

GENE FLOW AMONG SMALL POPULATIONS OF A SELF-INCOMPATIBLE PLANT: AN INTERACTION BETWEEN DEMOGRAPHY AND GENETICS¹

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We assessed the effects of population size and genetic relatedness on rates of pollen gene flow into experimental populations of the insect-pollinated, self-incompatible plant *Raphanus sativus*. We created synthetic populations of sizes 2, 5, 10, and 20 with three genetic structures (full siblings, half siblings, and unrelated plants). Following pollination in a natural setting, we conducted a simple paternity exclusion analysis using the allozyme genotypes of progeny to measure apparent gene flow and Monte Carlo simulations to estimate total gene flow. Estimates of apparent pollen gene flow rates ranged from 0 to 100% and were similar in rank to estimates of total gene flow. There were significant effects of population size and relatedness on the rate of apparent gene flow, and there were significant population size by relatedness interactions. Populations of size 2 had higher gene flow rates than larger populations, gene flow being negatively associated with the level of cross-compatibility (as measured by hand pollinations). Gene flow into populations of size 2 was also negatively associated with the distance to the nearest population of size 10 or 20. These results suggest that interactions among demography (population size), genetics (cross-compatibility), and ecology (pollinator behavior) are important influences on pollen gene flow rates into small plant populations.

Key words: Brassicaceae; gene flow; population size; *Raphanus sativus*; self-incompatibility.

The rate of migration or gene flow between populations directly influences the process of evolutionary change or stasis. Gene flow can ameliorate the long-term detrimental effects of inbreeding and reduced genetic variation resulting from genetic drift and, thus, is particularly important in the conservation of small populations of plants (Ellstrand, 1992a, b; Ellstrand and Elam, 1993, and references therein). For small populations of self-incompatible plants, gene flow also may have the more immediate benefit of providing compatible mates from other populations and may aid in maintaining mating type or S-allele diversity that can erode due to stochastic processes and lead to reduced reproductive output (e.g., Les, Reinhartz, and Essleman, 1991; Byers and Meagher, 1992; DeMauro, 1993; Elam, 1994; Byers, 1995). How ecological and demographic parameters, such as population size and mating system, influence rates of gene flow between small, spatially isolated populations is, therefore, of interest to both evolutionary biologists and conservationists.

Ecological and genetic theory predict that pollen gene

flow rates, as measured by the fraction of seeds sired by pollen from outside the population (out-sired seed) per generation, increase as the size of the target population decreases. In small populations, fewer targets (stigmas) exist for a fixed amount of "pollen rain," increasing the probability that foreign pollen will sire progeny (Handel, 1983). For animal-pollinated species, however, it is questionable whether the influx of foreign pollen remains invariant for large vs. small populations. Pollinators are likely to spend more time foraging in large populations and affect more within-population pollinations than in small populations (Pyke, 1984), suggesting a different basis for an increase in out-sired seed with decreasing population size. Small populations may attract fewer pollinators than large populations (Sih and Baltus, 1987), which could decrease the frequency of foreign pollen grains entering small populations relative to large populations. If the number of pollinators entering populations varies idiosyncratically with the size of the population, however, the effect of population size on gene flow may be difficult to predict.

For species with self-incompatibility systems, or any plants for which unrelated pollen has a selective advantage for other reasons (e.g., out-crossed pollen being favored over inbred local pollen), foreign pollen grains may be more likely than local pollen grains to affect fertilization and sire seed (Levin, 1981). The advantage of foreign pollen over local pollen will increase as the proportion of cross-incompatible individuals within the population increases. Because small populations experience higher rates of random genetic drift, they are likely to contain fewer incompatibility alleles and thus experience higher levels of cross-incompatibility, particularly if incompatibility is based on one or a few loci. Therefore,

¹ Manuscript received 6 November 1996; revision accepted 26 March 1997.

The authors thank Janet Clegg for her invaluable assistance in the field and in the lab, Jamie Joyner for help with field work, Drs. Ted Floyd and James Rohlf for advice on statistical analysis, Mark Brunell for assistance with computer programming, and Drs. Bernie Devlin, Jodie Holt, James Thomson, Andrew Schnabel, and Nicholas Waser and one anonymous reviewer for providing comments on earlier versions of this manuscript.

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we might expect smaller populations to set a higher proportion of out-sired seed, and that this proportion will increase as the diversity of incompatibility alleles decreases. Such interactions between population size and the level of cross-compatibility have not been accounted for in previous studies and may in part explain empirical results emphasizing the idiosyncratic nature of gene flow, which can vary between species, populations, and even individuals (Hamrick, 1978; Slatkin, 1987; Ellstrand, 1992a, b; Stacy et al., 1996).

Experimental studies of gene flow have attempted to tease apart factors influencing gene flow rate. Studies conducted in forestry and agricultural contexts have investigated the distance needed to isolate crops from each other to ensure seed purity (e.g., Williams and Evans, 1935; Bateman, 1947) and the isolation distance needed to prevent the hybridization of transgenic crops with their wild co-occurring congeners (e.g., Klinger, Elam, and Ellstrand, 1991; Klinger, Arriola, and Ellstrand, 1992; Till-Bottraud et al., 1992). These studies suggest that reproductive and genetic isolation vary both with isolation distance and the size of the source and target populations (but see Manasse, 1992). Yet these studies are only of limited application to natural systems because they typically measure unilateral gene flow from a single source or into a single, typically large, recipient population (but see Williams and Evans, 1935). Pollen flow into a natural population, in contrast, may come from a number of different sources or subpopulations with few or no large source or target populations. Few studies have directly assessed the effects that population sizes and spatial distributions found in nature have on patterns of multidirectional gene flow.

