

ANT-MEDIATED SEED DISPERSAL ALTERS PATTERN OF RELATEDNESS IN A POPULATION OF *TRILLIUM GRANDIFLORUM*

SUSAN KALISZ,^{1,3} FRANCES M. HANZAWA,² STEPHEN J. TONSOR,^{1,3} DENISE A. THIEDE,^{1,4} AND
STEVEN VOIGT¹

¹W. K. Kellogg Biological Station and Department of Botany and Plant Pathology,
Michigan State University, Hickory Corners, Michigan 49060 USA

²Department of Biology, Grinnell College, Grinnell, Iowa 50112 USA

Abstract. Seed dispersal creates the initial spatial distribution of individuals in a population and in conjunction with the mating system influences spatial patterns of relatedness. This spatial template of related individuals sets the stage for all subsequent density-dependent and frequency-dependent interactions. In this study we document how ant-mediated seed dispersal affects the number and relatedness of seeds in both dispersed and undispersed aggregations and how these patterns influence seedling emergence in the long-lived perennial, *Trillium grandiflorum*. Experimental hand-pollinations in two years demonstrated that selfing is extremely rare and suggested that self-incompatibility (SI) is a likely explanation. Our multi-locus outcrossing estimate ($t_m = 1.05 \pm 0.056$) confirms this result and also suggests that seeds within a fruit are likely to have the same pollen parent. Thus a highly outcrossing mating system is the initial determinant of relatedness among seeds within a fruit. We tracked uniquely coded, radiolabeled seeds from 30 and 40 fruits in 1991 and 1992, respectively, to determine how dispersal alters this initial relatedness of seeds. Of the 335 and 876 seeds labeled in these two years, we recovered 63% and 76% of the seeds postdispersal and found that 19% and 23% of the recovered seeds were dispersed >10 cm from the maternal parent in the first and second years, respectively. In both years, ant-mediated dispersal reduced the number of seeds near the maternal parent. However, the effect of seed dispersal on the number of seeds in aggregations varied among years. Ant-mediated dispersal increased the number of seeds in dispersed aggregations in the first year and decreased the number in the second year. The average seed dispersal distance also differed between years: 2.41 m (± 0.33) vs. 0.53 m (± 0.06) in years 1 and 2, respectively. Ant-mediated seed dispersal decreased the probability of a seed having a sibling as its nearest neighbor postdispersal by between one-third and one-half. In contrast, seedling emergence was related to neither dispersal nor seed aggregation size in our study. However, the fitness effects of dispersal may be important later in the life cycle of this long-lived species and as such were undetected. One scenario is that plants derived from seeds dispersed out of their sibling relatedness group may gain minority advantage both in terms of mating success (if the population is SI) and other frequency-dependent processes like disease resistance.

Key words: ant-mediated dispersal; dispersal distances; genetic structure; mating system; outcrossing rate; radiolabeled seeds; seed dispersal; self-incompatibility; *Trillium grandiflorum*.

INTRODUCTION

The dispersal pattern of seeds and the genetic properties of those seeds produce the initial population template that defines the context of future ecological, demographic, and genetic interactions of emerging cohorts of seedlings. Since seed dispersal plays a central ecological and evolutionary role, it is not surprising that the majority of terrestrial plants have seeds or fruits that are modified for dispersal, with animal dispersal

being especially common (van der Pijl 1972, Howe and Smallwood 1982). From the plant's perspective, dispersal agents can be viewed as moving seeds from the ecological neighborhood of the maternal plant (the site occupied following seed release), to another in which the seed must ultimately germinate, establish, and grow. The maternal neighborhood into which the seeds are initially released and the site into which seeds are subsequently dispersed can differ ecologically in several ways. These ways include the density of conspecific seeds (e.g., Janzen 1970, Howe and Primack 1975), the density of inter- and intra-specific competitors, including competition with the maternal parent (e.g., Handel 1978, Beattie 1985), the microhabitat conditions such as nutrient or light levels, and/or the presence of predators or pathogens (e.g., Augspurger

Manuscript received 23 February 1998; revised 20 October 1998; accepted 20 October 1998.

³ Present address: Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 USA. E-mail: kalisz@pop.pitt.edu

⁴ Present address: Center for Population Biology, University of California, Davis, California 95666 USA.

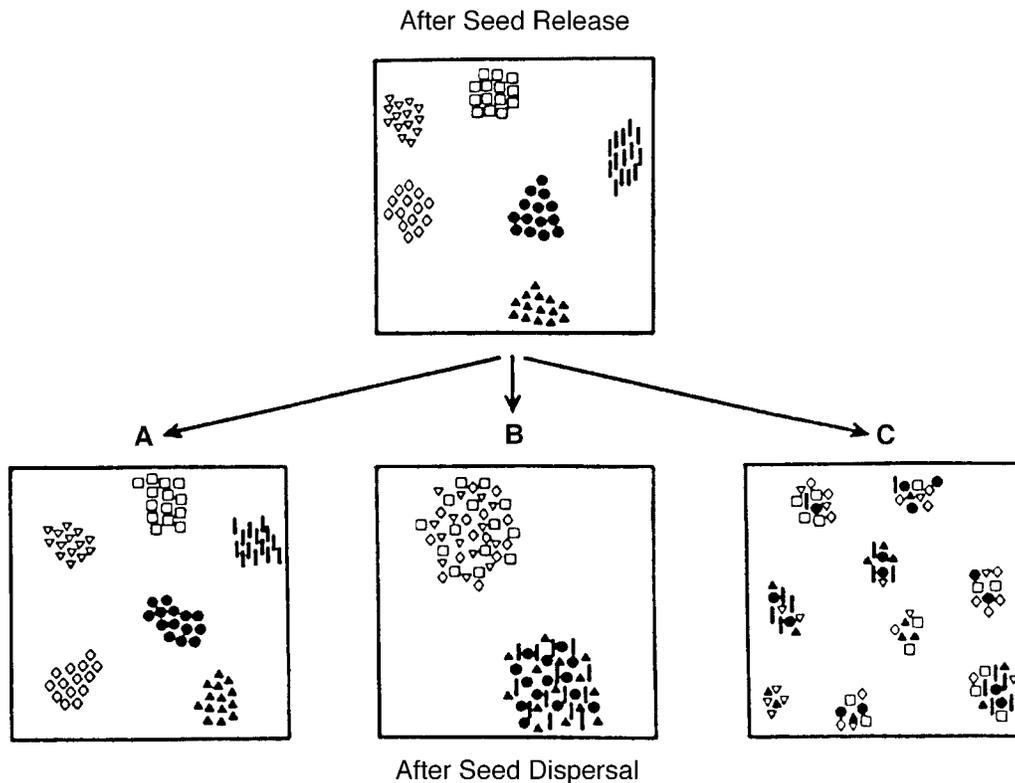


FIG. 1. Possible ecological and genetic patterns generated as a result of seed dispersal by ants. Each type of symbol represents seeds from a single maternal plant.

1983, Slingsby and Bond 1985, Kitijima and Augspurger 1989). Since dispersal changes the ecological conditions in which plants must establish, seed dispersal is expected to directly influence density-dependent components of plant fitness such as germination and survivorship (e.g., Handel 1978, Culver and Beattie 1980, Clark and Clark 1981, Davidson and Morton 1981, Horvitz 1981, Howe et al. 1985, Horvitz and Schemske 1986a, Hanzawa et al. 1988, Bergelson and Perry 1989).

In addition to moving seeds from one ecological environment to another, animal-mediated dispersal has the potential to move seeds from the genetic environment of the maternal parent to another, thus changing the degree of relatedness between conspecific seeds that are aggregated in space (Loveless and Hamrick 1984, Hamrick and Loveless 1986, Hamrick et al. 1993). Dispersal-mediated change in the genetic environment of seeds is important, because it can alter plant fitness through the operation of frequency-dependent selection. Examples of such frequency-dependent effects on the genetic environment have been found in studies of pathogen resistance (e.g., Jaenicke 1978, Augspurger 1983, Antonovics and Alexander 1986, Kitijima and Augspurger 1989), susceptibility to insect damage (e.g., Schmitt and Antonovics 1986), and performance under competitive conditions (e.g., Antonovics and Ellstrand 1984, Wilson et al. 1987,

Tonsor 1989). No studies have addressed how animal dispersal specifically changes both the ecological and genetic environments of seeds.

In addition to seed dispersal, the mating system will determine the degree of relatedness of seeds within a sibship and will interact with seed dispersal to determine the spatial distribution of related vs. unrelated individuals. Thus the mating system and seed dispersal jointly determine the evolutionary unit and the spatial scale over which gene flow and natural selection operate. Three possible changes in sib aggregations after animal dispersal that alter the genetic neighborhood and ecological neighborhood are given in Fig. 1. In panel A (Fig. 1), the foraging ranges of dispersal agents do not overlap, and each foraging range contains only one plant. In this case, neither the local genetic environment nor the conspecific seed numbers per aggregation are altered by dispersal, but ecological conditions may change. If most seeds are produced by selfing and dispersal by ants occurs at a low frequency, the relatedness of emerging seedlings in clusters will be as full sibs or higher. In panel B, the foraging ranges of dispersal agents do not overlap, and each contains >1 maternal parent. Dispersal causes an increase in the local seed number because multiple fruits within a single foraging range are aggregated. The local genetic environment diversifies as seeds from several sibships are mixed, and there is potential for changes in the

ecological environment as well. If seeds are produced by selfing and the probability of dispersal to multiple ant nests is high, then full sibs will be less likely to be interacting as seedlings. Conversely, if seeds are produced by outcrossing, the relatedness of emerging seedlings will be lower, owing to the potential for multiple paternity within fruits relative to selfed sibships. The relatedness of seedlings in aggregates will be further decreased if seeds are dispersed by ants to multiple nests. In panel C, the foraging ranges of dispersal agents overlap extensively, and each contains >1 maternal plant. Dispersal in this case results in a decrease in both local number per seed aggregation and in the relatedness of seeds in postdispersal aggregations. Aggregations of seeds contain individuals from multiple sibships, and seeds from any one sibship are likely to be dispersed to multiple sites. Again, the ecological environment is likely to be altered. These three patterns of seed aggregations (A–C) could be superimposed upon each other if multiple species are dispersing a single plant species. Clearly, both the spatial distributions of seeds post dispersal and their relatedness to other seeds within the aggregation can be expected to have significant consequences for events later in the life cycle.

