Isozyme variation in *Trillium nivale* (Liliaceae)

RANDALL J. BAYER[1]
University of Windsor, Department of Biological Sciences, Windsor, Ont., Canada N9B 3P4
JOHN C. LA DUKE
University of North Dakota, Department of Biology, Grand Forks, ND, U.S.A. 58202
AND
DANIEL J. CRAWFORD
Ohio State University, Department of Botany, Columbus, OH, U.S.A. 43210

Received October 29, 1986

BAYER, R. J., LA DUKE, J. C., and CRAWFORD, D. J. 1987. Isozyme variation in *Trillium nivale* (Liliaceae). Can. J. Bot. **65**: 2250–2254.

Trillium nivale Riddell is an early-blooming spring ephemeral, which occurs in widely scattered, small populations throughout the eastern United States. Nine populations from previously glaciated regions of Ohio were surveyed by enzyme electrophoresis to determine the extent of the genetic variation present in the species. Twelve enzymes, coded by 25 putative genetic loci, were assayed during the study. Within populations, either very low amounts of genetic variation or total genetic uniformity were detected. The low levels of genetic variation are perhaps correlated with the reproductive biology of *T. nivale*.

BAYER, R. J., LA DUKE, J. C., et CRAWFORD, D. J. 1987. Isozyme variation in *Trillium nivale* (Liliaceae). Can. J. Bot. **65** : 2250–2254.

Trillium nivale Riddell une éphémère fleurissant tôt le printemps, se rencontre en peuplements de faible densité mais très dispersés à travers les états de l'est des États-Unis. L'électrophorèse des enzymes a été utilisée dans l'étude de neuf peuplements de *T. nivale* des régions de l'état de l'Ohio ayant subi la glaciation, afin de préciser l'ampleur de la variation génétique présente chez cette espèce. Douze enzymes, codées par 25 loci génétiques probables, ont fait l'objet d'essais au cours de cette étude. À l'intérieur des peuplements, l'on a décelé soit de très faibles niveaux de variation génétique ou soit une uniformité génétique totale. Les faibles niveaux de variation génétique sont peut-être corrélés à la biologie de la reproduction de *T. nivale*.
[Traduit par la revue]

Introduction

Trillium nivale Riddell (Liliaceae) is a very early blooming species of the eastern United States and is commonly known as snow trillium because it often commences flowering when snow is still on the ground. The species is widespread in the eastern United States and extends as far west as extreme eastern South Dakota and Nebraska, although here it is rather rare and occurs as scattered populations. It is a diploid with a chromosome number of $2n = 20$. Nesom and La Duke (1985) have recently examined certain aspects of the biology of *T. nivale*, including its distribution and habitat, seasonal phenology, pollination, breeding system, seed dispersal, and vegetative reproduction. These authors found that *T. nivale* is self-compatible, weakly protogynous, and facultatively xenogamous. In addition, Nesom and La Duke (1985) determined that reproduction occurs via establishment of seedlings from sexually produced seeds and that there is little, if any, evidence for vegetative reproduction.

The purpose of the present investigation was to examine genetic variation in populations of *T. nivale* by enzyme electrophoresis to quantify genetic variation within and between populations. Two of the same populations examined earlier by Nesom and La Duke (1985) were included in the study to determine genetic variation within populations for which data on pollination biology and reproduction were available.

Materials and methods

Plants were examined from nine populations of *Trillium nivale* in Ohio (Table 1). In all instances, a small portion of a leaf was used as a source of enzymes. Plants were sampled at random and an effort was made to collect 50 individuals for electrophoresis. One population (Miami 1) was composed of only five individuals and thus all were sampled. Because of the relatively large size of the Delaware 1 site, nine transects (A–I) were made across the width of the largest population and leaf material was collected from plants along each transect. A transect was made along the length and width of the smaller populations at this site and plants were collected along each one. Crossing studies to confirm the genetic basis of the enzyme phenotypes (i.e., number of genes and alleles) seen on the gels could not be performed because the long time from seed to flowering and accessibility to the plants made such a study unfeasible at the current time. Interpretation of the enzyme phenotypes on the gels was therefore based on inferences from results of previous studies (see Gottlieb 1981*a*, 1982) and segregation patterns at polymorphic loci.

