The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae)

JOEL S. SHORE AND SPENCER C. H. BARRETT
Department of Botany, University of Toronto, Toronto, Ont., Canada M5S 1A1

Received July 6, 1983

SHORE, J. S., and S. C. H. BARRETT. 1984. The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). Can. J. Bot. **62**: 1298–1303.

Controlled pollination experiments were performed on the self-incompatible distylous herb *Turnera ulmifolia* L. to investigate the effects of pollination intensity and large amounts of incompatible pollen on seed set. In the first experiment, known numbers of compatible pollen grains ranging from 1 to 100 were applied to stigmas of the floral morphs. In both morphs, increasing amounts of pollen generally resulted in increased levels of seed set, although considerable variance was observed at all pollination intensities. Approximately two to seven pollen grains are required to produce a single seed and more than 95 grains are required to achieve maximum seed set in *T. ulmifolia*. Regression analysis of the seed set data failed to detect a difference in the response of the floral morphs to pollination intensity. In the second experiment, known proportions of compatible and incompatible pollen were applied to stigmas at various time intervals. Most treatments involving mixtures of compatible and incompatible pollen had no significant effect on seed set when compared with the controls. Clogging was only observed in the long-styled morph when one anther of compatible pollen was applied to stigmas 1.5 and 3.0 h after pollination with five anthers of incompatible pollen. The clogging of stigmas by incompatible pollen seems unlikely to have played a major role in the evolution and maintenance of distyly in *Turnera ulmifolia*.

SHORE, J. S., et S. C. H. BARRETT. 1984. The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). Can. J. Bot. **62**: 1298–1303.

Des expériences de pollinisation contrôlée ont été poursuivies chez le *Turnera ulmifolia*, une plante herbacée distylée auto-incompatible, dans le but d'étudier les effets, sur le rendement en graines, de l'intensité de la pollinisation et de la présence de grandes quantités de pollen incompatible. Dans la première expérience, des grains de pollen compatibles, de nombre connu et compris de 1 à 100, ont été déposés sur les stigmates des deux morphes. Une augmentation de la quantité de pollen amène une augmentation du nombre de graines chez les deux morphes, mais la variance est très élevée pour toutes les intensités de pollinisation. De deux à sept grains de pollen environ sont nécessaires pour produire une seule graine et plus de 95 grains de pollen sont nécessaires pour former le nombre maximum de graines chez le *T. ulmifolia*. Une analyse par régression du nombre de graines produites ne révèle aucune différence entre les deux morphes dans leur réponse à l'intensité de la pollinisation. Dans la deuxième expérience, des grains de pollen compatibles et incompatibles, dans des proportions connues, ont été déposés sur le stigmate à divers moments. La plupart des traitements par des mélanges de pollen compatible et incompatible n'ont pas d'effets sur le nombre de graines produites par comparaison aux témoins. Un colmatage a été observé seulement chez les plantes longistylées, lorsqu'une anthère de pollen compatible est déposée sur les stigmates 1,5 et 3,0 h après la pollinisation par cinq anthères de pollen incompatible. Le colmatage des stigmates par du pollen incompatible n'a probablement pas joué un rôle majeur dans l'évolution et le maintien de la distylie chez le *Turnera ulmifolia*.

[Traduit par le journal]

Introduction

Many factors influence seed set in outbreeding plant populations. These include the density, spatial pattern, and frequency of mating groups (Mulcahy 1967; Platt *et al.* 1974; Silander 1978; Wyatt and Hellwig 1979; Antonovics and Levin 1980; Barrett and Thomson 1982); the composition, abundance, and behaviour of the pollinator fauna (Levin and Berube 1972; Waser 1978; Barrett 1980*a*; Plowright and Hartling 1981; Motten *et al.* 1981; Bertin 1982*a*; Snow 1982); the environmental conditions during pollination, fertilization, and embryo development (Lewis 1942; Hagerup 1951; Barrett 1980*b*); as well as the availability of plant resources for fruit and seed maturation (Brink and Cooper 1947; Lloyd *et al.* 1980; Udovic and Aker 1981; Stephenson 1981). Several studies have demonstrated that during a single flowering season, seed set is, in part, limited by pollen availability (Schemske *et al.* 1978; Barrett 1980*b*; Willson and Schemske 1980; Weller 1980; Bierzychudek 1981; McDade 1983). This phenomenon is particularly likely to occur in habitats where pollinator service is unreliable owing to inclement weather.

