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Field and genetic studies testing optimal outcrossing in Agave schottii, a long-lived clonal plant

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Abstract In this study we combine field experiments, designed to test the predictions of optimal outcrossing theory in *Agave schottii*, with molecular genetic studies, using RAPD (random amplified polymorphic DNA), polymerase chain reaction to assess the underlying genetic hypothesis of optimal outcrossing theory. Initially, 48 "females" of *A. schottii* were hand-pollinated with pollen collected from 1 m, 10 m, 100 m, and 2500 m distances. Each female received all four distance treatments. Additionally, a subset of the focal females and their pollen donors were used in an analysis of genetic similarity across the four distances. Results of hand-pollinations showed that crosses of 1 m had significantly lower seed set than 10 m and 100 m crosses. Crosses of 2500 m had intermediate seed set. Combined relative fitness was significantly lower for 1 m crosses compared to 10 m crosses, while 100 m and 2500 m crosses were intermediate. Thus, *A. schottii* experiences inbreeding depression and a trend toward outbreeding depression. Genetic analyses showed a similar pattern: individuals 1 m apart had on average higher genetic similarity (proportion of bands shared) than individuals separated by greater distances, with a trend toward lower genetic similarity for plants located 2500 m distant. The observed spatial genetic patterns are likely maintained by the combined effects of clonal reproduction, clone longevity, limited seed dispersal and the substantial number of inbred progeny produced, counteracting distant allele transfer which tends to reduce population genetic structure. The correspondence between our ecological and genetic results indicates that RAPD markers are useful tools for assessing ecological phenomena.

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Introduction

When both inbreeding and outbreeding depression occur within a population, there is likely to be an intermediate distance at which two mating plants experience an optimal degree of outbreeding (Price and Waser 1979; Waser 1993). Inbreeding depression can be defined as a reduction in offspring fitness due to increased homozygosity from matings between relatives. There are two theoretical models to explain the genetic basis of inbreeding depression: the expression of deleterious recessive alleles (Grant 1975; Charlesworth and Charlesworth 1987) and heterozygote advantage at viability loci (Charlesworth and Charlesworth 1987; Ziehe and Roberds 1989; Agren and Schemske 1993). Because gene flow through pollination and seed dispersal is generally limited and leptokurtic in natural populations (Levin and Kerster 1974; Waser 1982), genetically similar plants are commonly found growing in close spatial proximity (Turner et al. 1982). Evidence that breeding between plants growing near each other can result in lowered fitness has been reported for a variety of species (Dobzhansky 1970; Koptur 1984; Levin 1984; Schemske and Paulter 1984; Redmond et al. 1989; Fenster 1991). At the other extreme, outbreeding depression can occur when the two parents are adapted to different enough environments that their offspring experience reduced fitness in either of the parental environments (Allard et al. 1972; Endler 1977). There is evidence that limited gene flow and environmental variation can lead to genetic differentiation within a single plant population (McNeilly and Antonovics 1968; Solbrig and Simpson 1974, 1977; Waser 1987). It has also been found, in some cases, that the offspring produced by matings over long distances suffer decreased fitness (Banyard and James 1979; Ritland and Ganders 1987; Dudash 1990; Fenster 1991; Waser 1993;

Waser and Price 1995), due perhaps to the breakup of coadapted gene complexes or favorable epistatic relationships (Mayr 1963; Shields 1982; Lynch 1991). It is generally accepted that inbreeding is important in maintaining genetic structure (Allard 1975; Loveless and Hamrick 1984; Ritland and Ganders 1985; Richards 1986), although it is not a universal relationship (Linhart et al. 1981; Carthew 1993). In a similar way, clonal growth may preserve genotypes that are well-suited to their local environment, thus producing or maintaining genetic structure. In contrast, outcrossing systems with extensive gene flow will serve to homogenize the genetic composition of a population, thus reducing genetic structure. The relative influence of these various factors is of interest to questions of ecological and evolutionary importance.

