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GENE FLOW IN *CHAMAECRISTA FASCICULATA* (LEGUMINOSAE) II. GENE ESTABLISHMENT

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Abstract.—The role of gene establishment in gene flow was investigated in a population of the annual legume *Chamaecrista fasciculata* by determining the effect of interparent distance on progeny fitness throughout the entire life history. A decelerating gain in progeny fitness with increasing interparent distance was observed. Selfed progeny suffered a 2-fold fitness disadvantage compared to progeny derived from mating events between individuals in the same neighborhood. Progeny derived from within neighborhood crosses had lower fitness than progeny from crosses between neighborhoods. Coupling the effect of interparent distance on gene establishment with information on gene dispersal resulted in a considerable increase in estimated gene flow. However, gene flow was still limited, as the average neighborhood area corresponded to a circle with radius of approximately 3.0 m. Yearly fluctuations in population size and variation in reproductive output lowered the estimate of N_e below the census estimate to approximately 100 individuals. The role of a seed bank in increasing the estimate of N_e was found to be insignificant. It is likely that genetic drift plays a major role in determining the distribution of genetic variation in this population.

The consequences of gene dispersal across different levels of population subdivision will depend on the role of selection and drift in determining the extent of local genetic differentiation. If there is a selective basis for local genetic differentiation, then matings beyond the scale of local differentiation may generate progeny that are less fit than either parent (Jain and Bradshaw, 1966; Slatkin, 1973; May et al., 1975; Price and Waser, 1979; Falconer, 1981; Waser and Price, 1983, 1989). If gene dispersal is limited, then genetic drift may be important and most mating events may be between relatives (Wright, 1943, 1977) with the consequent expression of inbreeding depression (Levin, 1977, 1981). Longer distance crosses may decrease the likelihood of consanguineous mating events, resulting in heterotic offspring (Price and Waser, 1979; Waser and Price, 1983, 1989; Levin, 1984).

Gene flow is the product of two forces, gene dispersal and gene establishment, with gene establishment conditional on gene dispersal (Endler, 1977). Gene dispersal was found to be limited in a natural population of *Chamaecrista fasciculata* (Leguminosae), with most pollen and seed dispersal events occurring within several meters (Fenster,

1991a). In this paper the role of gene establishment in gene flow is determined for the same population of *C. fasciculata* at Gooselake Prairie (GLP), in northern Illinois. Previously (Fenster and Sork, 1988), the first component of gene establishment was measured, i.e., the ability of a pollen grain that has traveled a given distance to penetrate the style and fertilize the ovule. In this study the effects of interparent distance on seed set, seed germination, seedling survivorship, and the reproductive output of adults were examined. Since most pollen and seed are dispersed locally (<2.5 m, Fenster, 1991a), there is a high probability of mating between related individuals in successive generations. To fully assess the effect of interparent distance on progeny fitness, the effect of successive years of short pollination distance was examined. The genetic neighborhood (Wright, 1943, 1946) was used as an estimate of the scale of gene flow.

The role of gene flow in determining the pattern of genetic variation will depend on the effective number of individuals (N_e), i.e., the number of individuals of an idealized population that would give rise to the observed increase in inbreeding (Wright, 1943, 1946). Although N_e is largely determined by neighborhood area in a subdivided population, two factors are responsible for reducing N_e compared to the actual number of individuals within the neighborhood:

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variation among individuals in the number of progeny produced and variation in population size with time (Crow and Denniston, 1988). In addition, the presence of a seed bank can increase N_e (Charlesworth, 1980).

The objectives of this study are 2-fold. First, the effects of interparent distance on progeny fitness are directly coupled to information on gene dispersal to give an adequate description of actual gene flow (Levin, 1981). Second, N_e is measured to quantify the role of inbreeding and drift as determinants of population structure of *C. fasciculata* at GLP.

METHODS

Study Site and Study Organism

The study site is located at Gooselake Prairie Nature Preserve (GLP), Grundy Co., Illinois, a 700-ha disturbed mesic prairie located in the floodplain of the Illinois River. The population of *C. fasciculata* at GLP is subdivided into discrete subpopulations consisting of 100s–1000s of adult flowering individuals. Each of the approximately 30 subpopulations is separated from its nearest neighbor by 50–200 m. From these, 14 subpopulations were chosen for study using stratified random sampling.

Chamaecrista fasciculata Michx., partridge pea, is a self-compatible annual legume of old field, disturbed prairie and savanna. *Chamaecrista fasciculata* is exclusively bee pollinated (Lee and Bazzaz, 1982; Fenster, 1991a) and seed dispersal is through explosive pod dehiscence.

The Effect of Interparent Distance on Progeny Fitness(1983–1984).—To determine the effect of interparent distance on progeny fitness, hand-pollinations were conducted in the greenhouse between plants collected from the field of known interparent distance. In late June 1983, juvenile plants were collected from the 14 randomly chosen subpopulations at GLP. A quadrat, 1.5 × 1.5 m, was randomly chosen at one end of a subpopulation. From this quadrat, two plants were randomly chosen to act as female parents (pollen recipients). Females were (1) selfed and cross-pollinated with pairs of male parents (pollen donors) chosen from (2) within the same quadrat as the fe-

male parents, (3) a quadrat 2 m from the first, and (4) a quadrat at the opposite end of the subpopulation, corresponding to interparent distances of <1.5 m ($\bar{x} = 0.8$ m), 3–5 m ($\bar{x} = 3.2$ m), and approximately 40 m, respectively (Fig. 1). Pairs of males from single quadrats served as pollen donors for crosses between (5) adjacent subpopulations (50–250 m, $\bar{x} = 120$ m), (6) intermediate subpopulations (300–800 m, $\bar{x} = 610$ m), and (7) far subpopulations (1,100–2,000 m, $\bar{x} = 1,320$ m) (Fig. 1). This sampling regime was replicated across the 14 subpopulations. Based on pollen and seed dispersal distances (Fenster, 1991a), the six categories of cross-pollination corresponded to neighborhood units of within a neighborhood (cross 2), between adjacent neighborhoods (cross 3), between far neighborhoods in the same subpopulation (cross 4), and between neighborhoods in different subpopulations of increasing distance (crosses 5, 6 and 7).

