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GENETIC VARIATION IN *ACIPHYLLA GLAUCESCENS* (APIACEAE)

Summary: Population structure, diversity and gene flow in four populations of *Aciphylla glaucescens* were studied using allozymes. Six of the seven putative loci were polymorphic in at least one population. Within populations the mean percentage of polymorphic loci was 68%. Gene diversity for *Aciphylla glaucescens* ($H_e = 0.258$) was greater at the species level compared with other outcrossing, wind pollinated plant species ($H_e = 0.162$). The mean diversity among populations of *A. glaucescens* ($F_{ST} = 0.256$) was also greater than that reported for most other species with similar breeding systems. Estimated gene flow (N_m) between populations was low, with only 0.72 migrants per generation. The high gene diversity of *A. glaucescens* may be due to the widespread distribution, obligate outcrossing and high fecundity of this species.

Keywords: *Aciphylla glaucescens*; allozymes; dioecious; gene diversity; gene flow; New Zealand; population genetics.

Introduction

New Zealand genera often contain many species and a high degree of intraspecific variation is common (Fenner, Lee and Wilson, 1997). This suggests that much of the speciation in the New Zealand flora is relatively recent and makes studies of the structure of genetic variation within these plants particularly relevant to an understanding of evolutionary processes.

The genus *Aciphylla* (Apiaceae) contains about 42 closely related species (Mitchell, Wagstaff and Webb, 1998; Webb, 1986; Dawson, 1971). *Aciphylla* species have shown adaptive radiation into new niches since mountain development and provide ideal subjects for the study of evolution in a recently evolved, rapidly speciating group. Species of *Aciphylla* are long-lived, dioecious, primarily wind and insect-pollinated, with many reduced compound umbels collected into narrow inflorescences (Webb, 1986, Dawson, 1971). Most *Aciphylla* species show considerable intraspecific variation and environmental effects can modify the phenotype markedly (Dawson and Le Comte, 1978).

While sex expression is affected by environmental conditions in some plant species, for many species sex is determined by genetic mechanisms (Dellaporta and Calderon-Urrea, 1993; Durand and Durand, 1990; Grant *et al.*, 1994). In order to understand the selective factors acting on the evolution of sex in plants, it would be useful to have a sex specific genetic marker, such as allozymes (Schnabel and Hamrick, 1990). This

would allow the study of ecophysiology, growth and survival of plants of known sex in the pre-reproductive stages, as well as the determination of population sex ratios including plants not in flower (*cf.* Godley, 1964). The greater investment in reproduction by females compared to males generally leads to lower survival and growth rates in females (Delph, 1998), and some species show physiological differences between the sexes that are ecologically important (Dawson and Bliss, 1989; Dawson and Ehleringer, 1993)

The pattern and rate of evolution is dependent in part on the existence of genetic variation and its distribution within and among populations (Hamrick *et al.*, 1991). To establish the basis for further studies of the genetic and environmental basis of intraspecific variation in this group, we present here a study of allozyme variation within and among populations of *Aciphylla glaucescens* W.R.B. Oliver, and between the sexes in a single population of this species. *A. glaucescens* is a widespread, montane to sub-alpine species that occurs in areas of moderate rainfall throughout the South Island of New Zealand, from sea level to about 800 m. This species is especially abundant in north-west Nelson. The genetic structure of populations has not previously been studied for *Aciphylla* species.

Several aspects of *A. glaucescens* biology could influence the distribution of genetic variation within and among populations of this species. These include 1) limited seed dispersal, which may reduce gene flow and tend to increase the amount of variation among populations relative to that found

within populations (Loveless and Hamrick, 1984); and 2) dioecism, which may lead to a lower gene diversity compared with hermaphroditic and monoecious species (Loveless and Hamrick, 1984). Land use changes following Polynesian and European colonisation may have also led to species range changes after land clearing, which could result in changes in the diversity within and among populations (Baker and Stebbins, 1965; McGlone and Moar, 1998).