Investigators have theorized that the phenomenon of "optimal outcrossing" is due to an inverse correlation between genetic similarity and physical proximity (Price and Waser 1979; Waser and Price 1989). Although evidence for optimal outcrossing has been found for a number of plant species (Price and Waser 1979; Waser and Price 1983; Schemske and Paulter 1984; McCall et al. 1988; Sobrevila 1988; Waser and Price 1989), only a single study has attempted to directly assess this basic underlying assumption to date. Using five polymorphic enzyme systems, Waser (1987) studied spatial genetic variation within a population of the montane wildflower *Delphinium nelsonii*. Results of his study failed to show the organized spatial genetic structure hypothesized. He concluded that "unless electrophoretic loci happen to be involved in local adaptation or are closely physically linked to selected loci, they will not exhibit clines". Thus, allozyme markers cannot be assumed to provide evidence of spatial genetic structure on such a small scale, particularly when limited in number.

Here we combine field experiments designed to test the theory of optimal outcrossing in *Agave schottii* with molecular genetic studies (using RAPDs; random amplified polymorphic DNAs) to directly test the underlying genetic assumptions. RAPDs are based on the amplification of unknown DNA sequences using single, short, random oligonucleotide primers (Williams et al. 1990; Hadrys et al. 1992). Overall, these markers should provide better estimates of genetic variation (or genetic "relatedness") than isozyme markers, covering a comparatively larger proportion of the total genome. In this study we specifically address the following questions: (1) is there an optimal outcrossing distance in *A. schottii* ?; (2) is there evidence of a negative correlation between genetic similarity and physical distance in this population of *A. schottii* ?; (3) are genetic results consistent with observed fitness measures?

Materials and methods

Study sites and organisms

Field studies were conducted along the Catalina Highway (mile 7.5) on Mt. Lemmon, near Tucson, Arizona. The site is hilly terrain in the Upper Sonoran region (McMahon 1988) at 1220 m elevation and is a Sonoran chaparral of manzanita (*Arctostaphylos* cf.*pringlei*), bear grass (*Nolina microcarpa*), oaks (*Quercus* spp.) and *A. schottii*. All *A. schottii* flowering stalks were counted in 5 m plots around each of the study plants; the mean density in these plots was 0.097 stalks/m^2 (range: 0.025–0.242 stalks/m^2).

A. schottii exhibits a typical "century plant" life cycle – after growing vegetatively for up to two decades, each rosette produces one large inflorescence, reproduces once, and then dies. During the vegetative stage, a rosette can produce an unknown number of daughter rosettes through rhizomes (Gentry 1982); thus the genet may survive indefinitely. In its reproductive year, a ramet produces a single spike inflorescence in the early summer that can reach 3 m in height and can produce 20–100 flowers (A.Trame, personal observation). The protandrous flowers reach anthesis sequentially, starting at the lower end of the inflorescence. The flowers are yellow, sweet-smelling, and open in the evening near sundown. The anthers also dehisce at this time, although both pollen and nectar are available the following morning before the stamens wilt. Stigmas become receptive during the second and third nights. Individuals in this population bloomed from mid-June through mid-July and population synchrony was quite high.

Potential pollinators included honeybees, bumblebees, hummingbirds, and sphinx moths. Effective pollination appears to occur at night (50% reduction in fruit set and a 50–65% reduction in seed set occurred during the day as compared to night-only pollinations; A. Trame, unpublished data); combined with the aforementioned flowering and anther dehiscence times, this suggests that the white-lined sphinx moth (*Hyles lineata*) is the effective pollinator on our study site. The moths were abundant and predictable (becoming active at dusk) and appeared to contact both anthers and stigmas with their movements (A. Trame, personal observation). Anecdotal observations suggest that flower-to-flower movements are commonly on the scale of a few meters (these observations are also consistent with those of Koptur 1984).

The fruits of *A. schottii* dehisce when mature, scattering tens to hundreds of seeds around the vicinity of the plant when wind or birds move the flower stalk. Although there is potential for secondary seed dispersal, recent experimental results suggest that no more than 15% of seeds are dispersed and/or eaten by seed predators (A. Trame, unpublished data).

