ELECTROPHORETIC VARIATION IN Arabis fecunda, A RARE ENDEMIC OF WESTERN MONTANA

February, 1991

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INTRODUCTION

Arabis fecunda Rollins is a candidate threatened species (Category 2) in Montana (Schassberger 1988). Management efforts to conserve this restricted species must include an understanding of the amount and kind of genetic variation present in A. fecunda. We have examined the level of genetic variability in six populations using protein electrophoresis (allozymes).

METHODS

We included six populations of Arabis fecunda in electrophoretic trials: 1) Charleys Gulch (EO 001), 2) Birch Creek Bluffs (EO 004), 3) Jerry Creek (EO 007), 4) Mouth of Quartz Hill Gulch (EO 006), 5) Vipond Park (011), and 6) Lime Gulch (EO 012). During 5-7 April 1990, 160 seeds from each population (960 total seeds) were planted in a randomized complete block design. Plants grew in a University of Montana greenhouse until harvest during 28-31 May 1990. Forty plants from each population were randomly placed into one of four grinding buffers (Soltis et al. 1983): 1) phosphate-PVP, pH 7.5, 2) tris-maleate-PVP, pH 7.5, 3) tris-HCl-PVP, pH 7.5, or 4) distilled water, pH 6.2. Plants in each grinding buffer were divided randomly into two grinding methods: 1) plant ground before freezing (-80C), or 2) plant ground after freezing (-20C). Specimens were kept on ice and pulverized in 0.5 ml grinding buffer.

Peter Lesica did a preliminary electrophoretic survey of Arabis fecunda (pers. comm. 1989). Our work expanded on number and kind of gel and electrode buffers and number of plants screened. We ran a total of 180 plants on 12% starch gels, using eight gel and electrode buffers from Soltis et al. (1983), Rieseberg and Soltis (1987), Clayton and Tretiak (1972) and Ridgeway et al. (1970). Twenty enzyme systems were screened. Enzyme abbreviations and locus scoring follow Gottlieb (1977) and Soltis et al. (1983). Data were analyzed using BIOSYS-1 (Swofford and Selander 1981).

RESULTS

Phosphate-PVP or tris-HCL grinding buffers and grinding method 1 (plant ground before freezing) consistently gave the best banding patterns. Tris-maleate grinding buffer caused bands to migrate slower, to separate less, and to be more diffuse. Distilled water gave no activity for any enzymes. Grinding method 2 (plant ground after freezing) showed no activity for most enzymes.

Given the low level of enzyme polymorphism in these samples (see below), we decided to screen a smaller number of individuals per population for as many enzyme systems as possible. Twelve enzyme systems coded by 18 putative loci were resolved (Tables 1 and 2). Eight additional enzyme systems were not used due to poor resolution and/or

interpretation of banding patterns (Table 3). All loci were monomorphic except for phosphoglucose isomerase, locus 2 (Pgi-2). Arabis fecunda expressed two alleles (slow=100 and fast=167) at Pgi-2. Jerry Creek and Lime Gulch populations were fixed for the slow allele (100). All other populations were polymorphic with both alleles present in homozygote and heterozygote plants. Progeny analyses have not been done, but banding patterns are similar to other plants (Gottlieb 1977 and 1981, Crawford and Wilson 1979, Adams and Allard 1977, and Loukas et al. 1983). PGI is a highly polymorphic, dimeric enzyme system in plants. Most plants have two to three isozymes in the chloroplast and cytosol. Pgi-1 is reported as monomorphic in most plants. Arabis fecunda had a very faint band at PGI-1. The faint expression of PGI-1 is possibly due to incomplete disruption of chloroplasts. Allozyme banding patterns suggest that these populations of Arabis fecunda exhibit disomic inheritance.

Genetic Variation

Summary statistics. The mean number of alleles per locus for the six A. fecunda populations is 1.06 alleles per locus. Mean heterozygosity 0.006. The percentage of polymorphic loci is 3.7%. Genetic distances were not calculated, since estimates based on a single polymorphic locus would have no biological meaning.

