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Rescue of stranded pollen grains by secondary transfer

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Abstract

Secondary transfer of pollen can occur when a second pollinator remobilizes grains that had already been transferred to a flower by a previous pollinator. We used a pollen-color dimorphism to measure components of secondary transfer by bumble bees visiting the lily *Erythronium grandiflorum*. Remobilization was surprisingly high, ranging from 20% of grains deposited on stigmas to 90% of grains deposited on inner tepal surfaces. Because most of the grains that are remobilized would otherwise have been stranded on non-stigmatic surfaces, secondary transfer has the beneficial effect of returning lost grains to circulation.

Keywords: bumble bee, *Erythronium grandiflorum*, pollen carryover, pollination, secondary pollen transfer.

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Introduction

Our understanding of pollen dispersal by animals has been transformed by the demonstration that substantial numbers of grains may be 'carried over' beyond the first recipient flower (Levin & Berube 1972; Thomson & Plowright 1980). Quantitative estimates of pollen carryover are important components in models of plant mating systems (e.g. Harder & Barrett 1995). Although there have been rather few attempts to characterize the shapes of pollen carryover distributions, and most of these have concerned bee or hummingbird pollinators (but see Campbell 1985; Svensson 1985, 1986; Robertson 1992), enough studies have accumulated for some attempts at synthesis (Morris *et al*. 1994; Harder & Wilson 1998). The principal conclusions are that pollen transfer is inefficient and that carryover distributions tend to have long tails; only a small percentage of the pollen grains removed from a donor flower are likely to be carried to a recipient stigma and the majority of those will be deposited on the first few recipients visited. Some grains, however, will travel much farther. In the case of bees, the rapid decline of carryover probably involves active grooming by the animals between successive flowers, and the long tails may owe their existence to 'safe sites' on the bees' bodies, where grooming is difficult (Kimsey 1984; Harder & Wilson 1998). More generally, long tails may reflect a broad spatial distribution of grains on the vector's body, with locally different probabilities of successful removal. The resulting long-tailed distributions frequently differ significantly from simple models of exponential decay and those far-traveling grains are of particular interest with respect to the spatial genetic structure of plant populations, the contamination of crop strains and the invasion potential of new genes (Ellstrand *et al*. 1999).

These models of pollen flow, and most of the empiric studies from which they derive, assume that grains stay where they are first deposited. This need not be the case, that is, consider the analogy to seed dispersal, where *primary dispersal* by wind from the plant to the ground may be succeeded by *secondary dispersal* by animals. With pollen grains, the distinction between primary and secondary dispersal need not involve different vector types. Instead, primary dispersal concerns the initial movement of grains from one donor flower to deposition on a recipient flower; secondary dispersal involves the remobilization of some of those deposited grains by a second pollinator and subsequent redeposition on still other flowers. In a comprehensive analysis of pollen fates, Inouye *et al*. (1994) discussed secondary transfer, pointing out that, in principle, grains can go through several cycles of mobilization, deposition, remobilization and redeposi-

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tion. Rademaker *et al*. (1997) showed that an average bumble bee visit to *Echium vulgare* picked up 4087 grains and moved 1204 pollen grains from a flower's anthers to its petals. Those petal-deposited grains constitute an appreciable pool that might undergo secondary dispersal.

Secondary pollen dispersal has received almost no quantitative study apart from Svensson's (1986) treatment of ant pollination of *Scleranthus perennis*. Its role in more typical systems, in which flying animals set large numbers of grains in motion, is completely unknown. Nevertheless, there are several reasons why secondary pollen dispersal might be important.

First, secondary dispersal might tend to equalize stigmatic loads among flowers. Especially when many grains are deposited on a stigma, the topmost grains may germinate slowly or not at all, as in *Erythronium grandiflorum* (Thomson 1989). However, unattached grains might be easily dislodged, picked up by a subsequent visitor and placed on a portion of the visitor's body that touches stigmas. Therefore, these grains might have a higher probability of redeposition on stigmas than would grains presented in anthers.

Second, grains carried to recipient flowers, but deposited on non-stigmatic surfaces, might be delivered to a stigma through secondary dispersal. Because grains adhere to most flower surfaces less than to stigmas, substantial numbers of grains could be rescued from non-stigmatic destinations, if visits are frequent and grains are long-lived.

