Oecologia (2001) 127:239–245
DOI 10.1007/s004420000586

Bradd Schulke · Nickolas M. Waser

Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*

Received: 28 January 2000 / Accepted: 25 October 2000 / Published online: 2 February 2001

Abstract Spatial processes in pollination biology are poorly understood, especially at levels above that of the local population. For example, little is known about how pollinators and pollen move among populations, although there is evidence that such movement can exceed what is predicted from intrapopulational movement. We explored pollination success in experimental isolates of the bumblebee- and hummingbird-pollinated wildflower *Delphinium nuttallianum*. We established a total of 15 arrays of potted plants isolated by 50–400 m from ten natural "source" populations, as well as control arrays embedded within each source. Flowers on potted plants were emasculated, so any pollen received could be assumed to come from source populations. A total of 69 h of observation suggested that pollinators were somewhat less abundant in isolates than in controls, but visited more plants and flowers once within an isolate. Consistent with this, 82.1% of all flowers in isolated arrays received pollen, versus 87.7% in controls. Mean receipt was more than 100 pollen grains per flower in most arrays, and seed set in isolates and controls respectively averaged 69.8% and 74.3% of ovules. Furthermore, pollen receipt in isolates declined relatively slowly with distance from the source. We conclude that pollinators of *D. nuttallianum* often will fly up to 400 m among populations, and that substantial pollination ensues. Thus isolated populations of this species often belong to metapopulations in terms of pollen dispersal, with important consequences for genetic differentiation, and potential implications for the management of endangered plant species.

B. Schulke · N.M. Waser (✉)
Department of Biology, University of California, Riverside, CA 92521, USA
e-mail: waser@citrus.ucr.edu
Tel.: +1-909-7873495, Fax: +1-909-7874286

B. Schulke · N.M. Waser
Rocky Mountain Biological Laboratory, Crested Butte, CO 81224, USA

Keywords Conservation · Fragmentation · Gene flow · Isolation · Metapopulation

Introduction

Ecological and evolutionary processes occur in dimensions of time and space. It is no wonder that the temporal domain served as an early focus in ecology, for example in the study of succession (Jackson 1981). The spatial domain entered only later, in part because it is so difficult to model analytically, but eventually penetrated first mathematical genetics (e.g., Wright 1943; Malécot 1948) and later ecology (e.g., Skellam 1951; Huffaker 1958; MacArthur and Pianka 1966). Spatial approaches have grown more explicit with time (Kareiva 1994), and increasingly allow us to explore the movement of individuals as they forage, search for mates, or disperse; the distance-dependent interactions of sessile organisms; and the spatial structuring of alleles and genotypes within and among populations, brought about by movements of individuals and gametes.

Pollination biology historically embraces many of these trends in spatial biology. Pollination systems have served as fruitful arenas for the development and testing of optimal foraging theory, for direct estimates of gene flow, and for comparing these measurements to indirect ones in the form of spatial genetic structure (e.g., Pyke 1984; Waser 1988; Williams and Waser 1999). Unfortunately, there are newer reasons as well for a spatial focus in pollination biology. These derive from human alteration of landscapes, which fragments ancestral habitats and isolates the fragments. Fragmentation and isolation seem likely to alter pollination services, with potentially severe consequences for the viability of plant populations (Rathcke and Jules 1993; Olesen and Jain 1994; Kearns et al. 1998; Richards et al. 1999).

In spite of a growing interest in the spatial domain among pollination biologists, we know the most about events at local scales, and much less about larger scales. Numerous workers have emphasized area-restricted for-

aging of pollinators and their predominantly-short movements between plants (e.g. Bateman 1947; Pyke 1978; Schaal 1980; Waser 1982), and the resulting prediction that gene flow will be highly restricted (e.g. Bradshaw 1972; Levin and Kerster 1974; Schaal 1974; Levin 1979). Some more recent studies, however, suggest that it can be misleading to extrapolate from within-population movements to those between populations. For example, Levin (1983) detected seed set in individuals of *Phlox drummondii* isolated by up to 100 m from the nearest pollen source. Ellstrand and Marshall (1985) reported that up to 18% of seeds within isolated populations of *Raphanus sativus* were sired by pollen from other populations 100–1,000 m distant. Godt and Hamrick (1993) concluded that 16–46% of seeds in the bumblebee-pollinated *Lathyrus latifolius* resulted from pollen movement from other populations up to 70 m distant. Using DNA markers, Dow and Ashley (1996) found that most paternity in an isolated stand of bur oak came from pollen imported from distant stands. Nason et al. (1998) used paternity assignment to infer that fig wasps routinely fly up to 14 km between trees in Panama. Results such as these led Ellstrand et al. (1989) to argue that pollen dispersal into isolates does not necessarily decline strongly with increasing distance to the nearest donating population, in contrast to pollen dispersal within continuous populations. Ellstrand and Marshall (1985) and Godt and Hamrick (1993) also emphasized how little is known about the movement of pollinators among populations.

