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Effects of Nectar Production and Pollinator Assemblies on Mating Patterns in Orchids

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Abstract

Pollinator visitation patterns should affect pollination success and mating patterns in flowering species. In the orchid family, about one third of the species do not provide any reward for their pollinators. Pollination by deceit is typically associated with low fruit set but may increase the chance of cross-pollination since the pollinator should soon leave the individual plant when there is no reward in the flowers. This may be beneficial if self-fertilisation results in inbreeding depression. I studied the mating patterns of one rewarding and one deceptive orchid in two closely related genera by tracking the fate of stained pollinia. I also conducted controlled crosses to estimate inbreeding depression. The results show that the deceptive orchid *Dactylorhiza lapponica* has lower pollination success, but higher cross-pollination rate (*ca.* 90%) than the nectariferous orchid *Gymnadenia conopsea* (*ca.* 18% cross-pollination). The results further suggest that in *G. conopsea*, nocturnal visitors mediate higher geitonogamous pollination rate (*ca.* 100%) than diurnal visitors (*ca.* 60%). In both study species, fruits produced from cross-pollination were heavier than fruits produced from selfing. Inbreeding depression for fruit mass did not differ significantly between the two species ($\delta = 0.21$ in *D. lapponica* and $\delta = 0.29$ in *G. conopsea*). These data support the hypothesis that pollination by deceit can enhance cross-pollination. A literature study including several rewarding and non-rewarding orchid species indicated lower geitonogamy in the deceptive orchids, but the difference was not statistically significant.

Key words

Inbreeding depression, mating system, nectar, nectarless, orchid, pollen staining, pollinator assembly.

Contents

Introduction

The mating system of plants, in particular the proportions of self-fertilization and cross-fertilization, strongly influences genetic diversity and plant population structure (Richards, 1986; Harder *et al.*, 2008). Since plants are sessile, they cannot directly control gamete dispersal, but instead need pollen vectors to achieve reproductive success. It is believed that 75% of the angiosperms depend on animal vectors to mediate pollination (National Research Council, 2007). As a result, pollinator assemblies and floral traits influencing pollinator behaviour are suggested to have large effects on mating patterns in flowering species.

Animal-pollinated species normally build reliable mutualistic relationships with their pollinators by offering a reward, such as food in the form of nectar or pollen (Dafni, 1992). However, some plant species do not produce any rewards, and instead cheat their flower visitors to accomplish pollination. Interestingly, this deceptive strategy, only occasionally found in other plant families, is pretty common in the family Orchidaceae (approximately 6500 orchid species out of 7500 deceptive species in total; Renner, 2006).

Pollinators may quickly learn to associate certain floral traits with a lack of rewards, and deceptive plants can be avoided after only a few visits (Internicola *et al.*, 2008). This should lead to low reproductive success, and suggests that a deceptive pollination strategy is evolutionary unfavourable (Darwin, 1862). Despite the fact that nectarless species have low fruit set compared to nectar-producing species (reviewed by Cozzolino & Widmer, 2005; Tremblay *et al.*, 2005), deceptive orchids make up about one third of their family (van der Pijl & Dodson, 1966; Dafni, 1984; Ackerman, 1986; Nilsson, 1992), and pollination by deceit has evolved independently several times in different lineages of the Orchidaceae (Johnson *et al.*, 1998; Cozzolino, et al., 2001), indicating some advantages that offset the disadvantage of low fruit set.

There are two main hypotheses that try to explain this intriguing phenomenon. One is the resource limitation hypothesis, which suggests that nectar production is costly, and that deceptive plants can allocate resources to other essential processes, such as fruit production and future flowering, rather than to nectar production (Southwick, 1984; Koopowitz & Marchant, 1998; Luyt & Johnson, 2002). However, it does not seem to be a plausible hypothesis, as seed production of non-rewarding orchids may be severely pollen limited instead of resource limited over their lifetime (Calvo, 1993; Tremblay *et al.*, 2005). It is hard to understand why deceptive species do not allocate some resources to a component of pollinator attraction, such as nectar production (Jersakova & Johnson, 2006).

The second main hypothesis is the outcrossing hypothesis. It states that rewards encourage pollinators to probe many flowers in sequence in rewarding plants, which could result in high self-pollination rates. In deceptive species, however, the pollinators are expected to visit few flowers within an inflorescence and leave quickly for another plant, leading to high cross-pollination rates (Ackerman, 1986; Dafni & Calder, 1987;

Johnson & Nilsson, 1999). Deceptive species may therefore avoid inbreeding depression, the commonly observed lower fitness of progeny produced by inbreeding (self-fertilization or breeding with their relatives) compared to those produced by outbreeding (breeding with unrelated individuals), (Lande & Schemske, 1985; Charlesworth *et al.*, 1990).

Generally speaking, there is a negative correlation between selfing rate and the magnitude of inbreeding depression in natural populations of angiosperms (Charlesworth & Charlesworth, 1987; Husband & Schemske, 1996) since the deleterious recessive alleles leading to inbreeding depression could be purged under selection over many selfing generations (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987). Thus, if deceptive species have a higher outcrossing rate than nectar-producing species, they are also predicted to experience higher inbreeding depression than closely related rewarding species (Charlesworth & Charlesworth, 1987; Ågren & Schemske, 1993).

In addition to nectar status, the composition of the pollinator assembly should directly influence pollination success and plant mating patterns (Dressler, 1981). Pollinator effectiveness and abundance will influence the quantity of pollination (e.g. the amount of pollen removal and receipt and seed production), while pollinator behaviour may also influence the quality of pollination through geitonogamy (among flower self-pollination) (Mitchell *et al.*, 2009). Most angiosperms have more than one pollinator species (Fishbein & Venable, 1996; Pellmyr & Thompson, 1996), and these species may differ in their foraging pattern and the degree of selfing they mediate (Schmitt 1980; but see Eckert 2002). Pollinator assemblies can vary both spatially and temporally. Flowers that are open more than 12 hours during a day can have both diurnal and nocturnal pollinators (e.g. Cruden, 1973; Young, 2002). The differences caused by diurnal and nocturnal pollinators can be examined by selective pollinator exclusions, where flowers are bagged either during the daylight hours or during the evening hours.

In order to reveal plant mating patterns, researchers have used a variety of approaches to mark and trace pollen. Some methods take advantage of a natural polymorphism in pollen colour, shape or size, but these are only applicable in a few specific taxa (Thomson & Thomson, 1989). Some methods use genetic markers based on the expression of easily recognized dominant genes in parent and offspring generations (Harder & Barrett, 1995). This requires cultivation of a large number of plants, and the development of genetic markers for the species of interest. Other methods include labelling of pollen grains by stains and dyes, by radioactive elements, or by neutron activation of pollen grains (see summary by Dafni, 1992). In orchids, pollen is packed in a structure called the pollinium, which makes pollen staining an easy and inexpensive technique to monitor pollen fate (Peakall, 1989).

