ORIGINAL PAPER

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The effectiveness of heterostyly in preventing illegitimate pollination in dish-shaped flowers

Abstract Heterostyly is thought to have evolved because it confers efficiency of cross-pollination. While it can be an alternative to an incompatibility mechanism in tube-shaped flowers, its effectiveness in dish-shaped flowers, such as those of *Fagopyrum esculentum*, has not been demonstrated. Stigmatic pollen loads were examined over the daily course of pollination in a planted field of this crop species. Natural pollination by bees resulted in 23% of the pollen on pin flowers and 69% on thrum flowers being legitimate. Both flower morphs therefore received the two pollen types in about the same proportions. Pin pollen constituted 74% of the pollen delivered to stigmata, even though the two morphs are present in equal numbers. Pin flowers collected about 75 pollen, and thrum flowers about 25 pollen by the end of the day of anthesis. The net result was about 15 compatible pollen per flower on both floral morphs. These results indicate that heterostyly is associated with excess pin-pollen delivery rather than excess legitimate-pollen delivery in *F. esculentum*.

Key words *Fagopyrum esculentum* · Heterostyly Pollen flow

Introduction

The adaptive significance of heterostyly has been thought to lie in promoting outcrossing (Darwin 1877), and in reducing pollen wastage through illegitimate pollination (Baker 1964). This pattern occurs in tube-shaped flowers of self-compatible *Eichhornia paniculata* where 77% of the mating is between morphs (Kohn and Barrett 1992). The bias in favor of inter-morph pollinations depends on the pollen from one morph sticking to the proboscis of the pollinator in a position so that it is trans-

ferred to the stigma in the other morph. Such a constraint is easily enforced in a tube-shaped flower that requires straight insertion of the proboscis. However, in a dish-shaped flower access to the nectaries can be from any direction. If pollen is to be selectively deposited on the opposite flower morph, a specific and consistent path of the insect's probe into the flower may substitute for the deeper corolla structure (Lloyd and Webb 1992). Specifically, the probe would make a curved path on the near side of the stamen in pin flowers but on the far side of the stamen in thrum flowers. Evidence for such behavior would be the selective deposition of pollen from the opposite flower types in the dish-shaped flowers of *Fagopyrum esculentum* (Polygonaceae), the cultivated buckwheat.

The contribution of heterostyly to reducing pollen wastage through intramorph pollination can be measured by counting the relative number of illegitimate pollen deposited. *F. esculentum* has strong intra-morph incompatibility (Schoch-Bodmer 1930) that precludes selfed progeny. Therefore, there is a cost of illegitimate pollination mainly if seed set is limited by insufficient legitimate pollen, or if illegitimate pollen blocks access to the stigmatic surface (Lloyd and Yates 1982; Shore and Barrett 1984).

This is a report of pollen transfer in a species with dish-shaped heterostylous flowers. Its aim is to determine whether heterostyly promotes efficient cross pollination when the anthers and stigmata are accessible to the pollinator from many angles.

Methods

Flowers were collected throughout each of 4 days during the flowering period. Collection was every half hour from 8:30 a.m. to noon, and hourly in the afternoon. The dates of collection were 6,

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Fig. 1 Accumulation of legiti-
mate and illegitimate pollen
grains on each flower type.
Open symbols indicate pin pol-
len, *closed symbols* indicate
thrum pollen. Data are from 6
August 1993, the day of maxi-
mum seed initiation. Anther
sacs dehisced at 9:30 a.m., and
pollen was completely removed
from the anther sacs by
12:30 p.m.

Time of Day (EDT)
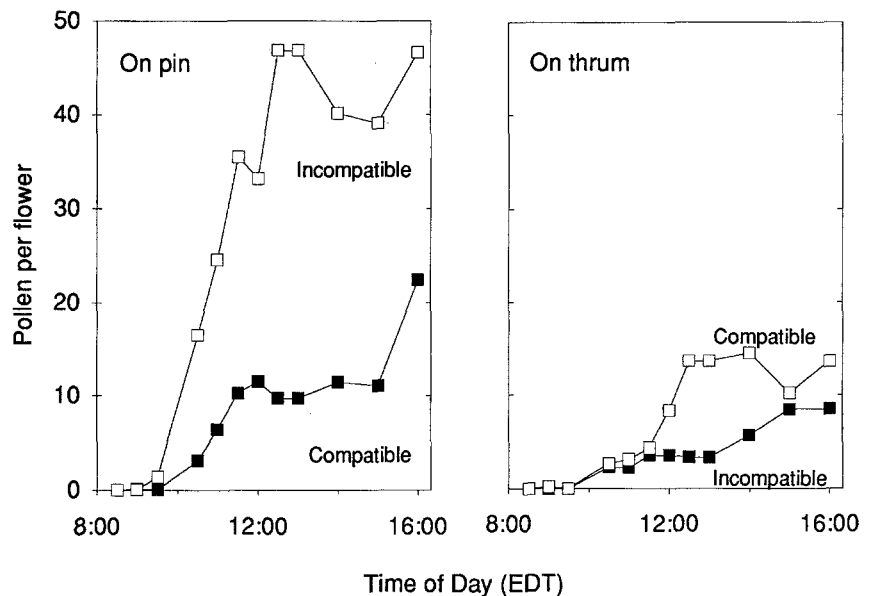
11, 16, and 20 August. The first flowers appeared 1 August, seed
initiation peaked on 6 August, and flowering peaked on 17 Au-
gust. Twenty flowers of each morph were collected at each collec-
tion time and preserved in 70% ethanol. Data from 16 August
were not included in calculations. On that day, it rained continu-
ously, bees were rarely seen, the flowers remained wet, and no
pollen was found on the collected flowers.

