

**POLLEN DISPERSAL IN *YUCCA FILAMENTOSA*
(AGAVACEAE): THE PARADOX OF SELF-POLLINATION
BEHAVIOR BY *TEGETICULA YUCCASELLA*
(PRODOXIDAE)¹**

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We investigated pollen dispersal in an obligate pollination mutualism between *Yucca filamentosa* and *Tegeticula yuccasella*. *Yucca* moths are the only documented pollinator of yuccas, and moth larvae feed solely on developing yucca seeds. The quality of pollination by a female moth affects larval survival because flowers receiving small amounts of pollen or self-pollen have a high abscission probability, and larvae die in abscised flowers. We tested the prediction that yucca moths primarily perform outcross pollinations by using fluorescent dye to track pollen dispersal in five populations of *Y. filamentosa*. Dye transfers within plants were common in all populations (mean \pm 1 SE, $55 \pm 3.0\%$), indicating that moths frequently deposit self-pollen. Distance of dye transfers ranged from 0 to 50 m, and the mean number of flowering plants between the pollen donor and recipient was 5 (median = 0), suggesting that most pollen was transferred among near neighbors. A multilocus genetic estimate of outcrossing based on seedlings matured from open-pollinated fruits at one site was $94 \pm 6\%$ (mean \pm 1 SD). We discuss why moths frequently deposit self-pollen to the detriment of their offspring and compare the yucca–yucca moth interaction with other obligate pollinator mutualisms in which neither pollinator nor plant benefit from self-pollination.

Key words: Agavaceae; fluorescent dyes; mutualism; plant–pollinator interactions; pollinator behavior; Prodoxidae; self-pollination; *Tegeticula*; *Yucca*.

One of the most central conflicts in plant–pollinator interactions is that plants benefit from pollen being transported among plants, whereas most pollinators benefit from collecting nectar, pollen, or other floral rewards regardless of whether they have effectively pollinated flowers (Waser and Price, 1983; Harder and Barrett, 1996; Kearns, Inouye, and Waser, 1998). For example, a pollinator may benefit from foraging at multiple flowers on a self-incompatible plant, but deposition of self-pollen wastes the plant's gametes. Most plant–pollinator interactions are generalized in that a single plant species often depends upon several pollinator species that differ in foraging behavior (Schmitt, 1980; Waser, 1983; Herrera, 1987; Waser et al., 1996). Differences in foraging behavior among floral visitors increases the probability that plants will receive outcross pollen. In addition, plants have evolved a number of mechanisms that increase the probability that visitors will move pollen among plants, such as timing of pollen availability (Harder and Wilson, 1994), nectar production (Marden, 1984; Zimmerman,

1988), and floral display (Bronstein and Patel, 1992; Di Pasquale and Jacobi, 1998). While generalization represents one way diverse selection pressures acting on plants and pollinators can result in mutual benefit, there are some highly specialized plant–pollinator interactions in which the fitness of both plant and pollinator depend upon effective pollination. In these cases, due to the dependence of both partners on pollination, one would expect less conflict between optimal dispersal of pollen for the plants and pollinator behavior.

Yuccas and yucca moths are an example of an obligate mutualism in which a pollinator's fitness directly depends upon its efficacy as a pollinator. A female yucca moth oviposits into the ovary of a yucca flower, then actively pollinates the flower by taking pollen that she has collected and pushing it onto the stigma. Her larvae feed on seeds in the developing fruit and will die if the flower fails to mature into fruit. Yuccas are exclusively dependent upon the pollination services of yucca moths (but see Dodd and Linhart, 1994). Due to resource limitation, usually over 80% of the flowers are abscised on an individual plant (Schaffer and Schaffer, 1979; Stephenson, 1981; Udovic, 1981; Addicott, 1986), and competition among flowers differing in pollen quantity and quality significantly affects which flowers become fruit (Aker and Udovic, 1981; Udovic, 1981; Fuller, 1990; Pellmyr and Huth, 1994; Richter and Weis, 1995; Huth and Pellmyr, 1997). Yuccas are self-compatible (Webber, 1953; Wimber, 1958; Aker and Udovic, 1981; James et al., 1993; Dodd and Linhart, 1994; Pellmyr et al., 1997; Richter and Weis, 1998), but flowers cannot autogamously self. Therefore, all selfing occurs from moths transporting pollen within or among flowers on a plant.