In this study, the mating patterns of one deceptive orchid, *Dactylorhiza lapponica*, and one nectariferous orchid, *Gymnadenia conopsea*, were studied by monitoring the fate of stained pollinia. Molecular data indicate a close phylogenetic relationship between

the two genera *Dactylorhiza* and *Gymnadenia* (Bateman *et al.,* 2003). Both study species are self-compatible, but depend on pollinators for successful pollen transfer, usually bumblebees in *D. lapponica* (Sletvold *et al.,* 2010a)*;* and butterflies, flies and diurnal and nocturnal hawkmoths in *G. conopsea* (Sletvold & Ågren, 2010). I specifically ask:

1) does the deceptive orchid *Dactylorhiza lapponica* have a higher cross-pollination rate than the rewarding orchid *Gymnadenia conopsea*? and if so,
2) does *D. lapponica* express higher inbreeding depression than *G. conopsea*?

Additionally, for *G. conopsea*, which is pollinated both by day- and night- active species,

3) do mating patterns mediated by diurnal and nocturnal pollinators differ?

Additionally, to further test the outcrossing hypothesis I reviewed published studies examining pollination success and inbreeding depression in rewarding and deceptive orchid species.

Material and Methods

Study site

The field study was carried out in Sølendet (62°40´N, 11°50´E), near Røros in central Norway. Sølendet Nature Reserve was established in 1974 and covers an area of 306 ha. on the transition between the middle boreal and northern boreal vegetation zones (Moen *et al.,* 1999). The growing season is short, usually from late May to late August (Sletvold *et al.,* 2010). Rich fens, especially rich lawn communities belonging to *Caricion atrofuscae,* and wooded (birch) grasslands and heaths are the common habitats here. Twelve orchid species, together with many other species, form the rich flora in the nature reserve (Moen *et al.,* 1990). The two studied orchids in this project, *Dactylorhiza lapponica* and *Gymnadenia conopsea*, are quite abundant in the area. The field study was carried out in Sølendet from mid July to early August 2010.

Study species

Dactylorhiza lapponica (Laest. ex Hartman.) Soó is a tuberous and non-clonal perennial herb belonging to the family Orchidaceae. It is found in calcareous fen habitats in moorland, grassy areas, pastures and meadows from lowlands to the subalpine zone (Moen, 1999). It is distributed in Fennoscandia, Scotland and alpine areas in Central Europe (Delforge & Harrap, 2006; Øien & Moen, 2002).

The individuals usually emerge above-ground in early June (Sletvold *et al.,* 2010). The single inflorescence opens acropetally with 3-19 flowers (mean ± SD, 8 ± 3, n = 281; this study). The flowers are in reddish violet to dark purple. Spurs are well developed but without any nectar production. Pollen massulae are tightly packed into two polliniaria with viscidium ends situated in the entrance of the spur. Each pollinium contains 88-127 pollen massulae (mean ± SD, 104 ± 9, n = 33). Pollinators are usually bumble bees: *Bombus pascuorum* and *B. lucorum* (Sletvold *et al*., 2010a). *D. lapponica*

usually has low fruit set (20%-40%), and seed production is severely pollen limited (Sletvold *et al*., 2010a).The fruits mature in August (Sletvold *et al*., 2010b) and the minute seeds are dispersed by wind.

Gymnadenia conopsea (L.) R. Br. S.l. is a tuberous and non-clonal perennial herb which occurs in damp calcareous soil, in meadows, pastures, and calcareous fens.

G. conopsea has more flowers per inflorescence compared to *D. lapponica* (range 10-38, mean ± SD, 21 ± 5, n = 219). It flowers from June to August. The flower is fragrant, and the color can vary from light pink to cerise red. The spur with nectar is long and narrow, extending behind the flower. Nectar is produced continuously in the blooming period (Stpiczynska & Matusiewica, 2001). Above the entrance of the spur, there are two pollinia, each of which contains about 52-118 pollen massulae (mean ± SD, 84 ± 14, n = 69). Diverse pollinators are recorded in *G. conopsea* (van der Pijl & Dodson, 1966). In Sølendet Nature Reserve, it has both diurnal and nocturnal pollinators, usually butterflies in the genus *Boloria* Moore (Nymphalidae) and the fly *Empis tessellate* F. (Empididae) during the day time, and the hawkmoth *Hyles gallii* Rott. (Sphingidae) during the night (Sletvold and Ågren, 2010). High levels of fruit set are typical (60-80%), but seed production is significantly pollen limited in the study population (Sletvold & Ågren, 2010). About 4-6 weeks after pollination, the fruits are mature. Then the capsule fruits dehisce and the minute seeds are dispersed by wind (Sletvold & Ågren, 2010).

Field experiment

Pollen Staining Experiment

In orchids, pollen staining is a convenient technique to follow the fate of pollen and get direct information about the mating pattern (Peakall, 1989). Previous studies have demonstrated that histochemical stains do not affect the rate of pollen removal and pollen deposition by insect vectors (Peakall, 1989; Johnson, 2005).

Experimental individuals were picked in the field by cutting at the base of the inflorescences and putting them in florist's foam at the bottom of a bucket with water for transport to the lab. In the lab, the histochemical stains fast green (1%), rhodamine B (0.2%), and gentian violet (0.2%), were randomly allocated to different individuals and carefully injected into anther sacs by the use of a 10 µl syringe under the stereo microscope. Both the number of open flowers and the number of stained flowers were recorded.

The stained plants were placed in marked plastic tubes with water. Tubes were translocated to the field within their natural environment. Groups of three focal inflorescences stained with different colours were placed together, and groups were separated by at least 20 meters to avoid pollen transfer among the groups.

After about 48 hours, the tubes were taken back to the lab and every flower was carefully checked to get the detailed information about the number of removed pollinia and the number of deposited massulae, as well as the color of the deposited massulae. All data were recorded at the flower level and then summarised for each plant.

The pollen staining experiment generally worked as described above for both study species. However, as the two species have some different features (e.g. *G.conopsea* has both diurnal and nocturnal pollinators; *G. conopsea* is a rewarding species and thus have higher level of visitation than the non-rewarding species *D. lapponica*; *D. lapponica* has larger pollinia; etc.), there are some differences in set ups for the two species.

Experimental set up for *Dactylorhiza lapponica*

In order to get virgin flowers of *Dactylorhiza lapponica*, the experimental plants were bagged prior to the experiment to prevent pollinator visitation. All the flowers of the experimental plants were stained with 2 μl histochemical stains per pollinium. In total, 138 individuals with 1110 flowers were stained in the experiment. The whole experiment was carried out in eight rounds from 18th to 26th July, 2010.

Experimental set up for *Gymnadenia conopsea*

For the nectar-producing species *Gymnadenia conopsea*, bagging might influence the nectar volumes and further affect pollinator visitation. Therefore, the experimental plants were directly picked in the field and all visited flowers (with pollinia removal and/or massulae deposition) were removed before pollinia staining.

Every 2nd flower in the inflorescence was stained with 1 μl histochemical dye per pollinium. This approach allows the separation of within- and among-flower self-pollination (see Appendix 1 for the detailed mathematical model).

To test whether diurnal and nocturnal pollinators mediate different mating patterns, I combined pollen staining with a selective pollinator exclusion experiment. Three focal plants with comparable flowers in each staining group were under different treatments: day pollination (plants were bagged during the night from 6pm to 6am), night pollination (plants were bagged during the day from 6am to 6pm), and control (plants without any bagging, exposed to pollinators both day and night). The histochemical stains switched among the treatments. A total number of 108 individuals with 2238 flowers in the staining experiment were allocated to the three treatments, each with 36 replicates. The whole experiment was carried out in nine rounds from 16th to 29th July, 2010.