The role of seed dispersal is central to the development of population genetic structure because it produces the original spatial distribution of individuals within populations (e.g., Furnier et al. 1987, Williams and Guries 1994, Gibson and Wheelwright 1995, Loisellet et al. 1995, Peakall and Beattie 1995). Determining the effect of animal dispersal on the spatial pattern, density, and genetic neighborhood of seeds requires that the movement and subsequent fate of individual seeds be followed. This is logistically difficult in most natural systems (Winn 1989, Bossard 1990) and as a result has rarely been attempted. Thus, ecological and genetic patterns apparent in many plant populations are assumed to be the consequence of seed dispersal, without data on the actual, initial seed dispersion pattern.

Myrmecochory, seed dispersal by ants, is a dominant dispersal syndrome in the understory of temperate deciduous forests (Beattie and Culver 1981, Handel et al. 1981), in the Australian scrub (Berg 1975, Hughes and Westoby 1992), South Africa (Milewski and Bond 1982), and elsewhere (see Beattie 1985, Handel and Beattie 1990). Ants are attracted to a lipid-rich body attached to the seed, termed the elaiosome, which is characteristic of myrmecochorous systems. In the act of dispersing seeds, ants first carry the seeds to their nest, where the elaiosome is removed. Afterward, the intact seed is deposited with other nest waste either in a nest midden or outside the nest. In this supposed mutualism, the ants receive elaiosomes as food. It has been suggested that the plants benefit from dispersal by ants because soil characteristics of ant nests provide a superior germination and establishment site for seeds (Culver and Beattie 1980, Beattie and Culver 1983, Hanzawa et al. 1988, but see Horvitz and Schemske

1986a). Dispersal may lower predation risks for seeds removed from the parent site (Bond and Slingsby 1984, Smith et al. 1989, but see Horvitz 1981). Since ants are relatively unaffected by the presence of experimenters and most ants have relatively small foraging ranges, myrmecochory is a highly tractable system for studying seed dispersal. We employed *Trillium grandiflorum* as a model system for studying the effect of animal dispersal on the pattern, seed aggregation size, and genetic structure of seed and seedling assemblages from the time of seed release through their secondary deposition and seedling establishment.

The major goal of this study was to determine the extent to which the original population structure template represented by the seeds within a fruit is changed by animal dispersal in the species *Trillium grandiflorum*. *T. grandiflorum* has been previously described as a highly selfing species based on two lines of evidence: little variation in chromosomal banding patterns (Fukuda and Grant 1980), and low observed pollinator visitation rates (Lubbers and Lechowicz 1989), but this has recently been disputed by Broyles et al. (1997). If seeds within fruits are indeed the products of self-pollination, the relatedness of seeds within fruits should have an outcrossing rate (t) close to zero, and the undispersed seedling aggregations around the maternal plant should be related as full siblings. Thus, the actual level of selfing sets our expectation for the genetic structure of seed aggregates prior to seed dispersal by ants. To quantify the degree of selfing in the natural population, we conducted controlled pollination experiments to test for the ability to self pollinate, and quantified the outcrossing rate using electrophoretic variation at five allozyme loci. To quantify changes due to ant dispersal in numbers of seeds in aggregations and relatedness of seed and seedling aggregations, we used a radiolabel to track the natural pattern of seed movement after release from their parents (70 fruiting plants, 875 total seeds, over two field seasons). We also determined the subsequent survival rate of seedlings derived from dispersed and undispersed seeds 2 yr post dispersal. We found that while patterns of seed dispersal varied between years in this highly outcrossing population, on the whole seed dispersal by ants decreased the average size and decreased the average degree of genetic relatedness of postdispersal aggregations of seeds.

MATERIALS AND METHODS

Study species

Trillium grandiflorum (Liliaceae) is a native, herbaceous perennial that occurs in the understory of deciduous forests throughout the Great Lakes region of North America and south through the Appalachian Mountains into Georgia and North Carolina (Gleason and Cronquist 1991). *Trillium* plants consist of a single stem (rarely two) 15–45 cm high, arising from a short, tuber-like rhizome. The stem bears a whorl of three

leaves and a single flower in May. We regularly observed large bumblebees visiting flowers. The solitary fruit, a fleshy capsule, matures, passively drops to the ground within 10 cm of the parent plant, and splits open, exposing the seeds. In our study population, the number of seeds within a single fruit averaged 16 (range = 3–60), and fruit and seed release occurred in early July. Gates (1940, 1941) first recorded seed dispersal by ants in *Trillium grandiflorum*. Two additional sources of long-distance seed dispersal noted in other *Trillium* species are harvestmen, Arachnida: Opiliones (Gunther and Lanza 1989) and yellow jackets, *Vespa vulgaris* (Jules 1996). In *Trillium*, the conspicuous elaiosome (Berg 1958) is attractive to insects because of a high lipid content and an abundance of oleic acid (Lanza et al. 1992). This fatty acid induces corpse-carrying behavior in ants, and its presence in the elaiosomes is the likely cause of ants' picking up and carrying *Trillium* seeds to their nest sites (Lanza et al. 1992). In the context of this study, we define the initial, passive drop of the fruit from the plant and the exposure of the seeds as seed release. We define secondary seed movement by ants after seed release as seed dispersal.

Study site

The experiments were conducted from 1991 to 1994 in Long Woods, a 9.5 hectare oak–hickory forest that is part of the Kellogg Biological Station of Michigan State University, Kalamazoo County, Michigan. *Trillium grandiflorum* is patchily distributed in the understory of Long Woods, with patch sizes ranging from a few to >2000 individuals. Our study population within Long Woods occupied an area roughly 28 × 19 m in size and contained 1855 *T. grandiflorum* plants, ranging from seedlings to 40-yr-old reproductive-stage plants (Hanzawa and Kalisz 1993). Other common understory species include *Podophyllum peltatum*, *Geranium maculatum*, *Parthenocissus quinifolia*, and *Isopyrum ternatum*. For a complete site vegetation description, see Burbank et al. (1992). Invertebrates observed visiting fruits in our study site include ants (*Myrmica americana*, *M. myrmica*, *M. apheno-gaster*, *Camponotus pennsylvanicus*, *Formica phenolepis*), slugs, and harvestmen (Opiliones) (F. M. Hanzawa, unpublished data).

Mating system of *Trillium grandiflorum*

We conducted two types of experiments to examine the initial genetic structure of seeds within fruits of *T. grandiflorum*. First, we quantified the extent of natural selfing with a field pollination experiment. In 1992, 66 flowering individuals of *T. grandiflorum* outside the dispersal plot in Long Woods were randomly assigned to one of four pollination treatments (Table 1). While the flowers were still in bud, their anthers were removed or buds opened with no anther removal, according to their treatment assignment (see Table 1). These flowers were then enclosed in a fine nylon mesh bag to exclude pollinators. When the flowers were fully

TABLE 1. Experimental manipulations used to test for automatic self-pollination and self-compatibility in *Trillium grandiflorum* (1992 and 1994).

Treatment	Anther removal	Pollinators excluded	Treatment/pollen source
Outcrossed	yes	yes	Hand pollinated/mixed pollen
Selfed	no	yes	Hand pollinated/self-pollen
Autogamy	no	yes	Unmanipulated/self-pollen
Open	yes	no	Open pollinated
Natural	no	no	Open pollinated

open, they received pollen from the appropriate source listed in Table 1. Plants in the outcrossed treatment received a mixed pollen load, obtained by mixing in a glass vial one anther from each of 18 plants outside the experiment. Pollen was applied to the stigma of each flower in the outcross treatment with a paintbrush. Pollen for plants in the selfed treatment was moved from anther to stigma within the flower with a paintbrush. The only pollen source available to flowers in the autogamy treatment was self pollen that naturally landed on the stigma. Plants in the open treatment could only receive pollen delivered by pollinators from outside sources, as the plants in that treatment had been emasculated but not bagged. Finally, to compare seed set in the experiment with natural seed set in control plants, we counted seeds in all fruits in the treatments and from the 124 naturally fruiting individuals in our study plots.

The two cases of fruit production in the 1992 self-treatment prompted us to suspect that self-pollination could change with floral age, as some studies with SI plants have shown a breakdown of self-compatibility with floral age (Ascher and Peloquin 1966). We repeated only the selfed (S) and outcrossed (O) treatments in 1994, but hand pollinated at three floral ages: when flowers were first open, midway through the floral life, and near the end of floral life. The unopened flowers were bagged on 11 May, and pollinations were conducted on 14 May ($N = 3$ S and 4 O), 20 May ($N = 6$ S), and 27 May ($N = 8$ S and 5 O). This pollination schedule resulted in flowers that were open for 1, 6, and 13 d, respectively.