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Opportunity for geitonogamy exists in that yuccas have a hermaphroditic breeding system, many flowers are open at the same time within an inflorescence, and anthers within a flower dehisce the same evening that stigmas are receptive. However, self-pollinated flowers have a lower probability of setting fruit compared to outcrossed flowers in populations of *Hesperoyucca whipplei* (Aker and Udovic, 1981; Richter and Weis, 1998), *Y. glauca* (Fuller, 1990), *Y. elata* (James et al., 1993), and *Y. filamentosa* (Huth and Pellmyr, 1997; Huth and Pellmyr, 2000), thus self-pollen contributes little to successful plant reproduction when in competition with outcross pollen. Selective abscission of self-pollinated flowers can affect yucca moths because larvae die in abscised flowers. For this reason, the survival rate of larvae from moths that perform outcross pollinations should be higher.

The idea that yucca moths would benefit from performing primarily outcross pollinations has been suggested by several authors for over a century (Darwin, 1881 in F. Darwin, 1903; Aker and Udovic, 1981; Abrahamson, 1989; Bertin, 1989; Powell, 1992; Addicott and Tyre, 1995; Fleming and Holland, 1998; Richter and Weis, 1998), but data on pollen collection and subsequent pollination behavior have been limited due to the difficulties of gathering an adequate number of observations of female moths. Determining the frequency with which moths deposit self-pollen requires observing both pollen collection and pollen deposition. Pollen collection behavior is rarely observed (Addicott and Tyre, 1995; Pellmyr, 1997); in one study, pollen collection constituted only 0.7% of the total activity time (Pellmyr, 1997). Information on the fate of pollen collections has also been limited. In a behavior study of a *Tegeticula* species that pollinates *Y. kanabensis*, Addicott and Tyre (1995) observed 70 female moths, only five of which collected pollen, and each of these moths left the plant after collecting pollen. The only extensive data set gathered for any yucca moth species was a 3-yr study in one population of *T. yuccasella* on *Y. filamentosa*. Seventy-eight pollen collections were documented, and 51% of these pollen collections were followed by oviposition and pollination on the same plant (Pellmyr et al., 1997). Despite the surprisingly high frequency of self-pollination events, the outcrossing rate of seedlings from open-pollinated fruit was near 100%. One possible explanation for the discrepancy between moth observations and high outcrossing rate is that moths selectively use pollen from their pollen load such that deposition of self-pollen is limited.

The purpose of this study was twofold. First, we test the assumption that moths collecting pollen and pollinating on the same plant are actually depositing self-pollen by tracking pollen dispersal using dyes as pollen analogues. Second, pollinator behavior can potentially vary among populations; therefore we have studied moth movement of pollen across several populations of *Y. filamentosa* to assess the frequency of self-pollen deposition by *T. yuccasella*. Specifically, we asked three questions relating to the movement of *Y. filamentosa* pollen by yucca moths: (1) How frequently do moths deposit self-pollen on *Y. filamentosa* flowers in five populations? (2) What distance do moths typically move pollen? (3) How do dye-based pollen transfer estimates compare

with the level of genetic outcrossing in viable seeds collected from open-pollinated fruit?

MATERIALS AND METHODS

Study species—The pollinator *Tegeticula yuccasella* (Riley) sensu stricto (Lepidoptera: Prodoxidae) is a member of a large species complex (Pellmyr, 1999). It was the only pollinator species observed at all sites in this study. Moths become active around dusk and remain active for 2–4 h. A female collects the sticky pollen with unique maxillary tentacles and compacts it into a loose pellet that is kept under the head. After a female has collected pollen, she oviposits into a yucca ovary and then takes some of the pollen from her batch and actively pushes it into the stigmatic cavity. A moth may oviposit into the ovary and pollinate a flower several times before walking or flying to another flower to repeat the procedure. Adult moths typically live <5 d (Kingsolver, 1984) and may drink water and trace amounts of nectar that sometimes collect at the ovary base, but otherwise do not feed as adults (Riley, 1892; Pellmyr, 1999).

