SPATIAL POPULATION GENETIC STRUCTURE IN *TRILLIUM GRANDIFLORUM*: THE ROLES OF DISPERSAL, MATING, HISTORY, AND SELECTION

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Abstract.—The roles of the various potential ecological and evolutionary causes of spatial population genetic structure (SPGS) cannot in general be inferred from the extant structure alone. However, a stage-specific analysis can provide clues as to the causes of SPGS. We conducted a stage-specific SPGS analysis of a mapped population of about 2000 *Trillium grandiflorum* (Liliaceae), a long-lived perennial herb. We compared SPGS for juvenile (J), nonreproductive (NR), and reproductive (R) stages. Fisher's exact test showed that genotypes had Hardy-Weinberg frequencies at all loci and stage classes. Allele frequencies did not differ between stages. Bootstrapped 99% confidence intervals (99%CI) indicate that F-statistic values are indistinguishable from zero, (except for a slightly negative F_{IT} for the R stage). Spatial autocorrelation was used to calculate f, the average kinship coefficient between individuals within distance intervals. Null hypothesis 99%CIs for f were constructed by repeatedly randomizing genotypic locations. Significant positive fine-scale genetic structure was detected in the R and NR stages, but not in the J stage. This structure was most pronounced in the R stage, and declined by about half in each remaining stage: near-neighbor $f = 0.122, 0.065, 0.027$, for R, NR, and J, respectively. For R and NR, the near-neighbor f lies outside the null hypothesis 99%CI, indicating kinship at approximately the level of half-sibs and first cousins, respectively. We also simulated the expected SPGS of juveniles post dispersal, based on measured R-stage SPGS, the mating system, and measured pollen and seed dispersal properties. This provides a null hypothesis expectation (as a 99%CI) for the J-stage correlogram, against which to test the likelihood that post-dispersal events have influenced J-stage SPGS. The actual J correlogram lies within the null hypothesis 99%CI for the shortest distance interval and nearly all other distance intervals indicating that the observed low recruitment, random mating and seed dispersal patterns are sufficient to account for the disappearance of SPSG between the R and the J stages. The observed increase in SPGS between J and R stages has two potential explanations: history and local selection. The observed low total allelic diversity is consistent with a past bottleneck: a possible historical explanation. Only a longitudinal stage-specific study of SPGS structure can distinguish between historical events and local selection as causes of increased structure with increasing life history stage.

Key words.—gene flow, population genetic structure, selection, spatial autocorrelation, spatial genetic structure, stage structure, *Trillium grandiflorum*.

Two major factors are thought to contribute to the extent of spatial population genetic structure (SPGS) either within or among plant populations: the patterns and distances of pollen and seed dispersal (Ennos 1994) and the extent of local selection (Hedrick 1986). The predicted effects of various patterns of pollen and seed dispersal are well understood (Slatkin 1985; Ennos 1994), as are the theoretical consequences of local selection (Felsenstein 1976; Slatkin 1985). However, the relative importance of local genetic drift versus local selection pressures as factors governing the formation and maintenance of within-population SPGS has been at issue since even before the time of Fisher and Wright (e.g., Provine 1986, pg. 288) and is yet to be resolved. There is considerable evidence for both forces acting in natural populations: local selection can produce SPGS (reviewed by Grant and Linhart 1996) and meta-analyses demonstrate that drift via local dispersal and mating system can contribute to SPGS as well (reviewed by Loveless and Hamrick 1984; Hamrick and Godt 1989). Clearly, patterns of SPGS could derive either from variable local selection acting within or among populations or the processes of local mating and dispersal, or both, because both processes have the potential to reinforce or accelerate the degree of spatial aggregation of relatives.

Despite their central roles in the evolutionary process, rigorous tests of the causes of extant within-population SPGS in natural populations are rare in the literature. This is likely because, although most studies of SPGS use putatively neutral markers in most circumstances, it is not possible to directly infer the *causes* of SPGS solely from marker locus polymorphisms (Slatkin and Arter 1991; Wade and McCauley 1988; Whitlock and McCauley 1999; but see Sokal and Wartenberg 1983; Slatkin 1985; Sokal et al. 1989). Nevertheless, assertions are often made about migration or dispersal rates based on SPGS measures (e.g., review by Slatkin 1985). Strong inferences about causes of local SPGS have been substantiated in only a handful of marker-based cases (Bergman 1975, 1978; Heywood and Levin 1985, McCauley 1994, 1997; Xie and Knowles 1992; Peakall and Beattie 1995; Loiselle et al. 1995; Schnabel et al. 1998, Ingvarsson and Giles 1999).

One powerful approach used to evaluate the development of SPGS examines SPGS with respect to distinct age- or stage-cohorts co-occurring in a population. This permits the detection of changes in SPGS over the life history with the expectation that various causal factors may contribute to SPGS at different stages. The SPGS of newly dispersed seeds

is a function of the parental stage SPGS, the effects of the dispersal distances of gametes (pollen) and mating system, and the dispersal distances of seeds. The post-dispersal SPGS of seeds establishes the initial template of SPGS, but is rarely measured. Subsequent stages' SPGS will reflect site-specific or random recruitment or mortality of individuals that can change the SPGS established at dispersal. A cohort approach has been taken in only a few studies and the patterns of SPGS differ among them. For example, Tonsor et al. (1993) found less structure in seedlings relative to adults and suggested postdispersal selection may be responsible for the adult SPGS. Alternatively, Hamrick et al. (1993) and Epperson and Alvarez-Buylla (1997) showed that the postdispersal juvenile cohorts were more structured than adult cohorts and emphasized the role of mating and dispersal in the development of SPGS. The initial SPGS of dispersed seeds has never been quantified, making inference as to the causes of postdispersal seed to adult changes in SPGS difficult.