The montane wildflower *Delphinium nuttallianum* may exemplify the pattern described above of restricted movement of pollinators and pollen within populations, coupled with relatively high levels of movement between populations. Direct estimates based on field studies of bumblebees and hummingbirds, and on the dynamics of pollen transport on the bodies of these pollinators, suggested that most genes move only a few meters within continuous populations (Pyke 1978; Price and Waser 1979; Hodges 1981; Hodges and Wolf 1981; Waser 1982). On the other hand, population genetic studies have failed to detect strong kinship structure within and among populations (Waser 1987; Williams and Waser 1999). Because sufficient connectance of populations should counteract genetic differentiation among them (Wright 1943; Green 1994), the genetic results suggest that pollen dispersal is more extensive than previously believed.

In this paper we explore pollinator movements and pollen dispersal between populations of *D. nuttallianum*. By constructing experimental isolates we were able to address two related issues. First, we explored the levels of pollinator activity in and near isolates, relative to those in large natural populations of the species. Second, we asked how much pollen is dispersed to isolates as a function of their distance from these natural populations, and what fraction of ovules is set as seeds. We conclude that isolates are surprisingly well connected by pollen and gene flow, and discuss the implications for genetic structure of plant metapopulations and for conservation of plants growing in fragmented landscapes.

Materials and methods

The study system

We worked near the Rocky Mountain Biological Laboratory (RMBL) in the Elk Mountains in west-central Colorado, at elevations of 2,700–3,100 m. During the late spring and early summer the larkspur *Delphinium nuttallianum* Pritzel (= *D. nelsonii* Greene, Ranunculaceae) flowers in open montane meadows, forming populations often separated by wide strips of riparian habitat and conifer forest, and by aspen forest containing scattered individuals of the species. Reproductive individuals usually produce 1–2 flowering stalks each 10–50 cm tall and each bearing 1–10 or more flowers. The bilaterally-symmetrical, deep blue or purple, hermaphroditic flowers are protandrous and mature from bottom to top of the stalk. Major pollinators at the RMBL are broad-tailed hummingbirds (*Selasphorus platycercus* Gmelin) and queens of several bumblebee species (*Bombus appositus* Cresson, *B. flavifrons* Cresson, *B. nevadensis* Cresson, and *B. californicus* Smith), the relative abundances of which vary across years (e.g., Bosch and Waser 2001). Hummingbirds and bumblebees contribute roughly equally to seed set (Waser and Price 1981, 1990) and exhibit similar foraging behaviors, including inter-plant flight distances (Waser 1982). Mean seed dispersal is restricted (mean =11 cm; Waser and Price 1983), as is pollen dispersal within continuous populations (mean and median <1 m, Waser 1988).

Experimental populations

During the summer of 1994 we located six natural populations *of D. nuttallianum*, and during the summer of 1995 located an additional four populations, along approximately 4 km of the East River near the RMBL. We chose large populations (estimated sizes ranging from 16,000 to 400,000 flowering individuals, covering areas of 7,000 to 83,000 m^2) that were discrete, with surrounding meadows containing few or no additional plants of the species. These ten populations are hereafter referred to as "source" populations, because they were the source of most pollen received by plants in experimental arrays at various distances away.

Near the edge of each of the ten source populations we chose an area from which to pot plants for a given replicate set of experimental arrays. Plants of average size and flower number were dug up, placed individually into 15 cm diameter flower pots, and watered. Within each replicate, 16 plants were assigned at random to construct an experimental isolate placed 50–400 m from the source population, and 16 were assigned to a paired control array embedded within the source population itself (Fig. 1). In a few cases the control array was associated with 2–3 isolates at different distances.

