genetic distances, 0.02–0.72, occurs within *palmatus*. Conversely, the narrowest range of genetic identities, 0.82–0.99, and distances, 0.005–0.202, occurs within *vitifolius*.

A cluster analysis derived from a matrix of genetic distances from pairwise comparisons of populations of all taxa of *Petasites* is shown in Fig. 1. The only taxon that forms a cluster is *vitifolius,* while populations of the other taxa do not form any distinct clusters but remain as an amorphous group. Low bootstrap values throughout most of the tree indicate that there is very little that can be called hierarchic

Table 2. Genetic variation within *Petasites*.

Population designation	A	P	H_{obs}	H_{exp}	F_{T}
frigidus complex					
AK-92026	1.69±0.92	0.4375	0.1553	0.1618	0.041
AK-92027	1.75±0.75	0.6250	0.2232	0.2395	0.068
AK-92028	1.84±1.14	0.4737	0.1968	0.2141	0.081
AK-92029	1.82±1.15	0.4706	0.2062	0.1822	–0.132
AK-92031	1.94±1.22	0.4444	0.2266	0.2241	–0.011
AK-92032	1.42±0.67	0.3158	0.1506**	0.0980	–0.536
AK-92033	1.53±0.82	0.3684	0.2156**	0.1606	–0.343
AK-92034	1.83±1.12	0.5000	0.2154	0.2034	–0.059
NW-92021	1.41±0.49	0.4118	0.1909**	0.1331	–0.434
NW-92022	1.58±0.88	0.3684	0.1753	0.1392	–0.259
YT-92015	1.68±0.92	0.4211	0.1285	0.1567	0.180
YT-920161	1.38±0.60	0.3125	0.1739**	0.1072	–0.624
YT-920162	1.44±0.49	0.4444	0.2941**	0.1872	–0.571
YT-92017	1.63±0.98	0.4211	0.1416**	0.2612	0.458
YT-92018	1.41±0.60	0.3529	0.2008**	0.1342	–0.496
YT-92019	1.37±0.48	0.3684	0.2354**	0.1614	–0.459
YT-92020	1.59±0.60	0.5294	0.2442**	0.1927	–0.267
YT-92023	1.47±0.85	0.2941	0.1704	0.1341	–0.271
YT-92024	1.68±0.98	0.4737	0.2258	0.1943	–0.163
YT-92025	1.76±1.06	0.4118	0.2500**	0.1852	0.600
YT-92036	1.80±0.91	0.5333	0.1138	0.1425	0.201
YT-92037	1.47±0.62	0.4000	0.0982*	0.1417	0.307
Mean	1.61±0.17	0.4263	0.1924	0.1707	0.122
SD		0.0779	0.0471	0.0409	0.333
palmatus					
AB-92001	1.20±0.40	0.2000	0.0487	0.0378	–0.290
AB-92002	1.38±0.60	0.3125	0.0424**	0.1231	0.655
AB-92003	1.44±0.70	0.3125	0.1247	0.0992	–0.258
AB-92004	1.41±0.97	0.2353	0.0338**	0.0950	0.644
AB-92008	1.27±0.45	0.2727	0.0278**	0.1154	0.759
BC-92010	1.35±0.59	0.2941	0.1111	0.1162	0.082
BC-92012	1.47±0.70	0.3529	0.1382	0.1254	–0.102
NW-92021	1.41±0.60	0.3529	0.1627	0.1326	–0.228
NW-92022	1.58±0.75	0.4211	0.1654	0.1474	–0.122
YT-92014	1.65±0.90	0.3529	0.1434**	0.0834	–0.719
YT-92023	1.71±0.89	0.4706	0.1549	0.1861	0.168
Mean	1.44±0.15	0.3252	0.1048	0.1147	0.054
SD		0.0741	0.0527	0.0359	0.444
sagittatus					
AB-92005	1.33±0.58	0.2778	0.0414**	0.0916	0.548
AB-92006	1.00±0.00	0.0000	0.0000	0.0000	—
AB-92007	1.15±0.36	0.1538	0.0941*	0.0471	–1.000
AB-92009	1.40±0.61	0.3333	0.0134**	0.0561	0.761
AK-92030	1.83±1.17	0.4444	0.1518	0.1838	0.174
BC-92011	1.31±0.58	0.2500	0.1062	0.1106	0.040
BC-92013	1.65±0.90	0.3529	0.1434**	0.0834	0.292
YT-92035	1.72±0.99	0.4444	0.1994	0.1847	–0.079
Mean	1.43±0.27	0.2821	0.0937	0.0947	0.092
SD		0.1403	0.0662	0.0604	0.492

Table 2. (*concluded*).

