John, Thanks for your help!
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Genetic Variation in Running Buffalo Clover (*Trifolium stoloniferum*, Fabaceae)

R. JAMES HICKEY
MICHAEL A. VINCENT
Botany Department
Miami University
Oxford, Ohio 45056, U.S.A.

SHELDON I. GUTTMAN
Zoology Department
Miami University
Oxford, Ohio 45056, U.S.A.

Abstract: *Starch gel electrophoresis was used to examine the genetic variation in four species of* Trifolium. *Two of these,* T. stoloniferum *and* T. reflexum, *are native, and, in Ohio at least, are considered rare. The genetic variation in these species was compared with that of two nonnative taxa,* T. hybridum *and* T. pratense, *which have similar life-history characteristics. The only known Ohio population of* T. reflexum *was completely homozygous across 14 loci. Three of the 20 loci examined for* T. stoloniferum *showed variation, with an average of 1.10 alleles per locus.* Trifolium hybridum *and* T. pratense *showed variation at 53.3% of the loci resolved with an average of 1.73 and 1.93 alleles per locus, respectively. Analyses of genetic structure show considerable differences between* T. stoloniferum *and the two nonnative species. In* T. stoloniferum *33.9% of the genetic variation is partitioned between populations, in contrast to 11.9% and 8.5% for* T. hybridum *and* T. pratense. *Genetic data suggest that gene flow in the endangered* T. stoloniferum *is severely limited, even across very short distances, and that conservation efforts should minimally include the preservation of genetic variation residing among various populations.*

Resumen: *La electrofóresis con gel de almidón fue utilizada para examinar la variación genética en cuatro especies de* Trifolium. *Dos de estas,* T. stoloniferum *y* T. reflexum *son nativas y, cuando menos en el estado de Ohio, son consideradas raras. La variación genética de estas especies fué comparada con dos taxa no nativos,* T. hybridum *y* T. pratense *las cuales tienen una biología similar. La única población conocida de* T. reflexum *en Ohio fué completamente homocigota en 14 loci. Tres de los veinte loci examinados para* T. stoloniferum *mostraron variación con un promedio de 1.10 alelos por locus.* Trifolium hybridum *y* T. pratense *mostraron variación en el 53.3% de los loci resueltos con un promedio de 1.73 y 1.93 alelos por locus, respectivamente. Los análisis en la estructura genética muestran considerables diferencias entre* T. stoloniferum *y las dos especies no nativas. En* T. stoloniferum *33.9% de la variación genética esta dividida entre las poblaciones en contraste con 11.9% y 8.5% para* T. hybridum *y* T. pratense. *Los datos genéticos sugieren que el flujo genético en la población en peligro de* T. stoloniferum *está limitada severamente, aún en distancias muy cortas, y que los esfuerzos de conservación deben mínimamante incluir la preservación de la variación genética presente entre varias poblaciones.*

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Introduction

Falk (1990) estimates that as much as 20% (5000 taxa) of the U.S. flora merits conservation concern. Presently, the Federal Register (USDI Fish & Wildlife Service 1990) lists 2125 U.S. plants whose status is of concern. For 1572 of these, there is some evidence of endangered or threatened status, for another 527 there is strong evidence, and 26 are currently listed as threatened or endangered. In addition, 94 taxa are known or thought to be extinct in the wild. While attempts to preserve all of these taxa would seem daunting, a greater emphasis is now being placed on conservation of natural resources (Falk 1990). Unfortunately, no consistent or explicit methods for conservation exist across all taxa (Fiedler 1986). As discussed in several recent papers (Ryder et al. 1981; Namkoong 1983; Fiedler 1986; Lesica et al. 1988; Sampson et al. 1988; Falk 1990), traditional maintenance programs should be augmented by information on the biology and, in particular, the genetic diversity and structure of the taxa in question.

Clovers are ideal for plant conservation. They not only have the esoteric importance of any native plant species as an integral part of the ecosystem, but they also have intrinsic worth as a food source for wild and domesticated animals. Bartgis (USDI Fish & Wildlife Service 1989) has outlined a recovery plan for the maintenance of *Trifolium stoloniferum* that includes protection and augmentation of natural populations, enforcement of protective legislation, conservation of germplasm, and publication of information on the species. In an attempt to gain insight into the genetic structure of rare plants in general and clovers specifically we have made electrophoretic studies of *T. stoloniferum* Muhl. ex A. Eaton and *T. reflexum* L. In addition we have performed similar studies on the common congeners, *T. hybridum* L. and *T. pratense* L., for comparison of both genetic structure and diversity.

