FLOWER VISITATION, POLLEN DEPOSITION, AND POLLEN-TUBE COMPETITION IN HIBISCUS MOSCHEUTOS (MALVACEAE)[1]

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The potential influence of pollen-tube competition on offspring "quality" has received considerable attention in recent years. Yet the prevalence of pollen competition in natural populations is largely unknown because few investigators have actually measured rates of pollen deposition on stigmas. In this study, we assess the potential for pollen-tube competition in natural populations of the self-compatible, pollinator-dependent herbaceous perennial, *Hibiscus moscheutos*. Individual flowers averaged two to four visits per 15 min by potential pollinators (*Ptilothrix* and *Bombus*), and about 34% of these visits involved contact with a stigma. The median number of pollen grains deposited on virgin stigmas per contact visit was 70 grains (values ranged from 0 to 889), and flowers averaged about four contact visits per hour. Approximately 360 pollen grains must reach stigmas for full seed set to occur in a typical flower (an average ovary has 139 ovules, and 2.6 pollen grains are required per seed). Within 2 and 3 hr exposure to pollinators, 65% and 97% of the flowers received excess pollen (>360 grains) and median stigmatic pollen loads exceeded the number of ovules by a factor of 4.0 and 5.4, respectively. Based on 3 yr observations, it is concluded that pollen competition may frequently occur in this species.

Gametophytic selection due to pollen competition may be an important component of natural selection in plants (Mulcahy, 1979; Snow, 1986; Mulcahy and Mulcahy, 1987; Walsh and Charlesworth, in press). One consequence of pollen-tube competition is its potential effect on seed "quality." Several investigators have found that seeds produced under intense pollen-tube competition have significantly better germination, seedling growth, or seedling survivorship than those produced with little or no pollen-tube competition (reviewed in Mulcahy and Mulcahy, 1987; but see Snow, 1990).

It is well documented that large numbers of genes are expressed by the microgametophyte (the germinated pollen grain) and that there is substantial overlap in gene expression between the gametophyte and sporophyte generations (Tanksley, Zamir, and Rick, 1981; Willing and Mascarenhas, 1984; Willing, Bashe, and Mascarenhas, 1988). Moreover, the genes expressed by the microgametophyte that contribute to fast-growing pollen tubes may also facilitate more vigorous sporophytes (e.g., Mulcahy, 1974; Ottaviano, Sari-Gorla, and Mulcahy, 1980; Winsor, Davis, and Stephenson, 1987). Consequently, pollen-tube competition may result in increased seed "quality" because the fastest growing pollen-tubes are

most likely to fertilize ovules. Another potential effect of pollen-tube competition is nonrandom mating, which could influence both outcrossing rates and paternal reproductive success (Snow and Spira, 1991a, b).

A prerequisite for pollen-tube competition is that flowers receive excess pollen relative to the amount needed for full seed set to occur. In some populations, only small amounts of pollen are deposited on stigmas, and seed set per fruit is actually pollen-limited (e.g., Bierzychudek, 1981; Spira and Pollak, 1986). In a review of the literature, Snow (1986) found that in most cases, pollinators delivered more than enough pollen to sire a full complement of seeds per fruit (see also Levin, 1990).

The fact that seed set is generally not pollen-limited suggests the potential for pollen competition but provides little insight into its magnitude (Mulcahy and Mulcahy, 1987). The intensity of pollen competition is influenced not only by the number of pollen grains reaching stigmas, but also by their rate of arrival. If pollen grains arrive gradually over time, then pollen tubes from separate pollen loads may not compete; consequently, many ovules may be fertilized from a random population of pollen grains rather than by those with the fastest growth.

With the exception of studies on *Geranium maculatum* (Mulcahy, Curtis, and Snow, 1983) and *Epilobium canum* (Snow, 1986), detailed studies on the timing and amount of pollen deposited on stigmas over time are rare. Moreover, we know little about other factors influencing the magnitude of pollen competition, such as the number of pollen grains required per seed, and the extent to which pollen-tube growth rates vary. Consequently, the prevalence of pollen competition in natural populations is largely unknown.

