Floral morphology affects seed productivity through pollination efficiency in radish (*Raphanus sativus* L.)

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Abstract

To examine the effect of stigma position and size on seed productivity through pollination efficiency in radish, the numbers of self and cross pollen grains on the stigmas and the seed productivity under insect-pollination were compared among 4 bred lines. Lines with a small stigma or a high stigma relative to the anthers on long stamens tended to receive fewer self and total (self + cross) pollen grains but showed a higher cross-pollination percentage (ratio of cross pollen grains to total pollen grains on stigmas) than those with a large stigma or a lower stigma. Additionally, a higher cross-pollination percentage was associated with a higher outcrossing percentage. This result suggests that variations in the two stigma traits affect the outcrossing percentage via the cross-pollination percentage. Therefore, it should be possible to prevent loss of F_1 purity by selecting new parental lines with floral morphology that favors cross-pollination percentage.

Introduction

Radish (*Raphanus sativus* L.) has self-incompatible entomophilous flowers and is categorized as allogamous (Bateman 1955). F_1 hybrids are predominant in the current commercial radish market, and F_1 seed production relies on self-incompatibility. This method results in some self seeds, lowering the F_1 purity (Horisaki *et al.* 2003; Niikura 2007). To elucidate how radish produces self seeds as well as hybrids, and thus to counter the loss of F_1 purity, it is essential to fully understand the reproductive process in radish.

Reproduction is generally divided into two main processes, pollination and fertilization (Namai *et al.* 1992). Molecular biological studies of *S* gene-controlled self-incompatibility in radish fertilization have made good progress (Niikura & Matsuura 1997, 1998, 1999), but the pollination process is less well understood (but see Namai *et al.* 1992). Horisaki *et al.* (2003) reported that the self seed set percentage, indicating the level of self-incompatibility, changed between artificial and insect pollination. This result suggests that seed productivity may be affected not only by self-incompatibility, but also by steps in the pollination process. Therefore, both pollinator behavior and pollinator interaction with floral traits should be considered in the context of seed production in radish.

Pollination in animal-pollinated plants has remained a well-studied topic since Darwin (1859). Most studies have reported clear relations between variation in floral characters and pollinator behavior or the resultant seed productivity. For example, different types of pollinators suited to species with different floral characters in *Mimulus* and more pollinators visit to wild radish plants with larger flowers (Young & Stanton 1990; Schemske & Bradshaw 1999; Bradshaw & Schemske 2003) or to a particular flower color (Lee & Snow 1998; Johnson & Midgley 2001). In addition, seed sets in pin-styled morph are higher than those in thrum-styled morph through more pollination by legitimate pollen grains in the former than in the latter in *Primula sieboldii* (Nishihiro *et al*. 2000). Thus, variations in floral characters could affect seed productivity by controlling pollen removal, deposition, morph, and so on. Radish has a wide range of variations in floral morphology (Kobayashi *et al.* 2006). These variations might significantly affect seed productivity through their effects on pollination efficiency via the amount and ratio of both self and cross pollen grains on the stigma (Kobayashi *et al.* 2006). That is, a pin-styled flower, in which the stigma is higher than the tips of the anthers on the long stamens, might avoid self-pollination and encourage cross-pollination, and a flower with a larger stigma might receive more pollen grains under open pollination, as reported in other species (Campbell *et al*. 1994; Anderson 1996; Nishihiro *et al*.

2000).

However, because the flower is composed of several organs, it is difficult to determine how much the variation in both stigma position and stigma size change pollination efficiency and seed productivity. In addition, multiple factors in field studies generate uncontrollable variation. To examine the effects of alleles at specific loci, recent studies have used near isogenic lines with different genotypes at a specific locus in the same genetic background (Bradshaw & Schemske 2003). To clearly show the effect of individual flower morphological traits, it is effective to compare the pollination efficiency and seed productivity among lines with different floral morphologies in a specific target trait but similar floral morphologies in other traits. Because the floral morphology in radish was highly heritable, selection by floral morphology based on the genetics behind each trait has been conducted (Kobayashi *et al.* 2007).

Self pollen grains landing on the stigma can lead to seed set, because self-incompatibility in radish is incomplete (Niikura & Matsuura 1999). Therefore, pollination efficiency of both self and cross pollen grains should be assessed. To clarify the mode of cross-pollination in species with hermaphroditic flowers, previous studies have used male-sterile lines as the recipient (Ohsawa & Namai 1988), emasculated recipient flowers (Young & Stanton 1990), used fluorescent powder in place of cross pollen grains (Campbell *et al*. 1994), or taken advantage of the difference in pollen grain sizes between heteromorphs (Nishihiro *et al.* 2000). In this study, to determine the ratio of self and cross pollen grains on stigmas under insect pollination, artificial tetraploid radishes with larger pollen grains than normal radishes were used as the donor to distinguish between self and cross pollen grains.

To clarify the effect of floral morphology on seed productivity through pollination efficiency in radish, the pollen grains on the stigmas and seed productivity were compared among lines with different stigma positions and sizes but similar genetic backgrounds.

Materials and Methods

Plant materials

Four lines with different floral morphologies but similar genetic backgrounds were bred from a hybrid population by crossing two accessions selected from the extremes of variations in each of stigma position and stigma size (Kobayashi *et al*. 2006). Cultivar ‘Manyo’, belonging to the ‘South China’ varietal group, has thrum-styled flowers (style shorter than long stamens) with large stigmas. Inbred line ‘SL19’, belonging to the ‘Risoh’ varietal group, has pin-styled

flowers (style longer than long stamens) with small stigmas (Table 1). Some F_2 plants with desirable stigma position and size were selected in April 2004. They were selfed repeatedly to the F_5 generation to derive the four lines 'Pin-Large', 'Pin-Small', 'Homo-Large', and 'Thrum-Small' (Table 1, Fig. 1). The stigma positions of 'Pin-Large' and 'Pin-Small' were both higher than the anthers, but 'Pin-Large' had a larger stigma. The stigma position of 'Homo-Large' was nearly equal to the anthers on the long stamens; these flowers were homostylous and had large stigmas. The stigma position of 'Thrum-Small' was lower than the anthers, and the stigma was small. Whole flower sizes were slightly different among the four lines, but only the difference between 'Pin-Small' and 'Homo-Large' was significant. Thus, the four lines had similar floral morphologies except in stigma position and size.

As a donor to examine pollination efficiency, an artificial tetraploid line, 'BmR', bred at Utsunomiya University (Tochigi, Japan) was used. Because its pollen size (ø 30–35 μm) was larger than those of the four lines (ø 25 μm) it was possible to distinguish between self and cross pollen grains on the stigmas. As a donor to examine seed productivity, cultivar 'Bei Jing Hong Xin-2' was used; the pubescence of its true leaves was dominant to the glabrousness of the four lines. Therefore, it enabled to distinguish between self seeds and hybrid seeds by pubescence.