Fresh leaf material was kept on ice after collection and stored at −60°C until it was examined electrophoretically. Material was ground in cold Tris–HCl extracting buffer: 0.1 *M* Tris–HCl, pH 7.5, 4.0 m*M* 2-mercaptoethanol, 1.0 m*M* EDTA (disodium salt), 10.0 m*M* KCl, and 10.0 m*M* $MgCl_2$ (Gottlieb 1981*b*). About 20 mg of polyvinylpolypyrrolidone was added to each sample at the time of grinding. The samples were centrifuged and the supernatant soaked onto filter-paper wicks before being loaded onto horizontal starch gels. Enzymes were resolved on 12.5% starch gels, using two buffer systems: (*i*) gel buffer consisting of 1 part 0.038 *M* lithium hydroxide – 0.188 *M* boric acid, pH 8.3, and 9 parts 0.05 *M* Tris – 0.007 *M* citric acid, pH 8.3, with the electrode buffer consisting only of the lithium borate component; (*ii*) gel buffer consisting of 0.02 *M* histidine–HCl titrated to pH 7.0 with NaOH; electrode buffer consisting of 0.4 *M* trisodium citrate titrated to pH 7.0 with HCl. The following enzymes were resolved with the lithium borate system: alcohol dehydrogenase (ADH), glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), superoxide dismutase (SOD), and triose-phosphate isomerase (TPI). Enzymes resolved on the lower pH histidine system include acid phosphatase (ACP), malate

[1]Present address: University of Alberta, Department of Botany, Edmonton, Alta., Canada T6G 2E9.

Printed in Canada / Imprimé au Canada

TABLE 1. Population designations, number of plants examined per population (in parentheses), and locality data for nine populations of *Trillium nivale*

1. Delaware 1 (149), Ohio: Delaware Co., ca. 1.0 km SE of White Sulfur on Hwy. 257, east side of road ca. 100 m from Scioto River, flat area in deep soil, perhaps more than 10 000 plants in two separate but close areas totalling ca. 1500 square m
2. Delaware 2 (50), Ohio: Delaware Co., area south and west of O'Shaughnessy Dam
3. Franklin 1 (48), Ohio: Franklin Co., 1.5 km south of Dublin on Hwy. 33, east side of Scioto River on steep, west-facing rocky slope
4. Greene 1 (50), Ohio: Greene Co., about 1.2 km west of Clifton along north side of Miami River
5. Highland 1 (50), Ohio: Highland Co., 0.3 km north of old bridge over Rock Fork on Ohio road 753, Beaver Mill
6. Miami 1 (5), Ohio: Miami Co., 0.1 km north of Ohio road 48 bridge over Ludlow Creek, Ludlow Falls
7. Miami 2 (50), Ohio: Miami Co., south bank of Ludlow Creek at crossing of Davis road NW 1/4 of section 8, Union twp.
8. Miami 3 (50), Ohio: Miami Co., centre of section 33, about 0.6 km south of Fener road
9. Miami 4 (50), Ohio: Miami Co., U.S. route 40, 0.4 km south of Sullivan road

NOTE: Vouchers are at OS.

dehydrogenase ((NAD) MDH), 6-phosphogluconate dehydrogenase (6PGDH), phosphoglucomutase (PGM), and shikimic acid dehydrogenase (SKDH). Isocitrate dehydrogenase ((NADP) IDH) was run and assayed using a continuous cellulose acetate gel system described by Hebert and Payne (1985), using a buffer consisting of 0.024 *M* Tris − 0.19 *M* glycine, pH 8.4. Procedures used for the assaying of all enzymes were those of Soltis et al. (1983), except for ADH, which followed Schaal and Anderson (1974). The locus specifying the most anodally migrating isozyme was designated as 1, the next 2, etc. Similarly, the most anodal allozyme at a given locus was labeled A and so on, consecutively.

Genetic variation was assessed using measures such as proportion of loci polymorphic (P), average number of alleles per locus, and expected and observed proportion of loci heterozygous. Observed and expected proportion of loci heterozygous were compared using chi-square tests to ascertain whether populations deviated from Hardy–Weinberg equilibrium expectations. Calculation of the statistics is outlined in Wright (1978).