Population designation	A	P	H_{obs}	H_{exp}	F_T
vitifolius					
AB-93038	1.68±0.92	0.4211	0.0859**	0.1243	0.309
AB-93039	1.59±0.75	0.4211	0.1307	0.1274	−0.026
AB-93040	1.79±1.06	0.4737	0.1793	0.1639	−0.094
AB-93041	1.37±0.58	0.3158	0.1503*	0.1189	−0.265
AB-93042	1.37±0.58	0.3158	0.1043	0.0991	−0.053
AB-93043	1.68±0.80	0.4737	0.1414*	0.1863	0.241
Mean	1.58±0.16	0.4035	0.1320	0.1367	0.019
SD		0.0656	0.0304	0.0294	0.197
Grand mean	1.54±0.20	0.3833	0.1506	0.1433	−0.027
SD		0.0924	0.0637	0.0488	0.395

Note: Variables are mean number of alleles per locus (±SD) (A); proportion of polymorphci loci (P); observed proportion of loci heterozygous (H_{obs}); expected proportion of loci heterozygous (H_{exp}); and mean fixation index (F_T). Population designations are as in Table 1.

*$p < 0.05$.

**$p < 0.01$.

Table 3. Nei's genetic diversity statistics for individual and pooled taxa of *Petasites*.

Locus	Taxon	H_T	H_S	D_{ST}	G_{ST}
6PGD-2	All taxa	0.6644	0.4104	0.2540	0.3823
G6PDH-1		0.3798	0.0161	0.3637	0.9576
G6PDH-2		0.6752	0.1559	0.5193	0.7691
LAP-1		0.4700	0.0808	0.3892	0.8281
LAP-2		0.4549	0.0786	0.3763	0.8272
ADH-1		0.6171	0.2278	0.3893	0.6308
ADH-2		0.4684	0.1142	0.3542	0.7562
SKD-1		0.4492	0.2366	0.2126	0.4733
SKD-2		0.5525	0.3599	0.1926	0.3486
PGI-2		0.7130	0.5701	0.1429	0.2004
PGM-2		0.4604	0.4111	0.0493	0.1071
PGM-3		0.4444	0.3890	0.0554	0.1246
All loci	*frigidus* complex	0.3035	0.1770	0.1265	0.4168
	palmatus	0.3483	0.1486	0.1997	0.5732
	sagittatus	0.3183	0.1381	0.1801	0.5660
	vitifolius	0.2533	0.1486	0.1047	0.4133
	All taxa	0.3342	0.1606	0.1736	0.5196

Note: Included are gene diversity statistics for individual polymorphic loci for pooled taxa, and gene diversity statistics over all loci for individual and pooled taxa. H_T, total gene diversity; H_S, gene diversity within populations; D_{ST}, gene diversity between populations; G_{ST}, coefficient of gene differentiation.

Table 4. Mean genetic identities (above diagonal) and mean genetic distances (below diagonal) for all pairwise comparisons of populations within four taxa of *Petasites*.

Taxon	*frigidus* complex	*palmatus*	*sagittatus*	*vitifolius*
frigidus complex	—	0.9484	0.9202	0.9258
palmatus	0.0530	—	0.9575	0.8836
sagittatus	0.0832	0.0434	—	0.8724
vitifolius	0.0771	0.1238	0.1365	—

structure among the majority of the populations, apart from a few pairs of terminal populations.