Finally, secondary dispersal can change the characteristics of the 'pollen shadow', that is, the total distribution of grains across potential recipients. Secondary dispersal increases the number of stigmas on which one plant's grains are deposited, thus increasing mate diversity. Depending on the movement patterns of pollinators, pollen shadows might become longer, more diffuse and less directional.

If secondary dispersal occurs only rarely, its effects can probably be ignored in most models. The goal of this paper is to measure secondary dispersal in a system for which primary dispersal has been well studied. We measured secondary pollen dispersal by bumble bee pollinators of *Erythronium grandiflorum*, using a naturally occurring pollen-color dimorphism that has formed the basis for a series of studies of pollen transfer (e.g. Thomson 1986; Thomson *et al*. 1986; Thomson & Thomson 1989).

Materials and methods

During May to June 1993, we collected buds of *Erythronium grandiflorum* Pursh (Liliaceae) in subalpine meadows near the Rocky Mountain Biological Laboratory, Gothic, CO, USA. The buds, nearly mature when picked, opened normally in the lab after being placed in water-filled florist's aquapiks. We caught bumble bee queens of three species, *Bombus terricola occidentalis* (Greene), *B. appositus* Cresson, and *B. bifarius* Cresson (for conformity with previous publications, we refer to the first species as *B. occidentalis*). These bees typically visit *Erythronium grandiflorum* for nectar only, but they move large amounts of pollen in the process. The first two species are larger and tend to make better contact with anthers and stigmas (Thomson 1986); *B. appositus* emerges later and is seen visiting *E. grandiflorum* less often than the other species. Thus, *B. occidentalis* has been the preferred subject in earlier studies, but we used all three species here due to the scarcity of *B. occidentalis* when the flowers were ready for experiments.

We induced bees to visit chosen sets of opened flowers in an indoor arena of approximately 0.5 m^3. One sequence of visits constituted a 'run', after which the bees were caught and set aside to clean themselves. Because primary carryover is known to be affected by the amount of pollen in the recipient flowers, we manipulated this variable, conducting 10 runs of each of two types (see Price & Waser 1982; Thomson *et al*. 1986). For all runs, the six anthers of yellow-pollen recipient flowers were allowed to dehisce completely. In *brushed-anther* runs, we removed most of the pollen by brushing the anthers with a small paintbrush, while covering the stigma to prevent contamination. In *standard* runs, no pollen was removed and we handled the recipients with care to avoid dislodging pollen from their anthers.

Fifteen such recipients were placed in the arena, spaced so that bees would have to fly between them. We then added a fully dehisced, unbrushed, red-pollen flower as the *primary donor*. The first bee, made hungry by confinement in a refrigerator, was then introduced to the arena on a 'warm-up bouquet' of yellow-pollen flowers. When the bee was warm and feeding normally, we removed the bouquet and presented the primary donor to the bee. After feeding on the red-pollen flower, the bee moved to the first of the yellow-pollen recipients and fed there. This flower, which would have received some red pollen from the primary donor, became the *secondary donor* for the secondary carryover run to follow.

Because the anthers of *Erythronium* flowers typically dehisce asynchrously over 2 days, our use of fully dehisced primary donors means that our bees received large loads of pollen. Natural visitation rates to *E. grandiflorum* near our study site are low enough, however, that it would not be unusual for a bee to encounter a flower that is presenting its entire amount of pollen.

Because the secondary donor was drained of nectar by the first visit, we replenished its supply by transferring nectar from another flower with a glass microcapillary tube. In nature, of course, such replenishment would not

occur so quickly after a visit and replenishment might not be complete. Still, *Erythronium grandiflorum* flowers do resecrete nectar after being thoroughly drained with filter-paper wicks (although the amounts have not been quantified; J. D. Thomson, unpubl. obs. 1993).

We introduced a second bee, clean and chilled, on a second warm-up bouquet. After this bee began to feed normally, we presented the secondary donor flower and then a minimum of 10 more recipient flowers. After each visit, we moved the flower to a clean plastic box, being careful not to dislodge pollen. We tape-recorded a narrative of the entire sequence, noting the duration of each visit and grooming session.