Pollination efficiency

All plants used in this examination were grown in a greenhouse at the Agriculture and Forestry Research Center, University of Tsukuba (Ibaraki, Japan). In the middle of December 2005, all lines were sown in 8-cm plastic pots filled with soil (Metro-Mix 350, Scotts-Sierra Horticultural Products Co., Marysville, Ohio). On 7 February 2006, they were transplanted into 24-cm plastic pots and grown until before the beginning of flowering. Four sets of plants, each set was composed of four plants in each recipient and four donor plants, were made. On 1 April 2006, four cages (2-m × 4-m) covered with gauze to exclude pollinating insects were built in the field and the each set was placed in each cage. Then, the plants for recipient and those for donor were transplanted at 50-cm spacing in a row, respectively, with 70-cm spacing between the two rows.

Two pollination treatments were conducted for 3 days to measure pollination efficiency. One was automatic self-pollination, which meant self-pollination without assistance from an insect pollinator, and the other was insect-pollination by bees from a hive (Katakura Industries Co., Ltd., Tokyo) set in the corner in each cage. After each treatment, three branches per plant were randomly selected and their flowers that had bloomed over the 3 days

were sampled. Prepared slides of the stigmas were immediately made to count all pollen grains on them. The automatic self-pollination treatment was performed twice and the insect-pollination treatment six times in all lines. To confirm that the bees visited both recipient and donor flowers, follow-up observation of the bees were conducted. A fling bee in the cage was focused on and its one movement between plants not within a plant was counted as one count. The follow-up observation on a bee was continued for a maximum of ten counts and next, another bee was observed. The observation was conducted for 15 min per trial; 13 or 14 trials were performed in each set throughout the 6 repeats of the insect-pollination treatment. The all counted numbers of movement were classified into three patterns in each set; within recipient, within donor, and between recipient and donor, respectively.

Pollen grains on the prepared slides were stained with acetocarmine and the self grains and the cross grains were counted under an Olympus BH2 fluorescence microscope (Olympus, Tokyo) at a magnification of 100×. Then, the number of self pollen grains under automatic self-pollination, as well as the numbers of self, cross, and total (self + cross) pollen grains and the cross-pollination percentage ([number of cross pollen grains] / [number of total pollen grains] × 100%) under insect-pollination were recorded.

To test whether movements of honey bees differed significantly among sets, chi-square tests with the lines and movement patterns as factors were conducted. Because the frequency distribution of the pollination efficiency was not normal but sloped downward, Kruskal–Wallis test which was one of the nonparametric analyses was used. To examine the effect of stigma size, comparison between ‘Pin-Large’ and ‘Pin-Small’ (pin-styled flowers but different stigma sizes) was conducted while to examine the effect of stigma position, comparisons between ‘Pin-Small’ and ‘Thrum-Small’ and between ‘Pin-Large’ and ‘Homo-Large’ (different stigma positions but similar stigma size) were conducted. All statistical analyses were done with JMP 4.0 software (SAS Institute Inc., Cary, NC, USA).

Seed productivity

As above, plants were arranged into four sets and were transplanted into cages on 4 April 2006. Three cages were randomly assigned as replications in each line. During flowering, a beehive was placed in the corner in each cage. To confirm that the bees visited the flowers of both recipient and donor during flowering, the movements were observed as noted before. Additionally, to determine the average number of ovules per flower in each line, four flowers from each plant were sampled and their ovules were counted. After flowering and seed set, all lines were harvested on 17 June 2006 and dried in a greenhouse. Five branches were

randomly selected from each plant and all seeds were collected from them. The seed set percentage as (total number of seeds) / ([average number of ovules per flower] × [total number of flowers]) × 100% was calculated. About 100 seeds randomly selected from each plant were grown and their genotypes were identified from their leaf pubescence. Then, the outcrossing percentage as (number of hybrid seeds) / (number of seeds grown) × 100% was calculated.

To determine the level of self-incompatibility in the lines, artificial self-pollination tests were conducted by using four plants per line in a greenhouse. About 10 flowers that opened 1 and 2 days before and were still blooming were self-pollinated. Then, pod set percentage, as (number of pods per plant) / (number of flowers pollinated) × 100% was calculated. This percentage was considered as the level of self-incompatibility.

The movements of bees were analyzed as above. The seed set, outcrossing, and pod set percentages were compared among lines by one-way ANOVA and Tukey's multiple-comparison test. The pollination efficiency examined by using the tetraploid radish as donor underestimates the pollination efficiency of normal radish because the pollen of the tetraploid radish is larger. However, it was assumed that the relative effects on pollination efficiency would be the same in the two experiments because the pollinator behaviors were almost the same (see Results). Therefore, the relationship between pollination efficiency and seed productivity was investigated by combining both data sets.

Results

Pollination efficiency in the four lines

Because the visitation behavior by honey bees differed among the four sets even on the same day, all data was shown together in Figure 2. The numbers of movements within a line (recipient or donor) varied from 0 to 26, whereas those between recipient and donor varied from 0 to only 8, resulting in low averages of movements in each set. Chi-square analysis showed that means did not differ significantly among the four sets, suggesting that the visitation behavior by honey bees was almost the same for the whole of flowering periods in the four sets.

Pollinated stigmas were obtained from all lines under both treatments. Figure 3 presents frequency distributions of the numbers of pollen grains on the stigmas sampled at all sampling times. Under automatic self-pollination, the distributions decayed and showed a mode of 0–50 pollen grains in all lines (Fig. 3a). 'Homo-Large' had more stigmas with >100

grains (up to 550 grains) than the others. Few stigmas had >100 grains in both 'Pin-Large' and 'Thrum-Small', and 'Pin-Small' received a maximum of about 100 grains. Under insect-pollination, average numbers of total pollen grains were 2–10 times as many as under automatic self-pollination in each line (Fig. 3d). 'Thrum-Small' (small stigmas) and both 'Pin-Large' and 'Homo-Large' (large stigmas) received >800 grains at a maximum, but 'Pin-Small' received 500 grains at maximum. In all lines, most stigmas received 0–2 cross pollen grains; stigmas with >10 cross pollen grains were rare (Fig. 3c). Therefore most of the grains on the stigmas were derived from the same or other plants in the same line (Fig. 3b). Cross-pollination percentages were also notably low (Fig. 3e); the maximum value was 70% in 'Pin-Small'.