Questions asked in this study were: (1) What is the gene diversity for *A. glaucescens* and what proportion of this diversity is found within compared with among populations? (2) What is the extent of gene flow among populations of *A. glaucescens*? (3) Is there a relationship between allozyme phenotype and sex among *A. glaucescens* individuals? (4) What factors have had the most impact on the gene diversity found in *A. glaucescens*?

Materials and Methods

Collection, extraction and storage

Leaf samples were collected from between 48 and 63 individuals from each of 4 populations of *A. glaucescens* (Table 1, Fig. 1). Population sizes were approximately 800-1000 plants at Black Birch, 300-500 plants at Nelson and Tiwai, and 100 plants at Kingston. Samples were kept cool and moist for a maximum of 2 hours until it was possible to snap freeze them in liquid nitrogen for the return trip to the laboratory. Samples were then transferred to a -80° freezer for a period of up to 1 month before extraction. Leaf material was removed from storage, and placed into a mortar for maceration. An equal volume of Laushman Crushing Buffer (25 ml pH 7.3, 0.1 M Tris-HCl, plus 0.05 g L-ascorbic acid, 0.07 g diethyldithiocarbamic acid, 0.025 g bovine serum albumin, 1 g Sucrose, 1 g PVP-40) was added to the leaf tissue, which was then macerated with ground glass at $4-10^{\circ}$. The resulting homogenate was centrifuged at 12000 X G for 3 min. Sample wicks were soaked in the supernatant and arranged in micro-titre plates for storage at -80° .

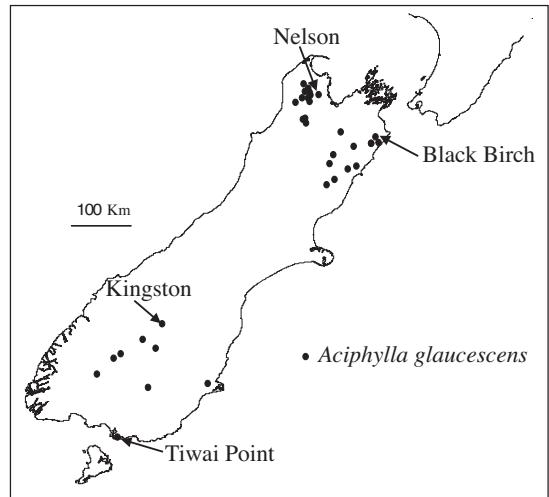


Figure 1. Map of locations for *Aciphylla glaucescens* specimens held by the Landcare Research Herbarium, (CHR). The four populations sampled for this study are shown.

Electrophoresis

Horizontal starch gel electrophoresis (HSE), carried out at $4-10^{\circ}$, was used to screen individuals from each of the *A. glaucescens* populations (Table 1). System 7 electrode buffer (3.2 g lithium hydroxide, 23.2 g boric acid brought up to 2 litres and adjusted to pH 8.3) and gel buffer (10.9 g Tris, 2.56 g Citric acid, brought up to 1.75 litres, plus 200 ml System 7 electrode buffer, adjusted to pH 8.3) were used (Selander *et al.*, 1971). Eleven-percent electrophoresis starch gels were prepared the day before use. Conditions for electrophoresis are given in Table 1. After the proteins had moved from the sample wicks into the starch, as indicated by a bromophenol blue dye marker, electrophoresis was interrupted, the wicks removed, and the gel pushed firmly back together. At the end of electrophoresis each gel was sliced horizontally and the gel slices stained with up to 40 enzyme systems in a preliminary search for those that provided scorable

Table 1: Origin of *A. glaucescens* populations used for allozyme study.

Location of population	Number of individuals sampled	Map reference (Info. Map Series)	Elevation	Herbarium (CHR) voucher specimen
Black Birch	63	P29/784393	750 m	CHR491136
Nelson	48	M27/775085	850 m	CHR492018
Kingston	48	F42/734298	300 m	CHR492019
Tiwai Point	48	E47/595927	5 m	CHR467984

allozymes. The individual enzymes selected for use are described in Table 2.

