Flowering phenology, floral display and reproductive success in the genus *Sirindhornia* (Orchidaceae): a comparative study of three pollinator-rewarding species

Kanok-orn Srimuang[1], Santi Watthana[2], Henrik Æ. Pedersen[3,*], Niramol Rangsayatorn[4] & Prapassorn D. Eungwanichayapant[1]

[1] *School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2] *Queen Sirikit Botanic Garden, P. O. Box 7, Mae Rim, Chiang Mai 50180, Thailand*
[3] *Botanical Garden & Museum, Natural History Museum of Denmark, University of Copenhagen, Gothersgade 130, DK-1123 Copenhagen K, Denmark (*corresponding author's e-mail: henrikp@snm.ku.dk)*
[4] *School of Science and Technology, Naresuan University, Phayao 56000, Thailand*

Numbers of flowers and their arrangement in time and space greatly influence levels of pollination and fruit set in natural populations of flowering plants. We examined relations between flowering phenology, floral display and reproductive success in the nectar-producing orchids *Sirindhornia mirabilis*, *S. monophylla* and *S. pulchella* in Thailand. We found each species to be self-compatible and dependent on insects for pollination. *Sirindhornia mirabilis* exhibited prolonged longevity of individual flowers and produced more flowers per inflorescence — features that may have evolved in response to low visitation rates. Patterns of reproductive success in relation to floral display suggest selection for increased inflorescence size in *S. mirabilis* and *S. monophylla*, but this may be counterbalanced by selection for a sustainable annual fruit set. In all three species, the female (and in *S. mirabilis* also the male) reproductive success decreased from the basal to the apical part of the inflorescence. Against this background, we propose a hypothesis for explaining differential fruit set in polinator-rewarding orchid species.

Key words: breeding system, fruit set, inflorescence size, natural selection, pollination

Introduction

Numbers of flowers and their arrangement in time and space greatly influence levels of pollination and fruit set in natural populations of flowering plants. Accordingly, conflicting demands such as pollinator attraction *versus* ovary competition, fruit dispersal *versus* fruit predation and reproductive *versus* vegetative allocation of resources imply species-specific evolutionary trade-offs that maximize the life-time genetic contribution of an individual to the next genera-
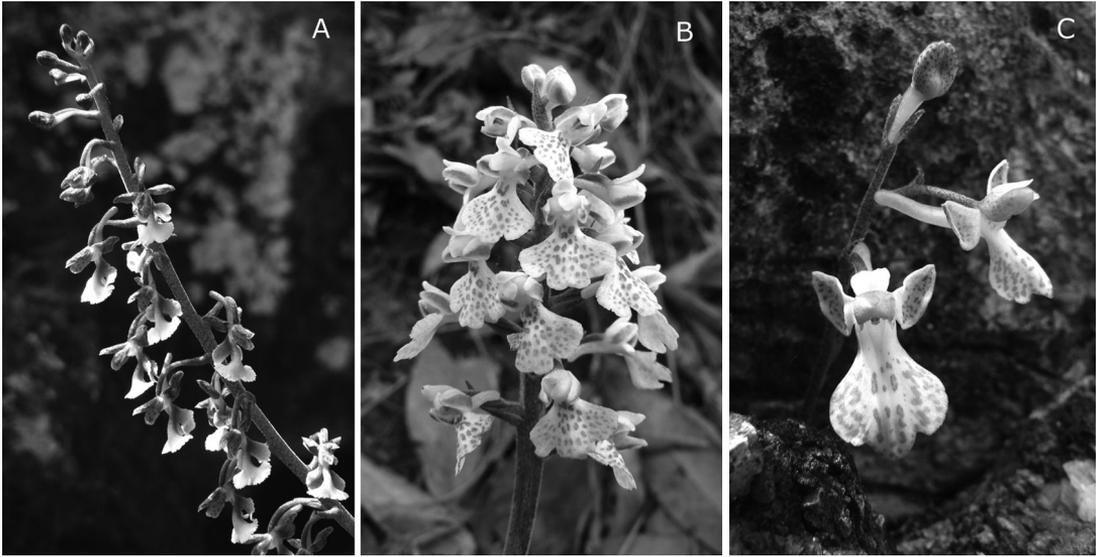
Fig. 1. Inflorescences from the members of the orchid genus *Sirindhornia*. — **A**: *S. mirabilis* (Thailand, Doi Hua Mot, 14 May 2008). — **B**: *S. monophylla* (Thailand, Doi Hua Mot, 7 May 2008). — **C**: *S. pulchella* (Thailand, Doi Chiang Dao, 27 April 2008). Photographs by K. Srimuang.

tion (Wyatt 1982). In line with this general finding, there is copious evidence that in the orchid family, natural selection is the principal process behind evolution of flowering phenology and floral display (Nilsson 1992) — although there is accumulating evidence that genetic drift is more important than previously recognized (Tremblay *et al.* 2005).

By far the majority of previous studies in the field of orchid reproductive success in relation to floral display addressed this topic in just a single species. Here, we perform a direct comparison of female and male reproductive success in relation to floral display, flower position and phenology between the three species constituting the orchid genus *Sirindhornia* (Fig. 1).