Because wind speed and direction differed between sampling times under automatic self-pollination (data not shown), comparisons between lines were conducted at each sampling time (Fig. 4). 'Pin-Large' had significantly more pollen grains than 'Pin-Small' only on 15 April ($\chi^2 = 4.08$, $P < 0.05$). 'Pin-Small' had significantly fewer grains than 'Thrum-Small' only on 6 May ($\chi^2 = 4.50$, $P < 0.05$). 'Pin-Large' had significantly fewer grains than 'Homo-Large' at both sampling times ($\chi^2 = 4.08$, $P < 0.05$ on 15 April, $\chi^2 = 5.33$, $P < 0.05$ on 6 May). Under insect-pollination, not only did the wind behavior differ among the six sampling times, but also the pollinator's behavior differed among sets at each sampling time. Therefore, the values were compared by using sampling times as replications (Fig. 5). 'Pin-Small' had significantly fewer self and total pollen grains but a higher cross-pollination percentage than both 'Pin-Large' and 'Thrum-Small' ($\chi^2 = 5.77–8.31$, $P < 0.05–0.01$). 'Pin-Large' and 'Homo-Large' did not differ significantly in any parameter. Any effect on the numbers of cross pollen grains on the stigmas was not shown because all lines received almost the same numbers of cross pollen grains.

Seed productivity in the four lines

The patterns and numbers of movements by honey bees were similar to those shown in Figure 2. Bees moved much less between recipient and donor (0–0.5 per 15 min) than within recipient or donor (2.6–4.8 per 15 min).

Table 2 shows the rate of pod set under artificial self-pollination and the rates of seed set and outcrossing under insect-pollination. The high average pod set percentage of 'Thrum-Small' (72.3%) indicates that 'Thrum-Small' had a very low level of self-incompatibility. On the other hand, those of 'Pin-Large', 'Pin-Small', and 'Homo-Large' were 6.4%, 28.2%, and 19.8%, respectively. Although the differences among these three lines were significant, they

were relatively small, implying a high level of self-incompatibility. The three lines averaged 5–7 ovules per flower (data not shown), and the proportions of stigmas on which more pollen grains landed than the number of ovules pollinated were 97% in 'Pin-Large', 90% in 'Pin-Small', and 100% in 'Homo-Large' (gray bars in Fig. 3d). Owing to the low level of self-incompatibility, 'Thrum-Small' showed a significantly higher seed set percentage than the others (13.1%) and a low outcrossing percentage (9.2%). The seed set percentages in the other three lines ranged from 3.1% to 5.3% (not significantly different), and the outcrossing percentages varied from 66.0% ('Homo-Large') to 93.9% ('Pin-Small'). A higher average cross-pollination percentage tended to increase the outcrossing percentage in the three lines (Fig. 6).

Discussion

The level of self-incompatibility in radish varies among lines and with the environment (Table 2; Niikura & Matsuura 1999; Horisaki & Niikura 2008). For this reason, self pollen grain can set seed, as observed in F_1 seed production fields (Horisaki *et al.* 2003; Niikura 2007). Therefore, it is essential to assess pollination efficiency by distinguishing between self and cross pollen grains on stigmas to understand the effects of floral morphology on seed productivity through pollination efficiency, and the reproductive process in radish. Use of the tetraploid radish made it possible to clearly determine pollination efficiency. In automatic self-pollination, the stigma receives pollen grains from anthers within the same flower by wind and gravity (Namai *et al*. 1992). The accidental effects by wind and gravity explain the downward-sloping distribution with a mode of 0–50 pollen grains, like a Poisson distribution, in all lines (Fig. 3a). Under insect pollination, the total number of pollen grains increased several fold and the distribution peaks shifted to the right (Fig. 3d). This indicates that pollinators increase the total number of pollen grains deposited on the stigmas under field conditions. But both the number and percentage of cross pollen grains on the stigmas were less than expected, and the majority of the grains on the stigmas were self pollen grains. The few number of cross pollen grains would be due to the limited movement of honey bees between recipient and donor lines (Fig. 2). Increased pollinator movement between recipient and donor would have facilitated cross-pollination. In addition, numbers of both self and cross pollen grains differed between sampling times under both pollination treatments (Figs. 4, 5) because of changes in environment and pollinator behavior during flowering (Fig. 2). Past studies of cross-pollination used only male-sterile recipients (Ohsawa & Namai 1988) or emasculated recipients (Young & Stanton 1990). However, these methods cannot reveal the

rates of self- and cross-pollination in a hermaphrodite flower. To our knowledge, this is the first study to show the numbers of both self and cross pollen grains on the stigmas in a species with homomorphic hermaphrodite flowers. The results show that events in the pollination process can affect seed productivity through pollination efficiency. Therefore, information on the conditions affecting pollination efficiency will improve understanding of the reproduction.

There are many reports of the effects of stigma position and size in other plants (Campbell *et al*. 1994; Anderson 1996; Nishihiro *et al*. 2000; Syafaruddin *et al*. 2006). This study also examined these effects by comparing lines with different stigma positions and sizes. Differences in the distribution patterns of the numbers of pollen grains among the four lines suggest an effect of floral morphology on pollination efficiency (Fig. 3). A larger stigma tended to increase the amount of self pollen grains received on the stigma and to decrease the cross-pollination percentage. A higher stigma position than the anthers on long stamens tended to reduce self-pollination and to increase the cross-pollination percentage. The effect of stigma position was not shown in the comparison between 'Pin-Large' and 'Homo-Large' under insect pollination. However, the distribution patterns of both self and total pollen grains in 'Pin-Large' were downward-sloping, whereas those in 'Homo-Large' were unimodal-like. This difference indicates that lower stigma position clearly increases the proportion of pollinated stigmas by the dropping and deposition of self pollen grains by the impact of pollinator visitation. Additionally, although it was expected that higher stigma position and larger stigma size would increase cross-pollination, the four recipient lines had almost the same number of cross pollen grains, probably because of the limited movement of bees between recipient and donor lines (Fig. 2). If pollinators moved more between recipient and donor lines, cross-pollination would be facilitated, and the effect of floral morphology on the number and percentage of cross pollen grains on stigmas would be clearer.

To examine the effects of floral morphology on seed productivity, the levels of self-incompatibility should be identical in all the lines to remove its effect on seed productivity. This study compared the seed productivity among 'Pin-Large', 'Pin-Small', and 'Homo-Large', which had high levels of self-incompatibility (Table 2). There were no significant differences in the seed set percentage among them (Table 2). The proportions of stigmas that received more pollen grains than the number of ovules pollinated were over 90% in all three lines (gray parts in Fig. 3d). Even in 'Pin-Small', which had the fewest total pollen grains among the four lines on account of its small stigma size and high stigma position, most stigmas received more pollen grains than the number of ovules under insect-pollination. For this reason or the small difference in cross-pollination percentages among the three lines, the

three lines had almost the same seed set percentages. Frequent pollinator movements will increase the variation in cross-pollination percentages among lines, thus highlighting the effect of floral morphology on seed set. There were significant differences in outcrossing percentages within the three lines, and 'Pin-Small' showed the highest percentage (Table 2). The outcrossing percentage tended to rise with average cross-pollination percentage in the same three lines (Fig. 6). Although the difference in either stigma position or stigma size did not change the outcrossing percentage significantly, differences in both might significantly change it by having more effect on cross-pollination percentage. This result suggests that outcrossing percentage is determined not only by self-incompatibility but also by pollination efficiency in radish, and that it increases with cross-pollination percentage. Why the higher cross-pollination percentage enhanced the outcrossing percentage remains unclear: further studies considering both pollination efficiency and self-incompatibility at the same time will be needed.