Each experimental array was laid out as a square with four plants on a side and 0.5 m spacing between them (Fig. 1). Holes were dug in the ground to contain each potted plant, so that pollinators encountered inflorescences at a natural height. For each control array these holes were dug at the site where plants had been potted in the source population, and additional flowering individuals were removed from this site and from a border of 0.5 m around the array. To ensure that isolates were truly isolated we searched the area around each experimental replicate, removing scattered *D. nuttallianum* individuals as needed and confirming that the nearest additional large populations of the species were at least twice as far from the isolate as was the source population.

Combining the 1994 and 1995 seasons, we constructed 12 control arrays and 15 isolates using the ten source populations (Table 1). The Willow and Snodgrass source populations were used in both years, but different individual plants were potted in each year. We established two isolated arrays 50 m and 100 m

Table 1 Reproductive success in control and isolated arrays. Isolation distance is the distance of each array from the source population; 0 m refers to control arrays embedded within source populations. One isolate in the Meadow site in 1995, indicated by *, was 150 m from our chosen source population but 50 m from another source discovered subsequently. Pollen load is the mean number of *Delphinium nuttallianum* pollen grains per flower, with SD and *n* (number of flowers). Non-zero flowers is the proportion of all flowers in an array that received some pollen. Seed set is the mean proportion of all ovules set as seeds; *N/A* means that no values were obtained because fruits did not expand (see text)

Plot	Year	Isolation distance	Pollen load (SD, *n*)	Non-zero flowers	Seed set (SD, *n*)
Big Falls	1994	0 m	284 (281, 77)	0.909	0.904 (0.135, 8)
		50 m	219 (252, 93)	0.892	0.775 (0.192, 5)
		100 m	129 (201, 82)	0.841	0.614 (0.163, 11)
Gothic	1994	0 m	304 (238, 79)	0.924	0.769 (0.205, 45)
		200 m	106 (133, 93)	0.828	0.363 (0.309, 55)
Meadow	1994	0 m	380 (310, 61)	0.918	N/A
		50 m	251 (292, 36)	0.917	0.758 (0.303, 11)
		100 m	226 (210, 30)	0.967	0.654 (0.194, 7)
		50 m*	281 (245, 52)	0.962	0.779 (0.140, 10)
Snodgrass	1994	0 m	394 (418, 65)	0.892	0.703 (0.174, 15)
		400 m	105 (173, 53)	0.925	0.620 (0.286, 4)
Swamp	1994	0 m	308 (254, 85)	0.965	0.704 (0.233, 31)
		50 m	107 (123, 71)	0.901	0.639 (0.167, 8)
Willow	1994	0 m	259 (288, 69)	0.913	0.702 (0.214, 22)
		50 m	170 (177, 69)	0.957	0.687 (0.148, 17)
Avalanche	1995	0 m	322 (265, 71)	0.887	0.771 (0.209, 13)
		200 m	101 (15,65)	0.769	0.786 (0.221, 17)
Bog	1995	0 m	225 (277, 123)	0.821	0.960 (0.208, 12)
		50 m	137 (192, 79)	0.709	0.764 (0.161, 9)
Rosy Point	1995	0 m	384 (266, 83)	0.928	0.785 (0.258, 16)
		100 m	174 (179, 91)	0.824	0.768 (0.255, 18)
Snodgrass	1995	0 m	231 (236, 74)	0.743	0.809 (0.157, 21)
		400 m	120 (185, 75)	0.693	0.737 (0.238, 8)
Vera Falls	1995	0 m	79 (113, 43)	0.674	0.343 (0.292, 2)
		100 m	4 (6, 48)	0.563	N/A
Willow	1995	0 m	305 (241, 61)	0.951	0.727 (0.137, 4)
		200 m	28 (62, 74)	0.568	0.832 (0.080, 7)
Control mean			289.6	0.877	0.743
Isolate mean			143.9	0.821	0.698

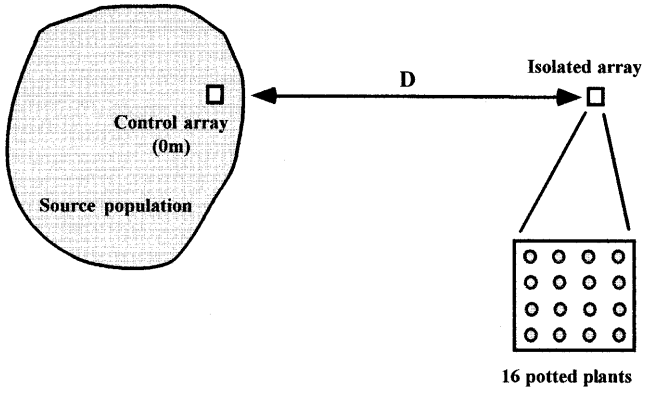

Fig. 1 Diagram of an experimental replicate. Embedded within the gray pollen source population is a "0 m" control array of 16 potted plants with emasculated flowers; an equivalent isolated array is placed a distance *D* from the source. Values of *D* were 50 m, 100 m, 200 m, or 400 m, depending on the replicate