Material and methods

Study species

Sirindhornia, which belongs to the subtribe Orchidinae and consistently occurs on limestone, comprises *S. monophylla*, *S. mirabilis* and *S. pulchella* (Pedersen *et al.* 2002). The former species (occurring at 800–2200 m a.s.l.) is widespread, ranging from northwestern Thailand (province of Tak) to northern Myanmar (Shan State) and southern China (province of Yunnan), whereas *S. mirabilis* and *S. pulchella* appear endemic to the mountains Doi Hua Mot (at 800–1100 m a.s.l.) and Doi Chiang Dao (at 1800–2100 m a.s.l.) in the northern Thai provinces of Tak and Chiang Mai, respectively. All these three species are terrestrial.

All *Sirindhornia* species flower in the beginning of the rainy season (April–June), with an inflorescence of mainly white to purple flowers that exude nectar in a distinct spur. The nectar contains 5–10 mg glucose per ml. It is difficult to characterize the floral scent of *Sirindhornia*; the flowers are generally perceived as fragrant, but in some individuals they emit an offensive odour. To the human nose, the scent is weak, being strongest in the early morning. The fruit is a capsule that produces numerous dust seeds; it ripens in 6–12 weeks and dehisces by longitudinal slits before September.

Sirindhornia mirabilis (Fig. 1A) is 13–70 cm tall, with 2–48 flowers in the inflorescence. Each flower is approximately 1.5 cm in diameter. The labellum is deeply three-lobed in its proximal part, glabrous, pink with purple markings in its basal and central part and with green sidelobes. The two pollinia have one common viscidium.

Sirindhornia monophylla (Fig. 1B) is 12–40 cm tall, with 6–29 flowers in the inflorescence. Each flower is approximately 0.7 cm in diameter. The labellum is more or less three-lobed in its distal part and rose-coloured to white with numerous tufts of purple papillae. The two pollinia have separate viscidia.

Sirindhornia pulchella (Fig. 1C) is 5–26 cm tall, with 2–13 flowers in the inflorescence. Each flower is approximately 1.5 cm in diameter. The labellum is shallowly three-lobed in its distal part, glabrous and pink with purple markings. The two pollinia have separate viscidia.

Study sites and plots

Our field work was conducted in northern Thailand on two mountains that were located ca. 360 km apart. We studied *Sirindhornia mirabilis* and *S. monophylla* on Doi Hua Mot (Umphang Wildlife Sanctuary), and *S. pulchella* in the southwestern part of Doi Chiang Dao (i.e. on the ridge called Doi Luang), Chiang Dao Wildlife Sanctuary. Plots measuring 50 × 50 m were demarcated for studying each species in detail. Due to the particularly scattered occurrence of *S. monophylla*, we included three different plots for this species (but for the sake of convenience, they are referred to as one plot below).

Flowering phenology

Progression of flowering was established from weekly observation of 31 inflorescences of *S. mirabilis* (2006), 24 of *S. monophylla* (2006) and 29 of *S. pulchella* (2008) during the study period. In each inflorescence (always one per individual), we counted the numbers of flower buds, fresh flowers and capsules plus withered flowers ten times (*S. mirabilis*) or six times (*S. monophylla*, *S. pulchella*).

Pollination experiments

In 2006, we transplanted six plants of *S. mirabilis* and four plants of *S. monophylla* with the total of 179 and 56 flower buds, respectively,

from their natural habitat to a site nearby the resort of Umphang and covered them with fine-meshed nylon net. Additionally, 25 individuals of *S. pulchella* with a total of 124 flower buds, remaining in their natural habitat on Doi Chiang Dao, were covered with nylon net in 2007. To test for autogamy through spontaneous self-pollination (and for apomixis) in the caged individuals, we left 96 flowers of *S. mirabilis*, 30 flowers of *S. monophylla*, and 72 flowers of *S. pulchella* untouched. To test for genetic self-compatibility, we manually self-pollinated 83 flowers of *S. mirabilis*, 26 flowers of *S. monophylla* and 52 flowers of *S. pulchella*.

Female reproductive success

We counted the number of inflorescences and the number of flowers per inflorescence in each study plot in 2006, 2007 (*S. pulchella* only) and 2008. At the end of flowering each year, we assessed the fruit set in order to determine the female reproductive success in each inflorescence. Using the program SigmaStat network ver. 1.01, we performed a one-way ANOVA on ranks (as the data set failed the normality test) to test for differences in the relative fruit set (pooled over the entire study period) among the three species. Subsequently, we used Dunn's test to reveal which species were mutually different in this respect. Using the program SPSS for Windows 16, we calculated Pearson's correlation coefficient to test for correlation between the fruit set and number of flowers per inflorescence.

To study the internal patterns of capsule production in inflorescences of *S. mirabilis* ($n = 14$), *S. monophylla* ($n = 11$) and *S. pulchella* ($n = 17$) in 2008, basal, middle, and apical parts were defined in each inflorescence (each part comprising the same percentage of flowers), and the fruit set was characterized accordingly. Using SigmaStat, we performed a one-way ANOVA on ranks (as all data sets failed the normality test) to test for differences in relative fruit set between the three inflorescence parts in each species. Whenever a statistically significant difference was found, we used Student-Newman-Keul's multiple comparison method to reveal which parts of the inflorescence that were mutually different in this respect.

