

The mating system in sympatric populations of *Carduus nutans*, *C. acanthoides* and their hybrid swarms

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Levels of outcrossing were compared in two sympatric weedy species of *Carduus*, *C. nutans* and *C. acanthoides* and their hybrid swarms from Grey County, Ontario, Canada in order to determine whether: (i) a departure from random outcrossing could be demonstrated in the Grey County populations; (ii) there were species differences in outcrossing rates and selfing potential; (iii) differences were evident between pure populations of the parental taxa and the hybrid swarms; and (iv) there was evidence for spatial substructuring of the populations or inter-plant variation in outcrossing rates. Self-fertilization studies, conducted in the greenhouse on individuals of both species, indicated varying degrees of self-fertility, up to 60 per cent among plants of each species. Two estimates of outcrossing, t and t_H , were obtained from analyses of three polymorphic enzyme loci, for three populations of each species and three hybrid swarms. Outcrossing rates, t_H , were based on Wright's inbreeding coefficient, F , calculated from the observed levels of heterozygosity pooled over all progeny per population, whereas outcrossing rates, t , were obtained from analyses of progeny genotype arrays using maximum likelihood estimation procedures. Both multilocus estimates of the outcrossing rate t and t_H averaged over all loci, were significantly less than 1.0 for all nine populations studied, indicating significant departures from random outcrossing. No consistent differences in outcrossing rates were detected for populations of the two parental species and the three hybrid swarms. However, considerable inter-plant variation in values of t were observed in both *Carduus* species, although the data did not provide evidence for population substructuring.

INTRODUCTION

Knowledge of the breeding system is important in understanding the genetic composition and evolutionary potential of plant populations (Richards, 1986; Brown and Burdon, 1988). In particular, the mating system will determine genetic structure, *i.e.*, how genetic variation is distributed within and among populations (Brown and Allard, 1970; Clegg, 1980; Hamrick 1982). Studies of plant breeding systems using polymorphic enzyme loci have demonstrated that levels of outcrossing vary considerably among species and among populations of a self-compatible species (Clegg, 1980; Hamrick, 1982; Schemske and Lande, 1985). Like other attributes of the genetic system, the rate of outcrossing is primarily under genetic control, but it is also plastic and responsive to selection and other environmental influences. For example, the mating structure of

plants that utilize insect pollinators will be affected by pollinator and floral availability, the latter determined by both plant density and the number and arrangement of flowers on plants being serviced by a given pollinator (Clegg, 1980; Richards, 1986). There are other factors in addition to self-fertilization that can contribute to a departure from random mating in plant populations, including spatial substructuring of populations and assortative mating or variation in outcrossing among maternal genotypes (Clegg, 1980; Ritland, 1983). Flexibility in the mating system may be an essential factor in determining colonizing success of a plant species (Brown and Burdon, 1988).

The purpose of this study was to compare levels of outcrossing in two sympatric weedy species of *Carduus*, *C. nutans* L. ($2n = 16$) and *C. acanthoides* L. ($2n = 22$) and their hybrid swarms from Grey County, Ontario, Canada. The two *Carduus* species show distinct differences in flower head size, number and arrangement, with the solitary, nodding flower heads of *C. nutans* being much

larger than those of *C. acanthoides*, which is characterized by clusters of erect flower heads. Such differences presumably affect resource availability and patterns of pollinator activity and may well be reflected in differential rates of actual outcrossing. Both species are introduced weeds, primarily of roadsides, disturbed ground, fields and pasturelands in Canada (Desrochers *et al.*, 1988a). Both are self-compatible and are visited primarily by *Bombus* and *Apis* spp., suggesting a high degree of outcrossing. Indeed, evidence for random outcrossing was obtained for two Kansas populations of *C. nutans*, while those from semi-isolated plants in the same area were highly variable (Smyth and Hamrick, 1984). Estimates of outcrossing rates are not available for *C. acanthoides*.