Information on the quantitative relationships between the number of compatible pollen grains deposited on a stigma and the level of fertilization is required to evaluate the importance of pollen limitation to seed set. Information of this type is available for relatively few species. These include *Passiflora* spp. (Akamine and Girolami 1959; Snow 1982), *Oenothera fruticosa* (Silander and Primack 1978), *Campsis radicans* (Bertin 1982*a*), and *Costus guanaiensis* (Schemske and Fenster 1983). In these studies, known amounts of compatible pollen were applied to stigmas, and the resulting seed set was recorded. Under field conditions stigmas can receive a mixture of compatible, incompatible, and interspecific pollen grains. To what extent the presence of noncompatible pollen grains on the stigmatic surface modifies the relationship between pollination intensity and seed set is unknown. Sukada and Jayachandra (1980) and Thomson *et al.* (1981) have, however, demonstrated the inhibitory effects of heterospecific pollen mixtures to fertilization and seed set in *Parthenium hysterophorus* and *Diervilla lonicera*, respectively.

Owing to the small number of mating groups in heterostylous plant populations (two in distyly, three in tristyly), the probability of stigmas receiving incompatible pollen grains is high. In addition, large floral displays, extensive clone size, and near-neighbour foraging by pollinators will increase incompatible pollen loads as a result of geitonogamy. Studies of the composition of pollen on naturally pollinated stigmas of heterostylous species confirm the presence of considerable amounts of incompatible pollen (reviewed by Ganders 1979). Several authors have suggested that this phenomenon, known as "clogging" (Yeo 1975; Lloyd and Yates 1982), might reduce

seed set through the inhibition of germination or tube growth of compatible pollen grains. However, there is no experimental evidence of the effect of clogging in heterostylous plants so it is difficult to evaluate its importance to the pollination process.

Here we examine the effects of pollination intensity and clogging on seed set in the two style morphs of *Turnera ulmifolia* L. (Turneraceae), a distylous, perennial herb. *Turnera ulmifolia* is particularly suited to studies of this type because it flowers profusely year-round under glasshouse conditions, has large flowers which are easily manipulated, and has a moderate number of ovules per flower. In addition, individuals produce a sufficient number of flowers each day for replicated trials. Of particular interest to us was the comparison of the response of the long- and short-sytled morphs to the pollination treatments since studies of pollen flow have demonstrated consistent differences in the number of compatible and incompatible pollen grains captured by stigmas of the floral morphs. Following the presentation of our results we discuss their implications in light of several recent proposals on the potential importance of clogging to the evolution of plant breeding systems.

Materials and methods

Material of *Turnera ulmifolia* used in the experiements was grown from a bulk seed sample collected from a population growing on open, sandy, waste ground in Barreirinhas, Maranhao State, Brazil, in August 1977. The varietal status of plants is unclear; they exhibit characters found in var. *elegans* Urb. and var. *intermedia* Urb. (Urban 1883). The population from which plants were derived corresponds to population 30 in Barrett (1978) where further details of the reproductive biology of *T. ulmifolia* are given. The population was chosen for study because individuals were strongly self-incompatible, were diploid ($2n = 10$) with no pollen sterility, and flowered prolifically. Flowers in *T. ulmifolia* are ephemeral, opening early in the morning and lasting for approximately 4 h.

In the summer of 1979, floral characters were measured from a sample of 29 plants (15 long styled, 14 short styled) grown under glasshouse conditions. The size of 20 pollen grains was measured for each individual by using a calibrated ocular micrometer on a compound microscope. Pollen production per flower was measured using the haemacytometer method (see Lloyd 1965). Three buds were used from each of seven individuals per morph.

Pollination intensity experiment

The experiment was conducted during August 1981, using 14 plants (7 of each style morph) of the same age grown in a pollinator-free glasshouse. All pollinations were performed in the laboratory. Each day one individual of each morph was selected as a pollen donor. Legitimate (compatible) pollinations were then made using a range of female parents. Flowers on each pollen recipient were assigned a random number of pollen grains between 0 and 101. Before pollination, part of the corolla was removed to allow deposition of a precise number of pollen grains per stigma. Although this was only necessary in the short-styled morph, because of its concealed stigma, excision of the corolla was also undertaken on the long-styled morph to serve as a control. Pollen from the donor was applied to a fine needle by touching the needle to an anther. While observing this needle under a dissecting microscope (×10), a second needle was used to remove a small number of pollen grains. This method enabled the transfer of small quantities of pollen. The pollen grains were then transferred to the recipient stigmas while observing them under the microscope. An effort was made to place an equal number of pollen grains on each of the three stigmas within a flower.

After pollination each flower was marked with a tag recording the date of pollination and the number of pollen grains applied. At least 6 days after pollination the capsules were harvested and dissected open, and the number of developing seeds and small shriveled ovules (assumed to be unfertilized ovules) were counted. Seed maturation in *T. ulmifolia* normally takes approximately 21 days. Capsules were harvested prior to dehiscence to enable the number of unfertilized ovules to be measured easily and to avoid the confounding effect of fruit abortion in capsules containing a small number of seeds.