Male reproductive success

To determine the male reproductive success in the population samples of *S. mirabilis* and *S. monophylla*, respectively, we checked 20 and 13 inflorescences (a subset of those examined for flowering phenology) for pollinarium removal in 2006. Thus, we recorded the total number of flowers on each inflorescence, together with the number of flowers from which the pollinarium was removed (no flowers were observed in which only one hemipollinarium was removed). Using the program SigmaStat, we performed a *t*-test to test for difference in pollinarium removal between *S. mirabilis* and *S. monophylla*. Using the program SPSS, we calculated Pearson's correlation coefficient to test for correlation between the number of pollinaria removed and the number of flowers per inflorescence.

We studied the patterns of pollinarium removal within inflorescences by comparing removal rates between the basal, middle and apical parts of each inflorescence, generally using the same sequence of statistical tests as indicated above for the patterns of fruit set. However, as the data set for *S. mirabilis* passed the normality test, a one-way ANOVA (rather than a one-way ANOVA on ranks) was applied for this species.

Using the program SigmaStat, we performed a Mann-Whitney rank sum test (as the data sets failed the normality test) in order to test for difference between relative fruit set and proportion of pollinaria removed in *S. mirabilis* and in *S. monophylla*.

Results

Flowering phenology

In all three species, flowering started from the proximal part of the inflorescence; for surveys of overall progression in flowering (*see* Tables 1–3 and Fig. 2). The longevity of individual flowers was about six weeks in *S. mirabilis* and about three weeks in *S. monophylla* and *S. pulchella*. However, it could be observed in all three species that, if a flower was pollinated it would wither in less than one week.

The 31 inflorescences examined in *S. mirabilis* carried 8–48 flowers (mean = 23, SD = 10.9). On average, 10 flowers would open before the the first opened flower started to wither, and a maximum of 14 flowers would be open at the same time (in early May). The 24 inflorescences examined in *S. monophylla* carried 6–27 flowers (mean = 15, SD = 6.1). On average, about seven flowers would open before the first opened flower started to wither, and a maximum of eight flowers would be open at the same time (in late May). The 29 inflorescences examined in *S. pulchella* carried 2–9 flowers (mean = 4, SD = 1.9). On average, three flowers would open before the first opened flower started to wither, and a maximum of three flowers would be open at the same time (in early May).

Pollination experiments

Among the caged *Sirindhornia* individuals, none

Table 1. Progression of flowering and pollinarium removal in *Sirindhornia mirabilis* throughout the study period. All values are given as mean ± SD.

Date	Flower buds	Fresh flowers	Capsules plus withered flowers	Proportion of pollinaria removed (%)
17 April	21.6 ± 11.2	1.1 ± 4.2	0.0 ± 0.0	2.9 ± 12.8
24 April	17.9 ± 10.4	4.6 ± 6.8	0.3 ± 1.3	5.8 ± 16.0
1 May	11.8 ± 10.2	9.8 ± 11.1	1.1 ± 5.1	13.0 ± 19.7
8 May	5.4 ± 7.2	13.9 ± 11.5	3.5 ± 7.6	20.0 ± 21.9
15 May	2.0 ± 3.5	12.7 ± 12.7	8.0 ± 10.3	31.4 ± 22.3
22 May	0.6 ± 1.6	7.5 ± 12.8	14.6 ± 10.9	36.4 ± 26.5
29 May	0.2 ± 0.8	5.2 ± 9.5	17.4 ± 11.0	39.9 ± 23.1
5 June	0.0 ± 0.0	2.3 ± 5.5	20.5 ± 10.0	39.9 ± 23.1
12 June	0.0 ± 0.0	0.4 ± 1.6	22.3 ± 11.8	39.9 ± 23.1
19 June	0.0 ± 0.0	0.2 ± 0.8	22.6 ± 11.5	39.9 ± 23.1

Table 2. Progression of flowering and pollinarium removal in *Sirindhornia monophylla* throughout the study period. All values are given as mean ± SD.