Flower constancy—repeated visitation of the same species by insect pollinators—has been observed in both natural populations and F_1 seed production fields (Yoshioka *et al*. 2005; Ishii 2006). Flower constancy may explain the limited movement between recipient and donor lines (Fig. 2) by bees using cues that we did not consider, such as floral morphology, nectar guides, and floral scent. Because this phenomenon might cause low F_1 purity in F_1 seed production fields, clarification of the causes is vital for the improvement of F_1 seed production and for clarification of the effect of floral morphology on seed productivity (Yoshioka *et al*. 2005). Because 'Pin-Small' showed the highest outcrossing percentage in this study (Table 2), genetic improvement for its floral morphology might raise F_1 purity and yields through the improvement of pollination efficiency and thus F_1 seed productivity.

In conclusion, floral morphology affected seed productivity through the pollination efficiency. Variations in floral morphology can change both pollination efficiency and seed productivity. But the effect on seed productivity through cross-pollination percentage was not as clear on account of rare visitations between recipient and donor lines by bees. Because flowers with a higher stigma position than anthers on the long stamens and a small stigma showed the highest cross-pollination percentage, genetic improvement for such floral morphology could improve F_1 seed productivity.

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Titles and legends for figures and tables

Figure 1. Floral morphology in (a) 'Pin-Large', (b) 'Pin-Small', (c) 'Homo-Large', and (d) 'Thrum-Small'. The stigma images are enlarged in the upper right boxes.

Figure 2. Numbers of movements of honey bees (mean ± SD) in the four sets of plants.

Figure 3. Frequency distributions of (a, b) self, (c) cross, and (d) total pollen grains on the stigmas, and (e) cross-pollination percentage under automatic self- and insect-pollination in the four lines. *n* indicates the total number of stigmas sampled at two or six sampling times. Mean ± SD was calculated in each line. In (d), shading indicates the proportion of stigmas on which fewer (black) and more (gray) pollen grains landed than the average number of ovules in each flower.

Figure 4. Number of pollen grains on stigmas under natural self-pollination in the four lines. Black, individual means sampled on 15 April; white, on 6 May.

Figure 5. Numbers of self, cross, and total pollen grains and cross-pollination percentages under insect pollination in the four lines. Each point is the average of four individuals sampled at each sampling time.

Figure 6. Relationship between cross-pollination percentage and outcrossing percentage in the four lines. Each point is the average of four individuals in each line.

Table 1. Stigma position, stigma size, and whole flower size in the four lines and their parents.

Table 2. Percentages of pod set, seed set, and outcrossing in the four lines.

Table 1. .

Line	Stigma position	Stigma size (mm^3)	Whole flower size (cm^3)
Pin-Large	1.07 ± 0.01 a	0.21 ± 0.04 b	5.15 ± 0.49 ab
Pin-Small	1.08 ± 0.02 a	0.08 ± 0.02 d	6.38 ± 0.13 a
Homo-Large	1.02 ± 0.04 a	0.29 ± 0.02 a	4.43 ± 0.82 b
Thrum-Small	0.80 ± 0.04 b	0.15 ± 0.03 c	5.28 ± 0.78 ab
Manyo[1)]	0.85 ± 0.02	0.23 ± 0.06	4.48 ± 0.44
SL19[1)]	1.02 ± 0.02	0.12 ± 0.01	6.56 ± 0.18

At spring 2006, sixteen flowers in each recipient (four flowers × four plants) were photographed by a digital microscope. Then, from these images the floral traits were measured (Kobayashi *et*

The means ± SD was calculated among four plants in each recipient.

Stigma position: pistil height / height of long stamen

Stigma size: stigma length × stigma width × stigma width

Whole flower size: tube length × corolla length × corolla length /1000

Means followed by different letters differ significantly ($P < 0.05$, Tukey's multiple-comparison

1) The floral traits of parents were measured at spring 2003.

Table 2.

Recipients	Pod set under artificial self-pollination (%)	Seed set under insect-pollination (%)	Outcrossing under insect-pollination (%)
Pin-Large	6.4 ± 6.1 c	5.0 ± 1.8 b	78.8 ± 9.8 ab
Pin-Small	28.2 ± 8.1 b	3.1 ± 0.8 b	93.9 ± 4.2 a
Middle-Large	19.8 ± 7.8 bc	5.3 ± 1.7 b	66.0 ± 6.7 b
Thrum-Small	72.3 ± 6.5 a	13.1 ± 2.7 a	9.2 ± 1.3 c

Means ± SD among four individuals in the pod set percentage and among three replications in the seed set and outcrossing percentages.

Means followed by different letters differ significantly ($P < 0.05$, Tukey's multiple-comparisc

Figure 1.

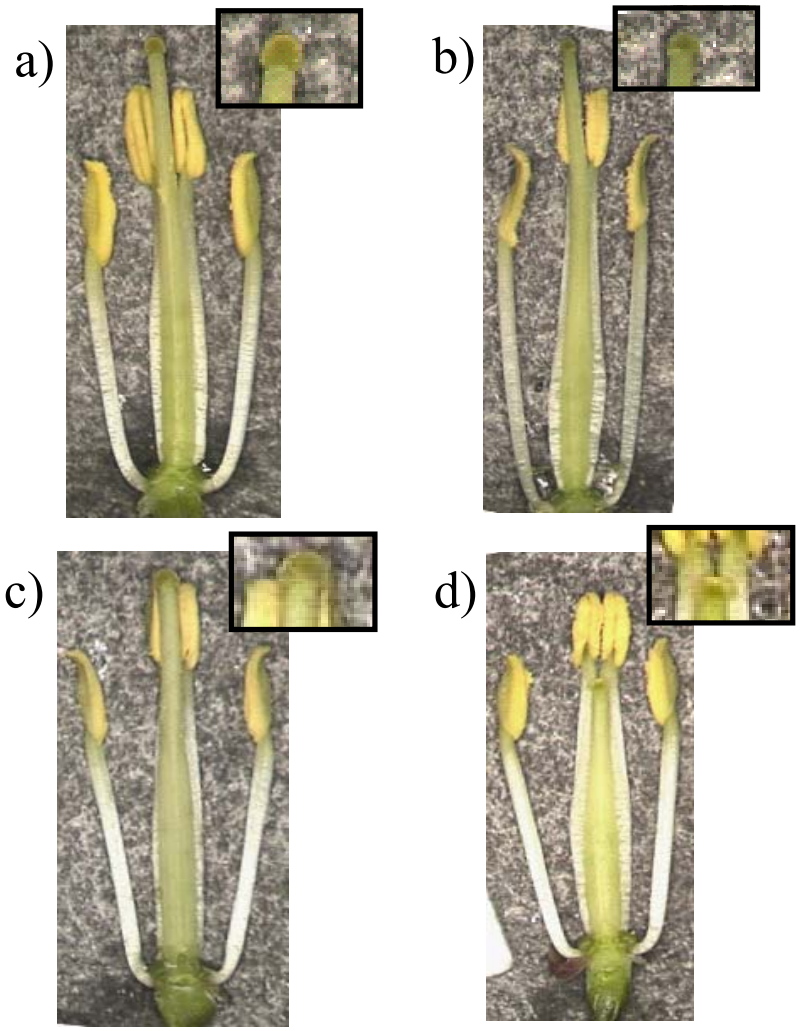


Figure 2.

