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Influence of pollinator grooming on pollen-mediated gene dispersal in *Mimulus ringens* (Phrymaceae)

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Abstract

Pollinator foraging patterns and the dynamics of pollen transport influence the quality and diversity of flowering plant mating opportunities. For species pollinated by grooming pollinators, such as bees, the amount of pollen carried between a donor flower and potential recipient flowers depends on how grooming influences pollen transfer. To investigate the relationship between grooming and pollen-mediated gene dispersal, we studied bumblebee (*Bombus fervidus*) foraging behavior and resulting gene dispersal in linear arrays of *Mimulus ringens*. Each of the 14 plants in an array had a unique multilocus genotype, facilitating unambiguous assignment of paternity to 1050 progeny. Each plant was trimmed to a single flower so that pollinator movements could be linked directly to resulting gene dispersal patterns. Pollen-mediated gene dispersal was very limited. More than 95% of the seeds sired by a donor flower were distributed over the first three recipient flowers in the visitation sequence. However, seeds were occasionally sired on flowers visited later in the pollinator's floral visitation sequence. Intensive grooming immediately following pollen removal from a donor flower significantly increased the decay rate of the donor flower's gene dispersal curve. These results suggest that the frequency and relative intensity of grooming can have significant effects on patterns of pollen-mediated gene dispersal from individual pollen donors.

Keywords: Bombus, gene dispersal, paternity analysis, pollen carryover, pollinator grooming.

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Introduction

In animal-pollinated plants, patterns of pollinator foraging and the dynamics of pollen transport strongly influence the genetic structure of populations (Wright 1931, 1938; Jain & Bradshaw 1966; Turner *et al*. 1982) and neighborhood size (Wright 1946; Levin & Kerster 1974; Crawford 1984; Karron *et al*. 1995b). The number of flowers receiving pollen from a donor flower depends on the extent to which pollen removed from the donor is available to be deposited onto successive flowers in the visitation sequence (Harder & Barrett 1996). This 'pollen carryover' between flowers separated by more than one flight (Crawford 1984; Waser & Price 1984) can lead to a leptokurtic distribution of pollen-mediated gene movements: substantial short distance dispersal and occasional longer distance dispersal. For species pollinated by grooming pollinators, such as bees, the amount of pollen 'carried over' between a donor flower and potential recipient flowers may also depend on the extent and timing of pollinator grooming (Morris *et al*. 1995; Rademaker *et al*. 1997). Intensive grooming is thought to reduce the amount of pollen from a donor flower that is available to be deposited onto later flowers in the visitation sequence (Thomson 1986; Harder & Barrett 1996; Castellanos *et al*. 2003). However, despite the potential importance of this mechanism for patterns of pollen transport, few studies have examined the effects of grooming on pollen carryover or pollen-mediated gene dispersal.

Our understanding of the patterns of pollen-mediated gene dispersal has been influenced by two traditions: ecological studies that emphasize pollinator behavior and the dynamics of pollen removal, transport and deposition (e.g. Price & Waser 1982; Thomson 1986; Thomson *et al*. 1986; Waser 1988; Willmer & Stone 2004) and population

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genetic studies that present cumulative pollen-mediated gene dispersal over an entire season (e.g. Ellstrand *et al*. 1989; Nason *et al*. 1998; Van Rossum *et al*. 2011). Studies combining observations of pollinator behavior with detailed data on the resulting patterns of pollen-mediated gene dispersal are rare, but can provide unique insights into the mechanisms influencing plant mating patterns (Harder & Barrett 1996; Mitchell *et al*. 2009).

Bateman (1947) proposed that pollen deposition from a donor plant declines exponentially as a pollinator visits flowers sequentially in a floral visitation bout. Empirical studies of pollen and/or pollen analogue transfer have shown that dispersal often declines more rapidly during pollinator visits to the first few recipients and much more slowly during later visits than is predicted by an exponential distribution (Price & Waser 1982; Waser & Price 1984; Thomson 1986; Thomson *et al*. 1986; Waser 1988). To address these limitations ecologists in the 1990s developed phenomenological continuous functions that allowed for these extended 'fat' tails (Kareiva *et al*. 1994; Morris *et al*. 1994; 1995).

