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RECOMBINATION SYSTEM AND GENETIC DIFFERENTIATION IN THREE *TRIFOLIUM* SPECIES

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A variety of hypotheses regarding the relationship of recombination system to population genetic structure ,were tested with allozyme genetic variation data for three biotically sysmpatric and closely related species of *Trifolium*, each with a different recombination system. The measures of gene deversity and genetic distance statistics revealed considerable interpopulation differentiation in the inbreeder (*T. arvense*) indicating a relative lack of gene flow between its populations. On the other hand, the populations of both the outbreeders (*T. repens* & *T. pratense*) showed remarkable genetic similarity which could sloley be attributed to the extensive gene flow which occurs in both the species.

INTRODUCTION

Reproduction involves one of three recombination systems i.e. outcrossing, selfing or cloning, or some combination of the three (Fryxell, 1957). How these reproductive modes affect the genetic structure of a species has been the subject of considerable discussion. The theory generated by this discussion predicts that different recombination systems ought to strongly influence the level and distribution of genetic variation. Patterns of heterozygosity and genetic differentiation may be determined by the recombination system (Wright, 1969; Brown, 1979; Ellstrand and Levin, 1980). Populations of predominant selfers are expected to have higher levels of interpopulation differentiation than the obligate outcrossers (Jain, 1976). Theoretical studies also show that clonal outbreeding species maintain higher levels of heterozygosity, both within population and species-wide, since they can retain mutant alleles by sheltering them from segregation (Lokki, 1976), but they are usually characterized by low level of interpopulation genetic differentiation (Levin and Kerster, 1971). The ideal test of these hypotheses would be to compare population structure of three closely related, sympatric species: an obligate outcrosser, a selfer, and a clonal outbreeder.

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The present study is an attempt to determine the population genetic correlates of different recombination systems in three species of *Trifolium*, which are closely related taxonomically but differ in their breeding systems. The species are *Trifolium arvense*, *Trifolium pratense* and *Trifolium repens*. The first is an annuul inbreeder. The second is a perennial obligate outbreeder and the third one is a clonal perennial obligate outbreeder.

MATERIALS AND METHODS

Two sites containing natural populations of each of the three *Trifolium* species were chosen for study, close to the Botanic Gardens of the University College of North Wales, Bangor, U.K. Fifty adult plants were chosen at random, labelled and leaf tissue collected from them in early spring of 1982 and stored for future electrophoresis. The mature seed heads were collected from the lebelled plants and sown in faimly units in a heated glasshouse. All families, which contained at least 8 progeny, were then electrophoresed. Electropheresis was carried out on 7.5% polyacrylamide slab gels using LKB Multiphor equipment, the details of which are given by Saleem (1984). The electrophoresis data for the parents and progeny were used to estimate the per locus effective level of outcrossing, using the method of Clegg (1980), in order to quantify the breeding system in each population of the three species. The data on electrophoretic gene frequencies were used to calculate different measures of gene diversity, interpopulation genetic differentiation and genetic distance statistics. Quantitative estimates of the mating system parameters in each population of the three species have already been reported (Saleem, 1985). On the basis of these estimates, the breeding system of *T. arvense* is one of predominant inbreeding with more than 65% effective self-fertilization. The amounts of natural outcrossing (not less than 90%) in both *T. repens* and *T. pratense* suggest that both the species are outbreeders.

RESULTS AND DISCUSSION

A battery of statistics is developed by Nei (1975) to partition gene diversity within a species into intra and interpopulation components. The most widely used measure of genetic variation is H_t, the total gene diversity, and calculated as $H_t = H_s + D_{st}$, where H_s is the average gene diversity within populations and D_{st} is the average gene diversity among populations. H_t varies from 0 for a monomorphic locus to nearly 1.0 for a highly polymorphic locus. When averaged over all loci, total gene diversity is the estimate for mean heterozygosity under the assumption of panmixia. This measure is also called "heterozygosity per locus" (Lewontin, 1974). The gene deversity data for the three *Trifolium* species are summarized in Table I. Summing over all loci, the total gene diversity of *T. repens* is 0.494 vs 0.497 in *T. pratense* and 0.247 in the selfing *T. arvense*. The values of D_{st} range from 0 (all populations have alleles at the same frequency) to 1 (when all populations are fixed for a different one of two or more alleles). The average D_{st} values for the three species are 0.002 (*T. repens*), 0.001 (*T. pratense*) and 0.044 for *T. arvense*.