Clearly, interpretation of the differences in SPGS among cohorts would be dramatically improved if a population's seed dispersal and mating system parameters were also quantified, permitting the estimation of the original SPGS template of dispersed seeds. This original template generates a post-dispersal, prerecruitment expectation of genetic structure to which later stages' SPGS can be compared. This approach has the potential to discriminate the effects on SPGS of dispersal versus postdispersal selection, but requires data on multiple stages' genotypes and spatial locations, actual seed dispersal distances, and the mating system, parameters which, to our knowledge, have never been simultaneously measured in wild populations.

Here we present analyses of SPGS of three life-history stages in a large, mapped, and genotyped population of the long-lived perennial herb, *Trillium grandiflorum* (Liliaceae). This life-history based examination allowed us to assess the causes of change in SPGS structure observed among stage classes. We used a spatial autocorrelation metric to test for within population spatial genetic structure and to test for differences in spatial genetic structure between stages. We compared SPGS estimates of juvenile, nonreproductive, and reproductive stages to determine the extent to which post dispersal forces influence SPGS. These analyses are coupled with a novel method for generating an expectation of SPGS at the postdispersal seed stage that allows a more rigorous interpretation of the potential factors responsible for altering SPGS over time.

MATERIALS AND METHODS

Study Species and Site Description

Trillium grandiflorum (Liliaceae) is a polycarpic perennial herb that occurs in the understory of deciduous woods throughout northeastern North America. Plants are nonclonal, single-stemmed, 15–45 cm high, arising from a tuber-like rhizome. Plants can be readily classified into three developmental stages (juveniles, nonreproductives, and reproductives) distinguished by leaf number and flower production. Juvenile plants produce only a single leaf and remain in this stage until a minimum size threshold for transition to the three-leaf nonreproductive stage is reached (Kawano et al.

1986). Reproductive individuals bear both a whorl of three leaves and a single flower and fruit. A minimum size threshold must also be met for nonreproductive three-leaf plants to transition to the reproductive stage (Hanzawa and Kalisz 1993). We classified all plants in our study area into the three stages and estimated their ages using the relationship of size and age determined for this population (Hanzawa and Kalisz, 1993). During this study, the population contained 128 juveniles (age range 1–8 years), 866 nonreproductives that did not flower at any time during the 1990–1993 field seasons (age range 9–26 years), and 328 reproductives that flowered at least once during 1990–1993 (age range 17–42 years). Hereafter, we refer to the three stages as J (juvenile), NR (nonreproductive), and R (reproductive).

The study population covered a 10 m × 20 m area in Long Woods, a 9.5 hectare oak-hickory forest in the Kellogg Biological Station of Michigan State University, Kalamazoo County, Michigan (for a site description, see Burbank et al. 1992). Each plant was permanently tagged and its location mapped (Fig. 1). Between 1990 and 1993, all plants were scored for leaf number, leaf area, and their stage class membership. To determine the multilocus genotypes of the plants, we collected 1-cm^2 sections of leaf tissue from R and NR plants and 0.2-cm^2 sections from J plants during 1989–1993 growing seasons for use in starch gel electrophoresis. Extraction and electrophoresis procedures were those of Gottlieb (1981, 1984). Fresh tissue was ground, the extracts absorbed onto wicks and stored at −80°C until run. Five polymorphic enzyme systems were assayed: Phosphoglucose isomerase (PGI2, 3 alleles [EC 5.3.1.9]), 6-Phosphogluconate dehydrogenase (PGD2, 3 alleles [EC 1.1.1.44]), Glutamate-oxaloacetate transaminase (AAT2, 3 alleles [EC 2.6.1.1]), and Malate dehydrogenase 1 and 3 (MDH1, 2 alleles and MDH3, 2 alleles [EC 1.1.1.37]). AAT2, PGI2, and PGD2 were scored on Tris-EDTA-borate pH 8 gel buffer system and MDH1 and MDH3 were scored on Morpholine Citrate pH 8.3 buffer system. These are the same systems used by Kalisz et al. (1999) to determine the outcrossing rate in this population.

