Mechanisms of Male-Male Interference during Dispersal of Orchid Pollen

Lawrence D. Harder,[1,*] Shane A. Richards,[2] Jon Ågren,[3] and Steven D. Johnson[4]

1. Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada; 2. School of Natural Sciences, University of Tasmania, Sandy Bay, Tasmania 7001, Australia; 3. Plant Ecology and Evolution, Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18 D, SE-752 36 Uppsala, Sweden; 4. Centre for Functional Biodiversity, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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ABSTRACT: Siring success of flowering plants depends on the fates of male gametophytes, which compete for access to stigmas, stylar resources, and ovules. Although rarely considered, pollen may often compete during dispersal, affecting the processes required for export to stigmas: pollen pickup, transport, and deposition. We quantified dispersal interference by tracking bee-mediated dispersal of stained *Anacamptis morio* (Orchidaceae) pollen from individual donor flowers and inferred the affected dispersal mechanisms on the basis of the fit of a process-based model. During individual trials, all recipient flowers were either emasculated, precluding interference with donor pollen, or intact, adding potentially interfering pollen to the pollinator. The presence of competing pollinaria on bees reduced pickup of additional pollinaria, doubled the overall proportion of lost donor pollen, and reduced total pollen export by 27%. Interference specifically increased loss of donor pollen between successive flower visits and variation in deposition among trials, and it likely also reduced pollen contact with stigmas and pollen deposition when contact occurred. Thus, by altering pollen removal, transport, and deposition, male-male interference during pollen dispersal can significantly—and perhaps commonly—limit plant-siring success.

Keywords: interference competition, male-male interference, orchid, pollen dispersal, pollination, sexual selection.

Introduction

Female promiscuity promotes male-male competition for mating and fertilization opportunities. Such competition directly influences the siring success of individual males and variation in siring among males (e.g., Collet et al. 2012), with implications for sexual selection on traits that influence competitive outcomes (Skogsmyr and Lankinen 2002; Shuster and Wade 2003). Male animals compete for mates via diverse mechanisms, including scrambles for receptive females, physical contests, and showy displays or control of breeding resources to attract females (Andersson 1994). Physical contests and resource control specifically involve intentional interference of one male with the mating opportunities of other males. Flowering plants also generally mate promiscuously (Pannell and Labouche 2013) if pollinators carry pollen from different sources. This condition occurs commonly because pollinators typically deposit only a fraction of the pollen they carry on individual stigmas, so some pollen is carried over to be dispersed to other flowers (Thomson and Plowright 1980; Morris et al. 1994). Consequently, pollen from multiple donor plants can accumulate on individual pollinators and be deposited simultaneously on individual stigmas (e.g., Johnson et al. 2005; Karron et al. 2006; Hasegawa et al. 2015). Such transport of mixed pollen loads introduces the possibility of male-male interference while pollen disperses on pollinators (Lertzman and Gass 1983; Minnaar et al. 2019), which has been detected in a few cases (Cocucci et al. 2014; Duffy and Johnson 2014). As dispersal interference occurs while pollen from different males travels together on individual pollinators, it must involve direct interaction, although intent is not involved.

Pollen interference during dispersal could have two important consequences. Most generally, dispersal interference could limit a plant's pollen export[1] and siring success

* Corresponding author; email: harder@ucalgary.ca.

ORCIDs: Harder, https://orcid.org/0000-0002-2303-5076; Richards, https://orcid.org/0000-0002-9638-5827; Ågren, https://orcid.org/0000-0001-9573-2463; Johnson, https://orcid.org/0000-0002-5114-5862.

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1. We use the term "export" in reference to successful transport of pollen between conspecific plants and the term "dispersal" in reference to the processes that generate export.

by reducing pollen pickup and/or aggravating pollen loss during transport (see the model of pollen layering in Harder and Wilson 1998). These effects would also contribute to pollen limitation of seed production by recipient plants because limited pollen dispersal reduces pollen deposition on stigmas (Harder and Aizen 2010). These fecundity problems should generally select for reduced interference. More specifically, if variation in pollen traits that influence interference contributes to differential siring among plants, those traits could be subject to sexual selection (Jennions and Kokko 2010; Beekman et al. 2016; Minnaar et al. 2019).

Both overall pollen export and the nature of sexual selection will depend on the mechanisms responsible for dispersal interference. Feasible mechanisms could act during four sequential stages of pollen dispersal from a specific donor flower by animals. First, the presence of pollen on a pollinator could reduce subsequent pickup of donor pollen by a visiting pollinator (Duffy and Johnson 2014), at best delaying its dispersal if pollinators visit commonly and at worst missing its sole dispersal opportunity if visits are rare. Second, interference between pollen from different sources on a pollinator may reduce the number of flower visits during which donor pollen remains on the pollinator (i.e., increased loss during transport), limiting its dispersal. Third, pollen from more recently visited competitor flowers may block contact of a specific donor's pollen with stigmas (Lertzman and Gass 1983; Harder and Wilson 1998). Fourth, the presence of more recent competitor pollen may reduce transfer of donor pollen during contact with individual stigmas by displacing or covering it. The latter three interference mechanisms could also indirectly affect average pollen export per pollen donor if interference alters variation among dispersal sequences in the associated process (e.g., pollen loss or deposition) and stigmatic deposition of donor pollen varies nonlinearly with the amount of donor pollen on a visiting pollinator (Jensen's [1906] inequality). Any combination of these pollen interactions could cause dispersal interference as long as their net effects reduce pollen export.

These mechanisms may apply widely, but documented cases of male-male interference during pollen dispersal involve only milkweeds (Apocynaceae, Asclepiadoideae) and orchids (Orchidaceae; Cocucci et al. 2014; Duffy and Johnson 2014). Two sets of shared floral traits that govern pollen dispersal by these clades (Harder and Johnson 2008) likely increase the incidence and intensity of interference. One set involves fusion of the style and stamens into a composite structure that promotes precise pollen transfer from anthers onto pollinators' bodies and then onto stigmas of other flowers (e.g., Ollerton et al 2003; Maad and Nilsson 2004). The other set involves production of pollen dispersal structures (pollinaria) composed of pollen aggregations (pollinia; see fig. 1) connected to a device that attaches mechanically (milkweed corpusculum) or adhesively (orchid viscidium) to a pollinator. Pollinaria enable efficient pollen dispersal by individual pollinators with relatively limited loss during transport (Harder and Johnson 2008). Together, these traits allow accumulation of multiple pollinaria either in close proximity on a pollinator's body or on each other (e.g., fig. 1*b*; Coombs et al. 2012; Cocucci et al. 2014; Duffy and Johnson 2014). Consequently, (competitor) pollinaria on a pollinator could interfere with pickup of additional pollinaria, loss of pollen during transport, and/or stigmatic deposition of pollen from other pollinaria on the pollinator (e.g., fig. 1*b*). Because of the unique dispersal characteristics of milkweed and orchid pollen, the details of this interference may often differ from that experienced by the pollen of most angiosperms, which disperses as separate grains. Nevertheless, the general mechanisms involved in interference should apply broadly.

Dispersal interference should be most similar for species with granular pollen and orchids in the Orchideae tribe, as in both groups pollen removed from a single donor flower can disperse to the stigmas of multiple recipient flowers (Johnson and Edwards 2000). The pollinium of an Orchideae pollinarium is subdivided into many units, or massulae, attached to a central elastoviscin core (caudicle), like kernels on a corn cob (see fig. 1*b*; Dressler 1981). Each massula includes multiple pollen tetrads bound together with elastoviscin (Dressler 1981). When a pollinium carried by a pollinator contacts a stigma, some massulae adhere firmly enough that their elastoviscin connection to the central core breaks as the pollinator exits the flower, leaving them on the stigma. Only a fraction of a pollinium's massulae is deposited per flower visit, so other massulae remain on the pollinator to be deposited on the stigmas of subsequently visited flowers, resulting in pollen carryover (Johnson et al. 2004). Thus, from the perspective of individual dispersal units, the dispersal of massulae is subject to the same general processes as that of granular pollen. This similarity has been demonstrated by studies that exploited the ability to stain and track massulae without affecting their dispersal (e.g., Peakall and Beattie 1996; Johnson and Nilsson 1999; Johnson et al. 2005), which has not been generally possible for granular pollen until recently (Minnaar and Anderson 2019).