Single donor hand-pollinations were performed in the greenhouse on 14 pairs of maternal plants, corresponding to the 14 subpopulations. All flowers on a plant were pollinated each day. Because of the time constraints of labeling each flower, only a subset of the flowers was followed for each pollen donor. On average, 11 flowers/cross type/plant were pollinated and followed.

All labeled flowers were examined for fruit maturation and the number of seeds produced. The effect of interparent distance on the probability of seed germination, survivorship, and fecundity of the F_1 was determined on a randomly chosen subset of the fruit. Seeds from each female parent in a given neighborhood were planted in flats containing soil from the maternal collection site and screened for native seed. On average 41 seeds/cross were planted in each subpopulation (20.5 seeds/cross/female × 2 females/subpopulation), totaling 4,032 seeds distributed among the 7 crosses and 14 subpopulations. The flats were covered with leaf litter collected from the respective subpopulations and allowed to overwinter in a protected outside area. In early April 1984, about 1 month prior to natural germination, the flats were transplanted to their respective maternal subpopulations. The flats were sunk to ground level and covered with leaf litter. All germinating seeds were
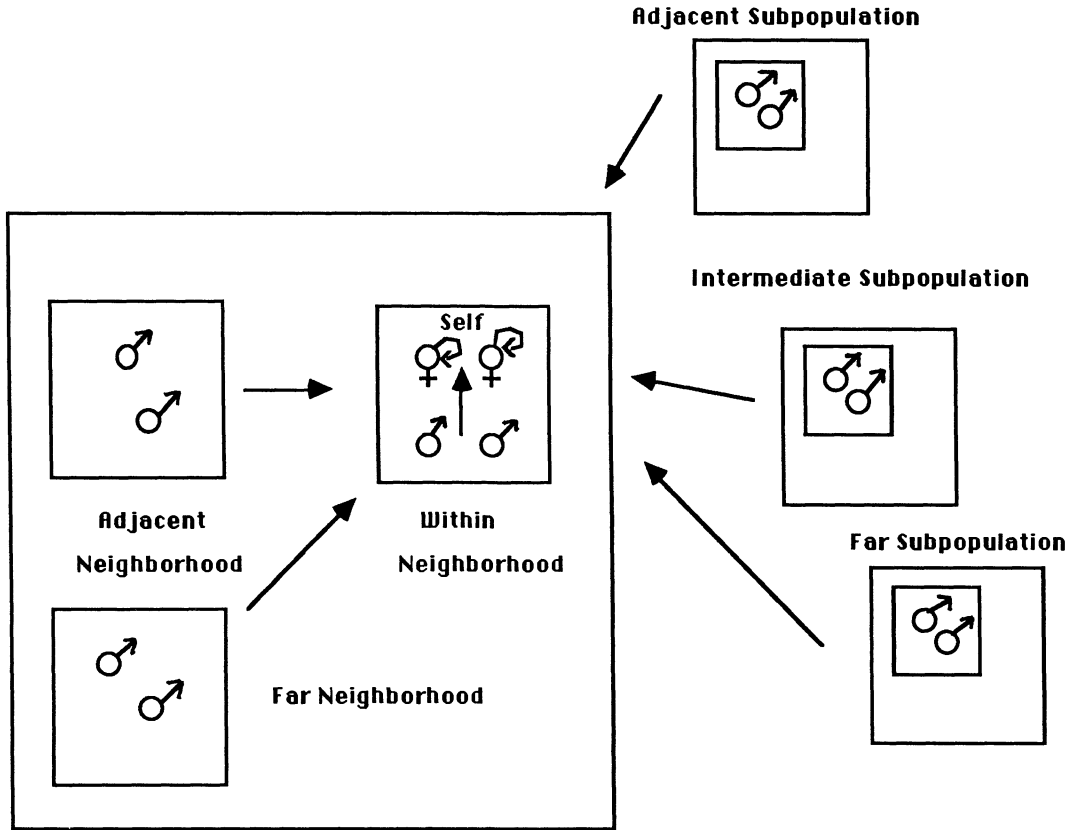
FIG. 1. Spatial distribution of plants used in the greenhouse crossing experiment.

transplanted into a random array (17 rows × 17 columns, each plant separated from its nearest neighbor by ≥9 cm) into the same 1.5 m² quadrat from which their maternal parents were collected. Survivorship and flower and fruit production were censused weekly from July 1 until the first killing frost (October 15). Roughly ⅓ of all seeds planted germinated, and ⅓ of the seedings survived to flower.

The Effect of Successive Generations of Inbreeding (1984–1985).—To determine the consequences of repeated bouts of short pollination distance four types of hand-pollinations were performed in the greenhouse in September 1984. F_1 plants were derived from lines established in the 1983 greenhouse pollinations. Progeny from within-neighborhood crosses (≤1.5 m) were selfed and crossed with plants from the same neighborhood that shared either 2 parents (full sibs), 1 parent (½ sibs), or were unrelated to the preceding 1983 generation of

crosses. The crosses were conducted between lines established from 8 of the 14 subpopulations.