Table I. *Interpopulation gene diversity in Trifolium species*

Species	Locus	H_t	H_s	D_{st}
T. repens	APH_1	0.487	0.486	0.001
	$ATPase_2$	0.5	0.497	0.003
	EST_1	0.492	0.488	0.004
	LAP_1	0.497	0.497	0.000
	MDH_4	0.496	0.495	0.001
Mean		0.494	0.493	0.002
T. pratense	APH_1	0.498	0.497	0.001
	EST_1	0.5	0.5	0.00
	EST_2	0.497	0.496	0.001
	LAP_1	0.5	0.5	0.00
	MDH_1	0.492	0.490	0.002
Means		0.497	0.497	0.001
T. arvense	APH_1	0.204	0.177	0.027
	EST_1	0.289	0.227	0.062
	MDH_2	0.269	0.218	0.051
	PER_1	0.226	0.192	0.034
Means		0.247	0.204	0.044

Since D_{st} involves comparing populations with themselves, a non-baised value of interpopulation gene diversity or the absolute degree of gene differentiation is defined as $D_m = D_{st}/s\text{-}1$ where s is the number of populations sampled.

As is evident the estimates of both the absolute measures of population differentiation i.e., D_m and D_{st}, calculated for the polymorphic loci are considerably higher in *T. arvense* than in either outbreeder (Tables 1-2).

Table 2. *Estimates of population differentiatiation by locus*

Species	Locus	D_m	G_{st}	R_{st}
T. repens	APH_1	0.002	0.002	0.004
	$ATPase_2$	0.005	0.005	0.01
	EST_1	0.008	0.008	0.017
	LAP_1	0.000	0.000	0.000
	MDH_4	0.001	0.001	0.002
Mean		0.003	0.003	0.007
T. pratense	APH_1	0.002	0.002	0.003
	EST_1	0.003	0.003	0.005
	EST_2	0.002	0.002	0.003
	LAP_1	0.003	0.003	0.005
	MDH_1	0.003	0.003	0.003
Means		0.003	0.003	0.004
T. arvense	APH_1	0.053	0.13	0.299
	EST_1	0.122	0.211	0.537
	MDH_2	0.102	0.191	0.471
	PER_1	0.068	0.149	0.351
Means		0.066	0.17	0.415

In considering the coefficient of differentiation (G_{st}), the polymorphic loci of *T. arvense* gave considerably higher values than either *T. repense* or *T. pratense*; while average R_{st} values which compare interpopulation gene diversity to intrapopulation gene diversity, were 0.007 in *T. repens*, 0.004 in *T. pratense* and 0.415 in *T. arvense* (Table 2). This implies that there was a gereater amount of between population gene diversity in the selfing populations than among the outbreeder's populations.

Table 3 : Genetic identity (I), and genetic distance (D) in populations of *Trifolium* species.

		Pop 1	Pop 2	
T. repens	Pop 1		0.0104	(D)
	Pop 2	0.9896		
		(I)		

		Pop 1	Pop 2	
T. pratense	Pop 1		0.0039	(D)
	Pop 2	0.9961		
		(I)		

		Pop 1	Pop 2	
T. arvense	Pop 1		0.1074	(D)
	Pop 2	0.8981		
		(I)		

To obtain quantitative measures of the number of gene differences between populations of the three species, Nei's genetic distance (Nei, 1972) was calculated for each pairwise combination of populations. Genetic distance represents the amount of between population differences in allele frequency over all loci tested. The data given in Table 3 show very little differentiation between populations of *T. pratense* (D = 0.0039) and to a lesser degree between populations of *T. repens* (D = 0.0104). The two populations of *T. arvense* were found to be highly differentiated genetically (D = 0.1074).

The patterns of gene diversity and genetic distances show that the populations of the selfing *T. arvense* are more highly differentiated than the related populations of outbreeders. Greater allozymic differentiation among populations of the self-compatible *Phlox cuspidata* than its self-incompatible relative has been reported by Levin (1978). Populations in the selfing group of *Gilia* were more differentiated than populations in the outcrossing group (Schoen, 1982). A similar trend was reported by Brown (1979) in a summary of geographical patterns of allozymic variation in outcrossing and selfing plant species.

Thus the genetic structure of the *Trifolium* species as determined by electrophoretic genetic variation revealed patterns of genetic variation predicated by simple theoretical models. The recombination system appears to be good predictor of the distribution of overall gene diversity within and among populations in the three *Trifolium* species studied.

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