Here, we test the pollen interference hypothesis, assess the responsible mechanisms, and consider their possible implications for plant mating and sexual selection. The study is based on an experiment that contrasted dispersal of stained massulae from individual donor orchid flowers to the stigmas of recipient flowers during trials when donor pollinaria traveled either alone on pollinators' bodies (emasculated recipients) or in the company of possibly

Figure 1: Example of pollen interference during dispersal illustrating *Bombus ruderarius* with single, pink-stained donor *Anacamptis morio* pollinaria (pollinia indicated by red arrows) in the absence (*a*; emasculated trial) and presence (*b*; intact trial) of unstained competitor pollinaria. The stigmas of both flowers lie hidden above each bee's head. Consequently, the stained pollinia in *a* were positioned to contact the stigma when the bee probes the flower in its mistaken search for nectar, whereas those in *b* were blocked from stigma contact by unstained pollinia. Features of the structure of *A. morio* pollen aggregations are also evident, including the paired pollinaria produced by a flower (*a*) and details of individual pollinaria (*b*), with a supporting central caudicle (white *c*) and a pollinium segmented into multiple pollen massulae (white *m*). Each massula comprises many pollen grains enclosed in elastoviscin. In *b*, the pollinaria labeled *f*, *p*, and *d* have full, partial, and depleted complements of massulae, respectively.

interfering competitor pollinaria from recipient flowers (intact recipients). Interference would be evident if (i) pickup of pollinaria from recipient flowers declined as pollinators visited more flowers during trials with intact recipients and/or (ii) more donor massulae were exported to recipient stigmas during emasculated trials than during intact trials. To identify which interference mechanism or mechanisms affect dispersal of removed donor pollen (i.e., outcome ii), we also derive a model of the possible effects of nondonor pollinaria on each dispersal stage, which we fit statistically to observed counts of donor-massula deposition during these trials. Although we focus on the effects of interference for focal donor pollinaria, those effects are not intrinsically unilateral; donor pollinaria should interfere in a similar—and hence reciprocal—manner with the dispersal of nondonor pollinaria. On the basis of the results, we consider the implications of the different interference mechanisms for siring success of species with pollinaria or granular pollen and for the selection of traits that might influence interference and its mating effects.

Methods

Data Collection

We studied dispersal of *Anacamptis morio* (R.M. Bateman, Pridgeon & M.W. Chase (syn. *Orchis morio* L.) massulae) by queen *Bombus ruderarius* (Müller) at the Ecological Field Station of Uppsala University at Skogsby on Öland, Sweden, from May 25 to June 1, 2009. Flowering *A. morio* plants produce single inflorescences with about eight flowers. This species engages in generalized food deception (not mimicry), providing no nectar for pollinators (Nilsson 1984; Jersáková and Kindlmann 1998; Johnson

et al. 2004). Consequently, individual bees typically visit only one or two flowers per plant (Nilsson 1984; Johnson and Nilsson 1999; Johnson et al. 2004). Even when an individual bee visits multiple flowers on an inflorescence, self-pollination between flowers is limited because a pollinarium must reorient for its pollinium to contact a stigma, which occurs about 18 s after pollinarium removal (Johnson and Nilsson 1999; Johnson et al. 2004). Each flower produces two pollinaria, with pollinia subdivided into a mean (±SD) of 100.0 ± 16.9 pollen massulae (i.e., 200 per flower, $n = 31$ flowers; fig. 1*b*) that can be deposited independently on stigmas. We quantified the dispersal of massulae rather than of individual pollen grains. Each massula includes ~360 pollen grains (Neiland 1994) and so contains less pollen than the number of ovules per flower (~8,200; Neiland 1994). A previous 5-year study of the same population by Nilsson (1984) reported that bees with *A. morio* pollen carried a mean of 3.4 pollinaria (range, 1–11); thus, pollinaria commonly have the opportunity to interact with each other. Nilsson also found that naturally pollinated stigmas received a mean of 25 massulae (SD = 17) and that an average of only 9.9% of flowers produced fruit (range, 4.3%–30.2%). For an *A. morio* population in Scotland, Neiland (1994) reported receipt of a similar mean of 23.7 massulae per stigma, which she estimated contained roughly the same total number of pollen grains as ovules per ovary. Thus, in addition to possible competition of *A. morio* pollen during dispersal, pollen tubes may often compete for access to ovules after pollination.

We quantified massula dispersal in a glasshouse at the field station. Bees without *A. morio* pollinaria were captured while foraging near the field station and maintained in individual vials in a refrigerator. We also collected *A. morio* inflorescences with intact pollinaria and clean stigmas from a nearby population and placed them in water-filled vials for use in the experiment. On each donor inflorescence, we stained both pollinia of one flower by injecting rhodamine pink histochemical stain into the anther sacs with a syringe (Peakall 1989; Johnson et al. 2004), which generally dyes all of a pollinium's massulae. This stain does not significantly affect transfer of orchid massulae (Johnson and Harder 2018), including those of *A. morio* (Johnson et al. 2004). Stain was allowed to dry >20 min before an inflorescence was used in the experiment. We also collected a neighboring flower from the inflorescence, for which we counted the massulae of one pollinium as an estimate of pollen availability in the stained flower. Massula production correlates very strongly between adjacent flowers within inflorescences ($r = 0.971$, $P < .001$, $n = 18$ plants). A bee was allowed to visit a donor flower, and if it removed the stained pollinaria it was left for a minute to reorient before the experimental trial began. Only *A. morio* flowers were present in the glasshouse.

The experiment contrasted two types of trials during which a bee with two stained donor pollinia visited a sequence of recipient flowers. For the 16 emasculated trials, the pollinaria had been removed from all recipient flowers, precluding pollen interference (fig. 1*a*). In contrast, for the 15 intact trials, all recipient flowers had unstained pollinaria that could transfer to the bee and interfere with dispersal of stained donor pollen that the bee already carried (fig. 1*b*). Both the intact state and the emasculated state occur naturally in *A. morio* populations (Nilsson 1984; Neiland 1994), depending on whether flowers have been visited previously. During intact trials, we allowed about 1 min to elapse between visits to successive recipient flowers to permit pollinarium reorientation. Such latency periods occur naturally, as bees typically visit flowers of rewarding species between visits to individual *A. morio* plants (Johnson et al. 2003). To minimize unintended effects of trial sequence, day, time of day, and so on, we conducted the two trial types in almost strictly alternating order. Most trials (11 emasculated, 10 intact) involved visits to ≥13 recipient flowers, but four trials involved <10 flowers (minimum of six flowers). The number of visits to recipient flowers did not differ significantly between trial types (Wilcoxon rank sum test, $\chi^2 = 2.04$, 1 df, $P > .15$). Except for one intact trial during which the bee escaped after visiting 10 recipient flowers, bees were caught at the end of each trial and placed in vials in a refrigerator to facilitate removal and counting of both recipient pollinaria and remaining stained massulae on the donor pollinarium (if still present). Most bees participated in only one trial each. We examined the stigmas of all visited recipient flowers with a dissecting microscope (24×) and counted both the stained (donor) massulae and, if applicable, the unstained (recipient) massulae. For intact trials, we also recorded the number of pollinaria removed from recipient flowers to quantify changes in a bee's maximal (cumulative) pollinarium load. Total loss of donor massulae was estimated as the difference between the initial number (based on an adjacent unvisited donor flower) and the sum of donor massulae deposited on all recipient stigmas and those remaining on the bee at the end of a trial. The data resulting from this experiment have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.41ns1rnc0; Harder et al. 2020).

Analysis of Pollinarium Removal and Overall Pollen Dispersal

The general hypothesis of dispersal interference was tested using generalized linear and linear mixed models (Stroup 2013). We assessed interference with pollen removal from flowers by pollinaria already on individual bees (pollinarium load) based on whether the incidence of pollinarium removal from recipient flowers declined as bees

accumulated pollinaria during intact trials. This relation was estimated using a generalized linear mixed model (binomial distribution, logit link function), with a bee's cumulative removal of prior pollinaria, including the two donor pollinaria, as the independent variable and trial as a random effect (glimmix procedure, SAS/STAT 14.2; SAS Institute 2016). Cumulative prior removal represents a bee's maximal, rather than actual, pollinarium load, as some removed pollinaria could have been lost before a bee visited a specific recipient flower.