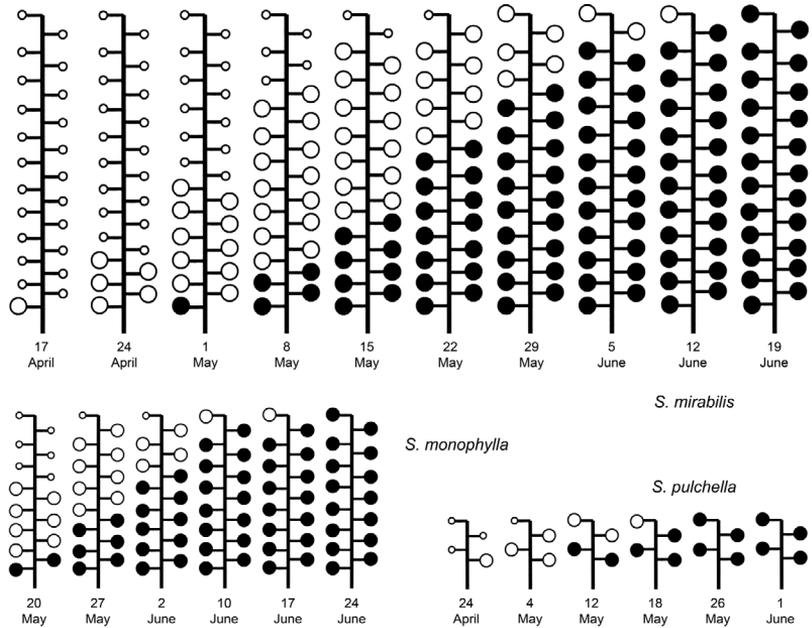| Date	Flower buds	Fresh flowers	Capsules plus withered flowers	Proportion of pollinaria removed (%)
20 May	5.7 ± 4.4	6.4 ± 4.9	2.0 ± 3.2	26.7 ± 30.4
27 May	1.0 ± 2.8	6.7 ± 4.4	6.4 ± 6.8	55.1 ± 27.9
2 June	1.0 ± 2.3	3.1 ± 3.5	10.0 ± 7.8	85.1 ± 17.1
10 June	0.2 ± 0.5	1.0 ± 2.3	12.9 ± 6.6	90.1 ± 11.2
17 June	0.0 ± 0.0	0.6 ± 1.8	13.4 ± 6.1	91.2 ± 10.4
24 June	0.0 ± 0.0	0.1 ± 0.4	14.0 ± 6.0	91.2 ± 10.4

Fig. 2. Phenological progression in *Sirindhornia mirabilis*, *S. monophylla* and *S. pulchella* during the periods of observation. Small white circle: bud, large white circle: fresh flower, large black circle: capsule or withered flower.

of the untouched flowers set fruit (*n* = 198; 0% fruit set), indicating absence of spontaneous autogamy and apomixis. On the other hand, each manually self-pollinated flower set fruit (*n* = 161; 100% fruit set), indicating genetic self-compatibility in all three species.

Female reproductive success

Overall relative fruit set (Table 4) depended on the species (*p* < 0.001) and was significantly different (*p* < 0.05) between *S. monophylla* on the one hand and *S. mirabilis* and *S. pulchella* on the other, but not between *S. mirabilis* and *S. pulchella*. Only in *S. monophylla* did we find the

fruit set to be correlated with the number of flowers per inflorescence (*r* = 0.52, *p* < 0.01; Fig. 3).

The female reproductive success depended on

Table 3. Phenological progression in *Sirindhornia pulchella* throughout the study period. All values are given as mean ± SD.

Date	Flower buds	Fresh flowers	Capsules plus withered flowers
27 April	3.2 ± 1.5	0.8 ± 0.9	0.0 ± 0.0
4 May	1.1 ± 1.1	2.9 ± 1.8	0.1 ± 0.4
12 May	0.0 ± 0.2	1.9 ± 1.6	2.1 ± 2.0
18 May	0.0 ± 0.0	0.8 ± 1.2	3.3 ± 2.1
26 May	0.0 ± 0.0	0.1 ± 0.4	4.0 ± 1.6
1 June	0.0 ± 0.0	0.0 ± 0.0	4.1 ± 1.6

Table 4. Survey of the number of inflorescences in each study plot, together with the overall relative fruit set (indicated as mean ± SD) for each study year. n = number of inflorescences

Species	Year	n	Relative fruit set (%)
S. mirabilis	2006	36	9.6 ± 12.3
	2008	34	4.5 ± 7.2
S. monophylla	2006	25	51.6 ± 31.2
	2008	12	38.0 ± 20.0
S. pulchella	2006	13	17.3 ± 20.8
	2007	23	25.9 ± 31.0
	2008	62	11.4 ± 19.7

the position of a flower in *S. mirabilis* ($p < 0.05$) as well as in *S. monophylla* and *S. pulchella* (both $p < 0.001$; Table 5).

Male reproductive success

The overall proportion of pollinaria removed (2006) was 39.9% ± 23.1% in *S. mirabilis* and 91.2% ± 10.4% in *S. monophylla* (both mean ± SD). The means were significantly different ($p < 0.001$). The number of flowers per inflorescence was positively correlated with the number of pollinaria removed in both *S. mirabilis* ($r = 0.67$, $p < 0.01$; Fig. 4), and *S. monophylla* ($r = 0.96$, $p < 0.01$; Fig. 3).