The seed set data were analyzed using the regression model: $Y = B_0 + B_1 \times X_1 + B_2 \times X_2 + B_3 \times X_1 \times X_2 +$ error (Neter and Wasserman 1974), where Y is the response variable (e.g., seeds per capsule), X_1 is the number of pollen grains applied to the stigma, and X_2 is an indicator (dummy) variable which has the value 0 for the long-styled morph and 1 for the short-styled morph. The regression parameters B_0–B_3 allow tests of the hypothesis that the style morphs respond differently to the varying pollination intensities.

Pollen clogging experiments

Three experiments were conducted in July and August 1982, using 18 plants of similar age grown under glasshouse conditions. In each experiment the following protocol was used. Each morning random pairs of morphs were selected from the sample, provided that the number of flowers on a plant was at least equal to the number of treatments to be applied. For each pair a single long- and short-styled flower was used as the pollen donor. Treatments were randomly assigned to flowers of the pollen recipient. Pollen was transferred using a single anther as the unit and rubbing the anther on the recipient stigmas until all pollen had been visibly removed from the anther. For each pair of plants all treatments were performed on both individuals. While the experimental design confounds daily environmental conditions with genotypic sources of variation, these effects were controlled by blocking in the analysis of variance.

The three experiments involved an examination of the effect of the order of application and the relative proportion of incompatible and compatible pollen grains on seed set. In the first experiment the two pollen types were applied in sequence with no significant time interval between the applications. The five treatments used were (*i*) one unit of compatible pollen (control), (*ii*) one unit of compatible pollen followed by one unit of incompatible pollen, (*iii*) one unit of incompatible pollen followed immediately by one unit of compatible pollen, (*iv*) one unit of compatible pollen followed by five units of incompatible pollen, and (*v*) five units of incompatible pollen followed by one unit of compatible pollen. The experiment was replicated 14 times, i.e., it consisted of 14 blocks. In the second and third experiments, only treatments 1, 3, and 5 were used and the time intervals between application of the two pollen types were 1.5 and 3.5 h, respectively. Control pollinations were performed after similar time intervals. Fifteen replications per morph were used in the second experiment and 22 in the third experiment. In all clogging experiments anthers were removed from recipient flowers and flowers were marked with tags indicating the treatment and date of pollination. Developing seeds and unfertilized ovules were counted as described above.

Results

The population of *Turnera ulmifolia* used in this study exhibits the floral polymorphisms common to most distylous species. Dimorphism occurs for style length, stamen length, pollen size, and pollen production (Table 1). The number of ovules per flower and the natural levels of seed set per capsule are not significantly different between the style morphs. Stigmas of the flora morphs of *T. ulmifolia* lack papillae but are highly dissected, thus providing a large surface area for pollen capture. The long-styled morph has a larger stigmatic surface than the short-styled morph.

Pollination intensity experiment

Data for seed set per capsule resulting from different pollination intensities are plotted as histograms in Fig. 1. A small number of pollinations at all pollination intensities failed to produce fruits. These were excluded from the analysis. While lack of fruit production at low pollination intensities may result

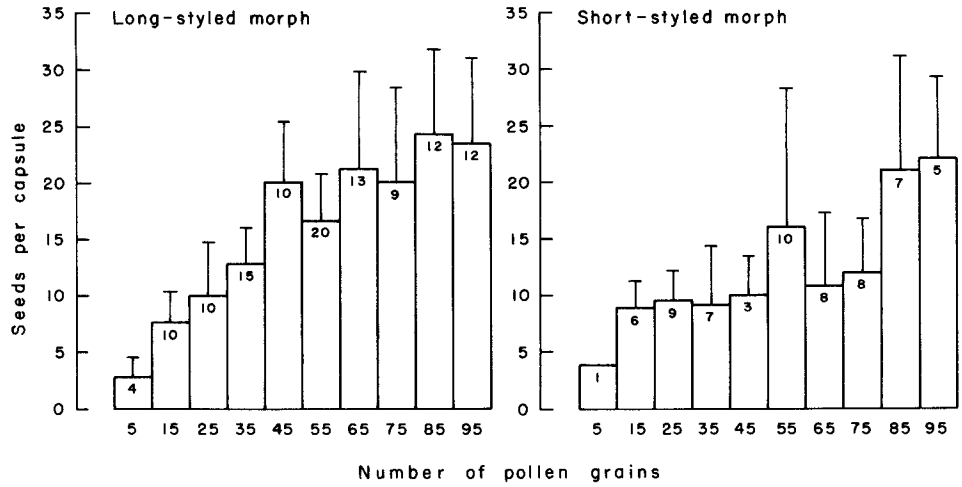

FIG. 1. The effect of pollination intensity on seed set in the floral morphs of *Turnera ulmifolia*. The vertical line over each bar indicates one standard deviation and the number in the bar indicates the sample size.