Immediately after each run, we counted red grains on the stigmas of all recipients under a dissecting microscope at ×50. We also counted all red grains on other floral parts, including the style, ovary, filaments, anthers and the inner surfaces of the tepals. Initially, we attempted these counts on the secondary donor, both before and after the second bee's visit, but the large numbers of grains prevented accurate 'before' counts without manipulating and disturbing the flower excessively.

To estimate the before counts, we added another experiment in 1995 in which we applied red grains directly to yellow-pollen donors. First, we chose one inner tepal and marked two circles of 3.3 mm diameter on it, using an inked piece of thin-wall plastic tubing as a rubber stamp. One circle (the 'proximal') was centered 7 mm from the base of the tepal; the other, or 'distal', was centered 7 mm from the tepal apex. The adjacent filament was also marked with a small ink dot. With a paintbrush, we then applied red grains to both circles, the filament and the stigma. We strove to spread the grains evenly, breaking up clumps to ease counting and to render the fates of grains more independent. We did not apply red pollen to anthers in this fashion, as the application process would have disrupted the existing yellow pollen and the abundant yellow grains would have obscured many red ones. Before counting the grains, we shook the flower to dislodge any loose grains.

We then counted the grains in each of the four application zones, secured a visit by a single *Bombus occidentalis* queen and immediately recounted the grains in the zones. Because we had previously shaken the flower, we assumed that none of the applied grains would be removed except by direct contact from the bee. In what follows, we assume that all of these grains adhered to the bee, at least briefly, rather than being knocked out of the flower. Therefore, we considered the difference between the pre-visit and post-visit counts to be the numbers of grains that were 'remobilized.' For analysis, we expressed remobilization as a fraction ([pre-visit count – post-visit count]/pre-visit count) and then transformed using logits. We scored the four zones for eight flowers.

To test for zonal differences in the remobilization fractions, we used generalized linear models after performing Hartley's test (Sokal & Rohlf 1995) to check for differences among variances in the four zones. In attempting to test zonal differences in a repeated-measures design with an unstructured covariance structure, SAS PROC GENMOD (SAS, Cary, NC) (Littell *et al*. 1996) was unable to converge on a solution. Therefore, we used GLIMMIX, a SAS macro that iteratively calls PROC MIXED, and then computed contrasts to evaluate pairwise differences between specific zones.

In 1997, we produced a small additional data set, to supplement the data gathered in 1993, on the numbers of grains deposited on flower parts other than the stigmas. A *B. bifarius* queen was enclosed in a cage containing clean red-pollen and yellow-pollen flowers. After the bee made an initial visit to a red-pollen flower, she was allowed to forage freely. As soon as a yellow-pollen flower received a single visit, it was removed from the cage; 10 such flowers were collected. We counted the number of red grains on the following organs or sets of organs: stigma, style, ovary, anthers, filaments and the proximal and distal portions of the tepals. The widest portion of the tepal served as the division between proximal and distal portions; grains on the abaxial surfaces of the tepal were not counted.

Results

At least one grain was secondarily transported to a stigma in all runs in 1993 (Table 1). The total number of such grains was significantly higher in brushed-anther runs (mean = 63.4) than in standard runs (mean = 14.9; Mann–Whitney *U*-test, $n_1 = n_2 = 10$, $P < 0.01$). Across all runs, secondary deposition was positively correlated with total number of red grains left on the secondary donor (product-moment $r = 0.499$, $P = 0.025$). Only a small fraction of the red grains remaining on the secondary donor were located on the stigma (standard runs 9.3%, brushed-anther runs 9.1%), leaving approximately 1000 grains stranded on non-target floral organs (Table 2a). These grains, together with those on the stigma, comprise a substantial pool for additional transport. The number of grains on the stigma of the secondary donor was not correlated with the total on all other parts of the flower (product-moment $r = 0.14$, $P > 0.05$).