Outcrossing rate

To determine the natural outcrossing rate in the population, we collected whole fruits and leaf tissue from 40 fruiting plants in 1992. The leaf tissue was kept cold until we returned to the lab, where it was ground and the wicks were stored at -80°C . Seeds from the fruits were planted into raised beds. As emergence in this species takes 2 yr, leaf tissue samples were taken for each maternal sibship from seedlings that emerged in 1994. Eight to 12 seedling leaf tissue samples/maternal

sibship were individually ground and wicks stored at -80°C . Maternal and seedling samples were subject to starch gel electrophoresis in 1992 and 1994, respectively, using protocols described in Tonsor et al. (1993). Five polymorphic loci (number of alleles) were resolved: mdh1 (2), mdh2 (3), got1 (3), got2 (3), and 6-pgd (3). Thirty-six to 39 maternal families were scored for each locus, with 246–308 samples per locus. We used Ritland's multilocus estimation program, MLT (1990), to determine t , the outcrossing rate, and r_p , the genetic correlation of mates for seeds within a fruit. We calculated t_s and t_m , the single locus and multilocus outcrossing rates, respectively. The means and standard errors of the t estimates were based upon 1000 bootstrap values obtained by resampling of the maternal sibships. The difference between the single and multilocus estimates of outcrossing ($t_m - t_s$) estimates the degree of biparental inbreeding (Ritland 1990).

Seed dispersal

We selected 30 of the 154 fruiting plants in the first year and 40 of the 127 fruiting plants in the second year, representing 20% and 32% of the fruiting plants in our study site per year, respectively. In mid-July each year, the fruit from each target plant was collected on the day it ripened and transported to the lab for labeling. To identify sibships (defined here as all seeds from a single parent), each target plant was assigned a unique two-color code. All seeds in a sibship were individually marked with their unique color code using two dots of Testors enamel paint placed on the seed coat opposite the elaiosome. C. Turnbull (*personal communication* to F. M. Hanzawa) observed ants moving *Viola* seeds marked with enamel paint spots and found no change in the attractiveness to ants or the ants' handling characteristics of these seeds relative to unpainted seeds.

To enable us to track the movement of seeds, one color of each two-color code was mixed with a radionuclide, Scandium-46 HCl. Scandium-46 has been used previously in studies tracing the movement of seeds (Lawrence and Rediske 1960, Watkinson 1978). It is a strong gamma-emitter, an essential property for detecting buried seeds since thin layers of soil or litter can block alpha and beta particles. Scandium-46 has a half-life of 85 d, which allows an adequate period for the relocation of dispersed, radiolabeled seeds, but is not unnecessarily long. We labeled each seed with $\sim 4 \mu\text{Ci}$ of activity, which does not impair germination of *T. grandiflorum* seeds (*personal observation*). Watkinson (1978) also applied $4 \mu\text{Ci}$ of Scandium-46 to seeds of *Vulpia fasciculata* and found no impairment of germination.

After the paint dried, the radiolabeled seeds were returned to the field (within 2 hr of fruit collection) and were placed within their fruit capsule on the soil surface. The capsules were placed within 10 cm of the maternal plant since, on average, *T. grandiflorum* fruits land within a 10-cm radius of the maternal parent during initial

fruit release (*unpublished data*). Natural seed dispersal was allowed to occur. Since 80–100% of *Trillium* seed dispersal occurs within 3 d of seed release (Smith et al. 1989), we waited for 1 wk after the last seeds were radiolabeled to begin the search. At that time, we systematically surveyed the 32×23 m search zone (Fig. 2A) using a 5×5 cm NaI scintillation probe attached to a Bicon Analyst rate meter (Bicon Corporation, Newbury, Ohio, USA). Working in 10-cm² sections at a time, we identified all radioactive locations, flagged them, and determined their coordinates within the search zone by triangulation. Radioactive sites were considered distinct if they were ≥ 10 cm apart. We classified seeds remaining within the 10-cm radius of their maternal parent as undispersed. Dispersed seeds were those found to be ≥ 11 cm from the parent plant.

At each radioactive location, we searched the soil surface for seeds, subsequently excavated the soil in 2-cm depth increments and sieved it to find seeds of *T. grandiflorum*. Recovered seeds were placed into labeled plastic containers and moved 20 m from the flagged site to improve our ability to detect labeled seeds. Further excavation at each flagged location continued until radioactivity readings returned to background levels. Seeds recovered at radioactive deposition sites were identified as to maternal sibship using the two-color code, then returned to the soil at the flagged location where they were found. This searching, excavation, and identification required daily effort for ~ 21 d each year. We mapped the spatial locations and recorded the stages (seedling, nonreproductive, and reproductive) of all *T. grandiflorum* in our study population. Mapping was done by triangulation from two fixed points on the study site. The locations of radioactive sites for both undispersed (< 10 cm from maternal plant) and dispersed seeds were mapped on this same coordinate system for the study site.

When ants dispersed seeds, the labeled seeds were commonly found with other *T. grandiflorum* seeds that were not radiolabeled. These unlabeled seeds were in all likelihood produced by nontarget plants during the same season as those that were radiolabeled. This is likely because, first, a severe drought during summer 1989 led to nearly total reproductive failure in that year; second, prior to 1989, sampling of the *T. grandiflorum* soil seed bank on this site showed that there were few seeds that persisted in the soil (Hanzawa and Kalisz 1993); and third, during 1990, as part of a different study, we removed most of the seeds that were produced in the study site. Thus, it is probable that the unlabeled seeds were produced in the current season by the remaining 68% (1991) or 80% (1992) of the population that were not radiolabeled and were not dormant seeds produced in previous years.

Distance

The distance that dispersed seeds were carried was determined by subtraction of the Cartesian coordinates

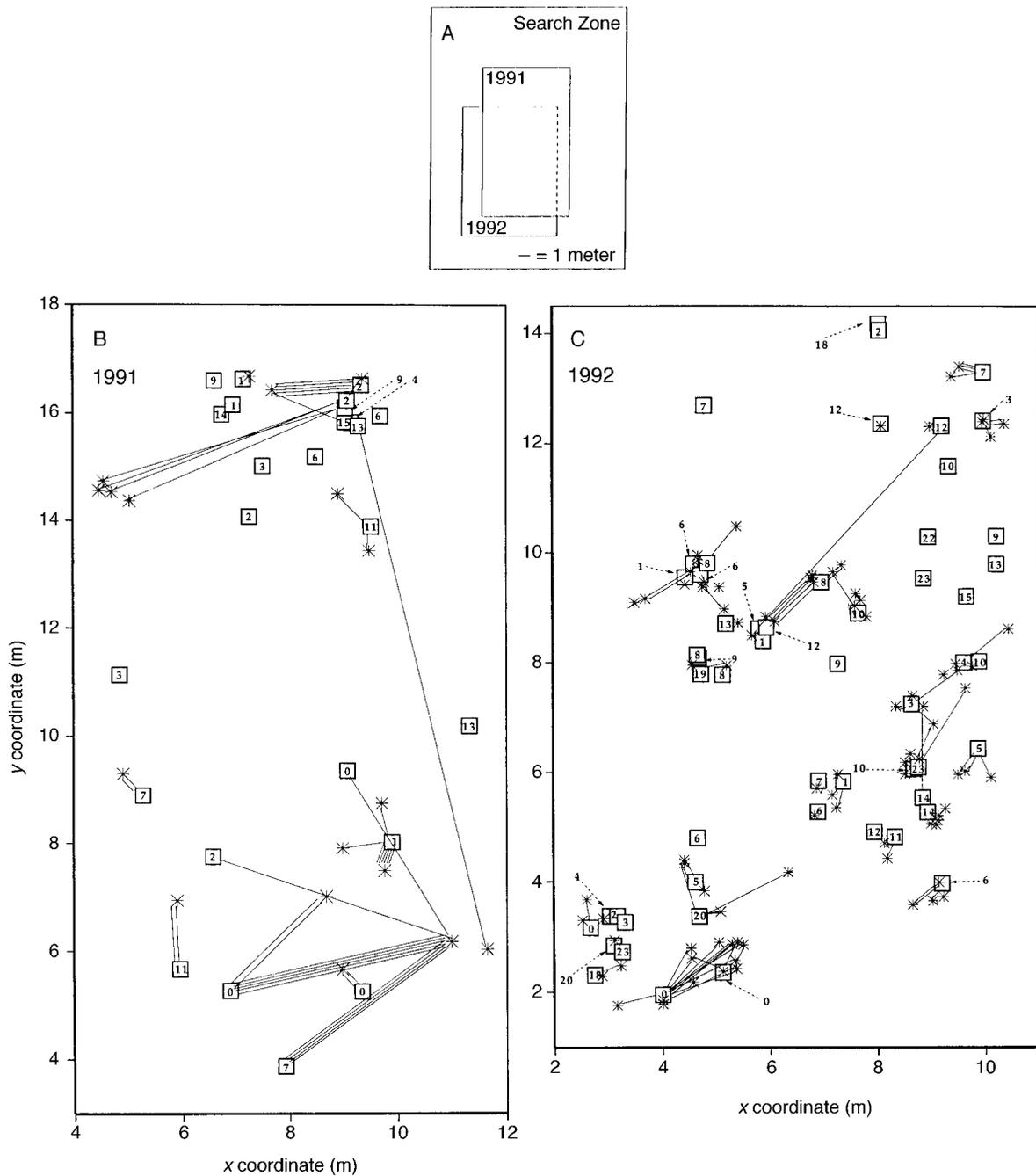


FIG. 2. (A) Schematic of zone searched for radiolabeled seeds (32 × 23 m). Within that search zone, the areas marked 1991 and 1992 indicate the areas in which seed sibships were radiolabeled and later recaptured in each of the two years. (B) Pattern of seed movement for 1991. Boxes indicate locations of the target plants whose seeds were radiolabeled. The value within each box is the number of seeds left undispersed near the target plant. Lines from boxes ending in asterisks indicate seed dispersal. Asterisks denote the seed recapture sites. Each line represents one seed. (C) Details of seed movement for 1992. Symbols are as in panel (B).

of the maternal location from the coordinates for each flagged radioactive location. All undispersed seeds were scored as having a dispersal distance of zero. Distance values for the dispersed seeds in each year were \log_{10} transformed to normalize the distributions,

and mean distances seeds were dispersed among years were compared with a single factor analysis of variance model $\log_{10}(\text{distance}) = \text{year}$. Analyses were conducted both with and without outliers. Removal of outliers did not alter the significance or statistical infer-

TABLE 2. Experimental pollination results for 1992 testing for self-pollination ability in *Trilium grandiflorum*.

