

VARIATION IN POLLINATOR EFFECTIVENESS IN SWAMP MILKWEED, *ASCLEPIAS INCARNATA* (APOCYNACEAE)¹

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The contribution of a pollinator toward plant fitness (i.e., its “effectiveness”) can determine its importance for the plant’s evolutionary ecology. We compared pollinators in a population of *Asclepias incarnata* (Apocynaceae) for several components of pollinator effectiveness over two flowering seasons to evaluate their importance to plant reproduction. Insects of the order Hymenoptera predominate in *A. incarnata* pollination, but there appears to be no specialization for pollination within this order. Pollinators varied significantly in nearly every component of effectiveness that we measured, including pollen load, removal and deposition of pollen, pollination efficiency (deposition/removal), flower-handling time, and potential for geitonogamy (fractional pollen deposition). The visitation rate of pollinators also varied significantly between years and through time within years. Pollination success and percentage fruit-set of unmanipulated plants in the population also varied significantly between years, and pollination success varied among sample times within years. Most components of effectiveness were weakly correlated, suggesting that the contributions of visitor species toward pollination varied among effectiveness components. Mean flower-handling time, however, was strongly correlated with several components, including pollen removal and deposition, pollination efficiency, and fractional pollen deposition. These findings highlight the significance of pollination variability for plant reproduction and suggest that time-dependent foraging behaviors may play an important role in determining pollinator effectiveness.

Key words: Apocynaceae; *Asclepias incarnata*; flower-handling time; fractional pollen deposition; geitonogamy; generalization; plant reproductive success; pollination; pollinator effectiveness; specialization.

Determining the effectiveness of pollinators can reveal their impact on the ecology and evolution of plant reproduction. We define “effectiveness” as any characteristic of a pollinator or pollinator’s behavior that contributes to its ability to affect plant fitness, including components of both “quality” and “quantity” (Herrera, 1987, 1989; Utelli and Roy, 2000). Effective pollinators can shape the evolution of floral characters (Campbell, 1989; Wilson, 1995; Schemske and Bradshaw, 1999) and the evolution of plant lineages (Grant and Grant, 1965; Stebbins, 1970; Crepet, 1983; Grimaldi, 1991).

Pollination is among the most variable of ecological interactions, which complicates the determination of pollinator effectiveness (Horvitz and Schemske, 1990; Fishbein and Venable, 1996; Waser et al., 1996; Aizen, 2001). Many plants are visited by multiple species of pollinators (Petanidou and Ellis, 1993; Waser et al., 1996), each of which may differ in effectiveness (Primack and Silander, 1975; Schemske and Horvitz, 1984; Fishbein and Venable, 1996). Pollinator effectiveness is also known to change with pollinator abundance across years (Herrera, 1989; Fishbein and Venable, 1996; Fenster and Dush, 2001). Pollinator effectiveness is likely to vary over even shorter time frames, such as within seasons (Ashman and Stanton, 1991; Pettersson, 1991; Totland, 1994), which may alter

patterns of selection for plant traits (Stebbins, 1970; Thomson and Thomson, 1992; Aigner, 2001). Unfortunately, this has seldom been examined.

The small size and large numbers of pollen grains carried by pollinators further complicates the task of measuring effectiveness (Snow and Lewis, 1993). Usually, it is accomplished by decomposing effectiveness into various components, which can include pollen load size (Primack and Silander, 1975; Kephart, 1983; Jennersten, 1984; Liede, 1994), visit duration (Fishbein and Venable, 1996; Pellmyr and Thompson, 1996), visit frequency (Primack and Silander, 1975; Herrera, 1989; Utelli and Roy, 2000), contact with anthers or stigmas (Cartwright, 1993), pollen removal (Young and Stanton, 1990; Conner, Davis, and Rush, 1995), pollen deposition (Thomson, 1986; Herrera, 1987; Pettersson, 1991; Hurlbert et al., 1996), effect on fruit- or seed-set (Spears, 1983; Richards, 1987; Dieringer, 1992), or contributions toward seedling recruitment (Herrera, 2000). Inferences about the role of pollinators in plant ecology and evolution are then drawn from some combination of these measurements. Differences among pollinators in components of effectiveness, however, will alter their relative contributions toward plant reproduction (Wilson and Thomson, 1991; Thomson and Thomson, 1992; Aigner, 2001). Examination of correlations among multiple components (Young and Stanton, 1990) can highlight conflicts in rankings among different effectiveness components. For example, the pollinator species that is most effective at pollen removal may not be the most effective at deposition (Thomson and Thomson, 1992; Aigner, 2001). In addition, correlations among components may yield insights into behaviors that influence effectiveness and suggest hypotheses for future research.

