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DISPERSAL OF *ERYTHRONIUM GRANDIFLORUM* POLLEN BY BUMBLEBEES: IMPLICATIONS FOR GENE FLOW AND REPRODUCTIVE SUCCESS

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Sexual reproduction in higher plants typically depends on the delivery of pollen to stigmas by external agents. For complete understanding of this phase of the reproductive process, one needs to document the patterns of pollen transport and delivery, the ecological forces that influence those patterns, and the evolutionary consequences of changes in the patterns. A basic but elusive first step is to specify the "pollen shadow" of a plant, i.e., to map the stigmatic destinations of all of its pollen grains that can participate in reproduction. It is, however, hard to mark all the grains of a donor so that they can be identified later (reviewed by Waser and Price [1982], Handel [1983], and Thomson et al. [1986]). Furthermore, the number of potential recipient stigmas in a population may be far too great for thorough screening to be practical.

In the work described here, on *Erythronium grandiflorum* Pursh (Liliaceae), we use a pollen color polymorphism (i.e., naturally marked pollen), and instead of attempting to map an entire pollen shadow, we restrict our attention to the contribution of one pollinator visit. By examining only those flowers visited by one bee that has picked up the distinctive pollen, instead of all the flowers within a corresponding radius, the screening effort can be reduced by several hundredfold. We will also demonstrate that a single flower visit in this species removes most of the available pollen and, therefore, is responsible for a large part of the pollen shadow. The contribution of later visits can be estimated from extrapolation.

By measuring pollen shadows and using the measurements to specify important functional relationships, such as the relation between the amount of pollen presented to a pollinator and the amount subsequently delivered to stigmas, we hope to show how the ecological mechanics of pollination determines variation in male reproductive success. This is the primary goal of the paper. Secondarily, pollen shadows also provide more accurate estimates of pollen dispersal and potential gene flow than are available from pollinator flight data alone (reviewed by Levin [1981]). Following recent tradition, we express this aspect of our data in terms of Wright's (1943, 1946, 1978) genetic neighborhood concept (see also Crawford [1984*a*, 1984*b*]).

Materials and Methods

The use of pollen color variants to measure pollen carryover and deposition in *Erythronium* has been described elsewhere (Thomson and Plowright, 1980; Thomson and Stratton, 1985; Thomson, 1986; Thomson et al., 1986). The "decay curve" is roughly exponential (see Thomson, 1986; Harder and Thomson, 1989), with the mean pollen grain travelling to about the sixth recipient. Runs of 40 recipient flowers are long enough for deposition of grains from the donor to be essentially complete.

We mapped partial pollen shadows in 1985–1987 in large populations of *Erythronium grandiflorum* at Kebler Pass, Colorado (ca. 3,050 m altitude). Unlike most populations of *E. grandiflorum* in this part of Colorado, the Kebler Pass populations are very nearly monomorphic for the yellow-pollen form; thus we could introduce red-pollen donors from elsewhere and confidently assume that red grains found on the recipient stigmas came from our donors.

We picked buds of red-pollen donors from populations near the Rocky Mountain Biological Laboratory (Gothic, Colorado; 2,990 m altitude) and placed them in individual florist's Aquapic cut-flower holders. After the tepals reflexed, we measured the length of the undehisced anthers to 0.1 mm with dial calipers, so that pollen production could be estimated via an independently obtained regression equation. The flowers were then left to open fully in a screen cage in the field.

The six anthers in an *Erythronium* flower do not dehisce simultaneously (see Harder et al., 1985). They are borne in two whorls of three, and the outer whorl typically completes dehiscence 12–24 hours before the inner whorl begins. Although dehiscence is relatively synchronized within each whorl, one can easily find plants with any number of dehisced anthers from one to six. We used flowers with six or three dehisced anthers, representing the most common conditions in the field, in all but two runs.

We captured bumblebee queens, primarily *Bombus occidentalis* Greene and *B. bifarius* Cresson. Both species are important local pollinators of *E. grandiflorum*, although the larger *B. occidentalis* tends to make better contact with the stigma (Thomson, 1986) and, therefore, this species was used whenever individuals were available. To avoid wasting floral material and field time, on three occasions we used other species (*B. nevadensis* and *B. californicus*) that inhabit lower altitudes and seldom if ever feed on *E. grandiflorum.* We successfully trained these bees to visit *Erythronium* flowers, but the extremely large size and the erratic behavior of the *B. nevadensis* queen led us to discard data for this bee from all analyses of pollen removal and deposition, although we do present summary statistics for comparative interest. For each trial, we released a bee in an intermediate-density, pure stand of *Erythronium* and observed her until she began foraging