TABLE 1. Reproductive parameters of the *Turnera ulmifolia* population used in pollination experiments (mean ±1 standard deviation)

Parameter	Morph		
	Long styled	Short styled	*P*
Style length (mm)	11.8±1.0	6.7±0.9	<0.001
Stamen length (mm)	7.4±0.7	11.5±0.5	<0.001
Ovule no./flower	43.6±2.5	49.9±8.4	NS
Pollen production/flower	13 010 ± 1090	9660±1190	<0.001
Pollen size (μm)	64.9±1.3	76.9±2.7	<0.001
Open-pollinated Fecundity (seeds/capsule)*	20.2±10.9	19.5±12.7	NS

NOTE: NS, not significant.
*As in Barrett (1978).

TABLE 2. Regression analysis of log seed number per capsule against the number of pollen grains applied to stigmas of the long- and short-styled morphs of *Turnera ulmifolia**

Regression coefficient	Estimate of coefficient	*t*-value	*P*
B_0	1.61	12.2	0.001
B_1	0.018	8.2	0.001
B_2	0.041	0.18	0.86
B_3	−0.006	1.49	0.14

*The model fitted is given in Materials and methods. For the long-styled morph the line will have the intercept B_0 and slope B_1. Corresponding values for the short-styled morph are $B_0 + B_2$ and $B_1 + B_3$.

from an absence of fertilization, other factors, such as resource limitation and damage to flowers during manipulations, likely were involved at greater pollination intensities.

In both the long- and short-styled morphs, increasing amounts of pollen applied to stigmas generally resulted in increased levels of seed set. Large variance in the number of seeds produced in each treatment was, however, a characteristic feature of the data. Visual inspection of data in Fig. 1 suggested that the responses of the two morphs to the pollination treatments may differ. This hypothesis was tested by regression analysis.

Preliminary analyses revealed that in both morphs the variance of seed number increased with the mean. Therefore, natural log transformation of the response variable was undertaken. Results of the regression analysis are presented in Table 2. The regression equation for the long-styled morph reduces to a line with intercept B_0 and slope B_1. B_2 represents the magnitude of the difference between estimates of the intercepts for the long- and short-styled morphs. Similarly, B_3 estimates the difference between the slopes of the lines for the two morphs. Tests of the hypothesis that parameters B_2 and B_3 differ from zero are not significant (Table 2). The percentage of variation accounted for by the model was 34.5. We also performed similar analyses on the ratio of seeds per capsule to ovules per flower with and without pollinations which gave no seeds. The results were qualitatively similar and did not allow rejection of the null hypothesis. Therefore we have no evidence that the morphs respond differently to increasing pollination intensity.

Pollen clogging experiment

As in the previous experiment analyses were performed on both the number of seeds set per capsule and on the ratio of seeds to ovules per capsule. Results were similar and only data for seeds per capsule are presented in Table 3. Although the same number and proportion of anthers were used in each treatment, the absolute number of pollen grains applied to stigmas of the two style morphs differed because flowers of the two morphs produced different amounts of pollen (Table 1). Hence, separate ANOVAs were performed on data from each morph.

In the first experiment involving sequential application of compatible and incompatible pollen and *vice versa*, seed set in both morphs was unaffected by the sequence or amount of pollen applied to stigmas. Earlier observations indicated that in both style morphs compatible and incompatible pollen germinates and penetrates the stigmatic tissue. We therefore hypothesized that clogging might occur in the style rather than on the stigmatic surface. Thus, two additional experiments were performed involving a 1.5- and 3.5-h delay between the application of the pollen types. The delay might allow incompatible pollen grains to germinate and penetrate the stigmatic tissue before the compatible grains were applied.

The results from the two experiments indicate that the long-styled morph is more sensitive to the inhibitory effects of incompatible pollen than the short-styled morph. In both experi-

TABLE 3. The effect of varying amounts of compatible and incompatible pollen on seed set per capsule in *Turnera ulmifolia*

Morph	One compatible (control)	One compatible, one incompatible	One incompatible, one compatible	One compatible, five incompatible	Five incompatible, one compatible	*F*	*P*
Experiment 1							
Long	29.9*	29.7	28.6	30.4	27.9	0.47	0.76
Short	24.9	24.1	24.3	29.3	24.1	0.64	0.63
Experiment 2							
Long	31.1		27.2		21.1	4.14	0.03
Short	28.0		27.6		29.9	1.73	0.10
Experiment 3							
Long	28.5		28.2		16.5	14.34	0.001
Short	33.3		32.4		27.7	2.15	0.13

NOTE: Experiment 1, immediate application (n = 14 flowers per treatment); experiment 2, 1.5 h between applications (n = 15 flowers per treatment); and experiment 3, 3.5 h between applications (n = 22 flowers per treatment).
*Mean seed set per capsule.

ments the treatment involving a 5:1 ratio of incompatible: compatible pollen resulted in a significant reduction in seed set compared with the control treatments. In contrast, in the short-styled morph there were no significant differences between treatments in either experiment. Thus clogging can occur in *T. ulmifolia*, but in our experiments it was only evident under restricted conditions.

