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Genetic variability and differentiation among founder populations of the pitcher plant (*Sarracenia purpurea* L.) in Ireland

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The pitcher plant (*Sarracenia purpurea* L.), a native North American species, presently grows on six sites in Ireland, these being the result of transplants (founder events) from an initial introduction to Termonbarry Bog in 1906. The extent and organisation of genetic variability among these populations was examined in this study.

Of 25 enzyme systems investigated by starch gel electrophoresis, 14 showed specific activity. Eighteen loci suitable for routine population screening were identified. Diallelic polymorphisms were observed at three loci, *Pgm*, *6Pg*, and *Mdh*-4. Genetic polymorphism at the *Pgm* locus has not previously been described.

The overall level of genetic variation (max $P = 17$ per cent; max $\bar{H}_{exp} = 0{\cdot}060$) in Irish populations was low, but nevertheless within the range recorded for native North American populations. While it was evident that the severe founder events (2–4 individuals) had reduced the number of polymorphic loci there was lesser impact on heterozygosity levels. Significant genetic differentiation among populations was found at all three polymorphic loci.

INTRODUCTION

Natural populations of plants and animals may go through bottlenecks where numbers are greatly reduced. Often this will constitute a founding event when a few individuals colonise a new territory, and is followed by a population "flush" as numbers increase sharply again, until the carrying capacity of the new environment is reached. During such "founder-flush" events there is potential for the genetic basis of the derived population to change dramatically, both as a result of sampling effect in the bottleneck itself and in subsequent generations through genetic drift and possible relaxation of selection pressures.

A critical part of the process involves the founding event, in particular how many individuals are involved—and to what extent genetic differentiation actually occurs as a result of the sampling effect. This has been well examined experimentally, under laboratory conditions, *e.g.*, in *Drosophila* (Powell, 1978). However, very little is known about wild populations as reliable data concerning the founding event are rarely available. An exception is a small study of one population of the pitcher plant (*Sarracenia purpurea* L.) in Ohio, U.S.A., known to have been founded (artificially, but since growing wild) from a single plant in 1912 (Schwaegerle and Schaal, 1979). Evidence of genetic differentiation, including a reduction in heterozygosity, compared with ten other North American populations, was noted. This work was based on a limited electrophoretic study, only seven enzymes (ten loci) being screened.

A similar founding event involving *S. purpurea* was carried out in Ireland early this century. Also, however, a number of subsequent transplantations (further founding events) to geographically isolated sites have been carried out. These have been well documented (summarised by Foss and O'Connell, 1985). This situation thus provided the opportunity to study the genetic consequences of repeated founding events in "wild" populations. The aim of the present investigation was to determine the levels of genetic variability and differentiation in introduced populations of pitcher plants in Ireland through an extensive electrophoretic survey.

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Table 1 History and present status of *S. purpurea* in Ireland

Ref.*	Date	Location	Source	Founders	Present status
1	1906	Termonbarry Co. Roscommon	Canada	Seeds and rootstock	Thriving, *ca.* 10 ha » 1000 plants
2	?	Coolatore Co. Westmeath	T'barry	"Some plants"	Thriving, *ca.* 1 ha » 1000 plants
3	1930	Woodfield Co. Offaly	C'atore	3 plants	Thriving, *ca.* 1 ha » 1000 plants
4	1963	Bellacorick Co. Mayo	T'barry	3 plants	Poor, 39 plants over 300 m^2
5	1963	Moud's Bog Co. Kildare	T'barry	2 plants	Thriving, *ca.* 250 plants over 400 m^2
6	1966	Abbeyleix Co. Laois	T'barry	"4 clumps"	Poor, 31 plants over 100 m^2
7	1966	Ballylough Co. Roscommon	T'barry	?	Not found, probably died out
8	?	Derrydoan Co. Westmeath	T'barry?	?	Not found, probably died out

* (1) Praeger (1932); (2) Kertland (1968); (3) Kertland (1968); (4) Kertland (1968); (5) Foss and O'Connell (1984); (6) Nelson and De Vesci (1981); (7) Newell (1968); (8) Foss and O'Connell (1985).

