
Comparative Studies of Pollen and Fluorescent Dye Transport by Bumble Bees Visiting Erythronium grandiflorum
Author(s): James D. Thomson, Mary V. Price, Nickolas M. Waser and Donald A. Stratton
Source: *Oecologia*, Vol. 69, No. 4 (1986), pp. 561-566
Published by: Springer in cooperation with International Association for Ecology
Stable URL: http://www.jstor.org/stable/4217985
Accessed: 08-12-2015 01:30 UTC

Oecologia (Berlin) (1986) 69:561–566

Oecologia

Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*

James D. Thomson[1,3], **Mary V. Price**[2,3], **Nickolas M. Waser**[2,3], **and Donald A. Stratton**[1,3]

[1] Ecology and Evolution Department, State University of New York, Stony Brook, NY 11794, USA
[2] Department of Biology, University of California, Riverside, CA 92521, USA
[3] Rocky Mountain Biological Laboratory, Crested Butte, CO 81224, USA

Summary. In the Colorado Rocky Mountains the glacier lily *Erythronium grandiflorum* exhibits a striking dimorphism in pollen color and is commonly pollinated by the bumble bee *Bombus occidentalis*. We induced bees to visit sequences of flowers in a flight cage, and compared dispersal of distinctively-colored pollen and fluorescent pigment ("dye") that the bee had picked up at a single donor flower. Nonparametric and parametric analyses showed that dispersal properties of pollen and dye differed; consistently less pollen was deposited and it was carried consistently shorter distances than dye. Dye thus does not provide an accurate means of assessing exactly where or how far pollen travels in this plant-pollinator system. On the other hand, both pollen and dye responded similarly to several experimental manipulations of donor and recipient flowers. Hence dye may well be of value for a qualitative investigation of how floral traits influence pollen dispersal.

In studies of plant reproductive ecology it often is of value to assess pollen dispersal. This assessment is usually hard to obtain, however, because pollen grains are small, numerous, and difficult to mark. Among techniques available for estimating dispersal (for reviews see Waser and Price 1982; Handel 1983), one that is straightforward and inexpensive is to apply finely-powdered insoluble fluorescent pigment (hereafter dye) to anthers of a pollen source and to examine recipient stigmas for the dye. The questions that arise are whether this technique is inaccurate or biased, and if so in what respects.

To our knowledge, the two studies cited above and one by Campbell (1985) are the only ones published to date that compare pollen and dye transport by animals. Waser and Price (1982) found good agreement between mean pollen and dye dispersal distances using hummingbirds and *Ipomopsis aggregata* (Polemoniaceae) as did Campbell (1985), with a smaller sample of solitary bee and bee fly visits to *Stellaria pubera* (Caryophyllaceae). Handel (1983) had less success in using dye to assess the exact spatial pattern of pollen dispersal from a patch of *Brassica campestris* (Brassicaceae). He found that flowers were more likely to receive dye and no pollen (29% of plants) than the reverse (11%), a result shared by a fourth study (Thomson, unpubl.) on bumble bees visiting *Erythronium americanum* (Liliaceae).

These studies are not exactly comparable. Whereas Waser and Price and Campbell counted pollen and dye on each stigma, Thomson counted pollen but only scored presence or absence of dye, and Handel scored presence of dye and inferred pollen deposition from marker genes in progeny. Along with these methodological differences, there were differences in criteria used to judge similarity of pollen and dye. Waser and Price compared average dispersal distance and carryover properties of each, whereas Thomson and Handel undertook more demanding comparisons of spatial dispersal patterns from individual plants. Such differences in methods and goals, in the way results are analyzed, or in the biology of specific plant-pollinator systems can lead to disparate conclusions about the usefulness of dye as a pollen analogue.

