

Pollinator-mediated selfing in two deceptive orchids and a review of pollinium tracking studies addressing geitonogamy

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Abstract Among the factors thought to have favoured the evolution of deception (rewardlessness) in orchids is the reduction of pollinator-mediated selfing when unrewarded pollinators visit fewer flowers per inflorescence. We obtained data on natural levels of geitonogamy in the deceptive orchids *Dactylorhiza sambucina* and *Himantoglossum hircinum* by monitoring the dispersal and receipt of colour-coded pollinia. As donors, we marked 185 flowers of *D. sambucina* and 956 flowers of *H. hircinum*. In *D. sambucina*, 30% of the pollinator-visited flowers and 62% of the marked inflorescences experienced geitonogamous pollination events. In *H. hircinum*, the respective percentages were 36 and 71%. The furthest pollen transport distance in the *Andrena*-pollinated *H. hircinum* was 6.9 m (median 1.27 m), while the furthest transport in the bumblebee-pollinated *D. sambucina* was 176 m (median 1.23 m), a record in Orchidaceae. An analysis of pollen-tracking studies in orchids revealed geitonogamy levels of around 40% (based on individuals; 19–37% based on flowers) in both rewardless species and rewarding ones. This is similar to geitonogamy levels in other animal-pollinated angiosperms, although the data basis for comparison may still be too small. So far, however, it is not evident that rewardless orchids experience particularly low levels of geitonogamy.

Keywords *Dactylorhiza* · Deceptive flowers · Geitonogamous pollination · *Himantoglossum* · Pollinia tracking

Introduction

Understanding the interplay between plant traits, pollinator behaviour, and resulting gene flow patterns is of key interest in evolutionary biology and conservation. Molecular approaches to the assessment of gene flow can demonstrate gene exchange, but cannot always distinguish maternal from paternal gene dispersal or events in the distant past from ongoing gene exchange (Avisé 2000). Direct monitoring of seed and pollen transport therefore is needed to complement the genetic picture of mating patterns among individuals and populations. It also is the only way to obtain data not modified by post-pollination processes. In flowering plants, monitoring pollen dispersal is possible through mark/recapture techniques, using histochemical dyes (Folsom 1987, 1994; Peakall 1989; Peakall and Beattie 1991, 1996; Tremblay 1994; Salguero-Farías and Ackerman 1999), fluorescent powders (Waser and Price 1982; Thomson et al. 1986; Snow et al. 1996 and references therein), micro-tagging of pollen packages (Nilsson et al. 1992), ¹⁴C labelling (Pleasants 1991), or backscatter scanning electron microscopy (Wolfe et al. 1991). Genetic assessments of gene flow patterns have been published for 70 species or subspecies of Orchidaceae (summarized in Tremblay et al. 2005), but we know of only ten species in which the fate of pollen packages has been monitored directly (*Dichaea potamophila*, Folsom 1987, 1994; *Prasophyllum fimbria*, Peakall 1989; *Microtis parviflora*, Peakall and Beattie 1991; *Caladenia tentaculata*, Peakall and Beattie 1996; *Aerangis ellisii*, Nilsson et al. 1992;

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Cypripedium calceolus, Tremblay 1994; *Comparetia falcata*, Salguero-Fara and Ackerman 1999; *Anacamptis morio*, Johnson et al. 2004; *Disa cooperi*, Johnson et al. 2005; *Disa pulchra*, Jersakova and Johnson 2006). These studies have reported maximum transport distances of 23 m (Tremblay 1994), 27 m (Peakall 1989), 58 m (Peakall and Beattie 1996), 64 m (Johnson et al. 2005), and 76 m (Nilsson et al. 1992), information that is useful in conservation efforts because type of pollinator and typical transport distances are correlated, allowing some extrapolation between species.

Another interest of pollen tracking studies lies in their unique ability to quantify pollinator-mediated self-pollination (Snow et al. 1996). Avoidance of geitonogamy, the transference of pollen between flowers on the same individual, has received considerable attention as a possible selective factor in the evolution of deception in orchids (Dressler 1981, 1993; Ackerman 1986; Johnson and Nilsson 1999; Johnson et al. 2004; Smithson 2002, 2006; Jersakova and Johnson 2006; Jersakova et al. 2006). The argument is that flowers with pollen packages instead of individual pollen grains should suffer strongly from pollen and stigma discounting because self-pollination completely eliminates such flowers' chance to export pollen and at the same the stigmas' chances to receive additional outcrossed pollen (Harder and Barrett 1995). As long as pollinators rapidly leave unrewarding inflorescences (without learning to avoid them completely), not producing nectar should decrease geitonogamy.