The purpose of this paper is to describe pollination rates in natural populations of *Hibiscus moscheutos* within the context of pollen competition. Data are presented on

flower visitation rates, the proportion of flower visitors that contact a stigma, and the amount of pollen deposited on stigmas per contact visit and over time. Based on stigmatic pollen loads and the number of pollen grains required per developed seed, the proportion of flowers that received excess pollen is estimated.

MATERIALS AND METHODS

The study species—*Hibiscus moscheutos* L. (Malvaceae) (hereafter referred to as *Hibiscus*) is a herbaceous perennial found in freshwater and brackish marshes of the eastern United States (Brown and Brown, 1984). The main research site is a freshwater wetland (locally known as Mill Swamp) at the Smithsonian Environmental Research Center in Edgewater, Maryland where more than 300 individuals of *Hibiscus* occur. Additional data were collected in large populations of *Hibiscus* near North Beach and off Hwy 450 adjacent to the North River (unless stated otherwise, data pertain to Mill Swamp; all three sites are in Anne Arundel County, MD).

Hibiscus plants have few to many upright stems, 1–2 m tall, which emerge each spring from a perennial rootstock. The flowering season extends from late July to early September, and the flowers are large (10–15 cm across) with white or pink petals that remain open for a single day. *Hibiscus* is self-compatible, but spatial separation of anthers from stigmas prevents automatic self-pollination (Spira, 1989). The main pollinator, *Ptilothrix bombiformis,* is a specialist anthophorid bee that utilizes *Hibiscus* pollen as its primary larval food and has a distribution that extends throughout the range of *Hibiscus* (Blanchard, 1976; Rust, 1980). Moreover, the emergence and foraging of adult *Ptilothrix* is closely synchronized with the flowering period of *Hibiscus* (Blanchard, 1976; Rust, 1980; Spira, personal observation). The large flat stigmas, 6-cm-long style, large pollen grains (150 μm diam), and five stylar branches per pistil makes this an attractive species for pollen competition studies.

Pollen-seed ratios—Unknown but variable amounts of outcrossed pollen were added to stigmas of pollinator-excluded (bagged) flowers shortly after anthesis. After approximately 24 hr, stigmas were harvested, stored in a refrigerator, and then stained with aniline blue. Pollen grain counts were made with the aid of a dissecting microscope. Developing fruits from pollinated flowers were monitored, and the number of developed seeds per fruit was determined.

Flower visitation rates—Visits to individual flowers were counted during 15-min observation periods from early morning until late afternoon when the flowers closed. There were 142 flowers observed over 23 d during the 1986 and 1990 flowering seasons (data combined for analysis). For each visit, the bee species were recorded and, in 1986, whether visitors foraged for nectar, pollen, or both was noted.

The open bowl-shaped flowers and large stigma lobes allowed assessment of whether flower-visiting insects contacted a stigma and thereby potentially pollinated the flower. For the primary pollinators (*Ptilothrix bombiformis* and *Bombus* spp.), the proportion of individuals that contacted a stigma during single flower visits ($N = 375$) was recorded and the rate that stigmas of individual flowers were contacted per 15-min observation period ($N = 129$) was quantified.

Stigmatic pollen loads—The amount of pollen deposited on stigmas was determined after flower visitors contacted a stigma once, twice, or three times ($N = 72, 31,$ and 13 flowers). The stigmas were then collected, stained with aniline blue, and the pollen grains counted as described above. Stigmas of 13 other flowers were harvested and pollen counts were made after the flower had been visited, but contact with the stigma did not appear to have occurred. A previous study had shown that few, if any, grains reach stigmas in the absence of pollinator visits due to spatial separation of anthers from stigmas (Spira, 1989).

