

Pollen fates and the limits on male reproductive success in an orchid population

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Male reproductive success in higher plants depends largely on the fates of pollen, but current methodologies have given only partial insights into this important aspect of plant mating. We present a detailed analysis of the proportions and absolute amounts of stained pollen involved in six key fates for the hawkmoth-pollinated African orchid *Disa cooperi*. Despite being packaged into pollinaria, high proportions (> 0.95) of the pollen removed from anthers were lost during transport by hawkmoths in both years. The proportion of pollen lost correlated positively with the number of pollinaria removed from a plant, so that pollen export did not vary with pollen removal. Most pollen was dispersed to neighbouring plants, with rare long-distance dispersal up to 65 m. Of the pollen that reached stigmas during both years, roughly equal amounts were involved in facilitated self-pollination vs. cross-pollination, but the relative proportions of these fates differed between years. Contrary to expectation, we found that self-pollination between flowers did not increase with the number of open flowers, even though moths probed significantly more flowers on larger plants. However, during both years the fraction of removed pollen exported to other plants declined significantly with increasing self-pollination on the source plant, indicating that once self-pollination occurred it reduced (discounted) subsequent pollen export opportunities. The packaging of pollen into pollinaria in orchids appears to increase overall transfer efficiency by at least an order of magnitude relative to plants with granular pollen. Nevertheless, considerable uncertainties remain in the male reproductive success of individual orchids. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, 86, 175–190.

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INTRODUCTION

'Fate is apparently not kind to the vast majority of pollen grains removed from flowers' (Stanton *et al.*, 1992: 81, commenting on dispersal of *Raphinus sativus* pollen by honeybees).

Male reproductive success in plants is highly uncertain and varies extensively in plant populations. For species with granular pollen, typically < 1% of the pollen removed from anthers reaches stigmas (Thomson & Thomson, 1989; Stanton *et al.*, 1992; Harder, 2000). Furthermore, some plants sire no seeds and others sire as much as a quarter of the population's seed pro-

duction (Meagher, 1986; Broyles & Wyatt, 1990; Devlin & Ellstrand, 1990; Campbell, 1998; Elle & Meagher, 2000; He & Smouse, 2002; Vassiliadis *et al.*, 2002). Much of this uncertainty and variance in siring success results from the reliance of plants on vectors to disperse pollen, which introduces many opportunities for pollen to reach destinations other than conspecific stigmas. For example, the widespread limitation of seed production by insufficient pollen receipt (Burd, 1994) could arise because too few pollinators visit plants, leaving pollen in anthers, or because sloppy pollinators dislodge pollen without carrying it away from the producing plant, or because pollinators lose pollen during transit between plants. Although these alternative mechanisms all result in pollen limitation, they require very different remedies and so select for

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very different floral traits. Thus the ecological and evolutionary consequences of uncertain and variable siring success depend on the complete spectrum of pollen fates.

Six general fates result from the interaction of pollen, pollen vectors and stigmas (Harder & Wilson, 1998a; Harder, 2000). Interaction with vectors initially divides a plant's pollen into four fractions: (1) pollen with the potential to be exported elsewhere (exportable pollen, x); (2) self-pollination that does not affect export opportunities (*non-discounting self-pollination*, a); (3) pollen lost during removal from flowers (*pollen-removal loss*, f), and (4) pollen left at the site of presentation (*pollen-removal failure*, $1-a-f-x$).

Before leaving the plant, a fraction of the exportable pollen (d) can be involved in self-pollination, reducing (discounting) the pollen available for cross-pollination (*discounting self-pollination*). Therefore, the total fraction of pollen involved in self-pollination ($a+dx$) can be heterogeneous, as it comprises both non-discounting and discounting components. Of the fraction of pollen that leaves the plant ($[1-d]x$), a proportion, $1-\pi$, is lost during transport (i.e. *transport loss* fraction equals $[1-\pi][1-d]x$) and the remainder ($\pi[1-d]x$) travels to the stigmas of other plants (*pollen export*).

This characterization of pollen fates identifies a web of interactions between fates, some of which can lead to counter-intuitive outcomes (Harder & Wilson, 1998a; Harder, 2000). In general, the fractions of pollen associated with removal failure, removal loss, non-discounting self-pollination and exportable pollen trade off against each other (except non-discounting self-pollination and exportable pollen), because a plant produces a fixed amount of pollen during each flowering season. In addition, subdivision of the exportable pollen creates a tradeoff between discounting self-pollination and the pollen that vectors carry away from individual plants. However, these two components need not vary negatively between plants if plants differ in their fraction of exportable pollen. For example, if plant A has a larger fraction of exportable pollen than plant B (i.e. $x_A > x_B$), then it will experience more discounting self-pollination (i.e. $dx_A > dx_B$) and have more pollen carried away by vectors (i.e. $[1-d]x_A > [1-d]x_B$) than plants with limited exportable pollen, even though they have the same probability of discounting per exportable pollen grain (d). Finally, transport loss necessarily reduces pollen export for individual plants. The chance that transport loss reduces successful export directly can depend on the time that pollen remains engaged by a vector, as a result of repeated pollinator grooming (see Harder & Wilson, 1998b), burial on the pollinator under pollen removed from subsequently visited flowers (see Harder & Wilson, 1998b), visits to other plant species (e.g. Campbell, 1985; Feinsinger, Busby & Tiebout,

1988), or the vector leaving the population. However, as with pollen discounting, differences between plants in the fraction exportable pollen (x) can cause transport loss ($[1-\pi][1-d]x$) to vary positively with pollen export ($\pi[1-d]x$). Harder & Wilson (1997) invoked this positive relation to explain how plants can benefit by having their pollen dispersed by pollen-collecting bees.

Even though recognition of the interactions between pollen fates provides insight into the complex relations of pollen dispersal and siring success to contrasting pollination conditions (e.g. Harder, Barrett & Cole, 2000), pollen fates have received little attention (although see Levin & Berube, 1972; Stanton *et al.*, 1992; Rademaker *et al.*, 1997, 1999; Cresswell, Osborne & Bell, 2002). Harder (2000) recently reviewed estimates of the six pollen fates and found no species for which all fates had been measured. This limited knowledge of pollen fates largely reflects the difficulty of tracking pollen (reviewed by Snow & Lewis, 1993). Fate analysis requires direct measurement of the relations of pollen outcomes to production and so cannot employ either pollen surrogates (e.g. dye particles), for which initial availability can neither be controlled nor measured, or genetic markers that must be assayed in seeds or seedlings after the intervention of various postpollination processes. Thus, it is no coincidence that the most complete fate data involve *Erythronium* species (Liliaceae), which have a pollen-colour polymorphism that can be used to track pollen directly (Harder & Thomson, 1989; Thomson & Thomson, 1989; Holsinger & Thomson, 1994). Given the rarity of such polymorphisms, the prospects for studying pollen dispersal in species with granular pollen are currently limited. In contrast, the ability to either stain (Peakall, 1989) or tag (Nilsson, Rabakonandrianina & Pettersson, 1992) the aggregated pollen masses of orchids (Orchidaceae) and milkweeds (Asclepiadaceae) makes these plants the most obvious candidates for a thorough analysis of pollen fates and the factors that determine their relative magnitude.

Several features of orchid pollinaria may alter the relative incidences of alternate pollen fates and the uncertainty and variance in pollination compared to species with granular pollen (Paulus & Gack, 1990; Harder, 2000; Johnson & Edwards, 2000). The sticky viscidium of many orchids glues the pollinarium to the pollinator, which probably reduces removal and transport losses, and increases pollen-transfer efficiency, or the proportion of removed pollen that reaches stigmas (cf. Harder, 2000). In addition, the aggregation of pollen into pollinia can result in pollen export from a donor flower to only a few stigmas (e.g. Peakall, 1989; Nilsson *et al.*, 1992), in contrast to the dispersal to > 50 flowers typical of many species with granular pollen (e.g. Morris *et al.*, 1994). Such limited pollen

carryover can increase the fraction of pollen involved in self-pollination through pollinator-mediated geitonogamy (Johnson & Nilsson, 1999; Johnson, Peter & Ågren, 2004) and should limit mate diversity. However, limited carryover may be less common for the 11% of orchid species that produce sectile pollinia comprised of numerous (*c.* 50–1000) individual pollen massulae, which successively break away into small units when adhering to stigmas (Dressler, 1990, 1993; Johnson & Nilsson, 1999; Johnson *et al.*, 2004). For example, Peakall & Beattie (1991) found that pollen from individual pollinia of *Microtis parviflora* reached as many as 11 flowers.

In this paper, we quantify the fates of stained and unstained pollen to identify the causes of uncertainty and variation in pollen dispersal in a population of *Disa cooperi* Reichb.f., a terrestrial, hawkmoth-pollinated orchid with sectile pollinia. Hawkmoth abundance varies notoriously between years, directly affecting reproduction by hawkmoth-pollinated plants (e.g. Miller, 1981; Pettersson, 1991), presumably because of changes in the relative incidence of alternate pollen fates. To assess the consequences of such between-year variation, we studied a single population of *D. cooperi* during 2001–03. During each year, we considered variation and covariation in pollen fates among plants, including pollen removal failure, self-pollination, combined pollen removal and transport losses, and pollen export. We are not able to distinguish explicitly between non-discounting and discounting self-pollination, but we propose that this species experiences little non-discounting self-pollination, because pollen removal requires the action of a pollinator. In support of this argument, we estimate the relative contributions of facilitated autogamy and geitonogamy to self-pollination. As geitonogamy always discounts pollen export (Lloyd, 1992), a high proportion of geitonogamy would indicate that most self-pollination reduced opportunities for male outcrossing. Finally, we examined the spatial extent of pollen export for individual plants and its relation to other pollen fates.