The hypothesis that nectarlessness evolved to reduce geitonogamy (because bees will rapidly leave unrewarding plants) has been tested in experimental studies that compared levels of geitonogamy in nectar-supplemented and nectarless flowers (Johnson and Nilsson 1999; Smithson and Gigord 2001; Smithson 2002; Johnson et al. 2004; Jersakova and Johnson 2006). These studies revealed previously unsuspected high levels of geitonogamy (Discussion). To enlarge and broaden the existing sparse data on natural levels of geitonogamous pollination in rewardless orchids we undertook a pollen mark/recapture study in two unrelated taxa, *Dactylorhiza sambucina* and *Himantoglossum hircinum*, from a geographic area (Central Europe) where no such study had previously been carried out (compare the list above of the ten Orchidaceae studied so far). For pollen tracking we relied on fluorescent dye powders. These have often been used as pollen analogues in other angiosperms (Waser and Price 1982; Thomson et al. 1986; Snow et al. 1996 and references therein), but had not been used in orchids. As with histochemical dyes, different colours of powder can be used to simultaneously label multiple plants. Besides providing data on natural levels of insect-mediated selfing, this study contributes data on the pollinator-mediated linkage of orchid populations in frag-

mented European landscapes, which may become relevant for conservation.

Materials and methods

Study species

Dactylorhiza sambucina (L.) Soo and *Himantoglossum hircinum* (L.) Sprengel belong to subtribe Orchidinae (Dressler 1993; Bateman et al. 2003). *Dactylorhiza sambucina* has simple, terminal inflorescences (mean height 16 cm; Kropf and Renner 2005) that bear several to numerous resupinate flowers with basally spurred lips, which serve as pollinator landing places. In *H. hircinum*, the median part of the lip is extended to a 5- to 6-cm-long strap, while in *D. sambucina* the labellum is maximally 1 cm long. The stigmas in both species are concave and sticky. In *D. sambucina*, the two pollinia are attached to separate viscidia and form a transport unit of two hemipollinaria that are removed together. The hemipollinaria are not deposited on stigmas as entire packages, however, but instead as clusters of pollen grains (massulae). A relatively fast caudicle bending time in *D. sambucina* (ca. 40 s; M. Kropf, personal observation) allows successful pollination shortly after the pollinaria have become attached to a pollinator. The most important pollinators of *D. sambucina* are bumblebees (Kropf and Renner 2005 and studies cited therein). Most populations of *D. sambucina* comprise yellow and purple individuals (Nilsson 1980), but all populations studied here consist exclusively of yellow-flowering individuals (Kropf and Renner 2005).

Himantoglossum hircinum inflorescences attained heights of 83 cm (mean 45 cm; this study) with an average of 61 flowers per inflorescence. Flowers have a single viscidium and pollinarium, and as in *D. sambucina* the pollen is organized in massulae. Caudicle bending in *H. hircinum* takes 1–3 min from the moment when pollinaria are removed from a flower to the moment when they are ready to be deposited on a stigma (Heusser 1915). The major pollinators of *H. hircinum* are species of *Andrena* (M. Kropf, personal observation).

Both studied orchids usually are rewardless (Heusser 1915; Nilsson 1980; Kropf and Renner 2005), although some populations of *H. hircinum* may produce nectar (Teschner 1980; M. Nickol, Botanical Garden Kiel, personal communication, 1996 and 2006; Teschner and Nickol both verified the presence of nectar physically and chemically). Schmid (1912) reports on bees taking nectar from *H. hircinum* in Thuringia, but without indicating how he verified the presence of sugar secretion in the spur. For this study, we tested both species for nectar visually as well as probing their spurs with micro-capillaries.

Distribution, habitats, and study locations

Dactylorhiza sambucina occurs throughout Central and Mediterranean Europe, with isolated populations in southern Scandinavia. *Himantoglossum hircinum* is restricted to the western part of Submediterranean Europe, with a number of recently established populations in England and Germany (Kropf and Erz 1996; Künkele and Baumann 1998; Carey 1999; Heinrich and Voelckel 1999). Both species typically grow in dry grassland communities on slopes (Kropf 1995; Kropf and Erz 1996; Carey and Farrell 2002), and both are listed in the German Red Data Book (Korneck et al. 1996).

Dactylorhiza sambucina was studied between April and May 1995 in six nature protection areas near the Nahe River in the vicinity of Bad Kreuznach (7°52'E, 49°50'N) in the Rhineland-Palatinate, Germany. The areas were Berlachsborg (BE; more than 100 flowering plants), Bremroth (BR; 84 plants), and Haarberg (HA; 500–600 plants). *Himantoglossum hircinum* was studied between May and June 1995 in the nature protection area Hasenkopf (HK; more than 100 flowering plants), Domberg—In der Amschel (AM; 23 plants), and Domberg near Steinhardt (ST; 35 plants). We piloted our tracking technique at the AM location in May 1994, using individuals of *H. hircinum* that were monitored again in 1995; the sole 1994 pollen recapture event observed (over a distance of 3.55 m) was pooled with the 1995 data.

Field experiments on pollen tracking

Each pollen source plant at the locations BE, BR, HA, AM, HK, and ST was marked with a different colour of fluorescent powder (Day-Glo Colour, USA), with pollinia in all newly opened flowers marked daily soon after flowers had opened. The individual dye particles had a size of 14–26 μm , and colours used were blue, green, orange, pink, and red. To apply the powder, pollinia were carefully pulled forward from their anther cap with the help of forceps and painted using a hairbrush of size zero (painted reinserted pollinia are shown in Fig. 1a). Labelled pollinia were reinserted under their anther cap. This handling did not affect the viscidia, which remained in their original position inside the bursiculae. Neither painted pollinia nor unpainted ones ever fell out of their anther caps unless pulled out by a pollinator, and the viscidia of labelled pollinia would stick to dissecting needles just like those of untreated pollinia (when tested after the end of the experiment).