away from the Big Falls population in 1994, and three isolates, one each at 50 m, 100 m, and 150 m away from the Meadow population in 1994. We subsequently discovered that the 150 m isolate in this latter case was within 50 m of a previously-undetected natural population of *D. nuttallianum*, and so this population was treated as a 50 m isolate in some analyses (labelled ''50 m*'' in Table 1) and was dropped from other analyses, as explained below. Overall, therefore, we obtained data from 14–15 isolates, depending on the analysis.

To ensure that all pollen received by potted plants came from outside their array, we removed initiated fruits and receptive female-phase flowers at the time of potting, and emasculated all male-phase flowers and large flower buds. Approximately every 2 days thereafter we returned to emasculate any new buds. We also watered all potted plants as needed, always treating isolated and control arrays in identical fashion. Emasculation and watering were continued until the last female flower had opened in any array within an experimental replicate.

Measures of pollination success

In 1995 only, we observed pollinators during a series of 1-h periods. When possible we deployed a pair of observers to simultaneously watch a control array and its paired isolate. In other cases a single observer rotated observations across arrays in random order and immediate succession. Observations were made at various times of day ranging from dawn to dusk. We noted the species, sex and caste of any visitor to flowers within the array, as well as the time of visit, number of plants visited within the array, and number of flowers visited on each plant. We also recorded "flybys" of pollinators that visited flowers near but not in our arrays, or that flew past them.

After the end of the 1994 and 1995 flowering seasons we checked experimental plants at regular intervals. When fruits were mature we collected them individually, removed the style and attached stigma from each fruit, and transferred these to microscope slides. Stigmas were heated with basic fuchsin jelly (Kearns and Inouye 1993) and squashed under a cover slip, after which we counted the stained pollen at 100× under a microscope. We dissected each fruit to determine the number of filled seeds and of undeveloped ovules.

Analyses

Analyses were carried out using JMP (Version 3) and Microsoft Excel. The experimental design yielded 14–15 paired comparisons for most response variables, and we therefore analyzed most variables with paired *t*-tests after confirming normality and homoscedasticity of residuals. Proportional data were arcsin-square root transformed before analysis. To assess how distance from the nearest pollen source affected pollen receipt we derived a "pollen loss" statistic for each isolated array and its paired control array:

Pollen loss = (Mean pollen load per flower in control)-(Mean pollen load per flower in isolate)

Pollen loss was then related to isolation distance using linear and power functions.

Results

Pollinator activity

Bumblebee and hummingbird pollinators of *D. nuttallianum* were active in or near all of our experimental arrays in 1995. The total number of encounters with pollinators, including flybys ("Pollinator activity" in Table 2), was higher in or near control arrays at four of the six sites used in 1995, and higher at or near isolates in the other two sites. In contrast, pollinator species richness was somewhat higher around the isolates in four of six sites (Table 2), and richness across replicates averaged 5.8 and 5.7 in the vicinity of isolates and control arrays, respectively (P=0.45, paired *t*-test). Over all sites, isolates and their surroundings averaged 9.4 episodes of pollinator activity per hour, whereas control arrays and their surroundings averaged 13.3; however the variance was large and this difference is not significant statistically (P=0.13, paired *t*-test).

In spite of this pollinator activity, 69 total hours of observation were rewarded with only 25 visits to arrays themselves, 16 foraging bouts in isolates and 9 in controls. Pollinators in these bouts visited on average 4.0 and 2.7 plants respectively in isolates and control arrays (P=0.14, 2-sample *t*-test), and 8.3 and 3.2 flowers per bout respectively (P=0.045, 2-sample *t*-test). Once they entered isolated arrays, therefore, pollinators seemed likely to visit more plants, and especially more flowers, than they did in control arrays.