Optimal outcrossing

To assess whether or not there is an optimal outcrossing distance in *A. schottii*, 48 reproductive ramets were randomly selected from the study population (within an area roughly 150 m × 400 m) to serve as "focal females". In June 1991, each of these was hand-pollinated with pollen from four distances: 1, 10, 100, and 2500 m. The pollen from 2500 m came from a second population of *A. schottii* located at a slightly lower elevation and on steeper terrain. This second population was located in a community dominated by bear grass, oaks, and sotols (*Dasylirion wheeleri*). Pollen from each distance was applied to a given plant on a single day, but due to the sequential flowering of the inflorescence, it took 4–8 days to complete all four pollen distance treatments on any given focal plant.

The focal flower stalks were systematically divided into four "sections", and received pollen from a single distance treatment on between one and five receptive flowers in each section. (Hereafter we refer to each section containing one distance treatment on one focal female as a "treatment unit"). The assignment of treatments to the four sections was made with a Latin square design, with each treatment appearing in each section on 12 different plants (Table 1). Each hand-pollinated flower was emasculated before the stigma became receptive; pollen was transferred directly from donor flower anthers onto receptive stigmas until the stigmas were saturated. Hand-pollinations were carried out between 4:30 and 10:00 a.m. and between 8:00 and 10:00 p.m., although each flower was pollinated only once. The flowers were protected throughout the experiment with handmade bags sewn from nylon mesh (wed-

Table 1 The Latin square experimental design. Each flower stalk was divided into four linear sections (A-D). One of the four distance treatments was applied to each section as shown, for a total of 48 flower stalks

Sections focal female	Pollen donor distances			
A	1	10	100	2500
B	10	100	2500	1
C	100	2500	1	10
D	2500	1	10	100
	N=12	N=12	N=12	N=12
	N (focal females)=48			

ding veil). This prevented uncontrolled pollination and retained seeds for collection after fruit dehiscence. Fruits from 41 of the 48 focal plants were collected at the end of September 1991. However, in some cases, fewer than four treatment units per flower stalk were retrieved. A total of 137 treatment units were collected, out of the potential 192 treatment units.

At the end of the season the following estimates of fitness were made: fruit set (percentage of mature fruits/treatment unit), absolute seed set, percentage seed set, seed mass, percentage seed germination, and a combined relative fitness estimate (see below). *A. schottii* seeds have a dark, shiny, thickened seed coat when mature. If ovules are not fertilized or aborted, the "seeds" are very thin, transparent and whitish in color. We estimated seed set as: (1) the average number of mature seeds/fruit within a treatment unit (absolute seed set); (2) the average percentage of the seeds within a fruit that were mature (percentage seed set). Seed mass is the average mass of an individual seed within a treatment unit (we weighed all seeds/treatment unit and divided by the number of seeds/treatment unit). Percentage germination was based on the number of seeds that produced seedlings out of the number that were planted per treatment unit. To assess germination success (percentage seedling emergence), up to 24 seeds per treatment unit (a total of 3048 seeds) were planted in a greenhouse at the University of Illinois. Each individual seed was randomly assigned to a single cell within a flat. If an experimental unit produced fewer than 24 seeds, then all seeds were planted.

Seeds were planted approximately 0.5 cm below the surface of a 1:1:1 soil, sand and calcified clay mixture in plastic flats that contained 96 cells (3.3 cm × 4.4 cm). The flats were set onto plastic trays to allow bottom-watering. They were checked daily, and any flat that was dry at the surface was bottom-watered. Once the water had soaked the soil surface, any extra water in the tray was discarded. The seeds and seedlings were not fertilized. Night-day temperatures in the greenhouse room ranged between 18 to 24°C. Natural sunlight was supplemented with metal halide lamps to produce a 16 h daylength. Post-emergence mortality was negligible (two seedlings throughout the 30-day experiment). Combined relative fitness was calculated by multiplying fruit set, absolute seed set, and percentage germination for each treatment unit. The result is the average number of seedlings emerged per pollinated flower. The data were analyzed by a blocked ANOVA, with female identitiy as the blocking factor (Zar 1984). The analysis was done on Systat (Wilkinson 1990).