Because they involve grains remaining after a single visit, instead of two visits, the 1997 data provides a more accurate picture of the extent to which bees deposit grains on non-stigmatic surfaces. Unfortunately, these data are available only for *B. bifarius*, a smaller bee and probably a weaker pollinator. Especially on larger *Erythronium* flowers, these bees can extract nectar without firmly contacting either anthers or stigma (Thomson 1986; Wilson & Thomson 1996). Furthermore, the 1997 experimental

Table 1 Numbers of secondarily deposited pollen grains on stigmas of a series of nine recipient flowers

	Recipient flower sequence number								
	1	2	3	4	5	6	7	8	9
Standard runs									
Run s1	8	1	0	0	0	0	6	0	0
Run s2	6	0	1	1	1	0	0	0	0
Run s3	5	0	10	6	0	0	6	1	0
Run s4	0	0	1	0	0	0	0	0	0
Run s5	2	0	0	1	0	1	0	1	4
Run s6	3	0	0	1	0	0	0	0	0
Run s7	3	2	0	2	0	24	0	1	0
Run s8	0	0	1	0	4	0	0	0	0
Run s9	1	0	7	0	0	0	0	1	8
Run s10	3	2	7	0	6	2	3	3	3
Mean	3.1	0.5	2.7	1.1	1.1	2.7	1.5	0.7	1.5
Brushed-anther runs									
Run b1	19	9	7	2	0	1	3	2	0
Run b2	35	26	22	7	12	1	1	9	9
Run b3	2	6	1	0	3	2	1	0	0
Run b4	1	0	3	0	1	1	4	0	1
Run b5	9	3	1	0	5	5	4	0	0
Run b6	22	18	24	30	18	11	22	13	8
Run b7	0	5	2	9	1	0	0	4	0
Run b8	19	9	11	4	5	0	3	0	8
Run b9	16	5	4	6	5	3	0	4	2
Run b10	31	18	25	5	19	17	1	5	4
Mean	15.4	9.9	10	6.3	6.9	4.1	3.9	3.7	3.2

design did not ensure that the bee had always visited a red-pollen flower immediately before visiting one of the yellow-pollen recipients. For these reasons, the 1997 flowers had lower total numbers of red grains (Mann–Whitney *U*-test, $U_s = 172$, $n_1 = 20$, $n_2 = 10$, $P = 0.005$), even though the 1993 flowers received one more pollen-removing visit. Nevertheless, the two data sets roughly agree regarding the distribution of grains across floral surfaces (see 'mean percentage' entries in Table 2), despite great variability within each data set. Grains on non-stigmatic surfaces outnumber those on stigmas, on average, by factors of 9.7 (1993) and 6.9 (1997). Anthers and filaments receive many of the non-stigmatic grains. The biggest discrepancy between the two data sets is the two-fold higher percentage of grains on anthers in the 1997 flowers. This is probably attributable to the second visit in the 1993 designs; that visit may have removed grains preferentially from anthers, but it may also have stirred the pollen on the anthers so that some red grains were covered by yellows and not counted. Regardless of details, there are usually many grains available for secondary pickup, with only a small fraction of them residing on the stigma.

In the 1995 experiments, second visits were surprisingly effective at remobilizing pollen from various floral surfaces (Fig. 1). The Hartley's test found no significant difference among the four variances. The GLIMMIX model indicated a highly significant overall effect of zone on remobilization ($F_{[3,7]} = 29.91$, $P = 0.0002$). Table 3 summarizes the pairwise contrasts among zones. The proximal zone of the tepal was cleaned particularly thoroughly; this area was usually contacted repeatedly by the top of the bee's head as she probed for nectar at the

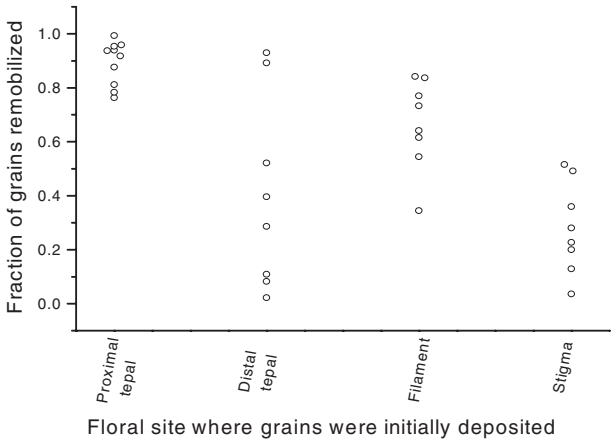

Fig. 1 Numbers of grains (expressed as fractions of numbers previously deposited on various surfaces of flowers of *Erythronium grandiflorum*) that were subsequently dislodged and presumably picked up by bumble bee visitors.