The flowers were scored by observation under a dissecting mi-
croscope at ×50 magnification. In alcohol, the styles were clear
and the pollen grains turned black. Germinated pollen grains were
firmly attached to the stigma. The two pollen types were easily
distinguishable by size, with pollen from long-style flowers having
a diameter of 40 μm, and those from short-style flowers having a
diameter of 50 μm (Schoch-Bodmer 1934).

Results

Pollination occurred soon after the anther sacs dehisced.
The anther sacs were normally emptied within 2 h, after
which there was little further pollen deposition. Flowers
were visited 3–12 times while pollen was available in the
anther sacs, based on bee counts on three days. The
counts (4, 13, 19 bee visits) were made on seven marked
inflorescences, each containing three to five flowers,
during the 15 min interval starting at anther sac dehis-
cence, and assumed to be constant as long as there was
pollen available on the anthers, about 1.5–2 h. Pollen
was delivered in nearly the same proportions and at the
same time to each morph (Fig. 1), but thrum stigmata re-
ceived only about one-third as much pollen as pin stig-
mata (Table 1).

Pin and thrum pollen was deposited in similar propor-
tions on the two flower types (Table 2). The small differ-
ence in the proportion is statistically significant ($\chi^2 = 197$,
$P > 0.999$), and the bias is in the direction favoring legiti-
mate pollination. However, heterostyly contributes little
to achieving legitimate pollination. The threefold higher
pollen load on pin flowers compensated for the threefold
lower proportion of legitimate pollen, resulting in a simi-

Table 1 Composition of pollen loads on stigmata and proficien-
cies of legitimate and illegitimate transfers in *Fagopyrum escu-
lentum*

Pollen source[a]	Pollen produced per flower[b]	Average stigma load[c]	
		Pin	Thrum
Pin	1354±164	46±21	**17±13**
Thrum	1080±339	**14±9**	7±6

[a] Mean±sd of 273 pin flowers and 246 thrum flowers. The stigma
loads were counted on all flowers collected after pollination was
complete for each of 3 days. Flowers collected while some pollen
was still present in the anther sacs were not included. The number
of legitimate pollen grains are shown in bold
[b] The two morphs are equally frequent and produce equal numbers
of flowers
[c] From Dulberger 1992

Table 2 Selectivity of pollen delivery to the two flower morphs.
The number of pollen on all 1440 perfect flowers collected (all
collection times on 6, 11, and 20 August 1993 were included to re-
flect the proportion of pollen delivered, rather than the absolute
pollen load)

Pollen source	Stigma	
	Pin	Thrum
Pin	17166	**6007**
Thrum	**5188**	2691
Proportion pin pollen	77%	69%
$\chi^2 = 197$		

lar number of legitimate pollen per flower on each
morph (Table 1).

There was a similar excess of pin pollen on both flow-
er morphs, rather than an excess of legitimate pollen (Ta-
ble 2). However, since more than two-thirds of the pollen
delivered to pin flowers was illegitimate, the amount of
legitimate pollen delivered to the two morphs was similar.

The pollen use efficiency was rather low. Each flower produced about 1100 pollen grains (Dulberger 1992), and collected about 20 (thrum stigmata) to 60 (pin stigmata) pollen grains by the end of the day. Of these, about 10–15 pollen per flower (1% of the amount produced) were compatible.