Hierarchical Analysis of Population Genetic Structure

Each stage class was tested separately for deviations from Hardy-Weinberg equilibrium (H-W) using Fisher's exact test (Weir 1996) calculated using the computer program GDA (Lewis and Zaykin 2001; http://alleyn.eeb.uconn.edu/gda/). A hierarchical analysis of genetic structure was conducted for each stage class using Wright's *F*-statistics to estimate the correlations of alleles within and between individuals. Visual inspection of the population maps (see Fig. 1) suggested that there could be two natural spatial aggregates, north and south. *F*-statistics were therefore calculated with respect to two levels of population spatial structure: the total population and the putative north and south spatial aggregates. Genetic differentiation between the north and south aggregates was examined using Weir and Cockerham's (1984) θ. Differentiation between the north and south aggregates was absent or weak (significant only for NR, but low; θ = 0.019), and thus the apparent north and south spatial structure was not given further consideration. The correlation
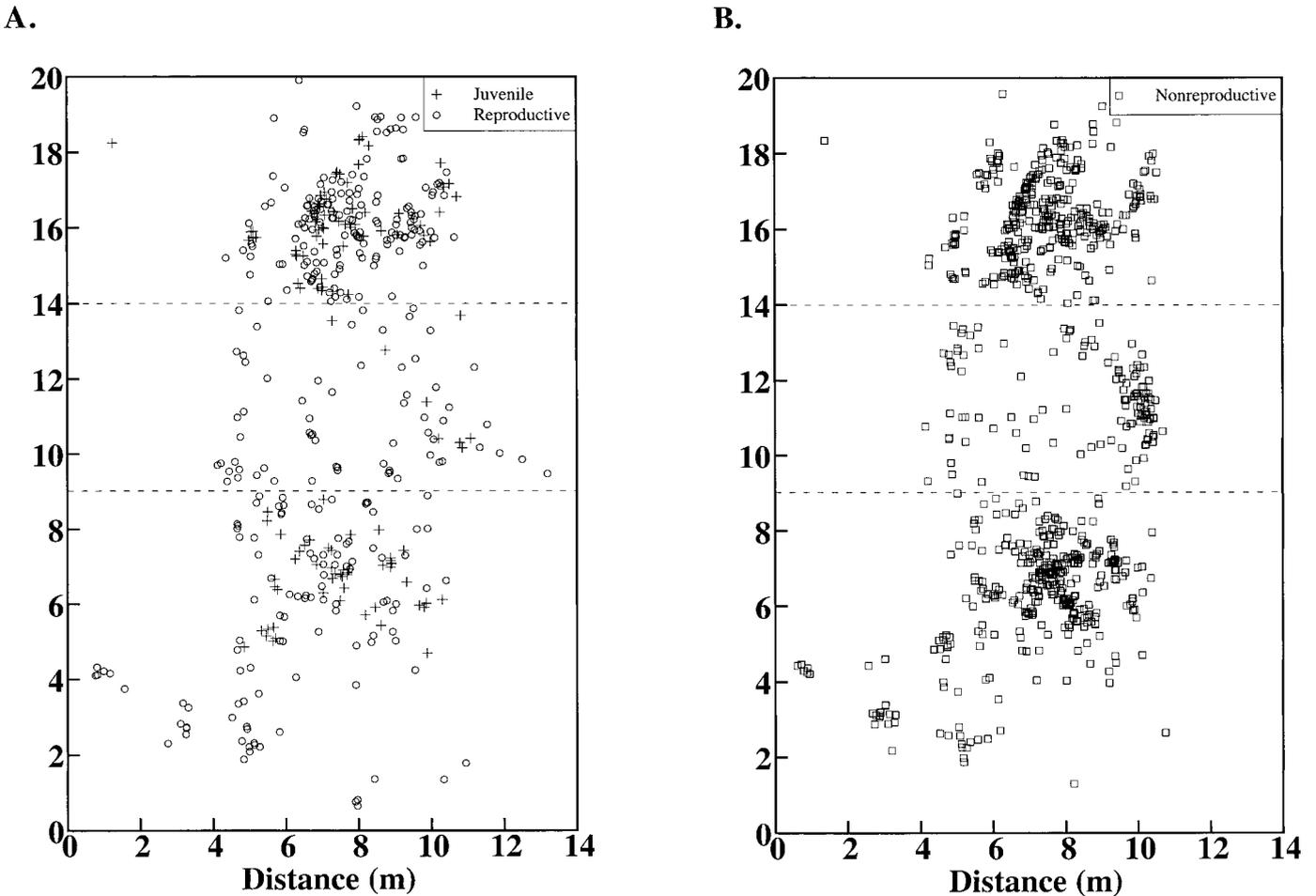
A.

B.

FIG. 1. Maps of all *Trillium grandiflorum* in the study population. A. Reproductive (o) and Juvenile (+) plants. B. Nonreproductive plants (□). Individuals above 14 m on the y-axis were in the north population aggregate while those below 14 m were in the south population aggregate (see text).

of alleles within individuals relative to the total population was measured by F_{IT} using the methods of Weir and Cockerham (1984) and Weir (1996) as implemented in the program GDA (Lewis and Zaykin). F_{IT} measures deviations from H-W expectations for homozygosity. Positive values indicate excess homozygotes whereas negative values indicate a deficit of homozygotes. Significance was determined by bootstrapping over loci to construct the multilocus 99% confidence intervals for F_{IT} using the program GDA.

Spatial Autocorrelation Analyses of Within-Population Genetic Structure

To identify differences in fine-scale genetic structure between stages, we used spatial autocorrelation techniques (Cliff and Ord 1981; Epperson 1989; Heywood 1991; Smouse and Peakall 1999). J. Nason has developed a multilocus estimator of kinship (Cockerham 1969; Barbujani 1987) that permits genetic correlations between individuals to be summarized over a range of distance intervals and was used in this study. In contrast to traditional single-locus estimators (e.g., Moran's I), genetic structure statistics like the kinship coefficient have a well-developed foundation in population

genetics theory and provide a natural means of combining data both over multiple alleles at a locus and over loci to obtain a more powerful test of genetic structure (Heywood 1991; Smouse and Peakall 1999). An individual-based spatial autocorrelation approach like the kinship coefficient permits the investigation of genetic structure at much finer distance intervals than estimators of genetic differentiation, such as Wright's F_{ST}, which only quantify gene frequency variation among sample plots or subpopulations. Spatial autocorrelation has the further advantage that it allows structure to be examined in populations at any spatial scales, even when no obvious spatial clustering of individuals exists. The kinship coefficient (f_{ij}) measures the correlation in the frequency of alleles, p_i and p_j, at a locus in pairs of mapped individuals i and j, and is, therefore, sensitive to the relatedness between paired individuals. In this study we measured kinship as a function of the distance between individuals using the estimator ($\hat{f}_{ij}$). For each allele

$$\hat{f}_{ij} = \frac{\sum_i \sum_{j>i} (p_i - \bar{p})(p_j - \bar{p})}{k\bar{p}(1 - \bar{p})}$$

where $\bar{p}$ is the population sample allele frequency and k is

the total number of possible pairwise connections between individuals located a discrete distance interval apart. For any discrete distance interval, the mean f_{ij} is obtained using all possible pairs of individuals located that distance apart. For a population in Hardy-Weinberg equilibrium with no spatial structuring the expected value of f_{ij} is zero. An artifact of sampling all possible pairs of individuals within a sample population, the expected value of $\hat{f}_{ij}$ for a Hardy-Weinberg population is biased by the amount $-(1/2[N - 1])$ leading to an adjusted estimator of

$$\hat{f}_{ij} = \frac{\sum_i \sum_{j>i} (p_i - \bar{p})(p_j - \bar{p})}{k\bar{p}(1 - \bar{p})} + \frac{1}{2(N - 1)}$$

where N is the total number of individuals in the sample population. For both estimators, results are combined over loci by weighting the result for each locus by its polymorphic index, $\sum p_a(1 - p_a)$, to obtain a multilocus measure of spatial genetic structure. Weights for individual loci are further adjusted for differences in sample size due to missing genotypic information.