Seeds from the crosses were collected in October 1984,and planted in soil from the site of the maternal grandparental generation. To contrast the effect of local inbreeding with wider crosses progeny derived from two 1983 crosses—interparent distances between far neighborhoods within the same subpopulation (40 m) and between far subpopulations ($\bar{x} = 1,320$ m), were planted with the 4 classes generated in 1984. The seed were treated similarly to seed collected in the 1983 experiment. Germination, survivorship, and flower and fruit production were measured throughout the spring and summer of 1985 at GLP.

Data Analysis

The Effect of Interparent Distance on Progeny Fitness (1983–1984).—The CAT-MOD procedure (SAS, 1985) was used to

examine the effect of female and interparent distance on fruit maturation. A mixed model, partially hierarchical ANOVA (Brownlee, 1965) was used to examine the effect of interparent distance on the number of seeds produced/fruit, with cross (fixed) and subpopulation (random) as the main effects. Cross was tested against the interaction between cross and subpopulation. Friedman rank tests were used to examine the effect of interparent distance on seed germination, seedling survivorship to reproduction, and flower and fruit production in the field, with subpopulation as the replicated block. Subpopulation means were also used as the dependent variable for a regression analysis of these later life-history traits on interparent distance and on the ln(interparent distance). The ln transformation of interparent distance was conducted to determine the contribution of a nonlinear relationship between interparent distance and the fitness related traits. If regressions on both interparent distance and ln(interparent distance) were significant then a stepwise regression was performed to determine which independent variable explained more of the variance. All 14 subpopulations were used to examine the effect of cross on fruit maturation, number of seeds/fruit, and germination. Sites with ≤10 individuals surviving to flower were excluded from the analysis where environmental factors were greatest. Only 9 of the 14 subpopulations had adequate sample sizes. Exclusion of the 5 subpopulations did not alter the results based on data pooled across subpopulations. A multiplicative fitness function was used to determine the cumulative effect of interparent distance throughout the entire life history of *C. fasciculata*. All measured parameters were used, including those that showed nonsignificant differences among treatments. The relative fitness of a cross was determined by dividing all fitness values by the observed maximum.

The Effect of Successive Generations of Inbreeding (1984–1985). — Because of poor survivorship in three of eight subpopulations and incomplete replication of the crosses, the data investigating the consequences of successive generations of local crosses could not be analyzed in the same manner as the 1983–1984 relative fitness

data. Instead, Spearman rank correlations between the overall mean germination rate across all 8 subpopulations, and the different levels of inbreeding were determined. Survivorship, flower production, fruit production, and relative fitness were also examined by conducting Spearman rank correlations using data from only 5 of the subpopulations where survivorship was high (>10 individuals surviving to flower/subpopulation). Again, exclusion of sites did not alter results based on data pooled across subpopulations.

Gene Establishment and Calculation of Neighborhood Area. — The estimates of pollen dispersal are over a continuous scale, while the fitness estimates are for discrete distances. Gene dispersal and gene establishment are combined in the following manner. The proportion of pollen dispersal events <3.0 m is determined by converting the distance of 3 to a unit normal standard variate $(3/\sigma_p)$ where σ_p was determined in Fenster (1991*a*). The proportion of pollen dispersal events <3.0 m is weighted by the relative fitness of the interparent distance cross of 1.5 m and the proportion of all pollen dispersal events ≥3.0 m is weighted by the relative fitness of the 3–5 m interparent distance cross, to give a new distribution of gene flow events based on pollen dispersal and gene establishment. The standard deviation of this new distribution is then calculated, and neighborhood area based on pollen dispersal, weighted by the effect of interparent distance on progeny fitness, is combined with estimates of seed dispersal to give a calculated neighborhood area combining information on gene dispersal and gene establishment. The effective outcrossing rate was determined by weighting the proportion of selfing events, measured at the mature seed stage with the multilocus outcrossing rate estimation procedure (Ritland and Jain, 1981; Fenster, 1991*a*), by their relative fitness compared to progeny derived from outcrossing events of <3.0 m and ≥3.0 m.

Density and Effective Density. — To determine the average effective number of individuals in a genetic neighborhood at GLP, yearly censuses were conducted from 1983 to 1986. In the spring of 1983, 4 permanent quadrats (1 × 1 m) were established at ran-

TABLE 1. ANOVA of effect of interparent distance on seed number/fruit.

Source	df	MS	F
Interparent distance	6	1.0549	3.35**
Subpopulation	13	3.6676	2.88*
Female (subpopulation)	14	1.2735	4.17***
Interparent distance × subpopulation	78	0.3148	1.03
Interparent distance × female (subpopulation)	83[a]	0.3055	1.06
Error	886	0.2817	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[a] 1 plant was male sterile resulting in the loss of 1 df.

dom points along a 40-m-long transect spanning each subpopulation. At the end of each season (late September–early October), the number of individuals in each quadrat was counted. Fruit production, an estimate of family size for each maternal individual, was determined for each individual in the censused quadrats from 1983 to 1986. Individuals with no fruit were included in the sample. In 1986, individuals outside the quadrats were used to supplement estimates of the variation in fecundity. The variance of family size was then calculated for each subpopulation, first adjusting to correspond to a population of stable size ($\bar{x}$ family size = 2) and a model of independence of intrafamily fate (Crawford, 1984). The mean variance in family size across all censused subpopulations was calculated and substituted into the following formula to determine the effective number of individuals (Crow and Kimura, 1970):

$$N_e = \frac{4N - 2}{V_k + 2} \qquad (1)$$

where N is the mean number of individuals across all subpopulations, V_k is the corrected variance, and N_e is the effective number of individuals. To take into account fluctuating densities across years, the harmonic mean number of individuals across years at GLP was calculated (Crow and Kimura, 1970).

