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 excensive 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 repductive 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 heteroxygosity and genetic differentiation may be determined by the recombination system (Wright, 1969; Brown, 1979; Ellstrand and Levin, 1980). Populations of predominant adfers are expected to have higher levels of interpopulation differentiation than the obligate outcrossers (J.4in, 1976). Theoretical studies also show that clonel outbreeding species maintain higher levels of heterozygosity, both within population and specieswide, 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 pratanse and Trifolium repens. The first is an annual 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. Pifty adult plants were chosen at random, labelled and lasf 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 own 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 Salvem (1984). The electrophoresis data for the parents and progeny were used to estimate the per locus effective level of outcrossing, using the method of Clogg (1980), in order to quantify the breeding system in each population of the three species. 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 cach 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. repeas and T. pratence suggest that both the species are outbreeders.

RESULTS AND DISCUSSION

A battery of statistics is developed by Nei (1976) to partition gene diversity within a species into intre 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 gens diversity is the estimate for mean heterotygosity under the assumption of panmixia. This measure is also called "heterotygosity 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. arrense. The values of Dat 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 Dat values for the three species are 0.002 (T. repens), 0.001 (T. pratense) and 0.044 for T. arrense.

Table 1. Interpopulation gene diversity in Trifolium species

Species	Locus	Нt	H	D_{st}
T. repens	APH ₁	0,487	0.486	100.9
	ATPase2	0,5	0.497	0.003
	EST_1	0.492	0.488	0.004
	LAP_1	0.497	0,497	0.000
	MDH4	0.496	0.495	0.001
Mean		0.484	0.493	0 002
T. pratense	APH,	0.498	0.497	0.001
	EST ₁	0 5	0.5	0.00
	EST ₂	0.497	0.498	0 001
	I.AP ₁	0.5	0.5	0.00
	ndH'	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,	0 226	0.192	0.034
Means		0,247	0.204	0.044

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

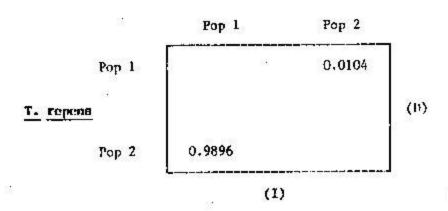
As is evident the estimates of both the absolute measures of population differentiation i.e., D_{m} and D_{st} , calculated for the polymorphic loci are considerably higher in T arvense than in either outbreeder (Tables 1-2).

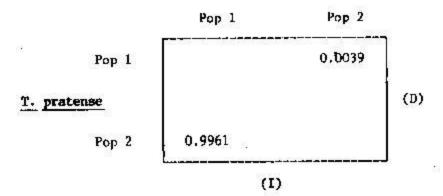
Table 2. Estimates of population differentiation by locus

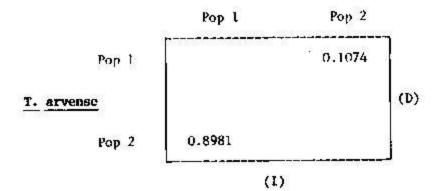
Species	Locus	$\mathbf{D}_{\mathbf{m}}$	Get	Rat
	APH ₁	0.002	0,002	0.004
	ATPaso2	0,005	0.005	0.01
Т. горена	EST ₁	0,008	0.008	0.017
	LAP ₁	0.000	0,000	0.000
	MDH₄	100.0	100.0	0,002
Mean		0,003	0.003	0.007
	APH,	0.002	0,002	0.003
	EST ₁	0.003	0,003	0,008
T. pratense	EST ₂	0.002	0.002	0.003
	LAP,	0.003	0 003	0.008
	MDH	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	0.068	0,149	0.851
Mesns		0.086	0.17	0.415

In considering the coefficient of differentiation (G_{ab}) , the polymorphic loci of T, arvense gave considerably higher values than either T, repease or T, pratense; while average R_{ab} values which compare interpopulation gene diversity to intrapopulation gene diversity, were 0.007 in T, repeas, 0.004 in T, pratense and 0.415 in T, errense (Table 2). This implies that there was a generator 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.







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, pratence (D=0.0039) and to a lesser degree between populations of T, repens (D=0.0104). The two populations of T, arrease 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 authreeders. Greater allozymic differentiation among populations of the self-compatible Phlox cuspidate 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 auterossing group (Schoen, 1982). A similar trend was reported by Brown (1970) 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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