Yucca filamentosa L. (Agavaceae) is a perennial native to the coastal areas of the southeastern United States and has been naturalized at least since the 1800s in the eastern United States (Riley, 1892; Trelease, 1902; Gleason and Cronquist, 1991). Plants can reproduce both clonally, forming clusters of rosettes, and sexually through seed production. Each rosette can produce 100–475 flowers on a single, 1–2 m tall paniculate inflorescence. Flowers open in the evening; anthers dehisce just before dusk, and stigmas are receptive upon flower opening. Flowers on an inflorescence open over the course of 10–20 d, and each flower is receptive to pollen for 1–2 d, but 2nd-d flowers are almost invariably rejected by pollinators. Capsular fruits mature and dehisce 6–8 wk after pollination, producing up to 300 seeds per fruit.

Study sites—Female moth behavior was studied during 1998 in five *Y. filamentosa* populations located in central Tennessee, USA (36°02' N, 86°22' W) and in Cincinnati, Ohio, USA (39°06' N, 84°30' W). In Tennessee, moth activity was measured throughout the flowering season in three populations: Harding Glade (Rutherford County) is a secluded cedar glade that had 57 flowering stalks, Vine (Wilson County) is a roadside population with 50 flowering stalks near the village of Vine, and Jones Mill Road (Rutherford County) is a gas pipeline clearing that had 70 flowering stalks. Distances between the Tennessee sites ranged from 6 to 12 km. In Ohio, moth activity was monitored for two nights at each of two planted populations in Cincinnati, Spring Grove Arboretum and Cincinnati Zoo. Observations were made at the beginning of the flowering period at Spring Grove Arboretum in an area containing 35 flowering stalks, a subset of a much larger population. At the Cincinnati Zoo, observations were made toward the end of the flowering period in an area with 26 flowering stalks. These sites are separated by 3 km. Dates of observation are provided in the legend of Table 1.

Outcrossing rate measured via moth behavior—We used fluorescent dye as a pollen analogue to track pollen movement by *T. yuccasella*. The yucca-yucca moth system is well suited for using dyes in that pollen must be pushed into the stigmatic cavity, therefore insects casually walking across the style will not result in dye being placed in the stigmatic region. All freshly opened flowers (mean per plant per night \pm 1 SE = 15.2 \pm 5.6 flowers) were marked on a focal plant with one dye color. For each evening of observation, a total of six focal plants were each marked with a different color of dye. Anthers were dusted with dye using a cotton swab that had been dipped into a vial with the appropriate dye. Anthers were marked once flowers had opened for the evening, between 1930 and 2030, but before moths became active. Moth activity was greatest between 2030 and 2200. Once we observed that all moths were resting in the flowers and no longer ovipositing and pollinating, we checked all inflorescences in the local population for dye transfers to the stigma with a black light. For each dye

TABLE 1. Frequency of self-pollen deposition by yucca moths based on dye transfers. Dates of observation for each site in 1998: Vine (29 May, 31 May, 3 Jun, 6 Jun, 8 Jun, 10 Jun, Harding Glade (6 Jun, 12 Jun, 15 Jun, 17 Jun), Jones Mill (1 Jun, 6 Jun, 13 Jun, 16 Jun), Cincinnati Zoo (20 Jun, 21 Jun), Spring Grove (23 Jun, 25 Jun). Two flowers received both self and foreign dye, and this was counted as 1 selfing and 1 outcrossing. One flower received two different colors of foreign dye, and this was recorded as one outcrossing event because we could not detect when two selfing events occurred. Standard errors were calculated using the binomial distribution.

Site	No. of dye transfers	No. of nights of observation	% selfing (mean \pm 1 SE)
Tennessee			
Vine	118	6	66 (4.3)
Harding Glade	38	4	50 (8.1)
Jones Mill Road	39	4	54 (8.0)
Ohio			
Cincinnati Zoo	51	2	33 (6.6)
Spring Grove	24	2	71 (9.3)
Total	270	18	55 (3.0)

transfer, we recorded the distance between the source and recipient plant and the number of flowering plants within the radius of this distance (potential recipients). Potential recipients were ranked; for example, a rank of 1 means that the flowering stalk nearest the dye source received dye and a rank of 5 means that there were four flowering stalks that were closer to the source plant than the recipient. Moths showed no behavior change when encountering marked flowers compared with unmarked flowers (personal observation). Moths preferentially visit 1st-d flowers, but sites were marked every other evening to make sure that new dye transfers were not confused with previous dye transfers. On most 2-d and all 3-d-old flowers, petals and anthers wilt such that it was easy to distinguish first day flowers from older flowers. The variance and standard error of self-dye transfers were estimated using the binomial distribution (Zar, 1996). The G statistic for goodness of fit was used to determine whether there were significant differences among sites in the frequency of self-pollen deposited by moths.