The presence of a seed bank of *C. fasciculata* was measured by survivorship of seed in the flats. Seeds were planted in the flats in the fall of 1983 for the fitness experiment, taken to the prairie about 1 month prior to natural germination, and then were left in the prairie through the summer of 1986. Each fall all dispersing adults were removed within 4 m of the flats, well beyond seed dispersal distances (Fenster, 1991*a*). Each

spring, 1984–1986, the number of seedlings germinating in the flats was recorded. If the proportion of seeds surviving in the soil beyond 1 year is large (mean age of seeds is $\gg 1$ year) then the effective number of observed individuals may be greater than the observed census count (Hill, 1979, cited in Falconer, 1981).

RESULTS

The Effect of Interparent Distance on Progeny Fitness (1983–1984).—There was no significant effect of interparent distance on the probability of fruit maturation [X^2 = 9.29, 6 df, ns, Fig. 2A]. A trend of increasing fruit maturation with longer crossing distances was observed but the effect of female parent was far more important [X^2 = 149.5, 27 df, $P < 0.001$]. Interparent distance had a significant effect on the mean number of seeds produced/fruit (Table 1, Fig. 2B), but a Tukey's multiple comparison of the means indicated that the effect is strictly a self vs. nonself phenomenon (Table 2). Progeny from self and short-distance crosses tended to have lower seed germination (Fig. 2C) and a lower probability of surviving to flower in the field (Fig. 2D) but in neither case was the effect of distance significant. There was a significant effect of interparent distance on flower production in the field [Fig. 2E, $S = 17.6$, 6 df, $P < 0.01$]; again the effect was due to lower fruit production in self progeny vs. all other progeny (based on Friedman contrasts of the ranks). Regression analysis did not reveal a significant relationship between flower production and interparent distance and demonstrated a marginal relationship with ln(interparent distance) [$r^2 = 0.04$, 62 df, $P < 0.10$]. Progeny of longer distance crosses tended to produce more fruit in the field than progeny from shorter distance crosses,
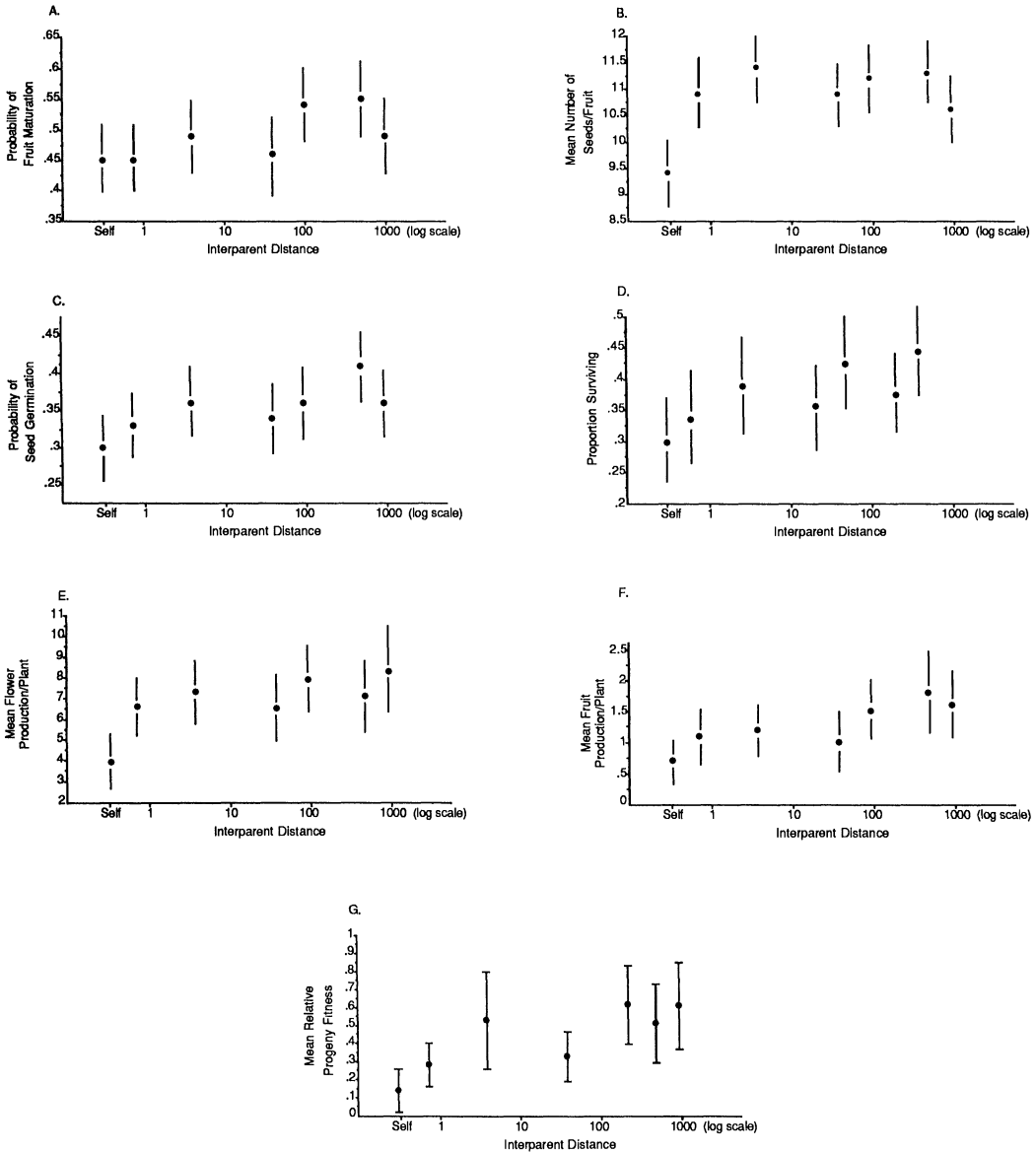
FIG. 2. 1983–1984. The effect of interparent distance on (A) the probability of fruit maturation, (B) the number of seeds produced/fruit, (C) the probability of seed germination, (D) the probability of germinated seed surviving to flower, (E) flower production, (F) fruit production, and (G) mean relative progeny fitness. The means with 95% confidence intervals are presented.

but the effect was not significant using either Friedman rank or regression analysis (Fig. 2F).