In the present study we examine variation in several components of effectiveness for multiple pollinators of swamp milkweed (*Asclepias incarnata* L.) between and within sea-

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sons. We also examine correlations among effectiveness components to determine the consistency of effectiveness rankings across different measures of effectiveness and to provide insight into behaviors that may influence effectiveness. Finally, we compare variation in pollinator effectiveness to concurrent patterns of variation in pollination success and fruit-set among unmanipulated plants to evaluate the impact of observed shifts in pollinator effectiveness on plant reproductive success.

MATERIALS AND METHODS

Species background and study site—Milkweeds (genus *Asclepias*) have proven to be useful subjects for studying pollination because of their unusual floral morphology (Wyatt and Broyles, 1994). Milkweed pollen grains are dispersed within discrete, aggregated packages, called pollinia. Pollinia are removed from milkweed flowers in pairs (pollinarium) after lodging onto an insect appendage. Pollination is completed when a pollinium slips into the stigmatic chamber of a subsequently visited flower (Wyatt, 1976). Each milkweed flower holds five pollinaria. A single pollinium contains enough pollen grains to ensure full seed-set (Wyatt, Broyles, and Lipow, 2000). In nature, insects are required for successful pollination of milkweeds, and only insects large enough to remove and carry pollinaria are capable of effecting pollination.

Asclepias incarnata is a perennial herb that occurs in wetlands throughout much of the United States (Woodson, 1954). Plants often have multiple stems up to 2 m tall that emerge from a single root crown; each stem has multiple paired umbels with an average of 22 pink flowers that open nearly synchronously. Over its 5-d mean lifespan, each flower produces about 1.5 μ L of nectar each day, with mean sucrose concentration of 30% (C. T. Ivey, unpublished data). This relatively rich nectar resource, available during the late summer (Kephart, 1987) when few other nectar-rich plants are in flower, attracts a broad diversity of insect visitors (Robertson, 1887; Kephart, 1983). As in all milkweeds, nectar is the only reward offered to pollinators.

The population of *A. incarnata* that we studied was located on the University of Virginia's Blandy Experimental Farm (Bowers, 1997) in Clarke County, Virginia, USA (30°04' N, 78°04' W; 190 m a.s.l.). The population was located on the margin of a seasonal pond surrounded by abandoned, early successional pasture. The surrounding landscape was a mixture of pasture, small woodlots, and agricultural crops. A voucher specimen from the population of *A. incarnata* studied is housed in the University of Georgia Herbarium (GA). Voucher specimens of insect species observed are deposited in the University of Georgia Collection of Arthropods (UGCA).

Statistical analyses—Many of the data that we collected involved counts or otherwise violated assumptions of normally distributed errors used in standard parametric statistical tests (Steel and Torrie, 1980). Transformations are sometimes used to mediate such violations, but interpreting the results from such tests can be less satisfying. Moreover, unbalanced designs and random effects can be problematical for many ANOVA tests (Shaw and Mitchell-Olds, 1993; Bennington and Thayne, 1994). To ameliorate these problems, we used the glimmix macro of SAS (Littell et al., 1996; SAS Institute, 1996), which applies an extension of generalized linear mixed model theory (McCullagh and Nelder, 1989). Glimmix allows users to choose a probability distribution appropriate for the data and, by applying a link function that relates the linear model to the data, uses restricted maximum likelihood to estimate variance components (Littell et al., 1996). The model deviance, which is a generalization of error sums of squares in ANOVA, can be used to test model goodness-of-fit; deviance is approximately distributed as a χ^2 with $n - p$ degrees of freedom, where n is the number of observations and p is the number of independent parameters in the model (Littell et al., 1996). Model deviance is corrected by an estimated extra-dispersion parameter, which adjusts the observed conditional error variance according to the assumed distribution (Littell et al., 1996). Random effects are evaluated using a likelihood-ratio test (Self and Liang, 1987). Glimmix iteratively calls PROC MIXED, which permits flexibility in modeling random effects and repeated-measures effects (Verbeke and Molenberghs, 1997). For example, repeated-