Variables	Treatments				
	Outcrossed	Selfed	Autogamy	Open	Natural
No. flowers tested	18	18	15	15	176
No. fruits produced†	18 (17)	2 (2)	0	15 (11)	176 (152)
Fruit set (%)	100	11	0	100	81
No. seeds/fruit (1 SD)‡	16.9 (9.3)	21.0 (9.9)	0	20.7 (6.8)	15.5 (6.9)§

Notes: Treatments are as in Table 1. Since each reproductive plant produces a single flower, the number of flowers tested also indicates the number of replicates of each treatment.

† Numbers in parentheses are the number of fruits harvested. The decrease in the number of fruits is due to loss of flowers and fruits to deer browsing.

‡ No significant difference in seeds/fruit among treatments.

§ Based on 124 fruits.

ences. Thus, distance analyses presented include outliers. This and all other analyses were performed using SAS (1989).

Aggregation size

To estimate the effects of dispersal on the average local number of seeds in a postdispersal aggregation, we summed the number of radiolabeled and unlabeled seeds in each aggregate. For each recovered seed, we compared the numbers in the aggregation before dispersal (i.e., the number of seeds in its sibship) with the numbers in the aggregation in which it was found after dispersal. Two types of aggregations existed: 1) undispersed seeds that remained singly or in aggregations of sibs near the maternal parent, and 2) dispersed seeds that were deposited singly or in aggregations at deposition sites. Mean sizes of the aggregations were compared with Type III ss from an analysis of variance: model (Aggregation size = DState + Year + [DState × Year]) where DState is the dispersal state of recaptured seeds (undispersed or dispersed).

Relatedness

To estimate the number of families that were dispersed to a site when both unlabeled seeds and radiolabeled seeds were found together, we scored all unlabeled seeds as the contribution of a single maternal plant. This is a conservative estimate of the number of sibships present at a deposition site and is likely in some instances to underestimate greatly the number of sibships actually present. The number of sibships per radioactive site was used to determine the proportion of sibs vs. nonsibs in postdispersal aggregations. The unlabeled seeds were also included in analyses of aggregation size after dispersal.

To estimate the effects of dispersal on the average relatedness among seeds in an aggregation, we calculated the proportion of seeds that shared the same maternal family as the labeled seed dispersed into that aggregation. We term this the proportion of sib near-neighbors (PSNN). Before dispersal, all aggregations contained only sibs; thus, all predispersal aggregations had a PSNN of 1.0. Dispersal could decrease the value

of PSNN if seeds were dispersed into sites with only nonsibs, or if seeds were deposited singly (some aggregations in Fig. 1C). Conversely, dispersal could maintain the predispersal value of PSNN if seeds from only one sibship were dispersed to any one location (Fig. 1A). We calculated the PSNN for each labeled sibship represented in a postdispersal aggregation and took the average over all sibships. These means were compared with a *t* test.

Finally, we determined the seed-dispersal-based neighborhood area for the study population. Wright (1946) introduced the idea of a genetic neighborhood for populations, in that the density of offspring is a declining function of distance from the parents. The neighborhood area, A_N , was calculated using the equation $A_N = 4 \sigma_d^2 \pi^2 \sigma_d$, where σ_d^2 is the axial variance in dispersal from a point source (Heywood 1991). Here, A_N measures the neighborhood area resulting from seed dispersal away from the maternal parent. We used this formula to estimate neighborhood area of *T. grandiflorum* based only on the gene flow estimate from seed dispersal quantified in this study. A full neighborhood area would also include pollen-mediated dispersal (Beattie and Culver 1979), which we did not measure.

Survival of seedlings

To determine differences in survival and emergence of dispersed vs. undispersed seeds, in May 1994 we surveyed each dispersed and undispersed location where radiolabeled seeds had been found during 1992. (Note that seedlings take 2 yr to emerge.) Seedling numbers per location were censused and the percent emerging was calculated.

RESULTS

Pollination experiments

The combined results of the 2 yr of experiments indicate that 95% of the plants (40/42) in the outcrossed or open pollinated treatments set fruit (Tables 2 and 3). This contrasts sharply with 6% of the plants (2/32) in the hand self-pollination or 0% autogamous selfing treatments (0/15) (Tables 2 and 3). These data suggest

TABLE 3. Experimental pollination results from 1994, testing for increasing selfing rate with floral age.

Variables	14 May		20 May	27 May	
	Outcrossed	Selfed	Selfed	Outcrossed	Selfed
Flowers tested†	4	3	6	5	8
No. fruits	4	0	0	3	0
Fruit set %	100	0	0	60	0
Mean no. seeds (1 SD)	18.5 (3.1)	0	0	14.0 (4.6)	0

Notes: Pollinations were conducted on three dates as shown and treatments are as in Table 1. Since each reproductive plant produces a single flower, the number of flowers tested also indicates the number of replicates of each treatment. No effect of floral age on selfing ability is detected.

† Sample sizes reduced from 10 flowers per date/treatment due to slug herbivory of the flowers.

that the study population of *T. grandiflorum* is not highly selfing, but rather is likely self-incompatible. (Two plants did set fruit upon hand self-pollination (Table 2). We suspect that either nonself pollen was inadvertently introduced to these plants, or that unobserved pollinators invaded the two enclosure bags). In the follow-up experiment, no temporal effect of flower age on selfing ability was detected (Table 3). However, older flowers have an overall decline in fruit set (100% fruiting of O treatment pollinated on 14 May vs. 60% fruiting of O treatment pollinated on 27 May). We see no evidence of pollinator limitation: 100% of the flowers in the emasculated, open pollinated treatment set fruit (Table 2). Deer may have a greater effect on seed production than pollinator limitation, as 14% of the fruits in the Natural treatment were lost to herbivory (Table 2).

The electrophoretic analysis of outcrossing rate mirrors the results of the pollination experiment. Both the single locus estimate $t_s = 0.998$ (SE = 0.061) and the multilocus estimate $t_m = 1.050$ (SE = 0.056) of the outcrossing rate indicate that this population of *Trillium grandiflorum* is highly outcrossing. In addition, $(t_m - t_s)$, the estimate of biparental inbreeding, is 0.052. This value is very low, indicating low levels of mating with near relatives. The estimate of correlated matings within a fruit, r_p (SD), was 0.818 (0.133), indicating a moderate to high probability that seeds within a fruit share the same pollen parent. This value of r_p indicates that seeds within a fruit have a relatedness value between full and half sibs.

Dispersal

The pattern of movements of seeds for both years is shown in Fig. 2B–C. In the first year, we recovered 63% of the 335 radiolabeled seeds. Of the recovered seeds, 81% were found ≤ 10 cm distance from their maternal parent and classified as undispersed. The remaining 19% were classified as dispersed. Surprisingly, only half of the 30 maternal parents had seeds that were dispersed; the other half had no seeds that were found greater than 10 cm away. In the second year, we recovered 76% of the 876 radiolabeled seeds. Of the

recovered seeds, 77% were undispersed and 23% were dispersed. In contrast with the first year's dispersal, only two out of 40 parents had no seeds dispersed. Dispersed seeds were located at a mean of 2.5 different sites in year 1 and 2.8 in year 2. The maximum number of dispersal sites scored for a single sibship was 12. None of the deposition sites contained seeds of other ant-dispersed species.

Distance

The distances seeds were dispersed differed significantly between years (Fig. 2B, C, Fig. 3). In year 1, dispersal distances for seeds moved by ants ranged from 0.12 to 10.00 m, with a mean dispersal distance of 2.41 m (SE = 0.33, $N = 39$, Fig. 3). In year 2, dispersal distances for seeds moved by ants ranged from 0.02 to 5.2 m, with a mean dispersal distance of 0.53 m (SE = 0.06, $N = 155$, Fig. 3). When the undispersed seeds are included, the mean distance from the maternal plant for all recovered seeds was 0.45 m (SE = 0.09, $N = 210$) in year 1 and 0.17 m (SE = 0.02, $N = 485$) for year 2. We calculated an identical seed-dispersal neighborhood area estimate for both years ($A_N = 20.96$ m²) because the standard deviation of dispersal distance for all recovered seeds was nearly identical in the two yr ($\sigma_d = 1.29$ in year 1 and 1.28 in year 2).