Outcrossing rate measured via genetic markers—We used protein electrophoretic markers from leaf tissue of seedlings and maternal plants to compare outcrossing rates with those derived from dye transfers. Fruits and maternal leaf tissues were collected at Vine because it was the major collection site for behavioral data. At Vine, 82% of the flowering stalks produced fruit, with 4.7 ± 3.8 (mean \pm 1 SE) fruits per rosette (median = 3 fruits/rosette). To ensure getting enough seeds for germination, we collected up to six fruits from each of 12 plants that had three or more fruits at the Vine site. Fruits were collected when ready to dehisce, with collection dates ranging from 27 July to 18 August 1998. Fruits were kept dry and stored in paper bags for \sim 1 mo. Twenty-five viable seeds were haphazardly chosen from each maternal plant and soaked in water for 48 h, then placed on wet filter paper in petri dishes. Seeds were kept moist in dark conditions until the first seeds had germinated (Baskin and Baskin, 1998), then brought into the light and planted in soil once the cotyledon and roots were 2 cm long. Most seeds germinated within 3 wk and had grown enough leaf tissue to collect for protein electrophoresis by 4 wk after planting. Basal leaf tissue (3 cm²) from maternal plants and leaf tissue from seedlings (1.5 cm²) were used for grinding. The difference in amount of leaf tissue reflects the ease of extracting soluble protein from seedling leaf tissue compared to the fibrous adult leaves.

Maternal and seedling genotypes were determined using the starch gel electrophoretic methods described in Pellmyr et al. (1997). Modifications from these methods included using morpholine-citrate buffer

TABLE 2. Distance between dye source and recipient. Only outcrossing events were included in the analysis.

Site	N	Range (m)	Median distance	Mean distance (\pm 1 SE)
Vine	39	1.6–48.9	11.0	18.3 (2.8)
Harding Glade	19	1.2–21.1	2.9	4.4 (1.0)
Jones Mill	17	0.9–50.6	12.9	13.7 (3.0)
Spring Grove	34	0.3–6.7	3.8	3.9 (0.3)
Cincinnati Zoo	7	1.3–36.5	7.8	11.6 (4.6)

pH 8.2 (Werth, 1985) for both electrode and gel buffer for 6-PGD, IDH, and SKDH, and using poulik buffer pH 8.7 (Werth, 1985) for PGI. Four polymorphic loci were scored, *6-Pgd-1* (E.C. [Enzyme Commission] 1.1.1.44), *Idh-1* (E.C. 1.1.1.42), *Skdh-1* (E.C. 1.1.1.25), and *Pgi-2* (E.C. 5.3.1.9) for up to 25 offspring per maternal parent. We used Ritland's multilocus mating system program (MLTR) to estimate outcrossing rate (t) at the population level (Ritland, 1990). Standard deviations were based on 250 bootstraps where family was the unit of resampling for the population outcrossing rate.

RESULTS

The proportion of dye transfers within plants ranged from 33 to 71% across the five populations, with an overall mean and median of 55% selfing (\pm 3.0) (Table 1). The frequency of self-pollen transfers was not significantly different among the three Tennessee sites ($G = 3.99$, $df = 2$, $P > 0.1$) where moth behavior was tracked over the course of the flowering season. The frequency of self-pollen transfers differed between the Ohio sites ($G = 9.2$, $df = 1$, $P < 0.002$), but these sites were tracked for only part of the flowering season.

The distance of dye transfers from the dye source to recipient plant ranged from 0 to 50.6 m (Table 2; Fig. 1A). The distribution for dye transfer distance was leptokurtic. The mean distance (\pm 1 SE) of pollen transfer across all sites, including both self and outcross transfers, was 4.66 ± 10.23 m with a median of 0 m (Fig. 1B). The number of potential recipients between the source and recipient ranged from 0 to 49, with an overall mean across all sites of 5 ± 8.74 plants and median of 0 (Table 3). There was no significant difference among the three Tennessee sites in terms of mean dye transfer distance as measured by number of potential recipients (Kruskal-Wallis chi square = 0.77, $df = 2$, $P > 0.68$).