MATERIAL AND METHODS

STUDY SPECIES

Flowering *Disa cooperi* plants produce a single spike up to 70 cm tall bearing up to 50 flowers, on which a mean of 13.3 flowers (SD = 4.8, $N = 36$) are open at once. Each flower has a spur about 4 cm long, which contains approximately 1.5 μ L of nectar with a sugar concentration of roughly 35% (Johnson, 1995). The flowers begin emitting a sweet scent at dusk. Hawkmoths, most commonly *Basiothia schenki* Möscher, visited *D. cooperi* flowers during the first 30 min after

dusk (Fig. 1: see Johnson, 1995 for details), although moths occasionally visited later. Each flower produces two pollinaria *c.* 5 mm long. A pollinarium includes a sectile pollinium connected to a viscidium by a strap-like caudicle. Well-developed rostellum arms between the anther and stigma prevent self-pollination without the action of a pollinator.

When a hawkmoth probes a flower for nectar, the narrow, funnel-like entrance of the corolla forces the moth's proboscis against the rostellum arms, which bear the sticky viscidia. If a viscidium adheres to the base of the moth's proboscis, the attached pollinium is extracted from its anther sac as the moth withdraws from the flower. Because of the long caudicle, removed pollinia dangle from the ventral base of the moth's proboscis (Fig. 1). In this position, pollinia can contact the stigmas of subsequently visited flowers, as long as they are not obscured by other pollinaria carried by the moth (Fig. 1B). Pollen massulae that adhere to the sticky mucilage on a stigma break away from the remainder of the pollinium when the moth departs from the flower (Fig. 1C). The sectile nature of the pollinium, which includes an average of 288 massulae (SD = 47.9, $N = 12$), allows a moth to disperse massulae from a single pollinium to the stigmas of many flowers.

We studied *D. cooperi* at the Himeville Nature Reserve in the foothills of the Drakensberg Mountains in KwaZulu-Natal, South Africa (29°45'S, 29°31'E) during January 2001, 2002 and 2003. The population included about 415 flowering plants in an open, grassy meadow with an area of 8.75 ha (mean density = 0.005 plants/m²). Plants were clustered into numerous denser subpopulations scattered throughout the meadow, with a median distance between neighbouring plants of 1.45 m ($N = 50$ plants) during 2002. No other plant species visited by hawkmoths were present at the study site.

HAWKMOTH BEHAVIOUR AND POLLEN LOADS

We observed hawkmoths during two evenings in 2003 to quantify their behaviour within and between inflorescences. For each inflorescence on which we observed a hawkmoth visit, we counted the numbers of open and probed flowers and measured the distance that the moth flew to its next plant. Using a handheld tape-recorder, we also recorded the duration of probes of individual flowers. We counted pollinarium loads on both captured hawkmoths and those that we photographed visiting flowers (e.g. Fig. 1A, B).

POLLEN TRACKING

To quantify pollen dispersal we used Peakall's (1989) technique to stain pollinia and then located stained

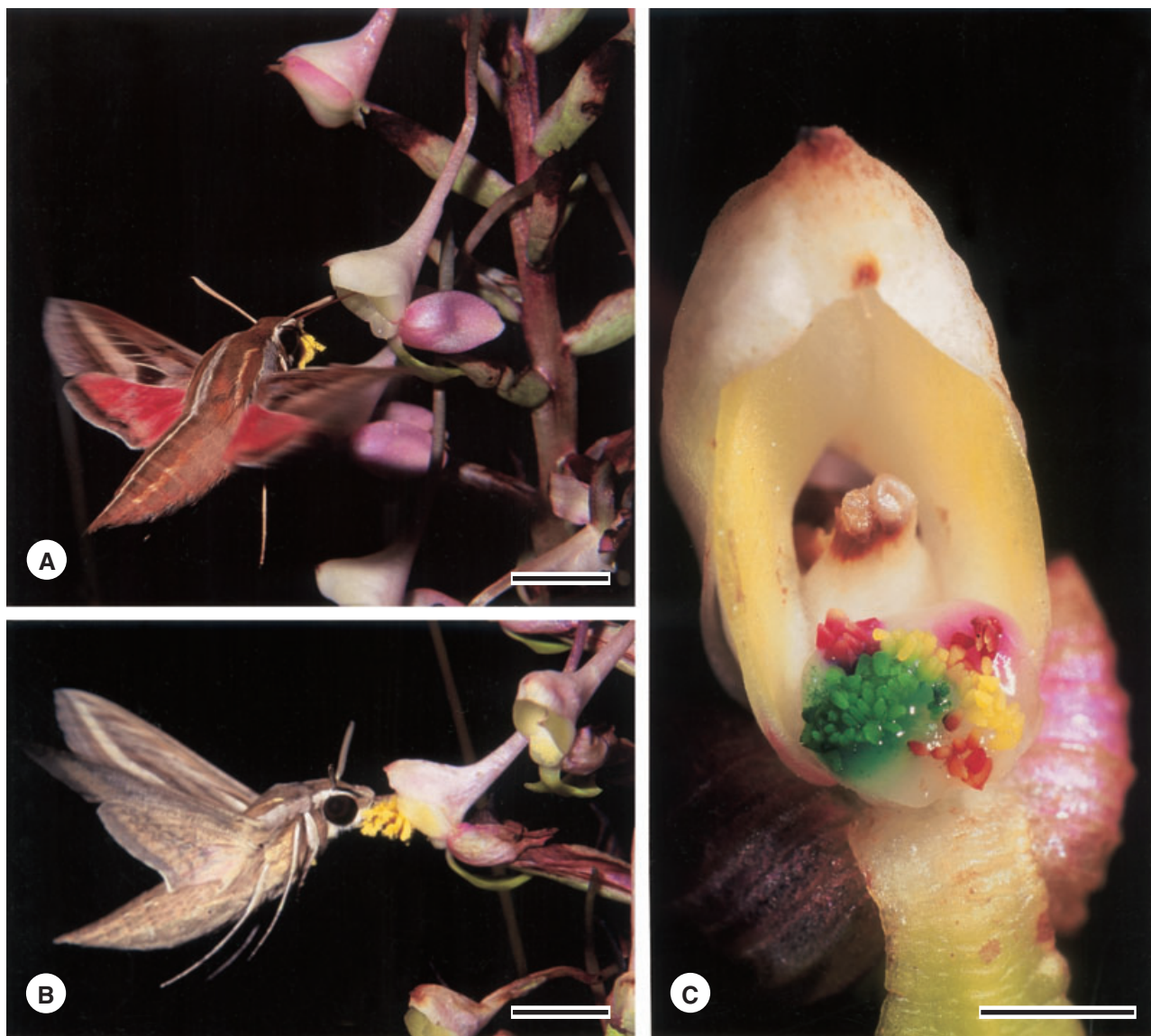


Figure 1. A, *Basiotbia schenki* visiting a *Disa cooperi* flower. Pollinia dangling from the base of the moth's proboscis will contact the stigma. B, *Hippotion celerio* carrying an estimated 18 pollinaria as it probes a *D. cooperi* flower, illustrating the potential for layering of pollinaria. C, unstained yellow and stained pink and green pollen massulae deposited on a *D. cooperi* stigma. Scale bars A, B = 10 mm. C = 2 mm.

massulae on stigmas 48 h later. We marked pollinia by injecting 4 μL of histochemical stain into individual anther sacs with a calibrated microsyringe (Hamilton 0–50 μL) or automatic pipette (Pipetman). During 2001, the stains used (and concentrations: mass per volume of water) included neutral red (1%), rhodamine (0.2%), fast green (1%), methylene blue (1%) and bismarck brown (1%). However, we excluded results with bismarck brown because of difficulty distinguishing stained massulae from old (brown) massulae on the stigmas. During 2002, we replaced

bismarck brown with gentian violet in a premixed medicinal preparation (Alpha).

We applied stain to all pollinia on an inflorescence, including 209 flowers on 24 plants divided equally among four colours during 2001, and 186 flowers on 50 plants divided equally among five colours during 2002. Plants stained with the same colour were separated by at least 20 m to limit the possibility of stained cross-pollen being mistaken for self-pollen on a plant that had also been stained with the same colour. After stained plants had been exposed to pollinators for

48 h, we examined the stigmas of all plants in the population and counted any stained massulae (Fig. 1C) with the aid of a 10× handlens. Quantification of stained pollen massulae on stigmas has not been attempted previously and greatly increases the resolution of pollen fates. We also measured the distance between any plant that had received stained massulae and the nearest plant whose pollinaria had been stained with the same colour.

Peakall (1989) showed that staining does not affect the probability of pollinarium removal by pollinators, but he did not test whether staining influenced the mechanics of pollen deposition by pollinia. Therefore, before using Peakall's technique we compared the number of massulae deposited on stigmas by stained vs. unstained pollinia using the following automated pollination technique. To prepare an individual pollinarium for this experiment, we applied the end of a 10-cm length of cotton thread to its viscidium and then withdrew the pollinaria from the flower by pulling on the thread. If the pollinarium was subject to staining, we then suspended it in one of the histochemical stains used in the field experiments for 5 s and then let it dry for 30 min. We then gently lowered each pollinium onto a virgin stigma, by means of the thread draped over a fixed steel bar (functioning as a pulley), and raised it again once the thread went slack. The pollinium was similarly applied to six additional virgin stigmas. This procedure simulated the action of hawkmoths hovering over a stigma and eliminated bias in the pressure applied to stigmas by pollinia. Pollen massulae that adhered to stigmas were counted under a dissecting microscope. We replicated the pollination sequence with ten pollinaria for each stain and with ten unstained pollinaria.