In this study, we assessed the importance of population size on gene flow rates among a group of small, experimental populations of *Raphanus sativus*, a self-incompatible plant species. Here we use “population” in a broad sense to denote a discrete, semi-isolated group of individuals. Synthetic populations were arranged to resemble a large, patchily distributed array of subpopulations. Specifically, we asked “What are the effects of target population size and the level of within-population cross-compatibility (as estimated by genetic relatedness) on pollen gene flow rate?” We used a simple, allozyme-based paternity exclusion analysis to measure proportions of out-sired seed as an estimate of pollen gene flow rate into each synthetic population. We expected that a synergistic interaction between population size and cross-compatibility would cause gene flow to be highest in the smallest populations of the most closely related individuals.

MATERIALS AND METHODS

Study system—Experimental populations were composed of *Raphanus sativus* L. (Brassicaceae), wild radish, an exotic, weedy annual that occurs throughout much of California. Like many brassicaceous species, wild radish has a sporophytic self-incompatibility system (Sampson, 1957; Karron, Marshall, and Oliveras, 1990). Karron, Marshall, and Oliveras (1990) estimated a minimum of 32 S-alleles per locus in two natural populations of *R. sativus*. Wild radish plants can produce up to several hundred flowers simultaneously, attracting a wide variety of floral visitors such as honey bees, small native bees, syrphid flies, varied lepidoptera, and even hummingbirds (Stanton et al., 1991; Klinger, Ar-

riola, and Ellstrand, 1992; K. Goodell, personal observation). Wild radish is polymorphic for several allozyme loci (Devlin and Ellstrand, 1989) and has been the subject of numerous gene flow studies (Ellstrand, Devlin, and Marshall, 1989; Devlin and Ellstrand, 1990; Klinger, Elam, and Ellstrand, 1991; Klinger, Arriola, and Ellstrand, 1992).

Experimental design—To investigate the effects of population size and level of cross-compatibility on pollen gene flow rates into small populations, we created synthetic populations in a factorial design with three relatedness treatments and four population size treatments. Relatedness treatments were populations composed of full siblings (full-sib), half siblings (half-sib), or unrelated plants that were collected from different localities around California (unrelated). We assumed that, on average, the level of cross-compatibility increased from full-sib to half-sib to unrelated treatments. Relatedness was used as a surrogate for the level of cross-incompatibility because of the considerable time and resources needed to compose populations of known levels of cross-incompatibility. The population sizes were 2, 5, 10, or 20 individuals. The population sizes were replicated in the following way: size 2, five replicates per relatedness class; size 5, two replicates; size 10, unreplicated; and size 20, unreplicated. This scheme, which includes 27 populations and 150 individuals, was repeated three times from November 1993 to March 1994 for a grand total of 81 populations and 450 individuals. The timing of the experiment was intended to reduce the possibility of gene flow from natural populations of wild radish that begin blooming in March. The scattered natural wild radishes that began blooming within a 1-km radius of the field site during the last few days of the experiment were eradicated immediately.

The experiment was conducted at the University of California Agricultural Experiment Station's Citrus Research Center in Riverside, California. The ~0.25-km² site was a mosaic of citrus groves, other agricultural tree and shrub plots, and fallow plots. The plants were raised from seed in the greenhouse, each in 8-L pots. When all of the plants needed for one temporal replicate (time) were flowering simultaneously, the 27 synthetic populations were placed into predetermined sites within the citrus groves. The populations were placed randomly with respect to relatedness class, but because of spatial constraints the size of populations placed into each site remained constant over times. We separated plants within populations by 0.75 m and arranged them so that branches of neighboring plants did not touch each other. Each population was isolated from its nearest neighbor by at least 100 m (Fig. 1).

Each branch on each plant was marked at the outset of the experiment to indicate the flowers that opened and were pollinated in the field. The synthetic populations were left in the field for 16 d to allow natural pollination. During this period, the plants were hand-watered approximately every 2 d. Each branch was remarked at the end of the experiment to ensure that only fruits produced from matings in the field under the experimental conditions were harvested and analyzed. Following the period in the field, the plants were brought back to the greenhouse to allow the fruits to mature.

Test of cross-compatibility—Following the field experiment, hand pollinations were performed on as many of the full-sib and half-sib populations of size 2 as possible to test for cross-compatibility within populations. Because of delayed flowering, the early death of some plants, and general time constraints, we were not able to test all populations of size 2. Buds were enclosed in bags of bridal veil until the flowers opened. Freshly dehiscid anthers of the second plant in that population were dragged across the stigmas of the first, and the bags were replaced. Three to eight recipient (maternal) flowers were pollinated on each plant. Reciprocal crosses were performed. The hand-pollinated flowers were scored for fruit set, and populations were designated “completely compatible” if fruits were set by both plants in the population, “partially compatible” if only one plant in the population set at least one fruit, and “completely incompatible” if neither plant set any fruit.

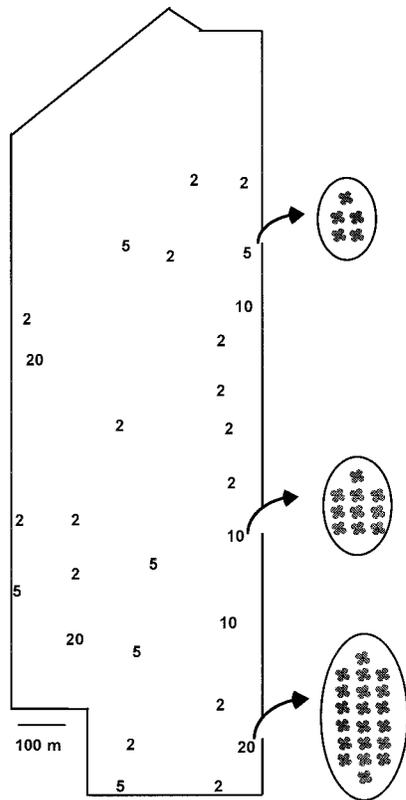


Fig. 1. Map of the field site showing relative locations of the 27 populations (sizes indicated by the numbers) for a sample temporal replicate (time) of the experiment. The magnified areas indicate the spatial arrangement of plants (flower symbols) within populations of 5, 10, and 20.