The Grey County hybrid zone offered a number of distinct advantages for this study. The two parental taxa occur sympatrically, ensuring that potential pollinator availability is similar for both. *Carduus* populations from this region have been intensively studied over a number of years, so that an extensive data base has been established (Moore and Mulligan, 1956, 1964; Warwick and Bain, 1987; Warwick *et al.*, 1989). Studies by Moore and Mulligan (1964) have provided evidence for variable and quite high rates of self-fertility among plants of both species from this region, suggesting the potential for departures from random mating. Since the region is a hybrid zone, characterized by both pure populations of each of the two parental species and numerous hybrid swarms, one is able to test whether hybridization and backcrossing, with their potential interruption of the plant's genetic system (Moore and Mulligan, 1964), have affected rates of outcrossing in the hybrid swarms relative to parental populations. Previous studies by (Warwick *et al.*, 1989)

have shown that although pollen fertility was high for all plants from the three hybrid swarms included in this study, differential seed set among plants was evident.

In the present study, levels of outcrossing were estimated for three populations of each of *C. nutans* and *C. acanthoides* and three hybrid swarms in order to determine whether: (i) a departure from random outcrossing could be demonstrated in the Grey County populations; (ii) there were species differences in outcrossing rates and selfing potential; (iii) differences were evident between pure populations of the parental taxa and the hybrid swarms; and (iv) there was evidence for spatial substructuring of the populations or inter-plant variation in outcrossing rates.

MATERIALS AND METHODS

Materials

Table 1 gives the location, habitat and relative density of the nine populations studied, including three populations of *C. nutans*, three populations of *C. acanthoides* and three hybrid swarms. Voucher specimens from each of the nine populations have been placed in the Herbarium at Agriculture Canada, Ottawa, Ontario.

Self-fertilization

Seed set under self-pollination was measured by bagging five flower heads from 22 and 17 individuals of *C. acanthoides* and *C. nutans*, respectively. These had been grown in the greenhouse from seed collected from each of the three populations of each species.

Table 1 Location, habitat, and population density of the nine populations studied; all were located in Grey County, Ontario, Canada

Species and population	Location, habitat, population density
<i>C. acanthoides</i>	A1 Lot 10, Conc. 2, Derby Township, Grey County. Pasture, plants widely spaced
	A2 Lot 12, Conc. 20, Keppel Township, Grey County. Disturbed wasteground, plants dense to widely spaced
	A3 Lot 26, Conc. 2, Sarawak Township, Grey County. Abandoned gravel pit, wasteground, dense stand
<i>C. nutans</i>	N1 Lot 24, Conc. 2E, Glenelg Township, Grey County. Gravel field, wasteground, plants dense to widely spaced
	N2 Lot 55, Conc. 2W, Bentinck Township, Grey County. Pasture, cattle, plants dense to widely spaced
	N3 Lot 15, Conc. 1E, Glenelg Township, Grey County. In and along edge of wheatfield, plants widely spaced
Hybrid swarms	H1 Lot 15, Conc. 1N, Artemesia Township, Grey County. Pasture, sheep, plants dense to widely spaced elsewhere
	H2 Lot 117, Conc. 1E, Artemesia Township, Grey County. Gravel pit, disturbed wasteground, dense stands
	H3 Lot 28, Conc. 1W, Bentinck Township, Grey County. Pasture, cattle, plants widely spaced

