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The effect of petal size manipulation on pollinator/seed-predator mediated female reproductive success of *Hibiscus moscheutos*

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Abstract The effects of petal-size manipulations on the behavior of pollinators and pollen/seed predators, and on pollen removal and deposition, were studied in *Hibiscus moscheutos* (Malvaceae) populations. The ultimate effects on the female reproductive success of flowers, such as fruit set, seed predation rate, and final seed set were also measured. We applied three levels of petal removal (100%, 50%, and 0% size reduction in radius) to flowers in natural populations. Two pollinators (*Bombus pennsylvanicus* and *Ptilothrix bombiformis*) ignored flowers without petals, suggesting that pollinators use petals as a visual cue to locate flowers. Consequently, 100% petal removal reduced female reproductive success considerably, mainly through a higher rate of fruit abortion due to failure of pollen deposition on stigmas. No significant differences between the 50% petal removal treatment and uncut control were detected in any components of female success examined. The results, therefore, suggest that differences in petal size have little influence on female reproductive success of *Hibiscus* flowers at our study site. Final seed set varied considerably depending on the larval densities of two coleopteran seed predators (*Althaeus hibisci* and *Conotrachelus fissunguis*). *A. hibisci* responded to petal size, and a higher density of adults was found in flowers in which petal size had not been reduced. Because *Althaeus* feed on pollen as adults and no effect of petal size on seed predation was detected, the preference of *Althaeus* for larger flowers may represent a foraging strategy for adult beetles and may exert counteracting selection pressure on petal size through male reproductive success of flowers.

Key words Corolla size · Fruit abortion · Floral display · Pollen predator · Pre-dispersal seed predator

Introduction

The role of floral display size has been addressed primarily in relation to pollinator attraction (e.g., Willson et al. 1979; Schemske 1980; Zimmerman 1980a; Bell 1985; Thomson 1988; Ohara and Higashi 1994). Natural selection should favor increased allocation to attractive floral features as long as the benefit of receiving more pollinator visits is greater than the cost of maintaining attractive organs and all the other costs associated with successful fruit/seed set. Substantial biomass may be invested in corolla tissue (Lovett Doust and Cavers 1982; Cruden and Lyon 1985), and larger flowers often attract more pollinators (Bell 1985; Galen and Newport 1987; Stanton and Preston 1988; Galen 1989; Galen and Stanton 1989; Young and Stanton 1990; Campbell et al. 1991; Eckhart 1991; Conner and Rush 1996).

Pre-dispersal seed predators can also affect the number of seeds produced (Augspurger 1981; Heithaus et al. 1982; Schemske and Horvitz 1988), and pollinator-mediated selection can be modified by seed predation (Campbell 1991; Ehrlén 1996). It is likely that the plant characters used as cues by pollinators to locate plants also attract seed predators, especially if seed predators, by using pollinator cues (Zimmerman 1980b), are able to oviposit selectively on flowers that set higher numbers of seeds. If seed predators use floral displays as cues to locate host plants, floral display size may be subject to opposing selective pressures from those due to pollinator activities (Brody 1992a; Ehrlén 1996). Thus, pre-dispersal seed predators may also play a role in shaping floral evolution. The interaction between seed predators and pollinators in affecting floral display evolution, however, has received relatively little attention (Beattie et al. 1973; Zimmerman 1980b; Augspurger 1981; Hainsworth et al. 1984; Campbell 1991) and only a few studies have addressed how floral displays that attract pollina-

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tors subsequently influence seed predator behavior (Hainsworth et al. 1984; Molau et al. 1989; Brody 1992a; Ehrlén 1996). Most studies have demonstrated that larger inflorescence suffer from higher seed predation (Hainsworth et al. 1984; Molau et al. 1989; Ehrlén 1996; Brody and Mitchell 1997). In most of these studies, the seed predators feed on seeds as larvae. If adult seed predators use flowers as mating or feeding sites, then, preference for larger floral displays by the seed predators may not necessarily result in higher seed predation.

Furthermore, in the case where adult seed predators are also pollen predators, the preference for larger flowers with more pollen could negatively affect reproductive success through its impacts on both male and female functions.

Two approaches have been applied to the question of how petal size variation affects pollinator behavior. One approach has been to examine correlations between flower size and pollinator visitation using phenotypic variation of flower size in natural and experimental populations (Bell 1985; Galen and Newport 1987; Stanton and Preston 1988; Galen 1989; Galen and Stanton 1989; Young and Stanton 1990; Cresswell and Galen 1991; Eckhart 1991; Stanton et al. 1991; Inoue et al. 1995). The second approach has been to investigate insect visitor responses by manipulating flower size artificially (Bell 1985; Stanton et al. 1992; Andersson 1994; Johnson et al. 1995; Conner and Rush 1996). The former approach allows one to assess the effects of variation in flower size on insect visitation. Artificial manipulations offer opportunities to expand the range of phenotypes and to test whether or not insect visitors directly use flower size as a cue by randomizing variation in other unmeasured correlated characters that may have large impacts on insect-visitor behaviors (Brody and Mitchell 1997).