Electrophoresis—Nine allozyme loci were used as genetic markers: aconitase (ACO), acid phosphatase (ACP), isocitric dehydrogenase (IDH), leucine amino peptidase (LAP), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM-1, 2, 3), and triosephosphate isomerase (TPI). Extraction buffers, gel buffers, and techniques are as described by Devlin and Ellstrand (1989).

Cotyledon and bud tissue from each of the 450 parent plants were assayed for the nine allozyme loci. When available, 40 seeds representing the progeny generation were sampled from each parent population and analyzed for the same nine loci. In populations that produced fewer than 40 seeds, all available seeds were used. Seeds from maternal plants within each population were sampled proportionally relative to maternal fruit set. Individuals producing few fruits may not have had compatible mates in the population, potentially resulting in an unusually high proportion of out-sired seed. Thus, as a result of proportional sampling, plants with relatively low seed set are not expected to overly influence population level estimates of out-sired seed. Because two or more seeds within the multiseeded fruits of wild radish often may be sired by the same pollen donor (Ellstrand and Marshall, 1986) so that out-sired seeds detected in a single fruit may not necessarily represent independent events, only one seed was sampled per fruit. When maternal plants did not have enough fruits for this sampling scheme, seeds were sampled over as many fruits as possible. In general, our intent was to obtain a representative sample of the population rather than of a subset of the plants within the population.

Estimating gene flow—Apparent pollen gene flow was detected using a simple paternity exclusion procedure (Ellstrand, 1984; Ellstrand and Marshall, 1986). This method identifies progeny that were not sired

by pollen from within the population by a direct comparison of maternal parent and progeny allozyme genotypes. A locus by locus comparison of the known progeny and maternal parent genotypes determined the maternal gametic contribution. Once the maternal contribution was identified, the set of possible paternal gametic contributions easily was inferred for each offspring. This set of paternal gametes was then compared, locus by locus, to the genotypes of all the potential pollen donors in the local population with donors excluded if they could not have produced a matching gamete. The maternal plant was also included as a potential pollen donor in the exclusion analysis because occasionally radish can produce self-fertilized seeds (J. Clegg, University of California, Riverside, personal communication). For each progeny, if none of the parent plants in the local population could have produced a gamete to match the inferred paternal contribution in the progeny, then it was attributed to interpopulation gene flow and designated an out-sired seed.

Apparent gene flow is the component of gene flow detectable by recognition of novel alleles or multilocus allelic combinations in the progeny genotype (Devlin and Ellstrand, 1990). However, *cryptic* gene flow may also result when the inferred genotype of a gamete is identical to a pollen genotype that could be produced in the target population. Therefore, such cryptic events cannot be measured directly (Devlin and Ellstrand, 1990). The sum of cryptic and apparent gene flow equals the effective (total) gene flow so that the rate of apparent gene flow serves as a minimum estimate of total gene flow. Apparent gene flow will constitute a higher proportion of, and be a more accurate estimator of, total gene flow as local population size decreases and levels of assayable genetic diversity increase. If gametic frequencies within a target population differ from those of potential sources of foreign pollen (e.g., due to small population size), then seed sired by foreign pollen is also more likely to be detected by simple paternity analysis (Adams and Birkes, 1990). Thus, in this experiment, a higher proportion of out-sired seed is expected to be apparent in the smaller and the full- and half-sib populations than in the larger populations of unrelated individuals.

We estimated the total proportion of out-sired seed on each maternal plant sampled using the program PollenGF (J. Nason, University of Iowa, unpublished program). This program is analogous to that described by Devlin and Ellstrand (1990) and uses Monte Carlo simulations to randomly draw pollen genotypes from a pool of gametes defined by the allele frequencies in the pool of potential foreign pollen donors. The program then creates progeny genotypes for each maternal plant in a target population using these pollen gametes. Therefore, all of the simulated seeds have foreign fathers, and the program calculates the expected proportion of apparent out-sired seed on each mother given 100% gene flow and the genotypes of pollen donors within the population. An approximate maximum likelihood estimate of the total proportion of out-sired seed on each plant is then obtained by dividing the actual proportion of apparent pollen flow events indicated by simple paternity analysis by the proportion of simulated pollen flow events expected to be apparent. The model assumes that pollen from outside the population is drawn at random from an infinitely large external source whose allele frequencies are accurately known; violations of this assumption could result in an overestimate of gene flow. By estimating total gene flow in this way, we assume that the individuals in the 26 remaining experimental populations are equally likely to contribute foreign pollen to the target population, although this assumption is probably violated to some degree. Because estimating total gene flow is not a central component of our analyses (it should closely parallel apparent gene flow in most cases), violations of this assumption should be unimportant.

Analysis—Pollen gene flow for each population was expressed as the odds of sampling an out-sired seed (odds = $\text{Pr}[\text{no. out-sired}]/\text{Pr}[\text{no. out-sired}]$). We used hierarchical logit modeling to statistically examine the effects of time, relatedness, and population size on the frequency of out-sired progeny. The logit transformation is required for these data

TABLE 1. Apparent gene flow rates (no. of out-sired seeds/sample size) for each experimental population of *Raphanus sativus*. Blanks represent populations that did not survive until the end of the experiment or that set no fruit (nfs).

