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EFFECT OF BREEDING SYSTEM ON THE GENOTYPIC STRUCTURE IN *TRIFOLIUM* SPECIES

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Abstract

The effect of breeding system on the genotypic structure was studied in 3 *Trifolium* species with contrasting breeding systems. The two out-breeder's populations were more variable genetically and more heterozygous than inbreeder's populations. However, the inbreeder's populations exhibited an excess of heterozygotes compared with panmictic expectations adjusted for the known amount of inbreeding; whereas the outbreeder's populations were as heterozygous as would be expected. Some of the several forces which might account for the so-called "heterozygosity paradox" are discussed.

Introduction

The genetic system of an organism is highly complex and comprises many components of evolutionary importance, such as rate of mutation, chiasma frequency and location, chromosome arrangement, degree of ploidy, generation time and breeding system. It is noteworthy that these components are interrelated with each other and affect the distribution of genetic variability in one way or an other. Due to this inter-relationship it is necessary to confound the effects of other components before one aspect can be studied in detail. This topic has been approached theoretically and treated extensively within mathematical and conceptual contexts by Wright (1965), Allard *et al.* (1968), Grant (1971), Kimura & Ohta (1971) and Brown (1979). The particular aspect of the genetic system was the effect of breeding system on the genotypic structure in natural populations of three *Trifolium* species which differ in their breeding systems.

Materials and Methods

Two sites for each of the three *Trifolium* species were surveyed around Bangor, U.K. to sample the maternal populations. The criteria used to choose a population were that it occured in a natural habitat and that it contained at least 100 plants making up a single continuous population, in order to be able to sample at least 50 plants which appears to be adequate sample size, large enough to provide about 50 wild genomes for each locus (Lewontin, 1974). Fifty random plants were sampled and labelled carefully at each site. Fresh leaves for electrophoresis were taken from each plant and placed

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separately in polythene bags with some moist cotton wool inside. The leaf tissue kept in this way remained suitable for use in electrophoresis for up to four weeks.

Preliminary electrophoretic runs were carried out to choose the gel and electrode buffer systems which gave satisfactory results and consistent zympgrams for the three species. This was to minimize differences in treatment between species which could confound estimates of differences in degree of genetic polymorphism. In all, eight different enzyme systems were assayed for each species representing different loci and the resulting data were used to determine various population genetics parameters. Wright's fixation index was calculated for each polymorphic locus. The details of the laboratory procedures are given by Saleem (1984).

Results and Discussions

The proportional increase of homozygosity (F) in excess of that expected under panmixia was calculated, using Wright's fixation index (Wright, 1965) which is considered a convenient measure of genotypic structure at a single locus. The value of F can range from −1 (extreme heterozygote excess) to +1 (no observed heterozygotes) in a polymorphic population. In general, positive F values indicate a deficit of heterozygotes and negative values of F an excess of heterozygotes compared with Hardy-Weinberg expectations. The F values alongwith their standard errors and the observed heterozygosities for each polymorphic locus for the three *Trifolium* species are given in Table 1. The mean F values for the two populations of *T. repens*, a clonal outbreeder, were slightly less than the mean F values for the two populations of *T. pratense,* an outbreeder (Table 1). However, in neither species was F much different from the equilibrium value of zero. The two populations of the inbreeding *T. arvense* showed high values of F, which were positive, indicating a dificiency of heterozygotes compared with random mating expectations (Table 1).

High positive values of F arise from either inbreeding due to self-fertilization or from differences in allele frequencies of the effective pollen pool of each plant within one population (Wahlund effect). This second mechanism represents inbreeding relative to the genotypic composition to be expected if the sampled population did constitute one panmictic unit (Brown *et al,* 1975). Since there was no heterogeneity or variation in the mating system as has been reported (Saleem, 1984), the observed departure of F from panmixia in populations of *T. arvense* can solely be attributed to inbreeding due to self-fertilization.

One significant feature of the observed F values (F_o) are the directions of their deviations from expectations (F_e). F_e values represent Hardy-Weinberg proportions adjusted for the known amount of inbreeding. The difference between the observed and the expected value of F is defined as ΔF (Brown, 1979). Negative F values indicate that

Table 1. Estimates of Wright's fixation index and observed heterozygosities for each polymorphic locus in two populations of *Trifolium repens, T. pratense* and *T. arvense.*

Locus	Pop 1 F-value	± S.E.	Heterozygosity	Pop 2 F-value	± S.E.	Heterozygosity
Trifolium repens						
APH_1	0.083	0.05	0.44	0.026	0.04	0.48
$ATPase_2$	–0.042	0.06	0.52	0.151*	0.06	0.42
EST_1	0.033	0.04	0.46	0.042	0.05	0.52
LAP_1	0.077	0.06	0.46	0.114*	0.05	0.44
MDH_4	0.026	0.05	0.48	0.157*	0.04	0.42
PER_3	–	–	–	0.062	0.05	0.46
Means	0.035		0.47	0.078		0.46
Trifolium pratense						
APH_1	0.160*	0.03	0.42	0.71	0.05	0.46
EST_1	0.118*	0.05	0.44	–0.010	0.04	0.50
EST_2	0.026	0.06	0.48	0.038	0.05	0.48
LAP_1	–0.042	0.04	0.52	–0.004	0.03	0.50
MDH_1	0.32	0.04	0.48	0.015	0.05	0.48
Means	0.059		0.47	0.022		0.48
Trifolium arvense						
MDH_2	0.448**	0.08	0.24	–	–	–
PER_1	0.273**	0.09	0.28	–	–	–
APH_1	–	–	–	0.266**	0.07	0.26
EST_1	–	–	–	0.341**	0.08	0.30
Means	0.36		0.26	0.303		0.28

*F significantly different from 0.0 at 5% level.

the population contains more heterozygotes than expected and positive ΔF values are indicative of fewer heterozygotes than expected.