In the absence of inbreeding or population genetic subdivision ($F_{IT} = 0$), f_{ij} is equal to the probability that a randomly chosen gamete from individual i and a randomly chosen gamete from individual j carry alleles that are identical by descent. This probability is, by definition, the inbreeding coefficient of a hypothetical offspring of i and j so that f_{ij} has expected values of 0.25 and 0.125 for full and half-sibling comparisons, respectively, and 0.25 for parent offspring comparisons. For two individuals that are not inbred, Wright's coefficient of relationship (r) is twice the inbreeding coefficient of the progeny of two individuals and is thus related to f_{ij} as $r = 2f_{ij}$. In the presence of inbreeding, r is defined as $2f_{ij}/\sqrt{(1 + F_i)(1 + F_j)}$, where F_i and F_j are the inbreeding coefficients of i and j (Crow and Kimura 1970). Computer programs for estimating f_{ij} and confidence intervals in a spatial autocorrelation framework are available upon request from J. Nason.

In this study we focused on the significance of individual and multilocus estimates of f_{ij} between pairs of individuals within the shortest distance interval (<25 cm), here after neighbors, and on the distance at which the correlogram crosses the zero axis. Within populations, significant positive values of f_{ij} are expected for the short distance intervals when localized seed dispersal results in the spatial aggregation of individuals related by recent common ancestry.

When $\hat{f}_{ij}$ values are combined over alleles or loci, standard normal deviates cannot be used as tests of significance, as is the practice with other autocorrelation measures based on the frequencies or join count statistics of individual alleles (Sokal and Oden 1978; Epperson 1989; Heywood 1991, Smouse and Peakall 1999). As a result, randomization procedures were used here to test the significance of estimated f_{ij} values by constructing a confidence envelope about the null hypothesis of no spatial genetic structure: H_0: $f_{ij} = 0$ (for a general discussion of this approach, see Slatkin and Arter 1991). In this procedure, map locations occupied by the individuals in the population or the subset of the population under examination, are randomly reassigned by randomly drawing locations with replacement from the set of observed locations. The approach

of utilizing extant genotypes, rather than simulating them, maintains any unrecognized gamete phase disequilibrium that may exist among loci. For a given distance class, the values of $\hat{f}_{ij}$ from the $N - 1$ simulation trials are ranked $\hat{f}_{(1)}, \hat{f}_{(2)}, \ldots, \hat{f}_{(N-1)}$, where is the $\hat{f}_{(1)}$ highest and $\hat{f}_{(N-1)}$ the lowest simulated values. The null hypothesis that there is no spatial genetic structuring of the sample population is rejected if the kinship coefficient based on the data, $\hat{f}_{data}$ (the Nth estimate), is greater than $\hat{f}_{(1-\alpha/2)N}$ or less than $\hat{f}_{(\alpha/2)N}$. In this study we conducted $N - 1 = 399$ simulation trials with $\alpha = 0.01$. Thus, $\hat{f}_{(399)}$ and $\hat{f}_{(2)}$ represent the upper and lower limits, respectively, of a 99% confidence interval on the distribution of simulated genetic structure statistics assuming no spatial genetic structure. We used 99% confidence intervals as a conservative critical value since the confidence envelope for the correlogram is essentially a test of significance at multiple distance intervals. An f_{ij} estimate falling outside this confidence envelope was considered ecologically significant only under two specific circumstances: if it occurred among the neighbor distance intervals (i.e., <25 cm), or if it occurred as part of a run of significant values over successive distance intervals. If genetic structure exists, then we expect a pattern of significant positive values at the shorter distance intervals becoming insignificant with increasing distance. Given this a priori assumption, differences between stage classes in the magnitude of these neighbor $\hat{f}_{ij}$ values were tested by using bootstrapping to construct 99% confidence intervals about the $\hat{f}_{ij}$ values themselves, as opposed to the null hypothesis of $f_{ij} = 0$. These bootstrap confidence intervals were used to test for significant differences between stage classes in the magnitude of genetic structure at the smallest distance interval.

Tests of the Homogeneity of Spatial Genetic Structure across Life-History Stages

We examined the relationship of spatial genetic structure across life-history stages in two ways. First, we compared the bootstrapped 99% confidence intervals about neighbor values, described above, to determine if stage-specific estimates came from the same sampling distributions. If the confidence intervals for two stages did not overlap, we rejected this hypothesis concluding they were drawn from different sampling distributions. Second, as described below, we assessed the extent to which dispersal alone explained J-stage spatial genetic structure by simulating seed dispersal around the R plants.

Simulation of Postdispersal Seed SPGS

We used seed dispersal and mating system data previously published for this *T. grandiflorum* population (Kalisz et al. 1999) to simulate the SPGS of the postdispersal seeds produced by the 328 mapped R plants.

Mating.—Using the methods of Ritland and Jain (1981) and Ritland (1989), this study population has been found to be highly outcrossing (multilocus $t = 1.05$, SE = 0.056) with seeds within fruits being primarily full siblings ($r_p = 0.82$, SE = 0.13) sired on average by $N = 1/r_p = 1.2$ effective males (Kalisz et al. 1999). In addition, field experiments conducted to test for self-pollination indicated a complete in-

ability of flowers to self-pollinate (Kalisz et al. 1999). Thus, the seeds within a fruit are likely the products of outcrossing among plants that do not share incompatibility alleles.