Mean seed germination rate was $85 \pm 15.1\%$ (\pm 1 SE; median 92%). Protein electrophoretic data for offspring from nine of the 12 plants were included in the outcrossing analysis; for one plant nearly 100% of seeds were eaten in all fruits that were collected, and for two other families no seeds survived long enough to be available for electrophoresis. The multilocus outcrossing estimate, based on 130 seedlings, was 0.94 ± 0.06 (\pm 1 SE) for the Vine Road population (Table 4), which is not significantly different from 1.0 ($t_{2,8} = 1.02$, $P = 0.339$). The mean single-locus outcrossing estimate was not significantly different from the multilocus estimate ($t_{2,8} = 1.85$, $P = 0.101$), indicating random outcrossing in that offspring genotypes were not strongly structured due to biparental inbreeding or microhabitat selection (Shaw, Kahler, and Allard, 1981). The outcrossing estimate based on viable seeds (94%) was significantly different from the

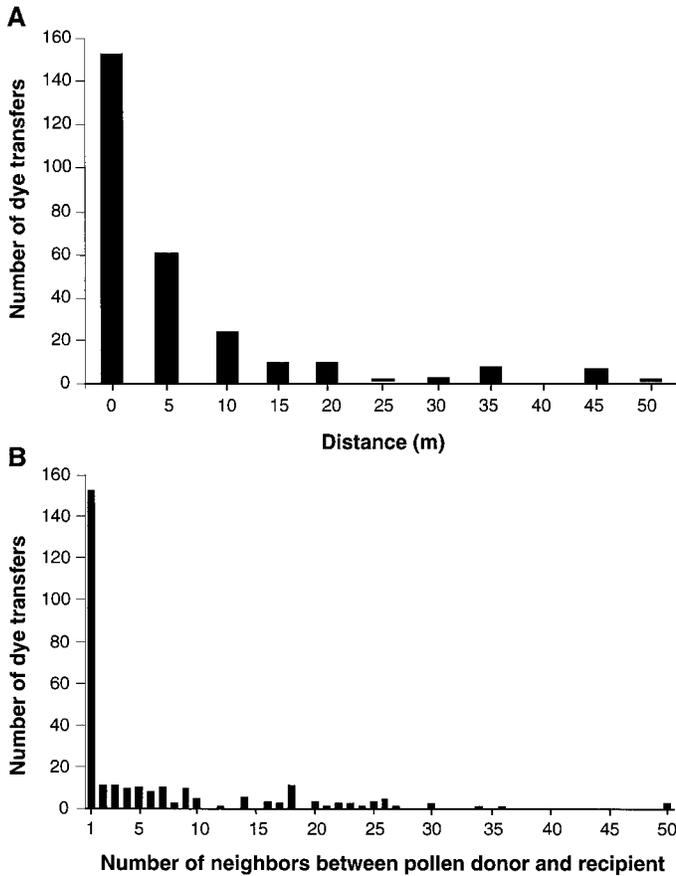


Fig. 1. The number of dye transfers by *Tegeticula yuccasella* in five *Yucca filamentosa* populations by (A) distance between pollen donor and recipient and (B) number of flowering neighbors between the pollen donor and recipient.

outcrossing estimate based on dye transfers (34%) at Vine Road ($t_{2,117} = 13.95, P < 0.0001$).

DISCUSSION

Yucca moths and yuccas represent one of a few obligate interactions in which pollination behavior is directly linked to the fitness of both pollinator and plant, yet tracking moth movement of pollen has been difficult. Previous studies of pollen dispersal by yucca moths have relied on direct observation of yucca moth behavior, which has several limitations in providing information about the frequency of self-pollination. As mentioned earlier, the main limitations are that pollen collection is rarely observed (Addicott and Tyre, 1995; Pellmyr, 1997), and usually only the first pollination is observed after pollen collection. In addition, moths may carry mixed pollen loads, so observing self-pollination may not accurately reflect the type of pollen the moth is actually depositing on the stigma. Using dyes to track pollen movement is an efficient and economic way to assess these limitations. Dyes make it possible to track the fate of pollen collections over an entire evening, provide a way to determine whether behavioral selfings result in the deposition of self-pollen, and do not obscure pollinator behavior by relying on assays of seedlings that may experience post-pollination selection.