Discussion

In recent years several workers have considered the potential importance of the inhibitory effects of incompatible pollen to seed set in self-incompatible plants. Bawa and Opler (1975) suggested that in tropical trees with large floral displays a plant's own pollen may interfere with the receipt of compatible pollen. They postulated that stigmatic clogging could result in reduced seed set and ultimately selection for dioecism. Similar arguments were made by Zapata and Arroyo (1978) in their consideration of low seed set in self-incompatible species, in comparison with dioecious species, from a tropical deciduous forest in Venezuela. Yeo (1975) suggested that the floral polymorphisms common to heterostylous plants could function as anticlogging devices which had evolved to compensate for the relatively "inefficient" diallelic incompatibility system. Lloyd and Yates (1982) have extended these ideas and have suggested that spatial segregation of anthers and stigmas within flowers of the style morphs may have evolved to reduce mutual interference between male and female function. In contrast, Charlesworth and Charlesworth (1979) did not incorporate stigmatic clogging into their model of the evolution of distyly and considered it unlikely to be an important selective influence. Finally, Cruden and Miller-Ward (1981) were of the opinion that competition between compatible and incompatible pollen grains on stigmas of heterostylous plants would be unlikely and should rarely limit seed set.

Given the potentially important role clogging may play as a force influencing plant breeding systems, it is necessary to review evidence for its occurrence. Three classes of study have been performed depending on whether the effects of (*i*) heterospecific pollen, (*ii*) dead pollen or foreign substances, or (*iii*) incompatible pollen were examined. Several authors have demonstrated that heterospecific pollen reduces seed set (Ockendon and Currah 1977; Waser 1978; Sukada and Jayachandra 1980; Thomson *et al.* 1981), while others failed to detect a reduction in seed set (Brown and Kodric-Brown 1979) or a marked reduction in the numbers of pollen tubes in styles (Sedgley and Blesing 1982). Dead self-pollen did not reduce pollen tube growth in *Brassica oleracea* (Ockendon and Currah 1977) or in *Cosmos bipinnatus* (Howlett *et al.* 1975). The latter study is often cited as providing evidence for pollen clogging (e.g., Zapata and Arroyo 1978; Lloyd and Yates 1982), but a misprint occurred in one of the tables (B. J. Howlett, personal communication) and no such effect exists. Pollen dilution with talc (Jennings and Topham 1971) reduced fruit set in raspberry cultivars. Ockendon and Currah (1977) demonstrated that self-pollen reduced the number of cross-pollen tubes in the styles of *Brassica oleracea*. However, this clogging effect disappeared if the compatible pollen was placed on the stigma at least 16 h after the application of self-pollen. Snow (1982) provides evidence, from a limited sample of open-pollinated intact and emasculated flowers of self-incompatible *Passiflora vitifolia*, which suggests that clogging may have occurred. She found that emasculated flowers set a higher percentage of seed than unmanipulated flowers, following natural pollination by hummingbirds. While these studies provide useful information to our understanding of the pollination process, they hardly constitute strong experimental evidence for clogging.

In this paper we have examined the effect of self-pollen (incompatible) on seed set in a distylous population of *Turnera ulmifolia* under controlled glasshouse conditions. Clogging was only observed under the most extreme treatments, i.e., clogging occurred when one anther of compatible pollen was applied 1.5 or 3.5 h (approximately the lifetime of a flower) after the application of five anthers of self-pollen. In addition this effect only occurred in the long-styled morph; short-styled plants did not exhibit reduced seed set (Table 3). The results suggest that clogging may occur within the style and requires the presence of a sufficiently large number of self-pollen tubes to reduce seed set. The absence of clogging in short-styled plants may have at least two explanations. The treatments applied to the two morphs are not equivalent since the morphs differ in total pollen production (Table 1). The short-styled morph received on average 25% fewer incompatible pollen grains than in the equivalent treatment applied to the long-styled morph. This difference could have affected the results. Furthermore, observations of pollen tubes in cleared styles and stigmas revealed that self-pollen tubes grew no farther than the stigma-style interface for the short-styled morph but at least half the length of the style in the long-styled morph. Thus clogging could occur in the stylar transmitting tissue of the long-styled morph, particularly if a smaller cross-sectional area

is available for pollen tube growth, as occurs in the long-styled morph of *Primula sinensis* (Dowrick 1956). For the short-styled morph, inhibition of pollen tube growth occurs in the highly branched stigmatic tissue where ample area may remain available for subsequent compatible pollen tube growth. At present we cannot distinguish between these two possibilities.