In contrast to the focus of pollination ecologists on individual episodes of pollen dispersal, population geneticists often estimate the cumulative effect of pollen-mediated gene dispersal across hundreds of individual floral visitation sequences. These patterns are often disproportionately influenced by rare long-distance dispersal events (Wright 1946; Crawford 1984). Population geneticists typically characterize the cumulative dispersal curve using a variety of continuous probability density functions known as 'dispersal kernels'. These curves are not usually fitted to pollen deposition data from single pollinator visits, but are instead applied to paternity data (Oddou-Muratorio *et al*. 2005; Devaux *et al*. 2007) or related to the genetic structure of offspring within and among maternal sibships (Smouse *et al*. 2001; Robledo-Arnuncio *et al*. 2006).

Despite fundamental differences in the scale (flower-to-flower versus deme-to-deme) over which dispersal is studied, and in the types of data and analytical tools used, both pollination ecologists and population geneticists are ultimately concerned with the shape of the distribution of total and/or effective pollen grains moved between donor and recipient flowers, inflorescences or populations. In the current study we combine these two approaches in order to enhance our mechanistic understanding of the factors influencing pollen transport in *Mimulus ringens*, a *Bombus*-pollinated wetland plant.

In our experimental study each plant had a unique multilocus combination of homozygous allozyme genotypes, facilitating unambiguous assignment of paternity. Inferring patterns of pollen carryover using paternity data enabled us to distinguish pollen-mediated gene dispersal from each donor flower in a pollinator's floral visitation sequence. With this information we could explore how the timing of grooming influenced patterns of pollen-mediated gene dispersal. To characterize the shape of the dispersal curve, we used a well-studied probability density function that population geneticists have previously used to describe landscape-scale pollen-mediated gene flow (Cresswell *et al*. 2002; Austerlitz *et al*. 2004; Robledo-Arnuncio *et al*. 2006).

We use this combination of behavioral and population genetic approaches to address the following questions: (i) what is the shape of the pattern of pollen-mediated gene dispersal in *Mimulus ringens*; (ii) how consistent are dispersal curves for different donor flowers; and (iii) how does pollinator grooming behavior affect patterns of pollen-mediated gene dispersal?

Materials and methods

Study species

Mimulus ringens L. (Phrymaceae) is a diploid perennial herb native to wetlands of central and eastern North America. Plants produce showy purple hermaphrodite flowers in displays ranging from one to more than 15 flowers. The zygomorphic flowers unfurl and the anthers dehisce before dawn. Stigmas usually close within 7 h following anthesis (Mitchell *et al*. 2004) and the corollas fall off by mid-afternoon. The flowers are visited primarily by bumblebee workers (Karron *et al*. 1995a,b; Mitchell *et al*. 2004) that forage for both pollen and nectar (mean volume = 0.729 μL; mean concentration = 25.1% sugar). *Mimulus ringens* is self-compatible and has a mixed mating system (Karron *et al*. 1995a; 1997, 2004). Nearly every flower produces a capsule containing 700–6000 seeds (Karron *et al*. 2004). In most capsules the seeds are sired by multiple pollen donors (Mitchell *et al*. 2005; Karron *et al*. 2006).

Arrays of plants with unique genetic markers

To facilitate paternity assignment, we established potted experimental arrays composed of 14 *M. ringens* plants, each with a different multilocus combination of homozygous genotypes at four unlinked allozyme loci (Karron *et al*. 1995a, 2004). In early June 2003 we transplanted 20 ramets of each of the 14 different 'genets' (plants with distinct multilocus genotypes) into 25.4 cm pots, and placed them in an experimental garden at the University of Wisconsin-Milwaukee Field Station (Saukville, WI, USA). The garden contained a diverse collection of old field vegetation, including several *Bombus*-pollinated species. Bees regularly visited the garden throughout the summer. The nearest natural population of *M. ringens* was >10 km from our site, so contamination from other populations was unlikely.

We used linear arrays, rather than two-dimensional arrays, to increase the likelihood that each pollinator flight segment had the same unit distance. Although our previous work with two-dimensional arrays demonstrated that bumblebees frequently move between adjacent *Mimulus* plants (Karron *et al*. 1995b); our pilot experiments indicated that we could increase this proportion from 70% to >90% using linear arrays. To ensure that the flowers in each array were not visited prior to our pollinator observations, we placed the *Mimulus* plants into one of two pollinator-free enclosures. These screened enclosures were also positioned on opposite sides of our plot to increase the likelihood that bumblebees would forage systematically along a linear array.