Effects of seed dispersal on aggregation size

In year 1, dispersed seeds, on average, experienced both a higher mean and a higher variance in local numbers than did seeds that were not dispersed. The converse was observed in year 2. The largest aggregations of dispersed seeds, containing 23, 38, and 73 *T. grandiflorum* seeds, were all found in year 1. All other dispersed seeds over both years were found groups of 1–7 seeds. The average number of seeds in an aggregation post dispersal decreased relative to the average aggregation size of all seeds after seed release (Table 4). However, a comparison of seed aggregation size for dispersed and undispersed seeds between years indicates annual variation in dispersal effects on numbers. In year 1, dispersed seeds experienced higher seed numbers relative to undispersed seeds, while in year

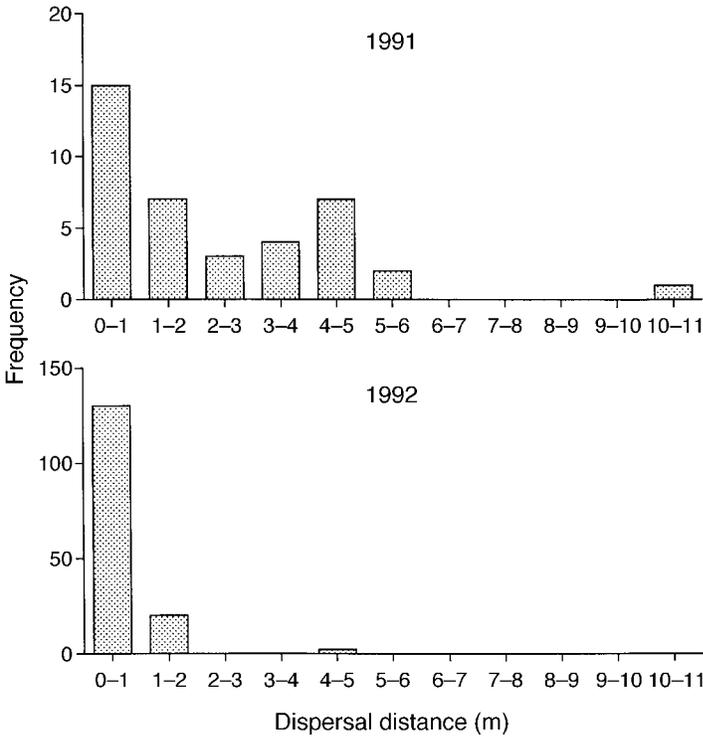


FIG. 3. Frequency distributions of *Trillium grandiflorum* seed dispersal distances (in meters) away from maternal plant for two years.

2, the opposite result is seen (Table 4). The ANOVA indicated that these differences were significant for the main effect of year and the year by dispersal status interaction (Table 5).

Relatedness effects of seed dispersal

To estimate the effects of dispersal on the average relatedness between seeds in an aggregation, we calculated the probability that a pair of seeds drawn from the aggregation, at least one of which is a labeled seed and the other drawn at random, were from the same

maternal family. In both years, all predispersal aggregations had a PSNN (the proportion of sib near-neighbors) of one at seed release. After dispersal in year 1 (Fig. 4), the mean PSNN for all aggregations was 0.64 (SE = 0.07, N = 43). These aggregations include 27 sites of undispersed seeds, each with a PSNN of one, and 16 sites of dispersed seeds. The dispersed seeds had a mean PSNN of 0.22 (SE = 0.08, N = 16). The PSNN differed significantly for dispersed and undispersed seeds (*t* test: $F = 104.9, P < 0.001$). Six of the deposition sites contained only one seed. Of the 11 sites that had >1 seed, all contained seeds from >1 family (one maternal parent). The mean number of families per deposition site was 1.94 in year 1.

In year 2, after dispersal, the mean PSNN for all aggregations was 0.52 (SE = 0.04, N = 143). This total included 52 undispersed seed sites and the 91 dispersed sites. These secondary dispersal deposition sites had a mean PSNN of 0.28 (SE = 0.04). The PSNN differed significantly for dispersed and undispersed seeds (*t* test:

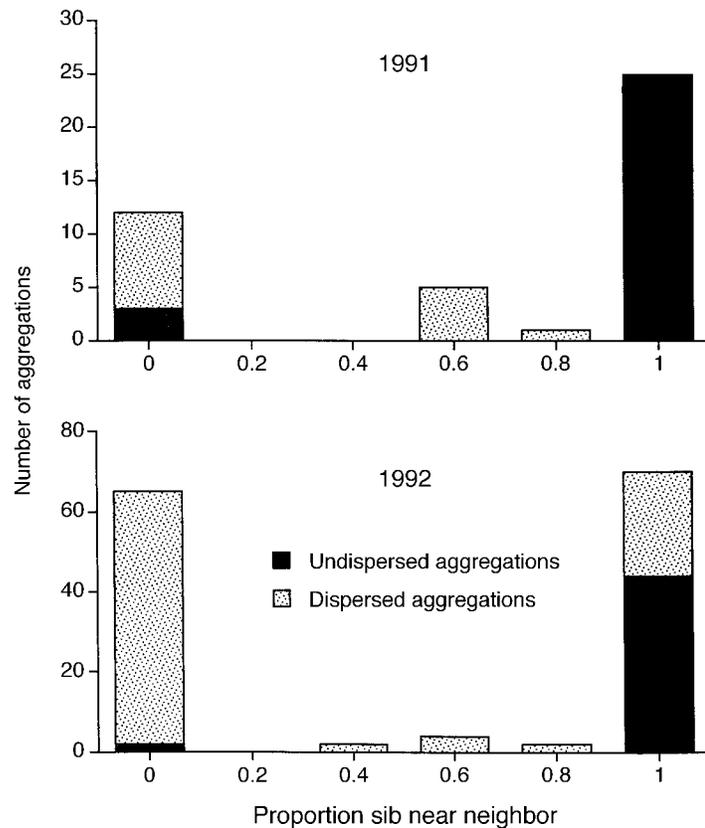
TABLE 4. Size of seed aggregations prior to and after dispersal for seeds that were recaptured. Mean, standard error (SE), range, and number of seeds for the 2 yr of dispersal studied.

	Pre dispersal All seeds	Post dispersal		
		All seeds	Undis- persed	Dis- persed
1991				
Median size	13	12	9.5	6
Mean size (1 SE)	13.95 (0.33)	12.46 (0.99)	9.39 (0.30)	25.87 (4.61)
Range of size	3-22	1-73	1-15	1-73
Number of seeds	210	210	171	39
1992				
Median size	19	10	12	2
Mean size (1 SE)	19.92 (0.25)	11.09 (0.28)	13.64 (0.27)	2.57 (0.14)
Range of size	3-30	1-23	1-23	1-7
Number of seeds	665	665	512	153

TABLE 5. Effects of year and dispersal state (dispersed or undispersed) on the size of seed aggregations. ANOVA performed on log-transformed data and presented with Type III sum of squares, R^2 of the model = 0.27.

Source	df	ss	F	P
Status	1	25 577	48.1	0.001
Year	1	28.4	0.5	0.47
Status × Year	1	758.7	4.3	0.0002
Error	265	14 094.2		

FIG. 4. Frequency distributions of the average proportion of sib near-neighbors in *Trillium grandiflorum* seed aggregations following dispersal for the two years of the study. Dispersed seeds (stippled bars) and undispersed seeds (solid bars) are presented.



$F = 507.3$, $P < 0.001$). Fifty-six deposition sites (62%) contained only one seed. Of the 35 sites that had >1 seed, 40% contained seeds from >1 family (one maternal parent). The overall mean number of families per deposition site in year 2 was 1.16. Over both years, seed dispersal significantly decreased the proportion of sib near neighbors experienced by individual seeds, on average by 75%. (Note that, if the seeds not recaptured were also deposited singly, the PSNN would dramatically decrease.) For many maternal plants, dispersal resulted in offspring being placed in several sites: Some remained aggregated near the parent, and others were dispersed to one or more deposition sites. For maternal parents that had a least one seed dispersed, their seeds were located at a mean of 2.5 different sites.

Emergence of seedlings

Of all the seeds recaptured in 1992, 14% of the undispersed seed and 12% of the dispersed seed survived and germinated in 1994. The difference in the fraction emerging as a function of dispersal status was not significant. In addition, neither the number of seeds in a postdispersal assemblage nor the minimum number of families in an aggregate (relatedness) was correlated with the proportion of seedlings emerging.

DISCUSSION

Proximate consequences of seed dispersal

Our study differs from previous work on seed dispersal in that we have determined the postdispersal locations of seed sibships and the consequences of that dispersal to the seeds and emerging seedlings. Overall, seeds of *T. grandiflorum* that were dispersed experienced lower relatedness to other seeds in the deposition site as well as a lower average number of seeds in those aggregations. This occurred because, before dispersal, all seeds from one maternal parent were aggregated in the vicinity of that parent. After dispersal, although many seeds remained in sib aggregations, dispersal frequently resulted in the mixing of seeds from different families at sites of seed deposition, reducing the relatedness of the seeds in aggregates. Most plants had only a fraction of their offspring dispersed (Fig. 2B–C), but those offspring that were dispersed were moved to an aggregation that was, on average, more genetically diverse (Fig. 4). These aggregations frequently contained one or more nonsib seeds (Fig. 4). In fact, dispersed seeds were more likely to be in an aggregation with a seed from another family than with a seed from their own family: 85% of the dispersed seeds were in an aggregation with ≥ 1 seed from another family, whereas only 67% of the dispersed seeds were in an

aggregation with a seed from the same family. The extent to which seed dispersal will actually lower the relatedness of seed aggregations will depend on, first, the mating system that produced the seeds and, second, the relatedness of nonsib seeds in postdispersal aggregations.