Here we apply some methodological refinements of Waser and Price (1982, 1984) and Price and Waser (1982) to a bumble bee – *Erythronium* system similar to that studied by Thomson (1985) and Thomson and Plowright (1980). Our primary goal is to assess similarity of pollen and dye in various dispersal and carryover properties. We also hope to infer something about "pollen transfer mechanics", the details of how pollen is picked up, transported, and deposited (Lertzman and Gass 1983; Thomson 1983; Waser 1983; Waser and Price 1984).

Methods

(1) Dispersal experiments

We worked at the Rocky Mountain Biological Laboratory (RMBL) in Colorado on the glacier lily *Erythronium grandiflorum* and the bumble bee *Bombus occidentalis*, a locally important pollinator (Thomson, in prep.). Like several congeners, *E. grandiflorum* exhibits pollen color dimorphism: most plants near RMBL have pale yellow pollen but a few have bright red pollen (hereafter "yellows" and "reds"). By using red donor flowers and yellow recipients we could measure pollen dispersal without emasculating recipients, which might itself influence dispersal (Price and Waser 1982).

We caught *B. occidentalis* queens on *E. grandiflorum* flowers and refrigerated them before they were used in experiments in an outdoor flight cage or indoor room. A

Offprint requests to: J.D. Thomson

bee was allowed to warm up on yellow-pollen flowers and then to visit a single red donor followed by a series of recipient yellows. Donor anthers had small amounts of dye (Radiant Color Corporation) applied to them with toothpicks. Dye particles are irregular in shape and size, and much smaller than pollen (longest linear dimension: $\bar{x}=6.9$ μ, $s=5.3$, $N=20$ for dye, vs. $\bar{x}=109.6$ μ, $s=12.4$, $N=20$ for pollen). Recipient flowers were placed individually into small vases spaced 5–10 cm apart. Flowers were collected in bud and allowed to open indoors; they contained enough nectar ($\bar{x}=0.47$ μl, $s=0.48$, $N=10$ in one sample vs. $\bar{x}=0.91$ μl, $s=0.58$, $N=20$ in a field sample) that bees usually fed readily. Bees broke off feeding part way through some runs; in these cases we held a recipient flower in the bee's flight path. If the bee landed we brought flower and bee back to the array; if not, we terminated the run. We forced bees to fly between runs to increase their hunger and to allow them to groom off residual pollen and dye; sometimes we refrigerated them between runs.

Immediately after each recipient was visited we excised the pistil and placed it in a tray with sequentially-numbered depressions. Because red pollen color is rapidly lost on germination, we counted pollen and dye within 30 min of each run. We obtained repeatable counts with a dissecting microscope at 50 × under incandescent light. We scored only particles and grains that adhered to the papillose stigmatic surfaces.

We conducted 3 kinds of experiments that differed in the condition of donor and recipient flowers. In the *Standard* run type all 6 anthers of the donor were freshly dehiscent and marked with dye. Recipient flowers had 3 dehiscent anthers, a stage at which stigmas are fully receptive. In the *Reduced Load* run type only 3 donor anthers were dehiscent. Two were brushed with a fine paintbrush to strip them of pollen (cf. Price and Waser 1982) and the third was marked with dye. Recipients were as before. In the *Brushed Anther* run type the donor was prepared as in Standard runs, but recipients had their 3 dehiscent anthers stripped of pollen. Stigmas of recipients were covered with a gelatin capsule during brushing to avoid contamination with self pollen.

During June 1983 we used 4 individual bees in 10 Standard, 5 Reduced Load, and 6 Brushed Anther runs, and scored a total of 795 recipient flowers. All runs were >23 flowers long and most were 40 flowers long.

(2) Analyses

Our experiments yielded paired dye and pollen counts for 21 runs. We analysed the results in several ways; we will supply raw data to anyone interested in further analysis.

First, in accord with many published studies of dye transport that report only presence or absence, we ignored particle counts, run type, and flower position and simply considered whether dye presence on a stigma accurately indicated pollen presence.