Stigmatic pollen loads were also quantified after various time intervals in 1985 and 1990. Freshly opened flowers from separate plants were tagged along transects, and stigmas from alternate flowers were harvested 1, 2, 3, or 10 hr after exposure to pollinators. Stigmatic pollen loads were assessed for 63 flowers sampled during 3 d in 1985 and 60 flowers sampled during 2 d in 1990. In 1991, flowers were sampled after about 10 hr exposure to pollinators at two additional sites—North Beach ($N = 20$) and North River ($N = 10$). Stigmas were harvested, stained, and scored as described above. Heterospecific pollen grains were rarely observed on stigmas and were not included in pollen grain counts.

RESULTS

Pollen-seed ratios—The number of pollen grains required per developed seed was 2.6 ± 0.3 ($\bar{X} \pm 1$ SE) based on 19 hand-pollinated flowers in which the ratio of pollen grains on stigmas to ovules in the ovary varied from 0.5 to 1.5 pollen grains per ovule. This suggests that fewer than half the pollen grains deposited on stigmas were capable of fertilizing an ovule (seed abortion rates are low; Snow and Spira, 1991a). If each seed requires about 2.6 pollen grains, and ovaries have an average of 138.5 ± 2.9 ovules ($\bar{X} \pm$ SE; $N = 25$), then approximately 360 pollen grains must be deposited on the stigmas of an average flower for full seed set to occur.

Flower visitation rates—*Hibiscus* flowers were actively visited by bees, particularly *Ptilothrix bombiformis* and *Bombus* spp. Individual flowers averaged 4.1 ± 0.5 and 2.0 ± 0.3 ($\bar{X} \pm 1$ SE) visits per 15-min observation period during the 1986 and 1990 seasons ($N = 75$ and 67 observations per year). Occasional visits to flowers by butterflies, moths, small bees, and flies were not included as these insects appeared to be ineffective pollinators. Most flowers received between one and five visits by *P. bombiformis* and *Bombus,* but about 27% received six or more visits per 15-min interval in 1986 (Fig. 1).

Ptilothrix bombiformis, which feeds almost exclusively on the pollen and nectar of *Hibiscus* flowers (Blanchard, 1976; Rust, 1980; Spira, personal observation), comprised 98% of 309 recorded flower visits in 1986. In contrast, only 24% of 137 flower visits were by *P. bombiformis* and 76% were by *Bombus* in 1990. Therefore, *P. bom-*
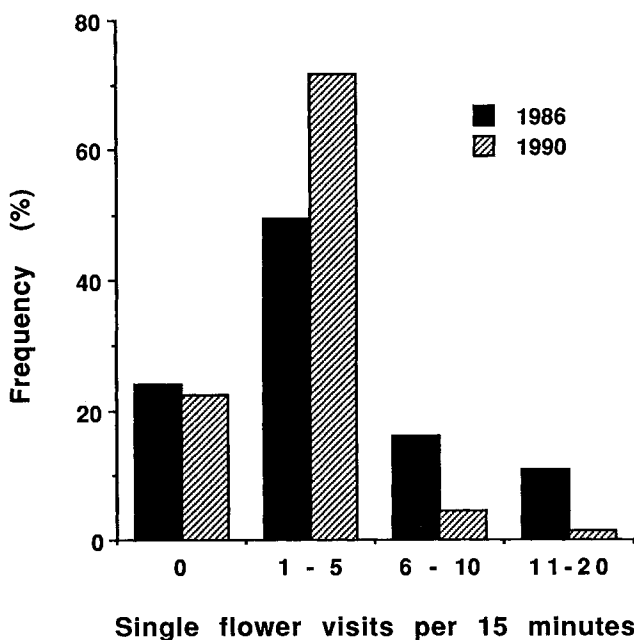
Fig. 1. Bee visitation rates to individual flowers in 1986 and 1990 (N = 75, 67 15-min observation periods).

Fig. 2. The proportion of bees (*P. bombiformis, Bombus,* and both) that contacted one or more stigmas per flower visited in 1986 (N = 238) and 1990 (N = 137).

biformis was essentially the sole pollinator in 1986, and bumble bees were the primary pollinator in 1990.