Pollen Staining Efficiency

Pollen staining efficiency measures how well the pollen staining performs in terms of labelling pollen massulae, i.e. the proportion of pollen massulae stained in each pollinium.

After the pollen staining experiment, the non-visited stained flowers were used to estimate pollen staining efficiency. Thirty-three stained pollinia (11 pollinia per color) of *D. lapponica* and 69 stained pollinia (23 pollinia per color) in *G. conopsea* were randomly chosen, and the stained and unstained pollen massulae were carefully counted under a stereo microscope.

Field Survey

In the pollen staining experiment, individual plants had only 48 hours to be visited. To estimate pollination success of the two studied species, I randomly picked 108 individuals of *D. lapponica* (on 20th and 21st July, 2010) and 111 individuals of *G. conopsea* (from 23rd to 27th July, 2010) throughout my study area. The total number of flowers was counted for each plant. Pollen removal and massulae deposition were quantified for every flower and summarised for each sampled individual.

Inbreeding Depression Experiment

To estimate the effects of inbreeding, a total number of 36 plants of *D. lapponica* were bagged at the onset of flowering. During the peak flowering, four flowers on each plant were randomly allocated to two treatments: self-pollination and cross-pollination. All experimental flowers were emasculated and pollinated with two pollinia. Pollinia for cross-pollination were collected from two different donors more than 5 meters away from the focal plant (to ensure outbreeding). These plants were bagged again after hand pollination. After fruit maturation, all focal fruits were collected and brought to the lab to determine the fruit mass.

Data on inbreeding depression for *G. conopsea* was collected by Nina Sletvold in Sødendet in 2008, when an identical experiment was carried out with a total number of 44 plants of *G. conopsea*.

Literature Study

The literature study was based on Smithson (2006) and Kropf & Renner (2008), where they summarised data on inbreeding depression and pollen tracking studies of rewarding and deceptive Orchidaceae. I also searched Google Scholar for related literature investigating pollination success, mating patterns and inbreeding depression in rewarding and/or deceptive orchids published until the year 2010. Results relevant to the outcrossing hypothesis were included, i.e. studies reporting one or several of the following response variables: the number of removed pollinia and deposited massulae, geitonogamy rate, and inbreeding depression. Inbreeding depression was recorded either from reports of inbreeding depression or based on reported differences in performance of selfed and outcrossed progeny. Only inbreeding depression in early life history stages (i.e. fruit mass, seed mass, the proportion of seeds with embryos) was included in this study.

Data analyses

I summed the number of visited flowers, and the number of removed pollinia and deposited massulae across flowers for all plants in the pollen staining experiment and the field survey. As the two species have different number of flowers per inflorescence, I calculated the proportion of active flowers and average pollinia removal and massulae deposition per flower to make the results comparable. Mann-Whitney U tests were performed to examine whether the proportion of active flowers and per flower pollinia removal and receipt differed significantly between the two species, and also between

the staining experiment and the field survey. Kruskal-Wallis tests were used to test for differences among the three pollination treatments in the rewarding species.

To estimate pollen staining efficiency (PSE), I used the formula:

$PSE = M_s/M_n*100\%$

M_s is the number of massulae that were stained successfully, and M_n is the total number of massulae in the sampled pollinium.

Pollen staining efficiency was calculated for all three colors in both species. For each species, a one-way ANOVA was performed to test whether PSE differs among colors.

To examine the mating patterns in the two study species, self- and cross-pollination rates were estimated with a mathematical model (detailed in Appendix 1).

To estimate pollen transfer efficiency (PTE), I used the formulae:

$PTE = M_d/(P_r*\overline{M}_n) * 100\%$

M_d is the total number of massulae deposited successfully on the stigma, P_r is the total number of removed pollinia, and $\overline{M}_n$ is the average number of massulae in each pollinium (Johnson *et al.,* 2004).

To calculate inbreeding depression (δ), I used the formula:

$\delta = 1-W_s/W_o$

where W_S is the fitness following self-pollination and W_o is the fitness following cross-pollination (Johnston & Schoen, 1994). Here, fruit mass was used as the fitness estimate. Fruit mass is strongly correlated with number of seeds with embryos in both study species (Sletvold *et al*., 2010a; Sletvold & Ågren 2010).

To examine the effect of cross type on fitness, I used a nested ANOVA, with the model 'Fruit mass = CrossType + Species + MatPlant(Species) + CrossType*Species. In this model, maternal plant is included as a random factor and a significant interaction between cross type and species demonstrates that the magnitude of inbreeding depression differs between species.

All the statistical analyses were carried out in Minitab 14 for Windows.

Results

Pollen Staining Efficiency

Pollen staining efficiency was high in both species, demonstrating that the staining technique worked well in the experiment. Almost all the pollen massulae were well stained in *D. lapponica* (Mean ± SD: 0.99 ± 0.03), and around 87% (SD = 0.13) of pollen massulae were successfully stained in the experiment with *G. conopsea*. There was no difference in staining efficiency among the different colors in either of the species (P = 0.726 for *D. lapponica*, P = 0.685 for *G. conopsea*; One-way ANOVA).

Nectar vs. Nectarless Orchids

In the pollen staining experiment, the rewarding orchid had both more visited plants and more visited flowers than the deceptive species. The proportion of visited individuals of *G. conopsea* (66.7%) was almost three times as high as that of *D. lapponica* (23.2%), and in each individual of *G. conopsea*, significantly higher proportions of flowers were active (Table 1). Pollination success differed substantially between the two studied species. Per flower pollinia removal was four times higher in *G. conopsea* compared to *D. lapponica*, and per flower massulae deposition was about 16 times higher (Table 1). In *D. lapponica*, a small proportion of the stained pollinia were successfully removed (2.4%) and only a limited part of the removed pollinia reached a conspecific stigma (6.6%). In *G. conopsea*, both pollen removal success and pollen transfer efficiency were considerably higher than in *D. lapponica* (13.0% and 20.2% respectively, Table 1).

In *D. lapponica*, the proportion of visited flowers and the number of removed pollinia and deposited massulae per flower was significantly higher (all P-values < 0.001, tested with Mann-Whitney U tests) in the field survey of non-experimental plants compared to plants in the staining experiment; while these values in *G. conopsea* did not change very much (all P-values > 0.1, tested with Mann-Whitney U tests).

In the field survey, the two species did not differ significantly in the proportion of visited flowers or pollinia removal per flower, resulting in comparable pollen removal success (Table 1). The significantly fewer massulae deposited in *D. lapponica*, however, lead to less efficient pollen transfer in this species compared to *G. conopsea* (Table 1).

Table 1. Pollination success (Mean ± SD) in *D. lapponica* and *G. conopsea*.

	Pollen Staining Experiment			**Field Survey**		
	***D. lapponica* (N=138)**	***G. conopsea* (N=36)**	**P-values**	***D. lapponica* (N=108)**	***G. conopsea* (N=111)**	**P-values**
Proportion of Visited flowers	0.051 ±0.116	0.214 ±0.255	<0.001	0.174 ±0.185	0.166 ±0.181	0.784
Pollinia Removal/Flower	0.053 ±0.134	0.270 ±0.396	<0.001	0.270 ±0.314	0.180 ±0.234	0.088
Massulae Deposition/Flower	0.242 ±1.393	4.660 ±9.310	<0.001	1.180 ±2.862	2.830 ±5.934	0.004
Pollen Transfer Efficiency	6.6%	20.2%	n.a.	4.0%	8.6%	n.a.