MATERIALS AND METHODS

History of introductions

S. purpurea was first successfully introduced into Ireland in 1906 (Praeger, 1932; Foss and O'Connell, 1985). An unrecorded number of seeds and rootstock of Canadian origin were planted in Termonbarry Bog, Co. Roscommon. The plants flourished on this site becoming established over some 35 hectares (Kertland, 1968). While commercial peat cutting has reduced its present range to *ca.* 10 ha. Termonbarry remains by far the largest pitcher plant colony in Ireland.

Seven transplants from this original founding stock have been documented, the details of which are summarised in table 1 and fig. 1. Extensive searches at Ballylough and Derrydoan failed to uncover any *S. purpurea.* It is probable, as reported by Foss and O'Connell (1985), that the plants have died out at these sites. Bog drainage at both sites is a likely cause. Of the five extant transplants four were founded directly from plants taken from Termonbarry Bog. The Woodfield population, founded from plants from Coolatore, is the result of a repeated founder event.

Sampling

Hood tissue from healthy pitchers was routinely taken though other tissues (petal, flower stalk, pollen, root and pitcher stem) were also investigated. Samples were stored at 5°C until used, usually within two days. Preliminary work to establish satisfactory electrophoretic protocols was confined to pitcher plant samples from Termonbarry. During the later systematic population screening the entire populations from Abbeyleix ($n = 31$) and Bellacorick ($n = 39$) were sampled.

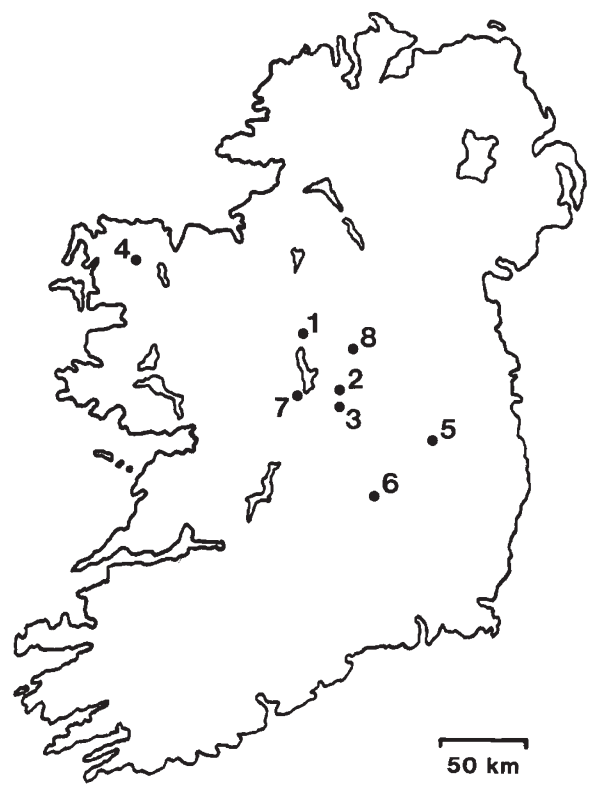

Figure 1 Documented sites for the occurrence of *S. purpurea* populations in Ireland. (Reference key is given in table 1.)

For each of the four much larger populations 100 plants were sampled. Each site was divided into four roughly equal sized sectors and 25 plants were sampled at random from within each sector.

Electrophoresis

Plant tissue was diced and then milligram quantities were hand homogenised in an equal volume of extracting buffer (0·5 M phosphate, *p*H 7·0, containing 0·5 per cent 2-mercaptoethanol). Before homogenising, an equal weight of caffeine (to tissue) was added to sequester phenolic compounds. Extracts not so treated yielded little enzyme activity.