In this study, we investigated the effect of petal-size manipulations on insect-visitor (pollinators and pollen/seed predators) behavior using the perennial wetland macrophyte, *Hibiscus moscheutos* L. (Malvaceae), referred to as *Hibiscus* hereafter. We also measured pollen deposition and removal, fruit set, seed predation, and seed set in response to petal-size manipulation. *Hibiscus* has large flowers (10–15 cm diameter) with showy petals, that open for a single day and are pollinated by a bumblebee, *Bombus pennsylvanicus*, and a specialist anthophorid bee, *Ptilothrix bombiformis* (Rust 1980; Spira 1989; Spira et al. 1992). Heavy pre-dispersal seed predation has been reported (Cahoon and Stevenson 1986; Spira 1989) and two coleopteran seed predators that feed on pollen grains as adults and on seeds as larvae are often found in open flowers (Weiss and Dickerson 1919). The majority of flowering shoots produce one flower per shoot per day (Spira 1989; Spira et al. 1992), suggesting that the size of individual flowers may be a major component in attracting insect visitors to plants. We addressed the following questions:

1. Do flowers with larger petals attract more pollinators?
2. Do flowers with larger petals attract more pollen/seed predators?
3. Does petal size affect pollen removal and deposition?
4. Do flowers with larger petals have higher fruit and/or seed set?
5. Do flowers with larger petals suffer from higher seed predation?

Materials and methods

Study system

H. moscheutos is an insect-pollinated herbaceous perennial native to wetlands of eastern North America (Brown and Brown 1984; Spira 1989). Plants have few to many upright stems, 1.5–2.5 m tall, which emerge from a root stock. The flowering season extends from late July to early September. *Hibiscus* is self-compatible, but spatial separation of anthers and stigmas prevents autopollination within flowers (Spira 1989). The main pollinators of *Hibiscus* at the study site are a bumblebee, *Bombus pennsylvanicus* Deeger (Hymenoptera: Apidae), referred to as *Bombus* hereafter, and a specialist anthophorid bee, *Ptilothrix bombiformis* Cresson (Hymenoptera: Anthophoridae), referred to as *Ptilothrix* hereafter (Rust 1980; Spira 1989; Spira et al. 1992). Pollinator activity is very high at the study site (individual flowers are visited two to four times per 15 min) (Spira et al. 1992). Most flowers receive more pollen (>360 grains) than is required for full seed set of fruits (139 ovules per fruit on average, and 2.6 grains required per seed) within 3 h exposure to pollinators (Spira et al. 1992). Fruit maturation takes 3–4 weeks.

Two insects are responsible for most seed predation at our study site (Spira 1989); a curculionid weevil, *Conotrachelus fuscunguis* Lec., referred to as *Conotrachelus* hereafter, and a bruchid seed beetle, *Althaeus hibisci* Olivier, referred to as *Althaeus* hereafter. Adults of both beetles are present throughout the flowering season at the study site. Larvae of both species feed on *Hibiscus* seeds in developing fruits. *Conotrachelus* larvae feed on seeds, leaving only the outer shell, which soon decays and turns black (Weiss and Dickerson 1919). Fully grown weevil larvae exit from fruits by late September through a circular hole that they cut in the capsule (Weiss and Dickerson 1919). *Althaeus* larvae enter ovules in developing fruits (Weiss and Dickerson 1919; Cahoon and Stevenson 1986). Infested ovules develop into normal sized seeds with no visible external evidence of infestation. *Althaeus* larvae mature and pupate within infested seeds (Weiss and Dickerson 1919; Cahoon and Stevenson 1986). Adults begin to emerge in the first week of October by cutting a hole in the seed coat, and emergence continues throughout October.

Study site

The research site is a freshwater wetland (locally known as Mill Swamp) at the Smithsonian Environmental Research Center, Edgewater, Anne Arundel County, Maryland, United States (38°53'N, 76°33'W). Average *Hibiscus* density at the site is 1.9 m⁻² with a mean of 13.6 shoots per plant, resulting in an average shoot density of 22.6 m⁻² (Kudoh and Whigham 1997). Random mating of *Hibiscus* in Mill Swamp has been suggested based on isozyme genotype frequencies of mature plants (Kudoh and Whigham 1997), and a 36% selfing rate has been estimated based on genotype frequencies of seed populations (Snow et al. 1996). The field experiments reported in this paper were conducted in August 1995.