We analysed the effects of staining on pollen deposition with a repeated-measures ANCOVA, which used an autoregressive model to depict covariation in massulae deposition between successive stigmas (Proc Mixed, SAS v. 8.02). Staining did not significantly affect the pollen-deposition properties of pollinia (Fig. 2). The number of pollen massulae deposited on a stigma from an individual pollinium declined significantly from an average (\pm SE) of 18.8 (\pm 1.03) massulae on the first stigma to 10.1 (\pm 1.04) massulae on the seventh stigma ($F_{1,220} = 41.93$, $P < 0.001$). The partial regression coefficients associated with this decline did not differ significantly among staining treatments (stain application–stigma position interaction; $F_{1,216} = 2.00$, $P > 0.05$). Furthermore, the average number of massulae deposited from pollinia did not differ among stains ($F_{5,125} = 1.10$, $P > 0.35$). This experiment did not include gentian violet, but other studies (Johnson *et al.*, 2004) have similarly shown that this stain does not significantly affect pollinium cohesion or massula deposition patterns.

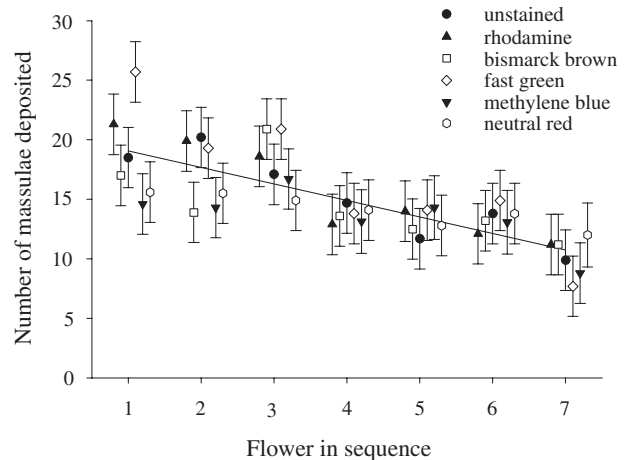


Figure 2. Decline in mean (\pm SE) pollen deposited on a sequence of virgin flowers for unstained pollinia and pollinia marked with different stains using an automated pollinating device (see text for methods).

ESTIMATES OF POLLEN FATES

To facilitate comparison between years in this study and with other species, we consider pollen fates as proportions. These fates can be estimated in several ways, which convey somewhat different information. In this paper, we present both median proportions, which represent typical performance of individual plants, and aggregate mean proportions, which depict overall population performance. Differences between these measures largely reflect asymmetry in the distribution of a particular fate among individual plants. We used bootstrap resampling (10000 samples) to calculate aggregate mean fates and their associated 95% confidence intervals.

We estimated pollen-removal failure and the overall efficiency of pollen transfer (proportion of removed pollen that reaches stigmas) during the 2002 flowering season based on the most recently wilted flower from each of 56 plants sampled from the Himeville population. For these flowers we counted the total number of pollinaria that pollinators had removed (R) and the total number of massulae deposited on the stigma (D). Given these counts, we calculated population pollen-removal failure as

$$F_R = 1 - (R/2N) \quad (1)$$

and population pollen-transfer efficiency as

$$E = D/MR \quad (2)$$

where M is the average number of massulae per pollinium and N is the number of flowers sampled. We applied bootstrap resampling (10 000 samples) to calculate F_R and E and their associated 95% confidence intervals.

We used the plants with stained pollinia that had been exposed to pollination for 48 h to estimate the proportions of removed pollen involved in self-pollination and export for individual plants, and to determine the proportions of self-pollination resulting from facilitated autogamy and geitonogamy in the population as a whole. For an experimental plant, we considered stained massulae on a stigma to have arisen from self-pollination if they were the same colour as had been applied to the plant's pollinia. To estimate the fraction of self-pollination resulting from geitonogamy we distinguished two classes of flowers that received stained self-massulae on a plant. A total of f_g flowers received m_g stained self-massulae, but did not have stained pollinia removed by pollinators, so that all self-pollen on these flowers must have been deposited geitonogamously. In contrast, the f_{ag} flowers with missing pollinia could have received their m_{ag} self-massulae by facilitated autogamy and/or geitonogamy. To estimate the fraction of self-pollination due to geitonogamy we must assume that each self-pollinated flower receives the same average number of self-massulae through geitonogamy, so that $m_{ag} = m_g + m_a$, where m_a is the unknown number of massulae involved in facilitated autogamous self-pollination. In this case, m_a can be estimated by

$$m_a = \left(\frac{m_{ag}}{f_{ag}} - \frac{m_g}{f_g} \right) f_{ag} = \frac{f_g m_{ag} - f_{ag} m_g}{f_g},$$

where the expression in parentheses estimates autogamous self-pollination per flower. Consequently, the total number of massulae involved in geitonogamy summed over all flowers equals

$$G = m_g + m_{ag} - \frac{f_g m_{ag} - f_{ag} m_g}{f_g}$$

and the fraction of self-pollination resulting from geitonogamy equals

$$g = \frac{G}{m_{ag} + m_g} = \frac{(f_{ag} + f_g) m_g}{f_g (m_{ag} + m_g)}. \quad (3)$$

[Note that eq. 3 overestimates g if facilitated autogamy and geitonogamy occur independently (i.e., $m_{ag} < m_g + m_a$.)] We used bootstrap resampling (10 000 samples) to calculate g and its associated 95% confidence interval.

STATISTICAL ANALYSES

We used general linear models (Neter *et al.*, 1996) for between-year comparisons of absolute outcomes (i.e. the number of open flowers, the number of massulae involved in self-pollination, and the number of massulae exported to other plants). In addition to considering year as a fixed categorical factor, the analyses of pol-

lination outcomes included one or two covariates. These analyses first considered all covariates and their interactions with year. However, these terms were excluded from the model by backward elimination if they did not explain a significant proportion of the variation in the dependent variable by themselves and they were not involved in a more complicated, significant interaction ($\alpha = 0.05$). We used the $\ln(x + 1)$ transformation for the numbers of pollinaria removed from flowers, self-massulae, and exported massulae to eliminate heteroscedasticity and create linear relations between dependent variables and covariates. Because we applied this transformation to both dependent variables and covariates, the partial regression coefficient (b) for a covariate indicates whether the ratio of the dependent variable to the covariate increases ($b > 1$) or decreases ($b < 1$) with increases in the value of the covariate. We used this approach to assess the proportionality of self-pollination in relation to display size and pollen removal, and of pollen export in relation to self-pollination. Tests concerning partial regression coefficients involved single-sample t -tests.

Our analysis of massula export detected significant effects of both the number of pollinaria removed and the number of massulae involved in self-pollination. We illustrate each of these independent effects graphically by isolating their effects on pollen export from those of other influences included in the general linear model. To this end, we calculated 'adjusted pollen export' by adding the residual from the overall general linear model for each observation to the export predicted by the estimated intercept and partial regression coefficient for the covariate of interest. This calculation resulted in slightly negative estimates of adjusted pollen export for a few plants.

To analyse responses with well-defined, but non-normal distributions (proportional outcomes, the number of pollinaria removed from plants, the number of plants receiving stained pollen, and pollen-export distances), we used generalized linear models, which tested for significant effects with likelihood-ratio (G) tests (McCullagh & Nelder, 1989). These analyses considered error distributions that reflected the characteristics of the observations (proportions: binomial distribution; pollinarium removal and number of recipient plants: Poisson distribution; pollen export distance: gamma distribution) and appropriate transformation (link function) of the dependent variables to linearize their relations to independent variables (proportions: logit transformation; pollinarium removal: ln transformation; pollen export distance: inverse transformation). We used the gamma distribution for pollen export distance because it can be used to test whether the probability of a massula being deposited on a stigma changes, depending on how far the pollinator has already travelled. If this probability is con-

stant, the shape parameter of the gamma distribution equals 1, resulting in the special case of the exponential distribution (McCullagh & Nelder, 1989). All of these analyses included year as a categorical factor. In addition, the analysis of pollinarium removal considered both the number of open flowers displayed by a plant as a covariate and the interaction between display size and year. The analyses of pollen-export distance between individual flowers and by individual massulae used generalized estimating equations (Liang & Zeger, 1986) to account for the repeated measurement of multiple massulae from the same donor plant on individual recipient plants.

We used non-parametric tests to compare responses between years for several variables with poorly defined distributions. These variables included the proportion of massulae reaching stigmas (Savage test: Hájek, 1969) and the proportion of dispersed massulae involved in self-pollination (Wilcoxon two-sample test: Hájek, 1969). We describe these variables using the median and interquartile range, because these statistics represent central tendency and dispersion most accurately for non-symmetric distributions and they are more representative of the comparisons considered by the Savage and Wilcoxon two-sample tests (Hájek, 1969).