Table 2 Distribution of deposited pollen grains across various floral surfaces. Tepal counts are from adaxial surfaces only

Flower ID	Run, bee type	Grains on: Style	Stigma	Ovary	Anthers	Filaments	Tepals		Total
(a) Data from 1993; grains remaining on secondary donor after second visit (see text).									
93–1	bru, app	118	185	116	218	367	516		1520
93–2	bru, app	113	478	39	584	1154	615		2983
93–3	bru, app	63	233	4	508	100	59		967
93–4	bru, bif	107	148	52	138	282	405		1132
93–5	bru, app	90	91	19	129	293	154		776
93–6	bru, app	163	86	78	77	546	1030		1980
93–7	bru, app	145	105	18	117	257	673		1315
93–8	bru, app	53	13	28	17	128	113		352
93–9	bru, app	114	64	8	110	165	378		839
93–10	bru, app	242	152	16	374	165	690		1639
93–11	stn, app	25	133	48	47	169	1004		1426
93–12	stn, app	120	76	66	90	180	603		1135
93–13	stn, app	121	210	87	145	814	629		2006
93–14	stn, app	8	10	13	5	24	24		84
93–15	stn, occ	285	111	6	149	203	150		904
93–16	stn, occ	53	312	42	255	483	307		1452
93–17	stn, app	58	108	54	81	333	483		1117
93–18	stn, occ	61	199	59	142	415	873		1749
93–19	stn, bif	88	134	37	111	337	451		1158
93–20	stn, app	77	191	43	156	282	634		1383
Mean percentage, 1993 data		9.33	11.43	3.93	13.17	25.23	36.89		100.00
(b) Data from 1997; grains deposited after a single visit.									
							Proximal*	Distal*	
97–1	bif	35	76	8	199	214	55	95	682
97–2	bif	149	75	1	210	371	78	204	1088
97–3	bif	326	89	3	131	144	82	49	824
97–4	bif	25	21	3	41	58	12	20	180
97–5	bif	62	18	1	71	144	15	18	329
97–6	bif	93	268	14	523	193	75	127	1293
97–7	bif	18	112	16	237	235	25	68	711
97–8	bif	2	16	1	36	77	20	31	183
97–9	bif	12	4	0	22	14	0	4	56
97–10	bif	12	25	5	122	125	42	89	420
Mean percentage, all 1997 data		12.62	10.43	0.87	27.05	30.38	6.67	11.99	100.00

Abbreviations: bru, stn, brushed-anther and standard runs; app *Bombus appositus*; occ, *B. occidentalis*; bif, *B. bifarius*
*Tepals divided into proximal and distal sections at widest part, 1997 only.

Table 3 Pairwise contrasts between four flower zones for the fraction of grains remobilized by a bee visit (following the demonstration of a significant effect of zone by SAS procedure GLIMMIX). The principal result of interest is that different zones of the tepals have different remobilization probabilities

Zone 1	Zone 2	*F*-value	*P*-value	Conventional Significance	Bonferroni Significance
Distal	Proximal	27.85	0.0012	**	**
Distal	Stamen	2.52	0.1567		
Distal	Stigma	0.68	0.4365		
Proximal	Stamen	9.75	0.0168	*	
Proximal	Stigma	42.72	0.0003	***	**
Stamen	Stigma	9.19	0.0191	*	

tepal base. Bees brushed the distal zone more sporadically, accounting for the significant difference between proximal and distal zones. The stickiness of stigmas probably accounts for their greater retention of pollen than the other surfaces that received frequent contact. Overall, when a second visit occurs, grains that have been stranded on non-stigmatic surfaces have a roughly 50% probability of being set in motion again; their chances depend on their specific location.

Discussion

Our data indicate that bumble bee visits to *E. grandiflorum* leave substantial numbers of pollen grains on various floral surfaces for secondary pollen transfer. During subsequent bee visits, those grains can be remobilized and delivered to stigmas. The probability of remobilization depends on where the grains are initially deposited. The extent of delivery varies with the amount of pollen available in the recipient flowers; we infer that secondary transfer was higher in the brushed-anther runs because bees were less stimulated to groom during those runs than during standard runs (in which they continually received new doses of pollen).