Horizontal starch gel electrophoresis (12 per cent starch) was performed as described by Ferguson (1980). Three main buffer systems were employed, detailed in table 2. Following electrophoresis gels were sliced into 3–5 horizontal slices and histochemically stained for specific enzyme activity. Twenty five enzymes were investigated, staining recipes being taken from Harris and Hopkinson (1976) and Vallejos (1983).

Genetic nomenclature

Following standardised nomenclature a gene locus is referred to by a set of initials, *e.g. Mdh*, while the capitalised abbreviation, *e.g.*, *MDH*, refers to its protein (enzyme) product. Multiple loci are identified by hyphenated numerals, *e.g. Mdh*-2, numbered sequentially from the most cathodally migrating protein product. Alleles, in parentheses, are designated according to the relative electrophoretic mobility of their protein products, the most common allele being arbitrarily termed "100". Thus, for example, *Mdh*-2 (100/50) refers to a heterozygote genotype at the *Mdh*-2 locus involving a variant allele whose homomeric enzyme product migrates half as far as the homomeric product of the common allele.

Genetic analyses

Allele frequencies were determined by direct allele counting. The *G* statistic (with Yate's correction where appropriate) was used to compare observed genotype numbers with those expected under Hardy-Weinberg equilibrium (Ferguson, 1980). Proportion of polymorphic loci (*P*, 99 per cent criterion), number of alleles per locus and observed and calculated heterozygosity (H_{obs} and H_{exp}) were computed by standard methods (Ferguson, 1980). Gametic phase equilibrium among loci was examined by linkage disequilibrium analysis, employing the maximum likelihood procedure of Hill (1974).

Inter-population genetic diversity was investigated using two standard measures. Single locus

Table 2 Details of electrophoretic buffers routinely used in this study

(1) TCB (Ferguson, 1980)

Electrode: (*p*H 8·6)	0·3M boric acid 0·1M lithium hydroxide
Gel: (*p*H 8·6)	0·076 Tris 0·005M citric acid 0·015M boric acid 0·005M lithium hydroxide

Gels were run for 5 hours at 40 mA constant current

(2) CAM (Clayton & Tretiak, 1972)

Electrode:	0·04M citric acid *p*H 6·1	Gel:	0·002M citric acid *p*H 6·3

Both buffers adjusted to *p*H with N-(3-aminopropyl)-morpholine
Gels were run for 6 hours at 20 volts/cm constant voltage

(3) TVB (modified from Schwaegerle & Schaal, 1979)

Electrode: (*p*H 8·0)	0·5M Tris 0·65M boric acid 0·016M EDTA, di sodium salt
Gel: (*p*H 8·0)	1 in 5 dilution of electrode buffer + 0·04 mM NADP

Gels were run for 4·5 hours at 40 mA constant current

comparisons were tested for homogeneity by a genic contingency chi-square analysis (Workman and Niswander, 1970). Multi-locus comparisons involved the computation of mean genetic identity ($\bar{I}$) values (Nei, 1972) for each pairwise combination of populations.

RESULTS

Zymograms

Initial screening for suitable enzyme systems was carried out on Termonbarry pitcher plants. Of the 25 enzymes investigated 11 showed no significant specific activity. These were (EC number): aconitase (4.2.1.3), acid phosphatase (3.1.3.2), alkaline phosphatase (3.1.3.1), adenosine deaminase (3.5.4.4), aldolase (4.1.2.13), fumarase (4.2.1.2), glycerol-3-phosphate dehydrogenase (1.1.1.8), lactate dehydrogenase (1.1.1.27), mannose phosphate isomerase (5.3.1.8), peroxidase (1.11.1.7) and shikimate dehydrogenase (1.1.1.25). These enzymes were not investigated further. The remaining 14 enzyme systems showed specific activity. These are detailed in table 3.

At least 100 individuals were screened for the 14 enzyme systems. From the resulting zymograms a conservative estimate of the number of encoding loci was made and those loci suitable for routine population screening (*i.e.*, giving clear, consistent and repeatable resolution in all individuals) were identified. This information is summarised in table 3.