Note: N is the sample size. Differences are examined with a Mann-Whitney U test, n.a. not applicable, results for *G. conopsea* in the pollen staining experiment are from the control group.

The mating patterns of the nectarless and nectariferous orchids differed. Most removed pollinia in *D. lapponica* contributed to cross-pollination, whereas pollinia in *G. conopsea* were mainly deposited on other flowers within the same individual (Figure 1).

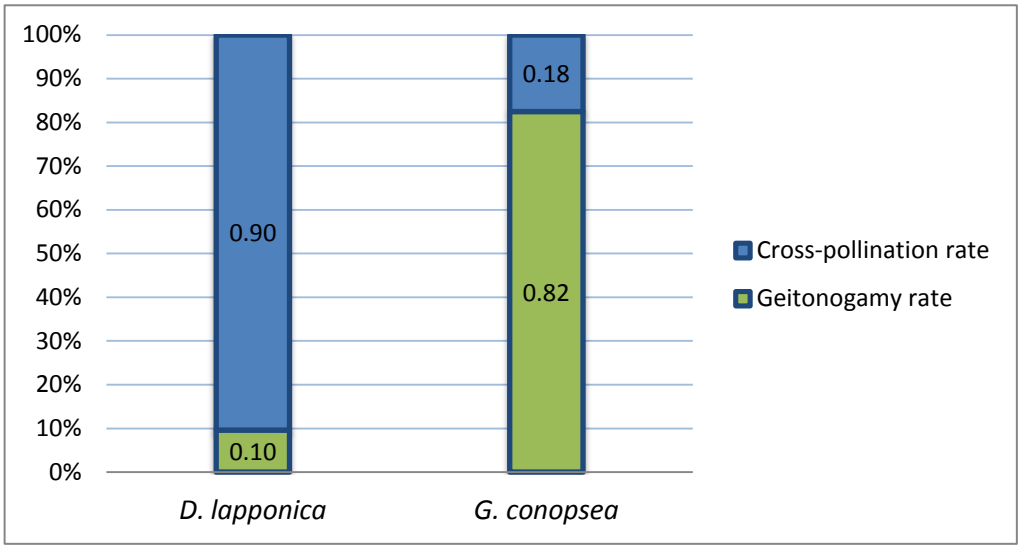

Figure 1. Mating patterns of the two studied species *D. lapponica* and *G. conopsea*.

Day vs. Night Pollinators

For *G. conopsea*, the three pollination treatment groups (control, day, and night) had comparable numbers of visited plants (24, 20, 24 respectively) and similar proportions of active flowers per plant (Table 2). Per flower pollinia removal and massulae deposition did not differ significantly among treatment groups (all P-values > 0.05, Table 2). The control group, which was exposed to both diurnal and nocturnal pollinators, had slightly higher pollen removal success (13%) and slightly higher pollen transfer efficiency (20.2%) than the groups only exposed in the day or at night (Table 2).

Table 2. Pollination success (Mean ± SD) of *G. conopsea* individuals exposed to flower visitors in the day or at night.

	Control (N=36)	**Day (N=35*)**	**Night (N=35*)**	**P-values**
Proportion of Visited Flowers	0.214 ±0.255	0.162 ±0.211	0.145 ±0.190	0.627
Pollinia Removal/Flower	0.263 ±0.397	0.139 ±0.233	0.244 ±0.586	0.360
Massulae Deposition/Flower	4.690 ±9.310	2.036 ±4.347	3.120 ±6.860	0.452
Pollen Transfer Efficiency	20.2%	17.3%	18.1%	n.a.

Note: N is the sample size; Differences in pollination success examined with Kruskal-Wallis test; n.a., not applicable. *, one plant in the day treatment and one plant in the night treatment died during the experiment.

Despite similar pollination success, mating patterns depended on whether plants were exposed to pollinators during the day or during the night (Figure 2). Pollination during day resulted in similar geitonogamy and cross-pollination rates (40% and 39%, respectively), with a rate of within flower selfing of about the half (20%). In contrast, only geitonogamous pollination (100%) was found at night. The proportions of the three mating types in the control group roughly corresponded to the averages of those in the day and night groups (Figure 2).

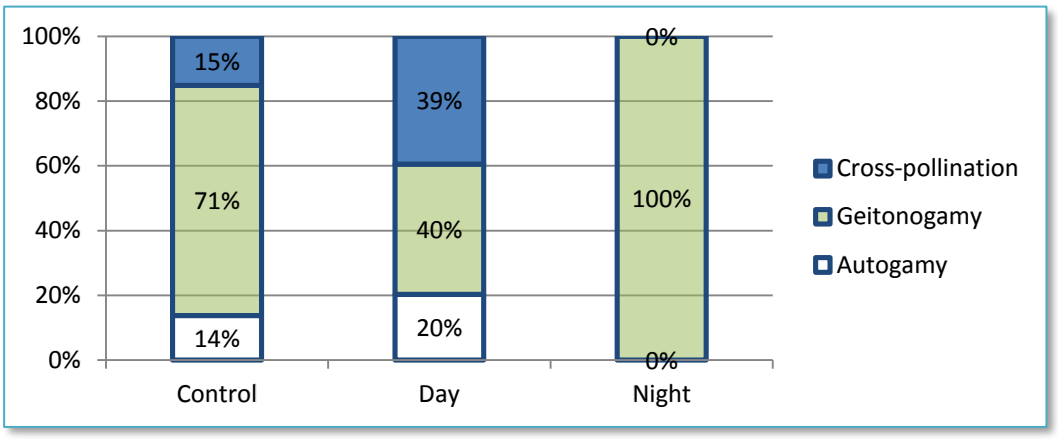

Figure 2. Mating patterns differ among *G. conopsea* individuals exposed to pollinators at different times during the day.

Inbreeding Depression

Cross-pollination resulted in heavier fruits than self-pollination did in both species. In *D. lapponica*, the average fruit mass (Mean ± SD) was 9.34 ± 4.01 mg after self-pollination, and 11.75 ±3.45 mg after cross-pollination. Fruit mass after Self- and cross-pollination in *G. conopsea* were 4.57 ±3.19 mg and 6.45 ±3.74 mg (Mean ±SD), respectively. Nested ANOVA (Table 3) showed significant differences for fruit mass both between cross types and between species. But there was no statistically significant interaction between cross type and species (P = 0.242), indicating that the two species had similar inbreeding depression in terms of fruit mass (0.29 ±0.20 in *G. conopsea* and 0.21 ±0.25 *D. lapponica*).