Discussion

Patterns of genetic variation

Variation within populations

A well-documented relationship exists between life-history characteristics of plants and their allozyme variability (Brown 1979; Gottlieb 1981; Loveless and Hamrick 1984). Hamrick and Godt (1989) compiled results from many of these studies to summarize the genetic diversity in different plant groups. Allozyme diversity within populations of *Petasites* (A = 1.54, P = 0.38; Table 2) is similar to previous results for dicotyledonous plants (A = 1.44, P = 0.29), long-lived herbaceous perennials (A = 1.44, P = 0.39), the geographic range of a regional species (A = 1.55, P = 0.364), temperate distribution (A = 1.51, P = 0.33), outcrossing-entomophilous breeding system (A = 1.54, P = 0.34), wind dispersal (A = 1.70, P = 0.43) to gravity (A = 1.45, P = 0.30) and gravity-attached (A = 1.64, P = 0.34) dispersal of fruits, and early (A = 1.46, P = 0.30) to mid-successional (A = 1.52, P = 0.34) status (Hamrick and Godt 1989). Studies of outcrossing, clonal, perennial species also exhibit results that closely parallel those of *Petasites* (Rajora 1989; Hughes and Richards 1988).

Mean H_{obs} values for taxa of *Petasites* range markedly (0.09–0.19; Table 2). Although the mean H_{obs} for all taxa in *Petasites* (0.15) is higher than that of *Populus deltoides* Marsh. (0.05; Rajora 1989), it is almost identical to diploid, outcrossing populations of *Taraxacum* (0.16; Hughes and Richards 1988). The mean values of H_{obs} and H_{exp} for each taxon of *Petasites* do not differ significantly from each other, although several populations within all taxa (22 of 47) exhibit significant differences (Table 2). Deviations from Hardy–Weinberg equilibrium expectations can result from inbreeding, selection, mutation, gene flow, and genetic drift in small populations (Schaal 1975).

Table 5. Nei's mean genetic identities and mean genetic distances for four taxa of *Petasites*.

	Genetic identity		Genetic distance	
Taxon	Mean	Range	Mean	Range
frigidus complex	0.83480	(0.6387–0.9888)	0.1841	(0.0113–0.4484)
palmatus	0.7399	(0.4884–0.9760)	0.3187	(0.0243–0.7167)
sagittatus	0.7667	(0.5581–0.9938)	0.2745	(0.0062–0.5833)
vitifolius	0.8774	(0.8171–0.9952)	0.1324	(0.0049–0.2020)

Of the 22 populations of *Petasites* that deviate from Hardy–Weinberg equilibrium, 13 populations exhibit higher levels of heterozygosity than are expected (Table 2). Values for F_T are negative in 26 of 47 populations (Table 2), indicating that outcrossing is occurring. Highly negative values of F_T are observed in four populations of the *frigidus* complex (YT-920161, YT-920162, YT-92025, and AK-92032) and one population of *palmatus* (YT-92014). It is probable that gene flow from nearby populations has influenced the number of heterozygotes in these populations. An excess of heterozygotes has also been postulated to be the result of heterozygote advantage, which confers adaptive superiority (Dobzhansky et al. 1977). This supposes that heterozygotes are superior in fitness because recessive genetic defects are masked in the heterozygous state.

The remaining nine populations of *Petasites* that deviate from Hardy–Weinberg equilibrium have lower levels of heterozygosity than expected, indicating the occurrence of mating among closely related individuals, i.e., functional inbreeding. The positive values of F_T in 45% (21 of 47) of the populations are also presumably the result of inbreeding effects (Hamrick and Godt 1989). It must be noted, however, that three isozymes with the potential for polymorphism were not scored in one of these populations because of poor resolution. With the addition of these data, the level of heterozygosity might have been elevated.

We emphasize that functional inbreeding alone cannot be responsible for deviations from Hardy–Weinberg expectations in *Petasites*. If inbreeding was solely responsible, deviations would be similar across all loci, and, consequently, the result would be a general deficiency in heterozygotes (Jelinski and Cheliak 1992; Silander 1984). This was not observed in *Petasites*. According to Schaal (1975), heterogeneous environments commonly result in the subdivision of a population. This is largely due to the restriction of movement of pollen or seeds, resulting in inbreeding or the mating of closely related individuals. Schaal (1975) and Silander (1984) maintain that selection may be different in these subdivisions; therefore, heterogeneity of allelic frequencies may reflect heterogeneity in the environment.