RESULTS

HAWKMOTH BEHAVIOUR AND POLLEN LOADS

During 2003, hawkmoths probed an average of 8.1 flowers (lower SD = 3.9, upper SD = 16.8, based on ln-transformed data) on 30 *Disa cooperi* inflorescences, which displayed an average of 12.1 open flowers (lower SD = 7.7, upper SD = 19.3). The number of flowers probed (n_p) by hawkmoths increased significantly with the number of open flowers (n_f : $n_p = 0.702n_f^{0.979}$, $r^2 = 0.382$, $P < 0.001$, based on ln-transformed data), but the mean proportion of flowers probed (0.70) did not vary with total display size ($t_{28} = 0.089$, $P > 0.9$). Hawkmoths probed individual flowers for an average of 2.0 s (lower SD = 1.01 s, upper SD = 3.77 s, $N = 28$) and spent an average of 15.5 s visiting a plant (lower SD = 7.18 s, upper SD = 33.26, $N = 28$). During 2001, we observed 11 flights between plants with a median distance of 2.5 m (lower quartile = 1 m, upper quartile = 5.5 m, Fig. 3A: light grey bars). Similarly, during 2003, we recorded 20 flights between plants with a median distance of 2.0 m (lower quartile = 0.88 m, upper quartile = 6.00 m). A median of 1.45 m separated neighbouring plants, so that moths tended to fly slightly beyond the closest plant. Hawkmoths foraged on the flowers of *D. cooperi* almost exclusively during the first 30 min after dusk (Johnson, 1995).

We counted pollinium loads of six captured hawkmoths (all *Basiothia schenki*) and four hawk-

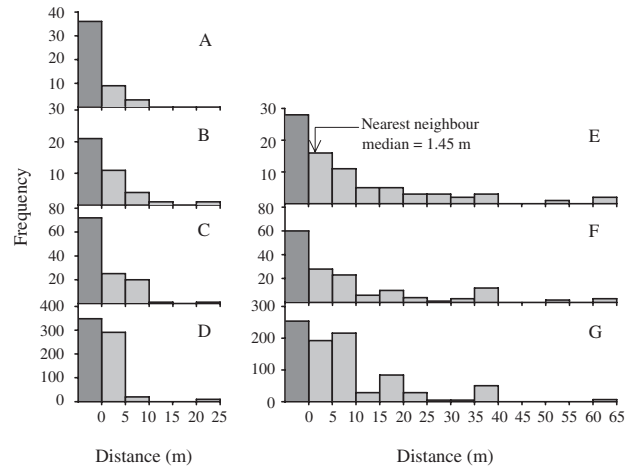


Figure 3. Distances flown by hawkmoths between *Disa cooperi* plants during 2001 (A) and pollen-dispersal distances during 2001 (B–D) and 2002 (E–G). Pollen dispersal is depicted from three perspectives: distance between source and recipient plants (B and E), distance between source and recipient flowers (C and F), and the distance travelled by individual massulae (D and G). The dark, leftmost bars depict either within-inflorescence flights by hawkmoths (A), or self pollination (B–F).

moths photographed while visiting *D. cooperi* (two *B. schenki*, one *Hyles lineata*, one *Hippotion celerio*). Seven of these moths carried pollinaria, with a median of 5 pollinaria per moth (range = 2–18). Captured hawkmoths had a mean tongue length of 42.0 mm (SD = 0.81), whereas the nectar spur of *D. cooperi* averages 48.8 mm in length (SD = 3.0, $N = 56$).

POLLINIUM REMOVAL AND TOTAL POLLEN DISPERSAL

The dispersal of stained pollen from *Disa cooperi* inflorescences during 48 h periods differed in many respects between the 2001 and 2002 flowering seasons (Table 1). Many of these differences seem to be a consequence of more moths visiting the population during the experimental period in 2001. Moths removed stained pollinaria from a larger proportion of the experimental inflorescences and they removed a larger proportion of stained pollinaria per inflorescence during 2001 than during 2002 (Tables 1, 2A, Fig. 4). In addition to apparent differences in overall visitation, differences in pollen dispersal seem to have arisen from contrasting moth behaviour on inflorescences during the two years. In particular, the relation of pollinarium removal to the number of open flowers differed between years (Fig. 4: year–display size interaction, $G_1 = 8.67$, $P < 0.005$), with removal rising sharply with increasing display size during 2001 ($G_1 = 19.58$, $P < 0.001$), but not 2002

Table 1. Comparison of pollination of *Disa cooperi* between 2001 and 2002, based on 48 h exposure to pollination after pollinium staining. Descriptive statistics for counts represent back-transformations of statistics estimated for ln-transformed data, whereas those for pollen export distances are based on inverse-transformed data. Numbers in parentheses indicate sample sizes

Characteristic	Year		Statistical comparison
	2001	2002	
Number of open flowers	8.1 7.5–8.7 (<i>N</i> = 25)	11.9 11.4–12.5 (<i>N</i> = 50)	$F_{1,73} = 21.28^{****}$
Proportion of plants from which moths removed pollinaria (interactive plants)	0.92 (<i>N</i> = 25)	0.72 (<i>N</i> = 50)	$G_1 = 4.52^*$
Number of pollinaria removed per plant	6.8 6.3–7.3 (<i>N</i> = 25)	1.4 1.2–1.6 (<i>N</i> = 50)	$G_1 = 140.70^{****}$
Proportion of interactive plants receiving self-massulae	0.87 (<i>N</i> = 23)	0.58 (<i>N</i> = 36)	$G_1 = 5.87^*$
Number of self-massulae received by self-pollinated plants	9.7 7.5–12.5 (<i>N</i> = 20)	7.7 5.9–9.8 (<i>N</i> = 21)	$F_{1,39} = 0.46$
Proportion of interactive plants exporting pollen	0.39 (<i>N</i> = 23)	0.53 (<i>N</i> = 36)	$G_1 = 1.05$
Number of massulae exported by exporting plants	19.0 11.6–30.7 (<i>N</i> = 9)	12.8 9.1–18.0 (<i>N</i> = 19)	$F_{1,26} = 0.43$
Number of plants receiving exported pollen	1.9 1.4–2.4 (9)	2.6 2.3–3.0 (19)	$G_1 = 1.51$
Distance between pollen source and recipient plants (m)	5.0 4.0–6.7 (17)	15.3 13.4–17.9 (51)	$G_1 = 11.39^{****}$
Distance between pollen source and recipient flowers (m)	4.1 3.5–4.9 (34)	14.6 13.1–16.5 (91)	$G_1 = 21.66^{****}$
Distance travelled by exported massulae (m)	2.7 2.5–2.9 (322)	10.6 10.2–11.2 (615)	$G_1 = 166.14^{****}$

* $P < 0.05$, **** $P < 0.001$.

($G_1 = 1.62$, $P > 0.2$). These contrasting relations were not affected significantly by differences among plants in the number of stained pollinaria ($G_1 = 0.0003$, $P > 0.95$).

Variation in the proportion of stained pollen removed from plants that reached stigmas was positively skewed (Fig. 5A, B), with many plants dispersing no massulae and a few having over 10% of their removed massulae dispersed during 2002. This disparity in pollen transfer efficiency among plants caused the aggregate mean efficiency to exceed the median by an order of magnitude (Table 2B). Variation in pollen-transfer efficiency was particularly extensive during 2002 (Fig. 5B), resulting in higher aggregate mean efficiency than during 2001 (Table 2B). Never-

theless, median pollen-transfer efficiency did not differ significantly between years (Table 2B), with a combined median of 1% (lower quartile = 0.1%, upper quartile = 3.7%). The contrasting conclusions for the aggregate mean and median reflect differences between years in the performance of the most successful plants, rather than that of the typical plant in the population.

SELF-POLLINATION

The proportion of dispersed massulae involved in self-pollination varied extensively among plants (Fig. 5C, D) and differed significantly between years (Wilcoxon two-sample test, $\chi^2 = 4.77$, 1 d.f., $P < 0.05$). During

Table 2. Comparisons of the fates of stained *Disa cooperi* pollen during 48 h of exposure to pollinators between 2001 and 2002 expressed as (A) fractions of total pollen availability ($N = 25$ and 50 plants, respectively), (B) the pollen removed from inflorescences ($N = 23$ and 36 plants, respectively), and (C) all pollen dispersed to stigmas ($N = 20$ and 26 plants, respectively). The median (lower and upper quartiles) depicts typical pollen fates for individual plants, whereas the aggregate mean (lower and upper 95% confidence limits) represents the typical fate of all pollen in the population. Aggregate means for 2001 and 2002 with non-overlapping confidence intervals differ significantly ($P < 0.05$)

Characteristic	Median		Statistical comparison	Aggregate mean	
	2001	2002		2001	2002
(A) Proportion of total pollen availability					
Pollen remaining in flowers	0.571 0.444–0.750	0.833 0.667–1	$Z = 3.14^{**}$ (Wilcoxon)	0.592 0.511–0.673	0.945 0.933–0.957
Self-pollination	0.0011 0.0002–0.0050	0 0–0.0022	$\chi^2_1 = 1.62$ (Savage)	0.0029 0.0018–0.0041	0.0007 0.0004–0.0011
Pollen export	0 0–0.0022	0 0–0.0041	$\chi^2_1 = 0.80$ (Savage)	0.0027 0.0011–0.0044	0.0017 0.0007–0.0031
Unaccounted lost pollen	0.427 0.247–0.535	0.150 0–0.333	$Z = 3.22^{**}$ (Wilcoxon)	0.402 0.324–0.482	0.0522 0.0411–0.0639
(B) Proportion of pollen removed from anthers					
Self-pollination	0.0035 0.0012–0.0113	0.0035 0–0.0135	$\chi^2_1 = 0.38$ (Savage)	0.0072 0.0043–0.0105	0.0128 0.0076–0.0189
Pollen export	0 0–0.0087	0.0014 0–0.0359	$\chi^2_1 = 2.53$ (Savage)	0.0068 0.0027–0.0116	0.0309 0.0134–0.0536
Total dispersal (pollen-transfer efficiency)	0.007 0.0014–0.0182	0.012 0–0.0618	$Z = 0.70^1$ (Wilcoxon)	0.0140 0.0081–0.0212	0.0440 0.0230–0.0713
Unaccounted lost pollen	0.993 0.982–0.999	0.988 0.938–1		0.986 0.979–0.992	0.956 0.929–0.977
(C) Proportion of all dispersed pollen					
Self-pollination	1.0 0.347–1.0	0.476 0.136–1.0	$Z = 2.17^*$ (Wilcoxon)		

¹This test result also applies to unaccounted lost pollen, which is the complement of total dispersal.
* $P < 0.05$, ** $P < 0.01$.