Gene diversities, genetic identities, and genetic distances were all calculated using the methodologies of Nei (1972, 1973), employing the GENESTAT programme of Whitkus (1985). All computations were done on the IBM computer at the Computer Centre of the University of Windsor. A distance phenogram based on the genetic distance matrix was generated using the unweighted pair − group method,using arithmetic averages (UPGMA; Sneath and Sokal 1973) from the TAXON subroutine of the NT−SYS programme of Rohlf et al. (1974).

Results

The 12 enzymes resolved in the present study are interpreted as being coded by a total of 25 putative genes. The genetics of the expression of these enzymes is inferred until F_1 and F_2 progeny can be obtained, but all, except acid phosphatases and malate dehydrogenases, may be compared with results from other diploid species with regard to number of structural genes present for each enzyme (see Gottlieb, 1981*a*, 1982, for summary and discussion). Two structural genes are apparently present for the following enzymes: ADH, LAP, 6PGDH (although 6PGDH-2 had low activity and poor resolution and was therefore not scored), PGI, PGM, SOD, and TPI. GDH and SKDH are each coded by one putative gene. The number of genes for these seven enzymes agrees with most reports from other diploid plants (Gottlieb 1981*a*, 1982). MDH appears to be specified by six genes and GOT by three, the number for both of these enzymes having been reported from other plants (Gottlieb 1981*a*). The number of genes that are present for each enzyme is apparently a feature that is highly conserved among flowering plants (Gottlieb 1982) and therefore conclusions on the number of loci present for each enzyme system based on the results of previous studies seem to be reliable deductions. Segregation at polymorphic loci provides evidence regarding the subunit composition of the particular enzyme. The subunit composition of those enzymes that were seen in the heterozygous condition was in agreement with results from previous studies (Gottlieb 1981*a*, 1982); ie., LAP (monomeric), MDH (dimeric), PGI (dimeric), and 6PGDH (dimeric). MDH appears to consist of six isozymes. The most anodal isozymes (MDH-1 and MDH-2) and the most cathodal isozyme (MDH-6) either were monomorphic (MDH-1 and MDH-2) or displayed polymorphism that was readily interpretable (MDH-6). However, MDH-3, -4, and -5 are difficult to interpret when heterozygous because of allelomorph overlap at these loci. Consequently, MDH-3, -4, and -5 were not included in the analyses because of the lack of allelic frequency data for several of the populations. Lastly, we were able to resolve the products of four putative loci coding for ACP.

Population designations, number of plants examined within each population, and locality data are presented in Table 1. Populations were surveyed for all enzyme systems (with a sample size as indicated in Table 1) with the following exceptions: Delaware 1, IDH, SKDH, and TPI were not assayed and 50 plants were assayed for MDH; Delaware 2, 48 plants were assayed for IDH; Franklin 1, IDH, SKDH, and TPI were not assayed; Greene 1, Highland 1, Miami 2, Miami 3, and Miami 4, 48 plants were assayed from each of these populations for IDH.

Populations were monomorphic at all loci assayed except for *6Pgdh-1*, *Lap-1*, *Mdh-6*, *Pgi-1*, and *Pgi-2* (Table 2). Polymorphism at these loci ranged from no variation in Delaware 1 (Table 2) to polymorphism at four loci in Delaware 2 and Highland 1. As a result, the proportion of loci polymorphic (P) is highest (0.16) in Delaware 2 and Highland 1 and lowest (0.0) in Delaware 1 (Table 3). The average number of alleles per locus was highest (1.20 ± 0.49) in Highland 1 and lowest (1.00 ± 0.0) in Delaware 1 (Table 3). All populations diverged significantly from the expected proportion of loci heterozygous (as would be expected under Hardy−Weinberg equilibrium) except Delaware 1 and Miami 3 (Table 3).