measures analyses usually assume a spherical variance-covariance structure (Winer, 1971; Potvin, Lechowicz, and Tardif, 1990), which is often restrictive. PROC MIXED permits users to choose a variance-covariance structure that best fits their data. For additional information about glimmix, PROC MIXED, and generalized linear models, consult McCullagh and Nelder (1989), Littell et al. (1996), and Verbeke and Molenberghs (1997). Details on models fitted to each response follow descriptions of the data collection. We did not report the results of significance tests of covariance components associated with random effects (see Results). These random effects were not the primary focus of our study; test results, however, suggested significant variation among individual plants and individual insects in nearly every model in which they were included.

Data collection—Pollen loads—Insects with larger pollen loads may be able to transfer more pollen between flowers (Primack and Silander, 1975; Willson and Bertin, 1979; Kephart, 1983; Liede, 1994). To estimate the number of pollinia carried by insects visiting swamp milkweed, we haphazardly captured foraging insects and chilled them to inactivity in glass jars on ice. Using headband magnifiers, we then counted the number of pollinia they carried, marked the insects uniquely with acrylic paint, and released them. Pollinia found inside jars after release were added to the pollinium count for that insect. We compared pollinium loads among all visitor species (hereafter "visitor species") using a single-factor generalized linear model. Because the data involved counts, we applied a Poisson distribution to the errors. Although we observed six species of bumble bees (*Bombus* spp., Hymenoptera: Apidae), all were uncommon (1.1% of total visits, see below). We therefore combined them into a single category for purposes of statistical analyses here and throughout the study.

Visitor abundance—Common pollinators can contribute more toward pollination success of a plant, even if less common species are better at transferring pollen on a per-visit basis (Herrera, 1987; Jennersten and Morse, 1991; Thomson and Thomson, 1992). To estimate population-level abundance of visitors, we recorded the frequency of arrival of all visitor species to a random subset of 13 plants in 1995. Eight of these plants flowered again in 1996; we randomly chose four additional plants for a total of 12 focal plants in 1996. Observations were confined to these focal plants to control variance due to spatial variation in pollinator abundance (Herrera, 1995) and among-plant variation in pollinator preference for plants. We observed focal plants at three sample times during the 1995 flowering season and four sample times during 1996. These times were chosen to span the entire flowering season for the population of *A. incarnata* during those years. Each sample time consisted of two consecutive days during which all focal plants were observed on multiple occasions in a randomized sequence. Each observation of a plant occurred at a distance of 1–2 m and lasted for 10 min, during which we counted the number of each species of visitor arriving to forage on plants. All observations occurred between 0730 and 1730 EDST, which captured the period of peak pollinator activity for *A. incarnata*.

As is often observed in plants (e.g., Willson and Bertin, 1979; de Jong, Klinkhamer, and van Staaldunin, 1992; Klinkhamer et al., 1992), we found visit frequency to be a positive function of flower number (see Results). We therefore divided the number of visits in each 10-min observation by the number of open flowers on each plant to remove the confounding effect of flower number on visit frequency. We compared visits per flower among all visitor species and among sample times using a repeated-measures generalized linear mixed model in which "visitor species," "time," and "visitor species \times time" were fixed effects and "individual plant," the random effect, was the subject of repeated measurements across sampling times. The best model fit was achieved when the covariance structure was permitted to vary among plant subjects ("variance components"; SAS Institute, 1996). During observations, we were unable to distinguish individual insects consistently, so "individual insect" was not included as a random effect in the model. Data were overdispersed, so we applied a Poisson error distribution to the model. Because sample times within a year are not likely to be statistically independent compared with sample times in different years, we conducted separate analyses for each year. In addition, we fit a similar model to compare visit frequency

between years; for this analysis, "year" was included as a fixed effect in place of "time."