Therefore, in addition to inbreeding, it is probable that deviations from Hardy–Weinberg expectations, in *Petasites*, are the result of differential selection, in various subpopulations, operating on different loci which are linked to isozymes.

Diversity among populations

The average amount of genetic diversity (H_T) maintained among all populations of *Petasites* (0.33; Table 3) is comparable to other herbaceous long-lived perennials (0.35) in addition to species with a widespread geographic range (0.35), and an early to mid-successional status (0.33 and 0.29, respectively) (Hamrick and Godt 1989). In accordance with previous results, H_T in selfing species (0.33; Hamrick and Godt 1989) is identical to that for *Petasites* (0.33).

The low values of H_T for *vitifolius* could be the product of examining too few populations from only a small segment of its geographic range. However, it is expected that a hybrid would possess a lower level of genetic diversity than is found in each of the parental species (Crawford 1990).

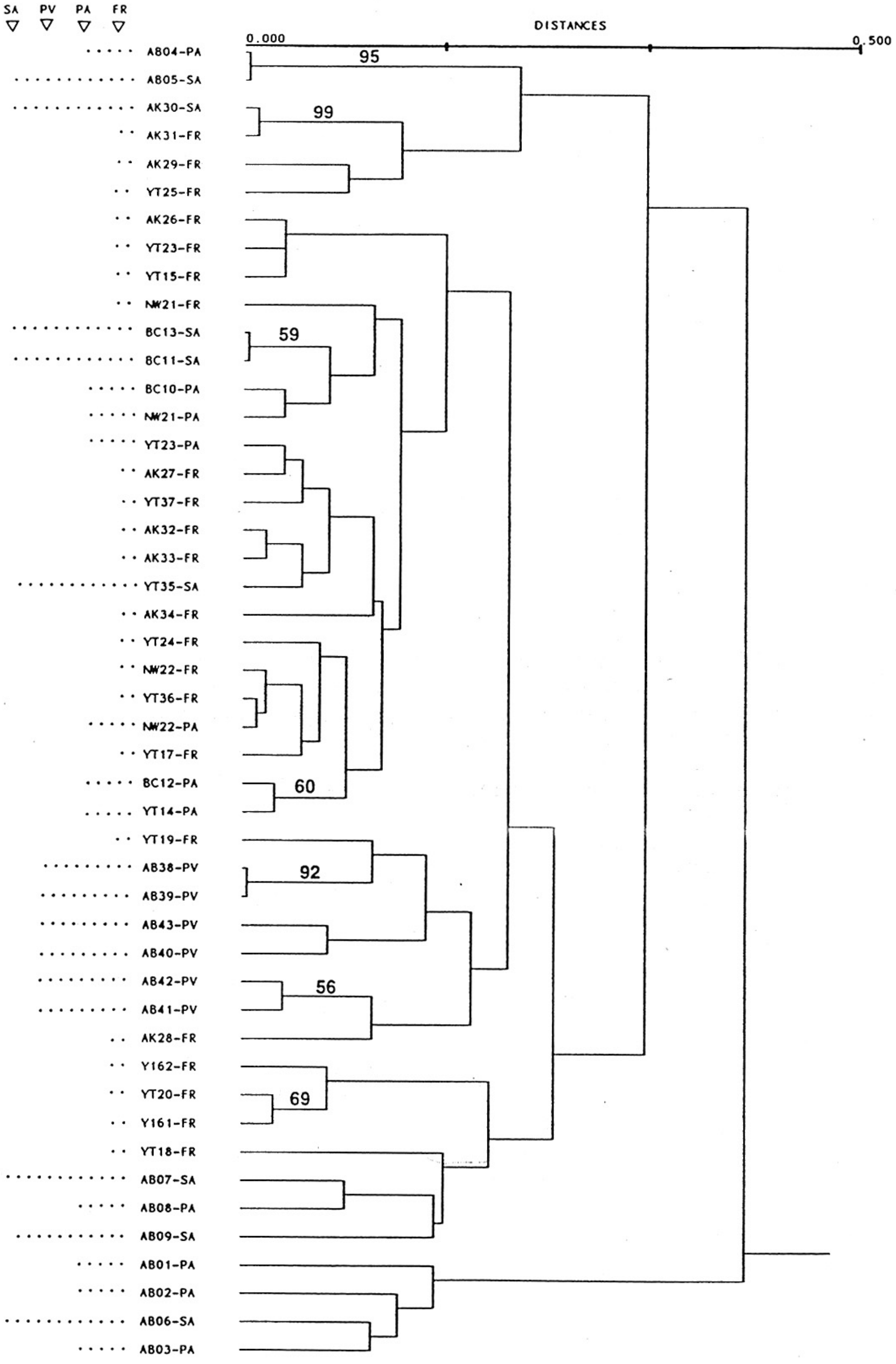
Perhaps, it is the clonal nature of *Petasites* that is the most salient feature that influences the amount of genetic diversity that its populations can maintain. Pleasants and Wendel (1989) discuss the advantages that asexual species have over sexual species in the preservation of genetic diversity in small populations. In sexual species, alleles can be lost by chance during sexual reproduction and by selection against individuals homozygous for rare alleles in the presence of inbreeding. Conversely, perennial asexual species may be less disposed to the loss of genetic variation because their capacity to live to an older age provides a greater time in which to pass on alleles (Pleasants and Wendel 1989).