The greatest gene diversity was found at *6Pgdh-1* (H_T = 0.44), with most of the diversity displayed within populations (H_S = 0.264) as compared with between populational differences (D_{ST} = 0.176; G_{ST} = 0.4). Gene diversity statistics for four other polymorphic loci were *Lap-1* (H_T = 0.190, H_S = 0.114, D_{ST} = 0.076, G_{ST} = 0.4); *Mdh-6* (H_T = 0.092, H_S = 0.069, D_{ST} = 0.023, G_{ST} = 0.25); *Pgi-1* (H_T = 0.225, H_S = 0.191, D_{ST} = 0.034, G_{ST} = 0.151); *Pgi-2* (H_T = 0.09, H_S = 0.067, D_{ST} = 0.023, G_{ST} = 0.26). The mean (D_{ST}) between populations and degree of genetic differentiation between populations (G_{ST}) for all nine populations of *T. nivale* are H_T = 0.207, H_S = 0.141, D_{ST} = 0.066, and G_{ST} = 0.319.

Table 4 presents pairwise comparisons of genetic identities and genetic distances for all combinations of the nine popula-

TABLE 2. Allelic frequencies for nine populations of *Trillium nivale*. Presented are the allelic frequencies for five loci that were found to be polymorphic. Numbers of individuals per population and an explanation of population locations are given in Table 1

Locus (allele)	Del. 1	Del. 2	Frn. 1	Grn. 1	Hgh. 1	Mim. 1	Mim. 2	Mim. 3	Mim. 4
6Pgdh-1-A	1.00	0.36	0.92	1.00	0.61	0.10	0.09	0.33	0.50
6Pgdh-1-B	0.00	0.64	0.08	0.00	0.39	0.90	0.91	0.67	0.50
Lap-1-A	0.00	0.47	0.00	0.02	0.00	0.00	0.58	0.00	0.00
Lap-1-B	1.00	0.53	1.00	0.98	1.00	1.00	0.42	1.00	1.00
Mdh-6-A	1.00	0.85	1.00	1.00	0.76	1.00	1.00	1.00	1.00
Mdh-6-B	0.00	0.15	0.00	0.00	0.24	0.00	0.00	0.00	0.00
Pgi-1-A	0.00	0.06	0.53	0.05	0.16	0.00	0.36	0.02	0.14
Pgi-1-B	1.00	0.94	0.47	0.95	0.84	1.00	0.64	0.98	0.86
Pgi-2-A	1.00	1.00	1.00	1.00	0.53	1.00	1.00	1.00	1.00
Pgi-2-B	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
Pgi-2-C	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00

NOTE: Del. 1, Delaware 1; Del. 2, Delaware 2; Frn. 1, Franklin 1; Grn. 1, Greene 1; Hgh. 1, Highland 1; Mim. 1, Miami 1; Mim. 2, Miami 2; Mim. 3, Miami 3; Mim. 4, Miami 4.

TABLE 3. Genetic variation within *Trillium nivale* including percent of loci polymorphic (frequency of most common allele less than 0.99), average number of alleles per locus, and observed and expected proportion of loci heterozygous

Population designation	Proportion of loci polymorphic	Average no. of alleles per locus	Observed proportion of loci heterozygous	Expected proportion of loci heterozygous
Delaware 1	0.00	1.0 ±0.00	0.000	0.000
Delaware 2	0.16	1.16±0.37	0.018**	0.053
Franklin 1	0.095	1.10±0.29	0.005**	0.031
Greene 1	0.08	1.08±0.27	0.0008**	0.018
Highland 1	0.16	1.20±0.49	0.50*	0.068
Miami 1	0.04	1.04±0.195	0.008**	0.072
Miami 2	0.12	1.12±0.32	0.006**	0.045
Miami 3	0.08	1.08±0.27	0.0152	0.020
Miami 4	0.08	1.08±0.27	0.008**	0.030

NOTE: *, significantly different at the 5% level; **, significantly different at the 1% level. Population designations are as in Table 1.

tions. Delaware 1 and Greene 1 are genetically identical, while Delaware 1 and Miami 2 are most distant (genetic identity = 0.937; genetic distance = 0.065). These relationships are more easily visualized by examining the phenogram (Fig. 1) derived from the cluster analysis (UPGMA) of the genetic distance matrix (Table 4).