Trifolium stoloniferum, running buffalo clover, is listed by the U.S. Fish and Wildlife Service (1987) as an endangered species, which had declined to the point that it was considered possibly extinct (Brooks 1983). It was rediscovered in West Virginia in 1983 (Bartgis 1985), and subsequently was found in Indiana (Homoya et al. 1989), Kentucky (Campbell et al. 1988), and Ohio (Cusick 1989). Based on herbarium specimens, the original range of this perennial species appears to have been Illinois, Indiana, Kansas, Kentucky, Missouri, Ohio, and West Virginia (U.S. Fish & Wildlife Service 1989). In Ohio, historic records exist for eight counties, with extant populations known from the southwestern counties of Clermont, Hamilton, and Warren (Cusick 1989). Detailed habitat descriptions of each of these populations are given by Cusick (1989) for the known Ohio sites, as is a lengthy discussion of the history of the taxon in Ohio.

Trifolium reflexum, buffalo clover, is a rare native annual with a historic range over much of eastern North America from Nebraska south to Texas in the west and from Florida to New York and Ontario in the east. It was thought to have disappeared from Ohio, Pennsylvania, West Virginia, Maryland, New York, and Ontario (Campbell et al. 1988; Roberts & Cooperrider 1982). Recently, *T. reflexum* was rediscovered (Vincent, unpublished data) in Ohio in a sandy, lightly wooded site in eastern Pike County that had been burned over less than a year before the plants were rediscovered.

Trifolium pratense and *T. hybridum* are both common weedy introductions from Europe. *Trifolium pratense* is an annual or biennial whereas *T. hybridum* is perennial. Both species have wide ranges and, as determined from previous studies (Guttman, unpublished data), have a surprising amount of genetic diversity. In lieu of common native taxa they represent the most appropriate comparison taxa for the more restricted native species.

Materials and Methods

Seventy-six plants of *Trifolium stoloniferum* representing four populations, 23 individuals of *T. reflexum* from the single known Ohio population, and 30 individuals each from two separate populations for both *T. hybridum* and *T. pratense* were collected in southwestern and south-central Ohio (Table 1). Attempts to gain permission to sample populations of *T. stoloniferum* outside of Ohio were initially unsuccessful. Voucher specimens from each population are deposited in the Willard Sherman Turrell Herbarium (MU) at Miami University. Collections for electrophoretic analysis consisted of single leaves, each separately bagged and transported to

Table 1. Collection data for *Trifolium* populations.

Species	*Population code*	*Locality*	*Voucher*
T. stoloniferum	WARREN	Warren Co.	No voucher
	FANK A	Clermont Co., Fankhauser Farm	*Lammers & Vincent 6705*
	CONGRESS	Hamilton Co., Congress Green Cemetary	*Vincent 3110, 4105*
	HAWN 1	Hamilton Co. Shawnee Lookout	*Vincent 4103, 4104*
	HAWN 3	Hamilton Co. Shawnee Lookout	*Vincent 4102*
T. reflexum	PIKE	Pike Co.	*Vincent et al. 3665*
T. hybridum	1874	Butler Co.	*Guttman, s.n.*
	TOYS	Hamilton Co.	*Guttman, s.n.*
T. pratense	COUNTRY	Butler Co.	*Guttman, s.n.*
	1875	Butler Co.	*Guttman, s.n.*

the lab on ice where they were stored at 4°C until they could be run, usually for a period of 3 to 4 days. In addition, a single leaflet from each plant was stored at −70°C for later line-up gels. For each run, one-half of a leaflet was ground in 4–5 drops of Microbuffer (Werth 1985) enhanced with 5% PVP-40 and 0.1% β-mercaptoethanol and absorbed onto Whatman #3 filter-paper wicks. These wicks were then inserted into 15% starch (Sigma Chemical Co., St. Louis, Missouri) gels and electrophoresed using standard techniques. A summary of gel buffers and corresponding enzyme systems is given in Table 2.