To assess possible treatment effects on pollinia removal, removal was also monitored in nearby unlabelled conspecifics. We did not test whether application of the fluorescent powders affected caudicle-bending times or the deposition of pollen massulae on the stigmas. The extremely small size and weight of the powder particles make the former

unlikely; if deposition was affected by powder application, this would affect all our results in the same direction since all our data come from painted massulae found on stigmas.

Pollen source plants for labelling were selected based on two criteria. First, they represented a range of distances to the next flowering conspecific that was typical of our study sites. Second, they came from patches with different densities of flowering conspecifics within a radius of 1 m. Pollen (massulae) receipt was monitored as follows. At four of the study sites, stigmas of all flowering conspecifics were checked every other day. At the remaining two locations, HA (*D. sambucina*) and HK (*H. hircinum*), flowering plants were too numerous to monitor all stigmas, and inspection therefore focused on areas around source individuals. Removed pollinia were counted, and for each recovered pollen load, the distance, direction from the source plant, and date of pollination event were recorded. “Pollination event” refers to a bee visit being responsible for the presence of marked pollen on one or more stigmas within the same inflorescence. Where several massulae of the same colour were found on a stigma, we conservatively assumed that they resulted from one bee visit. We also counted flowering *D. sambucina* and *H. hircinum* plants ignored by pollinators (i.e., not pollinated with labelled pollen) on their way from a labelled plant to a pollinated individual in 2-m-wide transects along the paths of observed pollen movements.

Statistical treatments

Treatment effects were tested with Student's *t*-tests. The randomness of pollinator flight direction was tested with circular statistics as described in Fisher (1993: p. 67, Eq. 4.13) and with a Rayleigh test for determining departure from uniformity (Fisher 1993).

Results

Treatment effects

There were no significant differences between pollen removal in labelled and unlabelled flowers, with results of *t*-tests at the six study sites all non-significant ($P > 0.05$): *D. sambucina*, BE, $t = -0.189$, $df = 17$; BR, $t = -1.851$, $df = 82$; HA, $t = -1.863$, $df = 40$; *H. hircinum*, AM, $t = 0.564$, $df = 14$; HK, $t = -0.557$, $df = 20$; ST, $t = -0.537$, $df = 35$ (data on pollen removal from unlabelled flowers of *D. sambucina* are from Kropf and Renner 2005). In *Dactylorhiza*, colour did not affect the proportion of pollinia removed ($\chi^2 = 1.54$, $df = 4$, $P = 0.82$), while in *Himantoglossum*, the proportion removed depended on the colour ($\chi^2 = 18.3$, $df = 3$, $P = 0.0004$) because of the low proportion



Fig. 1 **a** Flower of *Himantoglossum hircinum* with colour-coded pollinia. **b** *Apis mellifera* carrying labelled pollinaria. **c** The same honeybee on its flight to another plant. **d** Several colour-coded massulae deposited on the sticky surface of a stigma

of orange removed in one plant individual (ST no. 4). When red, blue, and green were compared, there was no difference in removal rate ($\chi^2 = 3.35$, $df = 2$, $P = 0.19$).

Dactylorhiza sambucina

Dactylorhiza sambucina flowers contained no nectar. Pollinia in 185 flowers on 13 inflorescences at three locations were painted (Table 1). Inflorescences produced 14 flowers on average, slightly more than the 9.5 found at other locations (Kropf and Renner 2005). Of the 185 labelled polli-

nia, bees removed 100 (54.1%; Table 1) of which we recaptured clumps of massulae on the stigmas of 47 flowers on 30 plants. Eight inflorescences (Table 1) received geitonogamous pollinations and 22 (Table 2) xenogamous pollinations. The maximum number of flowers on an inflorescence pollinated with labelled pollen was four (whether from xenogamous or geitonogamous pollinations). Because flowers were inspected every other day, pollinator visits could be deduced from consecutive observations of newly received marked pollen. Thirty-five pollination events could account for the deposited 47 clumps of

Table 1 Pollinia removal in 13 colour-labelled source inflorescences of *Dactylorhiza sambucina* at Berlachtsberg (BE), Bremroth (BR), and Haarberg (HA). Source plants with two inflorescences are indicated by A or B

Location	Source plant	Powder colour	Number of labelled flowers	Number of removed pollinaria (%)	Distance (cm) to next flowering conspecific	Number of flowering conspecifics within a radius of 1 m
BE	4	Blue	12	1 (8.3)	37	5
BE	9 ^a	Green	15	4 (26.7)	66	5
BE	10 ^a	Red	21	7 (33.3)	90	3
BR	1 ^a	Blue	10	7 (70.0)	37	1
BR	3	Green	11	9 (81.8)	24	12
BR	4	Red	13	4 (30.8)	11	9
BR	5	Pink	10	4 (40.0)	11	8
BR	6	Orange	9	5 (55.6)	6	10
HA	1A ^a	Red	22	15 (68.2)	29	10
HA	1B ^a	Red	15	15 (100.0)	29	11
HA	112 ^a	Blue	21	16 (76.2)	17	14
HA	117A ^a	Green	11	6 (54.5)	39	8
HA	117B ^a	Green	15	7 (46.7)	37	9
Total			185	100		
Mean			14.2	7.7 (54.1)	33.3	8.1
SD			4.5	4.8 (25.6)	23.3	3.7