Second, we used a nonparametric analysis to compare pollen and dye carryover within each run. We estimated the flower position reached by the mean and median particle (hereafter mean and median transport distance), scored whether pollen mean or median was greater than that for dye, and compared results across runs. We performed the same analysis on total amounts of pollen and dye deposited during a run. Because runs were of different length we summed counts from the first 5 flowers and used this "initial deposition" as an estimate of total deposition. Initial deposition is highly correlated with total deposition for runs of equal length.

Third, we fit exponential regressions to individual runs and compared regression parameters and other deposition and transport statistics using analysis of variance and correlation analysis. We examined effects of particle type (dye, pollen) and treatment (Standard, Reduced Load, Brushed Anther) on carryover. We performed analyses on mean and median transport distance, initial deposition, and exponential regression coefficient (hereafter termed "decay rate" because it estimates the rate with which counts "decayed" on successive stigmas). Because an exponential model will not accommodate zero values we added 1.0 to all raw counts. Adding constants smaller than 1.0 gave poorer fits, as judged by r^2 values.

Results

(1) Overall patterns

Amounts of pollen and dye deposited on stigmas declined as each run progressed (Fig. 1). Averaging across runs, mean and median numbers deposited as a function of flower position were well described by a negative exponential model ($r^2=0.79$ and 0.91 for pollen and dye means, and $r^2=0.86$ and 0.91 for medians). This model also fit data from individual runs relatively well. In 20/21 cases with pollen and 13/21 with dye, an exponential fit was better than linear or power fits, judging from r^2 values, and in all cases the exponent was negative (Table 1). The data deviated systematically from an exponential model, however, because decay

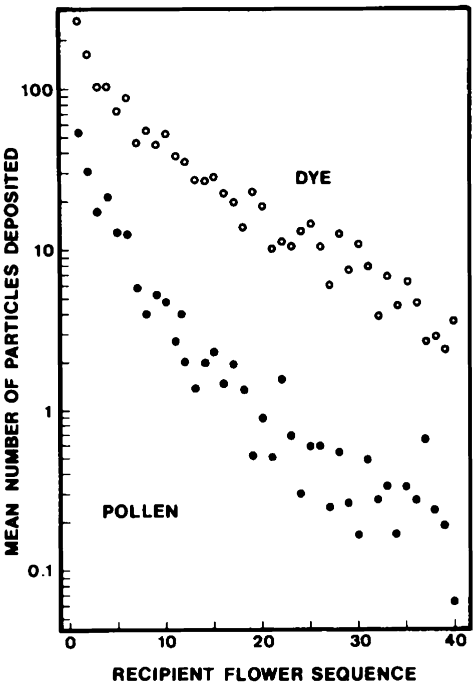

Fig. 1. The mean numbers of fluorescent dye particles and pollen grains deposited by *Bombus occidentalis* queens on the stigmas of *Erythronium grandiflorum* flowers in 21 carryover runs. The sample sizes are 21 for recipient flower sequence positions 1–23; 20 for positions 24–28; 19 for position 29; 18 for positions 30–37; 17 for position 38; and 16 for positions 39 and 40

Table 1. Summaries of pollen and fluorescent dye transport in 21 carryover runs. Mean transport distance is the number of recipient flower units that the average grain deposited in the run was carried before being deposited. Median transport distance is the number of the recipient flower by which one half of the grains have been deposited (analogous to an LD_{50}). Initial deposition is the total number of particles deposited on recipient flowers 1–5. The regression statistics are for log-transformed data (*ln*(y + 1))