The male reproductive success depended on position of flower in both species examined, that is *S. mirabilis* and *S. monophylla* ($p < 0.001$; Table 5). We found the proportion of pollinaria removed to be significantly higher than the relative fruit set in both *S. mirabilis* and *S. monophylla* ($p < 0.001$).
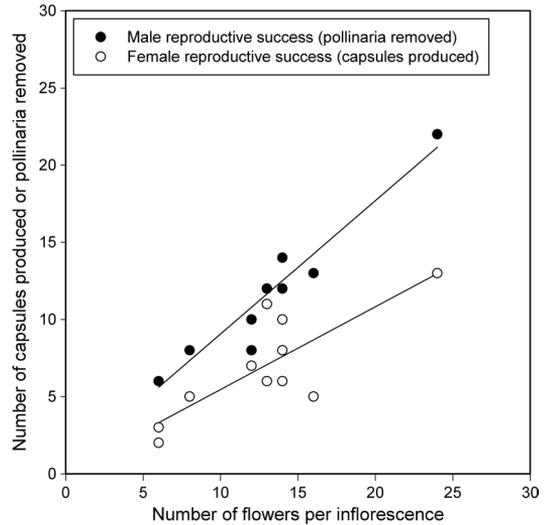

Fig. 3. Regressions indicating positive correlations between female reproductive success (measured as number of capsules produced) or male reproductive success (measured indirectly as number of pollinaria removed) and the number of flowers per inflorescence in *Sirindhornia monophylla*.

Discussion

Breeding system

None of the untouched *Sirindhornia* flowers on caged individuals set fruit, whereas all of the manually self-pollinated flowers set fruit. This demonstrates that *S. mirabilis*, *S. monophylla* and *S. pulchella* are genetically self-compatible, non-autogamous species, and that no apomixis occurs. Consequently, seed production in all three species is completely dependent on pollinators.

With their zygomorphic, mainly white to purple, more or less horizontal flowers (Fig. 1)

Table 5. Female and male reproductive success in population samples of *Sirindhornia*: surveys of relative fruit set (%) and proportion of pollinaria removed (%) in relation to flower positions in the inflorescence (i.e. basal, middle or apical). All values are means ± SDs. The relative fruit set or pollinarium removal was significantly different ($p < 0.05$) between inflorescence parts indicated by different letters.

Reproductive success		*S. mirabilis*	*S. monophylla*	*S. pulchella*
Female:	Apical part of inflorescence	4.8 ± 6.8[a]	13.6 ± 32.3[a]	0.0 ± 0.0[a]
	Middle part of inflorescence	8.9 ± 13.8[a]	28.6 ± 30.9[a]	47.2 ± 46.9[b]
	Basal part of inflorescence	19.4 ± 16.8[b]	78.9 ± 24.9[b]	70.4 ± 42.2[b]
Male	Apical part of inflorescence	14.3 ± 19.5[a]	74.2 ± 31.4[a]	–
	Middle part of inflorescence	33.4 ± 23.2[b]	100.0 ± 0.0[b]	–
	Basal part of inflorescence	60.5 ± 26.7[c]	100.0 ± 0.0[b]	–

that exude nectar hidden in a spur and offer an enlarged labellum as a landing platform (provided with markings or tufts of coloured papillae that serve as nectar guides), the flowers of all three *Sirindhornia* species fit the bee pollination syndrome of van der Pijl and Dodson (1966). Indeed, the only known observation of a pollination-related insect visit to *Sirindhornia* involved an unidentified halictid bee that, retreating from an *S. mirabilis* flower where it had probed the spur for nectar, extracted a pollinarium that was attached to the frontal part of its head (K. Srimuang unpubl. data). The flowering seasons of the sympatric study populations of *S. mirabilis* and *S. monophylla* overlap in May and June (Tables 1–2 and Fig. 2). The recent discovery of the hybrid *S. mirabilis × monophylla* in this colony (Pedersen & Ormerod 2009) demonstrates that at least one pollinator species must be shared between the two, at least occasionally.

Reproductive success in relation to floral display

The longevity of individual flowers in *S. mirabilis* was found to be about six weeks as compared with approximately three weeks in *S. monophylla* and *S. pulchella*. However, if a flower in any of the three species was pollinated, it would wither in less than one week. Prolonged longevity of flowers in *S. mirabilis* may have evolved in response to low visitation rates, in line with the findings of Sugiura *et al.* (2001) for *Cypripedium macranthos*, and the same explanation might well apply to the generally higher number of flowers per inflorescence in *S. mirabilis* (Tables 1–3 and Fig. 2). This hypothesis is congruent with the level of pollinarium removal in *S. mirabilis* (which was significantly lower than in the other examined species, *S. monophylla*) and partly congruent with the level of natural fruit set in *S. mirabilis* (which was significantly lower than in *S. monophylla* but not significantly different from the level in *S. pulchella* (*see* also Table 4).

Pollinarium removal in *S. monophylla* and *S. mirabilis* was positively correlated with the number of flowers per inflorescence (Figs. 3–4), whereas we found a positive correlation between
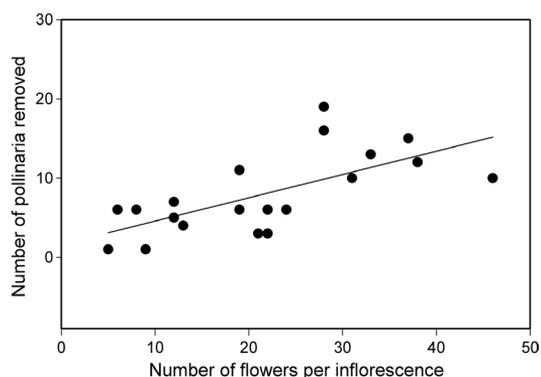