Similar to the relatedness effects, the local number of conspecific seeds was lowered by dispersal (Table 5). The lower seed aggregation size after the dispersal period was due to two factors: First, ants or other dispersal agents deposited seeds in smaller-sized seed aggregations than were initially present in fruits, and, second, after the dispersal period, undispersed seeds near their maternal parent were left in smaller aggregations. Thus, dispersal decreases the opportunity for sib competition in both types of seed aggregates. In our study, the overall aggregation size and genetic pattern of seeds post dispersal is most similar to Fig. 1C, with the addition of the many seeds that remain undispersed and seeds that are dispersed singly.

In other plant species, decreasing density of conspecific seeds has been associated with both reductions and increases in germination rate (e.g., Qadir and Abassi 1971, Bergelson and Perry 1989). Our expectation was that decreases in aggregation size and relatedness would result in enhanced numbers of seedlings emerging. Surprisingly, neither dispersal-mediated changes in seed aggregation size nor genetic relatedness significantly affected the probability of seedling emergence.

Dispersed fractions and dispersal distances

The greatest proportion of labeled seeds of *T. grandiflorum* (45% in year 1, 52% in year 2) remained at the location of seed release from the maternal parent (i.e., within a 10-cm radius). In total, the proportion of seeds moved from the parental location was 55% in year 1 and 48% in year 2. The seed movement documented here is similar both to that quantified for other genera of myrmecochores growing in similar habitats (range = 14–80%, Culver and Beattie 1978, Heithaus 1981, Smith et al. 1989) and to the proportion of seeds removed from ant-dispersed *Trillium* species in general. Removal from seed depots within *T. nivale* and *T. grandiflorum* ranged from 15 to 70% over a 3-d period (Smith et al. 1989), while the proportions of seeds removed after 90 minutes for *T. erectum*, *T. grandiflorum*, and *T. undulatum* were 35%, 22%, and 27%, respectively (Gunther and Lanza 1989). Relatively lower rates of removal have been found for other species of *Trillium* in different habitats (Mesler and Lu 1983, Higashi et al. 1989).

Dispersal distances recorded in this study indicated both longer average and maximum dispersal distances when compared to the few previous studies of myrmecochores in similar habitats. *T. tschonoskii* had a mean dispersal distance of 0.6 m and a maximum dispersal distance of 2.7 m (Higashi et al. 1989). Three

species of *Viola* had mean dispersal distances between 0.35 and 0.72 m, with maximum dispersal between 1.1 m and 1.5 m (Culver and Beattie 1978). The distances over which ants disperse seeds appear to vary with ant species and habitat. Relatively long-distance dispersal by ants (mean >2 m, maximum >9 m) has been reported for plants from the Sonoran Desert (O'Dowd and Hay 1980), Australian sclerophyllous heathlands (Andersen 1988), and South African fynbos (Slingsby and Bond 1985). It is possible that relatively long-distance dispersal occurs in other myrmecochorous plants growing in temperate forest habitat but has not been noted in previous studies owing to the general difficulty in tracking seeds the farther they are dispersed. Radiolabelling seeds, as was done in this study, may provide a more accurate metric of intermediate-distance seed movement relative to paint or fluorescent dyes. Our methodological difference could explain the higher average seed dispersal distances quantified in our study.

Variance in the pattern of dispersal among years

A number of abiotic and biotic factors are likely to influence seed removal by insects and the distances seeds are carried. Annual differences in weather patterns during the time of *Trillium* fruit drop may create conditions that are either conducive or detrimental to dispersal. Weather conditions could affect ants' or other dispersal agents' movements as well as the length of time the elaiosome remains attractive to dispersers. The summer of 1992, characterized by shorter dispersal distances, was an unseasonably rainy July in southwestern Michigan. This rain may have decreased both the frequency and length of ant-foraging bouts, and increased slug activity. Slugs are common in our site and are attracted to the elaiosomes of *Trillium*. Unlike ants, however, slugs consume the elaiosome in situ, leaving the seed where it was originally deposited (F. M. Hanzawa and M. Beilstein, unpublished data), rendering them unattractive to other dispersers. Satiation of potential dispersers (see Smith et al. 1989) or the presence of alternative or preferred food sources could result in lower rates of seed dispersal. There are no other myrmecochorous plant species in our study site that could have competed for the dispersers' attention, and we are unaware of alternate or preferred food sources for the ants.

Annual variation in the species composition of the ant community and the physical location of ant nests could significantly alter both the number of seeds removed and the distances that seeds are carried. Spatial and temporal variation in the ant community can affect both the probability of dispersal and the mean and variance in dispersal distances of seeds (e.g., Horvitz and Schemske 1986b). Herbers (1989), working with 14 species of forest ants in New York state, found that nest locations are regularly moved within the annual cycle and can even be ephemeral within a season (Her-

bers 1989, Tables 3 and 4 in Hölldobler and Wilson 1990). In our study, some of the annual variation in dispersal is likely to be attributable to variable activity of one ant species, *Camponotus pennsylvanicus*. A nest of this large-bodied ant was observed in the southeast corner of the study plot in the first year of this study (Fig. 2B). The largest aggregation of seeds ($N = 73$) and the longest seed dispersal distance (10 m) were in the vicinity of that active *Camponotus* nest in the first year of the study. In the second year, no seeds were dispersed to that location (Fig. 2C). Both low ant nest density and small colony size at the time of *Trillium* seed release could also result in lowered seed removal rates and affect average dispersal distances (e.g., Gomez and Expadaler 1994). We have no data on the relative densities of nest sites or on the species in the ant communities across the 2 yr of this study.

Foraging distances of ants will also directly affect seed dispersal distances. In Long Woods, foraging distances of a common small-bodied, territorial ant species, *Myrmica americana* were ~ 0.5 m (S. Uno and F. M. Hanzawa, unpublished data). These foraging distances are similar to those of other small-bodied, northern temperate forest ant species (J. Herbers, personal communication), while *Camponotus* foraging range is in excess of 6 m (J. Herbers, personal communication). It is likely that we have located all seeds dispersed by small ants in our study area (Fig. 2). It is possible, however, that other long-distance dispersers, including *Camponotus*, whose nests were located outside our search area, moved the marked seeds to remote locations.

Only 18 and 24% of the labeled seeds were unambiguously classifiable as dispersed in each year. The remaining 37% in year 1 and 24% in year 2 were never relocated. It is possible that some unrecovered seeds were eaten by seed predators or otherwise destroyed. Two instances of soil contaminated with radioactivity were found with no intact seeds present, suggesting seed predation. However, Smith et al. (1989:1653–54) in a 3-yr study found “virtually no predation of seeds of *T. grandiflorum*,” despite the fact that the study was conducted in a site where high predation on another myrmecochorous plant, *Jeffersonia diphylla*, had been detected (Smith et al. 1986). We consider predation an unlikely outcome for the missing seeds in this study. It is also unlikely that the unrecovered seeds were present in surface middens but overlooked. In general, temperate forest ant species do not have nests or middens deeper in the soil than would be detectable by our radiolabel and probe (J. Herbers, personal communication). The combination of the high level of signal from the β -emitting radiolabel and the thorough search protocol makes the probability of missing seeds in the search area low. However, seed dispersal by harvestmen or yellow jackets, which also feed on dead insects, may explain the high proportion of seeds that were dispersed singly and the seeds presumed to have been

dispersed longer distances. If the 37 and 24% unrecovered seeds were in fact dispersed singly by highly mobile insects beyond the borders of our 34×23 m search area (Fig. 2A), our calculations of postdispersal relatedness and aggregation size would be significant overestimates, and dispersal distance values would be significant underestimates.

Ultimate fitness consequences of animal-mediated seed dispersal in Trillium grandiflorum

Seeds of *Trillium* clearly are modified for dispersal by ants or other insects. Therefore, our expectations are that a fitness advantage will be gained that balances the cost of elaiosome production. Positive long-term effects of ant-mediated dispersal have been quantified in only one species, the biennial *Corydalis aurea* (Hanzawa et al. 1988). Getting results similar to ours, Hanzawa et al. (1988) found no difference in seed or seedling survival for seeds dispersed by ants relative to those undispersed. However, both survival through the first growing season and total reproductive output differed significantly between the two groups in *C. aurea*. Final fecundity of ant-dispersed seeds was 90% higher than that of the control group. Although no early life history benefit of seed dispersal was detected in the present study, long-term fitness benefits may accrue for those seeds dispersed away from the maternal parent. Both changes in density and relatedness of neighboring individuals have been shown to affect plant performance at different life stages. For example, in a 2-yr study Augspurger (1984) showed that long dispersal distance was positively correlated with survival of seedlings and that the dispersal-mediated decreases in seedling density lowered mortality from fungal pathogens in a wind-dispersed tropical tree. In a related study with two sibships of the tree *Tachigalia versicolor*, Augspurger and Kitijima (1992) found that decreased relatedness (i.e., with sibs from the same mother or mixed with sibs from another mother) significantly increased the period of survival from germination to 2-mo-old seedlings in general and germination to 2-yr survival at one site. We expect that, over the long term, a fitness benefit should be accrued by dispersed seeds if this mutualism is to be maintained in *Trillium*. Given that *T. grandiflorum* individuals are long lived, we expect that the positive effects of seed dispersal may appear later in the life history.