Visit duration—Pollination success can increase with the duration of a visit, but so can the potential for geitonogamous self-pollinations within a plant (Robertson, 1992; Klinkhamer and de Jong, 1993). We compared two components of visit duration among insect species foraging naturally on unmanipulated plants as an additional aspect of pollinator behavior that may contribute to effectiveness: (1) the number of flowers visited per plant and (2) the amount of time spent per flower. We focused our observations on the four most common visitors (see Results). We also collected observations on honey bees (*Apis mellifera*, Hymenoptera: Apidae) and bumble bees, even though these visitors were uncommon in our study (see Results). Honey bees and bumble bees are common visitors to *A. incarnata* in other populations (Robertson, 1887; Kephart, 1983); our study therefore provides comparative data. Hereafter, we refer to these six taxa as the "focal visitors."

From a distance of 1–2 m, we followed individual focal visitors from the time they began to forage on a plant and counted the total number of flowers they visited until the insect moved to a different plant. We also recorded the time spent foraging on each plant. If a focal visitor was unmarked, we collected and marked the insect as described above. We used a mixed-model analysis of covariance to compare the number of flowers visited among focal visitors. "Focal visitor" was the main effect and, because the number of open flowers on a plant typically influences the number of flowers visited (Robertson, 1992; Klinkhamer and de Jong, 1993), "number of open flowers" was included as a covariate. Random effects included "individual insect" and "individual plant." The data involved counts, so we permitted Poisson-distributed errors. To estimate foraging time per flower, we divided the amount of time spent foraging on the plant by the total number of flowers visited. We compared time per flower among species using a generalized mixed model in which "focal visitor" was the fixed effect and "individual insect" and "individual plant" were random effects. The best model fit was achieved when a Poisson distribution was applied to the errors.

Pollen transfer—One of the most important components of pollinator effectiveness is the ability of a visitor to transfer pollen between flowers (Schemske and Horvitz, 1984; Thomson and Thomson, 1992). In 1996 and 1997, we characterized the ability of the focal visitors to remove and deposit pollinia. Because honey bees and bumble bees were uncommon in our study population, we conducted supplemental experiments on these taxa at a nearby population. Observations from both years and both sites were pooled for analyses to increase sample sizes. In doing so, we assumed that differences among insect species in pollen removal and deposition outweighed differences between sites (for honey bees and bumble bees) or between years (for all taxa) within species. This would be true if variation in rates of removal and insertion of pollinia are influenced primarily by characteristics of body shape or species-specific foraging behavior as opposed to site-specific or season-specific behaviors (but see Thomson and Thomson, 1992; Fishbein and Venable, 1996).

We covered individual umbels in bud, initially using white bridal veil mesh, and waited until flowers had opened before making observations. After several days of these experiments, however, we observed some bees foraging on the open flowers enclosed by the mesh bags. We collected three of these "control" umbels and found 0.74 (0.14) pollinaria removed and 0.22 (0.09) pollinia inserted per flower (means and 1 SE). After this discovery, we used featherweight pellon bags to exclude insects. We observed no removals or insertions of pollinia in control flowers covered with pellon, and we never observed bees foraging on umbels covered with pellon. Furthermore, we excluded from analyses all earlier observations for which we had used mesh bags to exclude insect visitors. Both of these materials change internal humidity and temperature minimally (Wyatt, Broyles, and Derda, 1992). The bags were closed around the base of the peduncle with plastic-coated wire.