Self-compatibility

Ten "focal females" (not used in the optimal outcrossing study) were selected at random to evaluate the level of self-compatibility in *A. schottii*. One to five flowers per individual were emasculated and then pollinated with pollen from the same inflorescence to test for self-pollination. Five plants received the treatment on the lower half of their inflorescence while five received the treatments on the upper half of their inflorescence. Flowers were handled and bagged as described for the optimal outcrossing experimental plants.

Mature fruits from six of the ten focal plants were retrieved. Measures of combined relative fitness (fruit set × absolute seed set × percentage germination) of selfed treatments were compared with those of 10 m treatments via a Mann-Whitney test for unpaired samples (Statistical Graphics Corporation 1987).

Genetic analyses

Leaf tissue was collected from ten randomly selected focal female rosettes and the rosettes of their associated 1 m, 10 m, and 100 m pollen donors. All of these pollen donors were the same individuals used in the optimal outcrossing experiment. Leaf tissue was also collected from ten rosettes at 2500 m and haphazardly assigned to the ten focal females/pollen donor groups. Although the 2500 m plants are individuals that donated pollen to the outcrossing experiment, they did not necessarily pollinate the same female with which they were combined in the genetic study. This is because the 2500 m population bloomed earlier than the experimental population, so sometimes a single 2500 m pollen donor contributed pollen to more than one focal female in the outcrossing study. Nonetheless, genetic comparisons were made within ten experimental groups, totalling 50 plants. Genetic analyses were accomplished using RAPD-PCR (-polymerase chain reaction). Details of the techniques involved are described below.

Following collection, leaf tissues were placed on ice, shipped to the University of Illinois, and stored at –70° until extraction. DNA was extracted by grinding approximately 1 g of leaf tissue in liquid nitrogen with a mortar and pestle. The leaf powder was then added to a 65°C extraction buffer of 0.005 M 1,10-phenanthroline, 0.05 M Tris (pH 8), 0.02 M EDTA, 0.25 M NaCl, 1.0% w/v SDS, and 1% w/v PVP-40 with 20 µg/ml proteinase K, and incubated at 65°C for 30 min. After incubation, 2.5 ml of 5 M potassium acetate was added and the mixture was placed on ice for 30 min to remove carbohydrate and protein. Following extraction, the slurry was cooled and centrifuged to remove insoluble debris. The supernatant was then mixed with 0.65 volume of isopropanol to precipitate the DNA. The precipitation was then collected by centrifugation, dried, and then resuspended in 3 ml TE [10 mM Tris (pH 8),1 mM EDTA]. The DNA was then further purified by cesium chloride equilibrium centrifugation (Keim et al. 1989; Paige and Capman 1993).

Following extraction, each amount of DNA was quantified and diluted to a concentration of 5–25 ng/µl for PCR. Each reaction tube contained 0.5 U of Taq polymerase, 0.2 mM of each dNTP, 0.2 µM of an Operon 10-base pair primer, 1X PCR buffer, 10 mM MgCl2, 25 ng of DNA and sterile water to a final volume of 25 µl. Each reaction tube was then capped with 50 µl of mineral oil. The tubes were then placed in a thermal cycler and subjected to the following program: (1) 3 min at 94°C, (2) 1 min at 94°C to denature the DNA, (3) 1 min at 36°C to anneal the primer, (4) 3 min at 72°C for primer extension, and (5) a final extension for 3 min at 72°C (Williams et al. 1990). Steps 2–4 were repeated 44 times. The end product was run on a 1% agarose gel stained with ethidium bromide and visualized under UV light. Gels were then photographed and scored for percentage band sharing, combining data from all primers.

An average of 21 bands or "loci" (range 16–31) were scored per distance treatment. Three to six primers were used on each experimental unit. Each distance treatment within an experimental unit was compared to the focal female to assess genetic distance by counting the percentage of bands shared between the two individuals. Data were analyzed using regression analysis and one-way ANOVA (Statistical Graphics Corporation 1987).