Population	Relatedness class		
	Full	Half	Unrelated
Size 2	0.20 (8/40)	0.30 (12/40)	0.35 (9/26)
	0.58 (11/19)	0.87 (32/37)	0.19 (8/42)
	0.13 (5/40)	0.74 (32/43)	0.03 (1/40)
	0.75 (3/4)	0.71 (29/41)	0.72 (13/18)
	0.05 (2/40)	0.41 (17/42)	0.42 (13/31)
	0.77 (30/39)	0.86 (6/7)	0.28 (11/40)
	1.00 (39/39)	0.22 (6/27)	0.20 (8/40)
	0.36 (15/42)	0.85 (35/41)	0.26 (10/38)
	0.22 (8/37)	0.08 (1/13)	0.15 (6/40)
	0.23 (9/40)	nfs	0.05 (2/40)
	0.43 (17/40)	nfs	0.10 (4/40)
	0.88 (35/40)	nfs	0.14 (3/21)
	0.47 (15/32)		0.17 (7/41)
			0.38 (15/40)
			0.08 (3/40)
Size 5	0.13 (5/40)	0.03 (6/31)	0.08 (3/40)
	0.00 (0/38)	0.58 (21/36)	0.24 (10/38)
	0.26 (10/38)	0.12 (4/33)	0.15 (6/40)
	0.13 (5/39)	0.10 (4/40)	0.18 (7/39)
	0.23 (9/40)	0.05 (2/40)	0.05 (2/39)
nfs			
Size 10	0.06 (2/37)	0.08 (3/40)	0.23 (9/40)
	0.05 (2/40)	0.46 (16/35)	0.30 (11/37)
	0.06 (2/32)	0.25 (10/40)	0.05 (2/40)
Size 20	0.08 (3/40)	0.13 (5/40)	0.35 (14/40)
	0.10 (4/40)	0.18 (7/40)	0.28 (11/40)
	0.18 (7/40)	0.18 (7/40)	0.11 (4/38)

because they are distributed over a restricted range (0–1.0). The logit model is a categorical analog of an ANOVA that tests main effects and interactions of independent variables on a logit-transformed proportional response variable (DeMaris, 1992), stating that the natural logarithm of the odds of sampling an out-sired seed is a linear function of the independent variables. The simplest model that fit the data was accepted (that including the fewest number of factors), as indicated by a likelihood ratio with a nonsignificant probability (≥ 0.05). The significance of the main effects and the interactions for a particular model were tested using the likelihood ratio chi-square statistic (critical $\alpha < 0.05$). The analyses were done using Proc CATMOD in SAS (SAS, 1990). Relatedness groups for populations of size 2 were compared with a chi-square test using Proc FREQ in SAS (SAS, 1990). As a conservative measure, we lowered our critical α to 0.017 by dividing 0.05 by 3, the number of comparisons made (i.e., a Bonferroni adjustment of the critical α).

Proximity to a large pollen source population could also contribute to variation in pollen gene flow rates in small populations (e.g., Klinger, Arriola, and Ellstrand, 1992) resulting in elevated gene flow estimates. As an ancillary test, we assessed the effect of distance to the nearest large population (size 10 or 20) on the proportion of out-sired seed in populations of size 2 with a mixed-model logistic regression using Proc GENMOD (SAS, 1996). Logistic regression is a categorical analog of a least-squares regression that tests the effects of one or more continuous or categorical predictor variables on a logit-transformed proportional response variable. Time, relatedness, and the distance from each population of size 2 to the nearest population of size 10 or 20 were independent variables, and the logit-transformed proportion of out-sired seed was the dependent variable. This analysis was limited to only populations of size 2 because we lacked sufficient replication in larger size classes. The distance to populations of size 5 was not included because we specifically wanted to test the hypothesis of proximity to

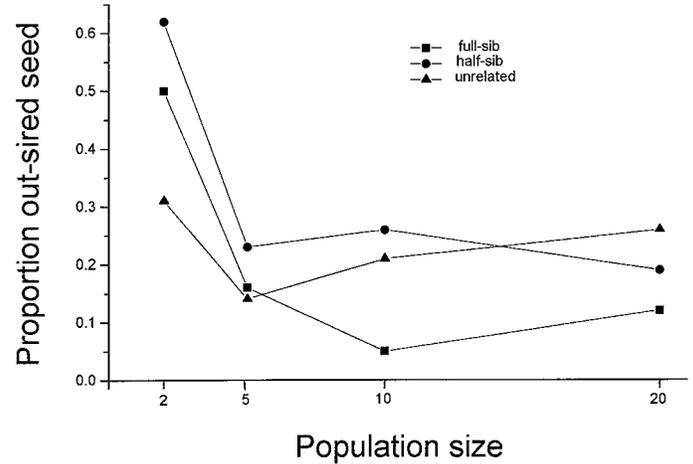


Fig. 2. Mean apparent pollen gene flow rates as measured by the proportion of out-sired seed sampled from experimental populations of *Raphanus sativus*.

“large” source populations and because including it would have effectively reduced the range of distances to a level not worth investigating.

For the populations of size 2 whose level of cross-compatibility had been tested by hand pollinations, we examined the effects of cross-compatibility on apparent pollen gene flow rates using a logit model (Proc CATMOD) with time and cross-compatibility as independent variables and the logit-transformed proportion of out-sired seed as the dependent variable. All zero values for the number of out-sired or not out-sired seed were converted to ones to avoid dividing by zero; these transformations are conservative and should have little effect on the results (Christensen, 1990).

RESULTS

Of the original 81 populations, 71 produced sufficient seed for the genetic analysis. Apparent gene flow rates, the number of out-sired seeds, and number of seeds sampled are given in Table 1. Apparent gene flow rates ranged from 0 to 100%. Populations of size 2 had the highest apparent gene flow rates (mean = 0.46 ± 0.05 SE, median = 0.38, $N = 36$). Gene flow rates varied little among populations of size 5 (mean = 0.17 ± 0.03 SE, median = 0.15, $N = 15$), size 10 (mean = 0.17 ± 0.05 SE, median = 0.10, $N = 9$), and size 20 (mean = 0.19 ± 0.04 SE, median = 0.18, $N = 9$). Gene flow rates for full-sib and half-sib populations of size 2 were on average higher than those for unrelated populations (mean = 0.50 ± 0.09 SE, median = 0.45, $N = 13$; 0.62 ± 0.09 SE, median = 0.73, $N = 9$; and 0.31 ± 0.06 SE, median = 0.24, $N = 14$, respectively). Apparent gene flow rates for each population size by relatedness class are shown in Fig. 2.

Total gene flow—The mean estimates of cryptic gene flow tended to be greater and spanned a larger range in populations of size 10 and 20 (0.006–0.13) than in populations of size 2 (0.009–0.03). Despite the higher estimates of cryptic gene flow in larger populations than in smaller ones, the qualitative ranking of total gene flow remained similar to that for apparent gene flow (Fig. 3).