We evaluated differences between intact and emasculated trials for total massula export, the proportion of removed massulae deposited on stigmas, the number of residual donor pollinaria on bees at the end of trials, and the number of massulae lost during transport (genmod procedure, SAS/STAT 14.2; SAS Institute 2016). These analyses considered sampling distributions and link functions appropriate for the dependent variables (residual donor pollinaria and proportional massula dispersal, overdispersed binomial distribution and logit link function; total pollen export and massula loss, negative binomial distribution and ln link function). The analysis of the loss of donor massulae (independent of pollinarium loss) was restricted to trials that terminated while a bee still carried at least one donor pollinarium and also considered the number of these remaining pollinaria (one or two) as a fixed factor.

Pollen Dispersal Model

The data from the emasculated and intact trials allow direct comparison of pollen dispersal sequences and total massula export and loss but not of the mechanisms responsible for differences between trial types. Nevertheless, the contributions of feasible mechanisms can be inferred by fitting a model that depicts the likely processes underlying pollen dispersal patterns to these data. We implemented this approach, using observations of the total number of massulae on intact donor pollinaria (M), the number of (stained) donor massulae deposited on stigmas of individual recipient flowers (m), and the numbers of donor pollinaria (R) that remained on a bee at the end of a trial.

The model incorporated biologically relevant variables and processes that might influence the number of donor massulae deposited on stigmas as bees visited a sequence, $v = 1, 2, \ldots, V$, of recipient flowers. The influences we considered included the inferred numbers of remaining donor pollinaria and massulae on the bee, whether the donor pollinia contact the flower's stigma, and, if so, the proportion of available massulae that was transferred (fig. 2). The proportion of massulae carried over on a bee between successive flower visits and the probability of stigma contact were assumed to vary independently of a flower's position in a visit sequence. In contrast, the proportion of donor massulae on a bee that transferred to a flower's stigma could vary with the number of stigmas that the donor pollinia had contacted previously. Each component could be subject to interference by recipient pollinaria. These processes could also vary stochastically during dispersal sequences, and the governing parameters could differ among sequences (within- and among-trial trial variation, respectively). The following model represents all

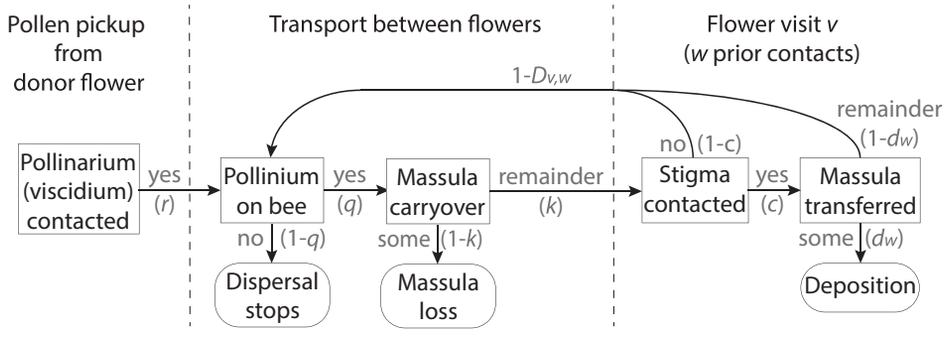

Figure 2: Processes represented in the model of dispersal of pollen from a donor *Anacamptis morio* flower. States identified in rectangles with square and rounded corners represent dynamic and terminal conditions, respectively. Dispersal involves initial pickup of two donor pollinaria followed by $v = 1, \ldots, V$ cycles of transport between and visits to recipient flowers. The number of donor pollen massulae deposited on the stigma of recipient flower v depends on the number of massulae on the pollinator at the beginning of cycle v; the proportion, k, of those massulae that remain resident during transport to flower v rather than being lost $(1 - k)$; whether the donor pollinia contact the stigma (probability c); and, if so, the proportion of donor massulae, $d_{v,w}$, transferred to the stigma. If stigma contact is uncertain (i.e., $c < 1$), the number of prior stigma contacts, w, may be less than the number of prior visits to recipient flowers $(v - 1)$, affecting pollen residence on the pollinator. Dispersal stops when no pollen remains on the pollinator, owing to pollinarium and/or massula loss and deposition on stigmas. All transitions between states could be affected by interference among pollinaria. See table 1 for symbol definitions.

of these features (also see fig. 2; see table 1 for definitions of all variables and parameters).

Pollinarium Residence. Deposition of donor pollen on the stigma of the vth recipient flower during a trial depends on whether the bee carries $r = 2, 1$, or 0 donor pollinaria. The probabilities of these three states, $a_{r,v}$, follow a binomial distribution if donor pollinaria on a bee are retained independently between flower visits with probability q. Specifically,

$$a_{r,v} = \binom{2}{r} q^{vr}(1 - q^v)^{2-r}. \tag{1}$$

The observed counts of donor pollinaria remaining on a bee at the end of a trial, R_V, provide two types of useful information for inferring $a_{r,v}$ for any visit, v. First, as q is assumed to be constant, it can be estimated on the basis of equation (1) by specifying $v = V$ and $r = R_V$ (see "Model Fitting"). Second, depending on the specific value of R_V, $a_{r,v}$ can be characterized more precisely, which we denote as $a_{r,v}(R_V)$. Specifically,

$$a_{r,v}(2) = \begin{cases} 0 & \text{if } r = 0 \text{ or } 1, \\ 1 & \text{if } r = 2; \end{cases} \tag{2a}$$

$$a_{r,v}(1) = \begin{cases} 0 & \text{if } r = 0, \\ \dfrac{a_{r,v}}{a_{1,v} + a_{2,v}} & \text{if } r = 1 \text{ or } 2; \end{cases} \tag{2b}$$

$$a_{r,v}(0) = a_{r,v}. \tag{2c}$$

Massula Residence. Fraction k of massulae on the resident donor pollinaria were assumed to persist (carry over) on the pollinator between consecutive flower visits and be available for stigma deposition, so fraction $1 - k$ was lost between visits (fig. 2). The carryover fraction was allowed to vary among trials (see "Model Fitting"), perhaps owing to differences in the placement of donor pollinaria on a

Table 1: Descriptions of symbols representing measured variables and model components, parameters and variables

Model component, parameter or variable	Description
Measured variables:	
m	Number of (stained) donor massulae deposited on stigmas of individual recipient flowers
M	Total massula number on intact donor pollinarium
R	Number of donor pollinaria remaining on a bee at the end of a trial
v	Position of a recipient flower in the sequence of visits during a single trial
V	Total number of recipient flowers visited during a trial
Model parameters and variables:	
Q	Pollinarium residence
q	Probability that a donor pollinarium remains on a bee between successive flower visits
r	Number of donor pollinaria resident on a bee
$a_{r,v}$	Probability that r donor pollinaria remain on a bee after v visits to recipient flowers
K	Massula carryover
k[a]	Carryover fraction (the proportion of massulae on donor pollinaria resident on a bee that persist between consecutive visits to recipient flowers)
l	Logit of the carryover fraction
σ_l[b]	Standard deviation of the carryover logit
C	Stigma contact
c	Probability that donor pollinia contact the stigma during a flower visit
w	Number of recipient stigmas contacted by donor pollinia during the preceding $v - 1$ visits
D	Massula deposition
d_0	Fraction of massulae deposited during the first stigma contact by donor pollinaria
d_w	Fraction of donor massulae on the pollinator deposited during the wth stigma contact by donor pollinaria
α	Per-contact fractional decline in donor massulae deposited during stigma contact
$D_{v,w}$	Expected fraction of the original M donor massulae deposited during the visit to the vth recipient flower, given that the resident donor pollinaria previously contacted w stigmas
W	Within-trial variation of massula deposition
ϕ	Dispersion parameter of the negative binomial distribution

Note: Some symbols may be accompanied by a subscript E or I to indicate that they apply specifically to emasculated or intact trials, respectively.

[a] $\tilde{k}$ and $\bar{k}$ are median and mean k, respectively.

[b] Represented by σ_E and σ_I for emasculated and intact trials, respectively.

pollinator's body or in a pollinator's tendency to groom between flower visits.

Pollinium-Stigma Contact. Donor pollinia on the pollinator were assumed to contact stigmas of recipient flowers with probability c regardless of their previous stigma contacts (fig. 2). A version of the model that allowed c to decline with successive visits fitted the data less well than the simpler model with constant c (Akaike information criterion [AIC] difference = 3.21), so we do not consider it further.