Petal size manipulation experiments

We conducted three separate experiments using the same petal-size manipulation. The general pattern for conducting each petal manipulation experiment was as follows. Plants to be used in an experiment were selected in the afternoon of the day before the experiments. A single flower that would open the next morning was chosen for each plant and covered with a paper bag. The next morning, the paper bags were removed before pollinators became active. Three levels of petal removal (100%, 50%, and 0% size reduction in radius) were then applied randomly to the flowers. The 50% treatment reduced the petal area of a circular *Hibiscus* flower to one-quarter of the original. Natural variation of petal area per flower ranged from 62 to 315 cm² with an average \pm SD of 194 ± 48 cm² ($n = 239$) at our study site (R. Shimamura, H. Kudoh, N. Kachi and D.F. Whigham, unpublished work). The 50% petal removal treatment, therefore, produced smaller or similar-sized flowers to the smallest flowers found at the study site. Cutting did not cause pollen to fall off, and no pollen grains were observed on stigmas when the petals were cut.

Experiment 1: pollinator visitation

Pollinator visitation was observed for 18 flowers for each level of petal-size manipulation. For each level of petal-size manipulation, observations were made for six flowers on 13 August and for 12 flowers on 20 August. Four 10-min observations, each within one of four different time periods (0850–1020, 1030–1200, 12:10–1440, and 1440–1610 hours), were conducted for each flower. In each 10-min observation period, three or six flowers, including an equal number of flowers from each treatment, were observed simultaneously by two observers. The total number of visits (V_t) and number of visits with stigma contact (V_s) were scored for *Bombus* and *Ptilothrix*, and the ratio of visits with stigma contact to the total (V_s/V_t) was calculated. The time it took to handle a flower per single pollinator visit (time/visit) was also recorded for all visits made by the two pollinators. A log-linear analysis with maximum-likelihood estimation of chi-square was applied to test for treatment, pollinator and their interaction effects on V_s/V_t using the frequency counts with the procedure CATMOD in the statistical package SAS (SAS Institute Inc., Cary, N.C., USA). Treatment, pollinator, and their interaction effects on time/visit was tested by a two-way ANOVA with a mixed model using log-transformed data (Sokal and Rohlf 1995; Zar 1996). The 100% petal removal treatment was excluded from the tests for V_s/V_t and time/visit because of the small number of pollinator visits.

Experiment 2: seed-predator visitation and pollen removal and deposition

For each petal size treatment 12 flowers were exposed all day to pollinators and seed-predators on 20 August. In addition, 12 other flowers were allowed to open in bags, to exclude the effects of all insect visitors. We collected flowers between 1730 and 1830 hours, and counted the number of *Althaeus* and *Conotrachelus* in the flowers. The numbers of beetles per flower were compared using a Kruskal-Wallis test followed by nonparametric multiple comparison tests (Zar 1996, Sect. 11.6). Stigmas and anthers of each flower were placed separately in plastic bottles with 5 ml and 15 ml 70% ethanol, respectively. Pollen removal was measured by estimating the relative amount of pollen left in anthers. The bottles with anthers were shaken with an electric mixer and the number of pollen grains in five 100 μ l-samples were counted and averaged for each flower. Pollen deposition was measured by counting the number of pollen grains deposited on three of five stigma lobes per flower. Pollen grains were dyed in 1% aniline blue prior to counting. Pollen removal and deposition were compared between treatments by the Tukey test for multiple comparisons (Zar 1996, Sect. 11.1) using log-transformed data.

Experiment 3: fruit and seed set, and seed predation

In this experiment 60 flowers were used for each treatment. Half of the flowers (30) for each treatment were available to pollinators, and the other half were hand-pollinated using the pollen collected from plants c. 10 m away. There were two reasons for including hand-pollinated controls in the experiment. First, petal cutting could have some direct effect on fruit development and/or seed set. This potential effect could be evaluated by comparing results from open-pollinated flowers and hand-pollinated controls. Second, we were concerned that our sample size might not be large enough to test for the effects of seed predation and seed set for the 100% petal removal treatments because of potentially higher abortion rates of flowers that received few or no pollen grains. In addition to the hand-pollinated controls, 30 flowers were allowed to open in bags that excluded pollinators. The bags were removed the next day after petals had wilted. The experiments were initiated on 13 August.

Fruit set. The fate of each treated flower and control was monitored for 28 days by recording whether fruits had aborted at 6, 14, 22, or 28 days after anthesis. The fruit set, the ratio of non-aborted mature fruits to the total number of observed fruits ($n = 30$), was calculated for each treatment. Because all fruits from bagged flowers aborted within 6 days, fruit abortion rates within 6 days and those between 6 and 28 days were analyzed separately. The effects of petal removal and pollination treatments, and their interactive effects on fruit set and abortion rates were tested by log-linear analysis with maximum-likelihood estimation of chi-square using frequency counts (the CATMOD procedure, SAS).