Main effects—The saturated logit model provided the only fit to the apparent pollen gene flow rates into all

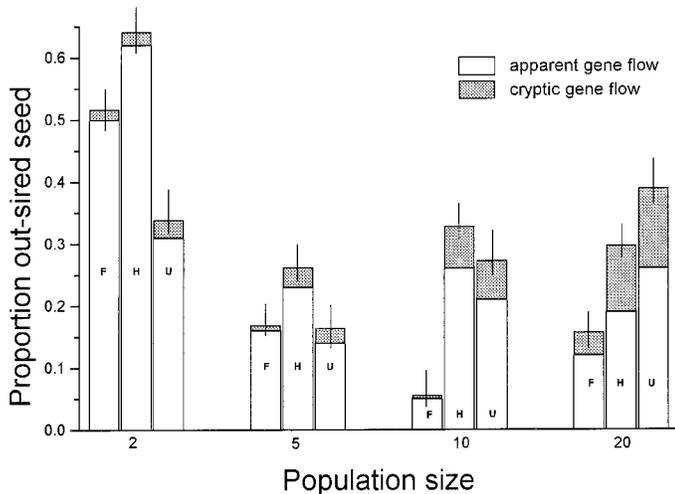


Fig. 3. Mean proportion of out-sired seed sampled from experimental populations of *R. sativus* where the length of the column represents the approximate maximum likelihood estimates of total pollen gene flow rate (see text) and is divided into components of apparent gene flow (white) and cryptic gene flow (shaded). The relatedness classes are indicated by letters on the columns (F = full-sib, H = half-sib, U = unrelated). The error bars for total gene flow rate represent the shortest unbiased 95% confidence intervals of a binomial distribution (Rohlf and Sokal, 1995). Note that the x axis is not drawn to scale.

populations, which means that all main effects (time, relatedness, and population size) and higher order interactions were required to explain the results obtained (Table 2A). The data were analyzed separately for each replicate time to better describe the patterns of gene flow over times. In each of these analyses only the saturated model fit the data, and all of the main effects and two-way interactions were significant (Table 3). The proportion of out-sired seed in experimental populations of *R. sativus* varied as a negative function of the population size (Figs. 2–4). The effect of relatedness among individuals within the population had a somewhat idiosyncratic effect on apparent gene flow rates but at least for populations of size 2, more closely related individuals showed higher gene flow rates (Figs. 2, 4). The interaction terms indicate that the effect of population size on apparent gene flow rates was also influenced by relatedness. The significant three-way interaction in the full analysis most likely is caused by the slightly higher chi-square value for the interaction term at time 2 ($X^2 = 15.88, 42.28, \text{ and } 16.47$ for times 1, 2, and 3, respectively) (Fig. 4).

The logit model analysis was repeated omitting populations of size 2 to determine whether this size class was driving the significant population size effect. A nonsignificant effect of population size when only populations of size 5, 10, and 20 were considered would indicate that populations of size 2 contributed disproportionately to the significant effect of population size in the full analysis. When populations of size 2 were omitted from the logit model, population size was no longer a significant factor, although relatedness was still significant (Table 2B). This result was obtained whether time was included as an independent variable or whether the analysis was done within times and suggests that a threshold exists between population sizes 2 and 5 above which target population

TABLE 2. Maximum likelihood analysis-of-variance table indicating the most parsimonious logit model to account for apparent pollen gene flow rates into experimental radish populations as a function of Time, Relatedness (Rel.), and Population size (Size). (A) All population sizes included. The likelihood ratio could not be tested because the model is saturated. (B) Populations of size 2 omitted. ** $P \leq 0.01$, *** $P \leq 0.001$.

Source	df	χ^2	P
A)			
Intercept	1	394.25	***
Time	2	14.82	***
Rel.	2	16.08	***
Size	3	148.07	***
Time \times Rel.	4	28.20	***
Time \times Size	6	27.14	***
Rel. \times Size	6	47.69	***
Time \times Rel. \times Size	12	20.87	0.052
Likelihood ratio	0		
B)			
Intercept	1	379.77	***
Time	2	11.52	**
Rel.	2	16.04	***
Size	2	1.81	0.40
Time \times Rel.	4	24.26	***
Rel. \times Size	4	15.00	**
Likelihood ratio	12	20.12	0.06

size has little influence over the proportion of out-sired seed (Figs. 2, 4).

Chi-square tests between pairs of relatedness groups for populations of size 2 showed that half-sib (H) populations had higher rates of apparent gene flow than both full-sib (F) and unrelated (U) populations (H vs. F: odds ratio = 2.26, $P_{\alpha = 0.017} < 0.005$; H vs. U: odds ratio = 4.01, $P_{\alpha = 0.017} < 0.001$). The full-sib populations had significantly higher rates of apparent gene flow than unrelated populations (odds ratio = 1.77, $P_{\alpha = 0.017} < 0.001$). The ranking of the full-sib and half-sib relatedness groups differed from our expectations in that the half-sib populations tended to have higher apparent gene flow rates than full-sib populations.

Isolation by distance and compatibility—There was a slight, but significant, negative relationship between the apparent gene flow rate in populations of size 2 and the distance to the nearest population of size 10 or 20 (parameter estimate = -1.01 , SE = 0.20, Wald $X^2 = 26.05$, df = 1, $P \leq 0.0001$). The rates of gene flow into populations of size 2 were also influenced by time and relatedness class, as well as by two-way and three-way interactions (Table 4).

Seven of 12 full-sib and three of seven half-sib populations of size 2 that were tested for cross-compatibility showed reciprocal cross-compatibility as indicated by both plants setting fruits. Only two populations (one half-sib and one full-sib) showed complete cross-incompatibility. The remaining seven populations (four full-sib and three half-sib) showed partial cross-compatibility (only one of the two reciprocal crosses produced fruits). The mean proportion of out-sired seed increased as level of cross-compatibility increased (apparent pollen gene flow rates were mean = 0.45, SE = 0.10, median = 0.38; mean = 0.61, SE = 0.11, median = 0.47; and mean =

TABLE 3. Maximum likelihood analysis-of-variance table indicating the most parsimonious logit model to fit apparent gene flow rates into experimental radish populations for each temporal replicate (time) 1, 2, and 3 analyzed separately. Rel. = relatedness. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. The likelihood ratios were unable to be tested because the models are saturated.