Massula Deposition. The fraction of donor massulae on a pollinator that transfers to a contacted stigma was assumed to depend on only the number of stigmas that the pollinator had contacted previously, w, and is denoted d_w. Accumulation of recipient pollinaria on the pollinator may increasingly reduce the deposition fraction, as it does for species with granular pollen (Morris et al. 1994; Richards et al. 2009). Therefore, d_w was allowed to decline with successive stigma contacts according to

$$d_w = d_0(1-\alpha)^w, \tag{3}$$

where d_0 is the deposition fraction during the first stigma contact (i.e., $w = 0$), which is reduced by fraction α during each subsequent contact. We estimated a common value of d_0, but α could differ for intact and emasculated trials. The expected fraction of the original M donor massulae deposited during the visit to the vth recipient flower, given that the resident donor pollinaria had previously contacted w stigmas ($0 \le w \le v-1$), is

$$\begin{aligned} D_{v,w} &= k^v \prod_{i=0}^{w-1}(1-d_i)d_w \\ &= k^v d_0(1-\alpha)^w \prod_{i=0}^{w-1}(1-d_0[1-\alpha]^i). \end{aligned} \tag{4}$$

In addition to describing the direct effects of massula carryover, k, and prior stigmatic contact, w, on average pollen deposition on stigmas of recipient flowers, equation (4) identifies characteristics of the indirect effect of variation in carryover among trials. Specifically, mean pollen deposition generally varies as an accelerating function of massula carryover, k, because $v > 1$ for all visits to recipient flowers except the first (e.g., black curve in fig. 3*a*). Consequently, among-trial variation in the fraction of massulae carried over between successive flower visits will generally increase average export of donor massulae over all trials owing to Jensen's inequality (fig. 3*a*; compare positions of the horizontal black dashed line and gray tick along the ordinate).

Patterns of Massulae Deposition. The numbers of donor massulae deposited on stigmas of recipient flowers depend on the combined effects of pollinarium residence (eqq. [1], [2]), pollinium contact (c), and massula carryover and deposition (eq. [4]). Given these components and initial removal of two donor pollinia with a total of M massulae, the expected (average) massula deposition if donor pollinaria contact the stigma during the visit to recipient v is

$$\bar{m}(v,w,R) = \left[a_{2,v}(R) + \frac{1}{2}a_{1,v}(R)\right] MD_{v,w}. \tag{5}$$

In this expression, the term in brackets represents the expected fraction of the two donor pollinia that remain on the bee, and $MD_{v,w}$ represents the expected number of donor massulae deposited on the stigma of recipient v if both donor pollinia remained on the bee, given stigma contact during w prior recipient visits.

In general, $\bar{m}(v,w,R)$ exhibits a characteristic deterministic decline with successive visits (i.e., as v increases; see fig. 4*c*), and deposition of donor pollen should vary distinctively around this trend. The decline occurs because transport loss and massula deposition on stigmas deplete the donor pollen on a bee. Stochastic variation arises from the probabilistic nature of pollinarium and massula loss, stigma contact, and pollen deposition (see fig. 2). The characteristics of this deterministic and stochastic variation depend on the specific values of the parameters controlling these processes. Therefore, the specific patterns of decline of observed average massula deposition and among-recipient variation can be used to infer statistically the most likely values of the controlling parameters.

Model Fitting

Analysis of variation in dispersal of donor massulae within and among trials was based on the model described above and three components of variation. The model includes five dispersal parameters, q, k, c, d_0, and α, of which all except d_0 could differ between intact and emasculated trials. In addition, k could vary among individual trials of each type, and pollen deposition could vary among visits to recipient flowers within trials. Analysis of the observations in light of this model involved two stages.

The first stage estimated the probability of pollinarium residence between flower visits (q) for the 28 trials (14 emasculated and 14 intact) for which we recorded the number of donor pollinaria on bees at the end of trials (i.e., after V visits). We used maximum likelihood methods (PROC NLMIXED, SAS/ STAT; SAS Institute 2016) based on a binomial distribution (eq. [1] with $v = V$) to estimate either q_E and q_I or a common q for both trial

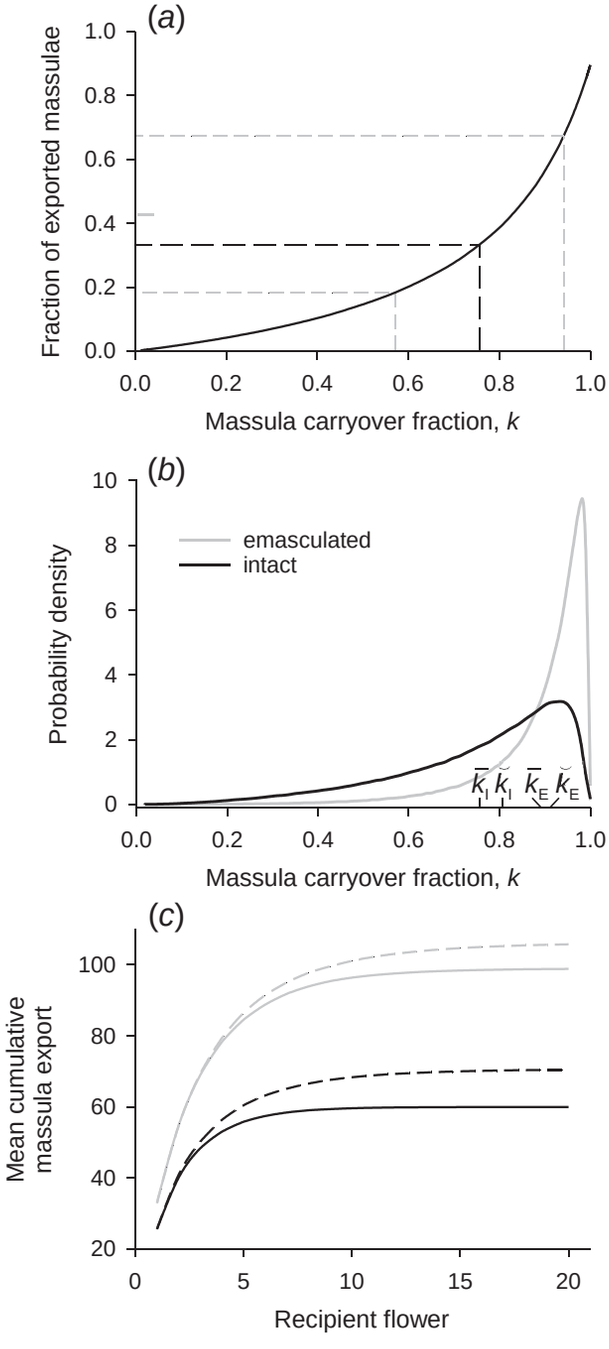

Figure 3: Effects of among-trial variation in the fraction of donor pollen carried over on a pollinator between successive flower visits on export of donor pollen, including an example of the cause of Jensen's inequality (*a*), probability density distributions of variation in the carryover fraction for emasculated (gray) and intact (black) trials based on empirical parameter estimates (*b*), and comparison of the average effects of Jensen's inequality for trials involving emasculated or intact recipient flowers (*c*). In *a*, the black curve depicts the relation of the cumulative proportion of donor pollen exported to the stigmas of 20 recipient flowers (i.e., eq. [4] summed over $v = 1, \ldots, 20$) to the fraction of donor massulae carried over on bees between successive flower visits if every visit involved stigmatic contact (i.e., $w = v - 1$), based on estimates of $\bar{k} = 0.757$, $d_0 = 0.188$, and $\alpha = 0.058$ for the intact trials. The black and gray dashed lines illustrate the relations of proportional export to the mean carryover fraction, $\bar{k}$ (black), and $\bar{k} \pm \text{SD}$ (gray; determined by simulation). The gray tick along the ordinate indicates average export for $\bar{k} - \text{SD}$ and $\bar{k} + \text{SD}$, which exceeds dispersal success for $\bar{k}$ (dashed horizontal line) owing to Jensen's inequality. In *b*, the median carryover fractions ($\breve{k}$; estimated from data) and associated means ($\bar{k}$; determined from 1,000,000 simulations) are indicated along the abscissa for emasculated and intact trials. The curves in *c* depict the change in mean cumulative export of an initial 200 donor massulae with successive visits to recipient flowers for intact (black curves) and emasculated (gray curves) trials when the massula carryover fraction was constant among trials (solid curves) or varied according to the estimated values of σ (dashed curves), based on 10,000 simulations.

types. Whether separate estimates or a common estimate best explained variation in pollinaria residence was assessed with a likelihood ratio test.