TABLE 1. Summary of 32 partial pollen shadows produced by bumblebee pollination of *Erythronium grandiflorum* during 1985–1987. The first two digits of each run number indicate the year of the run (e.g., 86-2 was the second run in 1986). Bee species are coded as follows: BIF = *Bombus bifarius,* CAL = *B. californicus,* OCC = *B. occidentalis,* NEV = *B. nevadensis.* The mean and maximum bee flight distances refer to distances flown between successive flowers. Directionality was calculated as the straight-line distance from the donor to the last recipient flower divided by the sum of the interflower flights. Other table entries are explained in the text. For Wright's effective neighborhood numbers (N_e), values marked by asterisks are not comparable to the others, as they are from short runs.

Run	Bee species and individual	Pollen presented	Pollen removed by bee (%)	Self pollen grains deposited on donor stigma	Number of recipient stigmas	Pollen grains deposited on foreign stigmas (adjusted)	Number of recipients	Mean (maximum) transport distance of outcross pollen (m)	Mean transport distance of outcross and self pollen (m)	Mean (maximum) number of flowers skipped	Mean (maximum) bee flight distance (m)	Local flower density (flowers/m^2)	Hypothetical neighborhood size (N_e)	Directionality
85-1	BIF-1	97,813	85	—	40	705	31	2.55 (30.39)	—	5.3 (71)	1.27 (8.76)	6.9	789	0.6000
85-2	BIF-1	108,214	86	—	40	453	29	2.48 (7.36)	—	1.4 (27)	0.46 (3.80)	6.8	209	0.4196
85-3	BIF-1	85,520	79	—	40	624	23	1.92 (8.13)	—	0.2 (77)	0.85 (8.15)	4.8	74	0.2402
85-4	OCC-1	78,901	71	—	40	503	16	2.32 (14.17)	—	2.2 (21)	0.65 (3.30)	7.4	225	0.5638
85-5	OCC-1	62,826	64	—	40	193	22	3.13 (8.23)	—	1.0 (4)	0.46 (2.89)	6.5	281	0.5225
85-6	OCC-1	74,173	82	—	40	407	14	1.06 (6.42)	—	2.5 (53)	0.49 (4.66)	7.0	37	0.2955
86-1	NEV-1	155,495	57	630	40	929	31	0.98 (4.04)	0.70	—	0.43 (1.86)	15.3	70	0.2567
86-2	NEV-1	47,015	5	48	40	80	20	5.49 (16.54)	3.43	8.0 (93)	0.97 (8.58)	17.5	2,508	0.4278
86-3	OCC-2	47,961	46	89	40	97	12	1.03 (6.95)	0.54	1.6 (15)	0.59 (7.53)	11.2	77	0.2924
86-4	OCC-3	138,474	37	10	40	114	9	1.13 (4.35)	1.04	5.6 (55)	1.19 (8.87)	11.2	61	0.1350
86-5	OCC-4	38,505	34	32	40	72	13	4.85 (20.88)	3.36	5.7 (51)	1.07 (12.31)	14.9	1,516	0.5249
86-6	OCC-5	125,236	44	458	40	245	18	7.52 (12.35)	2.62	2.9 (14)	2.28 (22.78)	5.6	1,039	0.1799
86-7	OCC-5	45,597	72	104	40	101	14	2.14 (8.27)	1.05	0.9 (7)	0.66 (3.44)	6.9	160	0.5325
86-8	OCC-5	47,015	36	274	40	484	26	4.26 (11.75)	2.73	3.4 (22)	1.18 (8.58)	9.9	640	0.2415
86-9	OCC-5	162,114	72	354	40	411	19	2.22 (21.94)	1.19	2.5 (12)	1.08 (4.17)	3.5	115	0.5068
86-10	OCC-5	116,725	67	85	40	322	19	1.23 (8.90)	0.98	1.3 (9)	0.46 (1.70)	7.2	110	0.5525
86-11	OCC-5	119,562	70	231	40	394	20	1.19 (21.95)	0.75	4.6 (92)	0.96 (5.22)	7.3	135	0.6001
86-12	OCC-5	81,530	87	288	40	311	21	2.31 (19.80)	1.20	3.0 (40)	0.99 (9.46)	4.5	206	0.5015
86-13	OCC-5	100,650	53	97	40	24	8	16.44 (36.30)	3.26	4.7 (78)	2.12 (32.01)	4.3	5,730	0.5449
86-14	OCC-5	29,049	68	59	40	138	13	1.32 (28.92)	0.92	4.5 (79)	1.04 (14.05)	7.3	421	0.6957
87-1	OCC-6	60,254	73	126	40	286	15	1.74 (17.74)	1.21	—	1.24 (9.73)	3.8	74	0.3968
87-2	OCC-7	66,539	59	284	19	370 (387)	14	3.59 (6.41)	2.03	2.7 (8)	0.60 (3.16)	10.6	476*	0.5650
87-3	CAL-1	56,944	65	462	40	184	20	1.20 (10.09)	0.34	2.5 (28)	0.81 (3.72)	5.8	59	0.3401
87-4	OCC-8	45,597	44	136	25	264 (270)	9	2.92 (26.68)	1.93	12.4 (169)	2.94 (17.52)	1.7	126*	0.3436
87-5	OCC-9	55,526	64	542	12	256 (286)	11	0.68 (2.00)	0.22	2.8 (66)	0.40 (1.39)	19.0	38*	0.3571
87-6	OCC-10	102,541	87	456	10	317 (363)	8	1.19 (4.25)	0.49	2.2 (7)	0.54 (1.65)	6.6	34*	0.7937
87-7	OCC-11	110,106	52	65	36	217 (218)	14	2.79 (24.39)	2.15	7.6 (161)	1.39 (18.32)	7.3	681*	0.4902
87-8	OCC-12	86,466	63	378	40	371	21	2.18 (6.94)	1.08	2.5 (24)	0.72 (4.06)	7.6	120	0.3096
87-9	OCC-12	139,420	73	168	40	484	24	2.54 (7.17)	1.89	1.5 (16)	0.48 (2.53)	12.7	328	0.5848
87-10	OCC-12	81,738	53	218	40	538	33	3.32 (4.30)	2.36	0.9 (14)	0.37 (2.32)	10.5	422	0.2801
87-11	OCC-12	23,464	37	65	40	105	13	7.93 (17.83)	4.90	4.8 (52)	0.91 (5.00)	12.1	2,644	0.6623
87-12	OCC-13	38,978	40	45	40	83	13	2.95 (20.62)	1.91	3.6 (18)	1.88 (21.60)	8.2	726	0.2415