Mean ΔF values, weighted by the number of polymorphic loci per population and averaged over all populations in a species were found to be 0.001 for *T. repens*, 0.01 for *T. pratense* and -0.18 for *T. arvense* (Table 2). Thus the outbreeders are as heterozygous as expected at the polymorphic loci, and the selfing *T. arvense* is considerably more

Table 2. Average observed fixation index (F_o), expected fixation index (F_e) based on average estimated outcrossing rate (t), and average ΔF values in *Trifolium* species.

Species	Population	F_o	$F_e = \frac{1-t}{1+t}$	$\Delta F = F_o - F_e$	t
Trifolium repens	Pop 1	0.035	0.053	−0.018	0.90
	Pop 2	0.078	0.058	0.02	0.89
Means		0.0565	0.0555	0.001	0.895
T. pratense	Pop 1	0.059	0.036	0.023	0.93
	Pop 2	0.022	0.026	−0.004	0.95
Means		0.041	0.031	0.01	0.94
T. arvense	Pop 1	0.36	0.53	−0.17	0.31
	Pop 2	0.30	0.49	−0.19	0.34
Means		0.33	0.51	−0.18	0.325

heterozygous than expected based on the observed amount of inbreeding. A general excess of heterozygotes over levels expected with no selection was found for six isozyme loci in two natural populations of *Avena barbata* (Marshall & Allard, 1970).

A variety of mechanisms have been put forward to explain "the heterozygosity paradox" (Brown, 1979). Finite population size and temporal fluctuations in outcrossing rate are two of the factors which can produce an excess of heterozygotes in inbreeders. It has been pointed out (Jain & Rai, 1974; Jain, 1975) that in the island model of subdivision, ΔF may be greater than +0.10 or less than −0.10 for moderate subpopulation sizes of 100. Neighbourhood size in populations of the inbreeding *T. arvense* is considerably more than 100 (494 in Pop 1 and 541 in Pop 2; Saleem, 1984) and it, therefore, seems unlikely that small population size has played a role in producing the excess of heterozygotes. Temporal fluctuations in outcrossing rate could also lead to a negative ΔF (Nei, 1975). This occurs because in any generation following one in which there has been an unusual burst of outcrossing, the frequency of heterozygotes can be much higher than the equilibrium value under constant outcrossing (Brown, 1979). Occasional bursts of outcrossing have been reported in predominantly inbreeding species (Stebbins, 1957). Theoretical work using computer models (Wu & Jain; cited in Jain, 1975) showed that such variation in outcrossing produces a higher than expected level of heterozygosity. Fluctuation in outcrossing leads to a biased estimate of ΔF because

the expected level of fixation for a given amount of inbreeding (F_e) may be over– or underestimated (Brown, 1979), and the bias can be reduced by making several temporally or spatially independent estimates of outcrossing and using the observed mean in a test of ΔF. In the present studies, outcrossing rates in populations of *T. arvense* were estimated for only one generation due to limited time period and were substantially higher than levels in many predominantly inbreeding species. If outcrossing rates in this species had been estimated over a period of time, it would be possible to determine whether fluctuations in outcrossing may affect estimates of ΔF.

An alternative explanation for an excess of heterozygotes in inbreeding species is that of heterozygote advantage at the loci sampled or at loci in strong linkage disequilibrium with the loci sampled (Brown, 1979). Thus, while selfers may produce fewer heterozygotes than outcrossers, viability selection in favour of heterozygotes would raise heterozygote frequencies among the surviving members of the population (Schoen, 1982). This phenomenon has been demonstrated in natural populations of different inbreeding species (Clegg & Allard, 1973; Fatunla & Frey, 1980; Schoen, 1982). During the course of the present studies, genotype frequencies were not assessed at different stages of the life cycle to determine whether heterozygote selection occurred between the seed and adult stages of the life cycle and, therefore, the possibility of heterozygote advantage cannot be ruled out.

Nevo (1978) in his review of genetic variation in natural populations, points out that the measures of genetic variation currently in use have large variances in widespread species and are applicable only to population estimates and not to species, although they are used in this way in many studies. It is therefore important to sample populations across a species range if it is desired to interpret variation in both percentage polymorphism and levels of heterozygosity. Hamrick *et al.* (1979) have shown that besides the effect of life-history and habitat characteristics in determining the patterns of genetic variation, there are "unknown variables such as past historical events which must play a significant role in the genetic structure of plant populations". However, it may be concluded that the patterns of genetic variation observed in the three *Trifolium* species are consistent with simple theoretical models, although they might be characterized by different life-histories or habitats.

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