The second stage analyzed variation in the observed number of donor massulae deposited on the stigmas of 379 individual recipient flowers, m_v, for the 31 trials (15 emasculated and 16 intact) for which we had massulae counts per donor pollinarium before trials started (M). The likelihood function for these analyses was based on equation (5) and also incorporated within- and among-trial variation. These analyses included the probability of pollinarium residence between flower visits (q) estimated during the first analysis stage as a fixed parameter. To characterize among-trial variation in massula carryover, the logit, l, of the carryover fraction, k, was considered to vary according to a normal distribution with mean $\bar{l}$ and standard deviation σ_l. Specifically, the logit of massula carryover for trial i was

$$l_i = \bar{l} + \sigma_l \varepsilon_i = \ln\left(\frac{\breve{k}}{1 - \breve{k}}\right) + \sigma_l \varepsilon_i, \qquad (6)$$

where ε_i is a random deviate from a standard normal distribution, $f_N(\varepsilon|0, 1)$ (i.e., mean = 0, SD = 1). The right-hand version of equation (6) identifies that the mean logit equals the logit of the median carryover fraction, $\breve{k}$. If either $\breve{k}$ or σ_l (or both) differ between trial types, then both the mean ($\bar{k}$) and the standard deviation (σ_k) of the probability density distribution of k also differ between trial types (for examples, see fig. S1, available online). Within-trial variation in the number of donor massulae deposited on stigmas of vth recipients, m_v, was characterized as a zero-inflated negative binomial distribution, $f_{\text{ZINB}}(m_v|c, \bar{m}(v, w, R), \phi)$ (see app. S1; apps. S1, S2 are available online). Allowance for zero inflation accommodates two processes that could cause a stigma to receive zero donor massulae during a bee visit: no contact with the donor pollinia with probability

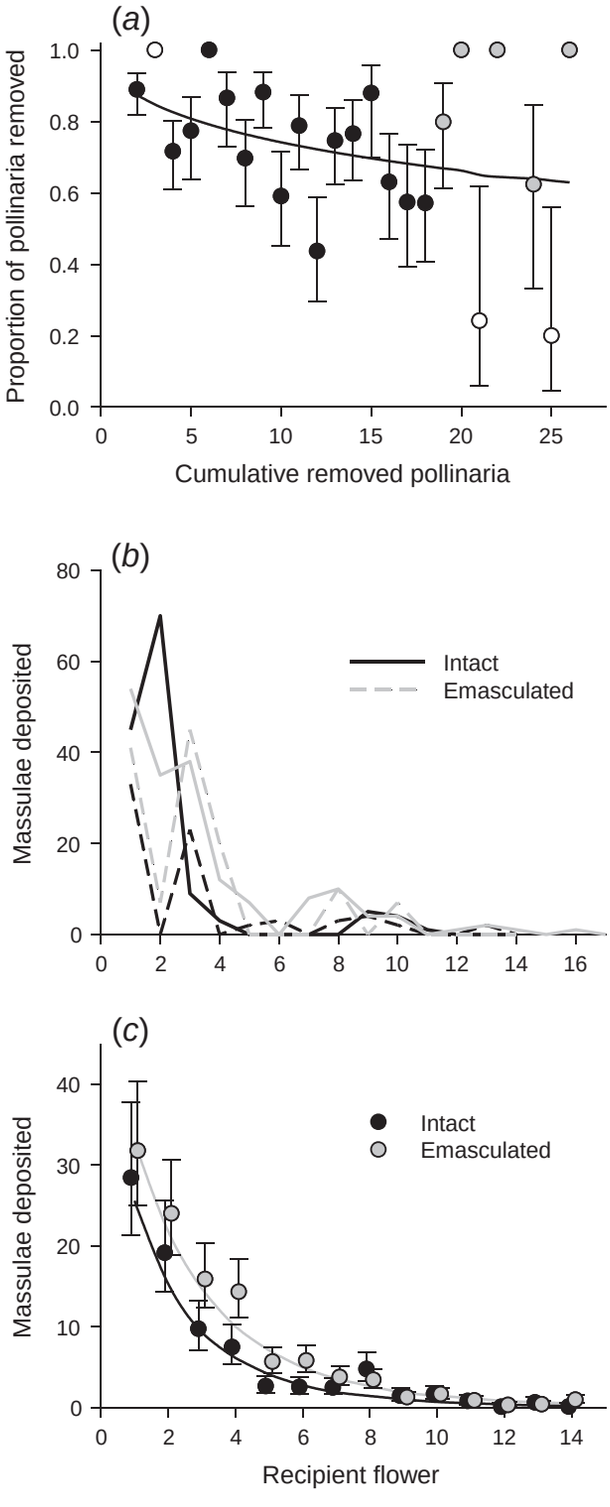

Figure 4: Effects of resident *Anacamptis morio* pollinaria carried by bumble bees on pollinarium removal from subsequently visited flowers by bees (*a*) and the export of stained donor massulae (*b*, *c*). In *a*, the cumulative number of pollinaria removed from previously visited flowers represents the maximum capacity for interference with pollinarium removal. In this panel, the curve illustrates the fitted regression relation, and symbol shading indicates sample size (white, one flower; gray, two to five flowers; black, more than five flowers). *b* depicts examples of pollen dispersal to individual recipient flowers during four individual trials, whereas *c* illustrates the least squares mean dispersal (±SE) over all trials. The trials illustrated in *b* were terminated after visits to 14 ($n = 3$) and 17 ($n = 1$) recipient flowers. The curves in *c* depict the relations for the means of each trial type based on 10,000 simulations of the model with the best Akaike information criterion, $K_k C$ (see table 2). Means ± SE in *a* and *c* are back transformed from the scale of the relevant link functions; hence, the asymmetric standard errors.

$1 - c$; or contact with probability c but no deposition as determined by negative-binomial variation (for details, see app. S1). The negative binomial component of this distribution was governed by its mean, $\bar{m}(v, w, R)$ (eq. [5]), and ϕ, which determines its variance (Richards 2008). We allowed ϕ to differ between emasculated and intact trials.

Including all sources of variation, the full statistical model comprised 11 parameters, $\boldsymbol{\theta} = \{\tilde{k}_I, \tilde{k}_E, \sigma_I, \sigma_E, c_I, c_E, d_0, \alpha_I, \alpha_E, \phi_I, \phi_E\}$ (see table 2), in addition to the estimate(s) of q from the first analysis stage. We denote this most complex model KCDW, indicating that it included separate parameters for intact and emasculated trials for massula carryover (K; $\tilde{k}_I, \tilde{k}_E, \sigma_I, \sigma_E$), stigma contact (C; c_I, c_E), massula deposition (D; α_I, α_E), and within-trial variation (W; ϕ_I, ϕ_E). We also analyzed 24 reduced models with common parameters for intact and emasculated trials for aspects of pollen carryover, stigma contact, pollen deposition, and within-trial variation. Separate parameters for at least one feature of pollen carryover ($\tilde{k}$ or σ) were included in all except the null model to account for the independent observation that intact trials involved more total massula loss than emasculated trials (see "Results"). For example, for model $K_{\tilde{k}}C$, $\boldsymbol{\theta} = \{\tilde{k}_I, \tilde{k}_E, \sigma, c_I, c_E, d_0, \alpha, \phi\}$, whereas for model $K_\sigma CD$, $\boldsymbol{\theta} = \{\tilde{k}, \sigma_I, \sigma_E, c_I, c_E, d_0, \alpha_I, \alpha_E, \phi\}$. For the null model, $\boldsymbol{\theta} = \{\tilde{k}, \sigma, c, d_0, \alpha, \phi\}$. Given a statistical model and observations of the total initial number of donor massulae (M), deposition of donor massulae on the stigma of the vth recipient flower (m_v), and the numbers of donor pollinaria remaining at the end of a trial (R_V), the likelihood of the data for a specific trial is approximated by

$$L(\boldsymbol{\theta}) \approx \int_{\varepsilon=-\infty}^{\infty} f_N(\varepsilon|0,1) \prod_{v=1}^{V} f_{\text{ZINB}}(m_v|c, \bar{m}[v, w_v, R_V], \phi)\, d\varepsilon. \tag{7}$$