Table 3. Nested ANOVA results for the effects of cross type (self vs. outcross), species identity (*Dactylorhiza lapponica* vs. *Gymnadenia conopsea*), maternal plant and the interaction between cross type and species identity on fruit mass.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
CrossType	1	0.0001737	0.0001765	0.0001765	87.73	0.000
Species	1	0.0009746	0.0009746	0.0009746	40.87	0.000
MatPlant(Species)	76	0.0018121	0.0018121	0.0000238	11.85	0.000
CrossType*Species	1	0.0000028	0.0000028	0.0000028	1.39	0.242
Error	76	0.0001529	0.0001529	0.0000020		
Total	155	0.0031161				

Literature Study

By combining my study results with related former studies, there seems to be a weak trend towards the pattern predicted by the outcrossing hypothesis (Figure 3), i.e. lower pollen removal rate, lower geitonogamy rate in deceptive orchids compared with rewarding ones. However, neither of the response variables differed significantly between deceptive and rewarding species (Figure 3).

Inbreeding depression for fruit/seed mass and seeds with embryos were very similar in rewarding and deceptive species (Figure 3).

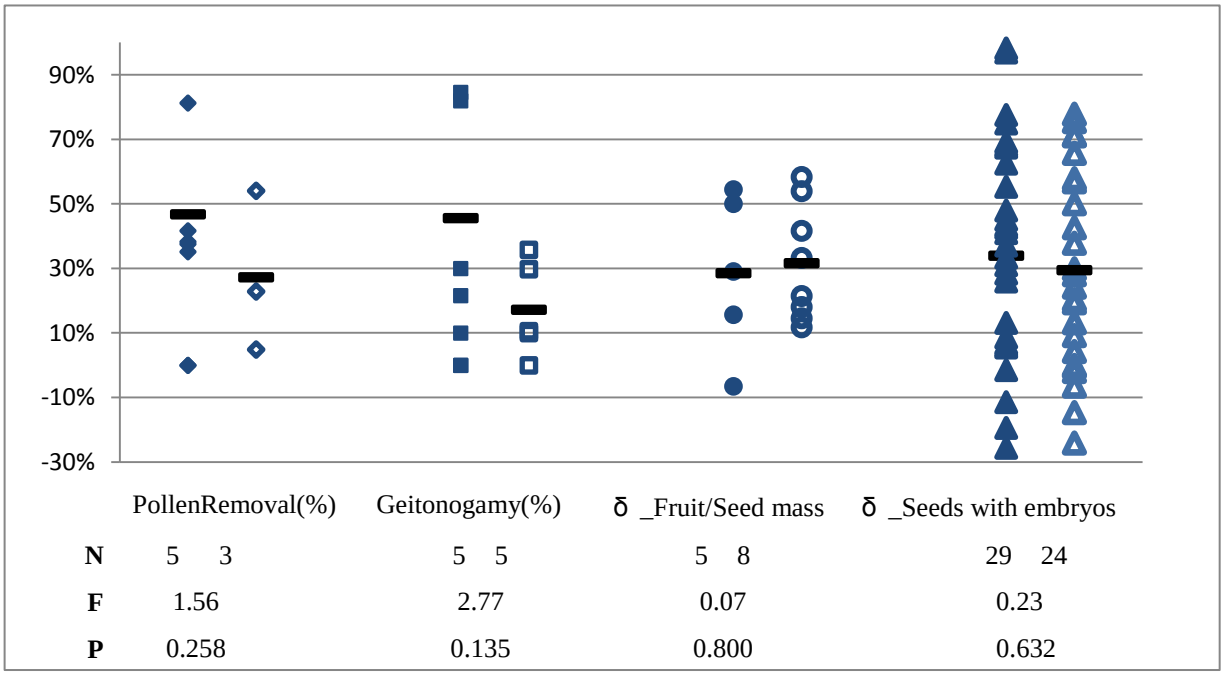

Figure 3. Results from empirical studies of rewarding and deceptive orchids. Closed symbols represent rewarding orchids, open symbols represent deceptive orchids, and bars stand for average values of each parameter. (N: sample size. P- and F- values are given by One-way ANOVA; Data resources: see Appendix 4 & 5).

Discussion

The present study comparing one deceptive and one rewarding orchid supports the hypothesis of lower pollination success and higher outcrossing rates in the deceptive orchid. Nectar production leads to high pollination quantity, while deceit ensures high pollination quality. Additionally, the results demonstrate that nocturnal pollinators of *Gymnadenia conopsea* mediate higher geitonogamy rates than diurnal pollinators. To sum up, there are effects of nectar production and pollinator assembly on the mating patterns in the studied orchids.

Do deceptive orchids suffer low pollination success?

In the 48 hours pollen staining experiment, the nectarless orchid *Dactylorhiza lapponica* had both a smaller proportion of active plants and fewer active flowers compared with the nectar-producing orchid *G. conopsea*. Both the number of removed pollinia and deposited pollen massulae per flower were significantly lower in the deceptive orchid. In other words, pollination success was lower in the deceptive orchid.

In the survey of non-experimental *D. lapponica* plants, whose flowers had been exposed to visitors for a long period, pollination was more successful than among

experimental plants that had been exposed to flower visitors for 48 hours. By comparison, the two categories of plants did not differ much in *G. conopsea*. Among non-experimental plants, pollen removal success was similar in the two species, while pollen receipt was lower in *D. lapponica*. This suggests the pollen transfer was not so efficient in the deceptive orchid.

The results suggest that the flowers of the deceptive species receive less pollen over their flowering time compared to the flowers of the rewarding species, which is consistent with a lower fruit set in *D. lapponica* (Sletvold *et al.,* 2010a,b) compared to *G. conopsea* (Sletvold & Ågren, 2010).

The results are also consistent with the review by Tremblay *et al.*(2005), who compiled a large data set and found halved per cent fruit set in deceptive orchids (20.7 ±1.7, N = 130) compared to rewarding orchids (37.1 ± 3.2, N = 84; P = 0.0008). The low pollination success and fruit set probably results from pollinators' foraging behaviour in deceptive orchids, where the pollinators probe a few flowers per inflorescence, spend little time per flower due to the absence of a reward (Smithson, 2003), and reduce their visits in total since pollinators learn to avoid the deceptive plants. Experimental studies show a significant increase in pollinator visitation after nectar supplementation in rewardless orchids (Smithson, 2001, 2002; Jersakova, 2006). Available data thus suggest that deceptive orchids generally have low pollinator visitation and low pollination success.

Do the deceptive orchids show higher cross-pollination than the rewarding orchids?

The present study reveals a higher proportion of cross-pollination in the deceptive species than in the rewarding species, indicating that pollinators visit fewer flowers in sequence in the deceptive species, which gives the pollen high chance to export to another plant. Although most orchid species are self-compatible, selfing always leads to inbreeding depression (Tremblay *et al.,* 2005). Therefore, a high level of cross-pollination in deceptive orchids elevates both female and male fitness and may serve as an explanation of the maintenance of deceptive orchids.

By now, data on rates of self-pollination are available for ten orchid species in total (five rewarding and five deceptive species). The average rate of self-pollination tends to be higher in the rewarding orchids than in the deceptive orchids, but rates do not differ significantly. This might be because the study species are too few, and also vary in many aspects besides nectar status. Nectar-addition and nectar-depletion experiments in deceptive and rewarding orchids, respectively, are designed to detect the influence of nectar production controlling for other factors. Geitonogamy increased significantly after nectar supplementation in two species of deceptive orchids, *Anacamptis morio* (Johson *et al.,* 2004) and *Disa pulchra* (Jersakova & Johnson, 2005). Clearly, studies of more orchid species are needed and more advanced experimental and analytic methods should be developed in order to reveal the general mating patterns of rewarding and deceptive species.