Seed predation and seed set. Fruits which remained on the plants for 28 days were collected and placed in individual plastic bags. Fruits were mature at the time of collection, seeds were fully developed, and most *Conotrachelus* damage to seed had occurred. We measured fruit diameter and counted the number of *Conotrachelus* exit holes on the outer surface of each fruit and the number of *Conotrachelus* larvae inside each fruit. The number of *Conotrachelus* larvae per fruit was estimated by adding the number of exit holes and the number of larvae still inside each fruit. The externally undamaged seeds in each fruit were counted and placed in separate plastic containers. Seeds were kept at room temperature and *Althaeus* adults began to emerge in mid-October. The number of infested seeds was determined for each fruit in early December, and we scored the number of undamaged seeds as the final seed set, an estimate of number of seeds per fruit available for dispersal.

We calculated the fruit infestation rates by *Conotrachelus* [number of fruits with *Conotrachelus* damage (f_{Cono})/number of fruits collected (f_m)] and by *Althaeus* [the number of fruits with *Althaeus* damage (f_{Alth})/number of fruits with seeds after *Conotrachelus* damage (f_s)]. Because we have no reason to assume that *Conotrachelus* larvae selectively avoid the *Althaeus*-infested seeds, the absolute number of damaged seeds in each fruit would not reflect the number of *Althaeus* larvae that had initially infested the fruit. Therefore, we assume that the seed infestation rate (i.e., relative initial density) of *Althaeus* was the ratio of seeds infested by *Althaeus* in undamaged seeds after *Conotrachelus* damage. The effects on petal removal and pollination treatments, and their interactive effect on fruit infestation rates were tested by log-linear analysis with maximum-likelihood estimation of chi-square using the frequency counts (the CATMOD procedure, SAS). The fruit diameter, numbers of *Conotrachelus* larvae per fruit, seeds per fruit after *Conotrachelus* damage, *Althaeus* seed infestation rate, and final seed set were analyzed by two-way ANOVAs with petal removal and pollination treatments, and their interaction, as factors (Sokal and Rohlf 1995; Zar 1996). If the interaction term in these analyses was significant ($P < 0.05$), the multiple comparison was performed within each pollination treatment using Tukey's method. The numbers of *Conotrachelus* larvae per fruit, seeds per fruit after *Conotrachelus* damage, and final seed set were log-transformed, and the *Althaeus* seed infestation rate was arcsine-transformed in the analyses.

Results

Pollinator response

The two pollinators, *Bombus* and *Ptilothrix*, showed similar responses to petal-size manipulations. Both ignored most flowers with 100% petal removal (Table 1A). In the analyses including the 0% and 50% petal removal treatments, neither the treatment effect nor the treatment×pollinator interaction were significant for both the ratio of visits with stigma contacts to total visits (V_s/V_t) and time per visit (Table 1B, C). *Bombus* made 78.8% of the total visits. In the 0% and 50% petal removal treatments, V_s/V_t was significantly greater for *Bombus* and time per visit was significantly longer for *Ptilothrix* (Table 1).

Pollen/seed-predator visitation to flowers

Adult *Althaeus* were found in most non-bagged flowers regardless of petal size; however, the number of *Althaeus*/flower differed significantly among treatments (Kruskal-Wallis test, $df=3$, $H=38.4$, $P<0.0001$) (Fig. 1). The number of beetles was largest in the 0% and smallest in the 100% petal removal treatments across the non-bagged treatments (Fig. 1). We found

only five *Conotrachelus* adults from five different flowers in the experiments (data not shown). This small sample size precluded statistical testing of treatment differences for this species.

Pollen removal and pollen deposition

Significantly more pollen remained in flowers with 100% petal removal compared to flowers with 0% and 50% removal, and there were no significant differences between the 0% and 50% removal treatments (Fig. 2). Significantly less pollen was counted on anthers in 100% petal removal treatment relative to bagged controls (Fig. 2). On average, *c.* 300 pollen grains were deposited on each stigma lobe in the 0% and 50% petal removal treatments, with a non-significant difference between the two treatments. (Fig. 2). Significantly fewer pollen grains were deposited on flowers with 100% petal removal, and almost no pollen was found on stigmas of bagged flowers (Fig. 2).

Fruit set

The interaction effect of petal removal and pollination treatments on fruit set was significant (Table 2B), and

Table 1 A Responses of two pollinator species to petal size manipulation, and the results of **B** the log-linear analysis with maximum-likelihood estimation of χ^2 on V_s/V_t , and **C** of the two-way ANOVAs on time per visit. In **A** log-transformed time/visit data were used in the calculation of mean and 95% confidence limits

(*lower, upper*), and these values are shown in the linear scale by calculating antilogarithms. In **B** the analysis of V_s/V_t used frequency counts. Because of the low frequency of bee visits, the 100% petal removal treatment was excluded from the statistical analyses shown in **B** and **C**

A Pollinator response (18 flowers × 40 min)