Because we only have data through seedling emergence for 1 yr that indicated that low numbers of seedlings were present, we can say little about the long-term demographic consequences of dispersal-mediated changes in the density or genetic environment of seedlings. In the present snapshot, dispersed and undispersed seedlings emerge with equal frequencies, and the density of seeds in a postdispersal aggregate is uncorrelated with seedling emergence and survival in the first season. Individuals of *T. grandiflorum* in our study population live ≥ 40 yr and have a prereproduc-

tive period of 16 yr (Hanzawa and Kalisz 1993). The frequency of recruits into the populations on an annual basis is clearly low, and their subsequent probability of survival to join the reproductive class is also low (S. Kalisz and F. M. Hanzawa, *unpublished data*). In a companion study in this population, we examined the spatial genetic structure expressed at different life history stages: one-leaved plants, three-leaved nonreproductive plants, and three-leaved reproductive plants. The degree of spatial genetic relatedness as a function of distance between plants at each stage was calculated using electrophoretic data. The results indicate that the seedling stage showed no genetic structure at any spatial distance interval. Because seeds in postdispersal aggregates were likely to have some sib near neighbors, this indicates that average nearest-neighbor relatedness drops from postdispersal aggregations to juvenile plants and closely adjacent seedlings were no more related to each other than those that were far apart. This could result from selection against sib near neighbors or simply be due to a reduction in density's leaving one or fewer individuals from a given aggregation that survive to the juvenile stage. Average near-neighbor distance drops from postdispersal seed aggregates to the one-leaf plant stage (*unpublished data*), corroborating the second interpretation.

The mating system generates the initial degree of relatedness among offspring within fruits. The results of our mating system study indicated that this *T. grandiflorum* population is highly outcrossing ($t > 99\%$) and is likely to be self-incompatible. The actual relatedness of seeds within fruits will be a function of the number of pollen donors present in a pollen load that sire seeds within that fruit. Although the number of polymorphic loci used in our study is not sufficient to conduct a paternity analysis, the presence of a self-incompatibility system would decrease the probability that mating occurs among close relatives, as they are likely to share incompatibility alleles. Self-incompatibility thus could create negative assortative mating (Hartl and Clark 1997). Correlated mating estimates, r_p , suggest that fruits are sired by one or a few pollen donors. Therefore, offspring from a single fruit are likely to be related as half to full sibs. As a consequence of the mating system, seedlings emerging from undispersed aggregates will be genetically similar and would share self-incompatibility alleles. In contrast, dispersed seeds are with nonsibs and thus would not share self-incompatibility alleles. The pattern of seed dispersal and seedling mortality described in this study sets the stage for minority advantage at the adult stage (e.g., Antonovics and Ellstrand 1984, Schmitt and Antonovics 1986, Wilson et al. 1987, Tonsor 1989).

The original purpose of Wright's genetic neighborhood was to estimate rates of inbreeding from effective population sizes, based on neighborhood areas and densities. As defined by Wright (1946), roughly 87% of an individual's relatives are found within the genetic

neighborhood determined by the dispersal distance. For *T. grandiflorum*, the seed dispersal mediated area of relatedness is 21 m². Without information on mating structure for a population, such inferences about inbreeding cannot be drawn. The "neighborhood" area calculated with our data estimates only the area or neighborhood of relatedness. The only previous study that calculated neighborhood areas resulting from seed dispersal by ants found neighborhood areas for three species of *Viola* that were an order of magnitude lower (Beattie and Culver 1979) than those reported here. In Beattie and Culver's study, pollen movement was deemed the more important mechanism of gene flow relative to seed dispersal. The relative importance of seed vs. pollen flow is likely to differ for *T. grandiflorum*. If SI alleles are responsible for the lack of seed set upon selfing in our study, individuals within the A_N would share SI alleles in direct proportion to their relatedness. Thus, if undispersed, seeds will share SI alleles and suffer reduced fecundity. It follows that seeds that are dispersed away from their maternal parent's genetic neighborhood and that survive to flower may have higher average fitness with respect to both male and female functions than those that remain within the relatedness neighborhood of their parent. An SI mating system could therefore confer a fitness benefit, selecting for the maintenance of ant-mediated dispersal; however, this benefit is not seen until plants reach reproductive status. Thus, if SI alleles are present, the long-term interaction of the SI system and ant-mediated seed dispersal may explain both the pattern of genetic structure and the maintenance of the mutualism.

Previous studies of animal-mediated seed dispersal have focused on the ecological consequences of the interactions. The results of our study demonstrate that the consequences of seed dispersal can be influenced by the specific *pattern* of movement of seeds. Seed dispersal by ants or other insect vectors clearly has the potential to affect plant fitness through frequency-dependent selection. This disperser-specific pattern of seed movement can also contribute to development and maintenance of genetic structure in the plant populations (Loveless and Hamrick 1984). Our results indicate that detailed studies of the interaction between disperser behavior and the spatial genetic characteristics of the reproductive plant population are necessary better to understand the demographic and population genetic consequences of various dispersal syndromes.

ACKNOWLEDGMENTS

We are grateful to D. Vogler, M. Servidio, T.-L. Ashman, S. Bliss, T. Knight, M. McPeck, M. Finer, and T. Getty for comments on the manuscript and P. Brautigan and T. Herman for help with the figures. M. A. McPeck, B. Black, S. Craig, K. Williamson, and P. Woodruff provided expert assistance in the field. M. Klug and Sandy Ford are thanked for allowing us to use their lab to radiolabel seeds. This research was supported by NSF grants BSR-9006715 to F. M. Hanzawa and BSR-9006647 to S. Kalisz and S. J. Tonsor, and REU support for S. Voigt. This is KBS Contribution #739.

LITERATURE CITED

- Andersen, A. N. 1988. Dispersal distance as a benefit of myrmecochory. *Oecologia* **75**:507–511.
- Antonovics J., and H. Alexander. 1986. The concept of fitness in plant–fungal pathogen systems. Pages 185–214 in K. J. Leonard and W. E. Fry, editors. *Plant disease epidemiology: genetics, resistance and management*, volume 2. McGraw-Hill, New York, New York, USA.
- Antonovics, J., and N. C. Ellstrand. 1984. Experimental studies on the evolutionary significance of sexual reproduction. 1. A test of the frequency-dependent selection hypothesis. *Evolution* **38**:103–115.
- Ascher, P. D., and S. J. Peloquin. 1966. Effect of floral aging on the growth of compatible and incompatible pollen tubes in *Lilium longiflorum*. *American Journal of Botany* **53**:99–102.
- Augsburger, C. K. 1983. Seed dispersal of the tropical tree, *Platygodium elegans*, and the escape of its seedling from fungal pathogens. *Journal of Ecology* **71**:759–771
- . 1984. Seedling survival among tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology* **65**:1705–1712
- Augsburger, C. K., and K. Kitijima. 1992. Experimental studies of seedling recruitment from contrasting seed distributions. *Ecology* **73**:1270–1284.
- Beattie, A. J. 1985. *The evolutionary ecology of ant–plant mutualisms*. Cambridge University Press, Cambridge, UK.
- Beattie, A. J., and D. C. Culver. 1979. Neighborhood size in *Viola*. *Evolution* **33**:1226–1229.
- Beattie, A. J., and D. C. Culver. 1981. The guild of myrmecochores in the herbaceous flora of West Virginia forests. *Ecology* **62**:107–115.
- Beattie, A. J., and D. C. Culver. 1983. The nest chemistry of two seed-dispersing ant species. *Oecologia (Berlin)* **56**:99–103.
- Berg, R. Y. 1958. Seed dispersal, morphology, and phylogeny of *Trillium*. *Skrifter Udfitt av Det Norske Videnskaps-Akademi-Oslo*, 1, Norway.
- . 1975. Myrmecochorous plants in Australia and their dispersal by ants. *Australian Journal of Botany* **23**:475–508.
- Bergelson, J., and R. Perry. 1989. Interspecific competition between seeds: relative planting date and density affect seedling emergence. *Ecology* **70**:1639–1644.
- Bond, W., and P. Slingsby. 1984. Collapse of an ant–plant mutualism: the Argentine ant (*Iridomyrmex humilis*) and the myrmecochorous Proteaceae. *Ecology* **65**:1031–1037.
- Bossard, C. C. 1990. Tracing of ant-dispersed seeds: a new technique. *Ecology* **71**:2370–2371.
- Broyles, S. B., S. L. Sherman-Broyles, and P. Rogati. 1997. Evidence of outcrossing in *Trillium erectum* and *Trillium grandiflorum* (Liliaceae). *Journal of Heredity* **88**:325–329.
- Burbank, D. H., K. S. Pregitzer, and K. L. Gross. 1992. *Vegetation of the W. K. Kellogg Biological Station*. Michigan State University Agricultural Experiment Station Research Report #510, East Lansing, Michigan, USA.
- Clark, D. A., and D. B. Clark. 1981. Effects of seed dispersal on the regeneration of *Bursera graveolens* (Burseraceae) on Santa Fe Island, Galapagos. *Oecologia* **49**:73–75.
- Culver, D. C., and A. J. Beattie. 1978. Myrmecochory in *Viola*: Dynamics of some seed–ant interactions in some West Virginia species. *Journal of Ecology* **66**:53–72.
- Culver, D. C., and A. J. Beattie. 1980. The fate of *Viola* seeds dispersed by ants. *American Journal of Botany* **67**:710–714.
- Davidson, D. W., and S. R. Morton. 1981. Myrmecochory in some plants (F. Chenopodiaceae) of the Australian arid zone. *Oecologia* **50**:357–366.
- Fukuda I., and W. F. Grant. 1980. Chromosome variation and evolution in *Trillium grandiflorum*. *Canadian Journal of Genetics and Cytology* **22**:81–91.
- Furnier, G. R., P. Knowles, M. A. Clyde, and B. P. Dancik. 1987. Effects of avian seed dispersal on the genetic structure of whitebark pine populations. *Evolution* **41**:607–612.
- Gates, B. N. 1940. Dissemination by ants of the seeds of *Trillium grandiflorum*. *Rhodora* **42**:194–196
- . 1941. Observations in 1940 on the dissemination by ants of the seeds of *Trillium grandiflorum*. *Rhodora* **43**:206–207.
- Gibson, J. P., and N. T. Wheelwright. 1995. Genetic structure in a population of a tropical *Ocotea tenera* (Lauraceae): influence of avian seed dispersal. *Oecologia* **103**:49–54.
- Gleason, H. A., and A. Cronquist. 1991. *Manual of the vascular plants of northeastern United States and adjacent Canada*, 2nd edition. New York Botanical Garden, Bronx, New York, USA.
- Gomez, C., and X. Espadaler. 1994. Seed dispersal by ants in *Euphorbia characias* L. and *Euphorbia nicaeensis* All. (Euphorbiaceae). *Ecologia Mediterranea* **20**:51–59.
- Gunther, R. W., and J. Lanza. 1989. Variation in attractiveness of *Trillium* diaspores to a seed-dispersing ant. *American Midland Naturalist* **122**:321–328.
- Hamrick, J. L., and M. D. Loveless. 1986. The influence of seed dispersal mechanisms on the genetic structure of plant populations. Pages 211–223 in A. Estrada and T. H. Fleming, editors. *Frugivores and seed dispersal*. Dr. W. Junk, Dordrecht, The Netherlands.
- Hamrick, J. L., D. A. Murawski, and J. D. Nason. 1993. The influence of dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetation* **107/108**:281–297.
- Handel, S. N. 1978. The competitive relationship of three woodland sedges and its bearing on the evolution of anti-dispersal of *Carex pedunculata*. *Evolution* **32**:151–163.
- Handel, S. N., and A. J. Beattie. 1990. Seed dispersal by ants. *Scientific American*, August 76–83.
- Handel, S. N., S. B. Fisch, and G. E. Schatz. 1981. Ants disperse a majority of herbs in a mesic forest community in New York State. *Bulletin of the Torrey Botanical Club* **108**:430–437.
- Hanzawa, F. M., A. J. Beattie, and D. C. Culver. 1988. Directed dispersal: demographic analysis of an ant–seed mutualism. *American Naturalist* **131**:1–13.
- Hanzawa, F. M., and S. Kalisz. 1993. The relationship between age, size, and reproduction in *Trillium grandiflorum* (Liliaceae). *American Journal of Botany* **80**:405–410.
- Hartl, D. L. and A. G. Clark. 1997. *Principles of population genetics*. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- Heithaus, E. R. 1981. Seed predation by rodents on three ant-dispersed plants. *Ecology* **62**:136–145.
- Herbers, J. M. 1989. Community structure in north temperate ants: temporal and spatial variation. *Oecologia* **81**:201–211.
- Heywood, J. S. 1991. Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* **22**:335–355.
- Higashi, S., S. Tsuyuzaki, M. Ohara, and F. Ito. 1989. Adaptive advantages of ant-dispersed seeds in the myrmecochorous plant *Trillium tschonskii* (Liliaceae). *Oikos* **54**:389–394.
- Holldöbler B., and E. O. Wilson. 1990. *The ants*. Harvard University Press, Cambridge, Massachusetts, USA.
- Horvitz, C. C. 1981. Analysis of how ant behaviors affect germination in a tropical myrmecochore *Calathea ovanensis* (P. and E.) Koernicke (Marantaceae): microsite selection and aril removal by neotropical ants. *Odontomachus*, *Pachycondyla*, and *Solenopsis* (Formicidae). *Oecologia* **51**:47–52.
- Horvitz, C. C., and D. W. Schemske. 1986a. Ant-nest soil