2001, self-pollination was the most common fate of dispersed massulae, with 60% of plants contributing massulae to stigmas only by self-pollination (Fig. 5C: median proportion of dispersed pollen involved in self-pollination = 100%, lower quartile = 34.7%, upper quartile = 100%). In contrast, during 2002 the fates of dispersed pollen varied more evenly from exclusive self-pollination to only pollen export (Fig. 5D: median = 47.6%, lower quartile = 13.6%, upper quartile = 100%).

On average, significantly more of a plant's massulae were involved in self-pollination during 2001 than 2002 ($F_{1,57} = 5.39$, $P < 0.025$). This difference resulted largely because a higher proportion of inflorescences experienced self-pollination during 2001, as self-pollinated plants received similar numbers of self-massulae during both years (Table 1). The overall difference between years in the number of self-massulae disappeared when the analysis included pollinarium removal as a covariate ($F_{1,56} = 0.16$, $P > 0.6$),

indicating that pollinarium removal largely governs opportunities for self-pollination. In general, self-pollination increased significantly with the number of pollinaria removed (Fig. 6: $F_{1,56} = 7.41$, $P < 0.01$). The partial regression coefficient associated with this effect ($b \pm SE = 0.75 \pm 0.277$) did not differ significantly from 1 ($t_{56} = 0.89$, $P > 0.35$), indicating that self-pollination used the same proportion of removed massulae, regardless of the number of massulae removed. The intensity of self-pollination did not vary significantly with the number of open flowers after accounting for the influence of pollinarium removal ($F_{1,55} = 2.94$, $P > 0.05$).

Based on (1), most self-pollination resulted from geitonogamy, rather than facilitated autogamy. During 2001, an estimated 67% of all self-pollination involved pollen transfer between flowers (lower 95% confidence limit = 40.8%, upper confidence limit = 97.1%) and the remaining 33% resulted from facilitated autogamy. Overall, these modes of

self-pollination represent 34.7% and 17.1% of all stained massulae deposited on stigmas. During 2002, geitonogamy accounted for an estimated 97% (LCL = 88.3%, UCL = 104.2%) of all self-pollination, so that facilitated autogamy represented only 3%. Overall, geitonogamy and facilitated autogamy

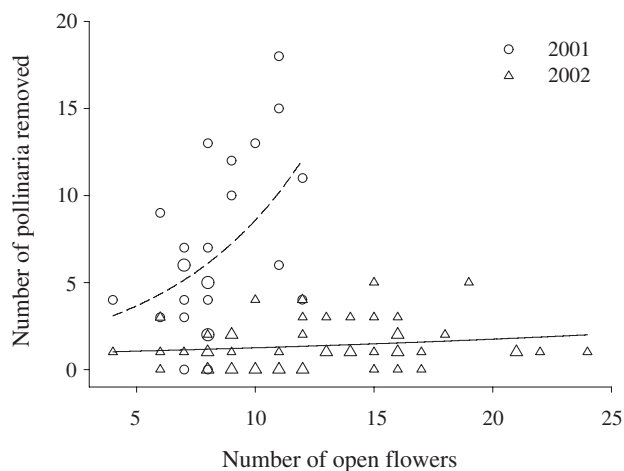


Figure 4. Contrasting influences of floral display size on pollinarium removal from *Disa cooperi* inflorescences during 2001 (circles and dashed regression line) and 2002 (triangles and solid regression line). Large symbols indicate two identical observations during the same year.

accounted for 28.2% and 0.8% of the stained massulae deposited on stigmas during 2002.

POLLEN EXPORT

Plants from which moths removed pollinaria exported pollen to as many as eight recipient plants, with an average of about two recipients (Fig. 7). The average number of recipient plants did not differ significantly between 2001 and 2002 (Table 1). Over both years, the number of recipients to which a source plant exported pollen varied positively with the number of self-massulae deposited on the source's stigmas ($G_1 = 6.03$, $P < 0.025$), but not with the number of pollinaria removed from the source plant ($G_1 = 0.90$, $P < 0.3$).

Most exported pollen reached nearby plants (Fig. 3B–G: light grey bars), particularly during 2001 when pollen travelled significantly shorter distances than during 2002 (Table 1). The most extensive observed dispersal occurred during 2002, when six massulae reached stigmas in three flowers on two plants 64 m from the nearest source plant. For both years, the mean export distance decreases as attention is shifted from the separation of source and recipient plants, to source and recipient flowers, to the dispersal of individual massulae (Table 1). This perspective of increasingly local dispersal probably reflects the combined effects of moths moving short distances between plants (Fig. 3B: light grey bars) and declining deposi-

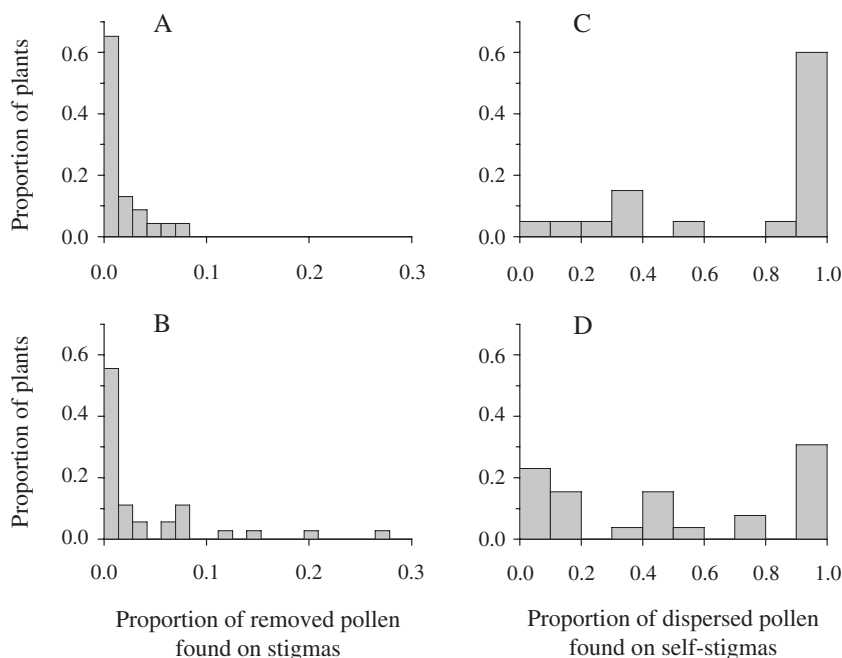


Figure 5. Frequency distributions of the proportion of stained massulae removed from flowers that reached stigmas during 2001 and 2002 (A and B, respectively) and the proportion of dispersed pollen that moths deposited on a plant's own stigmas during 2001 and 2002 (C and D, respectively).

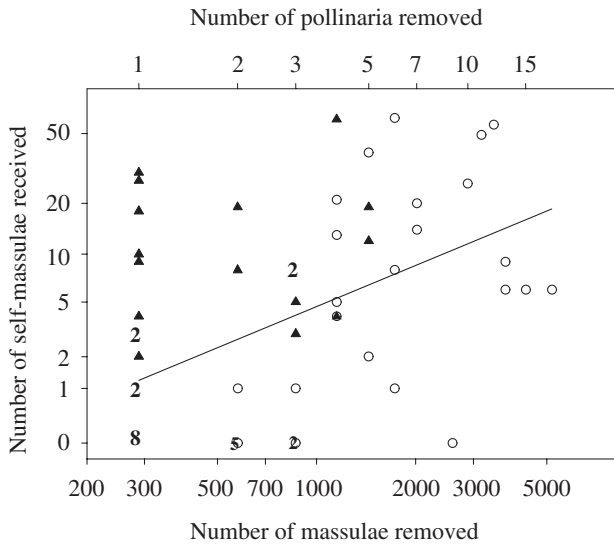


Figure 6. Relation of self-pollination of *Disa cooperi* inflorescences to pollinarium removal during 2001 (circles) and 2002 (triangles and numerals). The numerals for 2002 indicate the number of identical observations. The solid line represents the partial regression solution for both years combined. Both axes depict a natural-logarithmic scale.

tion of source pollen on stigmas of successively visited flowers (see Fig. 2).