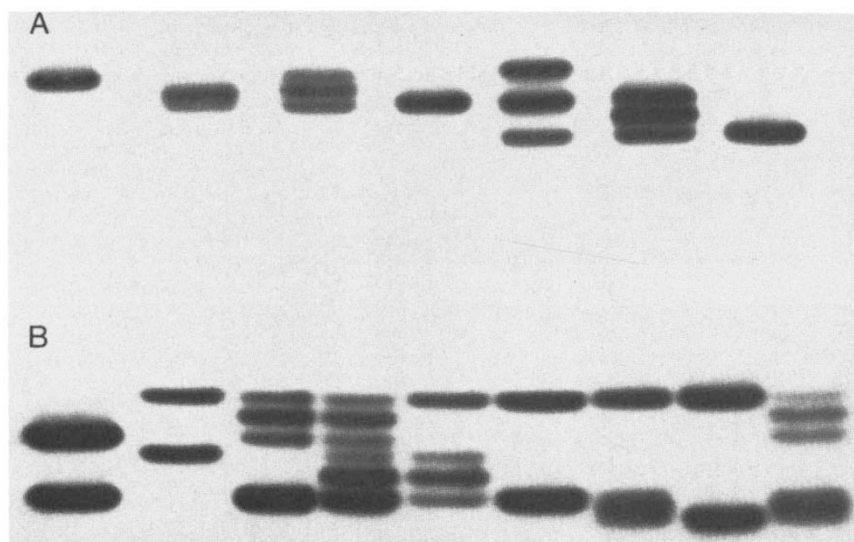


Figure 1 (a) Phosphoglucose isomerase variation patterns at locus 2. Homozygotes for alleles 0.49, 0.43 and 0.36 are shown in lanes 1, 4, and 7, respectively; heterozygotes for alleles 0.46/0.43, 0.49/0.43, 0.49/0.36, and 0.43/0.36 are shown in lanes 2, 3, 5, and 6, respectively; A homozygote for the allele 0.46 is not illustrated. (b) Triose phosphate isomerase variation patterns at locus 1 with alleles 0.70 and 0.61 and locus 2 with alleles 0.58, 0.49, and 0.41. At locus 1, homozygotes for allele 0.70 are shown in lanes 2, 5–8, and for allele 0.61 in lane 1 and the heterozygote 0.70/0.61 in lanes 3, 4, and 9. At locus 2, homozygotes for allele 0.58 is shown in lane 2, for allele 0.49 in lanes 1, 3, and 6 and for allele 0.41 in lane 8; heterozygotes for 0.58/0.49 in lanes 4 and 5 and for 0.49/0.41 in lanes 7 and 9.

Outcrossing rates

Estimates of outcrossing in the nine field populations were calculated using allozyme data at three polymorphic enzyme loci. These include phosphoglucose isomerase (*Pgi-2*, four alleles, fig. 1(A)) and triose phosphate isomerase (*Tpi-1*, two alleles and *Tpi-2*, three alleles, fig. 1(B)). The electrophoretic methods have been given in Desrochers *et al.* (1988b). Inheritance studies were carried out to verify simple Mendelian segregation of the alleles at each locus; the results are given in table 2. From 5 to 12 (usually 10) progeny were surveyed in each of 32 to 44 families (seedlings

grown from seeds collected from single mother plants in the field) for a total of 336 to 434 seedlings per population (table 3).

Proportional representation of a population's genotypes and equal pollen production was assumed in the analyses (Clegg, 1980). Allele frequencies in the progeny and ovule and pollen gene pools were estimated for the most common alleles at each of the three allozyme loci for each of the populations (table 3). Standard errors for progeny allele frequencies were obtained using statistical package S199 (Engineering and Statistical Research Centre, Agriculture Canada). Mean ovule and pollen pool allele frequencies and their

Table 2 Genetic bases for enzyme patterns. Segregation ratios for selfed heterozygotes and results of Chi-square analyses to test for Mendelian segregation of alleles a and b in a 1:2:1 ratio. ns: not significant at $P < 0.05$

Genotype ^{a/b}	No. of progeny			Total	Chi-square value	Significance level
	a/a	a/b	b/b			
<i>Pgi-2</i> ^{0.49/0.43}	64	139	67	270	0.44	ns
<i>Pgi-2</i> ^{0.46/0.43}	29	45	26	100	1.18	ns
<i>Pgi-2</i> ^{0.43/0.36}	14	36	14	64	1.00	ns
<i>Tpi-1</i> ^{0.70/0.61}	25	48	27	100	0.24	ns
<i>Tpi-2</i> ^{0.58/0.49}	20	52	18	90	3.04	ns
<i>Tpi-2</i> ^{0.49/0.41}	32	51	17	100	4.54	ns

Table 3 Progeny allele frequencies, and inferred ovule and pollen pool allele frequencies for *Pgi-2*^{0.43}, *Tpi-1*^{0.70} and *Tpi-2*^{0.49} in the three populations of *Carduus acanthoides* (A1, A2, A3), *C. nutans* (N1, N2, N3) and three hybrid swarms (H1, H2, H3). Standard errors are shown in parentheses. Number of families and total number of progeny surveyed is shown for each population