Pollination in arrays

All experimental arrays yielded reasonable samples of fruits (range 30–123) from which to assess pollen delivery and seed set. Based on these samples, the large majority of flowers in all arrays received *D. nuttallianum* pollen (Table 1). Over all isolates, 82.1% of flowers received pollen, versus 87.7% in control arrays (P=0.025, paired *t*-test). Twenty-four of the 27 arrays received mean pollen loads per flower an order of magnitude greater than the number of ovules per fruit, a surplus that should suffice for fertilization of most ovules (e.g., Waser and Price 1991; Bosch and Waser 1999). Only the 1995 Willow 200 m isolate, and the 1995 Vera Falls control and 100 m isolate, had mean pollen loads less than 100 grains (Table 1). However, the variation was substantial; ranges were 79–394 grains for control arrays (n=12), 107–281 for 50 m isolates (n=6), 4–226 for 100 m isolates (n=4), 28–106 for 200 m isolates (n=3), and 105–120 for 400 m isolates (n=2).

In accordance with these pollen loads, seed set was high in most arrays (Table 1). Seed set, expressed as the

Table 2 Pollinator activity and foraging in 1995 experimental arrays. Pollinator activity is the mean number of observations per hour of pollinators in or near each array, including flybys. Codes for pollinator identity are 1= *Bombus appositus* queen, 1b= *B. appositus* worker, 2= *B. flavifrons* queen, 2b= *B. flavifrons* worker, 3= *B. californicus* queen, 4= *B. nevadensis* queen, 4b= *B. nevadensis* worker, 5= cuckoo bumblebee (*Psythirus* sp.), 6= *Selasphorus platycercus* male, 6b= *S. platycercus* female, 7= *S. rufus* male, 8= *Stellula calliope* male. Array foraging is the mean number of plants and flowers visited in experimental arrays per hour, with *n* (number of times a pollinator was seen foraging in a plot) indicated; values of zero mean that no pollination of array plants was observed

Plot	Isolation distance	Pollinator activity	Pollinator identity	plants, flowers (*n*) Array foraging: mean,
Avalanche	0 m	8.25	1,2,4,4b,5,6	2.00, 4.00 (2)
	200 m	7.75	1,1b,2,5,6,6b,7	0
Bog	0 m	24.75	1,1b,2,4,5,6,6b,7,8	1.00, 6.00 (1)
	50 m	19.75	1,2,5,6,6b,7	1.00, 1.00 (1)
Rosy Point	0 m	5.13	1,1b,2,3,5,6,6b,7	2.75, 4.25 (4)
	100 m	6.60	2,5,6,7	3.67, 11.17 (6)
Snodgrass	0 m	27.30	1,4,6,6b	3.00, 7.00 (1)
	400 m	8.82	1,3,4,5,6,6b,7	4.33, 8.00 (3)
Vera Falls	0 m	2.50	1,2,5,6	0
	100 m	4.00	1,1b,2b,6,7	5.00, 15.00 (1)
Willow	0 m	12.00	1,6,6b	1.00, 2.00 (1)
	200 m	9.25	1,1b,4,6,6b,7	2.00, 4.60 (5)
Pooled means	Controls	13.32	5.67	2.4, 3.60 (10)
	Isolates	9.36	5.83	3.0, 7.76 (16)

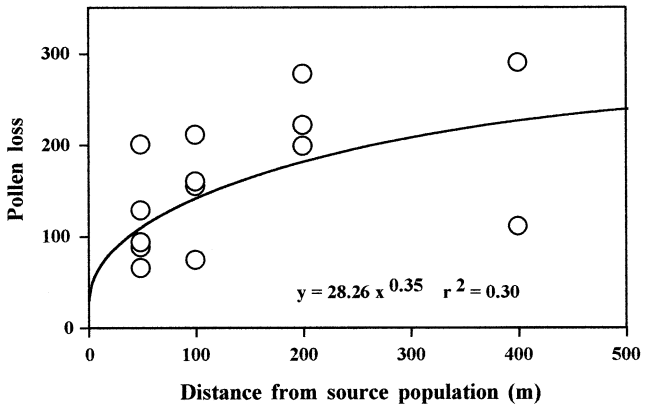

Fig. 2 Pollen loss as a function of isolation distance. The second 1994 Meadow 50 m isolate, which was 50 m distant from a *Delphinium nuttallianum* population we had initially overlooked, lacked a proper control in this unintended source population, and so was omitted from analysis

proportion of all ovules filled, averaged 69.8% for isolates and 74.3% for control arrays (P=0.019, paired t-test). These values do not include the 1994 Meadow control and 1995 Vera Falls 100 m isolated arrays because fruits never expanded, perhaps due to dry soil conditions (both of these plots had south-facing exposures and appeared to be very dry).