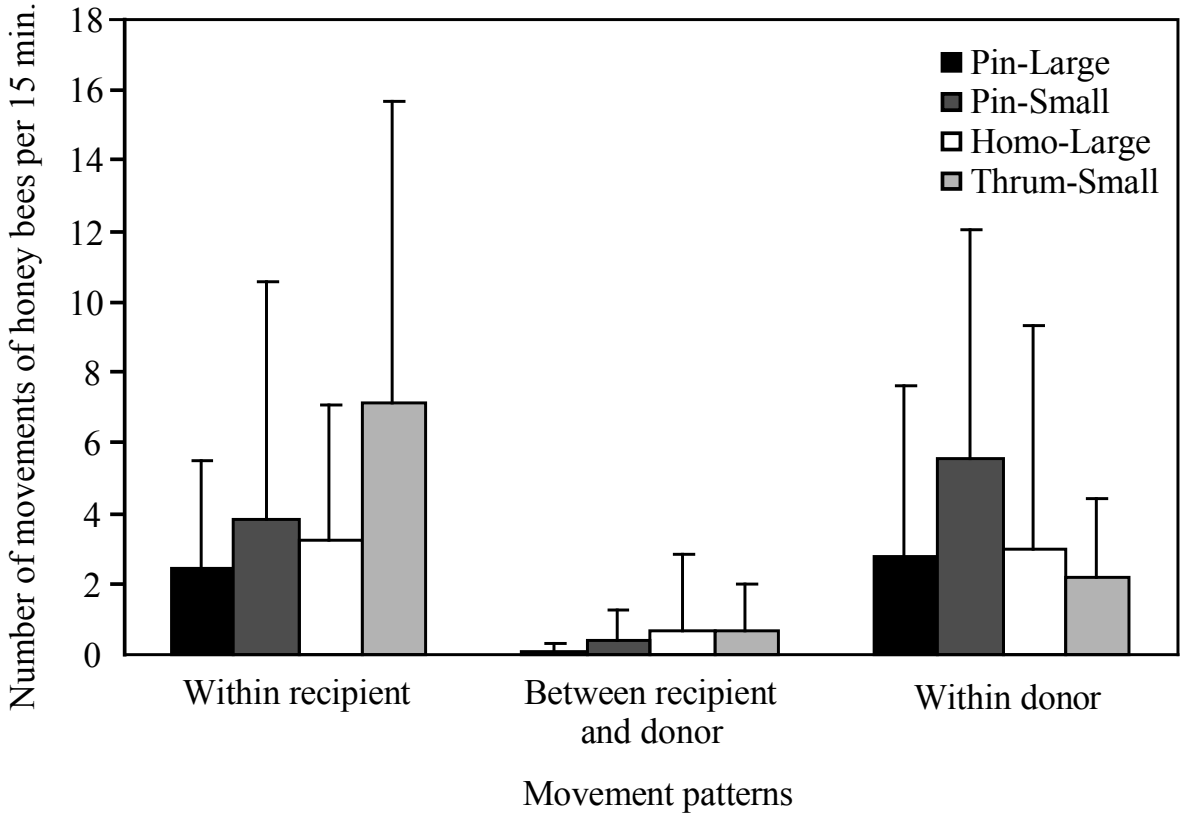
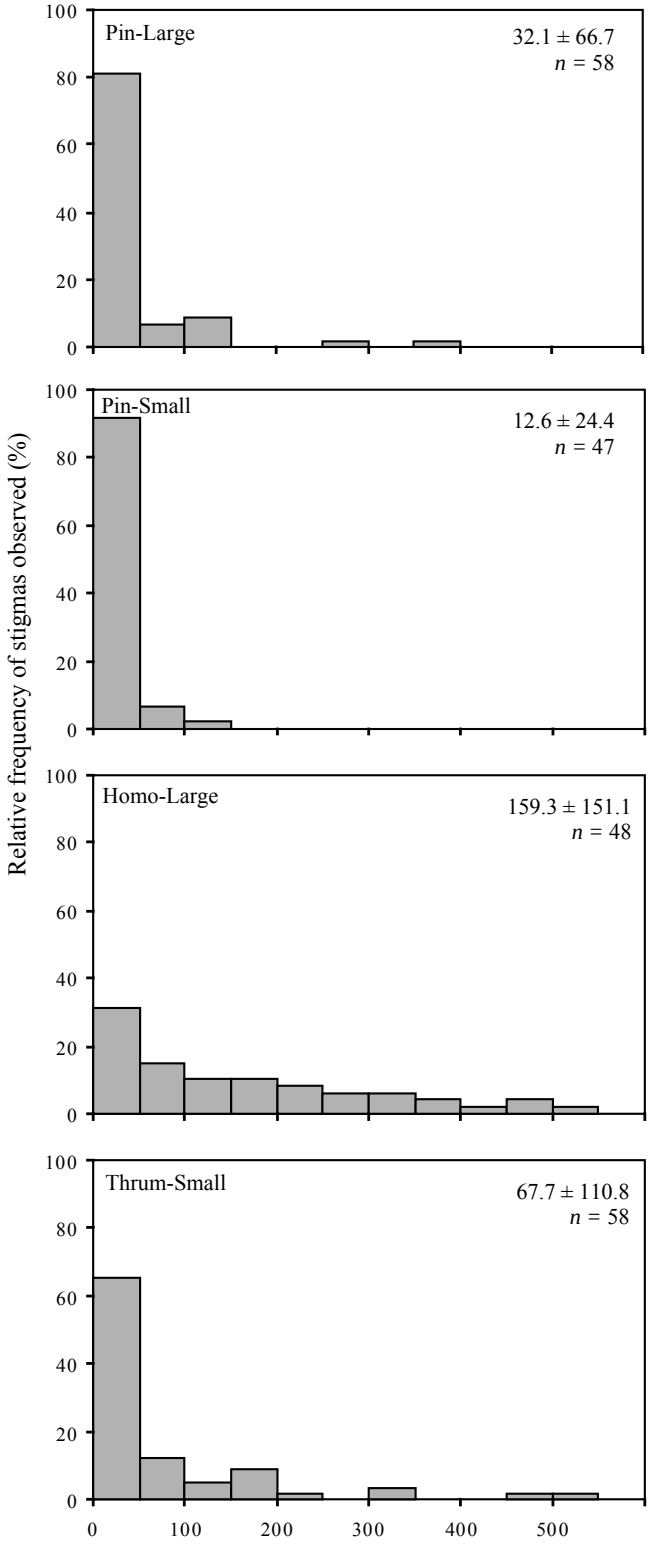
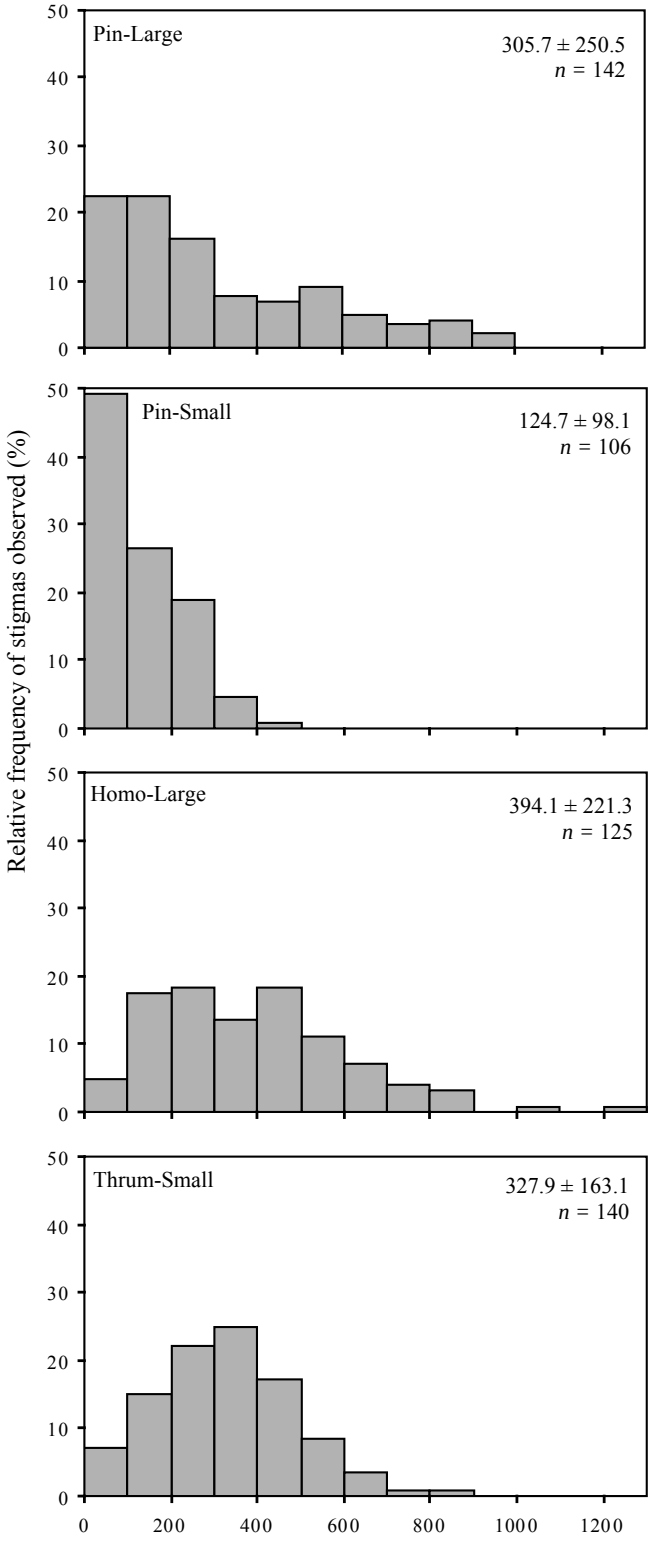