Table 2 The effect of outcrossing distance on six fitness estimates in A.schottii Non-normal data were transformed prior to analysis

Fitness estimates		*DF*	*F*	*P*
Fruit set	Block	40	1.616	0.025
	Distance	3	0.627	0.599
Seed set,absolute	Block	40	2.603	0.000
	Distance	3	3.611	0.016*
Seed set, percentage	Block	40	2.861	0.000
	Distance	3	3.852	0.012*
Seed mass	Block	40	22.056	0.000
	Distance	3	0.384	0.765
Germination	Block	40	6.722	0.000
	Distance	3	1.061	0.369
Relative fitness	Block	40	2.775	0.000
	Distance	3	2.944	0.036*
Genetic similarity	Distance	3,34	5.21	0.0046*

* Statistically significant effect

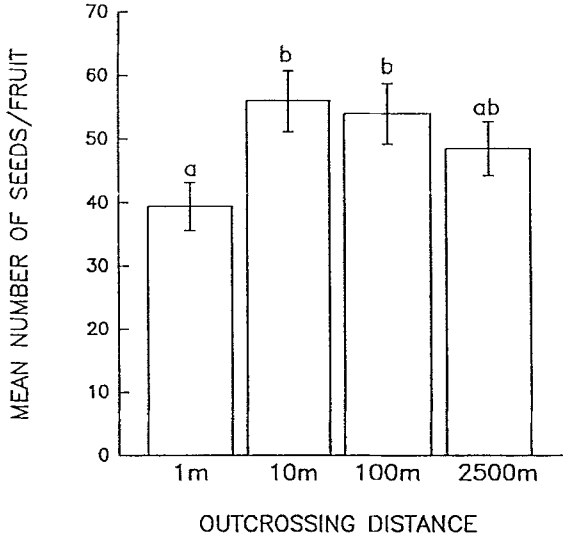

Fig. 1 Number of seeds matured per fruit (mean ±1 SE) at four outcrossing distances. Those treatments with the same letter were not significantly different from each other when compared using Tukey's test (blocked ANOVA, $P = 0.016$). Sample sizes were 33, 34, 34, and 36 treatment units for 1 m, 10 m, 100 m, and 2500 m, respectively

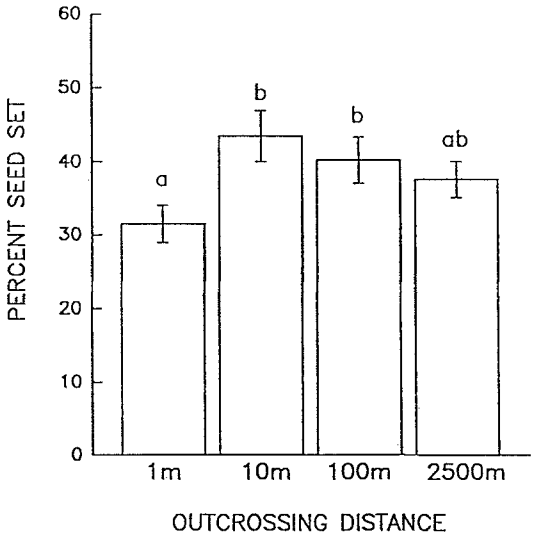

Fig. 2 Percentage mature seeds per fruit (mean ±1 SE) at four outcrossing distances. Those treatments with the same letter were not significantly different from each other when compared using Tukey's test (blocked ANOVA, $P = 0.012$). Sample sizes were 33, 34, 34, and 36 treatment units for 1 m, 10 m, 100 m, and 2500 m, respectively

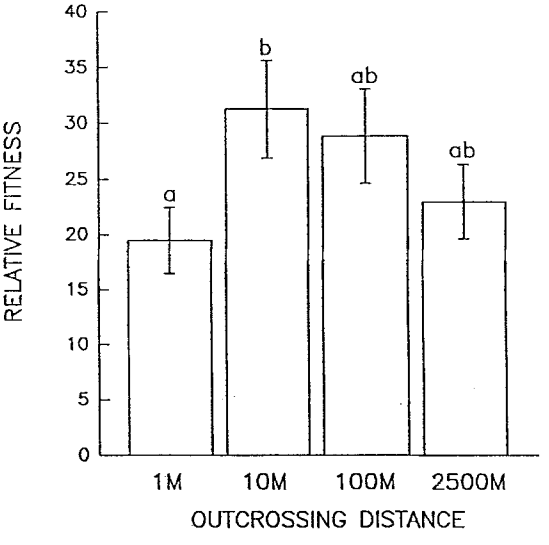

Fig. 3 Combined relative fitness (number of seedlings emerged per flower pollinated, mean ±1 SE) at four outcrossing distances. The treatments with the same letter were not significantly different from each other when compared using Tukey's test (blocked ANOVA, $P = 0.036$). Sample sizes were 40, 41, 40, and 40 treatment units each for 1 m, 10 m, 100 m, and 2500 m, respectively