Source	Time 1			Time 2			Time 3		
	df	χ^2	<i>P</i>	df	χ^2	<i>P</i>	df	χ^2	<i>P</i>
Intercept	1	115.34	***	1	88.04	***	1	152.26	***
Rel.	2	11.57	**	2	27.04	***	2	8.11	*
Size	3	9.18	*	3	80.29	***	3	112.61	***
Rel. \times Size	6	15.88	*	6	42.28	***	6	16.47	**
Likelihood ratio	0			0			0		

0.87, SE = 0.11, median = 0.87 for fully compatible, partially compatible, and incompatible populations, respectively). Both level of cross-compatibility and time had a significant effect on gene flow rates into those populations of size 2 that were tested for cross-compatibility (Table 5).

DISCUSSION

Rates of pollen gene flow—The highly variable pollen gene flow rates into our small, experimental populations of wild radish (Table 1) are consistent with previous studies of gene flow, which showed that gene flow is highly idiosyncratic in both time and space (Ellstrand and Marshall, 1985; Ellstrand, 1992a, b). The patterns of gene flow among our synthetic populations varied over the three times with significant population size by relatedness interactions (Table 3). The average magnitude of apparent pollen gene flow rates for the populations of sizes 5–20 (0.17–0.19) was generally higher than those estimated in prior studies at 150–1000 m isolation in similarly sized natural and artificial stands of wild radish (range 0.03–0.18, average = 0.08, SE = 0.02, $N = 10$) (summarized in Ellstrand, Devlin, and Marshall, 1989). Mean gene flow rates into our populations of size 2 (average = 0.46, SE = 0.29, $N = 36$) tended to be much higher than those reported in prior studies on wild radish (Ellstrand, Devlin, and Marshall, 1989). Klinger, Arriola, and Ellstrand (1992) reported comparable, if not higher, pollen gene flow rates (average = 0.80) to our populations of size 2 for similarly sized wild *R. sativus* populations at 1 m from a large source crop population, but at 200 m they dropped to below 0.10.

Higher pollen gene flow rates in this study may reflect differences in experimental design. First, our populations were separated by a minimum of 100 m, a shorter isolation distance than has been used in other studies. Second, this experiment was conducted in the winter when relatively few other species were blooming locally that might have drawn potential pollinators away from the radish flowers. Many have suggested that pollinator service may vary not only with the size and nature of the floral display but also with the overall floral environment (Thomson, 1981, 1982; Campbell, 1985a, b; Kunin, 1993; Westerbergh and Saura, 1994). The relatively poor floral environment encountered during the winter may have induced pollinators to fly longer distances between patches. It is also likely that the absence of *Raphanus* and other wildflowers provided decreased opportunities for pollen picked up by visitors in an experimental population to be deposited on other flowering plants before

reaching the next experimental population. Both the shorter interpopulation distances and the lack of other flowers could have increased pollen transfer between populations and therefore elevated levels of out-sired seed.

Population size effects—Despite the highly variable pollen gene flow rates, we found a significant decline in the proportion of out-sired seed with increasing population size (Figs. 2, 4). This decline was not a simple linear relationship, however, but a threshold in which increasing population size above five plants had little effect on the variation in pollen gene flow rate. A negative relationship between pollen gene flow and population size was also reported by both Klinger, Arriola, and Ellstrand (1992) and Williams and Evans (1935). Klinger, Arriola, and Ellstrand (1992) found that the negative relationship disappeared at distances greater than 1 m from the large source population, but Williams and Evans (1935) found that contamination rates of their small red clover plots were more closely associated with the comparative number of flowers in plots than with the isolation distance (the sizes of their populations, however, were much larger than ours). Manasse (1992) reported no significant differences in gene flow among patches of four or 16 individuals of *Brassica campestris* arranged at 4-m intervals in a linear array, although if pollen gene flow rates responded to a threshold population size as in our experiment, an effect of population size might not be apparent at the sizes used in her study.

Higher gene flow rates in smaller populations concur with Handel's (1983) prediction that smaller populations will produce a greater proportion of out-sired seed than larger populations, provided a constant influx of foreign pollen. The influx of foreign pollen, however, may vary with population size, particularly for animal-pollinated plants. Pollinator foraging patterns may be modified by several aspects of small population size (Handel, 1983; Sowig, 1989). The amount of foreign pollen entering a population relative to the amount available within a population will depend on how many visitors enter the population bearing conspecific foreign pollen and how many flowers they visit. Pollinator visitation to small populations is expected to be less frequent and more variable than that to larger populations because these populations are likely to have smaller floral displays and may lack sufficient pollen and nectar rewards for pollinators (Barrett, 1989; Barrett and Husband, 1990). Indeed, most empirical studies have reported negative relationships between pollinator visitation and population size (Sih and