TABLE 3. Number of flowering neighbors between dye source and recipient.

Site	N	Range	No. flowering neighbors (median)	No. flowering neighbors (mean ± 1 SE)
Vine	39	1–35	4.0	11.0 (1.9)
Harding Glade	19	1–49	7.0	8.9 (2.5)
Jones Mill	17	1–29	13.0	12.3 (2.2)
Spring Grove	34	3–25	15.5	14.0 (1.4)
Cincinnati Zoo	7	3–9	5.0	5.0 (0.8)

We found that dye transfers within plants were common at all of the sites studied in Ohio and Tennessee, suggesting that yucca moths frequently deposited self-pollen on the stigmas of *Y. filamentosa*. Pollen was moved primarily among flowers within plants and between plants in close proximity to each other; 80% of the dye transfers occurred within an 8 m radius of the source plant. The frequency of self-pollen deposition did not differ across the Tennessee sites. The difference in frequency of self-pollen deposition between the Ohio sites probably reflects small sample size and differences in sampling time more than biological differences in moth behavior.

Dye transfer data have limitations in that pollinators may respond differently to dyes or the dye may adhere to the pollinator’s body such that dye deposition does not accurately reflect pollen deposition (Waser and Price, 1982; Thomson et al., 1986; Craig, 1989). We observed yucca moths actively collecting dye and placing the dye particles underneath their heads in a manner that was similar to their pollen collection behavior. Furthermore, the correspondence of dye movement with direct observations of pollination behavior by individual female moths (Pellmyr et al., 1997) indicates that *T. yuccasella* commonly deposit self-pollen. Below we review patterns of pollen dispersal and outcrossing rates, address why yucca moths commonly perform self-pollinations, and compare yucca moth behavior to that of other specialized plant–pollinator systems.

Pollen deposition and realized gene flow—The discrepancy between 34% outcross dye transfers and the 94% outcrossing rate in viable yucca seeds at the Vine site is striking. Pollen movement often appears to be more restricted than genetic measures of sired offspring (realized gene flow) (Schaal, 1980; Levin, 1981; Campbell, 1991; Fenster, 1991; Broyles and Wyatt, 1991; Krauss, 1994; Karron et al., 1995; Nason, Herre, and Hamrick, 1998). For example, in *Chamaecrista fasciculata* (Caesalpiniaceae), which is pollinated by several bee species, the mean pollinator flight distance was half that

TABLE 4. Outcrossing estimate for *Yucca filamentosa* seedlings at Vine Road. Standard deviations are based on 250 bootstraps.

Outcrossing estimates	Mean (± 1 SD)
Multilocus <i>t</i>	0.94 (0.06)
Single-locus <i>t</i>	0.83 (0.06)
Difference ($t_M - t_S$)	0.11 (0.06)

of the mean gene dispersal distance (0.3 vs. 0.57 m) (Fenster, 1991). In *Y. filamentosa*, the near 100% outcrossing rate, in part, reflects selective maturation of outcrossed fruits (Aker and Udovic, 1981; Stephenson, 1981; Richter and Weis, 1998; Huth and Pellmyr, 2000), strong post-pollination selection from differences among pollen genotypes in fertilization success (as observed in *Delphinium nelsonii* by Waser and Price, 1993), or a combination of both factors.