Many orchid species show delayed pollinia bending mechanism. The caudicle (stalk) of removed pollinarium will stay straight for a while, during which duration the pollen massulae have few chances (if any) to stick to the stigma. Then the bending, the pollinarium's physical reconfiguration, makes the pollinarium correctly reach the stigma and thus achieve pollination. Peter and Johnson (2008) found that caudicle bending time is consistently longer than, and strongly correlated with, the duration time of pollinator visitation. It supports the prediction of Darwin (1862) who argued that delayed pollinia bending serves as a mechanism for reducing geitonogamous pollination. This is a challenge to the outcrossing hypothesis. If the delayed caudicle bending mechanism prevents orchids from geitonogamous self-pollination, rewarding orchids need not have higher self-pollination rates than deceptive species, even if pollinators stay longer and visit more flowers per inflorescence.

Do deceptive orchids express higher inbreeding depression than rewarding orchids?

In both study species, fruit mass following cross-pollination was significantly higher than that following self-pollination. However, inbreeding depression (measured by $\boldsymbol{\delta = 1 - W_s/W_o}$) did not differ between *D. lapponica* and *G. conopsea*. The results are consistent with those of my literature review, which did not detect a difference in inbreeding depression in early life stages between deceptive and rewarding orchids.

One potential explanation could be that only a very early life stage was considered in this study. It is possible that deceptive orchids suffer higher inbreeding depression in later stages. Husband *et al.* (1996) found that inbreeding depression in most selfing species (14 of 18 species) was mainly expressed late in the life cycle, at the growth/reproduction stages, whereas in outcrossing species it could be expressed either in early stages (17 of 40 species) or late stages (19 species). Early-stage and late-stage inbreeding depression may be weakly correlated (Carr, 1995). Ideally, inbreeding depression should be studied across the whole life cycle of orchids to get a better understanding of possible variation in its magnitude.

Another reason for the lack of a significant difference in inbreeding depression could be that the studied species differ in many other aspects than nectar status. And the differences in ecology and life history may influence the likelihood of purging of deleterious recessive alleles and affect the magnitude of inbreeding depression (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987). It would be ideal to compare closely related species pairs with contrasting nectar status.

Besides, the efficiency of purging should depend on factors such as population size (Charlesworth & Charlesworth, 1987). Therefore, orchids whose population sizes are usually small (Ackerman, 1986; Nilsson, 1992) might not meet the purging requirements, and thus show similar inbreeding depression in both rewarding and non-rewarding species.

Does the composition of the pollinator assembly influence the mating patterns in orchids?

In the present study, pollination success in terms of pollinia removal and massulae deposition was similar during day and night in *G. conopsea*. However, this need not result in identical male and female fitness, due to the different outcrossing rates mediated by diurnal and nocturnal pollinators. The dominance of geitonogamy at night should reduce the overall fitness because of inbreeding depression and pollen discounting (the decreased fitness associated with pollen 'wasted' on selfing, that could otherwise be exported for cross-pollination; Harder & Barrett, 1995).

These patterns are probably caused by the foraging behaviour of pollinators. The nocturnal pollinators (hawkmoths) may prefer to stay longer and visit more flowers on the same individual plant compared to the diurnal pollinators (butterflies and flies). This is supported by Brunet and Sweet (2006) who found that hawkmoths tend to visit several flowers per individual rather than travel long distances between plants. Although they suggested that the abundance of hawkmoths will enhance outcrossing because they prefer female-phase flowers in the protandrous plant *Aquilegia coerulea*, the hawkmoths will generally increase geitonogamy rate in the species whose anther and stigma are not separated such as *Gymnadenia conopsea*.

In contrast, butterflies often fly long distances between flowers and forage in short and often-interrupted bouts, because when they forage, they are also searching for mates and oviposition sites (Schmitt, 1980; Murawski & Gilbert, 1986; Herrera, 1987). Eckert (2002) found butterflies to move more frequently (64% of the time) and fly longer distances (50 cm) between inflorescences of *Decodon verticillatus* compared to bees, suggesting that butterflies should mediate high outcrossing rates, though Eckert did not find any effects on mating system. Flies have not been regarded as effective pollinators since most of them feed mainly on decaying organic matter rather than floral products such as nectar (Kearns, 1992). Surprisingly, Ginsberg (1984, 1985) found that dipteran pollinators are as good at pollination as small bees (halictids) in terms of pollination quantity, and both of them will visit more flowers in succession if available. They probably mediate some degree of geitonogamy (50% geitonogamy in *Myosotis* plants caused by flies, Robertson, 1992).

The various behaviours of pollinators may explain the different mating patterns we found in the pollinator exclusion experiment, but we should be cautious since the pollinator fauna in the study population is not known well enough to make powerful predictions. Therefore, more pollinator observations and experiments should be conducted to characterize the composition of the diurnal and nocturnal pollinator assembly, and evaluate their roles in deciding mating patterns of *G. conopsea*.

Additionally, we should be aware that the disruption of plant-pollinator interactions by human activity could greatly affect mating patterns in orchids, thereby threatening the species. For example, if we loose the diurnal pollinators for *G. conopsea*, the high selfing rate at night will strongly influence its genetic diversity and population structure.

More knowledge in this area is required to guide effective actions in orchid conservation.

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Appendix

1. The Model of Pollen Staining Experiment

Definitions:

Breeding can be divided into three types based on the source of pollen massulae:

1) Within-flower selfing: stigma receives pollen massulae from the same flower. **W** stands for the rate of within-flower pollination.
2) Geitonogamy: stigma receives pollen massulae from other flowers on the same plant. We use **G** to describe this rate.

Both 1) and 2) are self-pollination (**S**).

3) Cross pollination: stigma receives pollen massulae from other plants. I use **X** for this rate.

For Gymnadenia conopsea:

Every 2nd flowers were stained in *G. conopsea*. Here, we divided geitonogamy into four different types as shown in table 6.

Table 6. Four different types of geitonogamy in the pollen staining experiment of *G. conopsea*.

	Stained flower	Unstained flower
With Stained massulae	G_{ss}	G_{us}
With Unstained massulae	G_{su}	G_{uu}

Note: G_{ss} and G_{su} mean geitonogamy of stained flowers with stained or unstained pollen massulae from other flowers on the same individual; similarly in G_{us} and G_{uu}

The assumption of the mathematical model for *G. conopsea* is that the pollinators visit the flowers randomly; and there is no effect of stained color on pollinator behaviour and pollination success. Therefore, we have the relationship between different geitonogamy types as follows:

$G_{ss}/G_{su} = G_{us}/G_{uu} = P_{r\_St}/P_{r\_US}$;

$G_{ss}/G_{us} = G_{su}/G_{uu} = N_{fl\_St}/N_{fl\_US}$.

Where G_{ss}, G_{su}, G_{us}, G_{uu} is different geitonogamy types showed in table 6. P_{r_St} and P_{r_US} are the number of pollen removed in stained flowers, and unstained flowers, respectively; N_{fl_St} and N_{fl_US} mean the number of stained or unstained flowers.

Based on the directly collected data, we get the numbers of pollen massulae deposition with the same stained color (M_{d_SC}), with other stained colors (M_{d_OC}), with non stained color (M_{d_NC}). They indicate different pollination types depending on whether the focal flower is stained or not.