Allelic variation was inferred from banding patterns of population samples, and in no cases did the observed variation deviate from expected patterns based on known quaternary structures of the enzymes involved (Harris & Hopkinson 1976; Gottlieb 1981, 1982). Between-gel variation for individual species was initially assessed using Rf values relative to an internal standard (*Cercis canadensis* L.) and subsequently confirmed with line-up gels. On an individual species basis, loci and alleles were sequentially numbered and lettered, respectively, beginning with the most anodal form. Enzyme terminology follows the suggestions of Kendall (1989).

Genetic analyses on the various species were performed using Biosys I (Swofford & Selander 1981) and Type I (individual entry) genotypic data, as inferred from gel phenotypes. Summary statistics of genetic variation, Hardy-Weinberg statistics, corrected for small sample size using Levene's (1949) correction factor, and F statistics (Wright 1965, 1978) were derived for separate populations and species. In addition, the Shannon information measure of genetic diversity (H) was calculated for individual loci, populations, and species using the formula $H = -\sum_{i=1}^{n} p_i \log_e p_i$ and following the procedures outlined by Lewontin (1972), Griggs and Jain (1983), and Kubetin and Schaal (1979).

Table 2. Enzyme systems and associated electrophoretic buffers. Numbered systems are from Soltis et al. (1983), LiOH and T.C. 8.0 are as presented in Werth (1985).

Enzyme	*Buffer system*
Alcohol dehydrogenase—ADH (E.C. 1.1.1.1)	8
Aspartate aminotransferase—AAT (E.C. 2.6.1.1)	11
Glucose-6-phosphate isomerase—GPI (E.C. 5.3.1.9)	5,8
Glutamate dehydrogenase—GLUDH (E.C. 1.4.1.47)	7, LiOH
Hexokinase—HK (2.7.1.1)	7
Isocitrate dehydrogenase—IDH (E.C. 1.1.1.42)	11
Leucine amino peptidase—LAP (E.C. 3.4.11.-)	7
Malate dehydrogenase—MDH (E.C. 1.1.1.37)	11
Mannose-6-phosphate isomerase—MPI (E.C. 5.3.1.8)	8
Peptidase—PEP (E.C. 3.4.-.-)	T.C. 8.0
Phosphogluconate dehydrogenase—PGDH (E.C. 1.1.1.44)	5
Phosphoglucomutase—PGM (E.C. 5.4.2.2)	5
Triose-phosphate isomerase—TPI (E.C. 5.3.1.1)	8

Results

Overall, 13 enzyme systems and 23 loci were resolved. However, the number of consistently scoreable systems and loci varied between species (Table 3). Of the 11 enzymes and 20 loci analyzed in *T. stoloniferum,* variation was found only at GPI-1, PGM-1, and GDH-2. Only GDH was variable in all populations; variation in GPI and PGM was confined to populations HAWN 1 and FANK. All 14 loci in *T. reflexum,* representing eight enzyme systems, were monomorphic. In *T. hybridum,* 10 enzyme systems were encoded by 15 loci. Of these, variation was found at eight loci: AAT-1, ADH-1, GDH-1, GPI-2, LAP-1, MDH-1, PGM-1, and TPI-2, although GDH and MDH variation was confined to single populations. Ten enzyme systems and 15 loci were also resolved in *T. pratense.* Variation was found at eight loci: AAT-1, GDH-1, GPI-2, LAP-1, MDH-1, PGDH-2, PGM-1, and TPI-2. Variation at AAT, GDH, and MDH was confined to individual populations. Despite reports of polyploidy in *T. reflexum, T. hybridum,* and *T. pratense* (Moore 1973), all loci showed phenotypes and populational segregation patterns consistent with genetically diploid organisms. Chromosome counts for *T. stoloniferum* have only been reported at the diploid level (Campbell et al. 1988).

The Ohio population of *T. reflexum* is genetically more depauperate (Table 4) than the other clovers in this study, being completely uniform across all loci studied. While there is variation in *T. stoloniferum,* it is very low with an average of 1.10 alleles per locus and with only 15.0% of the loci polymorphic. *Trifolium hybridum* and *T. pratense* show considerably more variation, with 1.73 and 1.93 alleles per locus, respectively. Both species were variable at 53.3% of their loci.