Some of the difference between dye transfers and outcrossing rate may be attributable to estimate biases inherent to measuring pollen dispersal and realized gene flow. Dye transfers may underestimate outcrossing due to the difficulty of detecting long-distance pollen transfers (Campbell, 1991; Ellstrand, 1992). In this study we were limited to detecting outcrossing events that occurred within the local population and, consequently, may have missed some longer distance transfers. We did record a few long-distance pollen dispersal events, up to 50 m, but these transfers made up <1% of all observed dye transfers. Thus, even longer distance transfers that occur among populations are unlikely to raise the estimate of outcross pollen deposition by more than a few percent, assuming that the distribution is not bimodal. Taking this potential bias into account, within-plant dye transfers still represented >50% of all dye transfers across the five sites. Our estimate of outcrossing based on the seedlings may be inflated due to inbreeding depression that affects seed germination or survival. A previous study of inbreeding depression at the Vine population has shown relatively small differences in germination rate between hand-pollinated self and outcross seed at the Vine site (mean \pm 1 SE: selfed seed $54 \pm 16.7\%$ and outcrossed seed $61 \pm 10.9\%$) (Huth and Pellmyr, 2000). In their study, Huth and Pellmyr found that most inbreeding depression was observed in seedling survival. To minimize the effects of inbreeding depression in the present study, seedlings were genotyped as soon as enough leaf tissue was present (30–70 d). In addition, the outcrossing estimate at the Vine site (94%) is comparable to another study that estimated 96% outcrossing in an Ohio *Y. filamentosa* population (Pellmyr et al., 1997). Both outcrossing estimates are not significantly different from 1.0, indicating that even though moths frequently use self-pollen to pollinate, this pollen is contributing little to viable seed production. Moreover, a study of allozyme diversity in 18 *Y. filamentosa* populations showed high genetic diversity within populations and relatively low G_{ST} values across populations (Massey and Hamrick, 1998). This pattern of genetic diversity further provides evidence that *Y. filamentosa* is predominantly outcrossed and that long-distance gene flow occurs frequently enough to reduce the effect of genetic drift (Massey and Hamrick, 1998).

Why do moths self-pollinate flowers?—The level of disparity between pollen dispersal and realized gene flow is somewhat unexpected in the yucca–yucca moth interaction. Unlike many plant–pollinator interactions, both the seeds of yuccas and the progeny of yucca moths have a higher probability of survival when flowers receive outcross pollen. Flower abscission is a major factor in larval mortality (Aker and Udovic, 1981; Pellmyr and Huth,

1994), and the main factors that increase the risk of floral abscission are self-pollen (Richter and Weis, 1998; Huth and Pellmyr, 2000), low pollen amount (Pellmyr and Huth, 1994; Huth and Pellmyr, 1997), and feeding by the beetle *Carpophilus melanopterus* (Huth and Pellmyr, 1997). Hand-pollinations done at the Vine site, in which flowers were pollinated with similar amounts of either self- or outcross pollen, showed that outcrossed fruits were three times more likely to be retained compared to selfed fruit (Huth and Pellmyr, 2000). For yucca moths, depositing self-pollen undermines progeny survival by wasting pollen and eggs that could be used for outcross pollinations that have a higher probability of leading to retained fruit. Despite the disadvantages, one reason that selfing may continue to be prevalent is that selection against self-pollination behavior in individuals may be weak. The strategy of maximizing the number of ovipositions across flowers may outweigh the cost of time and risk involved in locating more distant flowers. The cost of self-pollination is likely to be density dependent in that at low moth densities selfed fruits have a higher probability of being retained due to lower competition with flowers receiving outcross pollen. In addition, we observed some flowers being pollinated by multiple moths, which would increase the chances that a flower would receive outcross pollen. The ability of plants to selectively use outcross pollen may reduce the strength of selection against moths that deposit self-pollen by weakening the connection between an individual's behavior and retention of that flower to fruit.

Plant–pollinator relationships in which pollinators “should” benefit from cross-pollination—There are three other obligate plant–pollinator associations in which pollen deposition behavior affects both pollinator and plant reproduction: figs and fig wasps, senita cacti and senita moths, and globeflowers and globeflower flies. In each case, pollinators oviposit into flowers and rely on those flowers to develop into fruits to provide food for their larvae. Specific information regarding the frequency of self-pollen transfer is lacking in these systems, therefore we compare the plant mating systems of these groups and discuss the opportunity and cost of self-pollination behavior for these pollinators.

Most species of fig (*Ficus* sp.) are pollinated by their own species of pollinating wasp (Hymenoptera: Agaonidae) (Ramirez, 1970; Bronstein, 1989; Rasplus, 1996; Anstett, Hossaert-McKey, and Kjellberg, 1997). Female wasps gather pollen from their natal fig, then emerge from the inflorescence and seek a flower that is in female phase. Female wasps usually only pollinate and oviposit into one flower (Janzen, 1979; Bronstein and Hossaert-McKey, 1996). In some fig species, females occasionally manage to escape the fig inflorescence and visit another flower (Bronstein and Hossaert-McKey, 1996; Gibernau et al., 1996), although oviposition success is much lower in second flower visits (Gibernau et al., 1996) and pollination success in second flowers has not been reported. Unlike yuccas, which are hermaphroditic, half of all fig species are functionally dioecious, and even in monoecious figs the timing of female and male phase within trees is such that opportunity for selfing is rare (Janzen, 1979; Bronstein and McKey, 1989; Anstett, Hossaert-