for nectar in a normal manner. (For unknown reasons, *Bombus* queens seldom, if ever, collect pollen from this plant.) We then presented one of the previously measured red-pollen donors, being careful not to dislodge any pollen before the bee's visit. After a normal feeding visit to the donor, we protected the donor from further visits and watched the next 40 flower visits. In 1987, we also accepted five sequences with fewer than 40 recipients. Two observers followed the bee at a distance large enough to avoid distracting her. We marked each recipient with a sequentially numbered surveyor's stake flag after the bee had moved on. After 40 recipients had been visited and marked, we recaptured the bee, mapped the visited flowers, and then carefully transferred the anthers of the donor to a vial for subsequent counting of the pollen left behind, using a Coulter electronic particle counter (Harder et al., 1985). In 1985, we counted red grains on the 40 recipient flowers only; in 1986–1987, we also counted the donor. Meanwhile the donor and recipient flower positions were mapped by triangulation to two or more fixed points (distances measured with a tape measure to the nearest cm). To save time, we did not attempt to map flowers that were not visited. However, we did estimate the number of intervening flowers that the bee "skipped over" on its flight between each pair of visited flowers. Because this estimation is subjective, especially for longer flights, skips were always scored by the same person for consistency. The distribution of "skips" provides a rough indication of how systematic a bee was. Finally, we laid out a belt transect along the flight path of the bee and counted all open *Erythronium* flowers to determine the local density. The width and length of the transect varied with the shape of the bee's flight, but the area was at least 45 m^2 in all cases. For each run, we also divided the straight-line distance from the donor to the last flower by the total distance along the bee's flight path, giving an index of directionality.

The number of grains delivered in a run will obviously depend on the number of recipients sampled. To make fullest use of our data, we have adjusted the deposition figures for the five short runs by estimating how many grains would have been delivered if these runs had been 40 flowers long. We used the full-length runs to calculate linear regression equations for the relationship between total deposition (on 40 flowers) and partial deposition (on the first 10, 12, 19, 25, and 36 flowers, as required for runs 87-6, 87-5, 87-2, 87-4, and 87-7, respectively [see Table 1]). Because most grains were deposited on the first few flowers, most of these adjustments were slight.