Analysis of genetic structure suggests that a greater proportion of the overall variation in *T. hybridum* and *T. pratense* is due to intra- than to interpopulational variation. In both of these species the F_{IS} component is considerably higher than F_{ST} and the low F_{ST} values indicate little difference in allelic frequencies between populations (Table 4). In contrast, F_{IS} and F_{ST} values for *T. stoloniferum* are quite similar, but with a higher F_{ST} value than in the preceding species, suggesting that a greater proportion of species variation is interpopulational. These F-value indices are mirrored by the Shannon Index (Tables 5 and 6). For both *T. hybridum* and *T. pratense* the amount of interpopulational genetic variation is minimal compared to that contributed within populations. Summed across the species, the amounts of genetic diversity that can be attributed to

Table 3. Summary of allele frequencies in populations of *Trifolium*. Allelic designations are comparable only within species.

SPEC. POP.		*Stoloniferum*					*Reflexum*	*Hybridium*		*Pratense*	
Enzyme	*Allele*	*WARREN*	*CONGRESS*	*HAWN 3*	*HAWN 1*	*FRANK*	*PIKE*	*1874*	*TOYS*	*COUNTRY*	*1875*
AAT-1	n	3	21	15	21	20		28	25	20	27
	A	1.000	1.000	1.000	1.000	1.000		0.911	0.840	0.975	1.000
	B							0.089	0.160	0.025	
AAT-2	n	3	21	15	21	20					
	A	1.000	1.000	1.000	1.000	1.000					
ADH-1	n						23	28	30	20	29
	A						1.000	0.018	0.017	1.000	1.000
	B							0.982	0.983		
GDH-1	n	3	21	15	21	20		19	30	19	29
	A	1.000	1.000	1.000	1.000	1.000		0.026		0.895	1.000
	B							0.947	1.000	0.105	
	C							0.026			
GDH-2	n	3	21	14	19	20					
	A	0.333	0.405	0.643	0.053	0.350					
	B	0.067	0.595	0.357	0.947	0.650					
GPI-1	n	3	21	15	21	20	23	28	30	20	29
	A				0.167	0.300	1.000	1.000	1.000	1.000	1.000
	B	1.000	1.000	1.000	0.833	0.700					
GPI-2	n	3	21	15	21	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000	0.089	0.117	0.025	0.034
	B							0.911	0.880	0.925	0.824
	C										0.034
	D									0.050	0.103
HK-1	n	3	21	15	21	20					
	A	1.000	1.000	1.000	1.000	1.000					
HK-2	n	3	21	15	21	20					
	A	1.000	1.000	1.000	1.000	1.000					
IDH-1	n	3	21	15	21	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-1	n	3	21	15	18	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000		0.017		0.125
	B							0.054	0.017	0.375	0.429
	C							0.929	0.917	0.375	0.411
	D							0.018	0.050	0.250	0.036
MDH-1	n	3	21	15	18	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000		0.083		0.017
	B							1.000	0.920	1.000	0.983
MDH-2	n	3	21	15	18	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-3	n	3	21	15	18	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MPI-1	n	3	21	15	21	20					
	A	1.000	1.000	1.000	1.000	1.000					
PEP-1	n	3	21	15	21	20					
	A	1.000	1.000	1.000	1.000	1.000					
PEP-2	n	3	21	15	21	20					
	A	1.000	1.000	1.000	1.000	1.000					
PGDH-1	n	3	21	15	21	20	23	28	30	20	29
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGDH-2	n	3	21	15	21	20	23	28	30	20	26
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950	0.923
	B									0.050	0.083
	C										0.083
PGM-1	n	3	21	15	19	20	23	28	30	20	27
	A	1.000	1.000	1.000	0.289	0.500	1.000	0.893	0.900	0.025	0.037
	B				0.711	0.500		0.107	0.100	0.950	0.963
	C									0.025	
PGM-2	n	3	21	15	19	20	23				
	A	1.000	1.000	1.000	1.000	1.000	1.000				
TPI-1	n						23	28	30	20	29
	A						1.000	1.000	1.000	1.000	1.000
TPI-2	n						23	28	30	20	29
	A						1.000	0.107	0.050	0.975	0.982
	B							0.893	0.950	0.025	0.018

Table 4. Summary of genetic variation and diveristy statistics for *Trifolium* species. (N = mean sample size per locus; A = mean number of alleles per locus; P = percentage of loci polymorphic; OBS = mean direct count of heterozygosity; EXP = mean expected heterozygosity, unbiased estimate.)