Number of movements of honey bees per 15 min.
18
16
14
12
10
8
6
4
2
0
Pin-Large
Pin-Small
Homo-Large
Thrum-Small
Within recipient
Between recipient and donor
Within donor
Movement patterns

Figure 3.



(a) Number of self pollen grains under automatic self-pollination

Figure 3. continued



(b) Number of self pollen grains under insect-pollination

Figure 3. continued

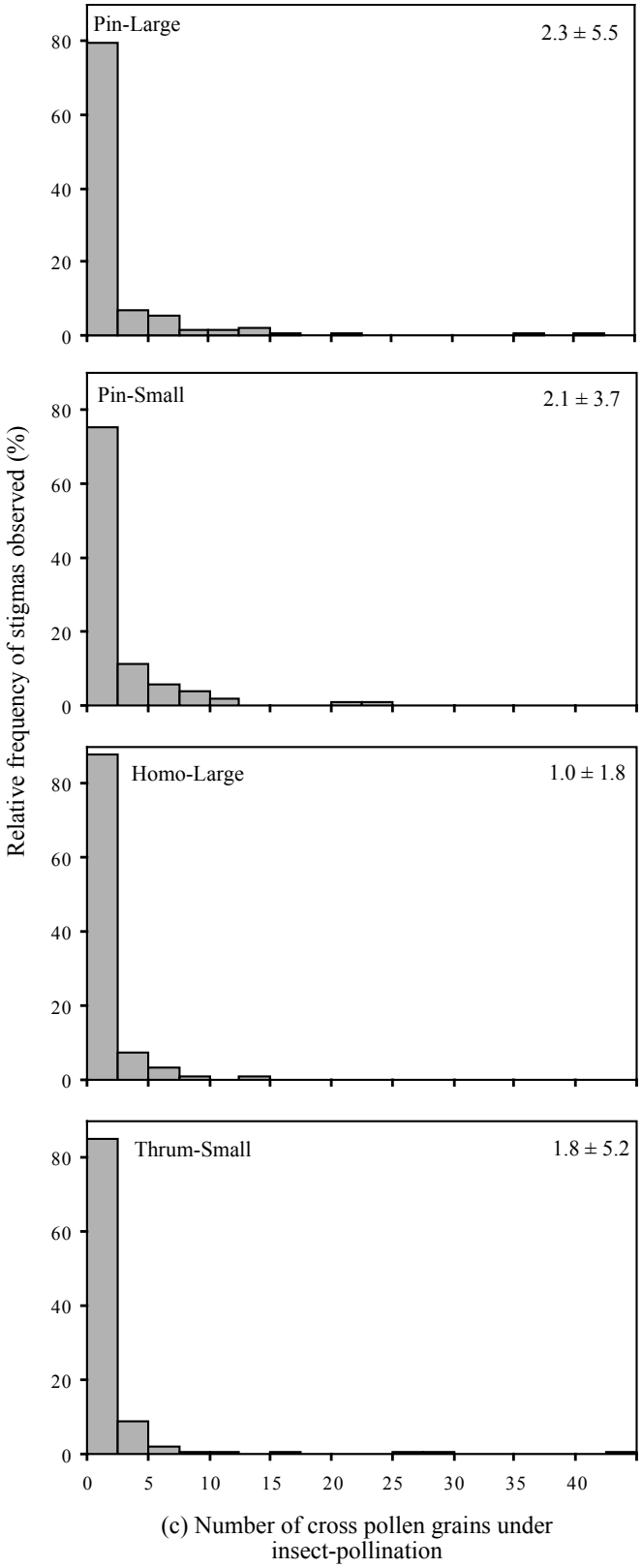
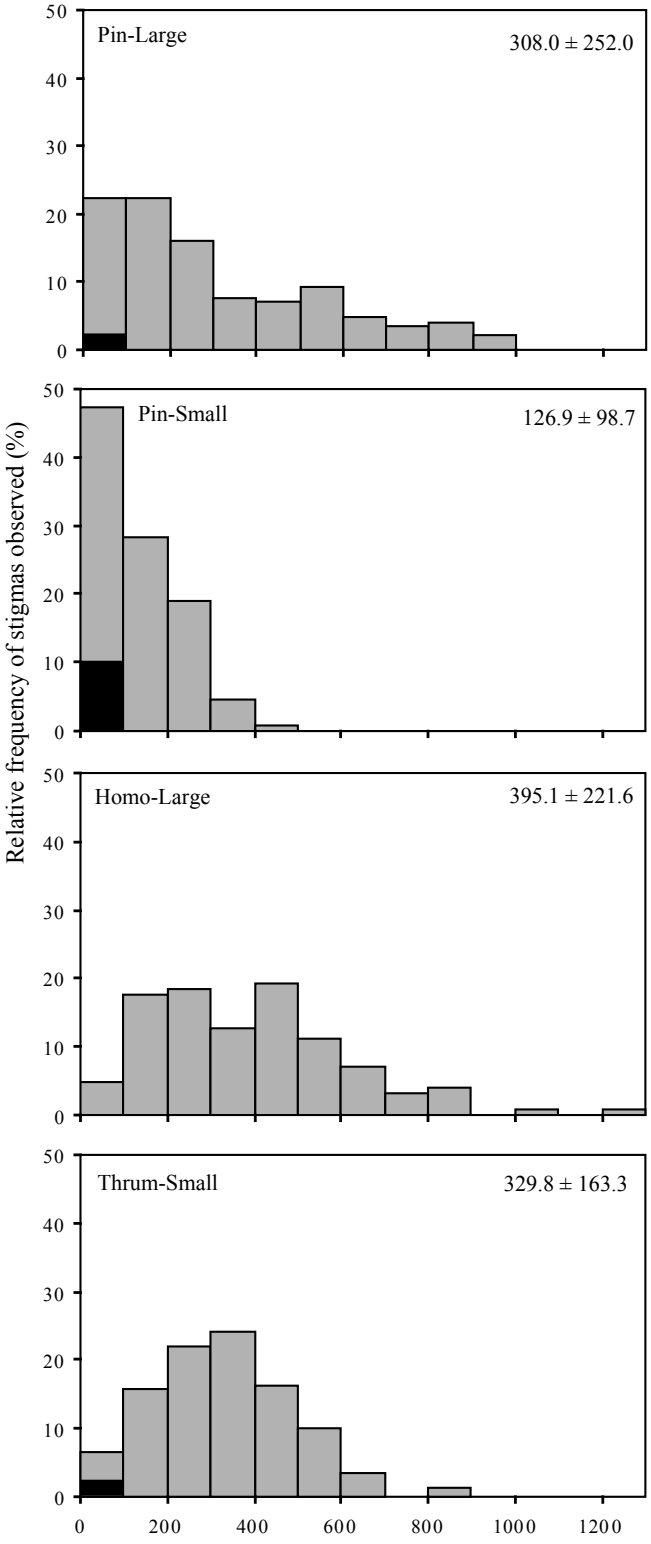


Figure3. continued



(d) Number of total pollen grains under insect-pollination

Figure 3. continued

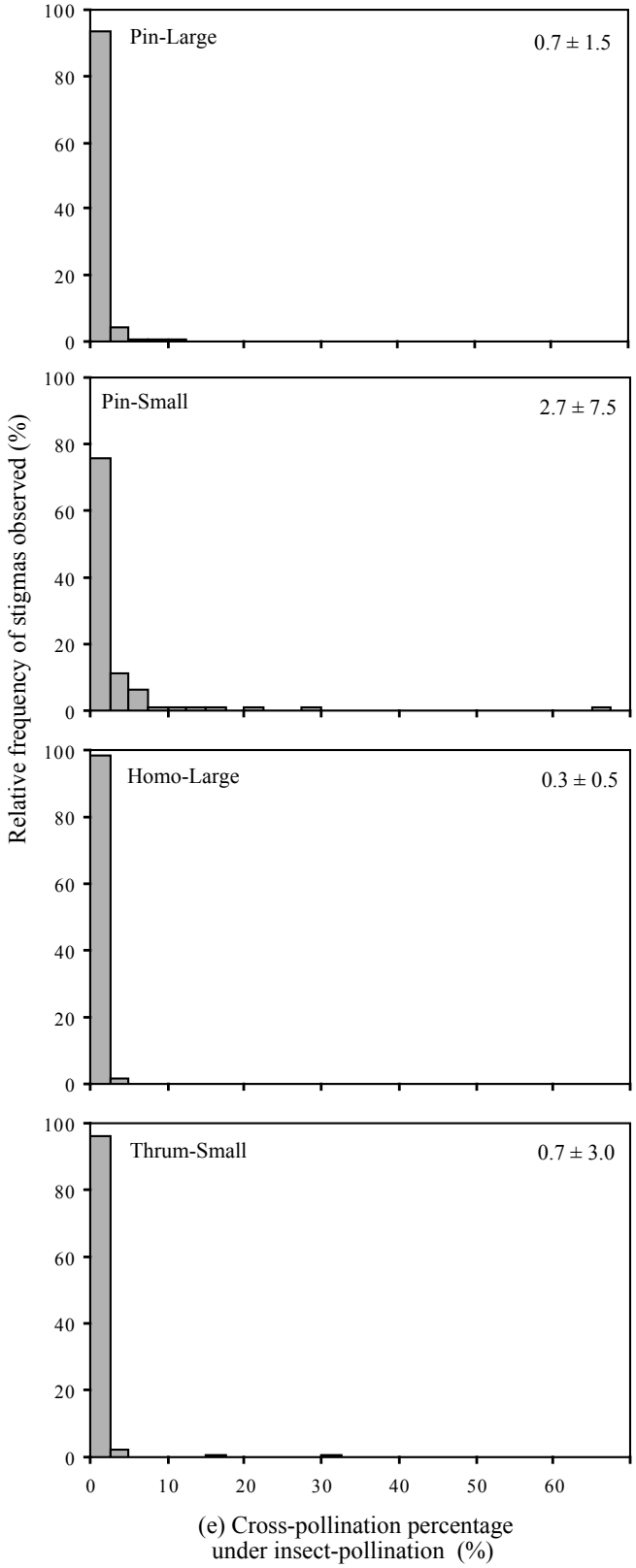


Figure 4.