Population	No. of families/ Total no. of progeny	<i>Pgi-2</i> ^{0.43}			<i>Tpi-1</i> ^{0.70}			<i>Tpi-2</i> ^{0.49}		
		Progeny	Ovule	Pollen	Progeny	Ovule	Pollen	Progeny	Ovule	Pollen
A1	35/349	0.88 (0.041)	0.86 (0.059)	0.89 (0.022)	0.87 (0.015)	0.83 (0.064)	0.95 (0.032)	1.00	—	—
A2	35/398	0.51 (0.018)	0.54 (0.084)	0.44 (0.039)	0.81 (0.016)	0.84 (0.064)	0.64 (0.047)	0.95 (0.008)	0.94 (0.039)	0.97 (0.015)
A3	34/348	0.65 (0.019)	0.69 (0.079)	0.65 (0.031)	0.91 (0.012)	0.90 (0.052)	0.91 (0.022)	0.85 (0.014)	0.79 (0.069)	0.87 (0.025)
H1	42/390	0.88 (0.012)	0.87 (0.052)	0.91 (0.025)	0.99 (0.005)	0.99 (0.017)	0.98 (0.010)	1.00	—	—
H2	41/404	0.82 (0.014)	0.84 (0.057)	0.78 (0.034)	1.00	—	—	0.85 (0.014)	0.78 (0.062)	0.99 (0.024)
H3	44/434	0.77 (0.016)	0.71 (0.069)	0.86 (0.034)	0.97 (0.017)	0.96 (0.031)	0.99 (0.008)	1.00	—	—
N1	35/336	0.87 (0.014)	0.84 (0.062)	0.91 (0.026)	1.00	—	—	0.89 (0.013)	0.84 (0.062)	0.93 (0.021)
N2	32/367	0.86 (0.014)	0.91 (0.052)	0.70 (0.037)	0.88 (0.013)	0.84 (0.064)	0.91 (0.022)	0.91 (0.011)	0.88 (0.058)	0.96 (0.017)
N3	38/380	0.87 (0.013)	0.84 (0.059)	0.92 (0.026)	1.00	—	—	0.81 (0.015)	0.83 (0.061)	0.78 (0.032)

standard errors were obtained from multilocus estimation programs described by Ritland and Jain (1981). Since parentage was unknown, the probability method of Brown and Allard (1970) was used to infer the most likely maternal genotype of each family. The standard error of the ovule frequency of allele, p , was estimated as the square root of $p(1-p)/2N$, where N is the number of families in the population. The pollen gene frequencies for each family and standard error was obtained via the Expectation-Maximization method (Ritland and Jain, 1981).

Two estimates of outcrossing, t and t_H , were obtained for each of the nine populations (table 4). These are the two basic approaches to mating system estimation for plants which utilize allozyme data. The first, outcrossing rate t_H , is the simplest and makes use of the heterozygosity present in a population at inbreeding equilibrium, whereas outcrossing rate t makes use of progeny genotype arrays or frequencies of genotypes derived from maternal genotypes. The latter generally provides more accurate and reliable information about outcrossing (Ritland 1983). Outcrossing rates t_H were calculated from Wright's inbreeding coefficient, F , (Wright, 1969) which was based on observed levels of heterozygosity present at these polymorphic loci. F measures the observed values of inbreeding or the average deviations of progeny genotypic proportions from Hardy-Weinberg expectations

and equals zero under panmixia. The genotype data were pooled over all progeny per population and F was calculated as $1 - (H_o/H_e)$, where H_o is the observed and H_e is the expected number of heterozygotes, that is, $2p(1-p)$, where p is the frequency of one allele. The variance of F was calculated by the methods of Phillips and Brown (1977). The outcrossing rate t_H was calculated as $(1-F)/(1+F)$ and the variance of t_H was calculated as $[4/(1+F)^4] \times \text{variance of } F$ (as described in Ritland, 1983). Outcrossing rates t and standard errors were calculated for each locus singly and as a multilocus estimate using the joint maximum likelihood estimation procedures of Ritland and Jain (1981) and Ritland (1986). The multilocus procedure is based on a maximum of three alleles per locus, since determining the maternal genotypes becomes more difficult with greater than three alleles. It was necessary to pool the allele frequencies at locus *Pgi-2* for two of the hybrid swarms, since a fourth allele, 0.46, was present at a very low frequency, 0.03 and 0.02 in populations H2 and H3, respectively. The frequency of this allele was pooled with the least frequent of the other alleles at this locus (Ritland, 1983). The number of loci included in the multilocus estimate varied from two to three loci for the different populations. A locus was excluded if the frequencies of one of the alleles was greater than 0.98 or if a chi-square test of heterogeneity performed on

Table 5 Progeny arrays of homozygous maternal plants (genotype inferred), are tested for deviation from a Poisson distribution in order to detect nonrandomness