Results

Optimal outcrossing

Our results do not provide conclusive evidence of an optimal outcrossing distance in *A. schottii*, but they do demonstrate that *A. schottii* suffers from inbreeding depression when crosses occur between plants at a distance of 1 m. Controlled crosses showed that absolute seed set was significantly lower for 1 m crosses than for 10 m and 100 m crosses (Table 2; Fig. 1). Absolute seed set from crosses at 2500 m was not significantly different from 1 m, 10 m or 100 m crosses (Fig. 1). Percentage seed set showed a similar pattern (Table 2; Fig. 2), suggesting that differences in seed set were not due to differences in ovule number but occurred at the fertilization stage. Fruit set, seed mass, and germination success, however, were not significantly affected by outcrossing distance (Table 2). Relative fitness (fruit set × absolute seed set × percentage germination) was significantly lower for 1 m crosses compared to 10 m crosses, while 100 m and 2500 m crosses were intermediate (Table 2; Fig. 3).

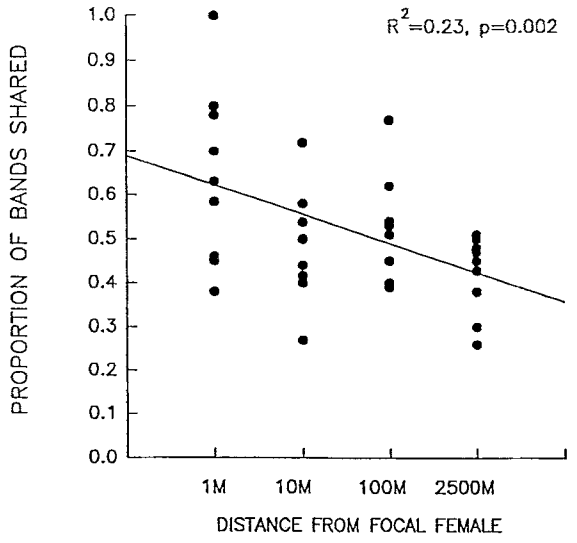

Fig. 4 Relationship between interplant distance and genetic similarity, as measured by percentage bandsharing (see text; $r^2 = -0.23, P < 0.002$)

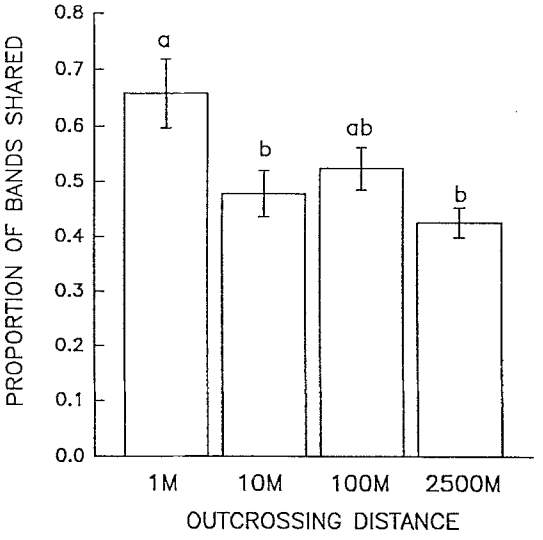

Fig. 5 Genetic similarity (bands shared, see text; mean ±SE) across four distances. Those treatments with the same letter were not significantly different from each other when compared using Tukey's test (one-way ANOVA, $P = 0.0046$). Sample sizes were 10, 9, 9, and 10 individual plants for 1 m, 10 m, 100 m, and 2500 m, respectively

Since significant inbreeding depression at 1 m outcrossing distances was found for absolute seed set and combined relative fitness, we used these data to calculate the severity of inbreeding depression as:

$$1 - \frac{(\text{fitness of 1 m crosses})}{(\text{fitness of 10 m, 100 m, 2500 m crosses averaged together})}$$

(modified after Barrett and Kohn 1991). Results showed that inbreeding depression was 25.4% for absolute seed set, and 29.7% for combined relative fitness in 1 m crosses.