Data analysis

BIOSYS-1.7 (Swofford and Selander, 1989) was used to analyse data. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. The following genetic parameters were estimated: mean number of alleles per locus (A_p), percent polymorphic loci (P), and expected panmictic heterozygosity or gene diversity, (H_e) (unbiased estimate according to Nei 1978). Wright's (1965) F -statistics (F_{IT} , F_{IS} and F_{ST}) were calculated over all populations of *A. glaucescens* and for each pair of populations over all polymorphic loci. From this an indirect measure of gene flow (N_m), assuming an island model of population structure, was derived from the relationship of neutral alleles: $N_m = ((1/F_{ST}) - 1)/4$ (Wright, 1951). Information on the sex of individuals sampled from the Black Birch population allowed analysis of relative contributions of the separate sexes to the genetic structure of this population. Genotype

(unique combinations of alleles) frequencies were calculated for male and female plants for the Black Birch population. A chi-square test was applied to investigate whether the frequency of homozygotes vs heterozygotes was the same between the two sexes. Male and female plants from the Black Birch population were mapped to determine if individuals were clustered together according to genotype.

Results

Fifteen alleles were scored across a total of 7 putative loci, with an average of 2.14 alleles at each locus. The most variable locus was *Acoh-1*, with 4 alleles, but the *Acoh-1* A and D alleles were only present in the Black Birch and Nelson populations. Six of the 7 putative loci were polymorphic in at least one of the populations sampled. *Est-1* was monomorphic for all populations sampled. *Est-2* and *Pgi-1* were monomorphic within the Tiwai population, *Pgi-1* within the Kingston population, and *6Pgdh-1* within the Nelson population. Genotype frequency was calculated for each population (Table 3), which

Table 2: Enzymes, loci studied, enzyme commission number (E.C. No.) and appropriate staining systems used in this study. All staining solutions were made up in 100 ml distilled water. Where indicated, staining solutions include 20 mg NADP⁺, 4 mg PMS, and 20 mg MTT. Recipes for stain solutions are based on Vallejos (1983). All gels were incubated at 30EC in the dark for 5-60 min.

Enzymes	Loci Studied	E.C. No	Conditions	Staining solution
Esterase	<i>Est-1, Est-2</i>	3.1.1.1	100V, 60mA 5 hr	0.1M Na phosphate, pH 6.2; 3 ml 1% α -naphthyl butyrate in acetone; 100 mg Fast Blue RR Salt.
Acid phosphatase	<i>Acp-1</i>	3.1.3.2	100V, 60mA 5 hr	50 mM Na acetate, pH 5.5; 1ml 1M, MgCl ₂ .6H ₂ O; 3 ml 0.5 % Na α -naphthyl phosphate in 50% acetone; 50mg Fast Garnet GBC Salt.
Aspartate aminotransferase	<i>Aat-1</i>	2.6.1.1	133V, 50mA 7 hr	<u>Solution A</u> ; 0.2 M Tris, pH 8.0; 200 mg α -ketoglutarate; 600 mg aspartic acid. <u>Solution B</u> ; 20 mg pyridoxal-5-phosphate; 150 mg Fast Blue BB Salt. Mix solution A at least 15 min. before adding to solution B.
Glucose-6-phosphate isomerase	<i>Gpi-1</i>	5.3.1.9	100V, 60mA 5 hr	0.1 M Tris, pH 7.5; 1ml, 0.1M MgCl ₂ .6H ₂ O; 80 mg fructose-6-phosphate (Na ₂); NADP ⁺ ; MTT; PMS; 20 units glucose-6-phosphate dehydrogenase.
Phosphogluconate dehydrogenase	<i>6Pgdh-1</i>	1.1.1.44	120V, 70mA 5 hr	0.1 M Tris, pH 8.0; 1 ml, 0.1M, MgCl ₂ .6H ₂ O; 50 mg 6-phosphogluconic acid; NADP ⁺ ; MTT; PMS
Aconitase Hydratase	<i>Acoh-1</i>	4.2.1.3	120V, 70mA 5 hr	0.1 M Tris, pH 8.0; 1 ml, 1M MgCl ₂ .6H ₂ O; 50 mg cis-aconitic acid; 20 units isocitric dehydrogenase; 20 mg NADP ⁺ ; MTT; PMS