For Stained flowers:

M_{d_sC} : $A+G_{ss}$

M_{d_OC} : X_1

M_{d_NC} : $G_{su}+X$

For Unstained flowers:

M_{d_SC} : G_{us}

M_{d_OC} : X'_1

M_{d_NC} : $A+G_{uu}+X'$

Where, X_1 is the cross pollination of the three experimental plants; X is the cross pollination of the other plants without any treatment. The prime (‘) indicates estimate obtained from unstained flowers.

Hence, we can calculate the values of **W**, **A** and **X** according to the formulae:

G = $G_{ss}+G_{su}+G_{us}+G_{uu}$

W = 2*W (Here, we also assume the rate of within-flower selfing is the same in stained and unstained flowers.)

X = $X_1+X'_1+X+X'$

For Dactylorhiza lapponica:

Model seems to be much simpler in *D. lapponica* because every flower of the whole inflorescence was stained. Here, self-pollination (**S**) and cross-pollination (**X**) can be directly estimated from the observed data.

S = M_{d_SC}

X = M_{d_OC} + M_{d_NC}

Where M_{d_SC} means the numbers of pollen massulae deposition with the same stained color; M_{d_OC}, with other stained colors; M_{d_NC}, with non-stained color.

In fact, there were just a few active flowers (flowers that has some visits) per plant, mostly one active flower, sometimes two or three active flowers, only one extreme individual case with six active flowers.

Thus, we can further distinguish within-flower pollination (**W**) and geitonogamy (**G**) from overall self-pollination (**S**) by a detailed examination for each focal plant. For example, if a stained flower has both pollinia unremoved, but has stained massulae on its stigma, we can conclude that the pollination represents geitonogamous pollination.

2. Individuals with pollination activities in pollen staining experiment for D. lapponica.

ID	Stained Colour	Total Flowers	Visited Flowers	PollenRemoval	MassulaDeposition	Selfing	Crossing
2	V	7	1	1	0	0	0
8	V	11	1	1	0	0	0
14	V	6	2	2	0	0	0
17	V	6	2	3	0	0	0
25	R	9	1	2	0	0	0
26	V	7	3	4	0	0	0
34	R	5	1	1	0	0	0
46	R	9	2	3	92	2	90
47	V	15	6	1	80	0	80
48	G	11	2	1	3	0	3
49	R	9	1	1	0	0	0
52	R	16	1	2	104	0	104
58	R	8	3	4	39	39	0
59	V	8	1	0	3	0	3
70	R	7	1	1	0	0	0
79	R	8	1	0	5	0	5
83	V	4	1	1	0	0	0
84	G	6	2	2	0	0	0
87	G	5	3	4	0	0	0
89	V	13	1	2	0	0	0
92	V	7	1	0	4	0	4
99	G	10	1	1	0	0	0
101	V	8	1	2	0	0	0
104	V	8	1	1	0	0	0
106	R	4	1	1	0	0	0
110	V	12	1	2	0	0	0
111	G	5	3	3	43	0	43
126	R	6	2	2	0	0	0
130	R	17	1	1	0	0	0
134	G	8	2	2	0	0	0
137	V	6	1	2	0	0	0
138	R	10	1	1	0	0	0

3. Individuals with pollination activities in pollen staining experiment for G. conopsea.

ID		**14**	**29**	**38**	**41**	**44**	**50**	**56**	**59**	**62**	**65**	**68**	**71**	**74**	**77**	**80**	**83**	**86**	**89**	**92**	**95**	**98**	**101**	**104**	**107**
Treatment		C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Color		G	R	R	G	V	G	R	G	V	R	G	V	R	G	V	R	G	V	R	G	V	R	G	V
Flower	**Number**	18	26	18	29	21	16	22	20	12	14	14	19	15	20	14	18	26	12	17	11	14	20	19	13
Visited	**Flower.**	1	2	1	8	1	1	7	5	2	6	12	5	9	3	6	11	20	7	13	1	4	4	4	2
Pollen	**Removal**	1	2	2	14	2	0	8	2	2	4	20	6	10	2	2	17	20	14	23	1	1	3	5	3
Massulae	**Deposition**	0	0	0	22	2	2	113	27	0	27	237	10	546	1	48	678	317	236	156	84	18	94	84	84
ID		**3**	**12**	**24**	**30**	**33**	**36**	**39**	**48**	**55**	**60**	**63**	**66**	**69**	**72**	**75**	**81**	**85**	**88**	**91**	**94**	**99**	**102**	**105**	**108**
Treatment		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Color		V	V	R	V	R	G	V	V	G	R	G	V	R	G	V	G	V	R	G	V	G	V	R	G
Flower	**Number**	11	20	10	19	13	30	20	12	19	20	14	14	16	22	14	18	22	11	21	10	14	19	31	14
Visited	**Flower.**	5	2	2	1	1	2	1	1	4	2	3	2	1	2	1	8	4	6	18	2	4	1	5	5
Pollen	**Removal**	1	3	1	1	1	0	1	1	3	0	1	1	2	3	1	8	8	11	31	3	2	0	5	2
Massulae	**Deposition**	90	0	25	0	0	41	0	0	23	7	88	85	0	0	0	214	0	77	740	0	96	3	19	106
ID		**7**	**16**	**25**	**28**	**52**	**57**	**58**	**61**	**64**	**67**	**70**	**73**	**76**	**79**	**84**	**87**	**93**	**96**	**97**	**100**				
Treatment		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
Color		R	R	R	G	R	V	V	R	G	V	R	G	V	R	V	R	V	R	R	G				
Flower	**Number**	21	12	21	20	18	20	23	15	21	16	16	15	19	14	18	24	16	14	14	19				
Visited	**Flower.**	1	2	1	1	1	7	2	5	5	6	4	5	5	4	12	16	11	6	4	1				
Pollen	**Removal**	1	3	2	1	0	5	2	4	1	7	6	7	9	1	14	25	18	2	3	0				
Massulae	**Deposition**	3	3	0	0	4	49	9	264	9	182	96	14	0	11	299	133	116	193	4	5				