From an ecological perspective we feel that pollen clogging is unlikely to be an important influence on the pollination process in this species since clogging may only be achieved under circumstances which rarely occur in nature. Clogging would require a pollinator to deposit large amounts of incompatible pollen on stigmas and no compatible pollen until at least 1.5 h later. Given the known population structure of the species, which frequently involves dense colonies of both floral morphs (Barrett 1978), this seems unlikely. However, we should point out that it is possible that treatment combinations not used in our study could lead to clogging. Studies attempting to demonstrate clogging should ideally include the range of compatible and incompatible pollination levels that can be achieved in a natural population. At best, a factorial design with differing levels of incompatible and compatible pollen should be applied and the resulting seed set determined. A time delay between the application of incompatible and compatible pollen may be included as an additional dimension. The implementation of a pollination programme this broad in scope is technically difficult and remains to be done.

The average number of pollen grains required to set a seed is an important parameter for studies attempting to demonstrate pollen limitation of fecundity. The pollen loading experiment performed here revealed the expected increase in seed set per capsule with increasing pollen number (Fig. 1). However, at all pollination levels the number of seeds set was less than the number of pollen grains applied. Further, if we compare the mean seed set per capsule at the highest pollination intensity (95 grains) with the controls of the pollen clogging experiment (Table 3), for which approximately 2000 pollen grains were applied, we find that with 95 grains per flower we have not achieved maximum seed set. Thus, more than 95 pollen grains are required to achieve maximum seed set and about two to seven pollen grains are required per seed.

The elevation of the number of pollen grains required per seed above the theoretical minimum of 1 has a number of explanations. The maternal parent may be able to prevent fertilizations by inferior male gametes or to abort ovules which have been fertilized by inferior or nonpreferred male gametes (see Willson and Burley 1983). Alternatively some pollen may be genetically defective. However, it is also possible that the experimental manipulations of the flower involving the excision of the corolla and the handling of pollen may have contributed to the observed effect. It is also worth noting that the mean ovule number per flower (Table 1) is greater than any of the mean seed set values observed (Table 3, Fig. 1). This suggests either that abortion of ovules, because of fertilization by nonpreferred gametes or because of limited maternal resources, has occurred or that some ovules are sterile. If our experimental results may be extended to natural situations, they suggest that pollen limitation might occur even though more pollen grains are present on a stigma than the number of ovules available in the ovary. For *Turnera ulmifolia* more than 95 pollen grains are required to achieve maximum seed set per capsule. Finally, simply counting the number of seeds and ovules produced in open-pollinated flowers will not necessarily provide evidence of pollen limitation as not all ovules may be capable of producing a seed.

Recently, Cruden and Miller-Ward (1981) have argued that large pollen grains are more efficient at achieving fertilization than small pollen grains (but see Baker and Baker (1982) and Plitmann and Levin (1983)). Cruden and Miller-Ward use evidence from pollen flow studies of distylous taxa to support their hypothesis. We feel that the use of total pollen load data is inappropriate as different ratios of compatible pollen to seed set among the morphs are the net result of asymmetries in pollen deposition among the morphs and provide no information on the relative efficiency of individual large and small pollen grains. If it is accepted that heterostylous taxa can be used as a test of their general hypothesis, then it would seem to us that a more useful approach would be to experimentally manipulate small numbers of pollen grains and observe seed set. With this in mind it would appear that our pollen loading experiment is a direct test of their hypothesis. From our results we cannot reject the null hypothesis that large and small grains perform identically. Although the curve for the long-styled morph, pollinated by large grains from the short-styled morph, seems to rise more quickly than that of the short-styled morph, there is no significant difference between the two curves (Table 2). As indicated above, the regression analysis accounted for only 34.5% of the observed variation in the data set. The requirement of corolla removal for the precise deposition of pollen on the stigmas may have been responsible for some of the unaccounted variation. In addition, differences in maternal and paternal preferences (Bertin 1982*b*) as well as environmental variation in the glasshouse may have also contributed to the unexplained variation. Despite these difficulties, we feel that this experimental approach is the best method for addressing hypotheses concerned with distinguishing the relative effectiveness of large and small pollen grains in achieving fertilization in heterostylous taxa.