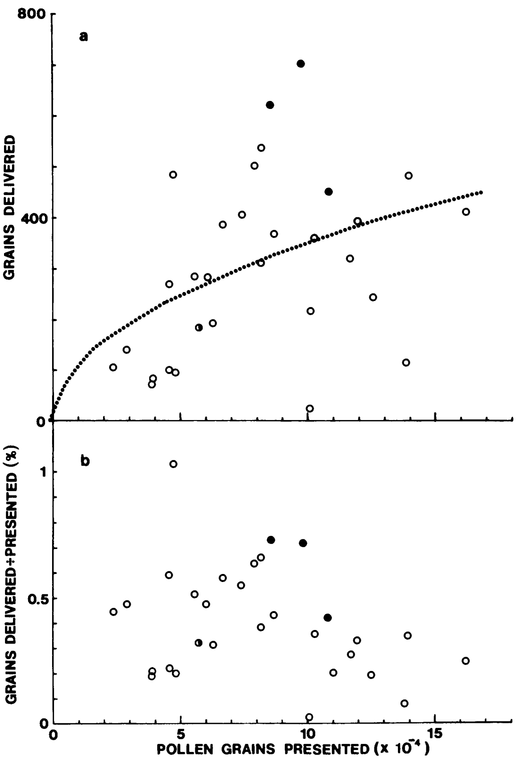

FIG. 1. a) The relationship between the number of pollen grains presented by an *Erythronium grandiflorum* donor flower and the number of grains delivered to the stigmas of the first 40 recipient flowers visited by a bumblebee pollinator. Data from runs with fewer than 40 flowers have been adjusted (see text and Table 1). Open circles indicate runs using *Bombus occidentalis,* filled circles indicate runs using *B. bifarius,* and the half-filled circle represents a run using *B. californicus.* The dotted line is the least-squares best fit of the simple nonlinear function $y = x^b$. For all data points, $b = 0.5086$, $R^2 = 80\%$; for *B. occidentalis* only, $b = 0.5000$, $R^2 = 81\%$. A linear function, also constrained through the origin, explains somewhat less variance (all data: $y = 0.003464$; *B. occidentalis* data: $y = 0.003110$; $R^2 = 76\%$ in both cases). b) The same data transformed to indicate successful pollen delivery per grain.

We follow Crawford's (1984*a*, 1984*b*) suggestions for estimating genetic-neighborhood parameters as follows. We denote the distance from the donor to recipient i as h_i and the number of grains deposited on recipient i as n_i. We then calculate the variance of absolute dispersal distances for outcross pollen as

$$\sigma^2 = \frac{\sum_{i=1}^{40} n_i h_i^2}{\sum_{i=1}^{40} n_i} .$$

We take one-half of this value as the variance of axial dispersal distances (σ^2_{axial}) and then calculate neighborhood area as $A = 4\pi[(1/2)\sigma^2_{\text{axial}}]$ (the ½ corrects for pollen being haploid) and neighborhood effective number (of plants) as $N_e = Ad$, where d is the local density of flowering plants. In addition to the usual assumptions inherent in neighborhood calculations (see Crawford [1984*a*, 1984*b*] for a review), these estimates are further compromised by not correcting for kurtosis of pollen dispersal and by ignoring seed dispersal and self-pollination. We present them primarily for comparison with previous studies of pollinator behavior and pollen flow, not as accurate genetic-parameter estimates.

To estimate interplant variation in pollen dispersal, we created synthetic pollen shadows by superimposing