Run number and type	Pollen						Fluorescent Dye						Dye/pollen comparisons	
	Mean transport distance	Median transport distance	Initial deposition	Regression statistics: y-intercept	Regression statistics: Regression coefficient	r^2	Mean transport distance	Median transport distance	Initial deposition	Regression statistics: y-intercept	Regression statistics: Regression coefficient	r^2	Dye mean transport ÷ Pollen mean transport	Dye median transport ÷ Pollen median transport
A. Standard														
1	9.56	5	64	5.84	−0.041	0.13	9.80	8	104	9.88	−0.050	0.12	1.03	1.60
2	5.12	2	145	7.62	−0.059	0.36	9.29	6	354	25.48	−0.065	0.26	1.81	3.00
3	4.41	1	72	4.70	−0.050	0.42	7.66	4	217	12.99	−0.062	0.30	1.71	4.00
4	11.83	6	37	3.68	−0.035	0.22	8.86	5	203	13.23	−0.056	0.26	0.75	0.83
5	5.03	2	163	10.74	−0.074	0.54	5.18	2	530	37.61	−0.104	0.56	1.03	1.00
6	6.25	5	200	15.56	−0.083	0.49	8.15	6	577	69.46	−0.098	0.50	1.30	1.20
7	6.77	3	48	3.31	−0.034	0.25	7.84	4	523	16.67	−0.050	0.14	1.16	1.33
8	4.50	1	79	5.09	−0.053	0.39	7.33	4	427	35.32	−0.082	0.41	1.63	4.00
9	3.72	2	186	8.27	−0.069	0.47	8.56	6	1,282	167.99	−0.094	0.51	2.30	3.00
10	3.77	3	312	10.38	−0.071	0.42	5.99	3	992	67.73	−0.101	0.53	1.59	1.00
$\bar{x}$	6.10	3.00	130.6	7.52	−0.057		7.87	4.80	520.9	45.64	−0.080		1.43	2.10
s.d.	2.66	1.76	87.0	3.84	0.017		1.43	1.75	366.4	48.12	0.022		0.46	1.27
B. Reduced load														
11	3.51	2	138	9.22	−0.077	0.59	4.70	3	243	24.64	−0.104	0.68	1.34	1.50
12	7.72	4	32	4.01	−0.041	0.47	8.48	5	115	16.15	−0.078	0.59	1.10	1.25
13	6.65	4	93	6.56	−0.056	0.38	7.76	5	226	15.20	−0.071	0.32	1.17	1.25
14	8.42	6	41	5.46	−0.046	0.41	9.66	6	282	21.61	−0.056	0.26	1.15	1.00
15	9.70	5	18	3.37	−0.035	0.35	6.67	4	437	53.62	−0.103	0.59	0.69	0.80
$\bar{x}$	7.20	4.20	64.4	5.72	−0.051		7.45	4.60	260.6	26.24	−0.082		1.09	1.16
s.d.	2.34	1.48	50.0	2.32	0.016		1.88	1.14	116.5	15.79	0.021		0.24	0.27
C. Brushed anther														
16	3.76	2	252	12.21	−0.078	0.52	8.73	4	2,956	274.38	−0.084	0.36	2.32	2.00
17	6.59	3	123	10.16	−0.067	0.48	7.39	4	673	49.61	−0.092	0.43	1.12	1.33
18	4.05	1	336	28.59	−0.119	0.42	7.18	6	1,262	167.48	−0.093	0.18	1.77	6.00
19	5.28	4	147	17.71	−0.113	0.53	9.61	8	1,046	248.35	−0.116	0.38	1.82	2.00
20	4.74	2	189	10.69	−0.068	0.50	9.99	6	1,231	97.10	−0.074	0.25	2.11	3.00
21	5.19	3	213	15.33	−0.080	0.47	8.33	7	1,341	157.46	−0.113	0.40	1.61	2.33
$\bar{x}$	4.94	2.50	210.0	15.78	−0.088		8.54	5.83	1,418.2	165.73	−0.095		1.79	2.78
s.d.	1.01	1.05	77.0	6.90	0.022		1.14	1.60	790.5	85.90	0.016		0.42	1.67
D. Total for 21 runs														
$\bar{x}$	6.03	3.14	137.5	9.45	−0.064		7.96	5.05	715.3	75.33	−0.083		1.45	2.07
s.d.	2.29	1.59	91.2	6.08	0.023		1.46	1.60	661.9	80.20	0.021		0.47	1.34

rate was inconstant: counts decayed rapidly for the first few flowers of a run and thereafter decayed more slowly (Fig. 1).