Pollinator	<i>Bombus</i>			<i>Ptilothrix</i>		
	0%	50%	100%	0%	50%	100%
Petal removal treatments						
Total visits (V_t)	72	66	3	22	16	0
Visits with stigma contact (V_s)	54	54	0	7	2	0
V_s/V_t	0.75	0.81	0.0	0.32	0.13	–
Time/visit (s)	8.3	7.9	–	11.9	10.2	–
95% confidence limits	(7.0, 9.8)	(6.6, 9.5)		(7.5, 18.8)	(5.8, 17.3)	

B V_s/V_t (log-linear analysis with maximum-likelihood estimation of χ^2)

Source	Degree of freedom	χ^2	Probability
Petal removal treatment	1	0.63	0.43
Pollinator species	1	29.5	< 0.0001
Petal removal × pollinator	1	2.64	0.10

C Time/visit (2-way ANOVA)

Source	Degree of freedom	Sum of squares	Mean squares	F	Probability
Petal removal treatment	1	0.0498	0.0498	3.02	0.33
Pollinator species	1	0.435	0.435	4.45	0.036
Petal removal × pollinator	1	0.0165	0.0165	0.169	0.68
Error	172	16.8	0.0978		

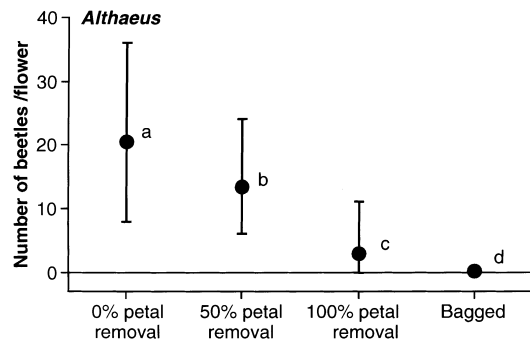


Fig. 1 Number of adult *Althaeus* on flowers with three petal removal and bagged treatments. Ranges are shown by vertical bars. Different letters indicate significant differences between treatments ($P < 0.05$)

except for bagged controls, the lowest fruit set was observed in the 100% petal removal treatment under open pollination (Table 2A). All fruits of the bagged controls aborted within 6 days after anthesis (Table 2A). Fruit abortion rates within 6 days after flowering were significantly greater for the open-pollinated 100% petal removal treatment (Table 2A), and the petal removal \times pollination interaction term was significant (Table 2B). Only 1 of 30 fruits aborted in the other open-pollinated flower treatments and all hand-pollinated flowers within the same period (Table 2A). For fruit abortion 7–28 days after flowering, there were no significant main or interaction effects (Table 2A, B).

Seed predation and seed set

High fruit infestation rates were scored for both beetles, and only 2 out of 112 fruits were uninfested. Fruit infestation rates by *Conotrachelus* were high under all conditions, ranging from 0.82 to 1.00 (Table 3A). Fruit infestation rates by *Althaeus* ranged from 0.71 to 0.89, and there was no significant variation among treatments (Table 3A). None of the petal removal and pollination treatments, or their interaction, had any significant effects on rates of fruit infestation by *Conotrachelus* and *Althaeus* (Table 3B). There was a significant petal removal \times pollination interaction for fruit diameter and the number of *Conotrachelus* larvae/fruit (Table 4B), and fruits in the open-pollinated 100% petal removal treatment had a smaller fruit diameter and fewer *Conotrachelus* larvae than those in the other treatments (Table 4A). The average number of seeds that survived *Conotrachelus* damage ranged from 11 to 26 per fruit across treatments, and *Althaeus* infested 15–26% of the available seeds (Table 4A). Final seed set was highly variable (0–151 seeds per fruit), and treatment averages ranged from 8.2 to 19.4 (Table 4A). None of the terms in the two-way ANOVA were significant for the number of seeds per fruit after *Conotrachelus* damage, seed infestation rate by *Althaeus*, and seed set (Table 4B).