However, the benefits of asexual reproduction are most fully exploited in combination with at least some degree of sexual reproduction. Many predominately asexual species alternatively reproduce sexually, thereby providing a continuous source of genetic variation (Pleasants and Wendel 1989). *Petasites* maintains a level of genetic diversity that is just below that for other clonal species (Cheliak and Dancik 1982; Silander 1984; Pleasants and Wendel 1989).

Several authors (Dobzhansky et al. 1977; Burdon 1980; Silander 1984) maintain that the high genetic diversity in clonal plants is the result of diversifying selection. Burdon (1980) suggests that many factors in an environment with high microsite heterogeneity, including competiton, exert strong disruptive selective forces on populations and thereby propel individuals in different evolutionary directions. It is most realistic to postulate that the high variability in *Petasites* is the result of differential selection in various subpopulations, in addition to inbreeding, genetic drift, and small amounts of gene flow.

The average value of G_{ST} (0.52) in *Petasites* (Table 3) indicates that 52% of genetic diversity resides among as opposed to within populations. The average G_{ST} value for *Petasites* is higher than most values in Hamrick and Godt (1989) for other outcrossing, long-lived herbaceous perennials (0.21). In the category of breeding system, the G_{ST} value for selfing species (0.51) closely parallels that of *Petasites* (Hamrick and Godt 1989). This high G_{ST} value is expected as it is characteristic of inbreeding populations to

Fig. 1. Average linkage tree diagram derived from Nei's genetic distances of all pairwise comparisons of 47 populations of *Petasites*. Bootstrap values above 50% are indicated above the branches. Populations consist of province, territory, or state; collection number; and taxon abbreviation. FR, *frigidus* complex; PA, *palmatus*; PV, *vitifolius*; SA, *sagittatus*.



partition most of their genetic diversity among populations (Huber and Leuchtmann 1992; Crawford 1990; Roberts 1983).

Selfing species typically have greater differences among populations because the frequencies of alleles at various loci vary considerably. Alleles in low frequency in some populations may occur in high frequency or become fixed in other populations (Gottlieb 1981). Allelic frequencies for each population *Petasites* show that this is indeed the case (Cherniawsky 1994). This, in combination with a lack of gene flow between geographically distant populations, provides a further contribution to the overall high proportion of genetic diversity among populations of *Petasites*. The fixation of alleles in *Petasites* may also be the result of the colonization of populations from a few founder individuals that originated from a larger population. Colonizing individuals have only a limited proportion of the total genetic variation that was present in the source population, promoting the occurrence of genetic drift, and, consequently, the fixation of different alleles in different populations.