There was no clear relationship between pollinator species richness and mean pollen load per array (pollen load =3.3× pollinator richness +138.7, P=0.49). Nor did we detect a slope significantly greater than zero for the relationship between pollinator activity and mean pollen load (pollen load =8.4× pollinator activity +127.5, P=0.69).

Pollen loss as a function of isolation

Pollen loss was greatest over the first 50 m, and then increased more slowly out to 400 m (Fig. 2). A power function yielded a higher model r^2 (0.30) than a linear fit constrained through the origin (0.23). The best-fit model is pollen loss =28.26× isolation in $m^{0.35}$ (P<0.05 for the overall fit). The upper confidence limit on the exponent is 0.68, indicating that the exponent is significantly less than one and the function is decelerating.

Discussion

Kunin (1997) stressed that different facets of plant rarity, including small population size, low density, small size of individuals, and increased intermixing with other plant species, may affect pollination services in distinct ways and so have different consequences for conservation. Another aspect of plant rarity, which Kunin (1997) did not discuss, is isolation of populations. Plant species occur as series of isolated to semi-isolated populations regardless of overall commonness or rarity, but naturally-rare species, and those made rare by anthropogenic habitat fragmentation, will often experience greater isolation than their common or undisturbed counterparts (Wilcove et al. 1986). Isolation is likely to have marked effects on the movement of foraging pollinators, and sufficient isolation may cause a loss of pollination service altogether, one form of an "Allee" effect (Lamont et al. 1993; Groom 1998; and see May 1981 for an isocline analysis).

What is the empirical evidence? Bowers (1985) found that marked *Bombus flavifrons* workers did not move among meadows isolated from one another by 400–1,100 m of forest, whereas queens did disperse over these distances. Sih and Baltus (1987) reported that patches of catnip isolated by 15–30 m or more from other populations suffered reduced visitation by bumblebees and honeybees, but not by solitary bees. Powell and Powell (1987) used scent baits to attract euglossine bees to fragments of tropical forest, and found that some species will not move to isolates as little as 100 m from intact forest. Jennersten (1988) compared pollination in "mainland" populations of *Dianthus deltoides* to that in two "island" populations separated by ca. 200 m of agricultural fields. Visits by insects, including butterflies and flies, declined by more than 50% in the islands. Kwak et al (1991a, b) found that marked bumblebees would not move between populations of an endangered Dutch plant separated by 150 m, and inferred that such movement would be promoted by the presence of intervening flowers of other species. Groom (1998) found that pollen receipt per stigma and seed set declined precipitously in tiny populations (≤10 plants) of *Clarkia concinna* that were more than 10 m from larger populations, and in medium-sized populations (10–50 plants) that were isolated by more than 100 m, but did not decline in large populations (>50 plants) isolated by up to 1,000 m; she did not report the relative contributions by specialized beefly pollinators versus generalized honeybees, bumblebees and butterflies. Steffan-Dewenter and Tscharntke (1999) performed an experiment similar to ours using mustard and radish and found that diversity and abundance of bees declined with distance from a source population, although abundance of beetles and flies did not. Finally, Richards et al. (1999) studied pollen dispersal and paternity in an experimental metapopulation of *Silene alba*, and inferred that the diverse insect pollinators move only about 15% of pollen beyond 40 m. From this review we conclude that the distance of movement between populations will depend on identity of the pollinator (and probably on how specialized it is for the plant in question), on features of the populations, and on the presence and identity of flowers of other species. It also seems apparent that there are few extant studies on effects of isolation, and only a subset of these consider pollinator decline in terms of behavioral mechanisms, from which general patterns might ultimately be understood.

It appears that the propensity of pollinators to move to isolates of *D. nuttallianum* was higher than in most of the studies cited above. In particular, bumblebees appear

to have moved farther between our populations than observed by Bowers, Sih and Baltus, Kwak and colleagues, Groom, or Richards et al. (although bumblebees moved as far as 1,000 m in the experiment of Steffan-Dewenter and Tscharntke; I. Steffan-Dewenter, personal communication). Indeed, we directly observed long-distance movements in a few cases. For example, one of us (B.M.S.) carried out a small mark-recapture experiment with bumblebees at the RMBL in spring 1993 and obtained recaptures at distances of 50 m, 100 m, and 300 m. The bumblebees in our study were queens, which may move over long distances, as suggested by the observations of Bowers (1985), whereas in most other studies cited above the bumblebees were workers. Furthermore, our meadows contained few other flowers during the experiments (personal observations). Thus queen bumblebees and hummingbirds, which are relative generalists in flower visitation, may have tended to fly to isolates because there was little scope for switching to other species in the proximity of source populations.