The approximation arises because the actual number of donor pollinaria on a bee during a specific flower visit, R_v, was unknown except for trials that ended with $R_V = 2$ (see eq. [2]). The log likelihood of a model for all trials is the sum of their individual log likelihoods. We calculated

Table 2: Maximum likelihood fits for the effects of massula carryover, stigma contact, and massulae deposition on dispersal of donor massulae during intact and emasculated trials for models with $\Delta_{AIC} < 6$ and no simpler nested model with a lower Akaike information criterion (AIC)

	Parameter													
	Massula carryover (K)				Stigma contact (C)		Massula deposition (D)			Within-trial variation (W)				
	$K_{\breve{k}}$		K_σ											
Model	$\breve{k}_E$	$\breve{k}_I$	σ_E	σ_I	c_E	c_I	d_0	α_E	α_I	ϕ_E	ϕ_I	Log likelihood	J	Δ_{AIC}
$K_{\breve{k}}C$	**.931**	**.807**	1.174	1.174	**.999**	**.906**	.188	.059	.059	8.692	8.692	−892.30	8	0
$K_{\breve{k}}$	**.930**	**.787**	1.116	1.116	.949	.949	.188	.041	.041	8.444	8.444	−893.69	7	0.79
$K_\sigma CD$	.905	.905	**.843**	**1.910**	**.999**	**.901**	.185	**.024**	**.172**	8.529	8.529	−891.93	9	1.27
$K_\sigma D$	.905	.905	**.792**	**1.933**	.947	.947	.183	**.003**	**.186**	8.212	8.212	−893.12	8	1.64

Note: Each model has four components (K, C, D, W) that describe massula carryover (K; parameters $\breve{k}_E$, $\breve{k}_I$, σ_I, σ_E), stigma contact (C; c_I, c_E), massula deposition (D; α_I, α_E), and within-trial variation (W; ϕ_I, ϕ_E). Models are distinguished by the sets of parameters that differed between emasculated and intact trials (boldface type; e.g., $K_\sigma CD$ involved different estimates for σ, c, and α, whereas $K_{\breve{k}}$ involved different estimates for only $\breve{k}$). For all models, $q = 0.951$. J indicates the number of free parameters, and Δ_{AIC} is the difference of a model's AIC from that of the best-AIC model (1,800.60). See table S1 for information on all fitted models. $\breve{k}$ = median carryover fraction; σ = standard deviation of the logit of carryover fraction; c = probability of stigma contact by donor pollinia; d_0 = fraction of donor massulae transferred during first stigma contact; α = proportional reduction of the fraction of donor massulae transferred during each successive stigma contact; ϕ = parameter governing the variance of the negative binomial distribution of within-trial variation in pollen deposition.

the AIC for each model on the basis of its maximum likelihood and used it to select parsimonious models. A model was selected if its AIC was within 6 units of the best-AIC model (i.e., $\Delta_{AIC} < 6$) and no simpler model involving a subset of the same independent variables had a lower AIC (Richards 2008). We implemented this fitting procedure in Microsoft Excel using the SOLVER add-in (available in the supplemental material) to find the maximum likelihood parameter estimates.

Simulated Massulae Deposition

Our characterization of pollinarium residence (eq. [1] or [2], depending on whether R was known), stigma contact (c), and massula carryover and deposition (eq. [4]) represents the direct effects of pollinarium interference on mean massula deposition on recipient flowers, $\bar{m}$, but not indirect effects associated with among-sequence variance in the proportion of massula deposited on stigmas (determined by σ_E and σ_I) and Jensen's inequality (fig. 3). Therefore, we used a simulation implemented in R (ver. 3.6.3; R Core Team 2020; available in the supplemental material) to obtain complete estimates of mean donor pollen deposition on the vth-visited recipient during emasculated and intact trials based on the parameter estimates for the model with the lowest AIC (10,000 simulations per v; see app. S2).[2] These simulations also verified that the best-AIC model described patterns of deposition that are consistent with the observations.

2. Code that appears in *The American Naturalist* is provided as a convenience to readers. It has not necessarily been tested as part of peer review.

Results

Overall Dispersal Characteristics

The presence of pollinaria on bees interfered with pollinarium removal (fig. 4*a*) and overall dispersal of donor massulae (fig. 5). As intact trials progressed, bees accumulated multiple unstained pollinaria from recipient flowers (see fig. 1*b*). Correspondingly, the average proportion of pollinaria removed during visits to individual intact flowers decreased from about 0.9 to 0.6 as bees' maximal (cumulative) pollinarium loads increased from two to 25 ($F_{1, 159} = 6.30$, $P < .025$, partial regression coefficient ± SE for ln(cumulative removal) = -0.549 ± 0.219; fig. 4*a*). Of the 28 trials for which we counted the donor pollinaria on bees at the ends of trials, zero, one, and two pollinaria remained for nine, nine, and 10 trials, respectively. Overall, the mean number of residual donor pollinaria did not differ significantly between intact and emasculated trials ($G_1 = 1.88$, $P > .1$; fig. 5*a*). On the basis of the 19 trials that ended while the bees still carried some donor pollinaria, the proportion of massulae lost during emasculated trials was half that during intact trials ($G_1 = 10.93$, $P < .001$; positive effect of residual pollinarium number: $G_1 = 7.09$, $P < .01$; fig. 5*b*). Correspondingly, 36% more donor massulae were exported to recipient stigmas during emasculated trials than during intact trials (likelihood ratio test, $G_1 = 4.18$, $P < .05$; fig. 5*c*). During trials with emasculated recipients, donor flowers exported a mean of 55.0% (lower SE = 4.7, upper SE = 4.6) of removed massulae, compared with 41.6% (lower SE = 4.7, upper SE = 4.8) during intact trials ($G_1 = 3.95$, $P < .05$). This pollen export involved

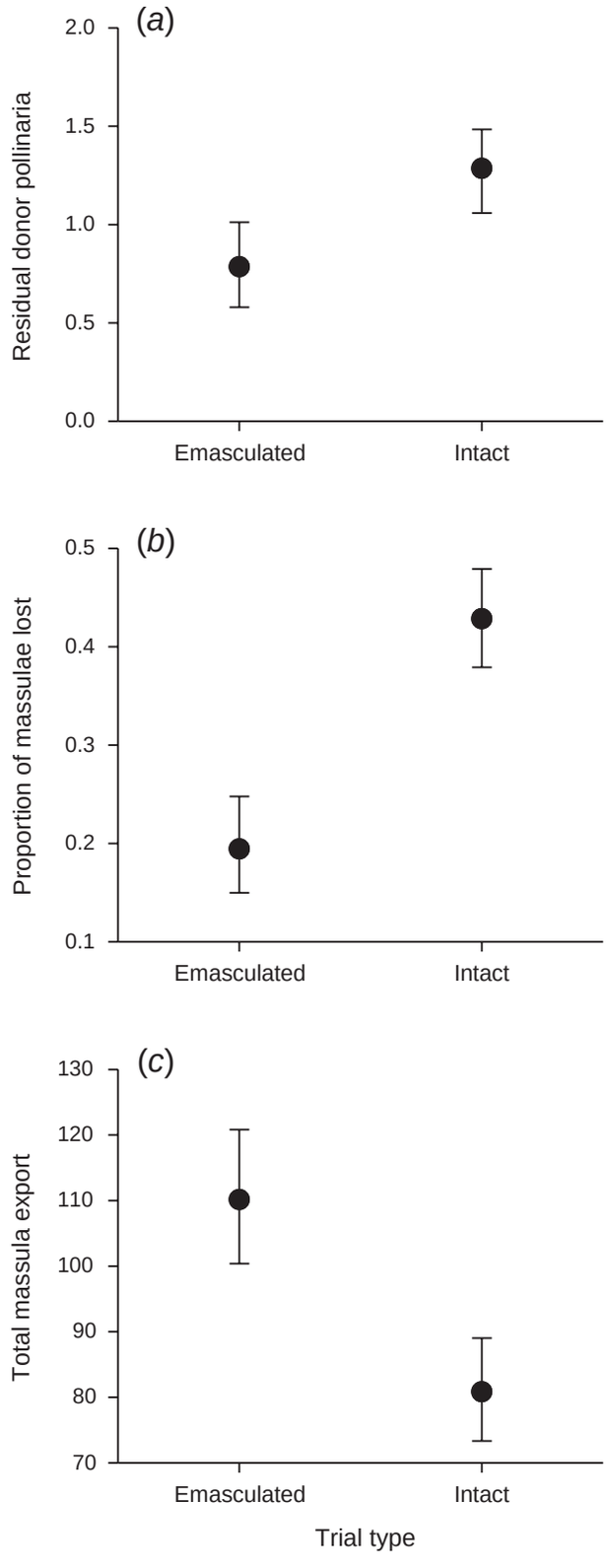

Figure 5: Comparison of overall mean (±SE) residual donor pollinaria remaining on bees at the ends of trials (*a*), total loss (*b*), and total export of donor massulae during emasculated and intact trials (*c*). Means ± SE are back transformed from the relevant link functions; hence, the asymmetric standard errors.

considerable pollen carryover on bee's bodies, as bees dispersed donor massulae to the stigmas of multiple recipient flowers (fig. 4*b*, 4*c*; maximum recipient, sixteenth of 17 visited flowers). Such carryover reflects three processes: protracted residence of pollinaria and of massulae and limited deposition of massulae from resident pollinaria on stigmas.