Neither the proportion of plants exporting pollen, nor the number of massulae that they exported differed significantly between years (Table 1); however, these overall findings obscure more interesting results. The number of massulae that a plant exported varied in a complicated manner with the number of massulae that moths removed (Fig. 8A). During 2001, when moths removed many massulae, pollen export tended to decline with increasing removal ($t_{54} = 1.28$, $P > 0.2$), whereas during 2002, when moths removed fewer massulae, pollen export tended to increase with removal ($t_{54} = 1.55$, $P > 0.1$). Although neither of these trends was statistically significant by itself, they differed significantly from each other (year–removal interaction, $F_{1,54} = 4.17$, $P < 0.05$). The implied peaked relationship between pollen export and pollen removal is confirmed by a significant quadratic regression for plants that exported at least one massula, which did not explicitly consider the differences in export between years ($r^2 = 0.298$, $P < 0.05$). This result suggests that plants from which moths removed three pollinaria during 48 h exported more pollen, on average, than plants that experienced either less or more removal (see Fig. 8A).

In addition to the effects of pollen removal, a plant's pollen export varied with its self-pollination. The chance that a plant exported pollen increased if it was also self-pollinated, as only 28% of the 18 plants that

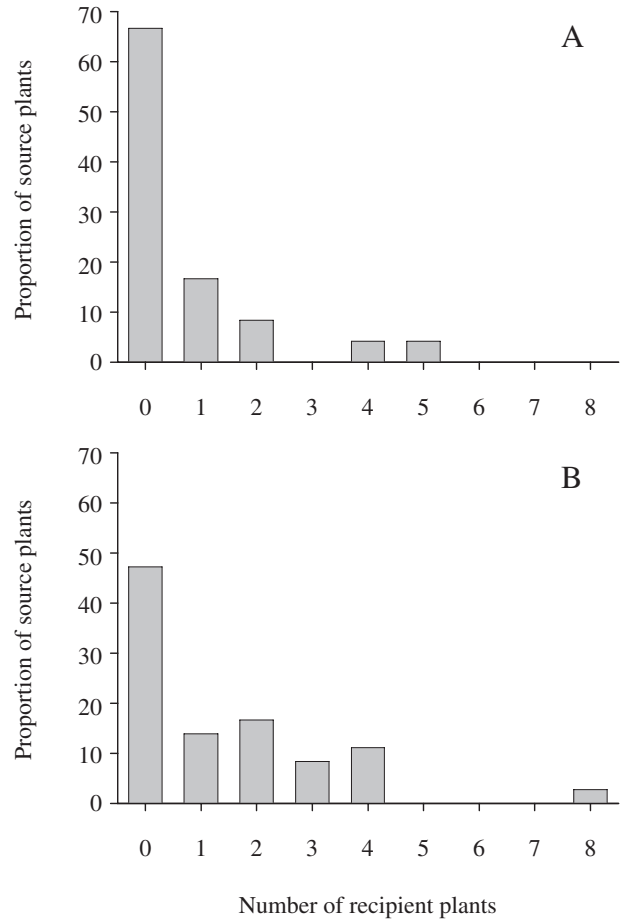


Figure 7. Frequency distributions of the number of *Disa cooperi* plants receiving pollen from plants with stained pollinia during (A) 2001 ($N = 24$ source plants) and (B) 2002 ($N = 36$ source plants). Only source plants from which moths removed pollinaria are included.

did not receive self-pollen exported pollen compared to 56% of the 41 plants that did receive self-pollen ($G_1 = 4.14$, $P < 0.05$). Furthermore, the number of massulae exported increased significantly with the number of self-massulae that a plant received (Fig. 8B: $F_{1,54} = 16.68$, $P < 0.001$), in a manner that did not differ significantly between years (year \times self-massulae interaction, $F_{1,53} = 0.93$, $P > 0.3$). The partial regression coefficient associated with the number of self-massulae ($b \pm SE = 0.643 \pm 0.158$) was significantly smaller than 1 ($t_{54} = 2.26$, $P < 0.05$), so the ratio of exported massulae to self-deposited massulae declined with increasing self-pollination. For example, plants on which moths deposited only one self-massula exported two massulae, on average, whereas plants that received 50 self-massulae exported only 20 massulae to other plants (Fig. 8B: compare solid and dotted lines). This declining ratio of pollen export to

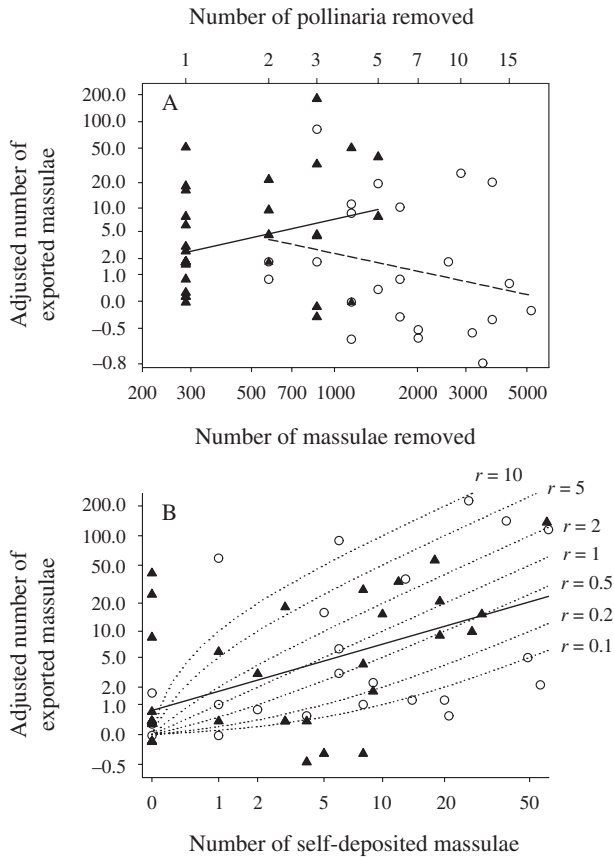


Figure 8. Effects of (A) pollinarium removal and (B) self-pollination on the number of massulae exported from individual *Disa cooperi* inflorescences during 2001 (circles) and 2002 (triangles). In (A) the dashed and solid lines represent partial regression solutions for 2001 and 2002, respectively. In (B) the solid line represents the partial regression solution for both years combined, whereas the dotted lines depict different ratios (r) of massulae export to receipt of self-massulae. In both (A) and (B), the number of exported massulae has been adjusted to illustrate only the effect of the independent variable plus the residual variation (see Methods for details). All axes depict a natural-logarithmic scale.

self-pollination indicates that self-pollination significantly reduced a plant's opportunities to sire out-crossed seeds.

ACCOUNT OF POLLEN FATES

The relative fates of stained pollen during 48 h exposure to pollinators showed contrasting patterns between 2001 and 2002 (Table 2). Based on medians, moths removed more than 40% of the available pollen from inflorescences during 2001, but only about 16% during 2002 (Table 2A). Despite these differences in removal, the small proportions of pollen involved in self-pollination or exported to other plants did not dif-

fer significantly between years (Table 2A). Furthermore, the proportions of removed pollen involved in self-pollination and export did not differ between years (Table 2B). Self-pollination accounted for most of the pollen that reached stigmas from individual plants during 2001 and about half of dispersed pollen during 2002 (Table 2C). Because a median of only about 1% of the pollen removed from plants reached stigmas, the vast majority of removed pollen was lost during either removal from flowers or transport between plants (Table 2B). Much more of the pollen produced by plants was involved in unaccounted loss during the year of high pollen removal (2001). This difference resulted largely from differences in pollen removal, as the fraction of removed pollen involved in unaccounted loss did not differ significantly between years (Table 2B).

Data collected from wilted flowers allow us to set the 48 h dispersal data in the context of a flower's entire lifetime. Estimates of total pollen dispersal during 2002 based on wilted flowers differed considerably from that determined from the fates of stained pollen during the 48 h experiment. Summed over all flowers, moths removed all but 35.7% of the pollen produced by flowers by the time they wilted during 2002 (lower 95% confidence limit = 27.7%, upper limit = 44.6%), whereas 95% of pollen remained in flowers after 48 h (Table 2A). The comparatively low rate of pollen removal during the 48 h experiment in 2002 is not surprising, given that flowers last about 8 days before wilting (S. D. Johnson, pers. obs.) and that moth visitation during the 48 h experiment was probably inhibited by persistent strong, cold winds. Nevertheless, the two flower samples provide similar estimates of pollen-transfer efficiency (based on overlapping 95% confidence intervals), with 4.4% of removed stained pollen reaching stigmas (Table 2B) vs. 6.3% efficiency based on wilted flowers (LCL = 4.7%, UCL = 8.1%). Figure 9 summarizes the overall accounting of pollen fates for *D. cooperi* during 2002, based on estimates of pollen removal and overall pollen-transfer efficiency from the wilted flowers, the proportions for stained pollen deposited on stigmas of source and recipient plants (Table 2), and the calculation of intrafloral vs. geitonogamous self-pollination (1).