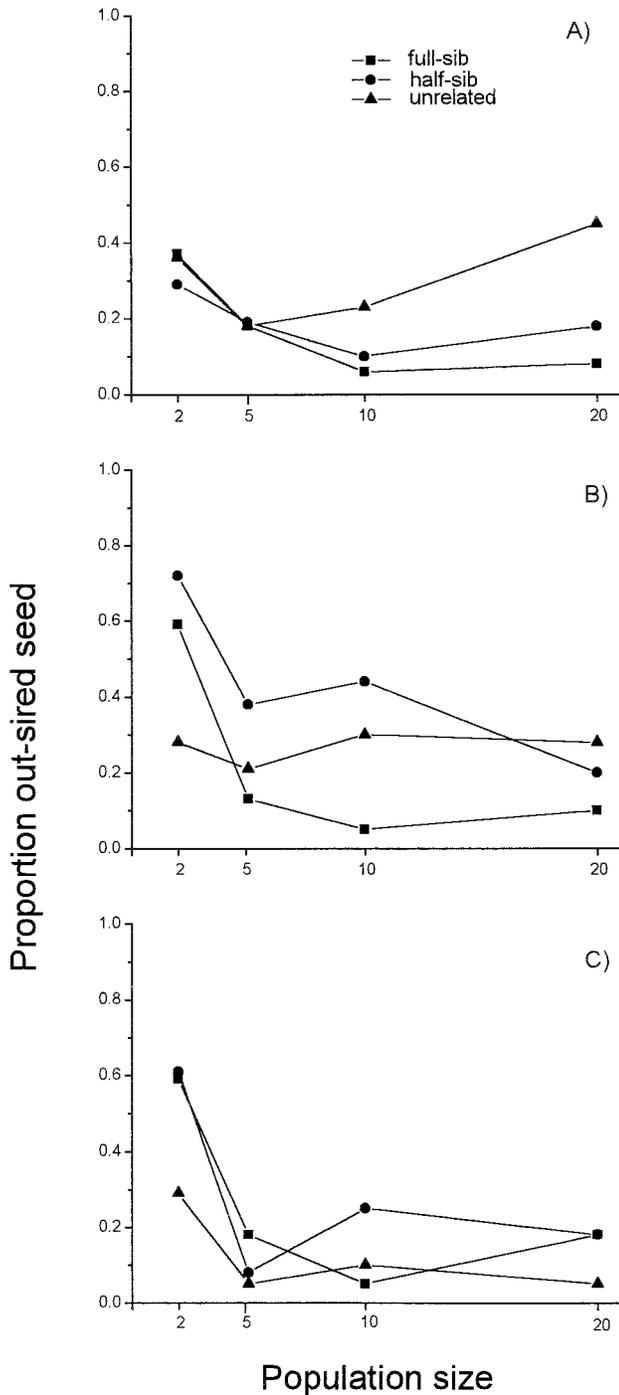


Fig. 4. Mean apparent pollen gene flow rates into experimental populations of *R. sativus* as measured for three temporal replicates (times). (A) Time 1, (B) Time 2, (C) Time 3.

Baltus, 1987; Klinkhamer and de Jong, 1990). Numerous studies have also investigated the effects of flowering plant density on visitation by pollinators and found in general that denser populations receive a greater number of visits, although on a per plant or per flower basis sparse populations may have higher visitation rates (e.g., Thomson, 1981; Schmitt, 1983; Klinkhamer and de Jong, 1990; Feinsinger, Tiebout, and Young, 1991; Ku-

TABLE 4. Logistic regression statistics for a mixed-model logistic regression of Time, Relatedness (Rel.), and the distance to the nearest population of size 10 or 20 (Dist.) on the proportion of out-sired seed in experimental radish populations of size 2. ** $P \leq 0.01$, *** $P \leq 0.0001$.

Source	df	χ^2	P
Intercept	0		
Time	2	62.59	***
Rel.	2	85.84	***
Dist.	1	14.77	***
Time \times Rel.	4	25.99	***
Time \times Dist.	2	48.06	***
Dist. \times Rel.	2	5.50	0.064
Time \times Rel. \times Dist.	3	11.88	**

nin, 1993). To the extent that density reflects population size, these studies suggest that the smaller populations may receive fewer visits. In order to draw a relationship between visitation rate and the amount of foreign pollen entering the population, however, we must know how many visitors enter the population (not how many flowers are visited) and preferably whether or not they move directly between conspecific stands. The amount of pollen carryover (Thomson and Plowright, 1980), which depends on the plant and pollinator species, may also influence rates of pollen gene flow but is unlikely to vary as a function of population size or density.

Two statistical effects are important to consider in comparing estimates of pollen gene flow rates between populations of different sizes. First, high rates of gene flow, especially in some populations of size 2, do not necessarily imply that more migrant pollen grains entered these populations. In some populations of two, the total number of seeds available was as low as four (Table 1) so that the variance in the estimated rate of gene flow was quite large. In these populations, the chance sampling of a few out-sired seeds can lead to high estimates of gene flow rates. Second, the detectability of apparent gene flow depends on local population genetic diversity and size. Pollen gene flow is less detectable when local genetic diversity is high relative to regional genetic diversity because many different pollen gametes could be produced within the local population. Populations of size 2 are likely to be the least genetically diverse, with a maximum of 29 different alleles (two alleles at each of the nine loci sampled, with two to six different alleles possible per locus). Therefore, gene flow events are more likely to be detected in smaller populations and apparent

TABLE 5. Maximum likelihood analysis-of-variance table indicating the most parsimonious logit model to describe the apparent pollen gene flow rates into experimental radish populations of size 2 as a function of time and the level of cross-compatibility (fully compatible, partially compatible, or incompatible) as determined by hand pollinations. The likelihood ratio indicates the fit of the model. *** $P \leq 0.001$.

Source	df	χ^2	P
Intercept	1	14.95	***
Time	2	44.32	***
Cross-compatibility	2	37.73	***
Likelihood ratio	3	2.33	0.51

gene flow rates likely to be higher than in larger populations. Our analysis of cryptic and total gene flow indicated that, indeed, a higher proportion of the total gene flow was apparent in smaller populations. The rank-order of gene flow rates estimated by simple paternity exclusion was little influenced by the inclusion of cryptic gene flow (Fig. 3), however, as is consistent with other studies (Ellstrand, Devlin, and Marshall, 1989; Devlin and Ellstrand, 1990; Broyles, Schnabel, and Wyatt, 1994).