Interference Mechanisms

Interaction between pollinaria on individual bees had diverse consequences for the processes that caused the observed interference during pollen dispersal. The first analysis stage detected little evidence that this interaction affected the residence of donor pollinaria on bees. Owing to their sticky viscidia, pollinaria detached infrequently from bees' bodies between flower visits, with an estimated between-visit residence probability of $q = 0.951$ (95% confidence interval, 0.940–0.959). On the basis of equation (1) and this estimate, zero, one, or two donor pollinaria remained on a bee as it visited the sixteenth recipient flower with average probabilities of 0.282, 0.498, and 0.220, respectively (fig. 6*a*). The estimated per-visit probability of pollinarium residence did not differ significantly between trial types (likelihood ratio test, $G_1 = 2.55$, $P > .1$), indicating that the presence of other pollinaria on bees did not interfere with persistence of donor pollinaria.

Of the 24 massula dispersal models that we considered during the second analysis stage, four satisfied our selection criteria and were retained as candidate explanations of differential dispersal of donor massulae (tables 2, S1; table S1 is available online). The candidate models (K_kC, K_k, K_σCD, K_σD) include differences between trial types in either the median carryover fraction (K_kC, K_k) or the among-trial standard deviation of the logit of the carryover fraction (K_σCD, K_σD) but not both. Because trial type differences in either parameter influence both the mean ($\bar{k}$) and the standard deviation (σ_k) of the carryover fraction (fig. S1), the two pairs of candidate models involve differences in details of variation in the carryover fraction rather than in its fundamental characteristics. Specifically, both pairs of models agree that emasculation of recipient flowers increased mean carryover and decreased among-trial variation in the carryover fraction compared with trials involving intact recipient flowers (figs. 3*b*, S1). Because massula loss is the complement of carryover (i.e., mean loss $= 1 - \bar{k}$), this result also identifies less average loss during emasculated trials than during intact trials. All candidate models detected extensive within-trial variation

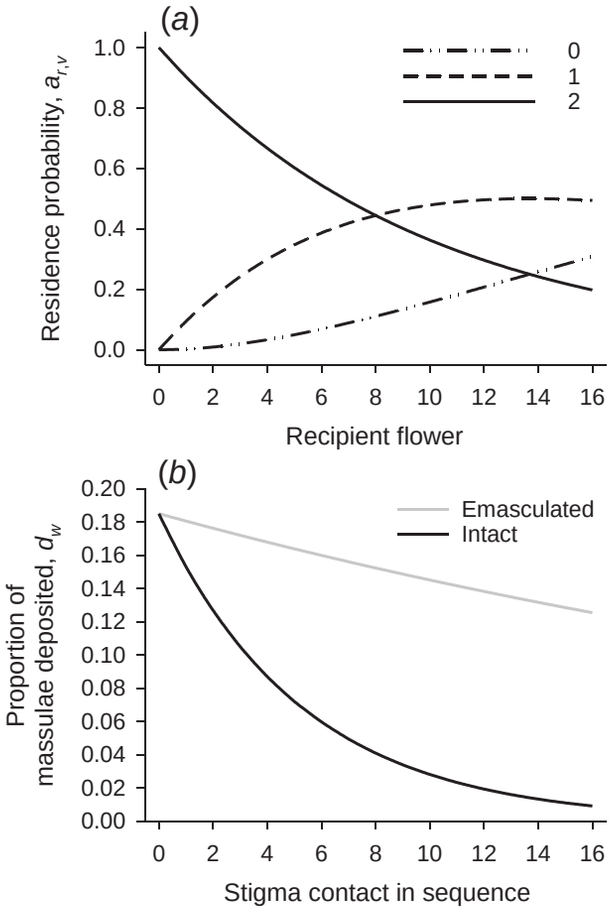

Figure 6: *a*, Changes in the binomial probabilities of the residence of $r = 2$, 1, or 0 donor pollinaria ($a_{r,v}$) during the sequence of visits, v, to recipient flowers (effect of $q = 0.951$; eq. [1]). *b*, Changes in the mean proportion of donor massulae on a pollinator deposited during the sequence of w stigma contacts, d_w (effects of α_I and α_E; eq. [3]). *b* is based on estimates of d_0, α_I, and α_E for model K_σCD (table 2).

(i.e., $\phi > 0$), but none included differences in this variation between emasculated and intact trials (table 2).

Owing to Jensen's inequality, among-trial variability in carryover enhanced average pollen export, especially for intact trials (compare solid and dashed curves in fig. 3*c*). However, the greater mean carryover for emasculated trials more than offset this variance effect (compare solid curves in fig. 3*c*). For example, on the basis of estimates of average massula loss (i.e., $1 - \bar{k}$) for the best-fitting model (K_kC), 10.8% of donor massulae were lost between successive visits to emasculated recipient flowers, on average, compared with 24.4% between visits to intact recipients. Correspondingly, total loss of donor massulae during intact trials was more than twice that during emasculated trials (fig. 5*b*).

Three candidate models also included differences between trial types in stigma contact (K_kC, K_σCD) and/or the decline of the probability of massula deposition on stigmas during dispersal sequences (K_σCD, K_σD; table 2). According to the differential contact models, donor pollinaria contacted stigmas during essentially every visit to recipient flowers during emasculated trials, compared with about 90% of visits during intact trials (table 2). According to the differential deposition models, the probability of massula deposition declined more rapidly with successive visits during intact trials (~18%) than during emasculated trials (<3%; table 2; fig. 6*b*). The ambiguity about which (if either) of these pollen-deposition processes was subject to interference likely reflects the functional correlation between stigma contact and pollen exchange from pollinarium to stigma when contact occurs. Owing to this correlation, statistical discrimination between the effects of these processes is challenging without observations of their distinguishing characteristics (e.g., documentation of stigma contact by donor pollinaria during each flower visit).

The mean dispersal patterns for simulations based on the best-AIC model, K_kC, are consistent with those observed for emasculated and intact trials (fig. 4*c*). This model included differences between emasculated and intact trials in massula carryover (and loss) and stigma contact but not in the proportion of donor massulae deposited per flower visit (table 2). As illustrated in figure 4*c*, the differences between trial types primarily manifest during the initial half of dispersal sequences, when bees still carried most donor pollinaria and massulae.

Discussion

Individual pollinators of most animal-pollinated plants typically carry pollen from multiple donors simultaneously (Johnson et al. 2005; Karron et al. 2006; Hasegawa et al. 2015), allowing male-male interaction that can alter export of a specific donor's pollen. Comparison of dispersal of *Anacamptis morio* massulae during intact and emasculated trials revealed significant interaction among pollinaria from different plants, reducing the male success of individual donors. In addition to supporting two previous demonstrations of intermale interference during pollen dispersal (Cocucci et al. 2014; Duffy and Johnson 2014), our study identified diverse effects of interference during the four stages of pollen dispersal. Specifically, interference hindered pollinarium removal by pollinators from *A. morio* flowers (fig. 4*a*), increased total massula loss during transport (fig. 5*b*), and decreased total massula export following successful pollinarium removal (fig. 5*c*). Pollinarium interaction also increased among-trial variation in the fraction of massulae carried over between successive flower visits, which enhanced average massula export owing to Jensen's

inequality (see fig. 3, especially 3*c*). However, this positive indirect effect was overwhelmed by the detrimental direct effects of pollinarium interference, reducing net pollen export during intact trials compared with emasculated trials (fig. 5*c*).