Ptilothrix bombiformis foraged solely for nectar in 87% of the flowers, only pollen was collected in 6% of the flowers, while both nectar and pollen were obtained in 7% of the flowers. Individuals foraging for pollen accumulated large loose masses of pollen on their ventral surfaces and on the well-developed scopae of their hind legs. Nectar-foraging individuals often crawled over the numerous anthers on their way to the nectaries at the base of the flower. Whether foraging for pollen, nectar, or both, individuals of *P. bombiformis* generally accumulated large amounts of pollen on their ventral surfaces, particularly in the morning hours when the anthers bore abundant pollen. Bumble bees foraging in flowers also often had large masses of pollen on their bodies.

Most visits by *P. bombiformis* and *Bombus* did not result in pollination because contact with the stigmas did not occur. The proportion of bees that contacted one or more stigmas per flower visited was 27% in 1986 and 46% in 1990 (N = 238, 137; Fig. 2). More visits were contact visits in 1990, presumably because a much higher proportion of visits were by *Bombus* (discussed above), and bumble bees contacted stigmas more frequently than did *P. bombiformis*. For example, in 1990, bumble bees contacted the stigmas of 57% of the flowers visited whereas only 12% of the flowers visited by the specialist bee, *P. bombiformis,* were contact visits (Fig. 2). Apparently this reflects bumble bees' more frequent use of the stigma lobes as a landing platform when entering flowers (Spira, personal observation).

Although flower-foraging bees often missed the stigmas, the high visitation rates to individual flowers resulted in frequent stigma contacts (Figs. 1, 3). Bees contacted the stigmas of individual flowers an average of 1.03 and 0.94 times per 15-min observation period (SE = 0.27, 0.14; N
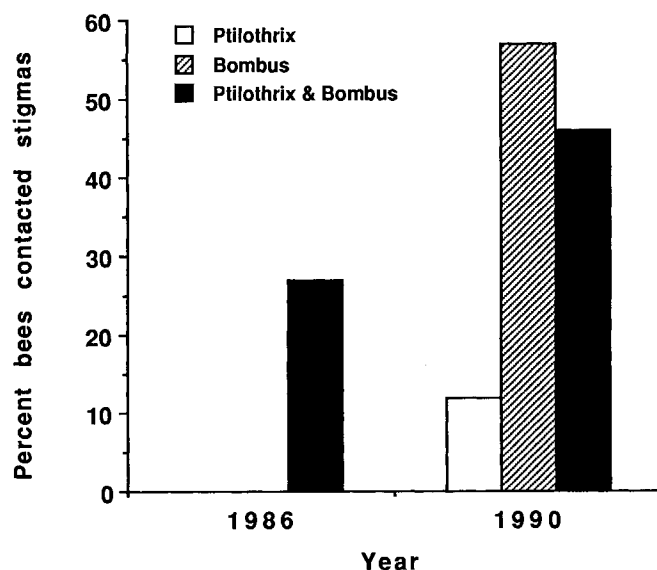
= 62, 67) in 1986 and 1990, or about four visits per hr in both years. Of the total flowers observed for 15-min intervals, 50% had 0 stigma contacts and 11% received three or more contact visits (Fig. 3).

Stigmatic pollen loads—Almost all flowers (>97%) had pollen on their stigmas after a single contact visit to a previously unpollinated flower (Fig. 4). The median stig-
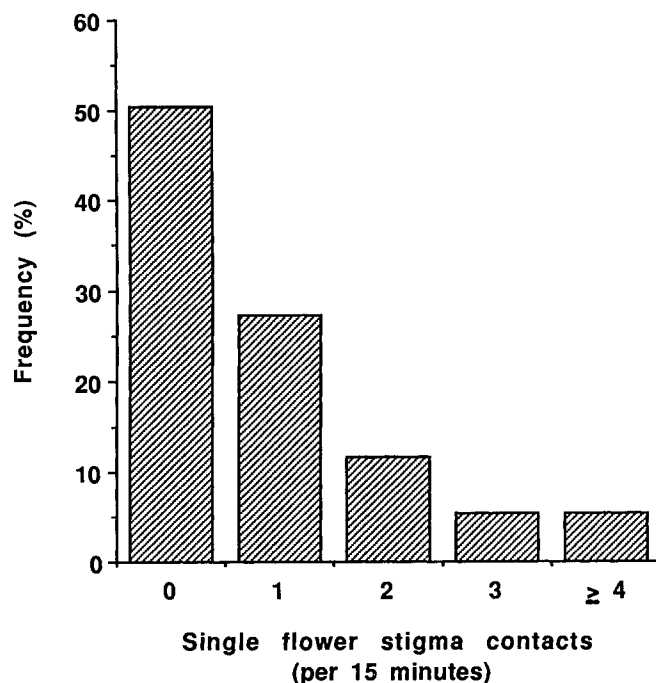