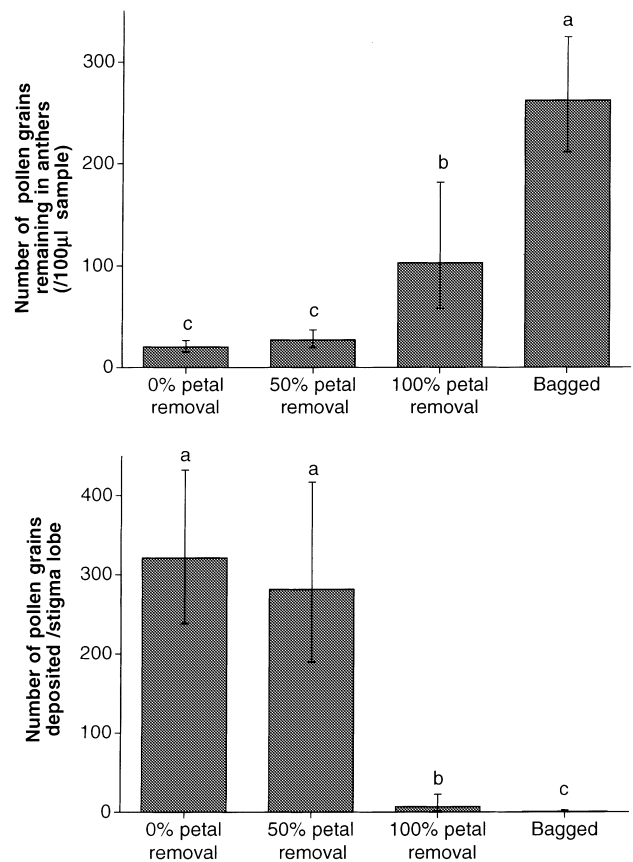


Fig. 2 Pollen removal estimated as the amount of pollen remaining in anthers (number of pollen grains counted in 100- μ l samples) and pollen deposition measured by the number of pollen grains per stigma lobe, for flowers with three different levels of petal removal treatments and bagged control. Vertical bars indicate standard deviation. Different letters indicate that means are significantly different between treatments at $P < 0.05$ (Tukey's multiple comparison test). The data were log-transformed in the analyses

Discussion

Effects on behaviors of insect visitors

The two pollinator species, *Bombus* and *Ptilothrix*, obviously used petals as a cue to locate *Hibiscus* flowers, because flowers with 100% petal removal were almost completely ignored (Table 1A). However, pollinators visited flowers with 50% petal removal as frequently as they visited uncut controls (Table 1). Furthermore, neither the ratio of visits with stigma contact (V_s/V_v) nor time per visit differed between the 50% petal removal and uncut-controls (Table 1). The high visitation rate of pollinators to *Hibiscus* flowers at our study site may explain why all flowers with petals, including small flowers with 50% petal removal, were visited frequently. In our study, we observed 7.8 visits h^{-1} on average, and Spira et al. (1992) previously reported even higher visitation rates, 16.4 and 8.0 visits h^{-1} in 1986 and 1990, respectively, at the same study site. Pollinator preference for larger flowers may not be detectable in plant

Table 2 A The effects of petal size manipulation on fruit abortion and fruit set and **B** the results of statistical tests on the rates of abortion and fruit set. In **B**, the log-linear analyses with maximum-likelihood estimation of χ^2 used frequency counts. The bagged treatment was excluded from these analyses

A							
Pollination treatments	Open			Hand-pollinated			Bagged
Petal removal treatments	0%	50%	100%	0%	50%	100%	
Number of fruits							
Observed (f_i)	30	30	30	30	30	30	30
Aborted 1–6 days after anthesis (f_{a1})	1	1	18	1	1	1	30
Aborted > 6 days after anthesis (f_{a2})	5	9	2	12	12	5	–
Matured (f_m)	24	20	10	17	17	24	0
Abortion rate							
1–6 days after anthesis (f_{a1}/f_i)	0.03	0.03	0.60	0.03	0.03	0.03	1.00
> 6 days after anthesis [$f_{a2}/(f_i - f_{a1})$]	0.17	0.31	0.17	0.41	0.41	0.17	–
Fruit set (f_m/f_i)	0.80	0.67	0.33	0.57	0.57	0.80	0.00
B Log-linear analysis with maximum-likelihood estimation of χ^2							
Variables Source	Degree of freedom		χ^2		Probability		
Abortion rate (1–6 days after anthesis)							
Petal removal treatment	2		6.45		0.040		
Pollination treatment	1		2.68		0.10		
Petal removal × pollination	2		6.45		0.040		
Abortion rate (> 6 days after anthesis)							
Petal removal treatment	2		3.69		0.16		
Pollination treatment	1		1.92		0.17		
Petal removal × pollination	2		1.42		0.49		
Fruit set							
Petal removal treatment	2		1.42		0.49		
Pollination treatment	1		0.29		0.59		
Petal removal × pollination	2		16.2		0.0003		

Table 3 A The effects of petal size manipulation on fruit infestation by *Conotrachelus* and *Althaeus*, and **B** the results of statistical tests on the fruit infestation rates. In **B**, the log-linear analyses with maximum-likelihood estimation of χ^2 used frequency counts

A						
Pollination treatments	Open			Hand-pollinated		
Petal removal treatments	0%	50%	100%	0%	50%	100%
Number of fruits						
Matured (f_m)	24	20	10	17	17	24
With <i>Conotrachelus</i> damage (f_{Conot})	22	20	9	17	14	22
With seeds after <i>Conotrachelus</i> damage (f_s)	19	14	9	14	15	23
With <i>Althaeus</i> damage (f_{Alth})	17	10	8	11	12	17
Fruit infestation rate						
By <i>Conotrachelus</i> (f_{Conot}/f_m)	0.92	1.00	0.90	1.00	0.82	0.92
By <i>Althaeus</i> (f_{Alth}/f_s)	0.89	0.71	0.89	0.79	0.80	0.74
B Log-linear analysis with maximum-likelihood estimation of χ^2						
Variables Source	Degree of freedom		χ^2		Probability	
Fruit infestation rate by <i>Conotrachelus</i>						
Petal removal treatment	2		0.87		0.65	
Pollination treatment	1		0.06		0.81	
Petal removal × pollination	2		3.02		0.22	
Fruit infestation rate by <i>Althaeus</i>						
Petal removal treatment	2		0.80		0.67	
Pollination treatment	1		0.64		0.42	
Petal removal × pollination	2		1.47		0.48	