Dispersal.—Kalisz et al. (1999) quantified the natural pattern of seed movement by ants away from individual *T. grandiflorum* maternal plants on our study site during 1991 and 1992. Seeds were uniquely colored coded and labeled using Scandium-46, a gamma- and beta-emitting radionuclide which permitted relocation and identification to sibship. In the two years, 19% (39) and 23% (155) of the seeds recovered had been dispersed at least 0.1 m, respectively. Mean dispersal distances for these seeds were 2.41 m (95% C.I. = 0.64 m) in the first year and 0.53 m (95% C.I. = 0.12 m) in the second. Mean dispersal distances for all seeds (dispersed and undispersed) were 0.446 m and 0.169 m. Pooling over years, the mean dispersal distances were 0.907 m for dispersed and 0.253 m for all seeds. The pooled dispersal distributions were used to simulate post-dispersal SPGS.

The simulation of mating and seed dispersal.—Simulations were conducted to test the null hypothesis that observed patterns of genetic structure in juveniles can be explained by the SPGS of reproductives, the mating system, and seed dispersal in the preceding generation. Confidence limits on the SPGS (f_{ij}) expected under the null hypothesis were generated by repeatedly simulating the production and dispersal of seed cohorts equal in size to the number of juveniles occurring in the actual study population ($N = 128$).

Dispersal about each reproductive was modeled probabilistically according to the empirically determined dispersal distribution (Kalisz et al. 1999). Two seed dispersal distributions were modeled, one based on total labeled seeds and one on dispersed seeds only. In both models dispersal direction was chosen at random. The dispersal process was simulated separately for each seed, since the probability of two seeds from a single sibship surviving to the juvenile stage was less than 0.5%. This process allowed us to construct a postdispersal juvenile population in the absence of selection. By constructing 399 simulated offspring cohorts and conducting spatial autocorrelation analyses of kinship within each simulated offspring cohort, we could place 99% confidence intervals on the SPGS that would be obtained if random mating and the observed seed dispersal distribution were the *only* causes of J population genetic structure. We then used the confidence envelope to test the null hypothesis that the actual fine-scale genetic structuring observed for J stage is caused by the observed mating system, pattern of seed dispersal, and spatial distribution and genetic structure of the R class. Rejection of this hypothesis requires that the actual pattern of fine-scale genetic structure for the J stage fall outside the confidence interval at neighbor distances (<25 cm), or at larger distances in some systematic way.

RESULTS

Hierarchical Analysis of Population Genetic Structure

Based on Fisher's exact tests, there were no significant deviations from Hardy-Weinberg equilibrium at any locus in any one of the three stage classes (Table 1, 15 tests). F_{IT} was significantly different from zero only for the reproductive stage (−0.02, 95% C.I.: −0.06, −0.01). Estimates were not

TABLE 1. Allele frequencies by locus for each of the three life stages. Exact tests of deviations from Hardy-Weinberg equilibrium were not significant at any locus (15 tests).

Locus	Allele	Juveniles ($n = 128$)	Nonreproductives ($n = 866$)	Reproductives ($n = 328$)
AAT2	1	0.149	0.179	0.159
	2	0.851	0.821	0.841
PGD2	1	0.750	0.719	0.728
	2	0.250	0.281	0.272
PGI2	1	0.370	0.358	0.390
	2	0.395	0.404	0.378
	3	0.235	0.238	0.232
MDH1	1	0.191	0.158	0.176
	2	0.809	0.842	0.824
MDH3	1	0.873	0.901	0.855
	2	0.127	0.099	0.145

significantly different between stages, however, and all values were negative. F_{IT} pooled over all stages was slightly negative but not significantly different from zero ($F = -0.013$).

Spatial Autocorrelation Analysis of Fine-Scale Genetic Structure

Spatial autocorrelation analyses revealed significant positive fine-scale genetic structure in two of the three stage classes (Fig. 2). This structure was most pronounced in the R class with a maximum value of $f_{ij} = 0.122$ at 0.125 m with values crossing the $f_{ij} = 0$ axis at 3.125–3.25 m (Fig. 2a). The structuring was about half as strong in the NR class, with a maximum value of $f_{ij} = 0.065$ at 0.125 m with values crossing the $f_{ij} = 0$ axis at approximately 3.75–5.5 m (Fig. 2b). The structuring in the J class was less than one quarter of that observed for the R class, with a maximum value of $f_{ij} = 0.027$ at 0.25 m with values crossing the $f_{ij} = 0$ axis between the first and second distance intervals (0.25–0.5 m; Fig. 2c). The R and NR classes exhibited a clear trend of decreasing f_{ij} values with increasing distance, and significant positive and negative values at shorter and longer distances, respectively. The J class, in contrast, did not exhibit a detectable trend in f_{ij} with distance. Differences in the distance interval employed in the analysis and the width of the confidence envelope within and between life stages are influenced by sample sizes (i.e., NR, $n = 866$; J, $n = 128$; see Fig. 2).