When flowers had opened, we removed the bags and inspected them for pollinaria. Any pollinaria attached to the bag upon removal were subtracted from the total removals counted for that observation. We then allowed an individual, freely foraging focal visitor to visit the flowers, recorded the total

number of flowers it probed, and the time it spent foraging on the umbel. Visitors arrived carrying pollinia collected from other plants, although we were unable to count the number of pollinia carried prior to visiting experimental umbels. When the visitor stopped foraging on the experimental umbel, we captured it, counted the number of pollinia it carried, marked it and released it as described above. Focal visitors that escaped before capture were categorized as "unidentified." All unidentified observations within a visitor species were regarded as a single individual in analyses. Experimental umbels were immediately collected, stored in 70% ethanol, and each flower was dissected and scored for the number of pollinia removed and inserted.

We compared the number of pollinia removed and inserted per flower visited among focal visitor species. We also compared "transfer efficiency" (i.e., insertion/removal of pollinia). Identification of the source of pollinia carried by freely foraging visitors was not possible, therefore both self and outcross pollinia may have been inserted during these experiments. Likewise, transfer efficiency may have included a combination of within-flower and geitonogamous pollinations, in addition to outcross pollinations. Estimates of transfer efficiency or pollinium insertion, however, provide no information about geitonogamy in isolation from other data. The extent of self-pollination will depend in part on the frequency of pollinium turnover (see below). We also compared the foraging time per flower on experimental umbels by dividing foraging time per umbel by the number of flowers probed. The best model fit for all models was achieved when we applied Poisson error distributions to the models. "Focal visitor" was the fixed effect, and "individual insect" and "individual plant" were included as random effects.

Geitonogamy potential—Pollinators that are adept at transferring pollen between flowers may have a detrimental impact on plant fitness if a high proportion of pollinations are geitonogamous (Broyles and Wyatt, 1993; Klinkhamer and de Jong, 1993; Harder and Barrett, 1995). We used data from the pollen transfer experiments to compare focal visitors with respect to their potential for geitonogamous self-pollination. Specifically, we compared "fractional pollen deposition" (k_i ; de Jong, Waser, and Klinkhamer, 1993), or the fraction of the pollen load deposited on each flower visited, among focal visitors. This approach involved several simplifying assumptions (de Jong, Waser, and Klinkhamer, 1993). In particular, we assumed that the number of pollinia carried by visitors was at equilibrium ("pollinator saturation" sensu Crawford, 1984; Robertson, 1992; de Jong, Waser, and Klinkhamer, 1993), that deposition and removal of pollinia were independent of the number of pollinia present in the flower (Lertzman and Gass, 1983; de Jong, Klinkhamer, and van Staalduinen, 1992), and that k_i did not vary with number of flowers visited (but see Morris et al., 1994). These assumptions, especially the second (Wyatt, 1978), may not withstand careful scrutiny, which could bias our estimates of k_i . In the absence of more rigorous, direct estimates, however, this model permitted a preliminary exploration of variation among focal visitors in their potential for geitonogamous pollination. We calculated k_i as $k_i = (I_i/F_i)/L_i$, where I_i is the number of pollinia inserted per umbel during an experimental trial for visitor species i , F_i is the number of flowers visited per umbel by visitor species i , and L_i is the number of pollinia carried by visitor species i during a foraging bout (de Jong, Waser, and Klinkhamer, 1993). For a given number of flowers visited, visitor species with a higher value of k_i will have a higher rate of pollinium turnover and will therefore be more likely to deposit pollen geitonogamously.

We compared k_i among focal visitor species using a generalized linear mixed model. "Focal visitor" was the main effect in the model and "individual insect" was included as a random effect. Because variation among individual plants did not significantly explain variation among insects in pollinium transfer in preliminary analyses, we did not include "individual plant" as a random effect in this model. The data were skewed left, with a long tail, so a Poisson distribution was assigned to the errors.

Correlations among effectiveness components—To compare the consistency of focal visitors' effectiveness ranks across different components of effectiveness and to explore how pollinator behavior may influence effectiveness, we calculated Spearman's rank-sum correlation coefficients (Steel and Torrie, 1980) between all taxon-level effectiveness components.