(2) Presence/absence

Amounts of pollen and dye deposited on individual stigmas were positively correlated (Pearson's product-moment $r = 0.71$; $P < 0.001$), despite considerable scatter. Many flowers received neither pollen nor dye. This occurred more commonly towards the end of a run, presumably because supplies on the bee became exhausted, but sometimes occurred early in a run, perhaps because bees failed to contact the stigma.

Despite the overall correlation just described, pollen and dye are transported somewhat independently. Many stigmas lacking pollen carried dye, while a smaller fraction carried pollen without dye (Table 2a). This agrees with the results of Handel (1983) and Price and Waser (1982) from other systems. That dye was deposited without pollen more often than the reverse reflects the fact that more dye particles than pollen grains were deposited during a run. Initial deposition was greater for dye than pollen in all 21 runs (Table 1; $\beta \ll 0.001$, two-tailed sign test); on average, total dye deposition was 7.6 times that of pollen.

Thus, the presence of dye is not a particularly good indicator of the presence of pollen. The association may be improved by discounting trace amounts of dye, for ex-

Table 2a, b. Occurrence of pollen and dye on 795 stigmas from 21 carryover runs. **a** Stigmas were scored as having pollen or dye present if they carried one or more particles. **b** To compensate for the 7.6-fold numerical dominance of dye over pollen, stigmas were scored as having dye present only if ≥8 dye particles were deposited. See text for further discussion. Discordant stigmas are those that have one particle type present and the other absent

		Dye Present	Absent	Fraction of discordant stigmas
a				
Pollen	Present	392	19	0.31
	Absent	225	159	
b				
Pollen	Present	299	112	0.23
	Absent	73	311	

Table 3a–c. Analyses of variance of the pollen and dye transport indices presented in Table 1. The model was fit by the GLIM 3.12 statistical package and chosen primarily to investigate the effects of variation in run type (Standard, Reduced Load, or Brushed Anther) and particle type (pollen or dye). In addition, the identity of the bee is considered. Because bee effects and run type effects are potentially confounded, we fit the bee effect first, so that run type effects will be assessed conservatively

Variable	Source of Variation	SS	df	MS	F	Significance
a						
Mean Transport Distance	Bees	34.16	3	11.39	5.56	$P<0.01$
	Run type	3.36	2	1.68	0.82	ns
	Run type × run	57.59	15	3.84	1.88	ns
	Particle type	39.23	1	39.23	19.17	$P<0.001$
	Particle × run type	15.55	2	7.78	3.80	$P<0.05$
	Error	36.83	18			
	Total	186.72	41			
b						
Initial Deposition	Bees	24,200	3	8,067	5.17	$P<0.01$
	Run type	21,540	2	10,770	6.90	$P<0.01$
	Run type × run	25,350	15	1,690	1.08	ns
	Particle type	45,568	1	45,568	29.21	$P<0.001$
	Particle × run type	30,201	2	15,100	9.68	$P<0.005$
	Error	28,081	18	1,560		
	Total	174,940	41			
c						
Decay rate	Bees	0.00516	3	0.00172	12.53	$P<0.001$
	Run type	0.00212	2	0.00106	7.73	$P<0.005$
	Run type × run	0.00923	15	0.00062	4.48	$P<0.005$
	Particle type	0.00375	1	0.00375	27.33	$P<0.001$
	Particle × run type	0.00076	2	0.00162	0.47	ns
	Error	0.00247	18	0.00014		
	Total	0.02350	41			

ample by considering dye counts less than 7.6 (the overall dye: pollen abundance ratio) as zeroes (Table 2b). Discordance between dye and pollen is reduced in this case but not eliminated.