In each case enzyme expression in pitcher hood tissue was found to be representative of the whole plant, no expression of additional loci being apparent from extracts of other tissues. Extracts from developing and mature pitcher hoods showed only quantitative differences in locus expression.

Three diallelic polymorphisms were observed. The variant alleles 6*Pg* (75) and *Mdh*-4 (110) (or similar) have previously been described (Schwaegerle and Schaal, 1979). The absence of a heterodimeric allozyme in zymograms of pollen (haploid tissue) from heterozygous plants confirmed the genetic bases of these polymorphisms in the present study. Phosphoglucomutase resolved as a single anodal zone of activity. Two single banded (homozygotes) and a double banded (heterozygote) phenotypes were observed. This was indicative of a diallelic polymorphism for a monomeric enzyme at a single locus. The variant allele was termed *Pgm* (85). This polymorphism has not previously been described in *S. purpurea.*

Population data

At the four sites where subsampling (4×25 individuals) was performed no statistical differences in genotype numbers among subsamples were found. Thus each site was treated as a single sample of 100 plants. The allele frequency data for all populations at each of the polymorphic loci is presented in table 4. Only one case of statistically significant departure from Hardy–Weinberg pro-

Table 3 Experimental conditions and genetic interpretations for the enzymes yielding specific staining following starch gel electrophoresis

Enzyme	EC number	Abbrev.	Buffer*	Loci†	Screen‡	Polymorphic
Alcohol dehydrogenase	1.1.1.1	ADH‖	TVB	1	1	No
Aspartate aminotransferase	2.6.1.1	AAT	CAM	1	1	No
Malate dehydrogenase	1.1.1.37	MDH‖	CAM	4	4	Yes
Malic enzyme	1.1.1.40	ME	TVB	2	1	No
Xanthine dehydrogenase	1.2.1.37	XDH	TCB	1	1	No
Glutamate dehydrogenase	1.4.1.2	GDH‖	TVB	1	1	No
Glucose-6-phosphate dehyd'ase	1.1.1.49	G6P‖	TVB	1	1	No
6-phosphogluconate dehyd'ase	1.1.1.44	6PG‖	CAM	1	1	Yes
Glucose phosphate isomerase	5.3.1.9	GPI‖	TVB	2	2	No
Isocitrate dehydrogenase	1.1.1.42	IDH	CAM	2	1	No
Phosphoglucomutase	2.7.5.1	PGM	CAM	1	1	Yes
Valyl-leucine peptidase	3.4.11.–	PEP‖	TCB	2	2	No
Diaphorase	1.6.4.3	DIA	CAM	3	1	No
Esterase	3.1.1.–	EST	TCB	2	0	?
			Locus total:	24	18	

* Electrophoretic buffer systems—see table 2.
† Putative number of loci.
‡ Usable in routine population screening.
‖ Screened in Schwaegerle and Schaal (1979) study (10 loci).

Table 4 Allele frequency data and test for homogeneity (Workman and Niswander, 1970) for each polymorphic locus among Irish *S. purpurea* populations

Population	*N*	*6Pg*		*Pgm*		*Mdh-4*	
		(100)	(75)	(100)	(85)	(100)	(110)
Termonbarry	100	0·555	0·445	0·990	0·010	0·820	0·180
Coolatore	100	0·460	0·540	1	0	0·815	0·185
Woodfield	100	0·365	0·635	1	0	0·760	0·240
Bellacorick	39	0·359	0·641	1	0	0·756	0·244
Moud's Bog	100	0·510	0·490	1	0	1	0
Abbeyleix	31	0·290	0·710	0·806	0·194	0·820	0·180
	χ^2	26·74		145·82		54·08	
	df	5		5		5	
	P	<0·001		<0·001		<0·001	

portions was noted (Abbeyleix, *6Pg*; $P < 0{\cdot}01$), where a deficit of heterozygotes was observed. Linkage disequilibrium analyses were performed for all pairwise combinations of polymorphic loci in each population. From a total of 13 analyses significant gametic phase disequilibrium was apparent in two cases (Abbeyleix, *6Pg*/*Mdh-4*, $P < 0{\cdot}05$; Bellacorick, *6Pg*/*Mdh-4*, $P < 0{\cdot}05$).