				Heterozygosity				
Species	*N*	*A*	*P*	*OBS.*	*EXP.*	*F(IS)*	*F(IT)*	*F(ST)*
T. stoloniferum	79.2	1.10	15.0	0.047	0.049	0.222	0.030	0.206
s.d.	(0.3)	(0.1)		(0.028)	(0.028)			
T. reflexum	23.0	1.0	0.0					
s.d.	(0.0)							
T. hybridum	57.1	1.73	53.3	0.066	0.069	0.178	0.208	0.036
s.d.	(0.7)	(0.2)		(0.021)	(0.022)			
T. pratense	48.4	1.93	53.3	0.079	0.085	0.041	0.061	0.021
s.d.	(0.3)	(0.3)		(0.039)	(0.045)			

the within-population component are 88.1% and 91.5%, respectively. The lower value for *T. hybridum* is largely the result of a single locus (GDH). The mean within-population apportionment without that system rises from 88.1% to 95.3%. H values for *T. stoloniferum* contrast with those of the other clover species. Approximately 34% of the genetic diversity in running buffalo clover can be attributed to interpopulation variation. Interestingly, GDH variation in this species is more strongly partitioned within populations, rather than between them as in *T. hybridum*.

Discussion

Data accumulated during this study suggest that clover species are quite variable in the amount and distribution

Table 5. Diversity components for variable loci in *Trifolium*.

Trifolium stoloniferum

	Population						
Locus	*WARREN HO*	*CONGRESS HO*	*HAWN 3 HO*	*HAWN 1 HO*	*FANK HO*	*HPOP*	*HSP*
GDH-2	0.636	0.675	0.652	0.208	0.647	0.564	0.643
GPI-2	0	0	0	0.460	0.611	0.214	0.365
PGM-1	0	0	0	0.602	0.693	0.259	0.499

Trifolium hybridum

	Population			
Locus	*1874 HO*	*TOYS HO*	*HPOP*	*HSP*
AAT-1	0.300	0.439	0.370	0.373
ADH-1	0.090	0.086	0.088	0.088
GDH-1	0.242	0	0.121	0.320
GPI-2	0.300	0.361	0.331	0.332
LAP-1	0.298	0.367	0.333	0.347
MDH-1	0	0.286	0.143	0.177
PGM-1	0.340	0.325	0.333	0.333
TPI-2	0.340	0.199	0.269	0.274

Trifolium pratense

	Population			
Locus	*COUNTRY HO*	*1875 HO*	*HPOP*	*HSP*
AAT-1	0.117	0	0.059	0.061
GDH-1	0.336	0	0.168	0.174
PGI-2	0.314	0.618	0.416	0.515
LAP-1	1.083	1.108	1.096	1.184
MDH-1	0	0.086	0.043	0.056
6PG-2	0.199	0.322	0.261	0.282
PGM-1	0.233	0.158	0.196	0.202
TPI-2	0.117	0.086	0.099	0.100

Table 6. Summary of genetic apportionment in *Trifolium*.

Trifolium stoloniferum

			Proportion	
Locus	*HPOP*	*HSP*	*Within*	*Between*
GDH-2	0.564	0.643	0.874	0.123
GPI-1	0.214	0.365	0.586	0.414
PGM-1	0.259	0.499	0.519	0.481
		Species mean	0.661	0.339

Trifolium hybridum

			Proportion	
Locus	*HPOP*	*HSP*	*Within*	*Between*
AAT-1	0.370	0.373	0.992	0.008
ADH-1	0.088	0.088	1.000	0
GDH-1	0.121	0.320	0.378	0.622
GPI-2	0.331	0.332	0.997	0.003
LAP-1	0.333	0.347	0.960	0.040
MDH-1	0.143	0.177	0.808	0.192
PGM-1	0.333	0.333	1.000	0
TPI-2	0.269	0.274	0.982	0.018
		Species mean	0.881	0.119
		Mean without GDH	0.953	0.047