(3) Pollen and dye carryover curves

Over all 21 runs, the mean (median) transport distances averaged 6.03 (3.14) flowers for pollen and 7.96 (5.05) for dye. This qualitative difference was consistent across runs: mean pollen transport distance was shorter than that for dye in 19/21 cases (Table 1, $P<0.001$, 2-tailed sign test), and median pollen transport was shorter in 16 cases, longer in 2, and equal in 3 (Table 1, $P<0.002$, 2-tailed, ignoring "ties"). Absolute differences were not large (about 2 flowers on average for both means and medians), but proportional differences were substantial. Within runs, the mean (median) dye transport distance averaged 1.45 (2.07) times greater than that for pollen.

That pollen and dye carryover differed consistently is also indicated by significant particle type effects for ANOVAs on mean transport distance, initial deposition, and decay rate (Table 3). In keeping with nonparametric results, mean pollen transport distance was significantly shorter, and initial deposition significantly less, than for dye. Pollen carryover was also characterized by smaller decay rates (Table 1).

(4) Effects of run type

We were unable to use exactly the same individual bees for all run type treatments. This creates a potential for partial confounding of bee and run type effects, and indeed each effect is always significant if entered first into ANOVAs. In order to obtain a conservative estimate of the significance of run type effects, we always entered bee effects first.

Despite overall differences in pollen and dye transport, there were significant run type effects on initial deposition and decay rate (Table 3b, c), suggesting that carryover of the two particle types was affected in qualitatively similar ways by our experimental treatments. Initial deposition of both pollen and dye was highest in Brushed Anther runs and lowest in Reduced Load runs (Table 1), but because the proportional response of dye exceeded that of pollen there was a significant run type – particle type interaction (Table 3b). Run type also influenced decay rate (Table 1, 3c), perhaps because decay rates were negatively related to initial deposition for both particle types (Table 4a). Again, differences between particle types in proportional response to treatments were reflected in a significant particle type – run type interaction (Table 3c). Particle counts decayed most rapidly for Brushed Anther runs.

Highly significant run type effects for initial deposition and decay rate (Table 3b, c) mean that run-to-run variation in these aspects of dye carryover was correlated with variation in pollen carryover. There were significant positive correlations between initial depositions of dye and pollen across all runs, and between regression coefficients in Standard runs (Table 4b).

There was no significant run type effect on mean transport distance (Table 3a), and no significant correlation across runs between mean transport distance of pollen and dye, although all correlation coefficients were positive (Table 4b). This could be a reflection of the conservative analysis

Table 4a, b. Pearson product-moment correlations among pollen and dye transport characteristics. Significance at the 0.05 and 0.01 levels is indicated by one or two asterisks, respectively. Pollen and dye counts were transformed as *ln* (Y + 1).

a Correlations, across all runs, within particle type, of four estimated transport parameters

	Pollen		
	Initial deposition	Regression coefficient	Median transport distance
Mean transport distance	−0.694**	0.634**	0.844**
Initial deposition		−0.797**	−0.479*
Regression coefficient			0.390

	Dye		
	Initial deposition	Regression coefficient	Median transport distance
Mean transport distance	0.138	0.471*	0.800**
Initial deposition		−0.362	0.053
Regression coefficient			0.111

b Correlations of transport parameters of pollen and dye for the various treatments

Treatment	Mean transport distance	Initial deposition	Regression coefficient
Standard $n=10$	0.52	0.73*	0.92**
Reduced Load $n=5$	0.64	−0.27	0.26
Brushed Anther $n=6$	0.15	0.45	0.43
Pooled data $n=21$	0.32	0.68**	0.68

mentioned earlier, but it could also mean that transport distance represents a complex balance between initial deposition and decay rate.

Discussion

(1) Dye as a pollen analogue

Dye transport by bumble bees between *E. grandiflorum* flowers resembles that of pollen in many respects, but not in others. The degree of similarity depends on the aspect of pollen transport considered and the methods used to measure it. The value of dye as a pollen analogue in this species thus depends on the goals of the study.