Self-compatibility

Experiments conducted to assess levels of self-compatibility showed that *A. schottii* are self-compatible; self-pollinated plants did not differ significantly in combined relative fitness from plants receiving pollen from 10 m (means were 18.4 ± 6.0 versus 31.3 ± 4.4; $P = 0.47$, Mann-Whitney).

Genetic analyses

Our genetic analyses uncovered some genetic structure within the study population. Regression results showed a negative relationship between genetic similarity and physical distance ($r^2 = -0.23$, $df = 3,34$, $P = 0.002$; Fig. 4). Percentage bandsharing ranged from 65.8 ± 6.1% (mean ±1 SE) between plants at 1 m distance to 42.6 ± 2.7% between plants at 2500 m distance. Plants at 10 m and 100 m distances were of intermediate similarity, averaging 47.8 ± 4.2% and 52.4 ± 3.9% bandsharing, respectively.

Although there is a general decline in genetic similarity with distance (Fig. 4), no statistical differences in genetic similarity were found among plants located 10, 100, and 2500 m distant (Fig. 5). Plants located at a distance of 1 m, however, were significantly more similar than plants separated by 10 m and 2500 m, but not the 100 m distance treatment (Table 2; Fig. 5).

Although *A.schottii* is clonal and there is the possibility that crosses were made between ramets of the same genet over short distances, genetic analyses demonstrated that nine of ten 1 m crosses were made with genetically distinct individuals (Fig. 4; band sharing <100%). In only one case was there the possibility that we crossed two ramets of the same genet. Exclusion of this point from the analysis, however, does not alter the negative relationship between genetic similarity and physical distance ($r^2 = -0.20$, $df = 3,33$, $P < 0.005$).

Discussion

Although genetic (e.g., Schaal 1975; Linhart et al. 1981; Gregorius et al. 1986; Van Treuren et al. 1991; Carthew 1993) and ecological data (e.g., Price and Waser 1979; Levin 1984; Galen et al. 1991) have been obtained to describe the genetic structure for a number of plant populations, and many researchers have found ecological evidence for optimal outcrossing (Waser 1993), our work represents the first to test the basic underlying assumptions of "optimal outcrossing" where both fitness and genetic data have been simultaneously collected from the same individuals within a plant population. Although we

do not conclusively demonstrate an optimal outcrossing distance in *A. schottii*, which, by definition, requires both inbreeding and outbreeding depression, our genetic results are consistent with the ecological patterns observed.

Our outcrossing experiment revealed significant inbreeding depression when plants were crossed at a distance of 1 m; however, there was no significant outbreeding depression when plants were crossed at the maximum distance of 2500 m. There was a nonsignificant trend toward outbreeding depression: every measure of fitness examined was, on average, lower for crosses of 2500 m compared to 10 m and 100 m crosses.

There is the possibility that outbreeding depression is subtle and would require the use of a larger sample size (Waser and Price 1989) to avoid making a type-II error (in our case, falsely accepting the null hypothesis that there is no outbreeding depression and hence no optimal outcrossing distance). Price and Waser (1979) and Waser and Price (1983, 1989) found significant optimal outcrossing in two species, *Delphinium nelsoni* and *Ipomopsis aggregata*, but only by hand-pollinating more than 1000 individual plants (with one distance treatment/plant) over a period of several years and then pooling the results via meta-analysis (see Waser and Price 1983, 1989). Application of all distance treatments within a female (using focal female as a blocking factor) represents a more powerful design, which should allow a smaller sample size. Nonetheless, our sample sizes may still need to be bolstered. Furthermore, Barrett and Kohn (1991) and Waser and Price (1989, 1995) suggest that stronger effects may be seen in measures of offspring fitness than in seed set alone. Therefore, it is important to assess as much of the life cycle as possible, including offspring fecundity (Svensson 1988; Fenster 1991; Waser and Price 1995). In our case this would require decades of study. Overall, it would be premature to conclude that *A. schottii* does not experience outbreeding depression and, thus, has no optimal outcrossing distance, based on these points and the trends from our experiments.