Fig. 4. Regression illustrating positive correlation between male reproductive success (measured indirectly as number of pollinaria removed) and the number of flowers per inflorescence in *Sirindhornia mirabilis*.

the fruit set and number of flowers per inflorescence in *S. monophylla* only (Fig. 3). Similar correlations between reproductive success and inflorescence size are known from several other orchid species, both rewarding (e.g. Schemske 1980, Inoue 1986, Piper & Waite 1988, Waite *et al.* 1991, Murren & Ellison 1996, Jersáková & Kindlmann 2004, Kindlmann & Jersáková 2006) and non-rewarding (e.g. Nilsson 1983, Firmage & Cole 1988, Fritz 1990, Waite *et al.* 1991, Jersáková & Kindlmann 2004, Kindlmann & Jersáková 2006, Watthana *et al.* 2006). Thus, we would expect directional selection towards an increase of inflorescence size through male function in *S. mirabilis* and through both male and female function in *S. monophylla* (although it should be remembered that pollinarium removal is only an indirect measure of male reproductive success; many pollinaria were evidently lost).

As noted by Calvo (1990), the potential for pollinator-mediated selection on floral display may be limited by the often low overall pollination level in orchids. Positive correlations between fruit set and floral display are sometimes limited to certain populations (Kindlmann & Jersáková 2005) and may be limited to favourable years because pollinators otherwise occur less predictably in time or space (Sutherland 1986, Jersáková & Kindlmann 2004). Furthermore, an increased fruit set in orchids may reduce future growth and/or reproduction (e.g. Montalvo & Ackerman 1987, Ackerman 1989, Snow & Whigham 1989, Zimmerman & Aide

1989, Ackerman & Montalvo 1990, Mattila & Kuitunen 2000, Meléndez-Ackerman *et al.* 2000, Primack 2002). Consequently, directional selection for increased floral display in *S. mirabilis* and *S. monophylla* may be counterbalanced by stabilizing selection for a sustainable annual fruit set that maximizes the lifetime reproductive success of the individual.

The consistently higher male reproductive success (as compared with female success) in *S. mirabilis* and *S. monophylla*, together with the fact that only pollinarium removal was found to be positively correlated with the number of flowers per inflorescence in *S. mirabilis*, suggest that large inflorescence size primarily evolved in response to selection on male reproductive success — in accordance with the so-called male function hypothesis (Willson & Rathcke 1974, Willson & Price 1977, Burd 1994). However, it should still be remembered that pollinarium removal is an indirect and over-estimating measure of male reproductive success (*see* also Nilsson *et al.* 1986, Snow 1989) and that orchid reproduction is more limited by pollination than by pollinarium removal (Nilsson 1992). For example, if the magnitude of pollinarium removal from *S. mirabilis* is compared with that of fruit set in the same population in 2006, it appears that ca. 76% of the removed pollinaria were lost. This is a high loss as compared with the corresponding figure for *S. monophylla* (ca. 43%). It may indicate lower pollinator specificity in *S. mirabilis*, as the risk of pollen wastage during transport generally increases with the taxonomic diversity of the pollinator fauna (Wilcock & Neiland 2002, Tremblay *et al.* 2005). The consistently lower female reproductive success in *S. mirabilis* and *S. monophylla* (as compared with male success) may be due to resource limitation (cf. Willson & Price 1977, Stanton *et al.* 1986). Limited support for this hypothesis in orchids has been provided for *Caularthron bilamellatum* (Fisher 1992), *Dactylorhiza maculata* (Vallius 2000) and *Platanthera bifolia* (Mattila & Kuitunen 2000). However, corresponding studies of several other orchid species (reviewed by Tremblay *et al.* 2005) did not give consistently similar results.

Reproductive success in relation to position of flower

Pollinarium removal in *S. mirabilis* and *S. monophylla* decreased markedly from the basal part to the apical part of the inflorescence (Table 5). This is surprising as the rewarding condition of the *Sirindhornia* flowers would be expected to induce pollinators to visit more flowers during each visit to an inflorescence (cf. Johnson & Nilsson 1999). In *S. monophylla* (contrary to *S. mirabilis*, Table 1), pollinaria were generally removed soon after the flowers had opened (Table 2).

Relative fruit set decreased on average by ca. 75% from the basal to the apical part of the inflorescence in *S. mirabilis*, by ca. 83% in *S. monophylla* and by 100% in *S. pulchella* (Table 5). This consistent trend of decrease matches previously reported observations of fruit set (or pollen deposition) in the non-rewarding, mainly bumblebee-pollinated *Anacamptis morio* (Nilsson 1984, Jersáková & Kindlmann 1998), *Dactylorhiza sambucina* (Nilsson 1980), *Orchis mascula* (Nilsson 1983, Johnson & Nilsson 1999) and *O. spitzelii* (Fritz 1990). Experimental pollinations performed by Fritz (1990) demonstrated that all flowers in inflorescences of *O. spitzelii* were capable of producing fruits — and in all the non-rewarding species above, differential fruit set was attributed to the pollinating bumblebees always starting their visit on one of the lowermost flowers and only probing a few flowers (in vain) before leaving the inflorescence. However, *Sirindhornia* flowers exude nectar in their spur, and similar patterns of differential pollination are known from the rewarding *Myrosmodes cochleare* (Berry & Calvo 1991), *Platanthera blephariglottis* (Cole & Firmage 1984) and *P. chlorantha* (Johnson & Nilsson 1999). In these species, like in *Sirindhornia*, pollinators searching for nectar may likewise, at least in some cases, start their visit on one of the lowermost flowers, but due to the nectar reward they have no reason to leave the inflorescence soon after — and indeed, Johnson and Nilsson (1999) demonstrated that pollinators stayed longer on each flower and visited more flowers per inflorescence when artificial nectar was added to the flowers of an otherwise non-rewarding species.