The spatial structure of populations is also believed to play a role in the degree of differentiation exhibited between populations. According to Loveless and Hamrick (1984) and Heywood and Levin (1984), divergence is enhanced between populations with a linear distribution. Additionally, a patchy distribution affects pollinator behaviour (Loveless and Hamrick 1984). Movement may be restricted within a patch, and gaps between patches may serve as barriers to gene flow, thereby enhancing differentiation. Since the majority of populations of *Petasites* we collected were distributed linearly along roadsides or in small patches, the effects of these spatial structures may have contributed to the high differentiation that is observed between populations.

Populations of the *frigidus* complex and *vitifolius* exhibit values of G_{ST} that are lower than those of the other taxa (Table 3). This indicates greater genetic diversity within populations as opposed to among populations, which is more typical of outcrossing populations (Hamrick and Godt 1989; Crawford 1990). There may have been greater gene flow between some of these populations because of seed and pollen movement. The many neighboring populations of these two taxa lend support to this possibility. Additional support for the occurrence of outcrossing is derived from the negative values of F_T in the majority of the populations of these taxa (Table 2).

The average value of H_S over all taxa of *Petasites* is 0.16. This is substantially lower than values of H_S in other long-lived, herbaceous perennials (0.28; Hamrick and Godt 1989) and is the consequence of high G_{ST} values (Table 3).

Genetic identities and genetic distances

Genetic identities (*I*) for conspecific populations approximate 0.95 (Gottlieb 1977). Subspecific taxa usually exhibit high genetic identities similar to those for conspecific plant populations (above 0.90; Crawford 1990). However, an *I* value as low as 0.71 has been shown to occur in infraspecific taxa of the diploid shrub *Solanum* (Whalen 1979), which is more characteristic of the average level of similarity that has been estimated for congeneric plant populations (*I* = 0.67; Gottlieb 1981). Furthermore, an *I* value of 0.75 has been observed between interfertile varieties of *Coreopsis cyclocarpa* Blake (Asteraceae), a diploid perennial (Crawford and Bayer 1981). The *I* values for pairwise comparisons between the various taxa of *Petasites* fall between those typical for subspecific and specific taxa (Table 4).

The highest *I* value between taxa of *Petasites* occurred between *palmatus* and *sagittatus* (*I* = 0.96) and the lowest occurred between *vitifolius* and *sagittatus* (*I* = 0.87) and *vitifolius* and *palmatus* (*I* = 0.88). Although it was unexpected to find lower genetic identities between the hybrid (*vitifolius*) and its parents than between *vitifolius* and the *frigidus* complex (*I* = 0.93), this *I* value is comparable with other studies of hybrids (Gallez and Gottlieb 1982).

Although electrophoretic results do not lend support for a hybrid origin for *vitifolius*, i.e., additivity of alleles from both parents (Gallez and Gottlieb 1982; Crawford 1990), its origin has been verified through crossing studies performed by Bogle (1961). It should be noted, however, that additivity arises only when the prerequisite of mutually exclusive alleles is met by the parental species, and it only occurs in the F_1 generation (Gallez and Gottlieb 1982; Crawford 1990). The results for the parental taxa of *Petasites* and their hybrid derivative are most probably due to the number of populations (6) electrophoretically assayed in *vitifolius*, in addition to only 8 and 11 populations for *palmatus* and *sagittatus*, respectively. The monomorphism of two additional alleles (*6Pgd-2*^{d} and *Adh-1*^{c}) in all populations of *vitifolius* undoubtedly contributed to the lower *I* values between this hybrid and its parents than are normally found in outcrossers. Furthermore, the sharing of the low frequency allele, *Adh-2*^{f}, with only the *frigidus* complex and *vitifolius* increased the similarity between them (*I* = 0.93; Table 4).

The mean and range of genetic identity values within each taxon of *Petasites* (Table 5) is lower and wider, respectively, than is generally found in other diploid, outcrossing perennials (Crawford and Smith 1984). *I* values between populations of *Petasites* appear to be more comparable with selfing plants than outcrossing plants, whereby the former usually exhibit higher levels of genetic variation (Crawford 1990). This was shown by McNeill and Jain (1983) who worked with four species of *Limnanthes* (Limnanthaceae) with infraspecific taxa. The breeding system of these diploid annuals ranged from autogamous to almost completely outcrossing. *Limnanthes floccosa* T.J. Howell proved to be especially interesting. This species is comprised of five subspecies that have fully autogamous to partially outcrossing breeding systems. It was found that the most autogamous subspecies, *L. floccosa* ssp. *floccosa*, had greater interpopulational differentiation than the more outcrossing taxa. The mean *I* value between pairwise comparisons of *L. floccosa* subspecies was 0.74 with a range from 0.58 to 0.99 (McNeill and Jain 1983).