Table 3: Genetic variability at 7 loci in all populations (standard errors in parentheses). The differences between direct count and expected heterozygosities were not significant.

Population	Mean heterozygosity				
	Mean number of plants per genotype	Mean number of alleles per locus (Ap)	Percentage of loci polymorphic (P)*	Direct count expected (He)	Hardy-Weinberg
Black Birch	1.24	2.10 (-0.30)	85.70	0.43 (-0.09)	0.40 (-0.07)
Tiwai	3.00	1.90 (-0.40)	42.90	0.11 (-0.05)	0.18 (-0.09)
Nelson	2.67	1.70 (-0.20)	71.40	0.31 (-0.14)	0.21 (-0.08)
Kingston	3.69	1.70 (-0.20)	71.40	0.23 (-0.14)	0.24 (-0.08)

revealed the Black Birch population to be the most heterogeneous, and the Kingston the most homogeneous. Within populations the mean percentage of polymorphic loci was 68%, ranging from 43% to 86% (Table 3). Mean heterozygosity for *A. glaucescens* (0.262), did not significantly differ from the mean expected heterozygosity (H_e) (0.258). Similarly, direct count heterozygosities for *A. glaucescens* populations did not significantly differ from the expected values (Table 3).

Analysis of fixation statistics (Table 4) at all polymorphic loci showed F_{IS} to differ widely among loci, ranging from -0.271 (*Aat-1*) to 0.402 (*Aco-1*), with a mean of -0.029. Similarly, F_{IT} values ranged from -0.179 (*Aat-1*), to 0.570 (*Aco-1*), with an average of 0.234 and F_{ST} values ranged from 0.073 (*Aat-1*), to 0.424 (*6Pgdh-1*). Estimated gene flow (N_m) between populations was 0.720 migrants per generation (Table 4).

Table 4: The fixation indexes of individuals relative to the total population (F_{IT}) and its subpopulations (F_{IS}). F_{ST} measures the amount of differentiation among subpopulations, relative to a hypothetical population in which all loci are fixed within subpopulations and the overall average allele frequency is the same as for the real population.

Locus	F_{IS}	F_{IT}	F_{ST}
<i>Est-2</i>	-0.113	0.162	0.247
<i>Aat-1</i>	-0.271	-0.179	0.073
<i>Pgi-1</i>	-0.189	0.178	0.309
<i>Aco-1</i>	0.402	0.570	0.280
<i>6Pgdh-1</i>	0.070	0.464	0.424
<i>Acp-1</i>	-0.106	0.079	0.167
Mean	-0.029	0.234	0.256
N_m			0.720

Analysis of the genotypes of the sexes in the Black Birch population revealed no genetic markers for sex. Allelic profiles for females and males at the Black Birch population of *A. glaucescens* are presented in Table 5. However, the frequency of

Table 5: Genotype frequencies for male and female *Aciphylla glaucescens* individuals from the Black Birch population. The observed frequencies were compared to those expected under Hardy-Weinberg equilibrium using a P^2 test.