Fig. 3. The rate flower-visiting bees contacted one or more stigmas per flower (N = 129 flowers observed for 15-min periods in 1986 and 1990).
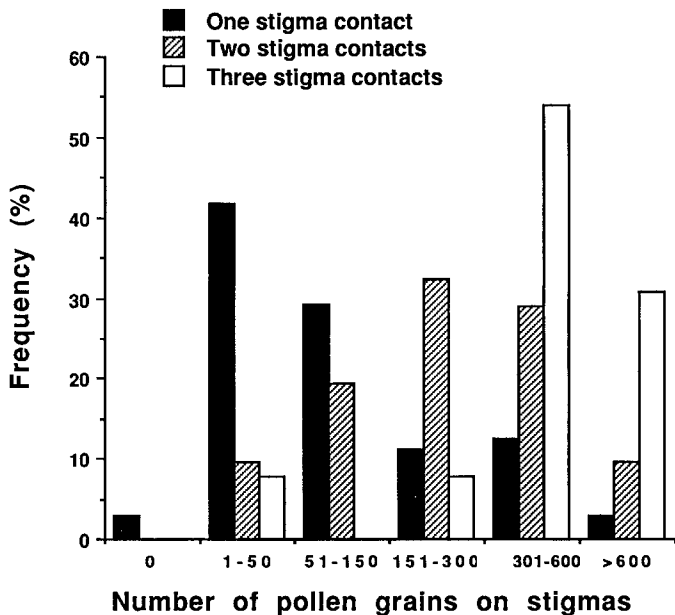
Fig. 4. Amount of pollen deposited after one, two, and three stigma contacts by flower-visiting bees (N = 72, 31, and 13, respectively). The median stigmatic pollen load after one, two, and three contact visits was 70, 266, and 501 grains, respectively.
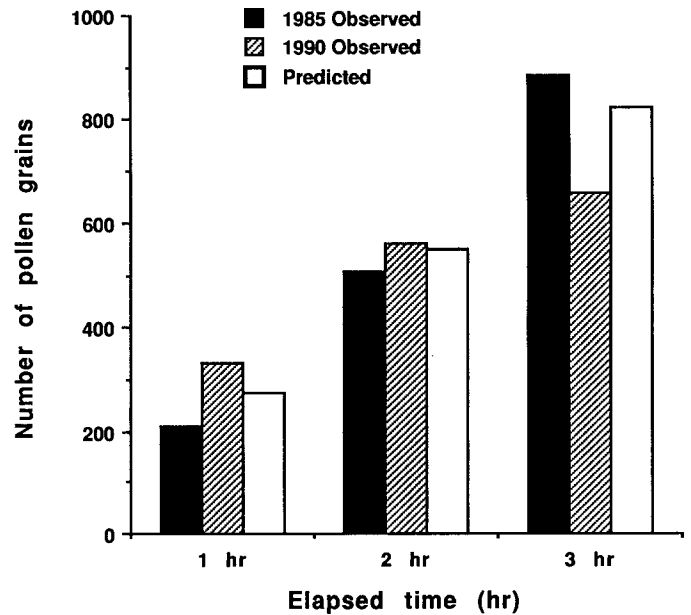

Fig. 5. Rate of pollen deposition on stigmas in 1985 and 1990 (median values; N = 14–18 flowers per sample interval). Predicted values are based on the amount of time flowers were exposed to pollinators (hr), multiplied by the average number of stigma contacts per hr, multiplied by the median number of pollen grains deposited after one stigma contact.