To determine whether the observed spatial patterns of fine-scale genetic structure were consistent across the five allozyme loci, we calculated separate f_{ij} estimates for each locus at each stage at the smallest distance interval used in the spatial autocorrelation analyses. In R and NR classes the smallest distance (0.125 m) f_{ij} estimates were significantly greater than zero for four of five loci. The exception in both stages, MDH1, was consistent with the other loci in having a positive f_{ij} value. The smallest distance interval (0.25 m) single-locus f_{ij} values for the J class, in contrast, were consistently positive and significant only for MDH1. Overall, the individual locus estimates do not indicate substantial heterogeneity between loci within-stage classes, but do suggest heterogeneity among-stage classes.
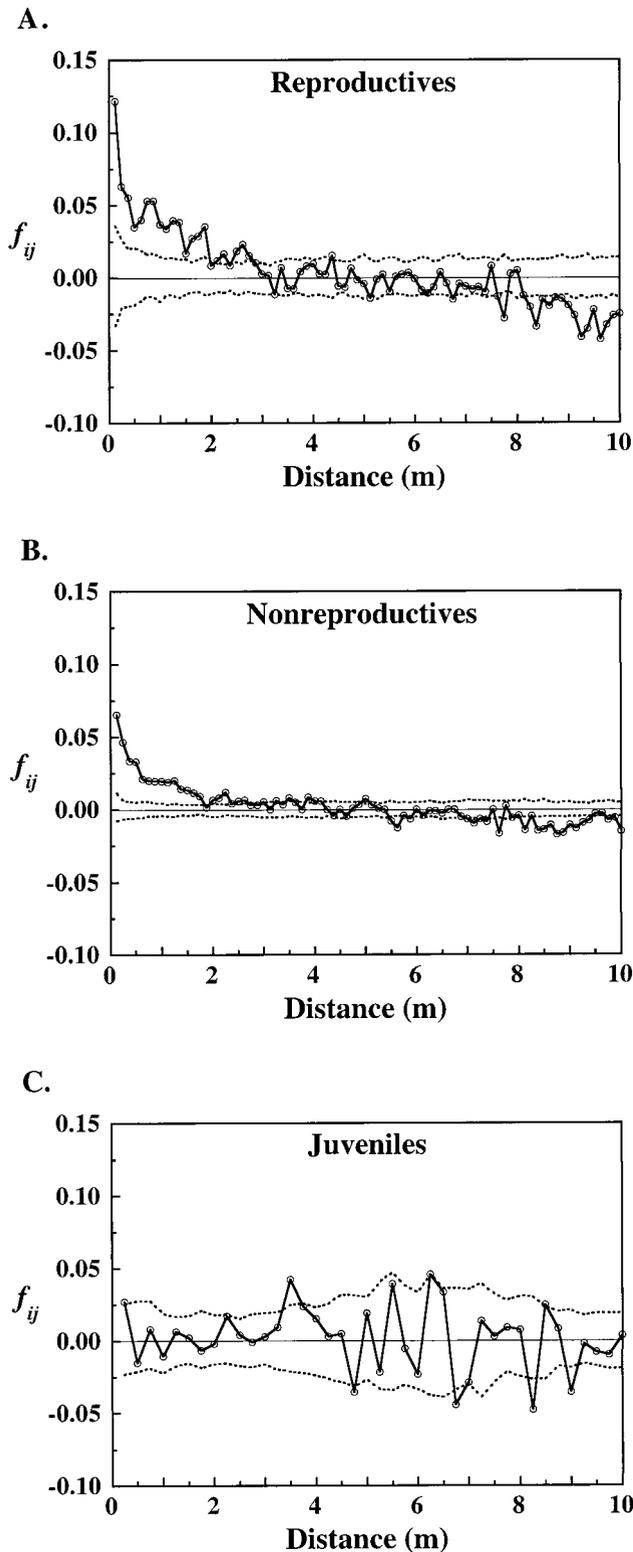
A.

B.

C.

FIG. 2. Spatial autocorrelation analyses of kinship, f_{ij}. A. Reproductive stage. B. Nonreproductive stage. C. Juvenile stage. Distance intervals are 0.125 m in the reproductive and nonreproductive and 0.25 m in the juvenile stages, respectively. The dashed lines represent a 99% bootstrap confidence interval about the null hypothesis of no genetic structure (the horizontal line at $f_{ij} = 0$).
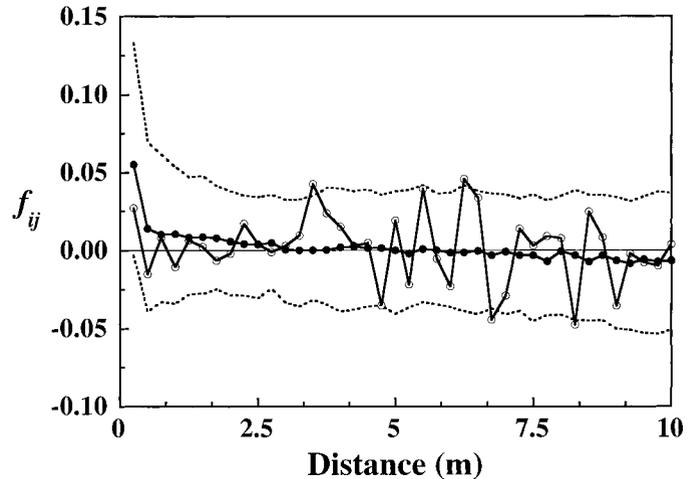

FIG. 3. Comparison of the correlogram based on juvenile stage genotypes (open circles) with the null hypothesis simulated correlogram (closed circles) and its 99% confidence envelope (dashed lines). See text for derivation of the null hypothesis correlogram.

Tests of the Homogeneity of Spatial Genetic Structure Across Life-History Stages

The mean f_{ij} for the 0.25 distance interval and the confidence envelopes around these kinship estimates were compared to determine if SPGS differs among stages. The 99% bootstrap confidence intervals for the R and the J stages do not overlap (0.122, 99% C.I.: 0.065, 0.192; 0.011, 99% C.I.: -0.048, 0.065, reproductive vs. juvenile, respectively). The NR stage confidence interval (0.065 99% C.I.: 0.050, 0.080), overlaps those of both the J and R stages. Differences in the sizes of the confidence intervals between stages were a function of the number of pairwise comparisons observed at this distance interval: 45, 1033, and 84 pairs in J, NR and R stages, respectively.