Locus	Alleles	Observed frequency		Significance
		Female n=23	Male n=40	
<i>Est-2</i>	A-A	2	9	$P = 0.09$
	A-B	20	27	
	B-B	1	4	
<i>Aat-1</i>	A-A	9	17	$P = 0.79$
	A-B	14	23	
	B-B	0	0	
<i>Pgi-1</i>	A-A	13	10	$P = 0.21$
	A-B	10	24	
	B-B	0	6	
<i>Aco-1</i>	A-A	0	1	$P = 0.01$
	A-B	0	0	
	A-C	0	0	
	A-D	1	0	
	B-B	6	25	
	B-C	4	8	
	B-D	4	3	
	C-C	6	0	
	C-D	1	2	
D-D	1	1		
<i>6Pgdh-1</i>	A-A	3	0	$P = 0.55$
	A-B	11	16	
	B-B	9	24	
<i>Acp-1</i>	A-A	6	16	$P = 0.07$
	A-B	12	11	
	B-B	5	13	

homozygotes vs heterozygotes was significantly different ($P=0.01$) between the two sexes at the *Aco-1* locus (Table 5), and close to significant at the *Acp-1* locus ($P=0.07$). Multilocus genotypes were apparently not clustered in space in the Black Birch population (Fig. 2).

Discussion

For comparative purposes it is interesting to consult information provided in the review of allozyme diversity in plants by Hamrick *et al.*, (1991), but a difficulty arises when comparing diversity measures between studies as they may have made use of different tissues and sampling strategies. We can, however, make the assumption of constant experimental design and only make comparisons between species of similar breeding system.

A. glaucescens maintained high levels of gene diversity ($H_e = 0.258$) at the species level compared with outcrossing, wind-pollinated species ($H_e = 0.162$) by Hamrick *et al.*, (1991). Levels of gene diversity were similar to the widespread, wind and insect-pollinated dioecious shrubs *Rhus glabra* ($H_e = 0.23$), *R. copallina* ($H_e = 0.29$) (Sherman-Broyles *et al.*, 1992), and *Eurya emarginata* ($H_e = 0.296$, Chung and Kang 1995), which were also considered to be high compared with the studies reviewed by Hamrick *et al.*, (1991). Individual populations of *A. glaucescens* varied widely in gene diversity, from $H_e = 0.175$ to 0.401 (Table 3). Even the lowest of these values was greater than the mean for outcrossing, wind-pollinated species ($H_e = 0.148$, Hamrick *et al.*, 1991). It is possible that the wide distribution (see Fig. 1 for *A. glaucescens*), obligate outcrossing, high fecundity, and long generation time for *A. glaucescens* and those species cited