4. Empirical studies in reward and rewardless orchids related to outcrossing hypothesis

Orchid species	References	Reward	Pollinators	Labelling technique	Labelled plants	Labelled flowers	Removed marked pollinaria (%)	Plants receiving geitonogamous pollen (%)	Flowers receiving geitonogamous pollen (%)
Aerangis ellisii	Nilsson *et al.* (1992)	Nectar	Hawkmoths	Micro-tags	151	1,329	n.a.	n.a.	8 (30.0)
Dichaea potamophila	Folsom (1994)	Oil	Euglossine bees	Histochemical stains	n.a.	88	31 (35.2)	1 (11.1)	1 (10.0)
(2001) ***Disa cooperi*****year**	Johnson *et al.* (2005)	Nectar	Hawkmoths	Histochemical stains	25	209	170 (81.3)	20 (80.0)	n.a.
(2002) ***Disa cooperi*****year**	Johnson *et al.* (2005)	Nectar	Hawkmoths	Histochemical stains	50	186	70 (37.6)	21 (42.0)	n.a.
Prasophyllum fimbria	Peakall (1989)	Nectar	Bees and wasps	Histochemical stains	16	139	58 (41.7)	n.a.	27 (21.6)
Comparettia falcata	Salguero-Faria and Ackerman (1999)	Nectar	Hummingbirds	Histochemical stains	n.a.	n.a.	n.a.	n.a.	22 (84.6)
Gymnadenia conopsea	This study	Nectar	Hawkmoths, fly and butterfly	Histochemical stains	36	428	6.8(38.3)	82.6(82.0)	n.a.
Caladenia tentactulata	Peakall and Beattie (1996)	None	Thynnine wasps	Histochemical stains	n.a.	n.a.	n.a.	7 (n.a.)	7 (10.6)
Cypripedium calceolus [c]	Tremblay (1994)	None	Bees	Histochemical stains	n.a.	81	n.a.	0 (0.0)	0 (0.0)
Dactylorhiza sambucina	Kropf, 2008	None	Bumble-bees	Fluorescent powder dyes	13	185	100 (54.1)	8 (61.5)	14 (29.8)
H. hircinum	Kropf, 2008	None	Solitary bees	Fluorescent powder dyes	14	1,059 (956 undamaged)	120 (22.9)	10 (71.4)	43 (35.8)
Dactylorhiza lapponica	This study	None	Bumble-bees	Histochemical stains	138	1,110	1.7(4.9)	1.3(10.0)	n.a.

Modified from Kropf, 2008 (Table 5 in that paper), and two more species in this study are added into the data pool. Note: n.a. not available.

5. Inbreeding depression review with different reward strategies

Species	Stages	δ	Reference	Nectar Status
Caladenia tentaculata	a	0.181	Peakall & Beattie (1996)	rewardless
Dactylorhiza lapponica	a	0.215	This study	rewardless
Dactylorhiza maculata	a	0.146	Vallius (2000)	rewardless
Disa ferruginea	a	0.584	Johnson (1994)	rewardless
Disa pulchra	a	0.540	Johnson (2000)	rewardless
Serapias vomeraceae	a	0.427	Bellusci *et al*. 2009	rewardless
Serapias cordigera	a	0.333	Bellusci *et al*. 2009	rewardless
Serapias parviflora	a	0.118	Bellusci *et al*. 2009	rewardless
N		**8**		
Mean		**1.172**		
SD		**2.566**		
Gymnadenia conopsea	a	0.291	This study	reward
Listera cordata	a	0.501	Melendez-Ackerman & Ackerman (2001)	reward
Mystacidium venosum	a	0.545	Luyt & Johnson (2001)	reward
Platanthera leucophaea	a	-0.065	Wallace (2003)	reward
Mystacidium venosum	a	0.157	Luyt & Johnson (2001)	reward
N		**5**		
Mean		**0.286**		
SD		**0.252**		
Caladenia tentactulata	b	-0.004	Peakall & Beattie (1996)	rewardless
Cleistes divaricata	b	0.281	Gregg (1989)	rewardless
Dactylorhiza praetermissa	b	-0.019	Ferdy *et al*. (2001)	rewardless
Dactylorhiza sambucina	b	0.427	Nilsson (1980)	rewardless
Disa atricapilla	b	0.132	Steiner *et al*. (1994)	rewardless
Disa draconis	b	0.299	Johnson & Steiner (1997)	rewardless
Disa ferruginea	b	0.579	Johnson (1994)	rewardless
Disa pulchra	b	0.501	Johnson (2000)	rewardless
Diuris maculate	b	-0.146	Beardsell *et al*. (1986)	rewardless
Epidendrum ciliare	b	-0.064	Ackerman & Montalvo (1990)	rewardless
Orchis mascula	b	0.204	Nilsson (1983a)	rewardless
Anacamptis (Orchis) morio	b	0.713	Nilsson (1984)	rewardless
Orchis spitzelii	b	0.379	Fritz (1990)	rewardless
Pleurothallis fabiobarrosii	b	0.657	Borba *et al*. (2001)	rewardless
Pleurothallis johannensis	b	0.779	Borba et al. (2001)	rewardless
Xylobium squalens	b	0.092	Pintau ́di et al. (1990)	rewardless
Leporella fimbriata	b	-0.241	Peakall (1989)	rewardless
Dactylorhiza praetermissa	b	0.043	Ferdy et al. (2001)	rewardless
Ionopsis utricularioides	b	0.000	Montalvo & Ackerman (1987)	rewardless
Leporella fimbriata	b	0.194	Peakall (1989)	rewardless

(Appendix 5 continued)

Species	Stages	δ	References	Nectar Status
Leporella fimbriata	b	0.569	Peakall & James (1989)	rewardless
Serapias vomeraceae	b	0.755	Bellusci et al. 2009	rewardless
Serapias cordigera	b	0.711	Bellusci et al. 2009	rewardless
Serapias parviflora	b	0.242	Bellusci et al. 2009	rewardless
N		**24**		
Mean		**0.295**		
SD		**0.308**		
Brownleea galpinii ssp.	b	0.982	Johnson et al. (2003a)	reward
Bulbophyllum involutum	b	0.260	Borba et al. (1999)	reward
Bulbophyllum ipanemense	b	0.055	Borba et al. (1999)	reward
Bulbophyllum weddellii	b	-0.256	Borba et al. (1999)	reward
Catasetum viridiflavum	b	0.310	Tremblay et al. (2005)	reward
Comparettia falcata	b	-0.015	Salguero-Farı´a & Ackerman (1999)	reward
Cynorchis uniflora	b	0.410	Nilsson et al. (1992b)	reward
Goodyera oblongifolia	b	0.333	Kallunki (1981)	reward
Goodyera oblongifolia	b	0.371	Ackerman (1975)	reward
Goodyera pubescens	b	-0.194	Kallunki (1981)	reward
Goodyera repens var. ophioides	b	0.433	Kallunki (1981)	reward
Goodyera tesselata	b	-0.114	Kallunki (1981)	reward
Listera cordata	b	0.061	Mele ńdez-Ackerman & Ackerman (2001)	reward
Listera ovata	b	0.086	Nilsson (1981)	reward
Microtis parviflora	b	0.067	Peakall & Beattie (1989)	reward
Mystacidium venosum	b	0.625	Luyt & Johnson (2001)	reward
Platanthera bifolia	b	0.480	Nilsson (1983c)	reward
Platanthera chlorantha	b	0.675	Nilsson (1983c)	reward
Platanthera ciliaris	b	0.132	Gregg (1990)	reward
Platanthera lacera	b	-0.426	Gregg (1990)	reward
Platanthera leucophaea	b	0.426	Wallace, 2003	reward
Platanthera stricta	b	0.451	Patt et al. (1989)	reward
Pleurothallis adamantinensis	b	0.692	Borba et al. (2001)	reward
Pleurothallis ochreata	b	0.682	Borba et al. (2001)	reward
Pleurothallis teres	b	0.970	Borba et al. (2001)	reward
Satyrium bicorne	b	0.776	Ellis & Johnson (1999)	reward
Satyrium coriifolium	b	0.553	Ellis & Johnson (1999)	reward
Satyrium erectum	b	0.750	Ellis & Johnson (1999)	reward
Vanilla claviculata	b	0.284	Tremblay et al. (2005)	reward
N		**29**		
Mean		**0.340**		
SD		**0.359**		

Note: a, fruit/seed mass; b, seeds with embryos.