matic pollen loads after one, two, and three contacts were 70 grains (range = 0–889; N = 72), 266 grains (range = 18–1,016; N = 31) and 501 grains (range = 40–957; N = 13). In contrast, previously unpollinated flowers in which bees visited the flower but did not appear to contact a stigma generally had no pollen grains on their stigmas ($\bar{X}$ ± SE = 0.6 ± 0.3; range 0–3; N = 13 flowers). As expected, the proportion of flowers with large stigmatic pollen loads increased with the number of contact visits (Fig. 4). For example, the proportion of flowers with stigmatic pollen loads in excess of 600 grains was 3%, 10%, and 31% after one, two, and three contact visits.

In a second experiment, the stigmas of newly opened flowers were harvested 1, 2, or 3 hr after exposure to pollinators. The median stigmatic pollen loads in 1985 and 1990 were 210 and 333 grains after 1 hr, 506 and 560 grains after 2 hr, and 883 and 658 grains after 3 hr (Fig. 5). The proportion of these flowers that received sufficient pollen to ensure full speed set (i.e., >360 grains) was 33%, 65%, and 97% after 1, 2, or 3 hr exposure (N = 33, 31, 30 flowers).

The empirical measure of pollination rates in 1985 and 1990 closely approximated predicted rates based on pollinator observations and data on the effectiveness of single visits. As discussed previously, individual flowers received about four contact visits per hr, and a median of 70 pollen grains were deposited per contact visit on previously unpollinated flowers. Observed and predicted pollen loads were remarkably similar after each of the three time intervals (Fig. 5).

The amount of pollen deposited on stigmas greatly exceeded the number of ovules per ovary in a typical flower (Table 1). Recall that about 2.6 pollen grains were required per developed seed and that the average number of ovules per ovary was 138.5. Given the stigmatic pollen loads

shown in Fig. 5, excess pollen had been deposited within 2 hr, and by 3 hr, the flowers had received about twice the amount of pollen needed for full seed set. Since these estimates are based on median values, many flowers undoubtedly received much larger pollen loads during the first 3 hr after anthesis. Flowers sampled after about 10 hr exposure to pollinators at the Mill Swamp, North Beach, and North River sites consistently had excess pollen on their stigmas and the median stigmatic pollen load exceeded the number of ovules by a factor of 8 to 15 (Table 1).

DISCUSSION

Seed production in *Hibiscus* is clearly not pollen-limited, since 100% of the flowers received excess pollen (>360 grains) by late afternoon. Also, natural levels of seed set per fruit are as high as those achieved by hand-pollination (Spira, 1989). In comparison, the proportion of flowers that received sufficient pollen for maximum seed set was 28% in *Passiflora vitifolia* (Snow, 1982), 50%–70% in *Epilobium canum* (Snow, 1986), and 96% in *Cassia reticulata* (Snow and Roubik, 1987). Although many other species typically exhibit high seed set per fruit (Snow, 1986), more information is needed about the timing of pollen arrival on stigmas in order to document competition among pollen tubes.

In this study, visitation rates were often high enough to foster pollen competition within a few hr of anthesis. Only about 34% of flower-foraging bees contacted a stigma, but high visitation rates resulted in an average flower receiving about one stigma contact per 15 min. About 25% of the flowers received ≥2 contact visits during this

TABLE 1. *The ratio of pollen grains deposited on stigmas to the number of ovules per ovary. Pollen loads based on data presented in Fig. 5 (except for 10-hr interval). Ovule number based on 138.5 ± 2.9 ($\bar{X}$ ± 1 SE; N = 25) ovules per ovary*

Year	Site	Ratio of pollen grains deposited on stigmas to ovules per ovary			
		Time (hr) flowers exposed to pollinators			
		1	2	3	10
1985	Mill Swamp	1.5:1	3.6:1	6.4:1	15.4:1
1990	Mill Swamp	2.4:1	4.0:1	4.8:1	7.6:1
1991	North Beach	—	—	—	10.2:1
1991	North River	—	—	—	11.7:1

interval. Frequent visits and the likelihood of pollen carryover (Thomson and Plowright, 1980) indicate that flowers may often receive pollen from several individuals (as well as self-pollen).