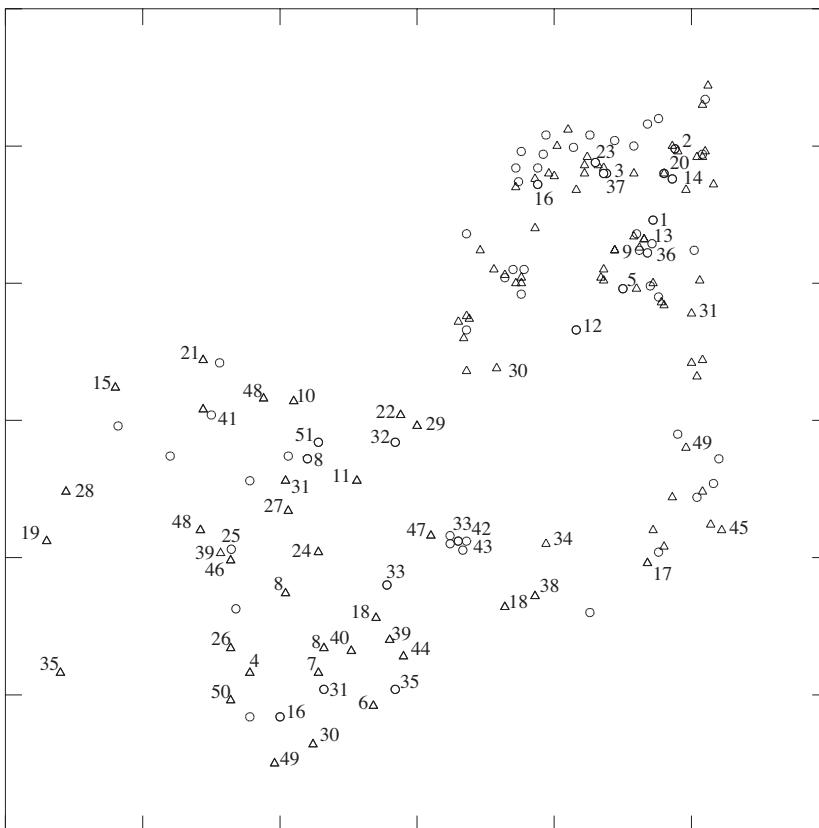


Figure 2. Map of plant location, sex (Δ = males, \circ = females), and genotype number for *Aciphylla glaucescens* at the Black Birch site. Each gap between grid marks on the axes represents 10m.

above may account for their high gene diversity. However, populations of *A. glaucescens* showed heterozygosities close to estimated Hardy-Weinberg equilibrium (Tables 3 and 4).

The mean diversity among populations of *A. glaucescens* ($F_{ST} = 0.256$) was greater than for other outcrossing, wind-pollinated plants ($F_{ST} = 0.100$, Hamrick *et al.*, 1991), i.e., almost 26% of the total variation is due to differences among populations, indicating low levels of gene flow. Gene flow (indirect measure based on F_{ST}) was low compared with 14 other species with similar breeding systems ($N_m = 0.720$ vs 5.380) (Hamrick, 1987), and may have contributed to population differentiation due to drift. A major influence on the low gene flow (N_m) in this study may have been the wide geographic separation of the Black Birch-Nelson populations (north of South Island) relative to the Tiwai-Kingston populations (south of South Island).

Kingston was the most genetically homogeneous population, which may reflect more recent establishment compared with the other populations. *Aciphylla* species are successful at colonising areas after clearing by fire (McGlone and Moar, 1998) and we found evidence of a fire event at the Kingston site. The Kingston population may be recently established following the fire and therefore likely to represent a relatively uniform gene pool, due to founder effects, compared with populations that have been established for longer. Early European colonists report that *Aciphylla* species were widespread and abundant (Allan, 1961), although for many species, populations may be eliminated by heavy grazing (Allan, 1961 and *pers. obs.*). Gene diversity might be higher in those populations that are remnants of formerly more extensive populations (Brown, 1979).

Significantly different frequencies of homozygotes vs heterozygotes between the sexes (Table 5) suggests that the effect of a given allele on fitness may depend on the sex of the plant. While we found no allozyme markers for sex, further studies should be made to test the relative contributions made by separate sexes to the genetic structure of *A. glaucescens* populations. A continuation of our work with *A. glaucescens* might be to grow plants of known genotypes to test their response to different environmental conditions.

Despite a lack of specialised seed dispersal mechanisms, the Black Birch population had 51 distinct multilocus genotypes among 63 individuals, and plants of the same genotype were distributed throughout the population, not in discrete clusters, (Fig. 2). This is consistent with a lack of clonal growth in *A. glaucescens* (*cf.* Fig. 2 in Mayes, McGinley and Werth, 1998). Although some

Aciphylla species in the Paniculatae group, e.g., *A. congesta* and *A. crosby-smithii*, form 'cushions' (Allan, 1961), which might undergo clonal fragmentation as a result of burning or grazing, this is not known in *A. glaucescens*, which belongs to the Elongatae subgenus. Three plants excavated from the Black Birch population showed no signs of clonal spread.

The present study has demonstrated that, compared with similar plant species, there are high levels of gene diversity at both the species and population levels with low gene flow among *A. glaucescens* populations. It is feasible that this species was once more widespread throughout the South Island and that populations have now reduced in number due to habitat fragmentation as a result of land use changes. In addition to vegetation history, the high gene diversity of this species may reflect biological factors such as the breeding system and seed dispersal mechanisms of this species. Genetic differences between sexes suggest the possibility of different selective regimes. This issue deserves further attention.

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