McKey, and Kjellberg, 1997). There are a few fig species in which flower development is more asynchronous within trees, providing some opportunity for selfing (Bronstein and Patel, 1992), although the extent to which this results in self-pollination has not been reported. Overall, compared to yuccas and yucca moths, the opportunity for wasps to self-pollinate figs is less, and because an individual wasp usually only oviposits into one flower, the cost of wasting eggs and pollen on a flower that fails to mature into fruit is greater. Realized gene flow is very high, especially considering the short life span of the wasps; paternity analysis in three fig species indicated that pollen dispersal routinely occurs over distances of 5.8–14.2 km (Nason, Herre, and Hamrick, 1998). Because wasps are essentially forced to outcross in seeking suitable oviposition sites, one would predict that mean distance of pollen dispersal may more closely match the mean distance of realized gene flow.

Senita moths (*Upiga virescens*) actively collect pollen, then pollinate and oviposit on flowers of the senita cactus (*Lophocereus schottii*). Female senita moths are not the exclusive pollinator of the senita cactus, but do account for 75–90% of the fruit set (Fleming and Holland, 1998). Female moths lay eggs on petals, and the resulting larvae depend on developing seeds for food. The senita cactus may be self-incompatible as hand-pollinated selfed flowers did not set seed in competition with open-pollinated flowers (Fleming and Holland, 1998). In comparison to yuccas, the plant mating systems of both the senita cactus and figs provide a stronger selection pressure against pollinators that deposit self-pollen.

In the case of globeflowers (*Trollius europaeus*) and globeflower flies (*Chiastocheta* spp.), flies are the exclusive pollinators and the fly larvae depend on seeds for food (Pellmyr, 1989). Pollination occurs incidentally from oviposition behavior and the activity of both male and female flies, including mating and moving within and among flowers (Pellmyr, 1989; Jaeger, 1998). Pollination behavior and oviposition are not as closely coupled in the globeflower-globeflower fly interaction compared to the insects that pollinate figs, senita cacti, and yuccas. The relative frequency with which flies deposit self-pollen is not known, but globeflowers are self-incompatible (Pellmyr, 1989). Thus, self-pollination would contribute nothing to seed set regardless of pollinator density.

For each of these specialized plant-pollinator interactions, the cost of selfing to the pollinator is higher than in the yucca-yucca moth system in that plant mechanisms, such as dioecy, dichogamy, and self-incompatibility, prevent selfed flowers from setting seed. Although globeflower flies do not benefit by selfing, it is unclear how effectively selection would act on the diverse behaviors that result in incidental self-pollination. Self-pollen deposition is not advantageous to either yuccas or yucca moth larvae, but larvae may survive in flowers that have been self-pollinated when outcross pollen is limited or when other moths carrying outcross pollen also visit the flower.

Summary—The results of this study indicate that individual moths self-pollinate *Y. filamentosa* flowers frequently even though such flowers have a lower probability of being retained as fruit. Using fluorescent dyes

enabled us to test whether moths collecting and depositing pollen on the same plant are actually depositing self-pollen and take a first step towards understanding the typical distance of pollen transfers across plants. The dye method also allowed us to gather information on pollen dispersal more efficiently and in more populations. For comparison, information on pollen dispersal collected by direct behavioral observation required combining three years of data from a single population in which each year 3–5 observers would record observations each night throughout the entire flowering season. The yucca-yucca moth relationship differs from other obligate plant-pollinator mutualisms in that yuccas seem to rely on post-pollination mechanisms rather than manipulating pollinator behavior through timing of pollen presentation or other mechanisms that would more strongly select against moths that deposit self-pollen. There is a rich diversity of interactions between different yucca and yucca moth species (Keeley et al., 1984; Addicott, 1986; Powell, 1992; Pellmyr, 1999), and yucca species differ in patterns of floral abortion and retention of selfed flowers (Aker, 1982; Huth and Pellmyr, 1997; Richter and Weis, 1998). More critical examination of pollen dispersal by related species of yucca moths that pollinate different yucca species would help elucidate the extent to which moth behavior has been shaped by the plant's need for gamete dispersal.

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