Species and population		Homozygous maternal genotype	No. heterozygous progeny										Poisson distribution χ^2 (df)		
			0	1	2	3	4	5	6	7	8	9		10	
<i>C. acanthoides</i>	A1	<i>Pgi-2</i> ^{0.43}	10	7	7	2	0	0	0	1	0	0	0	1.68 (3)	
		<i>Tpi-1</i> ^{0.70}	20	4	2	0	0	0	1	0	0	0	0	2.34 (1)	
	A2	<i>Pgi-2</i> ^{0.43}	0	3	2	2	1	2	3	0	0	0	0	4.05 (5)	
		<i>Tpi-1</i> ^{0.70}	6	6	8	5	2	1	0	0	0	0	0	1.12 (3)	
	A3	<i>Pgi-2</i> ^{0.43}	0	1	4	7	3	2	2	1	2	0	0	3.48 (6)	
		<i>Tpi-1</i> ^{0.70}	10	10	4	1	0	0	0	0	0	0	0	0.16 (2)	
		<i>Tpi-2</i> ^{0.49}	7	4	6	4	0	0	0	0	0	0	0	2.39 (3)	
	Hybrid swarms	H1	<i>Pgi-2</i> ^{0.43}	13	10	3	0	0	1	0	0	0	0	0	0.22 (2)
		H2	<i>Pgi-2</i> ^{0.43}	10	8	3	4	2	0	0	0	0	0	0	4.14 (3)
<i>Tpi-2</i> ^{0.49}			23	2	0	1	0	0	0	1	0	1	0	14.25 (2)***	
H3		<i>Pgi-2</i> ^{0.43}	8	4	7	2	0	0	1	0	0	0	0	3.42 (3)	
<i>C. nutans</i>	N1	<i>Pgi-2</i> ^{0.43}	13	4	2	2	0	0	0	0	0	0	0	2.18 (2)	
		<i>Tpi-2</i> ^{0.49}	14	6	1	2	0	0	0	1	0	0	0	2.76 (2)	
	N2	<i>Pgi-2</i> ^{0.43}	6	10	2	8	4	0	0	0	0	0	0	7.14 (4)	
		<i>Tpi-1</i> ^{0.70}	11	8	2	1	0	0	0	0	0	0	0	0.05 (2)	
		<i>Tpi-2</i> ^{0.49}	15	9	0	0	0	0	0	0	0	0	0	0.43 (1)	
	N3	<i>Pgi-2</i> ^{0.43}	15	9	2	0	0	0	0	0	0	0	0	0.10 (1)	
		<i>Tpi-2</i> ^{0.49}	7	7	7	2	4	0	1	0	0	0	0	1.24 (3)	

*** Significant at $P < 0.001$.

the progeny arrays was significant ($P < 0.01$), as for *Tpi-2* in population A1.

In multilocus estimation procedures, outcrossing is assumed to be randomly distributed among maternal genotypes within populations. Comparing the distributions of progeny of homozygous maternal genotypes, thus provides a means for testing for spatial heterogeneity in pollen frequencies as might arise if the populations were substructured. If outcrossing is randomly distributed, then the progeny arrays of homozygous maternal plants, i.e. the number of heterozygous progeny, would be expected to have a Poisson distribution. A total of 18 such progeny distributions were tested for goodness of fit to a Poisson distribution using a chi-square test (Sokal and Rohlf, 1969) (table 5).

RESULTS AND DISCUSSION

Self-fertilization

Our studies indicated a range of selfing potential among plants of both *Carduus* species. The mean seed set \pm standard error (S.E.) was 32 ± 5 and 68 ± 8 seeds per selfed head for *C. acanthoides* and *C. nutans*, respectively. Seed set values ranged from 10 to 60 and from 20 to 150 seeds per head for *C. acanthoides* and *C. nutans*, respectively. Based on average estimates of 120 and 250 flowers per head for plants of *C. acanthoides* and *C. nutans*, respec-

tively (Warwick *et al.*, 1989), the above seed set values correspond to ranges of 8 to 50 per cent and 8 to 60 per cent self-fertility among individuals of *C. acanthoides* and *C. nutans*, respectively. Our results are similar to that described by Moore and Mulligan (1964) for plants of *C. nutans* from Grey County, where self-fertility ranged from 0 to 42 per cent, but differed from those of Smyth and Hamrick (1984), who found low seed set in isolated greenhouse plants of *C. nutans* from Kansas. With respect to *C. acanthoides*, our values were higher than those found by Moore and Mulligan (1964), who reported 0–10 per cent self-fertility among plants originating from Grey County.