Is secondary carryover harmful or beneficial?

Our data show that the pools of grains deposited on non-stigmatic surfaces are substantially larger than stigmatic deposits in *E. grandiflorum*. Although Inouye *et al*. (1994) anticipated the possibility of repeated cycles of remobilization and redeposition, they focused on secondary pickup of grains that had already been deposited on a *stigma* (p. 1553). From this assumption, they logically argued that secondary removal of these grains should be harmful and should be opposed by selection on both pollen recipients and donors. Non-stigmatic deposits have received little attention in quantitative studies of pollen fates (but see Rademaker *et al*. 1997), but they are important to interpreting the value of secondary transfer, as our data show. Unlike grains on stigmas, these grains are stranded and can make no contribution to reproduction unless they are remobilized. Secondary transfer of grains that have landed on other surfaces must be beneficial, at least from the pollen grain's perspective.

How much secondary carryover takes place?

One would like the data to specify the relative magnitudes of primary and secondary pollen transport. In his study of ants visiting *Scleranthus*, Svensson (1986) did not count pollen grains, but rather compared primary and secondary dispersal of fluorescent dyes in terms of how many recipient flowers received dye particles. He reported that the slopes of carryover curves (regressions of *ln* [particles received] on recipient-flower sequence number) were statistically indistinguishable for primary and secondary carryover, but that fewer particles underwent secondary dispersal. He did not present counts that would allow easy estimation of the relative numbers of particles, but did report that secondary carryover moved particles over 1.2 recipients, compared to 4.5 for primary carryover.

Our data also do not permit direct calculation of the relative magnitudes of primary and secondary transfer in nature. First, we were unable to measure primary deposition on the secondary donor before the second visit occurred, and second, we did not obtain a primary carryover *sequence* from the first bee. Even if we had obtained these data, they might not represent natural conditions; the relative amounts of primary and secondary carryover in the field would depend substantially on the prevailing visitation rate. Our protocol modeled one particular situation in which all the secondary carryover came from a single visit to one particular flower, the first recipient. With a higher visitation rate, many flowers in the recipient series might have been visited secondarily and some of them might have been visited several times. Thus, total secondary carryover might be higher than our estimates indicate.

However, very infrequent visitation would nearly preclude secondary carryover, because hardly any second visits would occur. Suppose that, as in our experiments, each sequence of primary recipients receives one secondary visit on average; in the real world, it would not always be the pollen-rich first recipient that received that visit – nor need the visit occur immediately after the first bee had deposited the pollen. Later bees might encounter germinated grains that would adhere to stigmas, resisting secondary transport. If visits did occur soon, by chance, the flowers might have little nectar, resulting in short visits that would transfer little pollen (Thomson 1986). These considerations indicate that our experiments may overestimate secondary carryover when visitation is infrequent.

Nevertheless, it is still of some interest to compute such an estimate for our runs, subject to the caveats above. We took data on primary pollen carryover for *E. grandiflorum* from the experiments described by Thomson & Thomson (1989). Most of those runs used *B. occidentalis* queens rather than *B. appositus*. Also, they were done in the field, so the recipient flowers did not necessarily have clean stigmas and many recipients did not have six fully dehisced anthers. However, *B. appositus* and *B. occidentalis* are similar in size and the field recipients can be thought of as falling between the brushed-anther and standard treatments, so we feel justified in using the 1989 data for this crude comparison.

Specifically, for each of the 32 runs from 1989, we calculated the total number of grains from a red donor flower deposited on the first nine recipients. The mean of those values (279.3 grains, standard error = 38.5) represents an average level of primary pollen carryover across nine recipients; this can be compared to the means for secondary carryover sequences of the same length, as in Table 1. For standard runs, secondary carryover (to stigmas) was 5.3% of primary carryover; for brushed-anther runs, 22.7%.