We propose the hypothesis that in rewarding orchids, differential pollination is often due to a combination of (1) the flowers opening in sequence from the base towards the apex of the inflorescence with all flowers being simultaneously open for only a short period, if at all (Tables 1–3 and Fig. 2), and (2) the importance of floral display for attracting pollinators. At the beginning of flowering, only the flowers in the basal (to middle) part of the inflorescence are open and accessible to pollinators, but the coloured buds in the (middle to) apical part contribute to the floral display. Towards the end of flowering, the uppermost flowers are open and accessible to pollinators, but at this time the lowermost flowers are withered and do no longer contribute to the floral display. Furthermore, the lowermost flowers are usually larger than the uppermost, meaning that the floral advertisement consisting of open flowers only also differs between the basal and the apical part of the inflorescence. Admittedly, this description does not fit *Myrosmodes cochleare* in which the flowers open in the opposite sequence, but for this species Berry and Calvo (1991) revealed experimentally that the uppermost flowers were unable to produce fruits. Based on the consistently higher male than female reproductive success in all parts of the inflorescence in both *Sirindhornia* species examined, resource limitation also needs to be taken into account when explaining differential fruit set. Additional factors that can lead to differential pollination in orchids include (1) that a pollinator moving up the inflorescence may become satiated with the resources and leave before visiting the upper flowers (Tremblay *et al*. 2005), and (2) that pollinators are sometimes ineffective at depositing pollinia in the smaller upper flowers (Tremblay 2006).

Acknowledgements

References

Ackerman, J. D. 1989: Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). — *Syst. Bot.* 14: 101–109.

Ackerman, J. D. & Montalvo, A. M. 1990: Short- and long-term limitations to fruit production in a tropical orchid. — *Ecology* 71: 263–272.

Berry, P. E. & Calvo, R. N. 1991: Pollinator limitation and position dependent fruit set in the High Andean orchid *Myrosmodes cochleare* (Orchidaceae). — *Pl. Syst. Evol.* 174: 93–101.

Burd, M. 1994: Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. — *Bot. Rev.* 60: 83–139.

Calvo, R. N. 1990: Inflorescence size and fruit distribution among individuals in three orchid species. — *Am. J. Bot.* 77: 1378–1381.

Cole, F. R. & Firmage, D. H. 1984: The floral ecology of *Platanthera blephariglottis*. — *Am. J. Bot.* 71: 700–710.

Firmage, D. H. & Cole, F. R. 1988: Reproductive success and inflorescence size of *Calopogon tuberosus* (Orchidaceae). — *Am. J. Bot.* 75: 1371–1377.

Fisher, B. L. 1992: Facultative ant association benefits a Neotropical orchid. — *J. Trop. Ecol.* 8: 109–114.

Fritz, A.-L. 1990: Deceit pollination of *Orchis spitzelii* (Orchidaceae) on the Island of Gotland in the Baltic: a suboptimal system. — *Nordic J. Bot.* 9: 577–587.

Inoue, K. 1986: Different effects of sphingid and noctuid moths on the fecundity of *Platanthera metabifolia* (Orchidaceae) in Hokkaido. — *Ecol. Res.* 1: 25–36.

Jersáková, J. & Kindlmann, P. 1998: Patterns of pollinator-generated fruit set in *Orchis morio* (Orchidaceae). — *Folia Geobot.* 33: 377–390.

Jersáková, J. & Kindlmann, P. 2004: Reproductive success and sex variation in nectarless and rewarding orchids. — *Int. J. Pl. Sci.* 165: 779–785.

Johnson, S. D. & Nilsson, L. A. 1999: Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. — *Ecology* 80: 2607–2619.

Kindlmann, P. & Jersáková, J. 2005: Floral display, reproductive success, and conservation of terrestrial orchids. — *Selbyana* 26: 136–144.

Kindlmann, P. & Jersáková, J. 2006: Effect of floral display on reproductive success in terrestrial orchids. — *Folia Geobot.* 41: 47–60.

Mattila, E. & Kuitunen, M. T. 2000: Nutrient versus pollination limitation in *Platanthera bifolia* and *Dactylorhiza incarnata* (Orchidaceae). — *Oikos* 89: 360–366.

Meléndez-Ackerman, E. J., Ackerman, J. D. & Rodríguez-Robles, J. A. 2000: Reproduction in an orchid can be resource-limited over its lifetime. — *Biotropica* 32: 282–290.