While the potential importance of clogging as a factor influencing the evolution of unisexuality (Bawa and Opler 1975) or the spatial segregation of male and female reproductive functions in hermaphrodites (Lloyd and Yates 1982) has been stressed by previous authors, few data are available on the inhibitory effects of incompatible pollen to maternal parents. We have demonstrated that clogging can occur under extreme experimental conditions in long-styled individuals of *Turnera ulmifolia*, but it seems unlikely to have played an important role in the evolution and maintenance of distyly in this group.

Acknowledgements

We thank J. M. Anderson, D. E. Glover, and L. M. Wolfe, for advice and R. W. Cruden, L. D. Harder, and D. G. Lloyd for comments on the manuscript. This work was funded by a Natural Sciences and Engineering Research Council of Canada grant to S. C. H. Barrett.

AKAMINE, E. K., and G. GIROLAMI. 1959. Pollination and fruit set in the yellow passion fruit. Tech. Bull. Hawaii Exp. Stn. No. 39.

ANTONOVICS, J., and D. A. LEVIN. 1980. The ecological and genetic consequences of density-dependent regulation in plants. Annu. Rev. Ecol. Syst. **11**: 411–452.

BAKER, H. G., and I. BAKER. 1982. Starchy and starchless pollen in the Onagraceae. Ann. Mo. Bot. Gard. **69**: 748–754.

BARRETT, S. C. H. 1978. Heterostyly in a tropical weed: the reproductive biology of the *Turnera ulmifolia* complex (Turneraceae). Can. J. Bot. **56**: 1713–1725.

———1980*a*. Sexual reproduction in *Eichhornia crassipes* (water

hyacinth). I. Fertility of clones from diverse regions. J. Appl. Ecol. **17**: 101–112.

———1980*b*. Sexual reproduction in *Eichhornia crassipes* (water hyacinth). II. Seed production in natural populations. J. Appl. Ecol. **17**: 113–124.

BARRETT, S. C. H., and J. D. THOMSON. 1982. Spatial pattern, floral sex ratios, and fecundity in dioecious *Aralia nudicaulis* (Araliaceae). Can. J. Bot. **60**: 1662–1670.

BAWA, K. S., and P. A. OPLER. 1975. Dioecism in tropical trees. Evolution (Lawrence, Kans.), **29**: 167–179.

BERTIN, R. I. 1982*a*. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). Am. J. Bot. **69**: 122–134.

———1982*b*. Paternity and fruit production in trumpet creeper (*Campsis radicans*). Am. Nat. **119**: 694–709.

BIERZYCHUDEK, P. 1981. Pollinator limitation of plant reproductive effort. Am. Nat. **117**: 838–840.

BRINK, R. A., and D. C. COOPER. 1947. The endosperm in seed development. Bot. Rev. **13**: 423–542.

BROWN, J. H., and A. KODRIC-BROWN. 1979. Convergence and mimicry in a temperate community of hummingbird-pollinated flowers. Ecology, **60**: 1022–1035.

CHARLESWORTH, D., and B. CHARLESWORTH. 1979. A model for the evolution of distyly. Am. Nat. **114**: 467–498.

CRUDEN, R. W., and S. MILLAR-WARD. 1981. Pollen–ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: an hypothesis. Evolution (Lawrence, Kans.), **35**: 964–974.

DOWRICK, V. P. J. 1956. Heterostyly and homostyly in *Primula obconica*. Heredity, **10**: 219–236.

GANDERS, F. R. 1979. The biology of heterostyly. N.Z. J. Bot. **17**: 607–635.

HAGERUP, O 1951. Pollination in the Faeroes—in spite of rain and poverty in insects. D. K. Dan. Vidensk. Selsk. Biol. Medd. **18**: 15–48.

HOWLETT, B. J., R. B. KNOX, J. D. PAXTON, and J. HESLOP-HARRISON. 1975. Pollen wall proteins: physiochemical characterization and role in self-incompatibility in *Cosmos bipinnatus*. Proc. R. Soc. London, Ser. B, **188**: 167–182.

JENNINGS, D. L., and P. B. TOPHAM. 1971. Some consequences of raspberry pollen dilution for its germination and for fruit development. New Phytol. **70**: 371–380.

LEVIN, D. A., and D. E. BERUBE. 1972. *Phlox* and *Colias*: the efficiency of a pollination system. Evolution (Lawrence, Kans.), **26**: 242–250.

LEWIS, D. 1942. The physiology of incompatibility in plants. I. The effect of temperature. Ann. Bot. (London), **7**: 115–122.

LLOYD, D. G. 1965. Evolution of self-incompatibility and racial differentiation in *Leavenworthia* (Cruciferae). Contrib. Gray Herb. Harv. Univ. **195**: 3–134.