On 25 August 2003, all 20 potted ramets of each of the 14 genets were placed in the screened enclosures. The floral displays on each ramet were trimmed to a single open flower just after dawn on 26 August. Ten trial arrays of 14 unvisited genets were set up sequentially between 08.30 and 11.30 hours central standard time. In each array, the genets were spaced 0.75 m apart. The order of genets was consistent across arrays to facilitate comparison between foraging sequences. We allowed approximately 18 min between exposures of different arrays to ensure that any pollinators that had visited an array left the area before the next array was exposed.

Each array was visited by a single wild *Bombus fervidus* worker. As many *B. fervidus* workers were foraging in the vicinity, it is unlikely that the same worker visited multiple arrays. Entire foraging sequences (trials) were recorded by following the pollinator with a Sony Video 8 Handycam with 12× optical zoom (Park Ridge, NJ, USA). Following visitation, each array was immediately returned to the screened enclosure to prevent further visitation by a second pollinator. In three of the trials pollinators visited every flower once in sequential order, whereas in the other trials flowers were occasionally revisited. We selected the three trials where every flower received a single visit in sequential order for our paternity analyses. For these three trials, pollinator grooming behavior following each floral visit was scored from the videotape in the following three very distinct categories: (i) no grooming; (ii) light grooming; and (iii) intensive grooming. Intensive grooming bouts were conspicuous and could easily be scored from the video when played at full speed. The pollinator generally started grooming as it left the flower and continued grooming while either hanging from the flower or hovering. Light grooming bouts were extremely rapid and were confirmed by playing the video frame-by-frame. During these grooming bouts the bee generally scraped its head a few times with its fore- or middle legs after exiting the flower. The bee then immediately flew towards the next nearest flower.

Quantifying gene dispersal

In early October fruits from each flower in the three complete floral visitation sequences were collected, air-dried and stored in a dark, low-humidity chamber at 4°C. The total number of seeds in each of the 42 fruits was determined using a high-resolution scanner and image analysis software (Scion Corporation 2000). Seeds from each fruit were then germinated in separate pots. Germination and survival rates exceeded 90%. When the seedlings were 2 weeks old, they were transplanted to 5 cm square cells in plastic flats.

Five-week-old seedlings were genotyped at four unlinked allozyme loci using the methods of Karron *et al*. (2004): tissue from young leaves was ground in the extraction buffer of Ritland (1989). Shikimate dehydrogenase (Skd-1, EC 1.1.1.25) and aconitase (Aco-3, EC 4.2.1.3) were resolved on horizontal starch gels with a morpholine citrate pH 6.1 buffer (Ritland & Ganders 1987). We ran the morpholine citrate gels for 7.5 h at 35 mA. Glutamate oxaloacetate transaminase (Got-1, EC 2.6.1.1) and acid phosphatase (Acp-1, EC 3.1.3.2) were resolved on starch gels with a lithium–borate pH 8.1 buffer (Ridgeway *et al*. 1970). The lithium–borate gels were run for 5.5 h at 65 mA. Staining protocols followed Wendel and Weeden (1989).

Twenty-five seedlings were genotyped from each of the 14 fruits in each of the three replicate floral visitation sequences. Therefore, we assigned paternity to a total of 1050 seedlings. Following paternity assignment we calculated the number of offspring from each donor in each fruit. Approximately 18% of the seeds were the result of within-flower self-fertilization, and we therefore excluded them from our subsequent analyses of among-plant pollen-mediated gene movement.

Statistical analyses

All statistical analyses were carried out using R (R Development Core Team 2007). We used an analysis of covariance (ANCOVA) to test for differences in the number of outcross seeds sired by each pollen donor resulting from the donor's position in the bee's floral visitation sequence. In this analysis we used the position of the first intensive grooming event relative to pollen removal from the donor flower as the covariate using the *lm()* function of R (R Development Core Team 2007). We also tested for differences in the number of outcrossed seeds set by each hermaphrodite flower as a result of the flower's position in the floral visitation sequence. In this analysis we used the position of intensive grooms prior to visiting the recipient as the covariate.