The test for differences in genetic structure between the actual J SPGS and the confidence envelope based on the simulations are shown in Figure 3. The closest-distance f_{ij} estimates were inside of the simulation confidence envelopes whether all seeds or only dispersed seeds were used for the seed dispersal distance distribution. Although a few estimates were significantly positive or negative at larger distance intervals, there is no indication of decreasing genetic correlation with increasing juvenile interplant distance. The mean f_{ij} estimates from the simulation exhibited stronger positive correlations over close neighbor distances than did the actual SPGS of juveniles (Fig. 3).

DISCUSSION

Under classical models of isolation by distance, continuous plant populations are likely to develop genetic structuring because gene dispersal through pollen and seed is restricted about the maternal parent (Wright 1946; Levin and Kerster 1974; Brown 1979; Schaal 1980; Slatkin 1985). If the probabilities of survival and reproduction are independent of mating and seed dispersal patterns, then population genetic structure will be compounded over successive generations (e.g., Turner et al. 1982; Sokal and Wartenberg 1983). The evo-

lution of structure by this process will thus come to be characterized by significant genetic correlations in the form of positive associations between alleles, both within individuals (inbreeding, F_{IT}) and among neighboring individuals (population spatial genetic structure, f_{ij}). In this study there was no evidence for inbreeding. In conjunction with the mating system data from Kalisz et al (1999) the F_{IT}-values from this study strongly suggest that mating in this population is essentially random. Regardless of the seed dispersal distance distribution, random mating prevents the accumulation of inbreeding and population genetic structure through time.

Paradoxically, fine-scale genetic structure can exist within a nonsubdivided population, as demonstrated in this study of *T. grandiflorum*. Fine-scale SPGS can develop as a result of differential effects of pollen and seed dispersal on allelic correlations within and among individuals within a population. When the variance in seed dispersal is less than the variance in pollen dispersal, fine-scale genetic structure can occur without inbreeding. Four alternative scenarios are possible with these dispersal attributes: (1) When both pollen and seed dispersal are highly localized around the maternal parent and have similar variances, then inbreeding and genetic substructuring of a population will evolve as described by the isolation by distance model (Wright 1946; Sokal and Wartenberg 1983; Barbujani 1987). (2) Conversely, when both pollen dispersal and seed dispersal are random within a population, then neither inbreeding nor spatial genetic structure will develop. (3) When pollen dispersal is highly localized but seed dispersal is random, then neither inbreeding nor genetic structure will develop (if selfing is prohibited). (4) When pollen dispersal is random but seed dispersal is highly localized there will be no inbreeding but spatial aggregations of siblings will result in significant fine-scale genetic structure (Hamrick and Nason 1996).

In our study population, spatial autocorrelation revealed significant fine scale structure, which increases in magnitude with life-history stage. Had we only assessed SPGS at the adult stage, or had we pooled the data across life-history stages, the results would have been consistent with scenario four above: Seed dispersal is highly localized relative to pollen dispersal, homogenizing population genetic subdivision at the scale of the entire study population (~20 m). The SPGS of the juvenile stage, however, is not consistent with this explanation. Like the reproductives, the juveniles were not inbred. Unlike closely adjacent reproductive plants, closely adjacent juveniles were unrelated. Since the reproductive cohort of this *T. grandiflorum* population is significantly structured ($f_{ij} = 0.12$ at 0.125 m, approximately half sibs), we might expect their offspring to be spatially structured as well. What processes could be acting to create the lack of genetic structure seen in the juveniles? Our data show that random mating, high random mortality of seeds/seedlings, and moderate seed dispersal distances are the causes. We elucidate this conclusion by considering the range of possible juvenile f_{ij} values that could be observed under the range of possible mating, seed/seedling mortality and seed dispersal patterns for our study population.

The effects of random mating.—This population has been shown to be outcrossing (t ≈ 1) with maternal progenies containing primarily full-sibs ($r_p = 0.82$, Kalisz et al. 1999),

and is essentially random mating (this study). The reproductive stage exhibits positive spatial genetic structure (f_{ij}) to a distance of approximately 3 m. Despite this, juveniles are not inbred, indicating that reproductive plants mating within neighborhoods of relatedness do not influence the population genetic structure of the juvenile stage.

If seeds are not dispersed away from the maternal parent and only one offspring per maternal parent establishes each year, the f_{ij} of near-neighbor juveniles should be half that of the parental generation (i.e., ~0.06). This occurs because half the genes are inherited from the seed parent and do not move, and half are inherited from the pollen parent and are randomly moved during pollination. In contrast, under random mating, maternal full siblings will share 25% of their genes on average. If there is no seed dispersal and all juvenile near-neighbors are full siblings due to multiple full sib recruits per mother, the f_{ij} of near-neighbor juveniles would be 0.25, or twice the measured f_{ij} of the reproductive stage. These two scenarios establish the range of possible f_{ij} values for the juveniles if seeds did not disperse (f_{ij} between 0.06–0.25). Our estimate of *T. grandiflorum* juvenile near-distance f_{ij}, 0.01, (95% C.I. of 0.048–0.065) is considerably lower than the 0.06 lower bound of estimates based on random mating with no seed dispersal, suggesting that seed mortality and seed dispersal are likely to be involved.