LLOYD, D. G., C. J. WEBB, and R. B. PRIMACK. 1980. Sexual strategies in plants. II. Data on regulation of maternal investment. New Phytol. **86**: 81–92.

LLOYD, D. G., and J. M. A. YATES. 1982. Intrasexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). Evolution (Lawrence, Kans.), **36**: 903–913.

MCDADE, L. A. 1983. Pollination intensity and seed set in *Trichanthera gigantea* (Acanthaceae). Biotropica, **15**: 122–124.

MOTTEN, A. F., D. R. CAMPBELL, D. E. ALEXANDER, and H. L. MILLER. 1981. Pollination effectiveness of specialist and generalist vistors to a North Carolina population of *Claytonia virginica*. Ecology, **62**: 1278–1287.

MULCAHY, D. L. 1967. Optimal sex ratio in *Silene alba*. Heredity, **22**: 411–423.

NETER, J., and W. WASSERMAN. 1974. Applied linear statistical models. R. D. Irwin, Inc., Homewood, IL.

OCKENDON, D. J., and L. CURRAH. 1977. Self-pollen reduces the number of cross-pollen tubes in the styles of *Brassica oleracea* L. New Phytol. **78**: 675–680.

PLATT, W. J., G. R. HILL, and S. CLARK. 1974. Seed production in a prairie legume (*Astragalus canadensis* L.). Interactions between pollination, pre-dispersal seed predation and plant density. Oecologia, **17**: 55–63.

PLITMANN, U., and D. A. LEVIN. 1983. Pollen–pistil relationships in the Polemoniaceae. Evolution (Lawrence, Kans.), **37**: 957–967.

PLOWRIGHT, R. C., and L. K. HARTLING. 1981. Red clover pollination by bumble bees: a study of the dynamics of a plant–pollinator relationship. J. Appl. Ecol. **18**: 639–647.

SCHEMSKE, D. W., and C. FENSTER. 1983. Pollen-grain interactions in a neotropical *Costus*: effects of clump size and competitors. *In* Pollen: biology and implications for plant breeding. *Edited by* D. L. Mulcahy and E. Ottaviano. Elsevier Scientific Publishing Co., Amsterdam. pp. 405–410.

SCHEMSKE, D. W., M. F. WILLSON, M. N. MELAMPY, L. J. MILLER, L. VERNER, K. M. SCHEMSKE, and L. B. BEST. 1978. Flowering ecology of some spring woodland shrubs. Ecology, **59**: 351–366.

SEDGLEY, M., and M. A. BLESING. 1982. Foreign pollination of the stigma of watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai). Bot. Gaz. (Chicago), **143**: 210–215.

SILANDER, J. A, 1978. Density-dependent control of reproductive success in *Cassia biflora*. Biotropica, **10**: 292–296.

SILANDER, J. A., and R. B. PRIMACK. 1978. Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). Am. Midl. Nat. **100**: 213–216.

SNOW, A. A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. Oecologia, **55**: 231–237.

STEPHENSON, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. **12**: 253–279.

SUKADA, D. K., and JAYACHANDRA. 1980. Pollen allelopathy—a new phenomenon. New Phytol. **84**: 739–746.

THOMSON, J. D., B. J. ANDREWS, and R. C. PLOWRIGHT. 1981. The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). New Phytol. **90**: 777–783.

UDOVIC, D., and C. AKER. 1981. Fruit abortion and the regulation of fruit number in *Yucca whipplei*. Oecologia, **49**: 245–248.

URBAN, I. 1883. Monographie der Familie der Turneraceen. Gebruder Borntraeger, Berlin.

WASER, N. M. 1978. Competition for hummingbird pollination and sequential flowering in two Coloroda wildflowers. Ecology, **59**: 934–944.

WELLER, S. G. 1980. Pollen flow and fecundity in populations of *Lithospermum caroliniense*. Am. J. Bot. **67**: 1334–1341.

WILLSON, M. F., and N. BURLEY. 1983. Mate choice in plants: tactics, mechanisms and consequences. Princeton University Press, Princeton.

WILLSON, M. F., and D. W. SCHEMSKE. 1980. Pollinator limitation, fruit production and floral display in Pawpaw (*Asimina triloba*). Bull. Torrey Bot. Club, **107**: 401–408.

WYATT, R., and R. L. HELLWIG. 1979. Factors determining fruit set in heterostylous bluets, *Houstonia caerulea* (Rubiaceae). Syst. Bot. **4**: 103–114.

YEO, P. F. 1975. Some aspects of heterostyly. New Phytol. **75**: 147–153.

ZAPATA, T. R., and M. T. K. ARROYO. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica, **10**: 221–230.