To estimate the shapes of *M. ringens* dispersal kernels (the probability density function of pollen-mediated gene

dispersal) we limited our analyses to the first eight donor flowers so that we could examine siring success on a minimum of six recipient flowers. As pilot data indicated that almost all *M. ringens* pollen is dispersed to one of the first six recipient flowers in the visitation sequence, our design ensured that we could characterize the shape of the bulk of the dispersal curve for each donor flower. To generate data suitable for estimating dispersal kernels we first approximated the probability density of gene dispersal distances for each pollen donor. The classical approach to approximating probability density is a histogram re-scaled vertically (normalized) to have a total area of 1; known as the density histogram (Tukey 1972; Velleman & Hoaglin 1981). Owing to the symmetry of the dispersal kernel we selected (see below), the density histogram was scaled to have a total area of 0.5 so that we could carry out our curve fitting using only the 'positive' half of the distribution. We generated 24 density histograms (eight donor flowers in each of three runs.)

To characterize dispersal in terms of a continuous probability density function we fit the following symmetrical one-dimensional exponential-power model (Clark 1998) to the histogram data using least-squares non-linear regression (Gauss–Newton algorithm; R Development Core Team 2007):

$$f(r) = \frac{b}{2a\Gamma\left(\frac{1}{b}\right)} e^{-\left(\frac{|r|}{a}\right)^b}$$

In this model r represents the dispersal distance, a is the scale parameter, b is the shape parameter and $\Gamma()$ is the classically defined gamma function. When $b = 2$ the model generates a normal distribution (thin-tailed) and when $b = 1$ it generates an exponential distribution (thin-tailed). When $b < 1$ the dispersal kernel decays more slowly than exponentially (fat-tailed) and when $b > 1$ the dispersal kernel decays more quickly than exponentially (thin-tailed). To characterize the average dispersal curve for our plant-pollinator system, we first fit the model to the 24 pooled density histograms using the *nls()* module of R (R Development Core Team 2007). However, non-linear curve fitting based on ordinary least squares (OLS) and using the Gauss–Newton algorithm is relatively insensitive to observations that occur with very low frequency (the 'tails' of the distribution in this case). Therefore, in an effort to better fit the tails we also used an iteratively reweighted least squares (IRLS) regression with Huber and Hampel influence (psi) functions to robustly fit the distribution using *nlrob* in R (Hampel *et al*. 1986; R Development Core Team 2007; Huber 2009).

The non-linear curve fitting procedures did not converge on realistic estimates when we jointly estimated the scale (a) and shape (b) of the exponential-power distribution individually for each of the first eight pollen donors in the three trials (24 individual distributions). Therefore, to estimate the shape (parameter b) of the pollen-mediated gene dispersal distribution for each of the 24 individual distributions, we first estimated the scale parameter for each of the first eight donors (pooled between trials) by setting $b = 1$ (i.e. assuming that each distribution was exponential) using *nls()*. We then estimated b for each of the 24 individual unit area histograms by holding the scale parameter (a) fixed at the value estimated for each individual genet. For example, when estimating the shape of the dispersal distribution for pollen donor D in trial two, we fixed the scale parameter to the value estimated for genet D while assuming an exponential distribution over all three trials.

To examine how the timing of grooming influences the shape of the dispersal curve from a donor flower, we noted how many flowers were visited after the donor flower until the next high intensity grooming event. We then tested for differences in the estimated exponential-power shape parameters resulting from the pollen donor's position in the bee's floral visitation sequence using ANCOVA. In this analysis we considered the position of the first intensive grooming event relative to pollen removal from the donor flower as the covariate in *lm()* (R Development Core Team 2007). Because pollinators either groomed lightly or intensively after each floral visit in the present study, we did not need to consider floral transitions during which the pollinator did not groom.

Results

Bumblebees groomed lightly after every floral visit. The mean interval between intensive grooms was 3.71 floral visits (vertical arrows in Fig. 1). The position of intensive grooming events in the visitation sequence did not significantly influence the number of seeds per fruit or the number of seeds sired by individual pollen donors ($P > 0.35$).

Every flower in the three arrays set seed following a single bee visit (Fig. 1). The mean ± standard error number of seeds per fruit was 1596.2 ± 108.0. Most of these seeds resulted from outcrossing (outcrossing rate, $t = 0.82 \pm 0.04$).