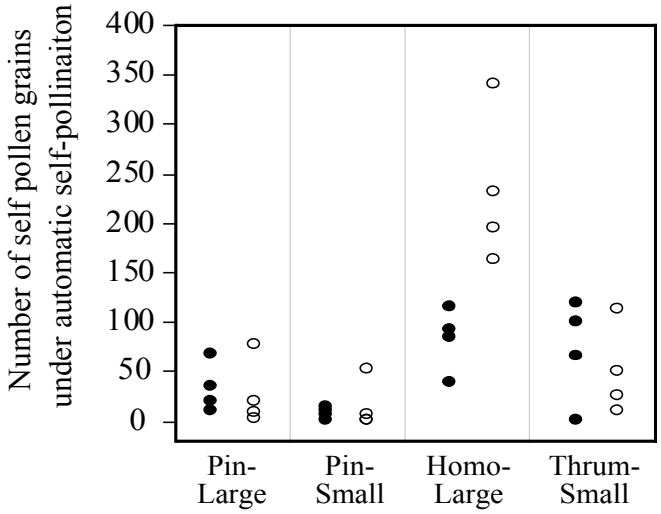

Number of self pollen grains under automatic self-pollinaiton
400
350
300
250
200
150
100
50
0
Pin-Large
Pin-Small
Homo-Large
Thrum-Small

Figure 5.

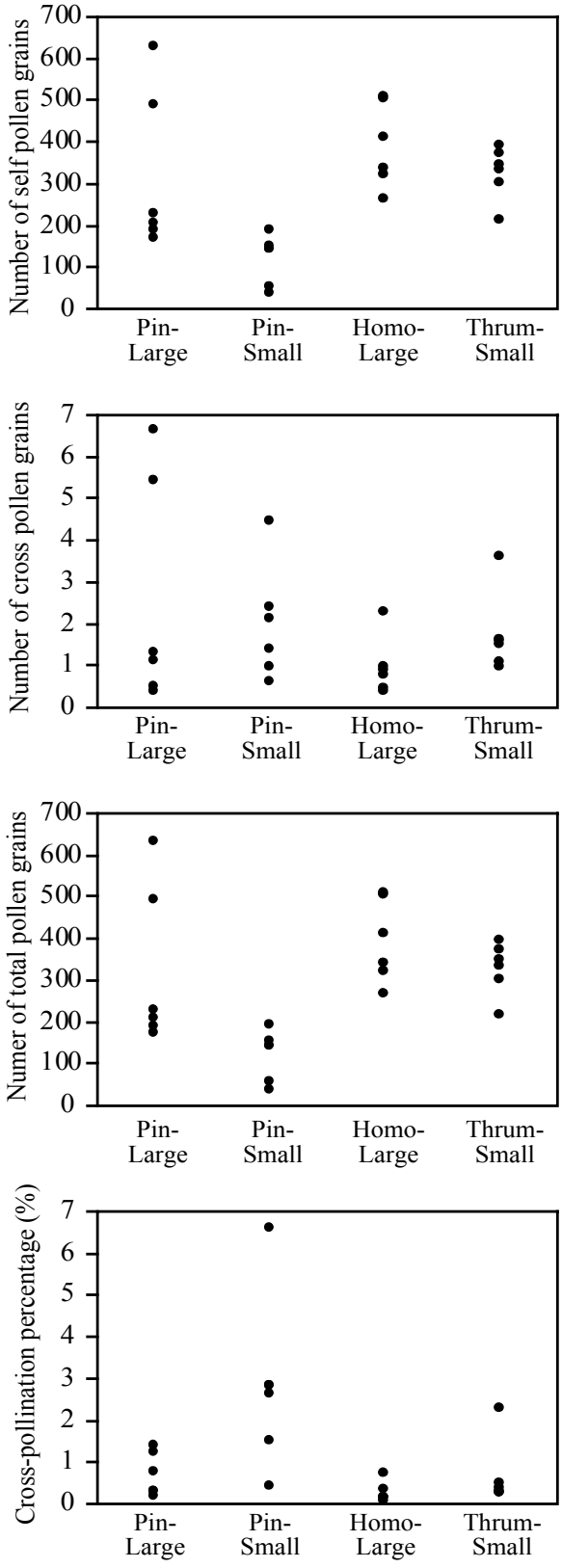
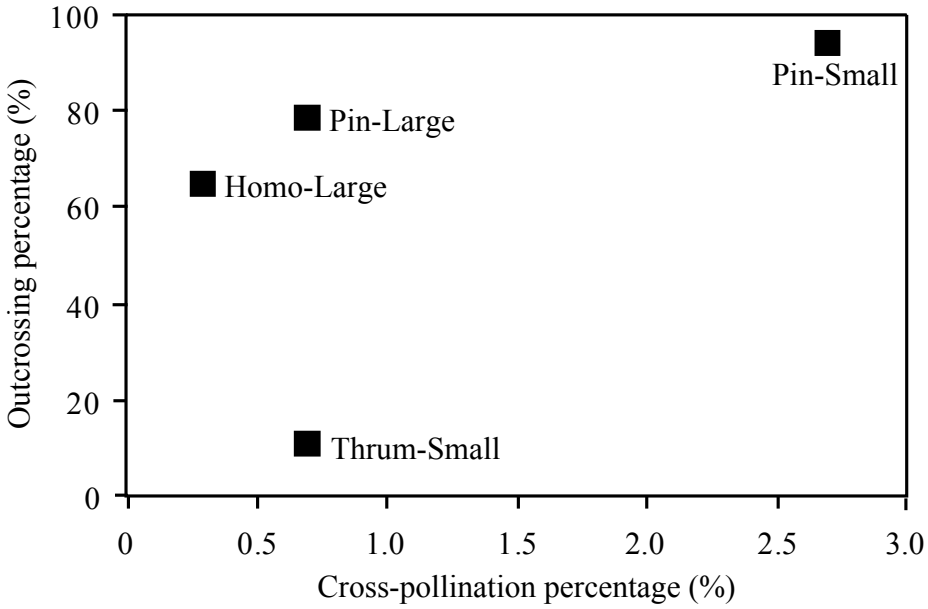

Number of self pollen grains
700
600
500
400
300
200
100
0
Pin-Large
Pin-Small
Homo-Large
Thrum-Small
Number of cross pollen grains
7
6
5
4
3
2
1
0
Pin-Large
Pin-Small
Homo-Large
Thrum-Small
Numer of total pollen grains
700
600
500
400
300
200
100
0
Pin-Large
Pin-Small
Homo-Large
Thrum-Small
Cross-pollination percentage (%)
7
6
5
4
3
2
1
0
Pin-Large
Pin-Small
Homo-Large
Thrum-Small

Figure 6.


100
80
60
40
20
0
Outcrossing percentage (%)
Pin-Small
Pin-Large
Homo-Large
Thrum-Small
0
0.5
1.0
1.5
2.0
2.5
3.0
Cross-pollination percentage (%)