The movement of pollinators to experimental isolates of *D. nuttallianum* implies that natural populations of this species, which commonly are separated by distances of tens to a few hundreds of meters, often will act as metapopulations in terms of pollen exchange. This genetic connectance of populations should be a powerful influence in preventing their differentiation at gene loci which are neutral in their contributions to individual fitness (e.g., Wright 1943; Malécot 1948; Green 1994). Furthermore, the movement of pollinators into populations bringing pollen from distant sources may tend to disrupt any intrapopulational genetic structure. In agreement with this, Williams and Waser (1999) failed to detect substantial genetic differentiation in and among populations of *D. nuttallianum* near the RMBL on a hierarchy of scales from several centimeters up to several kilometers. If pollinator movements in other systems resemble those in *D. nuttallianum*, the common expectation of small-scale genetic differentiation within and among populations (e.g. Levin and Kerster 1974; Schaal 1974) will need to be re-examined.

Finally, our results potentially bear on issues of the management of endangered plant species, which increasingly achieve this status through anthropogenic fragmentation of habitats. Fragmentation increases the isolation of populations, each of which suffers reduction in size and genetic diversity (Rathcke and Jules 1993; Olesen and Jain 1994; Richards 2000). As Kwak (1991a, b) and other have stressed, it is critical to study the movement of pollinators in such situations, rather than assuming how they will behave. The few available studies of pollinator movement to isolates cited above indicate a variable outcome, but it seems certain that pollinators sometimes will bring pollen to relatively distant isolates if conditions are correct, thus providing infusions of new genetic variability (Richards 2000); and that once within isolates they may provide substantial pollination services (see also Sork et al. 1999). More such studies are needed as we increasingly face the task of devising management strategies to enhance the movement of pollinators, pollen, and genes into populations threatened with extinction.

Acknowledgements We thank Norm Ellstrand, Julie McRobert Hardeman, Len Nunney, Mary Price, and Jacqueline Smith for advice and assistance. This work was supported by the National Science Foundation; the Newell Fund of the Department of Biology, University of California Riverside; the Snyder Fund of the Rocky Mountain Biological Laboratory; and the University of California, Riverside Academic Senate.

References

Bateman AJ (1947) Contamination of seed crops I. insect pollination. J Genet 48:257–275

Bosch M, Waser NM (1999) Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum*. Am J Bot 86:871–879

Bosch M, Waser NM (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. Oecologia 126:76–83

Bowers MA (1985) Bumble bee colonization, extinction, and reproduction in subalpine meadows in northeastern Utah. Ecology 66:914–927

Bradshaw AD (1972) Some of the evolutionary consequences of being a plant. Evol Biol 5:25–47

Dow BD, Ashley MV (1996) Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. Mol Ecol 5:615–627

Ellstrand NC, Marshall DL (1985) Interpopulation gene flow by pollen in wild radish, *Raphanus sativus*. Am Nat 126 606–616

Ellstrand NC, Devlin B, Marshall DL (1989) Gene flow by pollen into small populations: data from experimental and natural stands of wild radish. Proc Natl Acad Sci USA 86:9044–9047

Godt MW, Hamrick JL (1993) Patterns and levels of pollen-mediated gene flow in *Lathyrus latifolius*. Evolution 47:98–110

Green DG (1994) Connectivity and complexity in landscapes and ecosystems. Pac Conserv Biol 3:194–200

Groom MJ (1998) Allee effects limit population viability of an annual plant. Am Nat 151:487–496

Hodges CM (1981) Optimal foraging in bumblebees: hunting by expectation. Anim Behav 29:1166–1171

Hodges CM, Wolf LL (1981) Optimal foraging in bumblebees: why is nectar left behind in flowers? Behav Ecol Sociobiol 9:41–44

Huffaker CB (1958) Experimental studies on predation: dispersion factors and predator-prey oscillations. Hilgardia 27:343–383

Jackson JBC (1981) Interspecific competition and species' distributions: the ghosts of theories and data past. Am Zool 21: 889–901

Jennersten O (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conserv Biol 2:359–366

Kareiva P (1994) Space: the final frontier for ecological theory. Ecology 75:1

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. Annu Rev Ecol Syst 29:83–112