^a Pollinator-mediated self-pollination observed during field-work

Table 2 Xenogamous pollination events inferred from the recapture of colour-coded *D. sambucina* pollen on stigmas of unmarked plants at BE, BR, and HA. Receptor plants with two inflorescences are indicated by A or B. For abbreviations, see Table 1

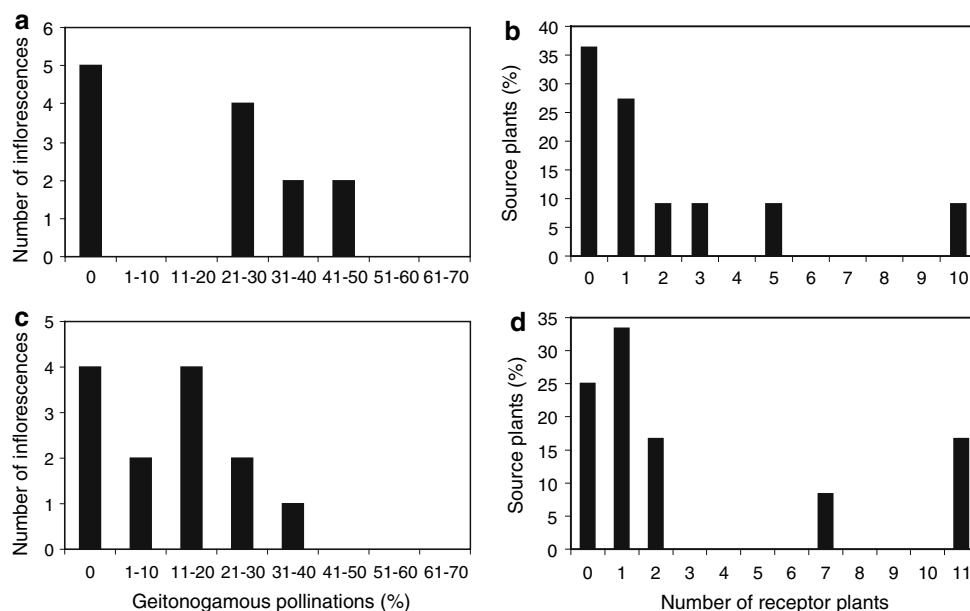
Location	Receptor plant	Colour of received pollen	Distance (cm) to source plant	Number of flowers receiving labelled pollen	Pollination events (pollinated flowers per event)
BE	16	Red	746	2	1 (2)
BE	21	Red	129	1	1 (1)
BE	22	Red	386	1	1 (1)
BR	45	Red	386	1	1 (1)
BR	51	Orange	27	4	1 (4)
BR	59	Green	35	1	1 (1)
HA	3	Red	29	3	2 (1,2)
HA	115	Blue	72	1	1 (1)
HA	116	Blue	234	1	1 (1)
HA	118	Red	3,900	1	1 (1)
HA	12	Red	75	2	1 (2)
HA	4	Red	30	1	1 (2)
HA	14	Red	123	2	1 (1)
HA	5A	Red	85	1	1 (1)
HA	5B	Red	90	1	1 (1)
HA	19B	Red	233	1	1 (1)
HA	130	Blue	38	1	1 (1)
HA	131 ^a	Blue	255	1	1 (1)
HA	131 ^a	Green	38	2	1 (2)
HA	132	Green	130	2	1 (2)
HA	133	Blue	348	2	1 (2)
HA	1 ^b	Red	17,600	1	1 (1)
Total			24,989.0 (25,018.0) ^c	33	23
Mean			1,135.86 (1,087.74) ^c	1.50	1.05
SD			3,765.87 (3,686.52) ^c	0.80	0.21

^a Plant no. 131 received pollen from two source plants

^b Plant no. 1 was located south of road L 420

^c The different distances *in parentheses* reflect that some of the 23 pollination events involved the same plant visited at different times

Fig. 2 Percentages of geitonogamous pollinations in **a** *Dactylorhiza sambucina* ($n = 13$ colour-coded inflorescences) and **c** *Himantoglossum hircinum* ($n = 13$ colour-coded inflorescences), and numbers of receptor plants receiving pollen from labelled donor inflorescences in **b** *Dactylorhiza sambucina* ($n = 11$) and **d** *Himantoglossum hircinum* ($n = 12$)



massulae, 12 of them involving geitonogamy, 23 xenogamy (Table 2). Geitonogamous pollinations occurred in 30% of the flowers on eight (61.5%) of the 13 inflorescences (these eight inflorescences are marked in Table 1), involving on average 1.75 flowers/inflorescence (based on the eight inflorescences that received at least one pollen package). Overall, 21.1% (± 19.3 SD) of the pollinations in the 13 marked inflorescences involved geitonogamy, with a few inflorescences experiencing 41–50% geitonogamy (Fig. 2a shows the frequency distribution of the geitonogamous pollinations). On average, source plants pollinated 3.3 receptor plants (± 3.3 SD), although one plant pollinated ten receptor plants (Fig. 2b), and another plant (HA no. 131) received pollen from two source plants (Table 2).