Relatedness effects—Relatedness significantly contributed to patterns of gene flow among populations, although not necessarily in the predicted manner. Out-sired seeds were about threefold as likely to be sampled from a full- or half-sib population as from an unrelated population of size 2 (Fig. 2). Unexpectedly, however, the odds of sampling an out-sired seed in a half-sib population was ~1.7-fold more likely than in a full-sib population of size 2. Assuming that pollinators do not discriminate between populations on the basis of their genetic composition, the effect of relatedness on gene flow is likely due to cross-incompatibility relationships. Although we expected the populations composed of closely related plants to have lower levels of within-population cross-compatibility than those composed of distantly related plants, relatedness is merely an analog for level of cross-compatibility and necessarily imprecise. Data from the hand-pollinations of some populations of size 2 confirm that half-sibs tended to have lower levels of cross-compatibility than full-sibs. Only 43% of half-sib populations compared to 58% of full-sib populations that were tested demonstrated reciprocal cross-compatibility, and there was a significant negative relationship between apparent gene flow rate and level of cross-compatibility (Table 5). Bearing in mind these results, it is not surprising that the multiple comparisons of relatedness groups that considered only populations of size 2 indicated higher apparent gene flow rates into half-sib populations than into full-sib and unrelated populations. The manner in which the crosses were made offers little insight into this unexpected result. All ten half-sib populations of size 2 used in the analysis were maternal half-sibs except two in which the maternal parent of the first was the pollen donor of the second. These populations of size 2 were formed from 12 different maternal plants and 16 different paternal plants; no two populations consisted of the same combination of genotypes. The 12 full-sib populations of size 2 represented 12 different families and shared no parents among them. Although we do not possess the pedigrees of the grandparents, we have no reason to believe that the grandparents of the half-sib populations were more closely related to each other than those of the full-sib populations. The unexpected ranking of the full-sib and half-sib groups may reflect a chance or inadvertent selection of half-sib populations with higher levels of incompatibility than the full-sib populations. The loss of six half-sib populations of size 2 (Table 1) may have contributed to these unexpected results. Nevertheless, we can generalize that populations of size 2 composed of completely unrelated individuals had lower rates of gene flow than those composed of more closely related individuals.

Population size by relatedness interaction—We anticipated that increased genetic relatedness would more strongly affect apparent gene flow rates into populations of size 2 than into larger populations because on average they are more likely to have fewer S-alleles and therefore higher levels of cross-incompatibility. In other words, we predicted significant population size by relatedness interactions. These predictions are based on the probability of cross-compatibility between individuals of given genetic relatednesses. For instance, a population of two randomly selected full siblings has only a 0.25 probability of cross-compatibility (assuming sporophytic self-incompatibility). If the two individuals are reciprocally cross-incompatible, then foreign pollen must sire all seed set in that population. Even if the two individuals are partially (non-reciprocally) cross-incompatible, one plant will require an influx of foreign pollen to set seed. When population size increases from two to five full siblings, there is a fourfold increase in the number of potential mates and a probability of 0.68 that at least one of those will be compatible. In populations of 20 full siblings, the probability approaches 1.0. The significant population size by relatedness interaction varied over the three times (Table 2A, Figs. 2, 3). Within each time, however, the two-way interaction was significant (Table 3). Therefore, interactions between population size and relatedness are important in determining gene flow rates into these small populations.

Combinations of small population size and low S-allele diversity, perhaps due to inbreeding or drift, have been cited as a potential cause of low reproductive output in some isolated natural populations of self-incompatible plants (Byers, 1995). In rare or endangered species, gene flow into such populations has been suggested as a management tactic to introduce new S-alleles into the population (DeMauro, 1993). Understanding how gene flow may vary with demographic and genetic parameters of populations, therefore, can provide useful information for developing management practices for rare plants. For example, our study suggests that maintaining an array of small subpopulations in a region may help maintain S-allele or mating-type diversity through pollen gene flow among subpopulations. The rate of pollen gene flow is likely to be highest in the smallest populations with the highest levels of cross-incompatibility. Despite the potentially higher rates of gene flow, however, the absolute numbers of seed set in small populations may be lower in smaller populations than in larger populations (Elam, 1994; Agren, 1996).

Isolation distance—Theoretical and empirical findings predict that gene flow rates into a target population will decrease with increasing distance from the nearest pollen source population (reviewed by Levin and Kerster, 1974; Handel, 1983). Many of these studies have investigated essentially unilateral gene flow from very large sources into relatively small populations and found that gene flow decreases with distance from the large source (Williams and Evans, 1935; Klinger, Arriola, and Ellstrand, 1992). Results of empirical studies measuring multilateral gene flow from multiple source populations that vary in size do not strongly support the predicted negative relationship between gene flow and isolation distance. Gene flow rates into a population of *Gleditsia triacanthos* were only

weakly correlated with isolation distance (Schnabel and Hamrick, 1995), and Ellstrand, Devlin, and Marshall (1989) reported that pollen gene flow into natural populations of *R. sativus* was not associated with isolation distances. In the present study, apparent pollen gene flow rates into the populations of size 2 were negatively associated with distance from the nearest population of 10 or 20 individuals, although other factors, such as relatedness and time, also had strong effects on apparent pollen gene flow. Pollen flow between populations in this study was probably not unidirectional, even into populations of size 2 whose closest neighbor was large. Nevertheless, distance to a relatively large pollen source cannot be ignored as a factor contributing to the rate of pollen gene flow.

Conclusions—A variety of ecological, genetic, and demographic factors may interact in determining the dynamics of pollen movement within and between populations, resulting in a departure of empirical observations from theoretical predictions derived from simple models. Results from empirical studies that emphasize the idiosyncrasy of pollen gene flow rates may, in part, reflect these interactions. This study provides experimental evidence that interactions between demographic (population size) and genetic factors (relatedness), as well as the spatial arrangement of populations, are important in determining gene flow rates into populations. The relationships of genetic and demographic factors to gene flow described here may be useful in better defining models of evolutionary change in small populations. These results may also prove useful to those making management decisions about how to allocate financial and natural resources to obtain a favorable distribution of rare, self-incompatible plant populations that will maintain mating type diversity. Clearly, more experimental work is needed not only to determine which ecological, demographic, and genetic factors may be important influences on patterns of gene flow between natural populations, but also how they may interact in their effects on gene flow.

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