Table 4 A The effects of petal size manipulation on fruit diameter, seed predation by *Conotrachelus* and *Althaeus*, and final seed set, and **B** the results of two-way ANOVAs. In **A**, means \pm SDs are listed for fruit diameter. For other variables, transformed data were used for calculation of means and 95% confidence limits (*lower, upper limits*), and these values are shown in the linear scale

Pollination treatments	Open			Hand-pollinated		
	0%	50%	100%	0%	50%	100%
Petal removal treatments						
Fruit diameter (mm) \pm SD	19.8 \pm 1.9	19.1 \pm 1.7	17.3 \pm 1.6	18.5 \pm 1.7	18.4 \pm 2.5	19.7 \pm 1.7
<i>Conotrachelus</i> larvae/fruit	3.1 (2.2, 4.3)	3.8 (1.7, 4.5)	1.7 (0.9, 2.9)	3.4 (2.6, 4.4)	2.8 (1.7, 4.5)	3.6 (2.5, 5.0)
Seeds/fruit after <i>Conotrachelus</i> damage	22.5 (9.6, 51.0)	12.5 (4.5, 32.0)	19.1 (7.6, 46.4)	11.4 (4.4, 27.6)	19.2 (8.3, 42.7)	26.3 (16.1, 42.6)
Seed infestation rate by <i>Althaeus</i>	0.26 (0.12, 0.44)	0.19 (0.05, 0.38)	0.25 (0.05, 0.53)	0.24 (0.06, 0.48)	0.17 (0.06, 0.31)	0.15 (0.07, 0.25)
Seed set	16.5 (7.1, 36.7)	8.8 (3.2, 21.6)	12.2 (4.2, 32.7)	8.2 (3.1, 19.8)	14.5 (6.2, 32.0)	19.4 (11.7, 31.6)
B Two-way ANOVA tables						
Variables	Degree of freedom	Sum of squares	Mean squares	F	Probability	
Fruit diameter						
Petal removal treatment	2	7.18	3.59	1.03	0.36	
Pollination treatment	1	0.59	0.59	0.17	0.68	
Petal removal \times pollination	2	62.2	31.1	8.91	0.0003	
Error	106	370	3.49			
<i>Conotrachelus</i> larvae/fruit						
Petal removal treatment	2	0.14	0.073	1.19	0.31	
Pollination treatment	1	0.079	0.079	1.30	0.26	
Petal removal \times pollination	2	0.43	0.22	3.53	0.033	
Error	106	6.46	0.061			
Seeds/fruit after <i>Conotrachelus</i> damage						
Petal removal treatment	2	0.44	0.22	0.46	0.64	
Pollination treatment	1	0.003	0.003	0.005	0.94	
Petal removal \times pollination	2	1.17	0.585	1.22	0.30	
Error	106	51.0	0.481			
Seed infestation rate by <i>Althaeus</i>						
Petal removal treatment	2	0.14	0.071	0.56	0.57	
Pollination treatment	1	0.074	0.074	0.58	0.45	
Petal removal \times pollination	2	0.041	0.020	0.16	0.85	
Error	88	11.3	0.13			
Seed set						
Petal removal treatment	2	0.29	0.15	0.32	0.73	
Pollination treatment	1	0.032	0.032	0.070	0.79	
Petal removal \times pollination	2	1.38	0.69	1.51	0.23	
Error	106	48.3	0.46			

populations in which pollinators are abundant relative to the amount of resource (nectar and/or pollen) provided by plants, because pollinators may need to visit all flowers to obtain sufficient amount of resource (Young and Stanton 1990; Cresswell and Galen 1991; Inoue et al. 1995). Lack of response to flower-size by bumblebees has been reported in *Raphanus sativus* (Stanton et al. 1991) and *Campanula* (Inoue et al. 1995), and these studies also reported high pollinator visitations. The positive response of pollinators to the larger flowers has been shown in *Polemonium viscosum* (Galen and Newport 1987; Galen 1989; Galen and Stanton 1989; Cresswell and Galen 1991). The combined results from the few species that have been studied indicate that whether or not flower size affects pollinator visitation may depend on pollinator abundance in the community where the studies are conducted. Therefore, the generality of our findings needs to be examined in populations where pollinators are less abundant.