There is a low correlation between *I* and geographical distance in *Petasites*. This is evident in populations of *palmatus*, which possesses the widest range of genetic identities (Table 5). Inspection of the genetic variation for pairwise comparisons of populations of this taxon (Cherniawsky 1994) reveals that the lowest *I* value (0.49) and corresponding highest *D* value (0.72) occurs between two geographically distant populations, one from Alberta (AB-01PA) and one from the Yukon (YT-14PA). Figure 1 portrays the largest genetic distance, and divergence, between these two populations. Such a high genetic divergence may reflect a lack of gene flow resulting from the geographical isolation between these two populations. The highest value of *I* (0.98) and the lowest value of *D* (0.03) in *palmatus* occurs between a population in British Columbia (BC-12PA) and one in the Yukon (YT-14PA) that are in relatively close proximity. The high genetic similarity, and shortest genetic distance (Fig. 1), between these two populations may be the result of gene flow.

The narrow range of I (0.822–0.995) and D (0.005–0.202) within *vitifolius* may reflect the geographical proximity of the populations. The isozymic similarity may be the homogenizing effect of gene flow from the movement of pollen and seeds. However, this may also be the product of examining only six populations of *vitifolius*, a small sample of the total variation that may actually be present in this hybrid entity.

Correlations between geographical distance and values of I and D are not always consistent, however. Two neighboring populations of *palmatus* from Alberta, AB-03PA and AB-04PA, exhibit an I value of 0.72 and a D value of 0.33 (Cherniawsky 1994). These populations also form the most distant clusters in the phenogram (Fig. 1). Similar observations were made in a study of *Bidens discoidea* (Torr. & Gray) Britt. where two populations from Connecticut had a low I value of 0.69 and two populations from Virginia and North Carolina had a high I value of 1.00 (Roberts 1983).

Furthermore, some populations of different taxa of *Petasites* that are geographically close are isozymically more similar than are some populations of the same taxon that are geographically more distant (Fig. 1 and Cherniawsky 1994). The I value between two populations of different taxa from British Columbia (BC-10PA and BC-13SA) is 0.91 (D = 0.04), whereas it is 0.56 (D = 0.58) between two populations of the same taxon, one from Alberta and the other from Alaska (AB-09SA and AK-30SA). Gene flow cannot be used to explain the high genetic identity between the two populations of *palmatus* and *sagittatus*. If the genetic similarity between them was representative of gene flow, some sort of morphological intermediacy would be expected, none of which is evident in these morphologically discrete taxa. Genetic drift may be a factor in the small population of *palmatus* (BC-10PA). Similar results were found between two species of *Aletes* (Apiaceae) (Linhart and Premoli 1993).

The only strong relationship that exists between isozyme variation and taxonomic boundaries occurs with the six populations of *vitifolius*. This is evident as a cohesive cluster in the genetic distance phenogram (Fig. 1). Reasons for this relationship were previously discussed. The results from this study indicate that morphological divergence in *Petasites* is not usually associated with divergence at isozyme loci (Fig. 1). Although there is some association between geographical proximity and genetic similarity, it is not correlated with morphological differentiation. Similar results were found by Crawford and Ornduff (1989) who examined genetic variation among three diploid, annual species of *Lasthenia* (Asteraceae) from California. No distinctions among separate entities in the *frigidus* complex have been made and no clusters of *sagittatus* exist exclusively from the other taxa. Except for *vitifolius*, the taxa are isozymically recognized as one group.