Discussion

Trillium nivale occurs in Ohio as small scattered populations of mostly 20−100 individuals (the Delaware 1 population is unusually large) on calcareous soil (Nesom and La Duke 1985; Stuckey 1976). The species is found primarily within glaciated territory (Nesom and La Duke 1985), and it is possible that the low genetic variability results from the founder effect during colonization following Wisconsinan glaciation. Numerous populations have been reported for *T. nivale* beyond the glacial maxima (for map, see Nesom and La Duke 1985); however, attempts to relocate some of these populations for this analysis were unsuccessful. These populations may have supplied the necessary propagules for the recolonization. A similar situation has been proposed by Schwaegerle and Schaal (1979) for *Sarracenia purpurea* L.

Nesom and La Duke (1985) have demonstrated that although *T. nivale* may be facultatively xenogamous, when an individual selfs, it is capable of producing abundant seed. Thus, it is possible that following dispersal of a few seeds into a favourable habitat, the number of plants could increase by seedlings resulting from autogamy. If this were the case, one would expect these populations to be rather genetically uniform, which is the situation with respect to genes coding for isozymes. Ihara and Endo (1981) found genetic uniformity for *Adh* in three species of Japanese *Trillium*. Four populations were examined (59−184 individuals per population) and there was no ADH variation within the species. Additional support comes from a study of *T. kamtschaticum* Pall. where Fukuda (1967) demonstrated inbreeding and the development of genetic subgroups within a population. However, these data are in conflict with chromosomal analysis of *T. kamtschaticum* reported by Narise (1956), who concluded that "... the breeding of *T. kamtschaticum* in almost all the natural populations was random" (Narise 1956, p. 73). Narise admitted, citing Hayaman (1953), that if selfing and random breeding take place together and homozygotes are selected against, then the values attained may be identical with those predicted by Hardy−Weinberg. Therefore, selfing may still be occurring in the populations studied by Narise (1956), but selection against homozygotes is affecting the interpretation of the data. Hence, genetic uniformity may be more common in *Trillium* species than would be predicted by Hardy−Weinberg equilibrium.

TABLE 4. Genetic identities (upper triangle) and genetic distances (lower triangle) for all pairwise comparisons of nine populations of *Trillium nivale*. The mean identity for all populations was 0.978 (range = 0.937−1.000) and the mean genetic distance was 0.022 (range = 0.000−0.065)

	Delaware 1	Delaware 2	Franklin 1	Greene 1	Highland 1	Miami 1	Miami 2	Miami 3	Miami 4
Delaware 1		0.968	0.968	1.000	0.981	0.961	0.937	0.978	0.987
Delaware 2	0.032		0.961	0.974	0.980	0.987	0.992	0.990	0.989
Franklin 1	0.014	0.039		0.989	0.977	0.954	0.948	0.970	0.984
Greene 1	0.000	0.026	0.011		0.984	0.967	0.949	0.982	0.990
Highland 1	0.019	0.020	0.023	0.016		0.979	0.963	0.987	0.990
Miami 1	0.039	0.013	0.047	0.033	0.021		0.981	0.998	0.993
Miami 2	0.065	0.008	0.054	0.052	0.038	0.019		0.979	0.977
Miami 3	0.022	0.010	0.030	0.018	0.013	0.002	0.021		0.998
Miami 4	0.013	0.011	0.016	0.011	0.010	0.007	0.023	0.002	

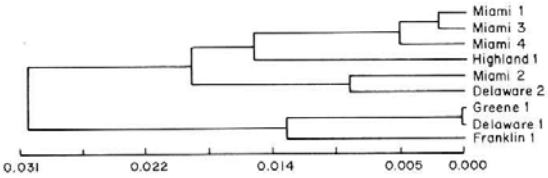

FIG. 1. Distance phenogram (UPGMA) derived from a matrix of genetic distances (Table 4) for nine populations of *T. nivale*. Population designations are those given in Table 1. Two populations (Greene 1 and Delaware 1) are genetically identical ($I = 1.00$; $D = 0.00$) and are illustrated on the phenogram as being linked very closely together.

The extremely low level of genetic variation in most populations and total uniformity in one (Delaware 1) are significant in understanding the biology of *T. nivale*. This genetic uniformity will be considered relative to certain aspects of the biology of the species. Two features of the present study are of some significance: (*i*) the 25 gene loci included in this investigation represent as large a sample as is normally included in isozyme studies of plants and (*ii*) a wide range of enzymes was included (Gottlieb 1981*a*, 1982; Hamrick et al. 1979). Thus, the lack of variation relative to other studies is not an artifact of a small sample of genes.