DISCUSSION

UNCERTAINTY OF POLLINATION

Pollination of *Disa cooperi* is fraught with uncertainty (Fig. 9). More than one third of the pollen produced by *D. cooperi* during 2002 remained in anthers when flowers wilted, and so had no opportunity to access stigmas. This result indicates strong pollinator limitation of pollen dispersal. In addition, only 6.3% of

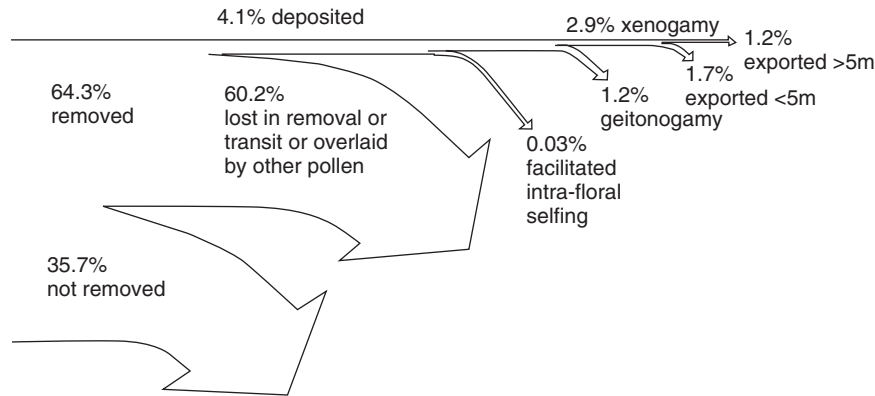


Figure 9. Relative incidence of alternate pollen fates for *Disa cooperi* during 2002. The proportions of pollen removed from anthers and lost during removal and transport are derived from wilted flowers and thus are representative of an entire flower lifetime. The partitioning of pollen into proportions that reach different types of stigmas is based on the results of the 48 h pollen staining experiment (Table 2) and the subsequent solution of eq. 3.

the pollen removed from these flowers reached stigmas in the population, despite being bound in a seemingly secure package (pollinium) that is literally glued to the proboscis of hawkmoths. Therefore, the majority (64.7%) of pollen produced by *D. cooperi* during 2002 was lost, either while being extracted from anthers (removal loss), or during transport (transport loss), or by being exported to stigmas in other populations. We consider export to other populations to be unlikely, because no other *D. cooperi* plants are known to occur within 5 km of the study population and pollen dispersed primarily from source plants to just a few neighbouring plants, even though moths visited dozens of plants and covered an extensive area during their foraging bouts. Therefore, most pollen seems to be dispersed locally to the first few plants that a moth visits after removing pollen from a source plant

We expect that most unaccounted pollen was lost during transport, although we could not distinguish removal and transport losses. Removal loss could occur if moths dislodged pollinaria from anthers, but the viscidia failed to attach firmly to the moths' proboscides, so that pollinaria fell before moths left source plants (e.g. Mosquin, 1970; Nilsson, 1978; L. D. Harder & S. D. Johnson, unpubl. data). However, removal loss seems unlikely to account for the equivalent pollen export during 2001 and 2002, despite greatly different pollen removal (Table 2, Fig. 9), because no obvious mechanism should cause the probability of removal loss to increase with pollen removal. In contrast, this outcome could result from two sources of transport loss. Removal of many pollinaria from individual plants by a single pollinator could limit pollen transport if pollinaria that accumulate in a large clump on a moth's tongue are more liable to fall off than small groups of pollinaria. That moths

typically carry relatively few pollinaria (median = 5; Fig. 1A, B), despite probing dozens of flowers, is consistent with this explanation. In addition, as pollinaria accumulate on a moth's proboscis (up to 18 were observed on an individual moth), they could obstruct the access of pollinaria from flowers visited earlier to stigmas of subsequently visited flowers. Both of these mechanisms would reduce pollen export most severely when a moth removes many pollinaria per plant, as is evident from the humped relation of pollen export to pollinarium removal (Fig. 8A). These mechanisms could also explain why plants typically export pollen to only two recipient plants (Fig. 7, Table 1), even though the number of massulae per pollinium should allow dispersal to many more recipients (see Fig. 2). Therefore, transport loss seems to be the primary mechanism limiting opportunities for pollen dispersal for *Disa cooperi*. Furthermore, our results illustrate that pollinarium removal can be a poor indicator of male success in orchids.

Self-pollination accounted for roughly half of the pollen reaching stigmas during both years that we tracked stained pollen. The incidence of self-pollination increased in direct proportion to the number of pollinaria removed from a plant (Fig. 6), as might be expected for a species in which pollen is removed from anthers only by the action of pollinators. We estimate that most (67–97%) self-pollination resulted as pollinators moved between flowers within an inflorescence. This large contribution of geitonogamy to both self pollination and overall pollen dispersal is consistent with observations for both other orchids with sectile pollinia (Peakall, 1989; Peakall & Beattie, 1991; Nilsson *et al.*, 1992) and species with granular pollen (Hessing, 1988; de Jong *et al.*, 1992; Schoen & Lloyd, 1992; Leclerc-Potvin & Ritland, 1994;

Harder & Barrett, 1995; Snow *et al.*, 1996; Eckert, 2000). Therefore, as with many other animal-pollinated plants, self-pollination in *D. cooperi* seems to be an inevitable consequence of the simultaneous presentation of many flowers. Fruits of *D. cooperi* that arise from self-pollination contain fewer seeds than those arising from cross-pollination (J. Jersakova & S. D. Johnson, unpubl. data). Many orchids are self-compatible, including *Disa* species (Johnson, Linder & Steiner, 1998; Johnson, 2000); however, self-pollination of orchids often results in lower fruit and seed production (e.g. Johnson, 2000) and inbreeding depression (e.g. Peakall & Beattie, 1996; Ortiz-Barney & Ackerman, 1999; Ferdy *et al.*, 2001).

Self-pollination, especially dichogamy, can also limit plant mating by reducing the opportunities for pollen export. Export of *D. cooperi* pollen increased with the incidence of self-pollination (Fig. 8B), which could be interpreted as evidence against such pollen discounting. However, the key issue is not whether pollen export declines with self-pollination, but whether pollen export would have been greater had self-pollination not occurred. Our results indicate that self-pollination reduced opportunities for pollen export, because the ratio of exported to self-deposited massulae declined with increasing self-pollination (Fig. 8B).

Overall, self-pollination accounted for only about 1% of the pollen removed from anthers, so that pollen discounting represents a much smaller component of the uncertainty of pollen export than transport loss (Fig. 9). However, if transport loss results from pollinaria layering and/or clumps of pollinaria falling off of moth proboscides, then opportunities for pollen export typically involve only the first few plants that a moth visits after removing pollen from a source plant. As self-pollination accounts for about half of all pollen deposited on stigmas, pollen discounting may severely reduce these limited export opportunities, and so represents a more significant influence on mating outcomes that is implied by the overall partitioning of pollen fates.

VARIATION IN POLLEN FATES

The partitioning of pollen into alternate fates differed considerably among plants in the Himeville population, even during a single flowering season. For most plants, none of the stained pollen removed by hawkmoths reached stigmas, whereas more than 10% of the pollen removed from a few plants reached stigmas (Fig. 5A, B). This strongly skewed distribution of pollen dispersal could greatly bias the representation of paternal alleles in the next generation, deviating strongly from the expectations of random mating. Such extreme disparity in mating success could

strongly affect evolution within populations by greatly reducing the effective population size (Nunney, 1993) and enhancing the relative impact of genetic drift.

High variance also characterized the proportions of dispersed stained pollen involved in self-pollination vs. pollen export, with some plants only self-pollinating, others only exporting pollen, and some doing both (Fig. 5C, D). As *D. cooperi* is susceptible to inbreeding depression (J. Jersakova & S. D. Johnson, unpubl. data), self-pollination will tend to increase the variance among plants in siring success, while reducing the variance in the proportion of offspring sired by selfing.

The quality of outcross mates may also be subject to high variance, depending on the relatedness of neighbouring plants (cf. Levin, 1989), because most pollen was exported locally (Fig. 3, Table 1). The more extensive dispersal during 2002 than during 2001 was accompanied by greater variance in dispersal distance (Fig. 3). This variation probably arose from differences in the distances flown by hawkmoths between plant visits, as neither the number of exported massulae nor the number of plants receiving stained massulae differed between years (Table 1). If neighbouring plants tend to be relatives (e.g. Peakall & Beattie, 1996), then the limited extent of pollen export during 2001 would have resulted in relatively higher biparental inbreeding in this year than during 2002. Although orchids produce tiny, wind-dispersed seeds, most seeds travel short distances from their maternal parent (e.g. Ackerman, Sabat & Zimmerman, 1996; Murren & Ellison, 1998) so that mate quality could vary significantly with the pollinator flight behaviour.

The considerable differences in pollen fates that we observed within a single population and between two reproductive seasons clearly illustrate that pollen fates are not fixed parameters. To what extent is the partitioning of pollen among fates stochastic, rather than mechanistically governed by (as yet unmeasured) interactions between plants and pollinators? We have argued above that associations between self-pollination and pollen removal, and between pollen export and self-pollination reveal essential features of the pollination process. At a gross level, such features seem to be influenced by floral characteristics, as the partitioning of pollen fates differs between orchids (relatively high removal failure, low transport loss) and species with granular pollen (Harder, 2000). However, the extent to which floral and pollinator characteristics affect pollen fates within these groups, between populations of the same species, or among plants within a population remains to be explored.

Packaging of pollen into pollinaria partly ameliorates overall transport losses, which are the greatest limitation on the male function for plants (cf. Harder,

2000). The percentage of removed pollen that reached stigmas in our *D. cooperi* population (6.3% in 2002) represents a level of efficiency that is at least an order of magnitude greater than that reported for plants with granular pollen (cf. Harder, 2000). However, export of pollen to other plants, particularly distant ones, appears to be constrained by geitonogamous pollen discounting and losses such as pollen layering that increase in direct proportion to the number of pollinaria removed. Thus the advantage of elevated efficiency of pollen transfer in orchids appears to be counterbalanced somewhat by a reduction in the number of mating partners and the consequent greater stochasticity in male reproductive success.