The relative fitness of the crosses, using each of the 9 subpopulations as replicates, is presented in Figure 2G and the average relative fitness of the crosses based on pooled data is presented in Table 2. In both cases there was a significant effect of interparent distance on progeny fitness associated with increasing interparent distance [Friedman rank $S = 23.4$, 6 df, $P < 0.002$; $r^2 = 0.10$, 62 df, $P < 0.01$, regression on interparent distance; $r^2 = 0.23$, 62 df, $P < 0.0001$, regression on ln(interparent distance)]. When both interparent distance and ln(interparent distance) were included in the same stepwise regression analysis only ln(interparent

TABLE 2. The effect of interparent distance on progeny fitness throughout the entire life-history of *C. fasiculata*.[1]

Interparent distance (m)	Life-history stage					Pro-duct	Mean relative progeny fitness
	1	2	3	4	5		
Self	0.45 A	9.4 A	0.30 A	0.30 A	3.9 A	1.52	0.21 A
≤1.5 (within neighborhood)	0.45 A	10.9 B	0.33 A	0.34 A	6.6 B	3.63	0.50 AB
3.0–5.0 (between neighborhood)	0.49 A	11.4 B	0.36 A	0.39 A	7.3 B	5.73	0.79 B
Approx. 40 (between far neighborhoods)	0.46 A	10.9 B	0.34 A	0.36 A	6.5 B	3.99	0.55 AB
50–200 (between adjacent subpopulations)	0.54 A	11.2 B	0.36 A	0.42 A	7.9 B	7.22	1.00 B
400–800 (between intermediate subpopulations)	0.55 A	11.3 B	0.41 A	0.38 A	7.1 B	6.87	0.95 B
1,000–2,900 (between far subpopulations)	0.49 A	10.6 B	0.36 A	0.44 A	8.3 B	6.83	0.95 B

[1] The mean probability of fruit maturation (column #1), the mean number of seed/fruit (2), and the mean probability of seed germination (3) are based on data collected from all 14 subpopulations. The mean probability of surviving to flowering (4) and the mean number of flowers and fruit produced per plant (5) are based on data collected from 9 subpopulations. The multiple fitness function, the product of the first 5 columns, and the relative progeny fitness are based on pooled data. Comparisons of number of seed/fruit were made by Tukey's contrasts, while all other comparisons were made by Friedman contrasts of the ranks. Means followed by the same letter are not significantly different from one another, $P < 0.05$.

distance) showed a significant relationship with relative fitness of progeny, independent of the order in which the variables were chosen. A Friedman contrasts of the ranks revealed that the effect was primarily a self versus nonself phenomenon (Table 2). However, a simple regression of the relative fitness of progeny, excluding the self progeny class, on ln(interparent distance) did show a significant relationship ($r^2 = 0.08$, 52 *df, P* < 0.05). A regression of relative progeny fitness on interparent distance excluding the self class was only marginally significant ($r^2 = 0.06$, 52 *df, P* < 0.10) and a stepwise regression with both interparent distance and ln(interparent distance) revealed a significant relationship only with the latter term.

Successive Generations of Short Interparent Distance Crosses (1984–1985). — There was a trend for the probability of germination to be lower in progeny derived from one generation of within-neighborhood crosses followed by one generation of selfing or full sib mating vs. crosses between less related individuals (Spearman rank $r = 0.77$, *P* < 0.10). There was no effect of inbreeding on the probability of seedlings surviving to reproduction, but less inbred progeny produced more flowers (Spearman rank $r = 0.87$, *P* < 0.06) and more fruit (Spearman rank $r = 0.94$, *P* < 0.05). Overall, the relative fitness of the progeny was marginally correlated with decreasing genetic relatedness among the parents (Fig. 3, Spearman

rank $r = 0.83$, *P* < 0.07). Progeny from the 40 m cross had similar relative fitness as the 1100 m or greater cross in the 1984–1985 experiment, in contrast to the previous year. Successive years of inbreeding did not result in increased inbreeding depression.

Effect of Gene Establishment on Gene Flow. — Weighting pollen dispersal by the effect of interparent distance on progeny fitness, increased the standard deviation of dispersal in each of the four years, from 17% (1983) to 25% (1984) (Table 3). The outcrossing rate determined by electrophoretic analysis of seedlings (Fenster, 1991*a*) was
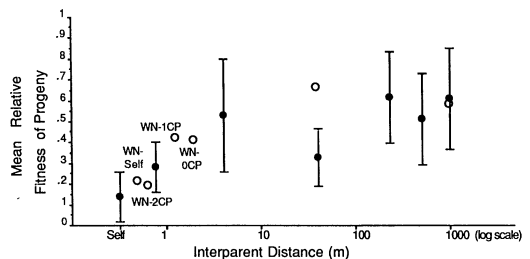

FIG. 3. 1984–1985. The effect of different levels of inbreeding on fitness of progeny (open circles). The results of the 1983–1984 experiment are superimposed (filled circles). WN-Self, within neighborhood cross followed by 1 generation of selfing. WN-2CP, within neighborhood cross followed by 1 generation of full sib mating. WN-1CP, within neighborhood cross followed by 1 generation of ½ sib mating. WN-0CP, within neighborhood cross followed by 1 generation of unrelated crosses between individuals with parents from the same genetic neighborhood. The means with 95% confidence intervals are presented for the 1983–1984 experiment.