Plant pollination success and fruit-set—To characterize patterns of plant reproductive success, we measured pollination success and fruit-set of unmanipulated plants in the population. Before each 2-d sample time during which we observed visitor abundance (see above), we marked the peduncles of all umbels with open flowers on all plants in the population ($N = 46$ in 1995, $N = 43$ in 1996, $N = 30$ plants that were used both years) using a small piece of colored tape. After observing visitor abundance, we collected a random sample of 15% of the flowers from 15% of the umbels on each plant (i.e., 2.25% of all flowers in the population). To accomplish this, we used a combined stratified and two-stage sampling design (modified from Thompson, 1992), in which each plant was treated as a stratum and flowers within umbels were sampled on all plants. Sampled flowers were stored in 70% ethanol in microcentrifuge tubes until they could be examined for pollen removal and deposition. Each flower was inspected and scored for pollinium removal and insertion as described above. We compared per-flower pollinium removal and insertion as well as “capture efficiency” (insertion/removal) among sample times within years using generalized linear mixed models, in which “sample time” was the fixed effect and “individual plant” and “umbel within plant” were included as random effects. The best fit was achieved with a Poisson error structure to the models. We also compared overall pollination success between years using a similar model in which “year” was the fixed effect and “individual plant” and “umbel within plant” were treated as random effects. At the end of the season we counted the total number of fruits matured on each plant. Fruit-set (number of fruits produced/number of flowers produced) was compared between years using a generalized linear mixed model in which “year” was the fixed effect and “individual plant” was the random effect. A binomial error distribution was applied to the model.

RESULTS

A wide taxonomic diversity of insects visited *Asclepias incarnata* plants during our study; we observed a total of 70 species of insects foraging on swamp milkweed flowers, representing 34 families in six orders of insects (this information has been archived at the Botanical Society of America website at <http://ajbsupp.botany.org/v90/>). Forty-one (58.6%) of these visitors were observed carrying pollinia and were therefore potential pollinators.

Pollen loads—Visitor species varied in the number of pollinia carried (Table 1). Carpenter bees (*Xylocopa virginica*, Hymenoptera: Anthophoridae) carried at least three times as many pollinia as any other species (Table 2). Excluding carpenter bees, Hymenoptera carried more than four times as many pollinia as Lepidoptera (Lep), which was the second most common order of visitors (<http://ajbsupp.botany.org/v90/>, and see below), and Hymenoptera (Hym) carried more pollinia than other insect orders (other) (lsmean [least-squares mean] (1 SE)_{Hym} = 18.8 (1.08), lsmean (1 SE)_{Lep} = 4.6 (1.21), lsmean (1 SE)_{other} = 2.0 (1.05); $F_{2,333} = 24.5$, $P < 0.0001$ in contrast comparison with *X. virginica* excluded).

Visitor abundance—For the most part, we successfully chose pairs of days for sample times that were sunny and warm, so that comparison of pollinator behavior across sample times would not be complicated by variation in weather. The one exception was the final sample time in 1996, during which we were confined to a single day of observations because the second day was rainy. We combined species of infrequent visitors into two categories: “Lepidoptera,” which included 21 species of butterflies and moths; and “other,” which included 39 species of visitors not placed in other categories. “Lepidoptera” represented 11.8% of all visits observed and was dominated (48.4%) by two species of skippers (family Hes-

periidae), neither of which were observed to carry pollinia. “Other” represented 20.9% of all visits observed; 63.3% of visits within this category were by two small *Cerceris* wasps (family Sphecidae), both of which rarely carried pollinia. None of the other species in these categories was a common visitor.

The number of visits to plants was positively influenced by the number of open flowers on plants ($y = 0.3x - 0.04$; $F_{1,281} = 80.2$; $P < 0.0001$; $r^2 = 0.22$). Weighting the number of visits by flower number therefore allowed us to discern variation in visitation rate apart from the complicating effects of variation in floral display size. The rate of number of visits per flower increased significantly during the last sampling time in both years (Table 1; Figs. 1 and 2) and although overall visits dropped about 28% from 1995 to 1996, this was not a significant decline (Table 1, Figs. 1 and 2). Visitor species varied in the frequency with which they visited *A. incarnata* flowers, and visitation rates of insects varied between years as well as among sample times within years (Table 1). The six focal visitors were responsible for the majority of visits (67.3%); all of these visitors were in the order Hymenoptera. Among these focal visitors, the rank of visit frequency was consistent between the two years ($r_s = 1.00$, $P = 0.0001$; Figs. 1 and 2), but not among sample times within years ($P > 0.05$, Figs. 1 and 2). The most frequent visitor in both years was *Tachytes crassus* (Figs. 1 and 2).