Hamrick et al. (1979) summarized plant data from a number of workers and correlated the values for P (percentage of polymorphic loci) and H (percent heterozygosity) with life-history features. Endemic taxa had values for P ranging from 0 to 71% with a mean of 26.52%, and narrowly distributed taxa ranged from 0 to 100% with a mean of 36.73%. *Trillium nivale* would be considered a narrowly distributed taxon and an average P value of 0.09 (range = 0.000−0.16) for all populations (Table 3) ranks among the lowest for that group cited by Hamrick et al. (1979). In addition, the only taxa listed by Hamrick et al. (1979) and Gottlieb (1981*a*) in the Liliopsida were members of the Poaceae. *Trillium nivale* is quite different from those taxa and comparisons between them would be of dubious value.

Loveless and Hamrick (1984) reviewed gene diversity statistics for ecological factors determining the genetic structure of populations and our values of H_T and H_S for *T. nivale* are lower for such categories as predominantly outcrossed breeding systems, hermaphroditic floral morphology, sexual reproduction, entomophilous pollination, animal seed dispersal (ants), long-lived perennials, polycarpy, and late successional stage. Mean diversity among populations G_{ST} for populations of *T. nivale* was higher or equal to the values presented by Loveless and Hamrick (1984) for all categories except floral morphology and seed dispersal. Considering geographic range, gene diversity values for *T. nivale* are most similar to other species that have a regional distribution, although Loveless and Hamrick (1984) note that geographic range seems to be a poor predictor of the genetic structure of a species.

A comparison of *T. nivale* with two species of *Camassia* (Liliaceae), another spring-blooming, perennial diploid, shows many similarities such as distribution pattern and life cycle (Ranker and Schnabel 1986). They differ because *Camassia* is primarily outcrossing (Ranker and Schnabel 1986), whereas *T. nivale* is primarily autogamous (Nesom and La Duke 1985). Genetic diversity statistics reflect the differences in breeding systems since *T. nivale* has higher D_{ST} and G_{ST} but lower H_T and H_S values than those given for *Camassia scilloides* and *C. angusta* (Ranker and Schnabel 1986). This could be a reflection of their different breeding systems, where higher D_{ST} and G_{ST} values and lower H_S and H_T values have been reported for inbreeders than for outcrossers (Loveless and Hamrick 1984; Brown 1979).

The interpopulational genetic distances and identities as well as the mean identity and distance for populations of *T. nivale* are within the range of values found by other investigators (Gottlieb 1981*a*) for outcrossing perennials, including two members of the Liliaceae, *Camassia angusta* and *C. scilloides* (Ranker and Schnabel 1986). The cluster analysis of the genetic distances (Fig. 1) demonstrates that there is little apparent regional differentiation of populations. Three populations, Miami 1, 3, and 4, are very similar geographically and isozymically; however, two populations that are geographically distant, Greene 1 and Delaware 1, are the least differentiated genetically (Fig. 1).

Gottlieb (1981*a*) summarized published data on the correlation between genetic variation in plant populations and species and breeding systems. Low levels of genetic variation occur in selfers and higher levels are found in outcrossers. Soltis (1982) has presented a similar explanation for low levels of isozyme variation in populations of *Sullivantia*. In addition, the low levels of genetic variation in *T. nivale* founders may have resulted in an edaphic preference for calcareous soils, an association noted by Stuckey (1976). A similar restriction was proposed by Babbel and Selander (1974) as one explanation for the edaphic restriction of *Lupinus subcarnosus* Hook. However, Nesom and La Duke (1985) found *T. nivale* was frequently found on calcareous soils but not restricted to them. This association may be a consequence imposed by the founding propagules which originated from populations successful on calcareous soils and thus were better colonizers of such

soils. The isozyme and life-history data support an hypothesis of single-seed colonization of *T. nivale* populations. The source of these propagules is yet to be determined. Additionally, the primarily self-pollinating breeding system of *T. nivale* is reflected by the gene diversity statistics.

