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GENE FLOW AND POPULATION STRUCTURE IN TWO SPECIES OF *TRIFOLIUM*

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ABSTRACT

Population structure was determined in natural populations of two *Trifolium* species which differ in their breeding systems. Gene flow was estimated from pollen and seed dispersal distributions, and the effective level of outcrossing. It is shown that the measurement of breeding system alone does not allow inferences to be made concerning the amounts of gene flow which occur in natural populations. The inbreeding *T. arvense*, had by far the largest neighbourhood size in this study, its relative lack of gene flow mediated by its breeding system being more than compensated by its long-distance seed dispersal.

INTRODUCTION

Gene flow, the movement or dispersal of genes within or among populations, is a crucial factor determining the structure and cohesiveness of species and populations. Spatial restriction of gene flow can lead to non-random mating and to subdivision of a population into genetic neighbourhoods. On the other hand, extensive gene flow over large geographical areas can overshadow the effects of localized selective forces, leading to genetic similarity among populations and increased uniformity within a species. The extent and magnitude of gene flow can therefore, significantly modulate the impact of evolutionary pressures impinging on a population.

Wright (1946) introduced the idea of a neighbourhood and defined it as an area from which the parents of central individuals would have come with equal probability. The breeding structure of plant populations is poorly under-

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stood, although it is clear that a plant population is not a panmictic unit and that gene exchange among populations may occur at very low levels (Levin and Kerster, 1974). Studies of population structure in plants, especially in herbaceous species, have revealed that the area of neighbourhood is sufficiently small to permit marked differentiation in response to selection over short to moderate distances (Levin, 1981). Neighbourhood size serves as a convenient parameter for measuring the decay of genetic variance under different reproductive systems (Levin and Kerster, 1971). This paper examines the nature of gene flow and describes population structure in two species of *Trifolium*, which are related taxonomically but have contrasting breeding systems. The species studied are *T. pratense* and *T. arvense*. The first is a perennial obligate outbreeder and novel for studies of gene flow through pollen as it is dependent exclusively on bumblebee species for pollination and fertilization. The seed dispersal component of gene flow in this species is rather poorly understood. *T. arvense*, is an annual inbreeder, on the other hand, and has a specialized feathery structure which encloses the seed and aids in long-range seed dispersal. The pollen component is expected to be less important in determining the population structure of this species.

MATERIALS AND METHODS

Two natural population sites for each of the two *Trifolium* species were selected around Bangor, U. K. to study neighbourhood structure. The populations were designated as Pop 1 and Pop 2 for each species. The pollen component of gene flow in both species was estimated from the pollinator flight distances. At each site, individuals pollinators were followed from the time they were first sighted, and the number of heads visited per plant, the flight distance between plants and the number of plants visited per foraging trip were recorded. All pollinator observations were made while most or all of the plants were in bloom, although the number of flowering heads per plant varied with date.

The seed dispersal component of gene flow in each of the two species was estimated on the basis of seedling germination, by measuring the distances from a mother plant. A 2-3 inch thick layer of John Innes compost was evenly spread on the ground to suppress weeds and provide substrate for seedling establishment on a 5m x 5m area free from vegetation. During the autumn several transplants of each of the two species were dug from their population sites and set up in the prepared plot. The height of the mature influorescence of each species was adjusted to the mean height measured in their natural habitats and left until the following spring to allow the seeds to disperse and germinate. Distances measured from the influorescence to the base of the seedling corresponded to the seed migration distances. To see whether the seed distribution was homogeneous, the area was divided into four equal parts and the observations recorded in each quadrant separately.

Assuming zero means, the two-way variances for pollen and seed in both *T. pratense* and *T. arvense* were estimated as :

$$\sigma^2 p = \Sigma p^2/n_p \text{ , and } \sigma^2 s = \Sigma s^2/n_s$$

where,

$\sigma^2 p$ = variance of pollen

Σp^2 = sum of sq. of pollen observations

$\sigma^2 s$ = variance of seed

Σs^2 = sum of sq. of seed observations

and n_p and n_s denote number of pollen and seed dispersal events, respectively.

The pollen and seed dispersal events were measured absolutely, thus the appropriate one-way variances for each were calculated as one-half of the true variance (Crawford, 1984).

Densities of flowering plants in populations of *T. pratense* and *T. arvense* were measured at the same time as pollen dispersal distances were recorded and the number of flowering individuals per square metre was counted. The genetically effective density (d_e) for each population was then calculated from Crawford's (1984) formula : $d_e = d/2\,(1 + t)$, where d is the density of flowering plants and t the mean outcrossing rate of the population. Outcrossing rates for each population of the two species were estimated by employing electrophoretic genetic markers and by using a computer programme which was written by Dr. C. J. Gliddon of the University College, North Wales, U.K. The programme is based on the mixed mating model of Hayman (1953) and use the joint maximum likelihood method of Clegg *et al.* (1978) to generate single-locus estimates of outcrossing rate (t) and pollen pool allele frequencies.