Visit duration—We found that a common slope analysis of covariance model best explained variation in the number of flowers visited on unmanipulated plants. We found no significant differences, however, among the six focal visitors in the number of flowers visited (Tables 1 and 3). There was a weak (slope = 0.0017), but significantly positive relationship between number of flowers visited and the number of open flowers on plants (Table 1). The six focal visitors varied more than twofold in foraging time per flower on unmanipulated flowers (Tables 1 and 3). In particular, *Myzinum carolinianum* had a longer flower-handling time than three of the other species (Table 3).

Pollen transfer—The six focal visitors varied over an order of magnitude in their ability to remove pollinia while foraging (Tables 1 and 4). Pollinators visited an average of 0.8 (for *M. carolinianum*) to 8.1 (for *T. crassus*) flowers before removing a pollinium (Table 4). Focal visitors varied nearly two orders of magnitude in their ability to insert pollinia (Tables 1 and 4). *Tachytes crassus* removed and inserted the fewest pollinia; although we observed at least 10 individuals of *T. crassus* visit 486 experimental flowers, we found only a single pollinium inserted by this species. Pollinators visited a mean of 6.3 (for *M. carolinianum*) to 500 (for *T. crassus*) flowers before inserting a pollinium (Table 4). Transfer efficiency (insertions/removal) varied nearly 50-fold among focal visitors (Tables 1 and 4). *Apis mellifera*, the exotic European honey bee, had the highest transfer efficiency, inserting more than 18% of pollinia removed, whereas *T. crassus* was the most wasteful pollinator, inserting only 0.4% of the pollinia it removed (Table 4). Foraging time per flower on experimental flowers varied almost fourfold among focal visitors (Tables 1 and 4). Flower-handling time increased between 2.5 and 4.2 times over unmanipulated flowers (Tables 3 and 4), which likely reflected increased nectar availability in experimentally bagged virgin flowers. An analysis of covariance, however, revealed that, after differences among focal visitors had been controlled, flow-

TABLE 1. Results of generalized linear models comparing pollinator effectiveness, plant pollination success, and fruit-set in a population of *Asclepias incarnata* from northern Virginia. Deviance is a generalization of error sums of squares from ANOVA and is used to test model goodness-of-fit. For additional information about model construction and tests, see Materials and Methods.

Experiment	Response	Year	Fixed effect	$F_{\text{inf,df}}$	P	Deviance	P
Pollen load	Pollinium count	Both years	Visitor species	55.2 _{13, 400}	0.0001	426.3	0.33
Visitor abundance	Visitation rate	1995	Visitor species	3.57 _{7, 1072}	0.0008	979.4	0.99
			Time	13.47 _{2, 1072}	0.0001		
		1996	Visitor species × Time	1.88 _{14, 1072}	0.02		
			Visitor species	37.40 _{7, 1136}	0.0001		
			Time	4.27 _{3, 1136}	0.005		
Both years		Visitor species × Time	1.68 _{21, 1136}	0.03	2065.8	0.99	
		Visitor species	17.28 _{7, 2248}	0.0001			
		Year	2.40 _{1, 2248}	0.1			
		Visitor species × Year	3.66 _{7, 2248}	0.0006			
		Focal visitor	0.6 _{7, 45}	0.7			
Visit duration	Number of flowers	Both years	Flowers	16.8 _{1, 52}	0.0001	152.7	0.95
			Focal visitor	8.07 _{5, 31}	0.0001	173.7	0.70
Pollen transfer	Time per flower	Both years	Focal visitor	14.0 _{5, 119}	0.0001	151.5	0.75
			Focal visitor	12.9 _{5, 100}	0.0001	138.9	0.92
		Both years	Focal visitor	4.8 _{5, 124}	0.0005	123.8	0.99
			Focal visitor	17.62 _{5, 119}	0.0001	82.7	0.99
			Focal visitor	5.7 _{4, 97}	0.0004	103.4	0.99
Geitonogamy potential	k_1 (see text)	Both years	Focal visitor	159.4 _{2, 1634}	0.0001	2009.3	0.0001
			Time ^a	41.8 _{3, 1019}	0.0001	1199.8	0.008
Pollination success	Removal	1995	Time ^a	182.1 _{1, 2692}	0.0001	3276.9	0.0001
			Year ^a	136.2 _{2, 1580}	0.0001	1673.7	0.48
		Both years	Time	23.4 _{3, 1032}	0.0001	954.4	0.99
			Year	112.9 _{1, 2667}	0.0001	2695.8	0.99
			Time	13.7 _{2, 1499}	0.0001	1340.4	0.99
Fruit production	Percentage fruit-set	Both years	Time	5.2 _{3, 1070}	0.002	746.8	0.99
			Year	11.22 _{1, 1697}	0.0008	2167.8	0.99
		Both years	Year	5.82 _{1, 42}	0.02	63.7	0.35