The decrease in fitness for crosses over short distances could indicate that we were simply crossing two ramets of the same genet since *A. schottii* is reportedly clonal. However, our DNA analysis demonstrates that the majority of ramets crossed at 1 m were genetically distinct individuals. Thus, *A. schottii* experiences inbreeding depression due to biparental crossing over short distances. It also indicates that clonal spread in *A. schottii* is limited to distances less than 1 m, at least in the individuals examined.

Genetic results reflect the fitness patterns revealed in the outcrossing experiment. Plants crossed at distances of only 1 m exhibited significant inbreeding depression; these plants were also genetically more similar to focal females than plants from more distant outcrossing treatments. Although there was no significant outbreeding depression when plants were crossed at the maximum distance of 2500 m, there was a trend toward outbreeding depression; every fitness measure examined was, on the average, lower for crosses of 2500 m compared to 10m and 100 m crosses. In a similar fashion, there were no significant genetic differences in percentage bandsharing among plants located 10, 100, and 2500 m from focal females. There was, however, a trend toward lower genetic similarity for plants located 2500 m distant.

The production and maintenance of the observed spatial genetic patterns are dependent upon gene flow within the population through pollination and seed dispersal, maintenance of genotypes through clonal growth and longevity, and the severity of inbreeding depression. If inbreeding depression is too great, the population should evolve towards an outcrossing system, which will reduce genetic structure. In our experimental population, 1 m crosses show fitness reductions that are less than the theoretical threshold of a 50% reduction in fitness (Lloyd 1979; Lande and Schemske 1985) which should select against fertilizations over short distances. Thus, inbred progeny probably do not experience strong negative selection. Inbred individuals will, therefore, continue to make up a substantial proportion of the population, maintaining the observed genetic structure.

In *A. schottii*, clonal growth, clone longevity, and apparently limited seed dispersal may cause genetically identical and related individuals to remain in close proximity. Sexually produced selfed progeny, siblings and halfsiblings probably germinate in the area surrounding their maternal parent due to limited seed dispersal. This structure is probably maintained through time by clonal reproduction. Although each ramet probably lives for an average of 20 years, production of a daughter rosette during that time will result in the perpetuation of that genotype through time. Thus, patches of sister plants will retain the genetic structure first created by seed dispersal.

The fact that genetic similarity (percentage bandsharing) is not significantly different between plants at 10 m, 100 m, and 2500 m distances suggest that gene flow is extensive rather than restricted, and that selection regimes are not substantially different over the spatial scale studied. It is possible that pollinator movement is a source of relatively significant gene flow in this population of *A. schottii*. Although Waser (1982) found mean interplant flight distances of hawkmoths to be less than 1 m in *Ipomopsis aggregata*, pollinator behavior and interplant distances are likely to change among different species (and even different populations) of plants and their animal pollinators. For example, different flowering stalk densities could influence pollinator movements. From our own observations, hawkmoth flight distances were commonly greater than 1 m; this is consistent with Koptur (1984). However, more data on pollination biology, especially pollinator behavior, will be needed before any firm conclusions can be offered. With the data currently available, the observed spatial genetic patterns are likely maintained by the combined effects of clonal reproduction, clone longevity, limited seed dispersal, and the substantial number of inbred progeny produced. Pollen transfer may serve to reduce population genetic structure, possibly up to distances of 2500 meters.

Although the ecological factors involved in the production and maintenance of the observed patterns are as yet unclear, our RAPD-PCR data suggest an underlying genetic basis for those patterns. Our work successfully used RAPDs to assess spatial genetic structure through patterns of genetic relatedness; the results shed light on observed fitness patterns. Thus, the correspondence between our ecological and genetic results indicates that RAPD markers represent a useful tool for assessing ecological phenomena.

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