Patterns of pollen-mediated gene dispersal

More than 95% of the seeds sired by a donor flower were distributed over the first three recipient flowers in the visitation sequence. However, individual pollen donors occasionally sired seeds on more distant recipient flowers (up to 13 flowers beyond the donor flower; Fig. 2). The exponential-power model explained more than 80% of the variation (R^2) in the pooled distribution of male fertility

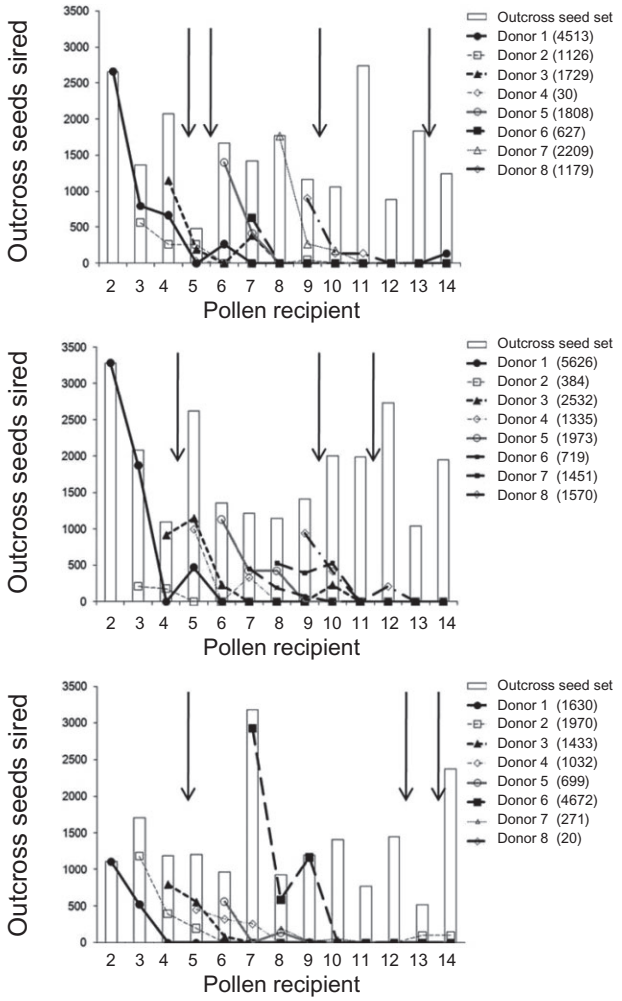

Fig. 1 Genet-specific gene dispersal distributions for the first eight flowers in three replicate floral visitation sequences. The vertical bars depict the outcross seeds for each of the 13 recipient flowers. The vertical arrows indicate intensive pollinator grooming. The legend shows the first eight pollen donating genotypes depicted by each line. The numbers in parentheses give the total number of seeds sired by each pollen donor across all recipients (excluding selfs).

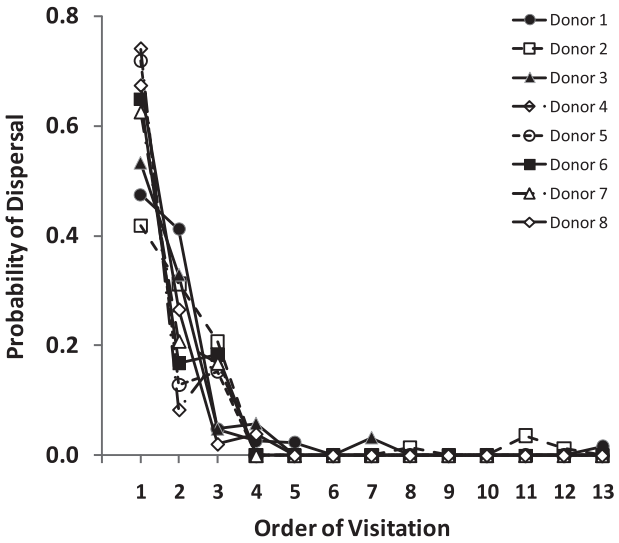

Fig. 2 Average probability of gene dispersal for the first eight flowers in three replicate floral visitation sequences. Values are back-transformed to total to 1.0.