Discussion

The large amount of illegitimate pollen in the dish-shaped heterostylous flowers of *F. esculentum* indicates that heterostyly alone did not result in efficient outcrossing, that heterostyly is not sufficient to eliminate the pollen wastage resulting from illegitimate pollination, and that the evolution of intra-morph incompatibility in addition to heterostyly has not been "incidental and purposeless" (Darwin 1877). Distributions similar to those seen here have also been observed in tube-shaped flowers. Most similar is *Amsinckia* (Ganders 1976) with pin pollen making up 67% of the pollen load on pin flowers and 61% on thrum flowers, with a slightly higher total pollen load on pin flowers. Other species showing comparable pollen flow are *Hedyotis caerulea* (Ornduff 1980) and *Pulmonaria obscura* (Olesen 1979). In the only other report on a dish-shaped, heterostylous and self-incompatible plant, *Turnera ulmifolia*, thrum pollen was preferentially moved; the pin pollen was 39% of the pollen load on pin flowers and 46% on thrum flowers (Rama Swamy and Bahadur 1984). In contrast, the Darwinian hypothesis is sometimes supported (Barrett 1990). Heterostyly effectively substitutes for self-incompatibility in *Eichhornia* (Kohn and Barrett 1992), *Jepsonia* (Ganders 1974), and *Pontaderia* (Barrett and Glover 1985) by limiting the amount of self-pollination so that cross-fertilization results even without self-incompatibility. Thus the intra-morph pollen flow in the dish-shaped flowers of *F. esculentum* appears to fall within the spectrum of variation found in tube-shaped flowers.

The total pollen load on pin flowers was about two and a half times higher than on thrum flowers (Table 1). Higher pollen loads on pin flowers have been attributed to the more readily accessible stigma of pin flowers, and to the fact that some pin to thrum flights carry little or no pollen (Levin 1968). The rate of pollen delivery to thrum flowers was consistent with pollinators carrying small pollen loads (Fig. 1). The low pollen efficiency (1% of pollen was delivered to compatible stigmata) is comparable to *Amsinckia* (Ornduff 1976) and *Primula* (Ornduff 1979), which have the same breeding system.

Heterostyly did not cause the pollinators to effectively cross-pollinate between flower types in *F. esculentum*. Both morphs received mainly pin pollen with the proportion nearly the same on both morphs (77 and 69%; Table 2). This can also be viewed as greater pollen flow from pin to thrum than from thrum to pin, but that can be due solely to lack of discrimination by the pollinator in the pollen it delivers. Such lack of discrimination may come from mixing of the proboscis pollen load

during cleaning (Olesen 1979). There is often an exceptionally large amount of pin pollen on pin stigmata (Levin 1968; Olesen 1979; Ornduff 1980) that may be due to intra-flower pollination. Such self-pollination has been identified by observing much less self-pollination in emasculated flowers (Ganders 1976; Barrett and Glover 1985; Ornduff 1993). Interestingly, far more pin pollen (75%) was delivered to both morphs than thrum pollen, even though both are produced in similar amounts (44% pin, Dulberger 1992). Therefore, self-pollination of pin flowers cannot account for the strong bias toward pin pollen delivery in *F. esculentum*. Furthermore, isolated flowers collected no pollen (Björkman 1994), so the observed proportion probably reflects the amount on bees' proboscises rather than movement through disturbance of the flower.

The pollen flow may be a consequence of an inappropriate pollinator, as has been suggested to explain similar results in *Jepsonia* (Ornduff 1971). The center of origin of *F. esculentum* was recently determined to be the mountains of Yunnan, China (Ohnishi 1990), but the local pollinators were not described. In New York State, the pollinators are almost exclusively honeybees (Björkman 1994), as they are in many other locations: Germany (Müller 1883), Japan (Hamakawa 1986), Russia (Elagin 1976), and Poland (Banaszak 1983). However, heterostyly in *F. esculentum* may have evolved with pollination by insects experiencing appropriately segregated contacts, but these insects have since been displaced by the ubiquitous honeybee. The honeybee is a generalist, and collects both nectar and pollen to maintain the hive (Westerkamp 1991). Bees therefore derive no advantage from pollinating *F. esculentum* in a manner that favors cross-pollination. Bees return pollen to the hive to feed the brood; if they favor the larger thrum pollen for this purpose it would account for the deficiency of thrum pollen. The sweet-to-fetid aroma of buckwheat nectar would be more consistent with fly pollination than with bee pollination, but pollination by the syrphid fly *Eristalis cerealis* is no more selective (Namai 1990). Generally, efficiency of cross-pollination in heterostylous species seems to be unrelated to pollinator specialization (Olesen 1979; Harder and Barrett 1993).

The purpose of heterostyly has been questioned when outcrossing is effectively assured through self-incompatibility, and heterostyly does not produce efficient transfer of pollen to the opposite flower type. One hypothesis is that having two different pollination pathways (pin-to-thrum and thrum-to-pin) makes the species more resilient to environmental fluctuations (Dommee et al. 1992). Conversely, stressful conditions appear to have favored the breakdown of heterostyly in some species (Levin 1968; Ornduff 1980).

In summary, in the dish-shaped flower of *Fagopyrum esculentum*, heterostyly is ineffective for ensuring cross-pollination when the insect pollinator is the honeybee. Pollen transfer favors delivery of pin pollen and delivery to pin flowers. Thus there is an excess of pin pollen rather than an excess of legitimate pollen.

146

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