The number of pollen grains deposited per stigma contact was quite variable, ranging from 0 to 889 grains on previously unpollinated flowers, with a median of 70. Pollen loads from two and three contacts were curiously nonadditive (medians were 266 and 501 grains). Since sample sizes were largest for single visits, these were used in the predicted pollen accumulation rates over time (Fig. 5).

Given considerable variation in both visitation frequency and amount deposited, it is important to note that average values of pollen deposition obscure large differences in rates of pollen accumulation. In a portion of the flowers, small, early pollen loads probably did not lead to competition. However, at least half of all stigmas received excess pollen (>2.6 grains per ovule) after about 1.5 hr (Table 1). Thus, it is likely that many seeds resulted from pollen competition. In addition, subsets of seeds within fruits could have been sired by later-arriving pollen that had competed for fewer available ovules. As discussed below, it is difficult to estimate what proportion of all seeds resulted from competition among pollen-tubes.

During the first 2 and 3 hr of exposure to pollinators, median stigmatic pollen loads exceeded the number of ovules by a factor of 4.0 and 5.4, respectively (Table 1; data combined for 2 yr). Pollen accumulation did not continue at this rate; after 10 hr, the stigmatic pollen load to ovule ratio was only 8 to 15. The reduction in pollen deposition rate later in the day may reflect saturation of the stigmatic surface. Also, because most pollen was removed from anthers in the early morning hours (Spira, personal observation), bees probably had less pollen on their bodies later in the day.

Pollen tube competition is influenced not only by the amount and timing of pollen reaching stigmas, but also by variation in pollen-tube growth rates. If pollen germination and pollen-tube growth rates are sufficiently variable, then fast-growing pollen-tubes from later visits may outpace slow-growing tubes from earlier visits (Mulcahy, Curtis, and Snow, 1983). Given enough of a head start, however, moderate amounts of early pollen could avoid competition completely.

Hibiscus pollen placed on stigmas germinates at a rate of about 60% (Snow and Spira, 1991a, in press), which in part explains why more than two pollen grains are required per developed seed. Most grains germinate within 1 hr of pollination, and by 3 hr, most pollen-tubes reach the base of the style (additional time may be needed to effect fertilization). Pollen-tube growth rates vary markedly among individuals, leading to nonrandom fertilization following the application of pollen mixtures to stigmas (Snow and Spira, 1991a). Differences between pairs of individuals in pollen tube growth rates are consistent across maternal plants, suggesting that sexual selection can occur since 'super males' with faster growing pollen-tubes could potentially sire a disproportionate number of seeds over a wide range of maternal plants (Snow and Spira, 1991b).

At present, we do not know the extent to which later but faster-growing pollen can overtake previously deposited pollen. We suspect that a head start of an hr or more is enough to guarantee precedence in the ovary. If this is the case, there should be strong selection for floral traits that encourages early bee visits because pollen from early visits would have a greater chance of fertilizing ovules than pollen from later visits. Anthesis is indeed very synchronous, and occurs about 1–2 hr prior to pollinator activity (Snow and Spira, personal observation).

In conclusion, detailed studies demonstrating pollen competition in natural populations are rare, despite considerable interest in the ecological and evolutionary significance of gametophytic selection. One reason for a lack of information is that it is extremely difficult to quantify the selection pressure engendered by ample pollen loads. Eventually, it should be possible to model this process using data on rates of pollen arrival, pollen-tube growth, and fertilization of available ovules within flowers. Lacking data on variation in pollen-tube growth rates and the time needed for fertilization, we cannot give a precise estimate of the selection pressure on microgametophytes in *Hibiscus*. Nonetheless, we suggest that pollen competition occurs frequently because within 2 and 3 hr exposure to pollinators, 65% and 97% of the flowers sampled had excess pollen on their stigmas.

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