Table 1 Non-linear regression analysis of pollen-mediated gene dispersal for 24 pooled unit-area approximations of the probability density of *Mimulus ringens* gene dispersal

	R^2	Parameter	Estimate
Gauss–Newton	0.87	*a*	1.6728
		b	2.0388
Huber Ψ	0.92	*a*	1.6617
		b	1.7752
Hampel Ψ	0.89	*a*	1.6772
		b	1.9289

The table shows the coefficient of determination (R^2) for the regression and best-fit parameter estimates for three different estimation procedures.

for *M. ringens* pollen donors (Table 1). Fits from each of the exponential-power model algorithms (Gauss–Newton, Huber Ψ and Hampel Ψ) gave similar parameter estimates and R^2 values (Table 1). The pooled exponential-power dispersal curves were thin-tailed ($b > 1$), suggesting that pollen-mediated gene dispersal decayed more quickly than exponentially in our linear arrays. Indeed, the fitted exponential-power distribution of pollen-mediated gene dispersal was approximately normal ($b \approx 2$), although this does not account for rare, but notable dispersal events beyond the third consecutively visited recipient (Figs 1,2; Holmquist 2005).

Individual dispersal curves were highly variable in shape, with tails that ranged from thin, to roughly exponential, to fat. Eleven of the 24 individual dispersal curves showed thinner than exponential tails ($b > 1$), reflecting very limited pollen carryover and gene dispersal. Eight curves showed fatter than exponential tails ($b < 1$). Five of the curves showed approximately exponential tails ($0.98 < b < 1.02$). However, no individual dispersal curve was normally distributed ($b \neq 2$). Furthermore, three of the 11 thin-tailed distributions showed gene dispersal only to the first recipient flower beyond the pollen donor.

Effect of pollinator grooming on dispersal distributions

Although the position of the pollen donor in the floral visitation sequence did not affect the shape of the dispersal distribution (Table 2), the position of high-intensity grooming events relative to pollen removal from the

Table 2 ANCOVA showing the effect of the donor flower's position in the bee's floral visitation sequence on variation in the shape (parameter *b*) of the exponential-power pollen-mediated gene dispersal distributions for individual pollen-donating *Mimulus ringens* flowers

Source	d.f.	MS	*F*	*P*
Position in the floral visitation sequence	7	0.0424	0.5463	0.7845
Intensive grooming	1	0.3959	5.0969	0.0434
Error	12	0.0777		

donor flower significantly influenced the shape of the dispersal curve ($F_{1,12} = 5.097$, $P = 0.043$; Table 2; Fig. 3). Grooming immediately after a floral visit increased the proportion of that flower's pollen dispersed to the next two flowers in the visitation sequence, and decreased the proportion exported to more distant flowers (Fig. 3; Tukey–Kramer test $P < 0.05$).