Although relatively low, the level of genetic variability in the initial Irish founder stock was within the range recorded for native North American populations (table 5(a)). (This comparison was based on the ten loci examined that were common to both studies—as indicated in table 3). A comparison among the Irish populations, based on the full 18 loci screened, indicates that, in general, the founding events have led to a reduction in the number of polymorphic loci, while heterozygosity levels have not been dramatically reduced (table 5(b)). However, the greatest reduction in heterozygosity was observed for the severest founding event (*i.e.*, Moud's Bog; $n = 2$).

The founder events have led to significant allele frequency differences among populations at all three polymorphic loci, table 4. A matrix of mean genetic identity values ($\bar{I}$) between populations is given in table 6, the values ranging between 0·9924 and 0·9999. A dendrogram constructed from these data is presented in fig. 2. The closer the value of $\bar{I}$ to unity the closer related are the populations. The Moud's Bog and Abbeyleix populations were shown to be the most genetically distinct.

Table 5 Genetic variability in Irish and American *S. purpurea* populations

Population*		Polymorphic loci		Mean number of alleles	Heterozygosity	
		No.	per cent		Obs.	Exp.
(a)						
Termonbarry		2	20	1·20	0·083	0·079
Ten	mean	2·6	26	1·31	0·094	0·087
North	min.	1	10	1·10	0·050	0·040
American	max.	4	40	1·50	0·162	0·173
(b)						
Termonbarry	(?)	3	17	1·17	0·047	0·045
Coolatore	(?)	2	11	1·11	0·036	0·044
Woodfield	(3)	2	11	1·11	0·038	0·046
Bellacorick	(3)	2	11	1·11	0·038	0·046
Moud's Bog	(2)	1	6	1·06	0·030	0·028
Abbeyleix	(4)	3	17	1·17	0·047	0·060

(a) Based on 10 loci common to both studies. American data from Schwaegerle and Schaal (1979).
(b) Based on full 18 loci.
* Number of founders in parentheses.

Table 6 Mean genetic identity values, $\bar{I}$ (Nei, 1972), for Irish *S. purpurea* populations. (Based on 18 loci)

		1	2	3	4	5
1	Termonbarry	—				
2	Coolatore	0·9993	—			
3	Woodfield	0·9977	0·9991	—		
4	Bellacorick	0·9975	0·9989	0·9999	—	
5	Moud's Bog	0·9982	0·9978	0·9956	0·9953	—
6	Abbeyleix	0·9940	0·9960	0·9976	0·9977	0·9924

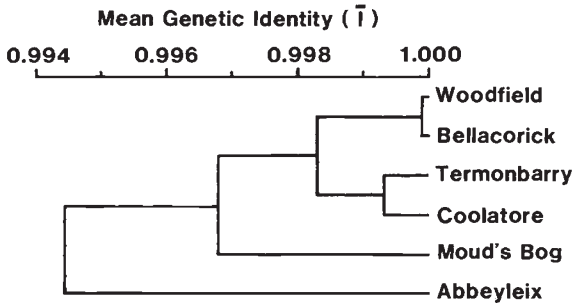

Figure 2 Dendrogram based on mean genetic identity values ($\bar{I}$, Nei 1972) for Irish *S. purpurea* populations.

DISCUSSION

Eleven enzymes failed to show specific activity following starch gel electrophoresis. Plant tissues present special problems in the isolation of enzymes owing primarily to the presence of inhibitory compounds such as phenolics and quinones (Loomis, 1974). Similar difficulties have been encountered in most plant electrophoretic studies (Gottlieb, 1981). However the number of loci available for study in *S. purpurea* was increased from ten (Schwaegerle and Schaal, 1979) to 18 in this study. Despite these additions the number of informative loci observed for Irish pitcher plant populations was low, only three polymorphic loci being scored. While this precluded a comprehensive study of the founder events, general underlying trends were evident.