Acknowledgments

We thank Guy Nesom for assisting us in the field on several occasions. Daniel Lebedyk is gratefully acknowledged for his technical assistance. This research was supported by Natural Sciences and Engineering Research Council of Canada grant A2924 to R.J.B., the Office of Research and Program Development, the University of North Dakota to J.C.L., and National Science Foundation grant DEB 82-04073 to D.J.C.

BABBEL, G. R., and SELANDER, R. K. 1974. Genetic variability in edaphically restricted and widespread plant species. Evolution (Lawrence, Kans.), **28**: 619–630.

BROWN, A. H. D. 1979. Enzyme polymorphism in plant populations. Theor. Popul. Biol. **15**: 1–42.

FUKUDA, I. 1967. The formation of subgroups by the development of inbreeding systems in a *Trillium* population. Evolution (Lawrence, Kans.), **21**: 141–147.

GOTTLIEB, L. D. 1981*a*. Electrophoretic evidence and plant populations. Progr. Phytochem. **7**: 1–46.

——— 1981*b*. Gene number in species of Asteraceae that have different chromosome numbers. Proc. Natl. Acad. Sci. U.S.A. **78**: 3726–3729.

——— 1982. Conservation and duplication of isozymes in plants. Science (Washington, D.C.), **216**: 373–380.

HAMRICK, J. L., LINHART, Y. B., and MITTON, J. B. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annu. Rev. Syst. Ecol. **10**: 173–200.

HAYAMAN, B. I. 1953. Mixed selfing and random mating when homozygotes are at disadvantage. Heredity, **7**: 185–192.

HEBERT, P. D. N., and PAYNE, W. J. 1985. Genetic variation in populations of the hermaphroditic flatworm *Mesostoma lingua* (Turbellaria, Rhabdocoela). Biol. Bull. (Woods Hole, Mass.), **169**: 143–151.

IHARA, M., and ENDO, T. 1981. Genetic control of alcohol dehydrogenase in the Japanese species of *Trillium*. Jpn. J. Genet. **56**: 397–407.

LOVELESS, M. D., and HAMRICK, J. L. 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. **15**: 65–95.

NARISE, T. 1956. Variation and evolution in *Trillium*. III. The breeding system of *Trillium kamtschaticum* Pall. in some natural populations. Jpn. J. Genet. **31**: 65–75.

NEI, M. 1972. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. **70**: 3321–3323.

——— 1973. Genetic distances between populations. Am. Nat. **106**: 283–292.

NESOM, G. L., and LA DUKE, J. C. 1985. Biology of *Trillium nivale* Liliaceae). Can. J. Bot. **63**: 7–14.

RANKER, T. A., and SCHNABEL, A. F. 1986. Allozymic and morphological evidence for a progenitor–derivative species pair in *Camassia* (Liliaceae). Syst. Bot. **11**: 433–445.

ROHLF, F. J. J., KISHPAUGH, J., and KIRK, D. 1974. Numerical taxonomy system of multivariate statistical programs. State University of New York, Stony Brook.

SCHAAL, B. A., and ANDERSON, W. W. 1974. An outline of techniques for starch gel electrophoresis of enzymes from the American oyster *Crassostera virginica* Gmelin. Ga. Mar. Sci. Centre Tech. Rep. Ser. No. 74-3.

SCHWAEGERLE, K. E., and SCHAAL, B. A. 1979. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. Evolution (Lawrence, Kans.), **33**: 1210–1218.

SNEATH, P. H. A., and SOKAL, R. R. 1973. Numerical taxonomy. W.H. Freeman and Co., San Francisco.

SOLTIS, D. E. 1982. Allozymic variation in *Sullivantia* (Saxifragaceae). Syst. Bot. **7**: 26–34.

SOLTIS, D. E., HAUFLER, C. H., DARROW, D. C., and GASTONY, G. J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern. J. **73**: 9–27.

STUCKEY, R. L. 1976. *Trillium nivale* (Liliaceae): the snow trillium in Ohio. Ohio J. Sci. **76**: 143–144.

WHITKUS, R. 1985. FORTRAN program for computing genetic statistics from allelic frequency data. J. Hered. **76**: 142.

WRIGHT, S. 1978. Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.