Outcrossing rates

No differences in the frequency of the most common allele at each of three allozyme loci was evident for the progeny, ovule and pollen gene pools based on comparisons of $2 \times$ S.E. of differences between means (table 3), providing no evidence of selection during the mating cycle.

Single locus and multilocus estimates of outcrossing rates t and t_H and inbreeding coefficient values (F) are given in table 4 for each of the nine populations. Multilocus estimates of the outcrossing rate, t , were significantly less than 1.0 for all nine populations studied (based on $2 \times$ S.E.), with values of 0.84, 0.75, and 0.71 for the three popula-

tions of *C. nutans*, 0.67, 0.60 and 0.87 for the three populations of *C. acanthoides* and 0.69, 0.69, and 0.63 for the three hybrid swarms. Outcrossing rates (t_H) estimated from inbreeding coefficient values, F_i , were also clearly less than 1.0. Single locus estimates of t and t_H were more variable, with locus *Pgi-2* providing the most consistent result with the multilocus estimates of t , especially for the three hybrid swarms and the three populations of *C. nutans*. Similarly single locus estimates of F were highly variable, with values ranging from -0.083 to 0.302 for the nine populations. Estimates were most consistent in the three hybrid swarms with four of the six single locus values showing significant deviations from zero, expected with nonrandom outcrossing. The mating systems of *C. nutans* and *C. acanthoides* in this region are, therefore, both characterized by departures from random outcrossing. Our results for *C. nutans* contrast with the evidence for random outcrossing in two Kansas populations obtained by Smyth and Hamrick (1984) using a similar two-loci, maximum-likelihood estimation procedure. Given the variability among populations in each species, no consistent difference in outcrossing rate was detected for the two parental species. Mean estimates of t and t_H averaged for the three hybrid swarms were consistently less than similar values for the populations of the parental species, although these differences were not statistically significant, and therefore did not provide evidence for differences between pure and hybrid populations of the parental taxa.

There are other factors in addition to self-fertilization that can contribute to a departure from random mating in plant populations. Spatial substructuring of populations, assortative mating or variation in outcrossing among maternal genotypes can all shift estimates of t downward (Clegg, 1980; Ritland, 1983). Inbreeding caused by population substructuring can be partly detected by a comparison of single-locus estimates with multilocus estimates, with inbreeding causing a greater downward bias in single-locus estimates (Ritland, 1986). The differences between the mean of the single locus estimates and the multilocus estimate (table 4) are small (0.00 to 0.06), with the exception of population H3 (0.17). The lack of a consistent deviation provides little or no evidence that population substructuring is responsible for the lower values of t . One of the assumptions in a mixed-mating model is that the distribution of progeny over families for a given maternal genotype should be identical. Comparing the distributions of progeny of homozygous maternal genotypes, thus pro-

vides a means for testing for spatial heterogeneity in pollen frequencies as might arise if the populations were substructured. Deviations from a Poisson distribution is expected with nonrandom outcrossing. Of a total of 18 such progeny distributions tested; only one family deviated significantly from a Poisson distribution (table 5). These results, therefore, do not provide evidence for spatial heterogeneity in pollen frequencies or substructuring in the populations studied. Recent studies by Smyth and Hamrick (1987) on realized gene flow via pollen and seed in four adjacent, artificial populations of *C. nutans* suggested that population differentiation due to random effects was unlikely in most populations of *C. nutans*. Estimates of effective neighbourhood sizes based on the movement of isozyme makers via pollen was around 252 plants at typical densities of one flowering individual per m^2 and was even greater if seed movement was included, ranging from 1281 to 3844 individuals at densities as above.

Heterogeneous outcrossing rates among maternal genotypes would also affect estimates of outcrossing. Variation in outcrossing estimates among individual maternal plants within each of the nine populations was considerable (fig. 2). For most populations, the values were not normally distributed but appeared to be trimodal, suggesting appreciable heterogeneity in outcrossing rates within populations. Smyth and Hamrick (1984) also found significant heterogeneity in apparent outcrossing rates among individuals within two Kansas populations of *C. nutans*.