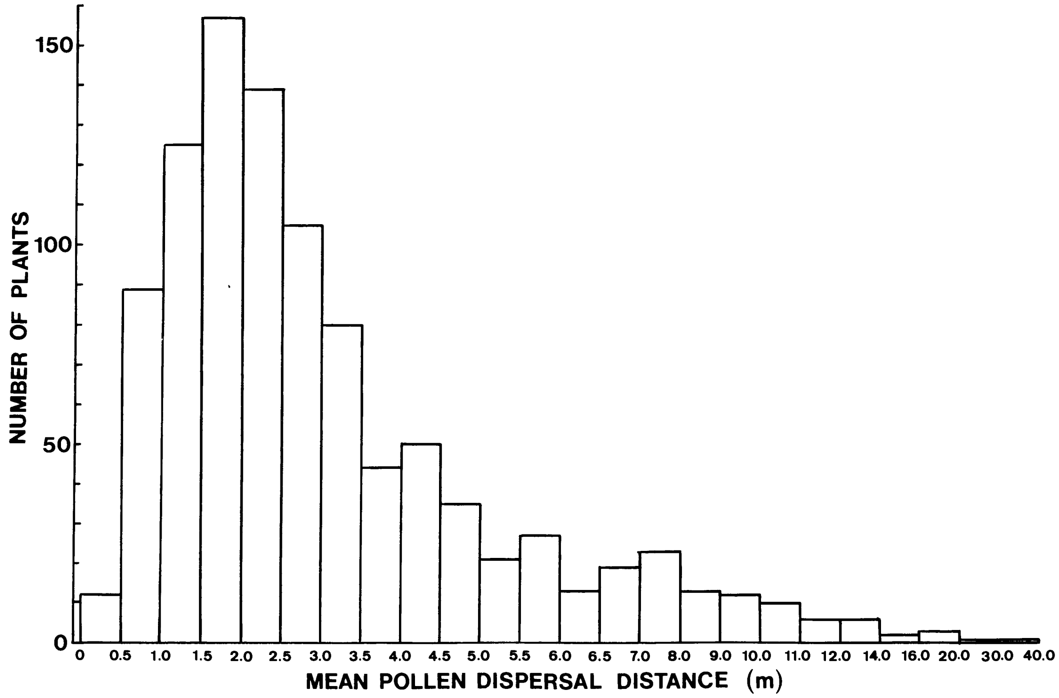

FIG. 2. The mean dispersal distances achieved by the pollen grains of individual single-flowered plants of *Erythronium grandiflorum* (see text). Note that the class marks are at unequal intervals.

observed pollen-deposition sequences on observed (but not matching) flight sequences. Within each of the 40-flower runs, instead of starting the deposition sequence at the true donor, we produced 40 simulated runs with the simulated donor at recipient positions 1, 2, 3, . . . , 40. We then calculated the mean dispersal distance of pollen from each position.

RESULTS

Bees removed a mean of 62.1% (SD = 16.7%) of the pollen presented by the donors and delivered a mean of 0.52% (SD = 0.43%) of the removed pollen (0.041% [SD = 0.023%] of the presented pollen) to the stigmas of other plants (Table 1). The relationship of pollen delivered to pollen presented shows considerable scatter (Fig. 1a), but given constraint through the origin, a single-parameter saturating model explains more variance than a linear one. (We follow Charnov [1982] and other theorists in using origin-constrained power curves to summarize the shape of reproductive success vs. investment curves.) Such a saturating curve has been predicted a priori for animal pollination (Lloyd, 1984). In terms of sex-allocation theory (e.g., Charlesworth and Charlesworth, 1981; Charnov, 1982; Lloyd, 1984) a saturating male curve suggests 1) that hermaphroditism will be stable to invasion by unisexuals and 2) that allocation to male function is likely to be low. Furthermore, the relatively poor pollen dissemination achieved by plants that present large amounts of pollen (Fig. 1b) suggests that male reproduction will often be enhanced by packaging pollen in sequentially presented subunits (Harder and Thomson, 1989; see also Thomson and Barrett [1981], Lloyd and Yates [1982], Haynes and Mesler [1984], Lloyd [1984], and Devlin and Stephenson [1985]).

Field observations and examination of pollen shadow maps indicate that most bees move with considerable directionality (mean = 0.44, SD = 0.16) and that they punctuate bouts of systematic near-neighbor movements with occasional jumps over tens of flowers (compare mean and maximum skips in Table 1). Directionality (see also Pyke [1978], Zimmerman [1979], Waddington [1983], and Plowright and Laverty [1984]), jumping (see also Bateman [1947]), and considerable pollen carryover (Thomson, 1986) cause most plants to have some long-travelling grains (see maximum transport distances in Table 1) and some plants (those visited before a jump) to have many (Fig. 2). Consequently, neighborhood estimates need not be small (Table 1); considering outcross pollen only, estimates for half of the plants exceed 200, a value that Wright (1946) believed would lead to only moderate levels of local differentiation. Estimates for six plants approach or exceed 1,000, corresponding to virtual panmixia. *Erythronium grandiflorum* is self-compatible in our populations and may have a substantial selfing rate, given that self grains typically constitute about half of a stigmatic load (Thomson and Stratton, 1985). If so, neighborhood-number estimates may need to be reduced by a fraction very roughly approximating the selfing rate (Crawford, 1984*a* p. 153); even so, they remain larger than values for *Bombus* pollination from other systems (see especially Schmitt [1980]; see also Levin and Kerster [1969, 1974] and Levin [1981]).

Seed-dispersal data would, of course, increase these estimates. Other *Erythronium* species (Motten, 1983; Harder et al., 1985) and other populations of *E. grandiflorum* (Fritz-Sheridan, 1988) show self-incompatibility and may have neighborhood sizes close to our "outcrossing-only" estimates.

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