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REFERENCES

- Ackerman JD, Sabat A, Zimmerman JK. 1996.** Seedling establishment in an epiphytic orchid: an experimental study of seed limitation. *Oecologia* **106**: 192–198.
- Broyles SB, Wyatt R. 1990.** Paternity analysis in a natural population of *Asclepias exaltata*: multiple paternity, functional gender and the 'pollen-donation hypothesis'. *Evolution* **44**: 1454–1468.
- Burd M. 1994.** Bateman's Principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* **60**: 83–139.
- Campbell DR. 1985.** Pollen and gene dispersal: the influences of competition for pollination. *Evolution* **39**: 419–431.
- Campbell DR. 1998.** Variation in lifetime male fitness in *Ipomopsis aggregata*: tests of sex allocation theory. *American Naturalist* **152**: 338–353.
- Cresswell JE, Osborne JL, Bell SA. 2002.** A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. *Oikos* **98**: 375–384.
- Devlin B, Ellstrand NC. 1990.** Male and female fertility variation in wild radish, a hermaphrodite. *American Naturalist* **136**: 87–107.
- Dressler RL. 1990.** *The orchids: natural history and classification*. Cambridge, MA: Harvard University Press.
- Dressler RL. 1993.** *Phylogeny and classification of the orchid family*. Portland, OR: Dioscorides Press.
- Eckert CG. 2000.** Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* **81**: 532–542.
- Elle E, Meagher TR. 2000.** Sex allocation and reproductive success in the andromonoecious perennial *Solanum carolinense* (Solanaceae) 2. Paternity and functional gender. *American Naturalist* **156**: 622–636.
- Feinsinger P, Busby WH, Tiebout HM III. 1988.** Effects of indiscriminate foraging by tropical hummingbirds on pollination and plant reproductive success: experiments with two tropical treelets. *Oecologia* **76**: 471–474.
- Ferdy J-B, Loriot S, Sandmeier M, Lefranc M, Raquin C. 2001.** Inbreeding depression in a rare deceptive orchid. *Canadian Journal of Botany* **79**: 1181–1188.
- Hájek J. 1969.** *A course in nonparametric statistics*. San Francisco: Holden-Day.
- Harder LD. 2000.** Pollen dispersal and the floral diversity of Monocotyledons. In: Wilson KL, Morrison D, eds. *Monocots: systematics and evolution*. Melbourne: CSIRO Publishing, 243–257.
- Harder LD, Barrett SCH. 1995.** Mating cost of large floral displays in hermaphrodite plants. *Nature* **373**: 512–515.
- Harder LD, Barrett SCH, Cole WW. 2000.** The mating consequences of sexual segregation within inflorescences of flowering plants. *Proceedings of the Royal Society of London B* **267**: 315–320.
- Harder LD, Thomson JD. 1989.** Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* **133**: 323–344.
- Harder LD, Williams NM, Jordan CY, Nelson WA. 2001.** The effects of floral design and display on pollinator economics and pollen dispersal. In: Chittka L, Thomson JD, eds. *Cognitive ecology of pollination*. Cambridge: Cambridge University Press, 297–317.
- Harder LD, Wilson WG. 1997.** Theoretical perspectives on pollination. *Acta Horticulturae* **437**: 83–101.
- Harder LD, Wilson WG. 1998a.** A clarification of pollen discounting and its joint effects with inbreeding depression on mating-system evolution. *American Naturalist* **152**: 684–695.
- Harder LD, Wilson WG. 1998b.** Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. *Ecology* **79**: 2789–2807.
- He T, Smouse PE. 2002.** Paternity analysis in *Ophiopogon xylorrhizus* Wang et Tai (Liliaceae s.l.): selfing assures reproductive success. *Journal of Evolutionary Biology* **15**: 487–494.
- Hessing MB. 1988.** Geitonogamous pollination and its consequences in *Geranium caespitosum*. *American Journal of Botany* **75**: 1324–1333.
- Holsinger KE, Thomson JD. 1994.** Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. *The American Naturalist* **144**: 799–812.
- Johnson SD. 1995.** Observations of hawkmoth pollination in the South African orchid *Disa cooperi*. *Nordic Journal of Botany* **15**: 121–125.
- Johnson SD. 2000.** Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behaviour. *Biological Journal of the Linnean Society* **71**: 119–132.

- Johnson SD, Edwards TJ. 2000.** The structure and function of orchid pollinaria. *Plant Systematics and Evolution* **222**: 243–269.
- Johnson SD, Linder HP, Steiner KE. 1998.** Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* **85**: 402–411.
- Johnson SD, Nilsson LA. 1999.** Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. *Ecology* **80**: 2607–2619.
- Johnson SD, Peter C, Ågren J. 2004.** The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. *Proceedings of the Royal Society of London B* **271**: 803–809.
- de Jong TJ, Waser NM, Price M, Ring RM. 1992.** Plant size, geitonogamy and seed set in *Ipomopsis aggregata*. *Oecologia* **89**: 310–315.
- Leclerc-Potvin C, Ritland K. 1994.** Modes of self-fertilization in *Mimulus guttatus* (Scrophulariaceae): a field experiment. *American Journal of Botany* **81**: 199–205.
- Levin DA. 1989.** Proximity-dependent cross-compatibility in *Phlox*. *Evolution* **43**: 1114–1116.
- Levin DA, Berube DE. 1972.** *Phlox* and *Colas*: the efficiency of a pollination system. *Evolution* **26**: 242–250.
- Liang KY, Zeger SL. 1986.** Longitudinal data analysis using generalized linear models. *Biometrika* **73**: 13–22.
- Lloyd DG. 1992.** Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences* **153**: 370–380.
- McCullagh P, Nelder JA. 1989.** *Generalized linear models*, 2nd edn. London: Chapman & Hall.
- Meagher TR. 1986.** Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most-likely parents. *American Naturalist* **128**: 199–215.
- Miller RB. 1981.** Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* **35**: 763–774.
- Morris WF, Price MV, Waser NM, Thomson JD, Thomson BA, Stratton DA. 1994.** Systematic increase in pollen carryover and its consequences for outcrossing in plant populations. *Oikos* **71**: 431–440.
- Mosquin T. 1970.** The reproductive biology of *Calypso bulbosa* (Orchidaceae). *Canadian Field-Naturalist* **84**: 291–296.
- Murren CJ, Ellison AM. 1998.** Seed dispersal characteristics of *Brassavola nodosa* (Orchidaceae). *American Journal of Botany* **85**: 675–680.
- Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. 1996.** *Applied linear statistical models*, 4th edn. Chicago: Irwin.
- Nilsson LA. 1978.** The pollination ecology of *Epipactis palustris* (Orchidaceae). *Botaniska Notiser* **131**: 355–368.
- Nilsson LA, Rabakonandrianina E, Pettersson B. 1992.** Exact tracking of pollen transfer and mating in plants. *Nature* **360**: 666–668.
- Nunney L. 1993.** The influence of mating system and overlapping generations on effective population size. *Evolution* **47**: 1329–1341.
- Ortiz-Barney E, Ackerman JD. 1999.** The cost of selfing in *Encyclia cochleata* (Orchidaceae). *Plant Systematics and Evolution* **219**: 55–64.
- Paulus HF, Gack C. 1990.** Pollinators as prepollinating isolating factors: evolution and speciation in *Ophrys* (Orchidaceae). *Israel Journal of Botany* **39**: 43–79.
- Peakall R. 1989.** A new technique for monitoring pollen flow in orchids. *Oecologia* **79**: 361–365.
- Peakall R, Beattie AJ. 1991.** The genetic consequences of worker ant pollination in a self-compatible, clonal orchid. *Evolution* **45**: 1837–1848.
- Peakall R, Beattie AJ. 1996.** Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentaculata*. *Evolution* **50**: 2207–2220.
- Pettersson MW. 1991.** Pollination by a guild of fluctuating moth populations: option for unspecialization in *Silene vulgaris*. *Journal of Ecology* **79**: 591–604.
- Rademaker MCJ, De Jong TJ, Klinkhamer PGL. 1997.** Pollen dynamics of bumble-bee visitation on *Echium vulgare*. *Functional Ecology* **11**: 554–563.
- Rademaker MCJ, de Jong TJ, van der Meijden E. 1999.** Selfing rates in natural populations of *Echium vulgare*: a combined empirical and model approach. *Functional Ecology* **13**: 828–837.
- Schoen DJ, Lloyd DG. 1992.** Self- and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Sciences* **153**: 381–393.
- Snow AA, Lewis PO. 1993.** Reproductive traits and male fertility in plants: empirical approaches. *Annual Review of Ecology and Systematics* **24**: 331–351.
- Snow AA, Spira TP, Simpson R, Klips RA. 1996.** The ecology of geitonogamous pollination. In: Lloyd DG, Barrett SCH, eds. *Floral biology: studies on floral evolution in animal-pollinated plants*. New York: Chapman & Hall, 191–216.
- Stanton ML, Ashman TL, Galloway LF, Young HJ. 1992.** Estimating mate fitness of plants in natural populations. In: Wyatt R, ed. *Ecology and evolution of plant reproduction*. London: Chapman & Hall, 62–90.
- Thomson JD, Thomson BA. 1989.** Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. *Evolution* **43**: 657–661.
- Vassiliadis C, Saumitou-Laprade P, Lepart Y, Viard F. 2002.** High male reproductive success of hermaphrodites in the androdioecious *Phillyrea angustifolia*. *Evolution* **56**: 1362–1373.