Kunin WE (1997) Population biology and rarity: on the complexity of density dependence in insect-plant interactions. In: Kunin WE, Gaston KJ (eds) The biology of rarity. Chapman & Hall, London, pp 150–173

Kwak MM, Kremer P, Boerrichter E, van den Brand C (1991a) Pollination of the rare species *Phyteuma nigrum* (Campanulaceae): flight distances of bumblebees. Proc Exp Appl Entomol NEV Amst 2:131–136

Kwak MM, van den Brand C, Kremer P, Boerrichter E (1991b) Visitation, flight distances and seed set in populations of the

rare species *Phyteuma nigrum* (Campanulaceae). Acta Hortic 288:303–307
Lamont BB, Klinkhamer PGL, Witkowski ETF (1993) Population fragmentation may reduce fertility to zero in *Banksia goodii* – a demonstration of the Allee effect. Oecologia 94:446–450
Levin DA (1979) The nature of plant species. Science 204:381–384
Levin DA (1983) Plant parentage: an alternate view of the breeding structure of populations. In: King CE, Dawson PS (eds) Population biology: retrospect and prospect. Columbia University Press, New York, pp 171–188
Levin DA, Kerster HW (1974) Gene flow in seed plants. Evol Biol 7:139–220
MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. Am Nat 100:603–609
Malécot G (1948) Les mathématiques de l'hérédité. Masson, Paris
May RM (1981) Models for two interacting populations. In: May RM (ed) Theoretical ecology, second edition. Sinauer, Sunderland, Mass., pp 78–104
Nason JD, Herre EA, Hamrick JL (1998) The breeding structure of a tropical keystone plant resource. Nature 391:685–687
Olesen JM, Jain SK (1994) Fragmented plant populations and their lost interactions. In: Loeschcke V, Tomiuk J, Jain SK (eds) Conservation genetics. Birkhäuser, Basel, pp 417–426
Powell AH, Powell GVN (1987) Population dynamics of male euglossine bees in Amazonian forest fragments. Biotropica 19:176–179
Price MV, Waser NM (1979) Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*, a montane wildflower. Nature 277:294–297
Pyke GH (1978) Optimal foraging: movement patterns of bumblebees between inflorescences. Theor Popul Biol 13:72–98
Pyke GH (1984) Optimal foraging theory: a critical review. Annu Rev Ecol Syst 15:523–575
Rathke BJ, Jules ES (1993) Habitat fragmentation and plant-pollinator interactions. Curr Sci 65:273–277
Richards CM (2000) Genetic rescue in *Silene alba*. Am Nat 155:383–394
Richards CM, Church S, McCauley DE (1999) The influence of population size and isolation on gene flow by pollen in *Silene alba*. Evolution 53:63–73
Schaal BA (1974) Isolation by distance in *Liatris cylindraceae*. Nature 252:703
Schaal BA (1980) Measurement of gene flow in *Lupinus texensis*. Nature 284:450–451
Sih A, Baltus M-S (1987) Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68:1679–1690
Skellam JC (1951) Random dispersal in theoretical populations. Biometrika 38:196–218
Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. Trends Ecol Evol 14:219–224
Steffan-Dewenter I, Tscharntke T (1999) Effects of habitat isolation on pollinator communities and seed set. Oecologia 121: 432–440
Waser NM (1982) A comparison of distances flown by different pollinators to flowers of the same species. Oecologia 55: 251–257
Waser NM (1987) Spatial genetic heterogeneity in a population of the montane perennial plant *Delphinium nelsonii*. Heredity 58:249–256
Waser NM (1988) Comparative pollen and dye transfer by natural pollinators of *Delphinium nelsonii*. Funct Ecol 2:41–48
Waser NM, Price MV (1981) Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. Evolution 35:376–390
Waser NM, Price MV (1983) Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 341–359
Waser NM, Price MV (1990) Pollination efficiency and effectiveness of bumble bees and hummingbirds visiting *Delphinium nelsonii*. Collect Bot (Barcelona) 19:9–20
Waser NM, Price MV (1991) Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set. Ecology 72:171–179
Wilcove DS, McLellan CS, Dobson AP (1986) Habitat fragmentation in the temperate zone. In: Soulé ME (ed) Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, Mass., pp 237–256
Williams CF, Waser NM (1999) Spatial genetic structure of *Delphinium nuttallianum* populations: inferences about gene flow. Heredity 83:541–550
Wright S (1943) An analysis of local variability of flower color in *Linanthus parryae*. Genetics 28:139–156