Distances between source plants and flowering conspecifics varied widely (6–90 cm, mean 33.3 ± 23.3 SD; Table 1) and so did flowering plant densities (range 1–14, mean 8.1 ± 3.7 SD; Table 1). Excluding geitonogamous events, the mean transport distance in *D. sambucina* was 10.88 m (± 37 m SD; median 1.23 m; Table 2), and shortest and longest distances were 0.27 and 176 m (Table 2). Longest transport distances were those between study locations, with bees even crossing a highway (L 420) south of one of the study sites. Excluding the two furthest distances, mean transport distance was 1.68 m (± 1.79 SD; median 0.90 m), and pollen deposition was strongly leptokurtic around the source plant (Fig. 3a). A circular statistical test indicated that flight directions did not differ from a random distribution ($\chi^2 = 9.3$, $df = 7$, $P = 0.23$). The mean number of ignored flowering *D. sambucina* inflorescences in 2-m-wide transects between successful donors and recipients was 6.1 (± 7.2 SD), with between zero and 26 flowering plants remaining unvisited by bees carrying labelled pollen (Fig. 4a).

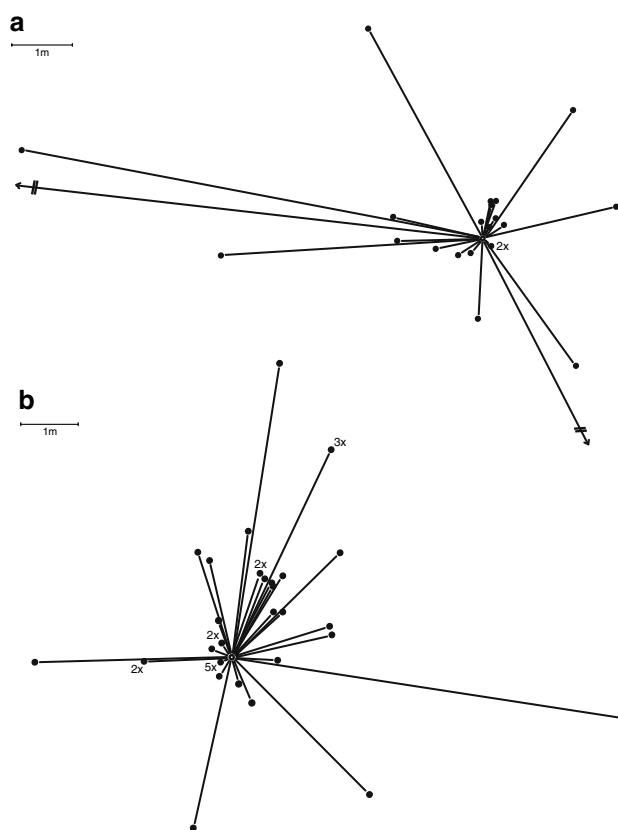
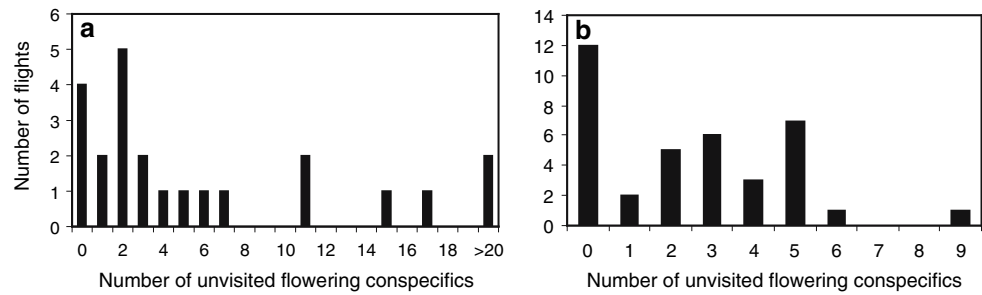


Fig. 3a, b Straight-line pollen movements superimposed on north-oriented maps. **a** *Dactylorhiza sambucina* ($n = 23$); **b** *Himantoglossum hircinum* ($n = 37$)

Himantoglossum hircinum

Himantoglossum hircinum flowers in our study populations contained no nectar. Pollinia of 1,059 flowers on 14 inflorescences were painted, but one plant with numerous

Fig. 4a, b Unvisited flowering conspecifics in 2-m-wide transects along pollinator flight routes to visited plants. **a** *Dactylorhiza sambucina* ($n = 23$); **b** *Himantoglossum hircinum* ($n = 37$)



labelled flowers was lost. Of the 956 remaining pollinia, 219 (30%) were removed and 120 were recaptured as clumps of massulae on as many stigmas in 36 plants; ten plants (Table 3) received geitonogamous pollinations and 28 (Table 4) xenogamous pollinations. (Of the 38 receptor plants, AM no. 19 was lost and AM no. 7 excluded because of incomplete data, leaving 36 plants.) The inflorescence with the highest success rate captured 15 labelled pollen loads from five consecutive xenogamous pollinations. Since flowers in the focal inflorescences were inspected at least every third day, we could estimate that 61 pollinator visits could explain the 120 pollen loads recaptured on stigmas. Excluding geitonogamy, there were 37 pollination events (Table 4). Geitonogamy occurred in 35.8% of the flowers on ten (71.4%) of the colour-coded inflorescences (these ten inflorescences are marked in Table 3). An average of 4.3 flowers/inflorescence received self-pollen (based on the ten inflorescences that received at least one pollen package), and overall 14.7% (± 14.1 SD) of all pollinations on the 13 colour-coded inflorescences involved geitonog-

amy, with most inflorescences experiencing 11–20% geitonogamy (Fig. 2c shows the frequency distribution of geitonogamous pollinations).