Seed and seedling mortality.—We have two lines of evidence supporting our hypothesis that mortality driven rarifaction contributes to the loss of spatial genetic structure by the juvenile stage. First, near neighbor distances are small for individuals in the seed stage. However, near neighbor distances are significantly greater at the juvenile stage, whereas they remain unchanged for the nonreproductive and reproductive stages (S. Kalisz, S. J. Tonsor, and F. M. Hanzawa, unpubl. data). This suggests thinning of the post-dispersal seed clusters to single juvenile individuals. Second, demographically speaking, few seeds survive the transition to juveniles. Although average annual seed production was 18.5 seeds per reproductive plant (Kalisz et al. 1999), only 0.39 juveniles per reproductive were present, indicating a seed to juvenile transition of approximately 2%, or about 0.36 seeds/year/sibship. This strongly suggests that very few maternal siblings exist among the juveniles. Thus high mortality alone could explain a large drop in near-neighbor f_{ij} from reproductive to juvenile stages observed in our study.

Seed dispersal.—In combination with random mating and low seed to juvenile survivorship, any amount of seed dispersal, even very short distance dispersal, could account for any remaining reduction in near-neighbor genetic structure between reproductive and juvenile stages. The average dispersal distance from 1991 (2.4 m; Kalisz et al. 1999) would carry a seed near the edge of the genetic neighborhood of its mother, suggesting that even the limited seed dispersal distances observed in *T. grandiflorum* should have a homogenizing effect on SPGS. This prediction is consistent with our simulations examining the effects of mating and seed dispersal on juvenile genetic structure (Fig. 3). Our simulations reveal that the expected f_{ij} based on empirically determined seed dispersal patterns is consistently stronger than the observed f_{ij}, up to a distance of 2 m (8 distance intervals, $P < 0.004$). This pattern holds whether all or only dispersed seeds

are used to model the seed dispersal process. Our results suggest that random mating and moderate seed dispersal likely explain the loss of SPGS from reproductive to juvenile stages. However, the processes underlying the increase in genetic structure from juvenile to adult stages remain unclear.

Reproductive stage.—Of the few other studies that have taken a life stage approach, Epperson and Alvarez-Buylla (1997) and Hamrick et al. (1993) have found evidence of the erosion of SPGS from the seedling stage to the adult stage consistent with random mortality during recruitment. In this study of *T. grandiflorum,* and a life-stage study of *Plantago lanceolata* (Tonsor et al. 1993), the observed increase in SPGS with life stage cannot be explained by spatially and/or genetically random mortality during recruitment.

Given that the original fine-scale genetic template in this *Trillium* juvenile population is not structured, what can create the strong pattern of structure seen in the reproductives? One possibility is that reproductive stage SPGS is a historical phenomenon. For example, a population founder event in the recent past could have established the current reproductive cohort, creating a pattern of genetic structure in adults that is in the process of decay in offspring cohorts. Genetic bottlenecks associated with founder events and/or rapid population growth are expected to result in a deficit of allelic diversity relative to populations at mutation-drift equilibrium (Nei et al. 1975; Cornuet and Luikart 1996). We used the Ewens' expectation (Chakraborty et al. 1988) to estimate the allelic diversity expected in an equilibrium population having the same heterozygosity as observed in the *T. grandiflorum* reproductives. The total observed allelic diversity over five loci ($n = 11$) was substantially less than expected ($n = 23$), a result consistent with the bottleneck hypothesis. Further, contemporary mating system and seed dispersal estimates are consistent with the decay of genetic structure from reproductive to juvenile stages. Thus, a recent founder event would be consistent with our data for *Trillium,* with the published data of Tonsor et al (1993) for *P. lanceolata,* and may be of sufficiently frequent occurrence to influence genetic structure in other species.

Another possible explanation for the high SPGS in adults relative to juveniles in both *T. grandiflorum* and *P. lanceolata* is that selection is increasing SPGS between the seed establishment and the reproductive stage. Such selection could result from microlocal environmental conditions or from microlocal genotype frequencies and positive frequency-dependent selection (review in Mitton 1995, Grant and Linhart 1996). Factors such as spatially structured disease (c.f., Parker 1985) and/or fine scale genetic interactions of mycorrhizal associations, as suggested by Taylor and Bruns (1999), could operate to favor the survival of spatial aggregates of relatives, resulting in a pattern of increasing fine-scale genetic structure for each successive life stage. In addition, balancing selection could be acting to maintain the high levels of heterozygosity seen with increasing stage. Thus, both of the historical factors discussed above and selection are potential explanations for the pattern observed in *T. grandiflorum,* whereas local dispersal and mating system are not.

Conclusions.—The majority of studies of fine-scale genetic structure have focused solely on the analysis of reproductive plants or of individuals not classified as to life stage or age.

Patterns of spatial structure in adults represent the culmination of ecological and evolutionary processes acting in the past and present. Moreover, fine-scale genetic structure and localized mating and seed dispersal combine to influence intra- and interindividual genetic correlations in the following generation and are thus important components of future population genetic structure. Nonetheless, as this study demonstrates, the causal ecological processes underlying observed patterns of genetic structure may be misinterpreted if adults alone are analyzed. The presence or absence of fine-scale genetic structure in adults cannot be interpreted to be representative of all life-history stages of the organism. The spatial and genetic structuring of seeds and seedlings established by pollen and seed dispersal may be modified by a number of ecological and evolutionary processes that may ultimately act to increase or decrease genetic structure. Although a life-stage specific approach cannot always distinguish between alternative mechanisms for the evolution of genetic structure, it can indicate how different forms of dispersal and selection may influence the process.

In the handful of life history based SPGS studies in plants, it is clear that spatial population genetic structure can vary significantly over the life history of the plant. This variation is sufficient in all the published studies of stage-specific SPGS cited here to cause SPGS structure to disappear in one or more stages. What is not clear is the extent to which this instability is driven by historical contingencies, local selection, or random processes. We know of no studies that have tested for long-term stability of SPGS. Long term life-history based studies of SPGS and its postulated causes are necessary before we can fully understand the balance of forces producing SPGS and how it evolves in natural populations.

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