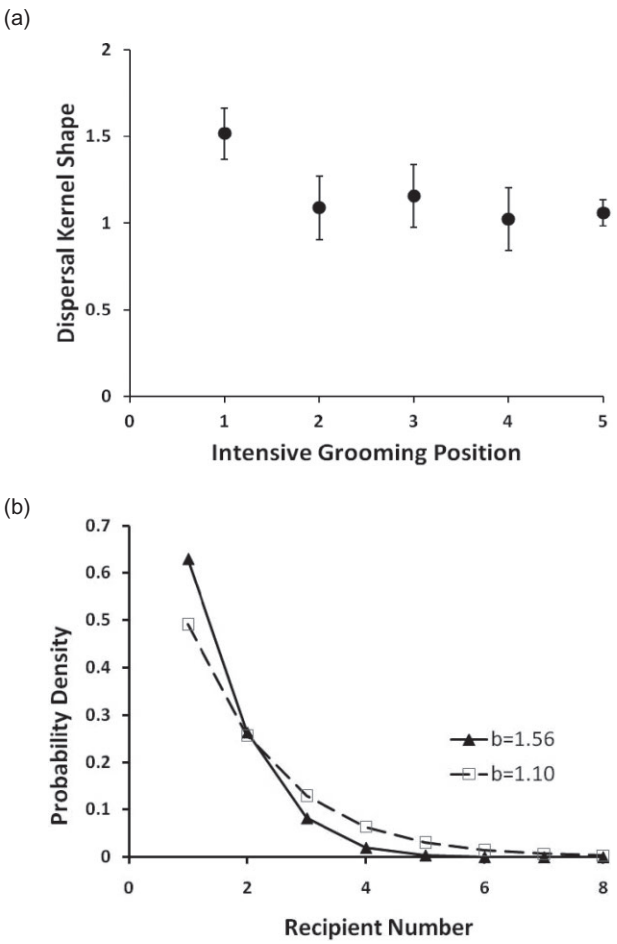

Fig. 3 Effects of the position of intensive pollinator grooming on the shape of the exponential-power dispersal kernel. (a) Comparison of values of the shape parameter across grooming positions. The *X*-axis denotes the position of the first intensive groom following a visit to a donor flower. The *Y*-axis indicates the least square mean ± standard error value of the exponential-power-shape parameter. (b) Effect of changes in the exponential shape parameter on the dispersal kernel; the dashed line shows the pattern of dispersal without grooming at position 1 (b = mean exponential shape parameter = 1.10) and the solid line shows the pattern with grooming at position 1 (b = mean exponential shape parameter = 1.56). Grooming at position 1 decreases the probability of dispersal beyond the second recipient from 25.2 to 10.7%

Discussion

Pollen-mediated gene dispersal is very limited in *M. ringens*. More than 95% of the seeds sired by a donor flower are distributed over the first three recipient flowers in a visitation sequence. This pattern of gene dispersal suggests that *M. ringens* populations are likely to have very small mating neighborhoods (Wright 1946). As *M. ringens* has a mixed-mating system, the frequency of geitonogamous self-fertilization will further reduce neighborhood area (Crawford 1984; Karron 1995b). *Mimulus ringens* floral displays usually have several open flowers and bumblebees typically visit 1–4 flowers consecutively on a single plant (Mitchell *et al*. 2004; Karron *et al*. 2009). Therefore, a large fraction of the pollen dispersed from a donor flower is likely to be deposited onto the stigmas of other flowers on the same plant (Karron *et al*. 2009).

Pollen-mediated gene dispersal is affected both by pre-pollination events, such as pollen carryover, and also by post-pollination events, such as pollen germination, pollen tube growth and ovule fertilization. We believe that pollen carryover is the major determinant of the patterns observed in our study for the following reasons. First, post-pollination events should not vary with position in the visitation sequence (e.g. pollen tube growth for a particular donor flower should not be faster on the first recipient flower than on the second). Second, the pattern of pollen-mediated gene dispersal we observed matches well with previously documented fluorescent dye (pollen analogue) carryover for *M. ringens* (Holmquist 2005). Our results suggest that pollen carryover in *M. ringens* may be among the most restricted reported (Robertson 1992; see also Castellanos *et al*. 2003). Remarkably, this restricted pollen carryover may be further reduced if competitors for pollination are present and *Mimulus* pollen is lost to heterospecific stigmas (Bell *et al.* 2005; Mitchell *et al*. 2009; Flanagan *et al*. 2010, 2011).

In our study, most of the individual gene dispersal curves had thinner than exponential tails when characterized using the exponential-power probability density function, and the overall fit to the data was distinctly thin-tailed ($b \approx 2$). This contrasts strongly with other

studies of pollen-mediated gene dispersal that typically find 'fat' tails ($b < 1$) (e.g. Morris *et al*. 1995). The thin-tailed fits of our work probably reflect two unique attributes of our study. First, we found extremely limited gene dispersal in our system, that is, there were only a few non-zero values beyond the first three flowers. Second, it is likely that we underestimated the extent of longer distance gene dispersal. Although we assigned paternity to >1000 progeny, our sample size of 25 seeds per fruit means that rare dispersal events (i.e. occurring at a frequency <0.04) may not have been detected. Despite this limitation, we nonetheless detected several matings between sires and mothers separated by up to 12 intervening flowers (Fig. 2). This suggests that although the typical pattern of pollen-mediated gene dispersal may be thin-tailed, rare long-distance dispersal events will occasionally occur. Relatively few studies have explored the effects of rare long-distance gene movements on plant mating neighborhoods (Crawford 1984; Mitchell *et al*. 2009). Often the tail of the distribution is ignored or the distribution is truncated at a distance more consistent with a normal distribution (e.g. Krauss *et al*. 2009). However, gene dispersal events that are rare and idiosyncratic within a single pollinator flight sequence may be predictable and important when summed over many separate visitation bouts. These occasional long-distance pollen movements may be of great importance in determining population genetic structure (Wright 1977; Crawford 1984; Ellstrand *et al*. 1989; Nason *et al*. 1998), even though they are hard to detect in a standard pollination ecology study.