Before considering the mechanisms and implications of pollen interference, we note that our experiment provided favorable conditions for pollen transport, perhaps influencing interference intensity. Specifically, the percentage of donor massulae exported to stigmas during intact trials (41.6%) was five times greater than natural pollen-transport efficiency in the source population of the experimental plants (8.1%; Johnson et al. 2004). Two aspects of our experiment could have enhanced pollen dispersal, with contrasting consequences for dispersal interference. On one hand, the intervals between successive flower visits in our experiment (~1 min) were likely much briefer than those under natural conditions (Johnson et al. 2003). Because *A. morio* flowers provide no pollinator rewards, pollinators visit them "by mistake," typically interspersed by many visits to flowers of rewarding species (Nilsson 1984; Neiland 1994). Extended intervals between successive conspecific visits and intervening heterospecific visits would reduce the probability that individual pollinaria and massulae remain on a pollinator, diminishing interference opportunities. On the other hand, we tracked dispersal of donor pollen picked up by clean bees, so interference was initially absent and intensified as trials progressed and bees accumulated competing pollinaria (see fig. 6*b*). This contrasts with natural conditions, during which focal pollinaria may often join pollinaria already resident on a pollinator and immediately experience interference. The relative influence of these opposing effects is unknown. Nevertheless, interference likely occurs commonly during natural dispersal of *A. morio* pollen.

Interference Mechanisms

Our analysis inferred pollen-pollen interference with four of the five transitions involved in pollen dispersal between *A. morio* plants (see fig. 2). Compared with emasculated trials, interference during intact trials reduced pollen pickup (fig. 4*a*), increased massulae loss (fig. 3*b*), and possibly impeded stigma contact (table 2, models K_kC and K_σCD) and pollen deposition on stigmas (fig. 6*b*). These effects reflect two possibly interacting consequences of interference by competitor pollen: general obstruction of transfer of donor pollen during flower visits (see fig. 1*b*) and an increased chance that donor pollen is dislodged from pollinators during transport. Obstruction reduces pollen exchange from donor flowers onto pollinators (fig. 4*a*) and of donor pollen from pollinators onto stigmas of recipient flowers (e.g., fig. 6*b*). Loss of focal pollen could be aggravated by the presence of competitor pollen if pollinators groom more when they remove or carry abundant pollen (Harder 1990). In addition, pollen loss could be exacerbated by two consequences of obstruction. Competing pollinaria may displace focal pollinia (compare fig. 1*a* with 1*b*) into positions that increase contact of focal pollen with other pollinia on a pollinator's body and/or flower structures other than stigmas that dislodge it from the pollinator. In addition, if obstruction reduces deposition of focal pollen on stigmas (e.g., K_kC, K_σD, and K_σCD), its continued residence on a pollinator increases its susceptibility to loss before later deposition. Whatever its cause, exacerbation of massula loss by pollen interference strongly curtailed dispersal of *A. morio* pollen (figs. 3*b*, 5*b*).

How commonly might male-male interference occur during pollen dispersal? Interference requires two conditions: residency (pollen from individual donors must remain on a pollinator long enough to interact with pollen from other plants) and proximity (pollen from different donors must be close enough to interact during pickup or transport). Residency is the essential characteristic of pollen carryover (Thomson and Plowright 1980; Morris et al. 1994), which is a widespread feature of animal pollination (Willmer 2011). Proximity probably occurs less commonly, as it depends on the area of pollen-carrying sites on pollinators and aspects of floral morphology that govern pollen exchange between flowers and pollinators. In general, pickup and dispersal interference should be more common and intense if suitable transport sites on pollinators are limited (Duffy and Johnson 2014; fig. 1). Precise exchange (and hence close proximity) are evident in species with fused stamens and styles, such as orchids, milkweeds, and trigger plants (Stylidiaceae, Stylidioideae; e.g., Armbruster et al. 1994; Ollerton et al. 2003; Maad and Nilsson 2004; see fig. 1*b*), and those that present pollen to pollinators on the style (e.g., Muchhala and Potts 2007), including large families such as the Asteraceae and Fabaceae (Howell et al. 1993; Yeo 1993). Interference may also occur in species with less precise pollen exchange. Particularly relevant are cases in which the pollen on pollinators remains relatively undisturbed by pollinator activity and so accumulates in layers as pollinators visit more pollen-donating flowers (see Lertzman and Gass 1983; Harder and Wilson 1998). Possible examples include species pollinated by animals that groom infrequently (e.g., hummingbirds; Castellanos et al. 2003) or for which pollen is carried on locations of pollinators' bodies that are difficult to groom (e.g., Vallejo-Marín et al. 2009; Koch et al. 2017; Tong and Huang 2018). In these cases, the outer layers of a pollinator's pollen load will be more prone to deposition on stigmas and so will shelter more deeply buried pollen and interfere with its deposition on stigmas (Harder and Wilson 1998; Minnaar et al. 2019). These considerations suggest that dispersal

interference may be relatively common among animal-pollinated angiosperms, despite having been demonstrated for only milkweeds and orchids (Cocucci et al. 2014; Duffy and Johnson 2014; this study).

Interference, Siring Success, and Sexual Selection

Male-male interference with pollen removal and export by individual pollinators, such as that demonstrated for *A. morio* (figs. 4*a*, 5*c*), clearly involves detrimental competition (also see Cocucci et al. 2014; Duffy and Johnson 2014). Interference with pollen removal causes lost dispersal opportunities. Whether that loss ultimately diminishes a plant's total pollen export depends on whether the affected pollen is removed later by another pollinator or remains unremoved when the producing flower wilts. In contrast, interference-induced pollen loss during dispersal by individual pollinators irretrievably reduces a plant's total pollen export. Both interference effects can decrease a donor's representation of pollen on stigmas and in pollen-tube races to fertilize ovules and, hence, its siring success.

Most pollen of outcrossing angiosperms fails to reach conspecific stigmas (Harder 2000; Harder and Johnson 2008), so selection should favor traits that promote pollen removal, transport, and/or deposition on stigmas (Delph and Ashman 2006; Minnaar et al. 2019; Lynn et al. 2020). If such traits promote ovule fertilization in competition with pollen from other individuals, they will specifically be subject to sexual selection (Jennions and Kokko 2010; Cocucci et al. 2014; Beekman et al. 2016; Minnaar et al. 2019). Traits related to dispersal interference that are amenable to such selection could influence pollen residency on pollinators (e.g., pollinarium characteristics of orchids, pollen stickiness of species with granular pollen; Johnson and Edwards 2000; Harder and Johnson 2008; Lin et al. 2013) or the proximity of pollen from different donors during transport (e.g., stamen orientation, filament length, anther size, and shape; Minnaar et al. 2019). Although this study did not address selection directly, the results strongly suggest that dispersal interference could influence selection of traits that promote a plant's own pollen export to the detriment of export by competitor plants.

Conclusion

Carryover of pollen from multiple donors on the bodies of individual pollinators promotes male-male interference during pollen dispersal and promiscuity for most angiosperms, with possible consequences for sexual selection on floral and pollen traits that govern dispersal characteristics. Interference arises to the extent that pollen from more recently visited donor plants on a pollinator's body precludes pickup or reduces transport of competitor pollen or obstructs stigma contact and deposition by resident pollen on stigmas. Our experiment with *A. morio* demonstrated net negative effects arising from all of these processes. Thus, male-gametophyte competition occurs before deposition on stigmas as well as after (see Skogsmyr and Lankinen 2002; McCallum and Chang 2016; Swanson et al. 2016).

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Statement of Authorship

S.D.J. and L.D.H. conceived the study. L.D.H., S.D.J., and J.Å. designed and implemented the experiment. S.A.R. and L.D.H. developed the pollen dispersal model and conducted the associated statistical analysis. L.D.H. drafted the initial manuscript, and all authors participated in its revision and completion.

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"The Southern Muscadine produces its fruit in clusters of from three to eight berries, on small branches put out from all parts of the vine, and, if the soil and other conditions be favorable, is often very prolific. The berries vary in size, from half-inch to an inch in diameter. They are brown-black and shining when commencing to ripen, but a dull-black, dotted and sometimes blotched with red when fully ripe. They vary much on different vines, being sometimes hard and sour, but often tender and deliciously sweet." From "The Southern Muscadine Grape" by D. H. Jacques (*The American Naturalist*, 1868, 1:638–641).