Distances between source plants and flowering conspecifics again varied widely (9–121 cm, mean 49.9 cm \pm 37.3 SD; Table 3) as did flowering density (range 1–8, mean 3.4 \pm 2.7 SD; Table 3). Excluding geitonogamous events, the mean transport distance was 1.59 m (± 1.44 SD; median 1.27 m; Table 4). The shortest distance was 0.16 m and the longest 6.88 m (Table 4). On average, source plants pollinated 4.1 receptor plants (± 4.3 SD), although one source plant exported pollen to 11 recipients (Fig. 2d). Pollen transport distances were leptokurtic, and the spatial spread of pollinaria (Fig. 3b) was not random ($\chi^2 = 22.9$, $df = 7$, $P = 0.0018$). However, the Rayleigh test for directional preferences showed no significant deviations from a mean direction ($Z = 1.937$, $P = 0.144$). The cause for these apparently contradictory results is the bimodality of the data. That is, bees tended to fly off either to the north or to the south, hence the data are non-randomly distributed; however,

Table 3 Pollinia removal in 14 colour-labelled source inflorescences of *Himantoglossum hircinum* at In der Amschel (AM), Hasenkopf (HK), and Steinhardt (ST). Source plants with two inflorescences are indicated by A or B

Location	Source plant	Powder colour	Number of labelled flowers	Number of removed pollinaria (%)	Distance to next flowering conspecific (cm)	Number of flowering conspecifics within a radius of 1 m
AM	8A	Green	25	9 (36.0)	41	3
AM	8B	Green	35	7 (20.0)	38	3
AM	10 ^a	Blue	44	7 (15.9)	38	3
AM ^b	19 ^a	Red	–	–	75	4
AM	20	Orange	25	7 (28.0)	90	2
HK	1 ^a	Orange	103	19 (18.4)	55	1
HK	2 ^a	Blue	86	37 (43.0)	121	0
HK	3 ^a	Red	86	18 (20.9)	16	6
HK	4A ^a	Green	86	16 (18.6)	12	8
HK	4B ^a	Green	86	20 (23.3)	9	8
ST	1 ^a	Red	87	29 (33.3)	111	0
ST	2 ^a	Green	80	18 (22.5)	14	6
ST	3 ^a	Blue	111	26 (23.4)	18	3
ST	4	Orange	102	6 (5.9)	60	1
Total			956	219		
Mean			73.5	16.8 (22.9)	49.9	3.4
SD			30.3	9.7 (9.5)	37.3	2.7

^a Pollinator-mediated self-pollination observed during field-work

^b The inflorescence of AM no. 19 with 27 labelled flowers was lost due to grazing

Table 4 Xenogamous pollinations inferred from the recapture of colour-coded *H. hircinum* pollen on stigmas of unmarked plants at AM, HK, and ST. Receptor plants with two inflorescences are indicated by A or B. For abbreviations, see Table 3

Location	Receptor plant	Colour of received pollen	Distance to source plant (cm)	Flowers receiving labelled pollen	Pollination events (pollinated flowers per event)
AM	8B	Blue	38	3	2 (2,1)
AM	33	Red	240	1	1 (1)
AM	7 ^a	Green	355	1	1 (1)
HK	12	Red	16	15	5 (1,1,2,4,7)
HK	13	Blue	157	2	1 (2)
HK	14	Blue	142	1	1 (1)
HK	15	Blue	420	1	1 (1)
HK	16B	Red	129	2	1 (2)
HK	17	Red	141	1	1 (1)
HK	18	Blue	121	1	1 (1)
HK	19	Red	31	2	1 (2)
HK	20	Green	63	1	1 (1)
HK	21	Red	114	3	1 (3)
HK	22	Red	133	1	1 (1)
HK	23	Red	32	1	1 (1)
HK	24	Blue	325	9	3 (2,3,4)
ST	1	Blue	160	3	2 (1,2)
ST	2	Blue	104	4	1 (4)
ST	4	Red	688	1	1 (1)
ST	5	Blue	116	1	1 (1)
ST	6	Blue	83	4	1 (4)
ST	7	Blue	49	2	1 (2)
ST	8	Red	232	2	1 (2)
ST	14	Blue	350	1	1 (1)
ST	18	Blue	280	1	1 (1)
ST	28	Blue	130	5	1 (5)
ST	29	Green	67	3	1 (3)
ST	29	Blue	127	5	2 (2,3)
Total			4,843.0 (5,882.0) ^b	77	37
Mean			172.96 (158.97) ^b	2.75	1.32
SD			147.45 (143.86) ^b	3.01	0.86

^a The pollination event on plant AM no. 7 was observed during the 1994 pilot study (see [Materials and methods](#))

^b The higher distances *in parentheses* indicate that some of the 37 pollination events involved the same plant visited at different times

one cannot identify a single direction since the distribution is bimodal (and therefore the *Z*-statistic, which tests for a directional preference, is non-significant). The mean number of unvisited *H. hircinum* inflorescences in 2-m-wide transects between donors and recipients was 2.49 (± 2.28 SD). A third of all pollination events involved closest neighbours (Fig. 4b).