TABLE 3. The effect of gene establishment on the standard deviation of pollen dispersal from 1983 to 1986.

Year	Pollen dispersal	Pollen dispersal weighted by gene establishment
1983	1.2	1.5
1984	2.1	2.6
1985	1.8	2.1
1986	2.0	2.5

increased from 0.8 to an effective outcrossing rate of 0.90 in 1983, and to 0.91 in 1984–1986 when the selfed progeny were discounted by their lowered fitness, compared to the progeny of the 1.5 and 3–5 m crosses. Weighting pollen dispersal by the effect of interparent distance on progeny fitness increased neighborhood areas by an average of 66% from 17.6 to 29.3 m^2 (Table 4).

Density and Effective Density. — The density varied greatly across subpopulations and across years, with the mean yearly density ranging from 3.4 to 10.5 individuals/m^2 (Table 5). The variance of family size among maternal plants at GLP was slightly more than expected (>2), based on a Poisson distribution (Table 6). The mean variance ranged from 2.3 to 3.1 across the four census years, reducing the effective density 7–22% below the observed density of individuals. The average effective number of individuals in the average genetic neighborhood of *C. fasciculata* at GLP varied from 100 to 166 individuals across the four census years (Eq. 1), with a harmonic mean number corresponding to 120 individuals.

TABLE 4. The effect of gene establishment on neighborhood area (N_a, m^2) and effective number of individuals (N_e) from 1983 to 1986.[1]

Year	N_a (pollen dispersal t = 0.80)	N_a (pollen dispersal weighted by gene establishment effective t = 0.90, 0.91)	N_a (pollen dispersal weighted by gene establishment + seed dispersal)	Effective density	N_e
1983	7.3	12.7	13.9	9.3	130
1984	22.8	38.7	39.9	3.5	105
1985	16.1	25.2	26.4	6.3	166
1986	19.6	35.8	37.0	2.7	100

[1] The N_a based on pollen dispersal has been weighted by a factor of ½ to take into account that total dispersal is the average of dispersal through the male and female components, and weighted by the outcrossing rate determined at the seed stage. The effective outcrossing rate t = 0.90 in 1983 and 0.91 in 1984–1986 reflects lower fitness of selfed progeny. Total N_a represents the combined contribution of pollen dispersal, weighted by gene establishment, and seed dispersal (1.2 m^2). Effective density represents the average density of individuals/m^2 multiplied by a factor to take into account a non-Poisson distribution of family sizes.

TABLE 5. Mean density (individuals/m^2) in each of 14 subpopulations from 1983 to 1986.

Subpopulation	Year 1983	1984	1985	1986
1	26.3	0	2.0	7.8
2	4.0	3.3	0.5	7.0
3	2.8	0.3	3.8	1.8
4	5.5	5.5	6.3	6.0
5	3.3	4.5	3.5	0.8
6	7.5	3.0	3.0	2.8
7	2.5	1.0	4.3	2.3
8	35.3	19.3	55.5	0
9	8.5	4.8	1.3	1.5
10	5.3	2.3	1.8	1.5
11	10.3	1.0	8.0	7.5
12	6.0	1.0	1.3	1.5
13	13.0	1.8	2.8	2.0
14	9.8	5.0	4.8	6.0
Mean	10.5	3.8	7.0	3.4

The presence of a short-lived seed bank was documented in GLP. In 1984, 36% of the seed planted the previous fall germinated, followed by 2 and 1% germination in the next 2 years. Assuming a linear decay of ½ of the seeds germinating in each additional year, then the mean age of plants at the time of seed formation is calculated as 1.2 years for the planted seeds. Therefore, the estimate of the effective number of individuals is only slightly increased by the presence of a seed bank.

DISCUSSION

The Effect of Interparent Distance on Progeny Fitness

Selfing of *C. fasciculata* was deleterious at different stages of the life history. Self-pollinations resulted in progeny with lower seed set, seed germination, juvenile survivorship, and reproductive output. The net effect was more than a 2-fold disadvantage compared to the nearest outcrossing distance (mean relative fitness of selfed progeny = 0.21 vs. 0.50 for the nearest outcrossed progeny. Table 2). Similarly, high levels of inbreeding depression have been observed in maize (Wright, 1977) and in other studies of natural populations (Jain, 1978; Price and Waser, 1979; Schoen, 1983; Schemske, 1983, 1984; Waser and Price, 1983; Schemske and Pautler, 1984; Waller, 1984; Ritland and Ganders, 1987). The high degree of inbreeding depression following

TABLE 6. Mean variance of fruit set, an estimate of family size, across 14 subpopulations for 4 years at Gooselake Prairie.

	Year			
	1983	1984	1985	1986
Mean variance	2.5*	2.3	2.5	3.1*
Range	1.4–3.9	0.6–7.0	0.8–12.8	2.6–4.4
Effective density/observed density	0.89	0.93	0.89	0.78

* $P < 0.05$ that mean variance = 2 (Sign test).

selfing correlates with the high outcrossing rate, which is approximately 80% (Fenster, 1991a). Both Falconer (1981) and Wright (1977) observed that outbreeders generally show greater inbreeding depression than selfers, presumably due to the maintenance of deleterious alleles in outcrossing populations (Lande and Schemske, 1985). In addition crosses within a neighborhood of gene dispersal (<3.0 m, Fenster, 1991a) result in lower progeny fitness compared to crosses between parents in different neighborhoods within the subpopulation, which in turn have progeny of lower fitness compared to progeny of crosses between subpopulations.