Because total amounts of dye dispersed are much greater than amounts of pollen, and because dye and pollen are transported somewhat independently, dye is an unreliable pollen analogue when one needs to measure how many pollen grains are moved and their exact destinations. Moreover, simply scoring presence/absence of dye will grossly overestimate the extent of pollen dispersal, because stigmas often receive dye but no pollen. Dye may also be an unreliable analogue when one needs to characterize pollen carryover precisely. Pollen carryover differs from that of dye in having lower initial deposition and faster initial decay. The net result is that the average (mean or median) pollen grain is carried over fewer flowers and travels less far than the average dye particle.

The systematic bias that dyes introduce may not always be critical, however, for two reasons. First, although the relative bias is substantial, the absolute magnitude is not. Mean dye transport distance in *E. grandiflorum* is 145% that of pollen, but the absolute error from using dye would be only about 2 flowers. This would correspond to an error of 0.5 m in a natural population with 0.5 m spacing between plants, assuming that bees visit 2 flowers per plant on average and move between neighboring plants. Second, many questions depend on *relative* dispersal of pollen from plants receiving different treatments, rather than absolute dispersal distance. In such cases dye may be useful so long as treatments affect dye and pollen carryover in qualitatively similar ways.

Our results suggest that experimental treatments do affect dye and pollen carryover in similar ways. There was a significant run type effect on initial deposition and decay rate for both pollen and dye (Table 3b, c), and the significant 2-way interaction between run type and particle type were functions of different magnitudes rather than signs of pollen and dye responses in Brushed Anther runs. Furthermore, mean transport distance was not affected by run type for either particle type (Table 1). Not only did pollen and dye respond in similar ways to different run types, but variation among individual Standard runs in decay rate and initial deposition for dye was positively correlated with variation in these parameters for pollen (Table 4b).

Because dye tends to travel farther, reports of restricted pollen flow distances based on dye flow (e.g., Price and Waser 1979) may well be conservative. However, inferences of extensive pollen dispersal from observations of extensive dye dispersal (e.g., Webb and Bawa 1983) may not be fully warranted. Webb and Bawa (pp 1266-7) stress that they scored only pollen grains with adherent dye particles, rather than stray particles, but there is no way to be certain that such "dyed grains" are actually from the dye donor flower. Free dye particles on a pollinator can presumably adhere to pollen at any point in a foraging bout.

(2) Pollen transfer mechanics

Our experimental treatments allow us to investigate how the initial pollen load picked up by a pollinator affects carryover (compare Standard and Reduced Load runs, Table 1). High initial loads increased the initial and total amounts deposited as well as decay rate. The net effect was to decrease mean transport distance in Standard relative to Reduced Load runs. Dye responded in a qualitatively similar way to these two treatments. We do not know what mechanism causes this effect, but speculate that large pollen and dye loads may be less firmly attached to the bee so that clumps are deposited on the first few flowers or that the bee may more actively groom large loads off its body as it flies between flowers.

We also can examine how the state of recipient anthers affects carryover (compare Standard and Brushed Anther runs, Table 1). Pollen in recipient anthers affected both pollen and dye carryover, but to different extents. Initial deposition of both particle types was strongly increased in Brushed Anther relative to Standard runs (a result also found by Price and Waser 1982), but the effect was proportionally greater for dye. The decay rate also increased for both particle types in Brushed Anther runs, but here the effect was proportionally greater for pollen. The net effect was to decrease mean pollen transport distance but to increase dye transport distance. Perhaps pollen from recipient flowers layers over or mixes with pollen or dye from a donor flower and hence interferes with subsequent deposition (see also Price and Waser 1982), but the small particle size of dye somehow means that its initial deposition and decay rate are reduced less than those of pollen by this interaction. If initial dye deposition is affected slightly less strongly than dye decay rate, then transport distance, which reflects a balance between the two, will increase; the opposite situation would explain why pollen transport distance decreases.