Using pollen and seed dispersal variances, outcrossing rate estimates and genetically effective density, neighbourhood area (A) and size (N_e) for each population of the two species will be calculated from Crawford's (1984) formula which is a modification of Wright's (1969) isolation-by-distance model.

$$A = 4\pi\,((t\sigma^2p)/2 + \sigma^2s)$$

$$N_e = 4\pi\,((t\sigma^2p/2 + \sigma^2s)\,d/2\,(1 + t)$$

RESULTS AND DISCUSSION

An accurate appraisal of pollen component of gene flow in both species may be obtained from pollinator foraging behaviour which effects plant gene dispersal (Schmitt, 1980). Mean flight distances are given in Table 1. The majority of flights were between neighbours, an observation consistent with the predictions of optimal foraging theory (Pyke, 1978). The observed flight distances have important general implications for the genetic structure of populations. Gene flow will be restricted in species which are principally pollinated by bumblebees because they are energetically constrained to a near-neighbour foraging pattern (Levin and Kerster, 1969). Reduced pollen flow may result not only from pollinator behaviour, but also from asynchronous flowering times among already limited flowering individuals. The mean distances at which seed migrated from the mother plant are shown in Table 1. The Chisquare test for heterogeneity of seedling distributions revealed no significant deviation from a uniformdistribution ($P > 0.05$) of seedling over quadrants, which implied that there was no directional dispersion of seeds in both species. The one-way pollen and seed variances are given in Table 1. A comparison of seed dispersal variances shows that the inbreeding *T. arvence* disperses its seeds over greater distances than the outbreeding *T. pratense*. This may be attributed to the fact that seeds of this species are enclosed in a feathery structure, so that wind currents help in long-range seed dispersal. Restricted pollen and seed dispersal, with associated limited gene flow, are expected to reduce levels of genetic variation because they result in small neighbourhood sizes which are more responsive to selection and more susceptible to random genetic drift (Hamrick *et al.*, 1979). Plant density affects neighbourhood area because flight parameters of pollinating insects are a function of density (Crawford, 1984). Actual standing crop densities are regarded as greater than genetically effective densities by an order of magnitude (Dobzhansky Wright *et al.*, 1942; and Wright, 1941). Self-fertilization leads to a reduction in neighbourhood number not only because neighbourhood area is reduced but also because of the inbreeding effect upon genetically effective density.

Table 1. *Means and one-way variances of pollen and seed dispersal distances, density of flowering plants (d) and mean outcrossing rate (t) in populations of Trifolium species*

Species	Population	Pollen		Seed		d	(t)
		Mean (m)	Variance (m^2)	Mean (m)	Variance (m^2)	(plants/m^2)	
	Pop 1	0.59	0.277	—	—	16.4	0.93
T. pratense		—	—	0.54	0.231	—	—
	Pop 2	0.66	0.38	—	—	13.5	0.95
	Pop 1	0.44	0.161	—	—	105.8	0.31
T. arvense		—	—	0.84	0.542	—	—
	Pop 2	0.38	0.136	—	—	113.7	0·34

The subdivision of a population into smaller neighbourhoods leads to genetic drift if the neighbourhood size is small. If the entire population is not much larger than the neighbourhood size, drift will affect the whole population. Considerable differentiation will occur if neighbourhood size is 20 or less, and a moderate amount with about 200 individuals (Wright, 1946). Neighbourhood sizes in populations of *T. pratense* are only slightly smaller than the total number of individuals (Table 2), and the entire populations of this species might have been affected by a moderate amount of genetic variation. On the other hand, in the inbreeding *T. arvense*, there is extensive gene flow through seed dispersal. Neighbourhood sizes determined in this species were surprisnigly greater than the total number of individuals of its populations and therefore the possibility of random genetic drift can be ruled out. The population structure of the two species suggests that extensive gene flow through seed dispersal and high plant densities in *T. arvense* and limited seed dispersal and short-range pollen migration in *T. pratense* are the main factors which determine their different population structure. It is clear, therefore, that understanding of the factors responsible for the genetic structure of populations may require examination of the entire genetic system, rather than only one of its components such as breeding system.

Table 2. *Estimates of neighbourhood area (A) and neighbourhood size (N) and total number of plants in T. pratense and T. arvense populations*

Species	Population	A (m^2)	N_e	Total No. of plants
T. pratense	Pop 1	4.53	72	98
	Pop 2	5.18	68	81
T. arvense	Pop 1	7.12	494	423
	Pop 2	7.10	541	456

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