Other evidence also indicates that progeny fitness increases rapidly with interparent distance over short distances, and then plateaus. First, within-neighborhood crosses resulted in lower progeny fitness compared to between-neighborhood crosses (Fig. 3). Also the progeny of the 40 m cross have the same fitness as progeny from the longest crossing distance (1,100–2,000 m, 1984–1985 experiment). Second, assuming the lowered vigor of the progeny from selfed and within neighborhood crosses is due to inbreeding depression resulting from mating between related individuals, then the rapid increase in fitness with increasing interparent distance fits the expectation of limited gene dispersal documented in Fenster (1991a). The degree of heterosis expressed in progeny depends on the amount of genetic differentiation between the parents (Falconer, 1981). Thus the decreasing gains in progeny fitness suggest that genetic relatedness falls off exponentially with distance, in accordance with the stepping stone model of limited gene flow (Malecot, 1969; Kimura and Weiss, 1964). Studies of *Drosophila* (Wright et al., 1942; Wallace, 1966) and *Phlox* (Levin, 1977, 1984) also demonstrated a rapid dropoff of lethal allelism

with distance, suggesting that the study populations were not panmictic but were subdivided into a number of breeding units with mating among relatives common.

The expected rate of allelism of lethal genes in one- and two-dimensional stepping stone models of gene flow falls off as a negative exponential with distance (Yokoyama, 1979). The rate of decrease is most affected by the magnitude of gene flow between colonies and is fastest when gene flow is small. In *C. fasciculata* at GLP, progeny fitness appears to begin to plateau at or slightly beyond the 3–5 m distance class. The beginning of the plateau corresponds with the estimated boundary of the neighborhood (Fenster, 1991a), and is further evidence that gene flow is limited and that mating events of short distances are between close relatives. Additional genetic evidence for extreme population subdivision in *C. fasciculata* based on an isozyme survey will be presented in a subsequent paper.

Since there was no increase in inbreeding depression following two generations of local crosses, no additional weighting of short distance crosses on pollen dispersal was necessary. Increased homozygosity may not lead to significantly increased inbreeding depression if the effects of homozygosity for 2 or more loci do not contribute additively to the decline of a trait (Crow and Kimura, 1970).

Self and short distance crosses result in the expression of inbreeding depression of the progeny and thus are not weighted as heavily as longer distance crosses. Because of the low fitness of the self progeny, the proportion of reproducing individuals in the next generation derived from outcross mating events increases from 0.8 at the seed stage to approximately 0.9 at the adult stage. Variation in the effective outcrossing rate between 1983 and 1984–1986 reflects the

increased likelihood of pollen dispersal events occurring beyond 3.0 m in 1984–1986 due to lower density of flowering individuals in these years (Fenster, 1991*a*). A similar increase of the outcrossing rate over the life history has been observed in tree species that retain their seed over a number of years. By genotyping different aged cohorts of alpine ash (Moran and Brown, 1980), lodgepole pine (Hamrick and Schnabel, 1985), and jack pine (Cheliak et al., 1985) it was found that the outcrossing rate increased steadily with the age of the cohort, implying that the seeds derived from selfing events had a lower survivorship on the maternal parent. Therefore determination of outcrossing rates based solely on pollinator movement or electrophoresis of progeny at an early stage in the life history may not reflect the effect of outcrossing on the genetic structure of the population (but see Loveless and Hamrick, 1984). Populations which show intermediate levels of outcrossing as determined by progeny analysis at early life history stages may actually have genetic loads associated with much higher outcrossing rates, if the selfed progeny suffer considerable inbreeding depression (Schemske and Lande, 1985).

Outbreeding depression, the lowered fitness of progeny following wide crosses, has historically been associated with crossing events of very long distance at the level of populations, races or subspecies (Kruckeberg, 1957; Hughes and Vickery, 1974; Dobzhansky, 1970). The lowered fitness of progeny observed on a far smaller scale, on the order of 100's of m by Price and Waser (1979), Waser and Price (1983, 1989) and Schemske and Pautler (1984), suggests spatial variation of selective pressures on this scale. However, no evidence of optimal crossing distances was found in this study, suggesting that drift and not selection is responsible for local differentiation of *C. fasciculata* at GLP. Sobrevila (1988) observed variation among populations of *Espeletia schultzii* in the effect of interparent distance on seedling survival and found that pollinations conducted between populations at different altitudes along an elevational gradient had lower seed set than crosses between populations at the same altitude. These results suggest that the magnitude of selection and drift vary among the *E. schultzii* populations.

The Effective Number of Individuals.—According to Wright (1951, 1965) drift should play an important role in determining the genetic structure of a population if the effective number of individuals is fewer than 200 and spatial heterogeneity in selection pressures is low. In *C. fasciculata* at GLP, the estimate of the variance of family size, on average, was slightly greater than the random expectation, and the effective number of individuals was reduced by 17–22%. In other studies, (cited in Crawford, 1984) the ratio of the effective number of individuals to the observed number of individuals ranges from 0.01–0.95.

An equally important force in reducing the N_e of *C. fasciculata* at GLP was the frequent occurrence of population bottlenecks. The density of *C. fasciculata* varies among subpopulations, and among years, depending on water availability and the time since disturbance (fire). In years of low density, greater inbreeding occurs which is not affected by the expansion of numbers in later years (Falconer, 1981), resulting in the low estimate of N_e of 120 individuals across the four census years.

A seed bank can influence the effective number of individuals since loss of genetic variation can be buffered by the release of "lost" genetic variation from the seed bank (Epling et al., 1960; Templeton and Levin, 1979). However, the mean generation length of 1.2 years suggests that the seed bank of *C. fasciculata* at GLP has a small effect on the effective number of individuals, aside from reducing the probability of extinction in the immediate area. As a result, the effective number of individuals in the average neighborhood at GLP appears to be well below the threshold number of 200 at which the role of drift becomes less important.