The variety of run type effects on properties of carryover curves emphasizes the complexity of the events that determine transfer mechanics for a single pollen grain (Waser 1983, Waser and Price 1984). Interaction with other grains in the parental anther and with pollen already on the pollinator may determine whether the grain adheres to the pollinator. Once there the grain is part of a dynamic pollen pool that is augmented by donations from subsequent flowers. There may be interactions among grains in the pool, such as layering or mixing, and interactions involving the pollinator, such as grooming. These, in addition to interactions with recipient stigmas, will determine whether the grain is eventually deposited in a suitable place. The fates of pollen grains vary, so that transfer mechanics should influence not only the proportion of grains successfully reaching a stigma but also the spatial pattern of dispersal around the pollen parent. The roles of layering, mixing, grooming, *etc.*, need further investigation in *Erythronium* and in other plants.

Well-designed experiments with dye may be useful for these studies, as long as the sort of qualitative similarity to pollen seen in *E. grandiflorum* turns out to be general. At the same time, substantial overestimates of pollen deposition may turn out to render dye inappropriate for quantitative estimates of pollen carryover and dispersal in many species.

Conclusion

Fluorescent dyes can be useful pollen analogs, but caution is required in using them and in interpreting the results. We recommend: (1) that dye particles be counted, rather than scored as "present"; (2) that dye be applied to donor flowers consistently and carefully, ideally in controlled amounts; (3) that experiments be designed to infer relative pollen transport from measures of relative dye transport in different experimental treatments; and (4) that dye and pollen be calibrated in each system for which absolute pollen dispersal is to be measured.

Acknowledgements. For assistance with experiments we thank R. Patten and B. Thomson. The research was supported by NSF grants DEB-8102774, and BSR-8313522 (to NMW), DEB-8206959 and grants from the Penrose Fund of the National Academy of Sciences and the American Philosophical Society (to JDT). R. Armstrong, C. Plowright, and L. Harder provided useful suggestions. Publication No. 568 in Ecology and Evolution from the State University of New York-Stony Brook.

References

Campbell DR (1985) Pollen and gene dispersal: the influences of competition for pollination. Evolution 39:418–431

Handel SN (1983) Pollination ecology, plant population structure, and gene flow. In: Real L (ed) Pollination biology, Academic Press, Orlando, Florida

Lertzman KP, Gass CL (1983) Alternative models of pollen transfer. In: Jones CE, Little RJ (eds) Handbook of Experimental Pollination Biology. Van Nostrand-Reinhold, NY. pp 474–489

Price MV, Waser NM (1979) Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. Nature 277:294–297

Price MV, Waser NM (1982) Experimental studies of pollen carryover: hummingbirds and *Ipomopsis aggregata*. Oecologia (Berlin) 54:353–358

Thomson JD (1983) Component analysis of community level interactions in pollination systems. In: Jones CE, Little RJ (eds) Handbook of Experimental Pollination Biology. Van Nostrand-Reinhold, NY. pp 451–460

Thomson JD (1986) Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. J Ecol (in press)

Thomson JD, Plowright RC (1980) Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. Oecologia (Berlin) 46:68–74

Waser NM (1983) The adaptive nature of floral traits: Ideas & evidence. In: Real LA (ed) Pollination biology. Academic Press. pp 241–285

Waser NM, Price MV (1982) A comparison of pollen and fluorescent dye carryover by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae). Ecology 63:1168–1172

Waser NM, Price MV (1984) Experimental studies in pollen carryover: effects of floral variability in *Ipomopsis aggregata*. Oecologia (Berlin) 62:262–268

Webb CJ, Bawa KS (1983) Pollen dispersal by hummingbirds and butterflies: a comparative study of two lowland tropical plants. Evolution 37:1258–1270

Received July 29, 1985