Gottlieb (1984) has indicated that only one or a few genes can control some morphological differences. This makes it possible for speciation to occur without isozymic divergence. Conversely, isozymic divergence can occur without speciation (Crawford 1985; Gottlieb 1981). Rapid speciation events, as in quantum speciation (Crawford 1990), may lead to rapid differentiation in morphology, although it usually takes a longer period of time to accumulate allelic differences at isozyme loci. This could be the case for *Petasites*. A slower, more gradual process of speciation, such as geographical speciation, would more likely result in changes at genes coding for isozymes, in addition to changes in morphology (Crawford 1990).

Not only the kind of speciation that has occurred, but the amount of time since the speciation event, influences the amount of diversity that is present in a new species. The three genera comprising the Hawaiian silversword alliance (Asteraceae) provide a classic example of adaptive radiation in an insular environment where species arose from a rapid and recent speciation event (Witter and Carr 1988). Although the species are extremely divergent in morphology, they are similar with respect to isozymes because there has not been sufficient time for the accumulation of genetic variation. Similar results occurred with Hawaiian *Tetramolopium* (Asteraceae) (Lowrey and Crawford 1985), continental genera of *Heuchera* (Saxifragaceae) (Soltis 1985), *Antennaria* (Asteraceae) (Bayer 1988), and presumably *Petasites*.

Taxonomic relationships

The similarity in isozymes, high frequency allozymes, and high genetic identities, in conjunction with the close association of morphological characters (Cherniawsky and Bayer 1998), the absence of reproductive barriers, and the similarity in chromosome number (Cherniawsky 1994; Morton 1981; Löve 1975) among all entities of *Petasites*, suggests a very close relationship and a rapid and recent origin in North America. The absence of correlations between divergence at isozyme loci and morphological features suggests there was insufficient time for the diverging taxa to accumulate mutations. The genetic similarity may be indicative of a previous, more continuous distribution of populations prior to the evolution of separate taxa. The populations that are most divergent do not have a taxonomic basis but have likely been influenced by various factors relating to the population genetic structure of *Petasites*. The clonal nature and breeding system of *Petasites* have been the most influential factors. The strong partitioning of genetic variation among populations in this outcrossing species results from the mating of close relatives because of restricted movement of pollen and seeds. Populations such as these, which have also been geographically separated for longer periods of time than other populations, exhibit the greatest differentiation.

The morphometric analyses showed that leaf characters provided the best taxonomic discrimination in North American *Petasites* with the separation of four ill-defined entities: the *frigidus* complex, *palmatus*, *sagittatus*, and *vitifolius* (Cherniawsky and Bayer 1998). Only *vitifolius*, which is not strongly differentiated by leaf characters, forms a separate cluster based on genetic distances (Fig. 1). This supports its recognition as a separate hybrid taxon. The high similarity between *vitifolius* and the *frigidus* complex lends support for a close relationship among all taxa of *Petasites*. The three entities comprising the *frigidus* complex are not considered to be sufficiently differentiated, morphologically or isozymically, to be recognized as separate taxa. As a group, the *frigidus* complex does maintain a certain degree of morphological integrity in leaf form; therefore, the entities in this group should be united under one taxon. Although the *frigidus* complex was the only group to possess two unique alleles, this created separation from the other taxa, not separation within the *frigidus* complex itself. Because of the absence of leaf lobes and sinuses, *sagittatus* is the most morphologically distinct taxon. However, similarities with the other taxa, with respect to floral morphology and isozymes, do not warrant taxonomic ranking which differs from them. Similar conclusions have been drawn for *palmatus*, the taxon with the greatest interpopulational isozymic differences, and the only one, which may possess orbicular shaped leaves.

In agreement with the morphometric analyses (Cherniawsky and Bayer 1998), we suggest the recognition of one species of *Petasites* in North America with infraspecific taxa and one hybrid taxon: *P. frigidus* var. *frigidus*, *P. frigidus* var. *palmatus*, "*P. frigidus* var. *sagittatus*," and "*P. frigidus* var. ×*vitifolius*" in ed. It is believed that the taxa of *Petasites* continue to differentiate rapidly. Environmental factors, such as light (Andersson 1989; Tetramura 1983), temperature (Cutter 1971), and nutrient and soil conditions (Gossler et al. 1994; Andersson 1989), acting differently on different genotypes may be responsible for the high degree of leaf polymorphism that characterizes *Petasites*.

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