Levels of pollinator-mediated geitonogamy in orchids

To place these geitonogamy levels in context, we compiled data from all species in which pollinium fate has been monitored via direct tracking (Table 5). It appears that about 40% (based on individual plants) or 19–37% (based on flowers) of pollen transport events in nectar-producing as well as deceptive orchids typically involve geitonogamy.

These levels are similar to those reported from rewarding non-orchid angiosperms (Snow et al. 1996).

Discussion

Natural levels of pollinator-mediated geitonogamy in rewarding and deceptive orchids

This study revealed unexpectedly high levels of geitonogamous pollination, with 62% of the *D. sambucina* inflorescences and 71% of the *H. hircinum* inflorescences experiencing this form of selfing and about a third of all visited flowers (30 and 36%) receiving pollen from neighbouring flowers on their own inflorescence. Seen from the male perspective, almost 8% of the pollinaria produced by

Table 5 Pollen tracking studies of rewarding and deceptive Orchidaceae. n.a. Non applicable

Orchid species	<i>Aerangis ellisi</i>	<i>Dichaea potamophila</i>	<i>Disa cooperi</i> year 2001	<i>Disa cooperi</i> year 2002	<i>Prasophyllum fimbria</i>	<i>Comparentia falcat</i> ^a	<i>Caladenia tentaculata</i> ^b	<i>Cypripedium calceolus</i> ^c	<i>Dactylophiza sambucina</i>	<i>H. hircinum</i>
References	Nilsson et al. (1992)	Folsom (1994)	Johnson et al. (2005)	Johnson et al. (2005)	Peakall (1989)	Salguero-Faria and Ackerman (1999)	Peakall and Beattie (1996)	Tremblay (1994)	This study	This study
Country	Madagascar	Colombia	South Africa	South Africa	Australia	Puerto Rico	Australia	Canada	Germany	Germany
Reward	Nectar	Oil	Nectar	Nectar	Nectar	Nectar	None	None	None	None
Pollinators	Hawkmoths	Euglossine bees	Hawkmoths	Hawkmoths	Bees and wasps	Hummingbirds	Thymine wasps	Bees	Bumble-bees	Solitary bees
Labelling technique	Micro-tags	Histochemical stains	Histochemical stains	Histochemical stains	Histochemical stains	Histochemical stains	Histochemical stains	Histochemical stains	Fluorescent powder dyes	Fluorescent powder dyes
Labelled plants	151	n.a.	25	50	16	n.a.	n.a.	n.a.	13	14
Labelled flowers	1,329	88	209	186	139	n.a.	n.a.	81 (i.e. 162 pollinaria) ^c	185	1,059 (956 undamaged)
Labelled plants that exported pollen (%)	n.a.	n.a.	23 (92.0)	36 (72.0)	12 (75.0)	n.a.	n.a.	n.a.	13 (100.0)	14 (100.0)
Labelled plants with pollen recaptured on a stigma (%)	n.a.	n.a.	9 (36.0)	19 (38.0)	n.a.	n.a.	n.a.	n.a.	9 (69.2)	11 (78.6)
Removed marked pollinaria (%)	n.a.	31 (35.2)	170 (81.3)	70 (37.6)	58 (41.7)	n.a.	n.a.	n.a.	100 (54.1)	219 (22.9)
Plants that received marked pollen	n.a.	9	17	51	47	n.a.	66	n.a.	30	36
Flowers that received marked pollen	27	10	n.a.	n.a.	125	26	66	25	47	120
Plants receiving labelled pollen (mean)	n.a.	n.a.	1.9	2.6	n.a.	n.a.	n.a.	n.a.	3.3	4.1
Maximum number of flowers receiving labelled pollen in a single pollination event	n.a.	2	5	8	6	n.a.	n.a.	n.a.	4	7
Plants receiving geitonogamous pollen (%)	n.a.	1 (11.1)	20 (80.0)	21 (42.0)	n.a.	n.a.	7 (n.a.)	0 (0.0)	8 (61.5)	10 (71.4)
Flowers receiving geitonogamous pollen (%)	8 (30.0)	1 (10.0)	n.a.	n.a.	27 (21.6)	22 (84.6)	7 (10.6)	0 (0.0)	14 (29.8)	43 (35.8)
Minimum transport distance (non-geitonogamous; cm)	<100	100	<500	<500	<100	<100	<100	95	27	16
Maximum transport distance (m)	76	9	25	64	27	n.a.	58	23	176	7
Mean transport distance (m)	ca. 10.5	5.7	5.0	15.3	8.0	n.a.	17.2	5.2	10.88 (1.68) ^d	1.59

^a Pooled data from 2 study years

^b *C. tentaculata* has solitary flowers, and the number of pollinated flowers therefore equals that of plants