F-statistics. Wright's F statistics were estimated for the one polymorphic locus, Pgi-2: $F_{IS} = 0.57$, $F_{ST} = 0.44$, and $F_{IT} = 0.76$. The four polymorphic populations showed a significant deficit of heterozygotes at the Pgi-2 locus ($F_{IS} = 0.57$, chi square = 12.009, df = 4, $p = 0.0173$).

DISCUSSION AND CONCLUSIONS

We examined 20 enzyme systems, and found 12 systems that could be resolved consistently (Tables 2 and 3). Based on allozyme banding patterns and previously published work, we infer that these 12 enzyme systems code for 18 putative loci. Only one locus was polymorphic in our population samples.

These populations of Arabis fecunda appear to be partially inbred, and have very low levels of genetic variability. There is a significant deficit of heterozygotes ($F_{IS} = 0.57$, $p = 0.017$), as is commonly observed in self-compatible plants with self-pollination or local mating among relatives. Arabis fecunda is autogamous (habitually self-pollinating, Roberta Walsh, unpublished data). At the Pgi-2 locus there has been substantial genetic differentiation between populations (Wright's fixation index, $F_{ST} = 0.57$). Again, these results are typical of geographically isolated populations of partially inbred plant species. We have not calculated indices of genetic distance between populations, since an estimate

based on a single polymorphic locus would have no biological meaning.

These data indicate a partially inbred species with little allozyme variation. However, these data do not permit identification of populations that may have unusually high or low susceptibility to the deleterious genetic effects of small population size, such as inbreeding depression or inability to response to natural selection. Such questions can and should be addressed in the future.

Table 1. Allelic mobility relative to the most common allele (100).

Locus	Mobility		System[1]	N
AAT-1	100		RW	17
ALD-1	100		S1,AC,S7	41
APH-1	100		S6	34
APH-2 anodal	100		S6	15
EST-FE-1	100		S7	36
EST-FE-2	100		S7	36
EST-COLOR-1	100		S6,RW	34
IDH-1	100		S1,AC,S4	44
MDH-1	100		S7,AC,S4	90
MDH-2	100		S7,AC,S4	89
ME-1	100		S1,AC	30
6PGD-1	100		S1,S2,S4	19
6PGD-2	100		S2,S4	19
6PGD-3	100		S2,S4	19
PGI-2	100	167	S8	37
SKDH-1	100		S1,AC,S4	44
TPI-1	100		S7,S2,S4	73
TPI-2	100		S2,S4	36

[1]Systems preceded by S are from Soltis et al. 1983. AC is from Clayton and Tretiak 1972; RW is from Ridgeway et al. 1970.

Table 2. Resolved enzyme activity for Arabis fecunda electrophoretic analyses.

Enzyme Abbreviation	Enzyme (Enzyme Commission no.)
AAT	aspartate aminotransferase (E.C. 2.6.1.1)
ALD	aldolase (E.C. 4.1.2.13)
APH	acid phophatases (E.C. 3.1.3.2)
EST-FE	esterase fluorescent (E.C. 3.1.1.-)
EST-COLOR	esterase colormetric (E.C. 3.1.1.-)
IDH	isocitrate dehydrogenase (E.C. 1.1.1.42)
MDH	malate dehydrogenase (E.C. 1.1.1.37)
ME	malic enzyme (E.C. 1.1.1.38)
6PGD	6-phosphogluconate dehydrogenase (E.C. 1.1.1.44)
PGI	phosphoglucose isomerase (E.C. 5.3.1.9)
SKDH	shikimate dehydrogenase (E.C. 1.1.1.25)
TPI	triosephosphate isomerase (E.C. 5.3.1.1)

Table 3. Unresolved or no enzyme activity for Arabis fecunda electrophoretic analyses.

Enzyme Abbreviation	Enzyme (Enzyme commission no.)	System
ACN	aconitase (E.C. 4.2.1.3)	AC
CAT	catalases (E.C. 1.11.1.6)	S7
FDP	fructose-1,6-diphosphate dehydrogenase (E.C. 3.1.3.11)	AC
GDH	glutamate dehydrogenase (E.C. 1.4.1.2)	S7
G3PDH	glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8)	S1,AC
G6PDH	glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49)	S7
HK	hexokinases (E.C. 2.7.1.1)	S6,RW
PGM	phosphoglucomutase (E.C. 1.1.1.44)	S1

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