Evolutionary Implications of Limited Gene Glow.—A low N_e suggests that a considerable amount of biparental inbreeding is occurring. The observation that 1 generation of within neighborhood crosses result in progeny that are intermediate in fitness compared to full and half-sib crosses further suggest that mating events within the neighborhood are between fairly close relatives. A lower frequency of deleterious

alleles may be maintained in the population as a consequence of biparental inbreeding (Uyenoyama, 1986; Waller, 1986). However, despite the high likelihood of mating between relatives, considerable genetic load is maintained within the population, enough to maintain a 2-fold difference in fitness between self and within neighborhood pollinations. Depending on the genetic basis of inbreeding depression limited gene flow need not result in the reduction of inbreeding depression (Charlesworth and Charlesworth, 1987), an important selective agent for the maintenance of an outcrossing breeding system (Lande and Schemske, 1985). A consequence of fitness differences among progeny with different levels of inbreeding from within the neighborhood (Fig. 3) is greater representation of progeny derived from less related individuals. Limited seed dispersal at GLP (Fenster, 1991a) may also promote sib competition, thus contributing to this differential representation.

The positive correlation of progeny fitness with interparent distance suggests there should be intense selection on floral characters associated with the avoidance of inbreeding by increasing pollen dispersal distance. In GLP relative flowering time of *C. fasciculata* influences pollen dispersal because interparent pollinator flight distances increase during periods of lower floral density (Fenster, 1991a). Therefore relative pollen dispersal distance may be an important selective agent on flowering time. However, periods of low floral density were also associated with a low probability of fruit production. Within-population variation in pollen morphology (Tonsor, 1985) and stamen and style lengths (Campbell, 1989; Campbell and Waser, 1989) can also be associated with differential pollen dispersal ability. The advantage associated with increased pollen dispersal will depend on the origin of genetic differentiation occurring at various levels of population subdivision. Differentiation due to divergent selection pressures may lead to selection to reduce pollen dispersal (Jain and Bradshaw, 1966; Slatkin, 1973; May et al., 1975; Price and Waser, 1979; Falconer, 1981; Waser and Price, 1983, 1989). Pollinator grooming behavior may also be an important constraint to the evolution of high pollen dispersal in

◨ - Seed Dispersal

▤ - Pollinator Flight Distance

▨ - Pollen Carry-Over
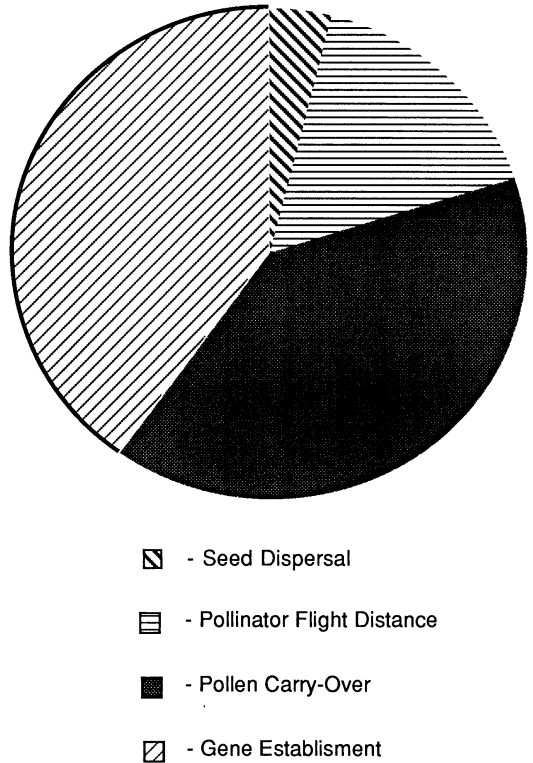
▨ - Gene Establisment

FIG. 4. The relative contribution of gene dispersal and gene establishment to gene flow.

animal pollinated plants (Harder and Thomson, 1989).

Limited pollen dispersal constrains the opportunity for female mate choice via gamete competition (Willson, 1977; Willson and Burly, 1983; Stephenson and Bertin, 1983; Lee, 1984). The paternity pool, i.e., 99% of the potential male parents that can sire offspring on a given female (Levin, 1988), was sometimes as large as 150 individuals. However, because plants are sessile, males in the paternity pool do not have equal access to females. The probability of mating events between individuals is negatively correlated with interparent distance. No variation in male fitness was observed in *C. fasciculata* either at the gametophyte stage (Fenster and Sork, 1988) or in the ability of pollen donors to draw resources differentially from seed parents (Fenster, 1991b). Variation in viability of offspring associated with different male parents is a consequence of population subdivision and results from differences in genetic relatedness among pairs of mating individuals.

CONCLUSION

Most of gene dispersal is through pollen dispersal (Fenster, 1991*a*) and weighting pollen dispersal by the effect of interparent distance on progeny fitness nearly doubles the estimate of gene flow for *C. fasciculata* (Fig. 4). The increased fitness of progeny from crosses outside the neighborhood has the effect of opening up the population structure (Levin, 1981), resulting in larger neighborhood areas and lowering the degree to which genetic drift will cause local differentiation. Due to the combined effects of pollen carryover and gene establishment, gene flow probably approximates a normal distribution. However, limited pollen and seed dispersal in *C. fasciculata* constrain the role of gene establishment in gene flow. Despite the role of gene establishment, neighborhood areas are still small, as predicted by Levin (1981) and suggest that inbreeding and drift should play an important role in determining the spatial pattern of genetic variation in the population of *C. fasciculata* at GLP.

ACKNOWLEDGMENTS

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