The level of genetic variation in the initial founder stock at Termonbarry was within the range recorded for other North American populations. Therefore, although low, there is no evidence to suspect that this was primarily the result of a severe founder event. Indeed the presence of a rare allele (*Pgm* (85), frequency = 0·01) would suggest the number of original founders numbered at least 30–50 individuals. With the original planting in 1906 consisting of "seeds and rootstock" (Praeger, 1932), is is plausible that such relatively large numbers were involved.

It is a generally held view that founder events substantially reduce genetic variability. However theoretical considerations would suggest otherwise (Nei *et al.*, 1975; Chakraborty and Nei, 1977). While rare alleles are undoubtedly lost during such events these contribute only minimally to average heterozygosity (the most commonly reported measure of genetic variability). $\bar{H}$ is mainly influenced by "mid frequency" alleles. Such alleles are likely, by chance sampling, to be well represented in founding stocks, maintaining relatively moderate levels of heterozygosity. This view was supported by the pitcher plant data. While, in all but the Abbeyleix sample, the founding event had resulted in the loss of the rare *Pgm* (85) allele, $\bar{H}_{exp}$ was only substantially reduced in the severest founder event (Moud's Bog). The latter reduction can be attributed to the loss of a "mid frequency" allele (*Mdh*-4 (110)), in this population. The repeated founder event experienced by the Woodfield population had no apparent additional effect on heterozygosity level.

It is clear from the above considerations that the effects of founder events on genetic variability measurements are largely dependent upon the pattern of variation present in the original stock. In this respect the Termonbarry population, with one rare and two mid-frequency diallelic polymorphisms, presents an atypical, and analytically restrictive, situation. Had a number of low frequency polymorphisms (*i.e.*, variant allele frequency of around 0·1) been identified a more varied range of heterozygosity values across the founded populations could have been expected.

The founding events and subsequent drift/selection have had a more dramatic impact on differentiation, all three loci showing highly significant allele frequency differences among the populations. The two most genetically distinct populations (lowest $\bar{I}$ values) illustrate the two basic mechanisms by which differentiation can

arise in such situations: (a) by overall loss of polymorphic loci—Moud's Bog; and (b) by the chance occurence and proliferation of a rare allele—Abbeyleix. Considering their recent derivations from a single population it was not surprising that $\bar{I}$ values for the Irish populations were generally higher than those recorded for widely geographically separated North American populations (range 0·934–0·999, mean = 0·974; Schwaegerle and Schaal, 1979).

The occurrence of heterozygote deficiency and linkage disequilibrium among loci in the two smallest populations probably reflects non random sampling, *i.e.*, from distinct family groups. Such inbreeding was particularly evident in the Abbeyleix population where 28 of the 31 individuals present were found in four distinct groups of around one metre diameter, possibly reflecting the four "clumps" originally transplanted (Nelson and De Vesci, 1981). Each group had a unique genotype combination for the three polymorphic loci. Despite their close proximity (max. distance between groups being six metres) there was no evidence of significant gene flow among the separate groups. For example the *Mdh*-4 (110) allele was restricted to one group, the *Pgm* (85) allele to two groups. This could not be solely attributed to vegetative reproduction, a number of distinct seedlings being sampled.

From the limited data available there was no indication that the present status of the *S. purpurea* populations in Ireland was correlated to either the presence or absence of any particular allele or to overall heterozygosity. Rather, observations would suggest that environmental factors were the main influence. The largest colonies were those in the wetter bogs and within sites the major concentrations of plants were to be found in the wettest regions. Therefore the allele frequency differences may be attributed solely to the founding events and subsequent genetic drift.

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