Genotypic differences in the amount of outcrossing may well be related to genetic differences in self-fertility as observed in greenhouse grown plants or may reflect nonrandom pollinator patterns (Smyth and Hamrick 1984, 1987). Plant population densities may interact with pollinator behaviour to produce considerable inter-plant variation in gene flow. Studies by Smyth and Hamrick (1987) suggested a trend towards greater gene flow with increased density of flower heads of *C. nutans*. There was some correlation between stand density and degree of outcrossing in our *Carduus* populations. Among populations of *C. acanthoides*, A3 was the most dense population, forming an almost impenetrable solid stand on the highly disturbed site of an abandoned gravel pit, and had the highest rate of outcrossing. In comparison, plants in the two pasture populations of *C. acanthoides*, A1 and A2, were more widely spaced and had correspondingly lower outcrossing rates. Among populations of *C. nutans*, population N3 had the lowest outcrossing rate and the lowest

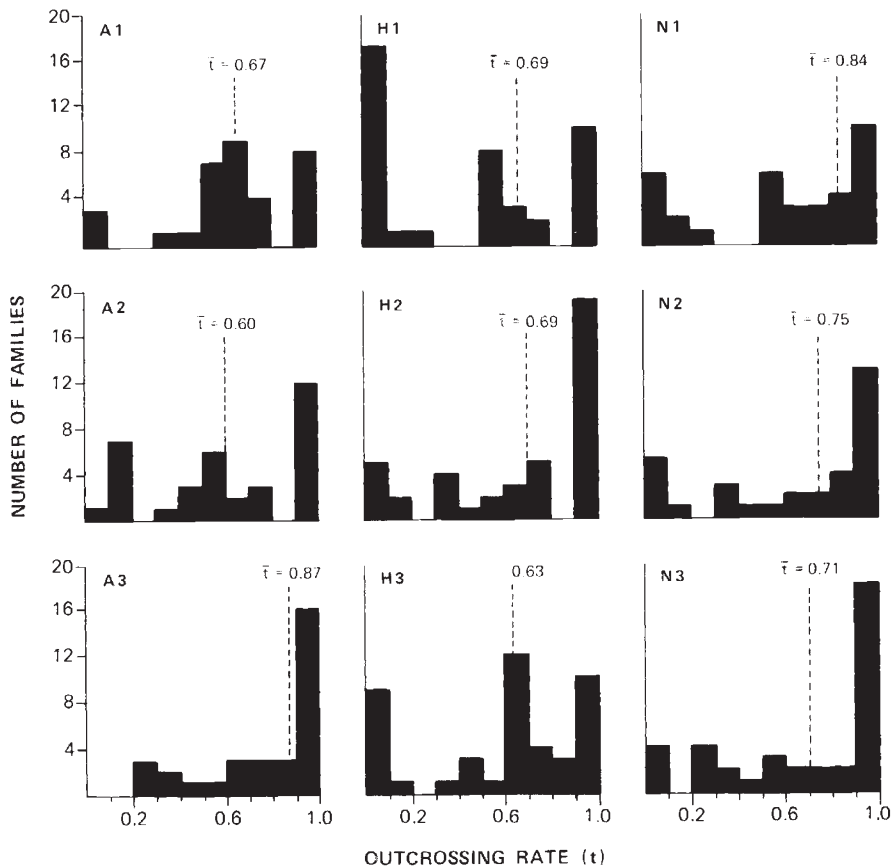


Figure 2 Histograms illustrating variation among maternal genotypes in outcrossing rate (t) for each of three populations of *C. acanthoides* (A1, A2, A3), *C. nutans* (N1, N2, N3) and the three hybrid swarms (H1, H2, H3). Mean outcrossing rate is given for each population and the sample size for each population is given in table 3.

population density. Estimates of outcrossing in the hybrid swarms were very similar to each other even though density levels varied among populations.

In conclusion, the mating system of pure populations of *C. nutans* and *C. acanthoides* and their hybrid swarms from Grey County, Ontario exhibited significant departures from random outcrossing, *i.e.*, outcrossing rates were significantly lower than 1.0, presumably due to higher rates of selfing. No consistent differences in outcrossing rates were detected either between the two parental taxa or between the hybrid and pure parental populations. Heterogeneity in outcrossing rates was evident both among populations as well as among maternal plants within populations and may well reflect genetic differences in self-fertility. Such non-obligatory outcrossing should be adaptive in a colonizing species. The ability to self-pollinate when isolated enhances successful colonization following long distance dispersal or at the margin

of a distribution range when pollinator frequencies may be reduced, or when seed production may be disrupted as, for example, in hybrid swarms.

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