Althaeus adult density responded to petal removal treatments (Fig. 1), demonstrating that the beetle uses petal size as a cue to select host plants. It is often difficult to directly detect a seed predator's response to floral display size, mainly because of infrequent visitation due to low density of adult seed predators, such as that found in *Conotrachelus* in this study. Several previous studies have documented that seed predation increases with increasing inflorescence size (Zimmerman 1980b; Hainsworth et al. 1984; Molau et al. 1989; Ehrlén 1996). Based on these observations, it is assumed that seed predators exert counteracting selection pressure on floral display size, if seed-predators use floral displays as cues to locate host plants (Zimmerman 1980b; Brody 1992a; Ehrlén 1996). Experimental studies are still required, however, to test whether seed predators use floral displays as cues to locate host plants in these systems. For example, in the studies of pre-dispersal seed predation of *Ipomopsis aggregata*, seed predators chose flowers with a higher probability of seed set (Brody 1992b), but showed no response to manipulation of floral-display size (Brody 1992a). Because *Althaeus* feeds on *Hibiscus* pollen as adults and we detected no effects of petal size on seed predation, the preference of *Althaeus* for larger flowers may represent a foraging strategy for adult beetles rather than a behavior to optimize oviposition.

Effects on reproductive success of flowers

Pollen counts on stigmas reflected the responses in pollinator visitation, and no effect of petal-size reduction on pollen deposition was found unless all petals were removed (Fig. 2). After *c.* 11 h exposure to pollinators, *c.* 300 pollen grains per stigma lobe (*c.* 1500 pollen grains per flower) had been deposited. This amount of pollen deposited in flowers greatly exceeded the number of pollen grains needed to fertilize all ovules in *Hibiscus* flowers (*c.* 360 pollen grains; Spira et al. 1992).

We found no evidence for a negative effect of small petal-size on fruit set unless all petals were removed. Significantly higher fruit abortion within 6 days after flowering for flowers in the 100% petal removal treatment was due to failure of pollen to reach the stigmas. This was verified by the fact that fruit set for flowers without petals increased to the levels of unmanipulated flowers when they were hand-pollinated (Table 2). We also have support for the conclusion that fruit abortion occurs within 6 days due to pollen limitation because all fruits aborted during the same period from bagged flowers (Table 2). Fruit abortions of flowers which were pollinated occurred later (7–14 days) but were not significantly related to petal size (Table 2). Pollen limitation in flowers in the 100% petal removal treatment further reduced fruit size, and consequently reduced the number of *Conotrachelus* larvae per fruit. This conclusion is supported by the observation that fruit size and density of *Conotrachelus* larvae for fruits developed from flowers without petals increased to levels of unmanipulated flowers when they were hand-pollinated (Table 4). We found no evidence that petal-size manipulation influenced fruit or seed infestation rates by *Althaeus* or by *Conotrachelus* (Tables 3, 4) or final seed set (Table 4).

Overall, the study has shown that 100% petal removal reduced female reproductive success considerably, mainly through a higher rate of fruit abortion due to failure of pollen receipt. No significant differences between the 50% petal removal treatment and uncut controls were detected in any components of female reproductive success examined in this study. The 50% petal removal treatment produced flowers of smaller or similar size to the smallest flowers in natural populations at the study site. The results, therefore, suggest that differences in petal size have little influence on female reproductive success of *Hibiscus* flowers at our study site. Flower-size independence of fruit or seed set has also been reported in *Impatiens capensis* (Bell 1985), *Raphanus sativus* (Stanton and Preston 1988), and *Nemophila menziesii* (Andersson 1994). In contrast, it has been reported that larger flowers attracted more pollinators and had higher seed set in *Polemonium viscosum* (Galen 1989) and *Campanula americana* (Johnson et al. 1995).

It has been suggested that large showy petals contribute more to male than female fitness because pollen removal is generally more limited by pollinators than is seed set (Bell 1985; Stanton and Preston 1988). In our study population, the total amount of pollen removed from flowers was not limited by pollinators. The reduction in petal size had no effect on the total amount of pollen removal unless all petals were removed, and almost all pollen were removed from un-manipulated controls and flowers with 50% petal removal (Fig. 2). It has been reported in several other plant species that, when pollinators are abundant, they quickly remove all pollen that can be removed (Wilson and Thomson 1991; Stanton et al. 1992; Wilson et al. 1994). Synchronous

opening of flowers, high pollinator visitation rates, and rapid saturation of stigmas with pollen found in our study population suggest that there may be a strong male-male competition based on the speed of pollen export, rather than on the total amount of pollen exported over the life time of the flower (Stanton 1994). Furthermore, we do not know how much of the removed pollen was eaten by *Althaeus* and *Conotrachelus*. We need further research to determine whether or not a higher density of *Althaeus* in larger flowers has negative effects on the male reproductive success of flowers, and counterbalance any positive relationship between flower size and pollen removal rate.

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