Rescue of stranded pollen as a selective force

Here we speculate that the beneficial aspects of secondary mobilization play a role in floral evolution. The large flower of *E. grandiflorum* has flaring, recurved tepals that do little to constrain a visitor's posture; in moving about the flowers, *Bombus* queens deposited grains over all the floral parts (Table 1). We are unaware of comparable data from other species, but it seems reasonable that non-stigmatic deposition may often equal or exceed stigmatic deposition, at least when bees are heavily laden, as these were. Given that most floral surfaces other than stigmas are not sticky, the non-stigmatic grains may be held in place less tenaciously, creating a substantial pool of grains that may be remobilized by a second visit. Depending on the site within the flower, remobilization may be high.

Because of effective grooming by the bees, these remobilized grains are not too likely to reach stigmas (Thomson & Thomson 1989), but their chances should be comparable to those of grains that are directly removed from anthers. Their chances may be somewhat diminished by positional effects – for example, grains removed from tepals onto a bee's head are not in the best position to be redeposited on stigmas, but positional disadvantages may be weakened by redistribution of grains on the bee's body. For example, in the process of grooming *Erythronium* grains off their bodies, *Bombus* generally pass the pollen backward to the hind legs, which often do contact stigmas. Any positional disadvantage may also be opposed by the tendency of secondarily visited flowers to present fewer grains in total (because the first visit will have removed most of the anther contents). To the extent that the probability of a grain being delivered to a stigma declines with the number of grains presented in a flower (i.e. diminishing returns on pollen presentation; Thomson & Thomson 1989; Harder 1990), secondarily deposited grains may gain a per-capita advantage simply because they are sparse. Certainly, the 1993 experiments show that such grains do occasionally succeed in reaching a stigma on the second try.

Grains are less likely to be remobilized from stigmas than from other flower sites. In nature, this effect is probably stronger than indicated in Figure 1, because in our experiments we counted the grains and elicited the second visit immediately after applying the grains. If more time separated the first and second visits, as we would expect in nature, grains on stigmas would start to become anchored by pollen-tube growth.

From the premise that stigmas anchor grains, but other surfaces do not, one could model remobilization as a Markov-like process wherein grains on different floral surfaces have surface-specific probabilities of being remobilized. If there were no losses from the system, *all* grains would eventually arrive at stigmas as the number of visits approached infinity. Although this fantasy is drastically unrealistic, it may not be far-fetched to think that some of the adaptive significance of, say, corollas may lie in their ability to catch stray pollen grains and give them second chances. Tubular corollas with inserted anthers would be particularly effective in this role, particularly those that are large enough to enclose the entire pollinator. Substantial pollen losses could be prevented. For example, the first *Bombus* visit to an *E. americanum* flower with fully dehisced anthers removes about 62% of the available pollen, 14% of which immediately falls out of the open, pendant flower to the ground (Thomson & Thomson 1989). If the same process took place in a horizontal, tubular corolla, those grains would probably stay in the flower and thereby stay in circulation.

Our data on pollen rescue present an apparent contradiction. Given the high remobilization rates in the 1995 experiment, why did so many red grains remain on the non-stigmatic sites of the secondary donors in the 1993 experiment? The most likely explanation is that in 1993, many of the red grains were initially deposited on the *anthers* of the secondary donors. We hypothesize that the second bee may have knocked these anthers against the tepals *after* she had already drained the corresponding nectaries. Alternatively, secondary removal of red grains may have been equally high in both experiments, but the initial deposition may have been very high in 1993 in comparison to the small numbers of grains we applied by paintbrush in 1995.

Conclusion

Most treatments of pollen dynamics ignore grains that do not land on stigmas and assume that grains landing on stigmas remain. A more inclusive view recognizes that grains may go into and out of circulation several times. In diverse plant families, various floral surfaces other than anthers are clearly and precisely adapted for secondary pollen presentation (summarized by Yeo 1993). Our studies indicate that substantial secondary presentation can also occur, imprecisely, on generalized floral surfaces that show no obvious special adaptations for this role. There should be a net movement toward stigmas

from other surfaces. In the case of bumble bees, grooming losses prevent recirculation from being numerically important, but this need not be true generally. The contribution of recirculated grains to overall pollination should be greatest in species with enclosing corollas, high visitation rates, and pollinators that are slow to groom pollen off their bodies. Under such conditions – which characterize, for example, some hummingbird–plant interactions – imprecise secondary pollen presentation and pollen rescue may be prevalent enough to exert previously unsuspected selection on floral morphology and mechanism.

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