Montalvo, A. M. & Ackerman, J. D. 1987: Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). — *Biotropica* 19: 24–31.

Murren, C. J. & Ellison, A. M. 1996: Effects of habitat, plant size, and floral display on male and female reproductive success of the neotropical orchid *Brassavola nodosa*. — *Biotropica* 28: 30–41.

Nilsson, L. A. 1980: The pollination ecology of *Dactylorhiza sambucina* (Orchidaceae). — *Bot. Notiser* 133: 367–385.

Nilsson, L. A. 1983: Anthecology of *Orchis mascula* (Orchidaceae). — *Nordic J. Bot.* 3: 157–179.

Nilsson, L. A. 1984: Anthecology of *Orchis morio* (Orchidaceae) at its outpost in the north. — *Nova Acta Regiae Soc. Sci. Upsal.* ser. V:C 3: 167–179.

Nilsson, L. A. 1992: Orchid pollination biology. — *Trends Ecol. Evol.* 7: 255–259.

Nilsson, L. A., Jonsson, L., Rason, L. & Randrianjohany, E. 1986: The pollination of *Cymbidiella flabellata* (Orchidaceae) in Madagascar: a system operated by sphecid wasps. — *Nordic J. Bot.* 6: 411–422.

Pedersen, H. Æ. & Ormerod, P. 2009: Notes on the orchid flora of Thailand (I). — *Taiwania* 54: 213–218.

Pedersen, H. Æ., Suksathan, P. & Indhamusika, S. 2002: *Sirindhornia*, a new orchid genus from Southeast Asia. — *Nordic J. Bot.* 22: 391–403.

Piper, J. G. & Waite, S. 1988: The gender role of flowers of broad leaved helleborine, *Epipactis helleborine* (L.) Crantz (Orchidaceae). — *Funct. Ecol.* 2: 35–40.

Primack, R. B. 2002: An eleven year experimental study of the pink lady's slipper orchid (*Cypripedium acaule*). — In: Kindlmann, P., Willems, J. H. & Whigham, D. F. (eds.), *Trends and fluctuations and underlying mechanisms in terrestrial orchid populations*: 239–249. Backhuys Publ., Leiden.

Schemske, D. W. 1980: Evolution of floral display in the orchid *Brassavola nodosa*. — *Evolution* 34: 489–493.

Snow, A. A. 1989: Assessing the gender role of hermaphroditic flowers. — *Funct. Ecol.* 3: 249–250.

Snow, A. A. & Whigham, D. F. 1989: Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). — *Ecology* 70: 1286–1293.

Stanton, M. L., Snow, A. A. & Handel, S. N. 1986: Floral evolution: attractiveness to pollinators increases male fitness. — *Science* 232: 1625–1627.

Sugiura, N., Fujie, T., Inoue, K. & Kitamura, K. 2001: Flowering phenology, pollination, and fruit set of *Cypripedium macranthos* var. *rebunense* a threatened lady's slipper (Orchidaceae). — *J. Pl. Res.* 114: 171–178.

Sutherland, S. 1986: Patterns of fruit-set: what controls fruit-flower ratios in plants? — *Evolution* 40: 117–128.

Tremblay, R. L. 2006: The effect of flower position on male and female reproductive success in a deceptively pollinated tropical orchid. — *Bot. J. Linn. Soc.* 151: 405–410.

Tremblay, R. L., Ackerman, J. D., Zimmerman, J. K. & Calvo, R. N. 2005: Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. — *Biol. J. Linn. Soc.* 84: 1–54.

Vallius, E. 2000: Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). — *Funct. Ecol.* 14: 573–579.

van der Pijl, L. & Dodson, C. H. 1966: *Orchid flowers: their pollination and evolution*. — The Fairchild Trop. Garden & Univ. Miami Press, Coral Gables.

Waite, S., Hopkins, N. & Hitchings, S. 1991: Levels of pollinia export, import and fruit set among plants of *Anacamptis pyramidalis*, *Dactylorhiza fuchsii*, and *Epipactis helleborine*. — In: Wells, T. C. E. & Willems, J. H. (eds.), *Population ecology of terrestrial orchids*: 103–110. SPB Acad. Publ. bv, The Hague.

Watthana, S., Pedersen, H. Æ. & Suddee, S. 2006: Substrate diversity, demography, and fruit set in two populations of the epiphyte *Pomatocalpa spicatum* (Orchidaceae) in Thailand. — *Selbyana* 27: 165–174.

Wilcock, C. & Neiland, R. 2002: Pollination failure in plants: why it happens and when it matters. — *Trends Pl. Sci.* 7: 270–277.

Willson, M. F. & Price, P. W. 1977: The evolution of inflorescence size in *Asclepias* (Asclepiadaceae). — *Evolution* 31: 495–511.

Willson, M. F. & Rathcke, B. J. 1974: Adaptive design of the floral display in *Asclepias syriaca* L. — *Am. Midl. Naturalist* 92: 47–57.

Wyatt, R. 1982: Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. — *Am. J. Bot.* 69: 585–594.

Zimmermann, J. K. & Aide, T. M. 1989: Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. — *Am. J. Bot.* 76: 67–73.