Pollinator grooming and pollen dispersal

Like most pollen-foraging bees (Thorp 1979, 2000; Harder 1990a,b), the visitors in our study groomed to some extent after every flower visited, packing most of the removed pollen into corbiculae, and therefore reducing the amount of pollen available for transfer to stigmas. These grooming events varied considerably in relative intensity; as bees accumulated pollen on their bodies during a foraging bout, intense grooming became more frequent (see Harder 1990a). In our study, as in other studies (Thomson 1986; Rademaker *et al*. 1997; Harder & Wilson 1998; Castellanos *et al*. 2003), grooming reduced pollen dispersal and the reduction in pollen carryover depended on the timing and intensity of grooming. Grooming immediately following removal of pollen from a donor flower should reduce pollen carryover considerably because the largest loads of pollen from a particular donor are usually deposited on the first few recipient flowers (Thomson 1986; Rademaker *et al*. 1997; Castellanos *et al*. 2003).

Mechanisms of pollen-mediated gene dispersal in M. ringens

The simple exponential-power model we studied can approximate a more detailed discrete-time, mechanistic 'two-compartment model' (Harder & Wilson 1998) of pollen dispersal that can more fully describe the dynamics of pollen transport by bumblebees. In the two-compartment model the pollen collecting areas on a bee (e.g. the frons, as opposed to pollen storing areas such as scopae or corbiculae) are divided into two compartments referred to as 'safe' and 'exposed' sites (Harder & Wilson 1998). Pollen residing in both safe sites and exposed sites is slowly depleted by deposition of small fractions onto successive recipient stigmas. The pollen residing in exposed sites is also rapidly depleted by grooming. If stigmas contact safe and exposed sites with equal probability, the total pollen deposited will be the sum of pollen from these two compartments. However, to characterize patterns of pollen carryover using the two-compartment model for grooming pollinators ('horizontal heterogeneity'; Harder & Wilson 1998), pollination ecologists must not only estimate the number of pollen grains removed from a donor flower (R) and the proportions of pollen deposited (ρ) from the two interacting compartments, but also the amount of pollen re-distributed (Γ) among the two compartments and the pollen storing structures of the pollinator. By using the phenomenological, continuous-time exponential-power probability density function to describe the dispersal curve, we were able to overcome the limitations imposed by the many extremely difficult to measure parameters and transition probabilities specified in the two-compartment models.

Support for the mechanisms suggested by the two-compartment model comes from our field observations of pollen removal, transport and deposition. As a bumblebee enters a *Mimulus* flower, its face contacts the stigma as the bee forces itself into the closed corolla. The stigma brushes along the head and receives pollen lodged there. The probability that a pollen grain makes the transition from the pollinator to the stigma depends primarily on the extent of pollinator grooming. As a bumblebee forages, corbicular pollen loads rapidly accumulate and therefore 'exposed site' pollen is rapidly depleted. The dispersal distributions in our study showed steep (faster than exponential) decay. However, a patch of white *M. ringens* pollen is often visible on a restricted region between the antennae, extending from the midpoint of the clypeus to a point immediately anterior to the ocelli of foraging bumblebees (see Karron *et al*. 2006, p. 1308; Flanagan *et al*. 2009, p. 811). This region appears to be a 'safe site' that is only depleted during intensive grooming. It is possible that pollen in this region becomes buried by subsequent deposition, but re-emerges following grooming. The

re-emergence of buried pollen grains by pollinator grooming may be one of the primary mechanisms of occasional long-distance pollen dispersal (Lertzman & Gass 1983).

In conclusion, our study highlights the value of combining observations of pollinator behavior with detailed data on the patterns of pollen-mediated gene dispersal. Our results suggest that pollen-mediated gene dispersal is very limited in *M. ringens* and that pollinator grooming is an important source of variation in the shape of the dispersal curve for individual flowers. The use of a similar approach in other animal-pollinated species will further enhance our understanding of the mechanisms influencing gene dispersal and the resulting fine-scale patterns of spatial genetic structure.

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