^c *C. calceolus* has two stamens per flower

^d Mean transport distance in *D. sambucina* after excluding the two furthest pollen transport distances of 39 and 176 m

D. sambucina ended up on a flower of the plant producing the pollen, and in *H. hircinum*, that percentage was almost 5%. Geitonogamy levels in other orchids in which pollinium fate has been monitored via direct tracking (Table 5) are generally similar, with about 40% (based on plants) or 19–37% (based on flowers) of all pollinations involving geitonogamy. Geitonogamy levels in rewarding angiosperms other than Orchidaceae are equally high (Snow et al. 1996), although as pointed out by Snow and colleagues, geitonogamy has received relatively little attention from pollination ecologists outside orchid circles. This is probably because the most reliable method to assess geitonogamy is direct tracking, which is difficult in angiosperms with individual pollen grain deposition. [Geitonogamy in orchids has also been inferred indirectly from the reduction in pollen receipt in emasculated flowers (Maad and Reinhammar 2004).]

Pollinator-mediated geitonogamy (and likewise pollinator-mediated autogamy) should reduce fitness because of the resulting seed and pollen discounting (Harder and Barrett 1995; Goodwillie et al. 2005), yet a recent survey found that 42% of flowering plant species may engage in mixed mating, reproducing via outcrossing as well as selfing, including pollinator-mediated selfing (Goodwillie et al. 2005). Of the two types of selfing, autonomous selfing should be beneficial in species that commonly face large seasonal or inter-annual fluctuations in pollinator visits because it removes dependence on an insect vector, thus providing reproductive assurance. In orchids, autonomous selfing is relatively common (Kirchner 1922; Hagerup 1952; Catling 1990; Tremblay et al. 2005). In contrast, pollinator-mediated selfing (geitonogamy) may be beneficial in species occurring in sparse populations where flowering conspecifics are scarce, but pollinators are present (Lloyd 1979, 1992; Goodwillie et al. 2005). With flowering conspecifics far apart, geitonogamy may not be strongly selected against because it provides reproductive assurance (just like autogamy). Whether these considerations apply will depend on the costs of the resulting inbreeding. Of the species studied here, *D. sambucina* suffers strongly from inbreeding depression, reflected in a smaller percentage of embryos formed after selfing (Nilsson 1980), lower seed mass, viability, and germination rates (Jersáková et al. 2006), and greenhouse survival after 1 year (Juillet et al. 2007). *Himantoglossum hircinum* has not been studied with respect to possible inbreeding depression.

So far, patterns of pollen dispersal and levels of geitonogamy (20–40%) found in rewarding and rewardless species are similar (Table 5), but few species have been investigated. Based on the limited current data, indications are that contrary to predictions rewardless orchids may not necessarily sustain lower geitonogamy than other species.

Apparently, some percentage of individual bees revisits the same inflorescence and persists in probing its flowers for nectar even when disappointed during earlier visits. Indeed, complete avoidance of rewardless plants is unlikely to be selectively favoured because the polylectic bees that pollinate most orchids will typically encounter temporarily depleted flowers of other plant families that it pays to revisit, and only a few consistently empty orchids (that should not be revisited). In such fluctuating nectar markets, rapid strong avoidance would be a poor strategy (cf. Renner 2005).

Transport distances in mark/recapture studies of orchid pollinaria

Previous studies that have quantified pollen transport distances in orchids are summarized in Table 5. Studies that used the “presentation stick method” in which focal flowers are experimentally presented to pollinators (Thomson 1988; Johnson et al. 2004; Jersáková and Johnson 2006) were excluded because they do not address natural distances between pollen receipt and deposition. The single studied ant-pollinated orchid, *Microtis parviflora* (Peakall and Beattie 1991), experiences extremely low pollen flow distances (12.4 ± 14.9 cm), and we decided not to include them with the remaining fly-, wasp-, bee-, and bumblebee-pollinated species in Table 5. Maximum transport distances are 58 m in a wasp-pollinated species (Peakall and Beattie 1996), 64 m and 76 m in two hawkmoth-pollinated species (Nilsson et al. 1992; Johnson et al. 2005), and 176 m in the bumblebee-pollinated *D. sambucina* studied here. Larger insects forage over wider areas than smaller ones, which fits roughly with the transport distances recorded so far (Table 5). There were two predominant directions of pollen movement (see Results, circular statistics), and the longest pollinator flights recorded in *D. sambucina* (39, 176 m) both ended at target plants at the periphery of dense, visually conspicuous patches, in agreement with earlier results demonstrating the positive correlation between inflorescence height and male and female fitness in *D. sambucina* (Kropf and Renner 2005). In the bee-pollinated, deceptive *Orchis pallens*, successful pollinations also occur at the periphery of populations where incoming pollinators first alight (Vöth 1982), perhaps due to inherent bee foraging behaviour involving runs of consecutive short-distance flights within flower patches, interrupted by occasional long-distance switches between patches (Keasar 2000). For the two deceptive species studied here, this foraging behaviour results in a mix of geitonogamy, near-neighbour pollination (Fig. 3), and occasional long-distance outcrossing not fundamentally different from the situation in many other insect-pollinated perfect-flowered rewarding angiosperms.

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