Trifolium pratense

			Proportion	
Locus	*HPOP*	*HSP*	*Within*	*Between*
AAT-1	0.059	0.061	0.967	0.033
GDH-1	0.168	0.174	0.966	0.034
GPI-2	0.416	0.515	0.808	0.192
LAP-1	1.096	1.184	0.926	0.074
MDH-1	0.043	0.056	0.768	0.232
6PG-2	0.261	0.282	0.926	0.074
PGM-2	0.196	0.202	0.970	0.030
TPI-2	0.099	1.000	0.990	0.010
		Species mean	0.915	0.085

of their genetic diversity. The two native species are considerably less diverse than either introduced congener. In some ways this was not anticipated, considering that the analyzed populations of introduced clovers would seem to be geographically isolated from their original gene pools. Such data suggest that the original gene pool was initially quite diverse, that both taxa were introduced a number of times, or both. The high levels of intrapopulational variation in each taxon do not preclude the former.

Both native species are characterized by low genetic diversity. In the case of *T. reflexum,* no variation was detected, although certainly there is insufficient data to determine whether this is true for the species as a whole. Our population sample is from an area where the species was thought to be extinct and may represent a recently founded population or one emerging from a genetic bottleneck. Additional studies on this species from throughout its range are being planned.

In the case of *T. stoloniferum,* the presence of even a limited amount of genetic variation was both surprising and promising. This species was thought to have been extinct since the early part of the century. Bartgis (USDI Fish & Wildlife Service 1989) had hypothesized that the newly discovered populations would be genetically depauperate. Thus the potential for survival might be limited as in the case of *Howellia* (Lesica et al. 1988) and certain other rare or endemic taxa (Hamrick 1983). The presence of high interpopulational variation in *T. stoloniferum* is similar to that found in a number of annual species (Clegg & Allard 1972; Levin 1978; Babbel & Selander 1974) but is in contrast to the situation found in *Polygonum* (Kubetin & Schaal 1979) and *Chenopodium* (Crawford & Wilson 1974) and most notably in the annual or biennial *Trifolium pratense* and the perennial *T. hybridum.* Furthermore, the presence of localized common alleles, especially at GDH-2 and PGM-1, is analogous to the situation seen in *Eucalyptus crucis* (Sampson et al. 1988). The difference in PGM-1 and GDH-2 frequencies between populations HAWN 1 and HAWN 3, which are separated by only a short distance, further supports our evidence that individual

populations are quite distinct genetically. The PGM and GDH data also support the contention of Campbell et al. (1988) that gene flow between populations is highly restricted. In *T. hybridum* and *T. pratense* there is no statistical evidence of strong interpopulational differentiation and there is no evidence of localized common alleles.

Lesica et al. (1988) have argued for a conservation strategy with *Howellia* that includes protecting all currently known populations. Their reasoning was that *Howellia*'s endangerment is due in great part to its narrow ecological niche and loss of suitable habitat. Compounding this situation is a complete lack of genetic variation in the species. The history of *T. stoloniferum* (Campbell et al. 1988) as well as its genetic structure indicates that similar conservation strategies are needed. Campbell et al. (1988) hypothesized that the decline of native clover species might be attributed to several factors, including initial habitat destruction, differential habitat maintenance, competition from exotics, poor dispersal, and a lack of rhizobial infection. Our data would suggest one additional concern: the low genetic diversity of running buffalo clover (as well as buffalo clover) is in distinct contrast to that of other species of *Trifolium* with similar life-history characteristics. At present it is difficult to tell whether this low level of variation was an initial cause of decline or if it is a result of genetic bottlenecks caused by loss of habitat, etc. The strong interpopulational variation seen in the study populations coupled with the presence of localized common alleles, however, could be the result of one or more phenomena such as drift or independent population genetic bottlenecks of varying severity. It is therefore important to locate and genetically characterize as many populations of *T. stoloniferum* as possible to identify rare or locally common alleles before those populations are extirpated. Such studies, for both *T. stoloniferum* and *T. reflexum,* are currently under way in our laboratory. The establishment and periodic genetic monitoring of new, genetically composite populations would be of interest not only scientifically but to provide a genetic reservoir for future conservation management.

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