- and seedling growth in a neotropical ant-dispersed herb. *Oecologia* **70**:318–320.
- Horvitz, C. C., and D. W. Schemske. 1986b. Seed dispersal of a neotropical myrmecochore: variation in removal rates and dispersal distances. *Biotropica* **18**:319–323.
- Howe, H. F., and R. B. Primack. 1975. Differential seed dispersal by birds of the tree *Casearia nitida* (Flacourtiaceae). *Biotropica* **7**:278–283.
- Howe, H. F., and J. Smallwood. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics* **13**:201–228.
- Howe, H. F., E. W. Schupp, and L. C. Westley. 1985. Early consequences of seed dispersal for a neotropical tree (*Vireola surinamensis*). *Ecology* **66**:781–791.
- Hughes, L., and M. Westoby. 1992. Fate of seeds adapted for dispersal by ants in Australian sclerophyll vegetation. *Ecology* **73**:1285–1299.
- Jaenicke, J. 1978. An hypothesis to account for the maintenance of sex within populations. *Evolutionary Theory* **3**:191–194.
- Janzen, D. H. 1970. Herbivores and the number of trees in the tropical forests. *American Naturalist* **104**:501–528.
- Jules, E. S. 1996. Yellow jackets (*Vespula vulgaris*) as a second seed disperser for the myrmecochorous plant, *Trillium ovatum*. *American Midland Naturalist* **135**:367–369.
- Kitajama, K., and C. K. Augspurger. 1989. Seed and seedling ecology of a monocarpic tropical tree, *Tachigalia versicolor*. *Ecology* **70**:1102–1114.
- Lanza, J., M. A. Schmitt, and A. B. Awad. 1992. Comparative chemistry of elaiosomes of three species of *Trillium*. *Journal of Chemical Ecology* **18**:209–221.
- Lawrence, W. H., and J. H. Rediske. 1960. Radiotracer technique for determining the fate of broadcast Douglas-fir seed. *Society of American Forestry Proceedings*. **1959**:99–101.
- Loiselle, B. A., V. L. Sork, J. Nason, and C. Graham. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* **82**:1420–1425.
- Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* **15**:65–95.
- Lubbers, A. E., and M. J. Lechowicz. 1989. Effects of leaf removal on reproduction vs. belowground storage in *Trillium grandiflorum*. *Ecology* **70**:85–96.
- Mesler, M. R., and K. L. Lu. 1983. Seed dispersal of *Trillium ovatum* (Liliaceae) in second-growth redwood forests. *American Journal of Botany* **70**:1460–1467.
- Milewski, A. V., and W. J. Bond. 1982. Convergence of myrmecochory in mediterranean Australia and South Africa. Pages 89–98 in R. C. Buckley, editor. *Ant-plant interactions in Australia*. Dr. W. Junk, The Hague, The Netherlands.
- O'Dowd, D. J., and M. E. Hay. 1980. Mutualism between harvester ants and a desert ephemeral: seed escape from rodents. *Ecology* **61**:531–540.
- Peakall, R., and A. J. Beattie. 1995. Does ant dispersal of seeds of *Sclerolaena dicantha* (Chenopodiaceae) generate local spatial genetic structure? *Heredity* **75**:351–361.
- Qadir, S. A., and M. Abbasi. 1971. Chemical interaction between seeds of common plants. *Pakistan Journal of Scientific and Industrial Research* **14**:211–218.
- Ritland, K. 1990. A series of FORTRAN computer programs for estimating plant mating systems. *Journal of Heredity* **81**:235–237.
- SAS Institute. 1989. SAS language and procedures: usage, version 6, first edition. SAS Institute, Inc. Cary, North Carolina, USA.
- Schmitt, J., and J. Antonovics. 1986. Experimental studies of the evolutionary significance of sexual reproduction. IV. Effect of neighbor relatedness and aphid infestation on seedling performance. *Evolution* **40**:830–836.
- Slingsby P., and W. J. Bond. 1985. The influence of ants on the dispersal distance and seedling recruitment of *Leucospermum conocarpodendron* (L.) Buek (Proteaceae). *South African Journal of Botany* **51**:30–34.
- Smith, B. H., P. D. Forman, and A. E. Boyd. 1989. Spatial patterns of seed dispersal and predation of two myrmecochorous forest herbs. *Ecology* **70**:1649–1656.
- Smith, B. H., M. L. Ronsheim, and K. R. Swartz. 1986. Reproductive ecology of *Jeffersonia diphylla* (Berberidaceae). *American Journal of Botany* **73**:1416–1426.
- Tonsor, S. J. 1989. Relatedness and intraspecific competition in *Plantago lanceolata*. *American Naturalist* **134**:897–906.
- Tonsor, S. J., S. Kalisz, J. Fisher, and T. Holtsford. 1993. A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*. *Evolution* **47**:833–843.
- van der Pijl, L., 1972. Principles of dispersal in higher plants, second edition. Springer-Verlag, Berlin, Germany.
- Watkinson, A. R. 1978. The demography of a sand dune annual: *Vulpia fasciculata*. II. The dynamics of seed populations. *Journal of Ecology* **66**:35–44.
- Williams, C. E., and R. P. Guries. 1994. Genetic consequences of seed dispersal in three sympatric forest herbs. I. Hierarchical population genetic structure. *Evolution* **48**:791–805.
- Wilson, M. F., W. G. Hoppes, D. A. Doldman, P. A. Thomas, P. L. Katusic-Malmborg, and J. L. Bothwell. 1987. Sibling competition in plants: an experimental study. *American Naturalist* **129**:304–311.
- Winn, A. A. 1989. Using radionuclide labels to determine the post-dispersal fate of seeds. *Trends in Ecology and Evolution* **4**:1–2.
- Wright, S. 1946. Isolation by distance under diverse systems of mating. *Genetics* **31**:39–59.