^a Test of main effect was adjusted to accommodate overdispersion according to methods of Littell et al. (1996).

TABLE 2. Least-squares means of number of pollinia per individual (1 SE) carried by insects foraging on swamp milkweed (*Asclepias incarnata*) in northern Virginia. Means followed by the same letter are not significantly different at $\alpha \geq 0.05$ by Bonferroni-corrected *t* tests.

Insect species	N	Pollinia carried
<i>Apis mellifera</i>	41	31.3 (2.8) ^B
<i>Bombus</i> spp.	42	16.4 (1.8) ^{BC}
<i>Cerceris clypeata</i>	16	1.7 (0.7) ^{BC}
<i>Danaus plexippus</i>	10	2.5 (1.2) ^{BC}
<i>Hemeris diffinis</i>	7	2.1 (0.7) ^{BC}
<i>Myzinum carolinianum</i>	77	28.9 (2.0) ^B
<i>Papilio glaucus</i>	9	14.0 (5.8) ^{BC}
<i>P. polyxenes</i>	8	1.4 (0.5) ^{BC}
<i>Sphex ichneumoneus</i>	23	16.1 (2.5) ^{BC}
<i>Tachytes crassus</i>	39	2.2 (0.4) ^C
<i>Xylocopa virginica</i>	78	113.8 (7.9) ^A
Other Hymenoptera	25	11.1 (2.5) ^{BC}
Other Lepidoptera	23	3.8 (1.5) ^{BC}
Other insects	16	2.0 (1.0) ^{BC}

er-handling time on experimental flowers had no significant effect on pollen transfer, either overall (time per flower: pollinium removal, $F_{1, 143} = 0.14, P = 0.7$; pollinium insertion, $F_{1, 52} = 1.81, P = 0.2$) or within visitor species (time per flower \times focal visitor: pollinium removal, $F_{6, 134} = 0.7, P = 0.6$; pollinium insertion, $F_{6, 74} = 1.40, P = 0.2$).

Geitonogamy potential—Estimates of k_1 varied almost 15-fold among focal visitors (Tables 1 and 4). These values suggest that *M. carolinianum* and *A. mellifera* have the potential to effect more geitonogamous self-pollinations per flower visited than other pollinators. We were unable to include *T. crassus* in our analyses of this parameter because we observed only one pollinium insertion by this species during our experiments.

Correlations among effectiveness components—Visitors that removed more pollinia were also more likely to insert more pollinia per flower visited (Table 5). Pollen transfer was also highly correlated with estimates of fractional pollen deposition. Visitors that had a longer flower-handling time were more likely to pollinate flowers, more likely to do so with a higher transfer efficiency, and were also more likely to have a high rate of pollinium turnover. Although flower-handling time increased in experimental flowers relative to unmanipu-

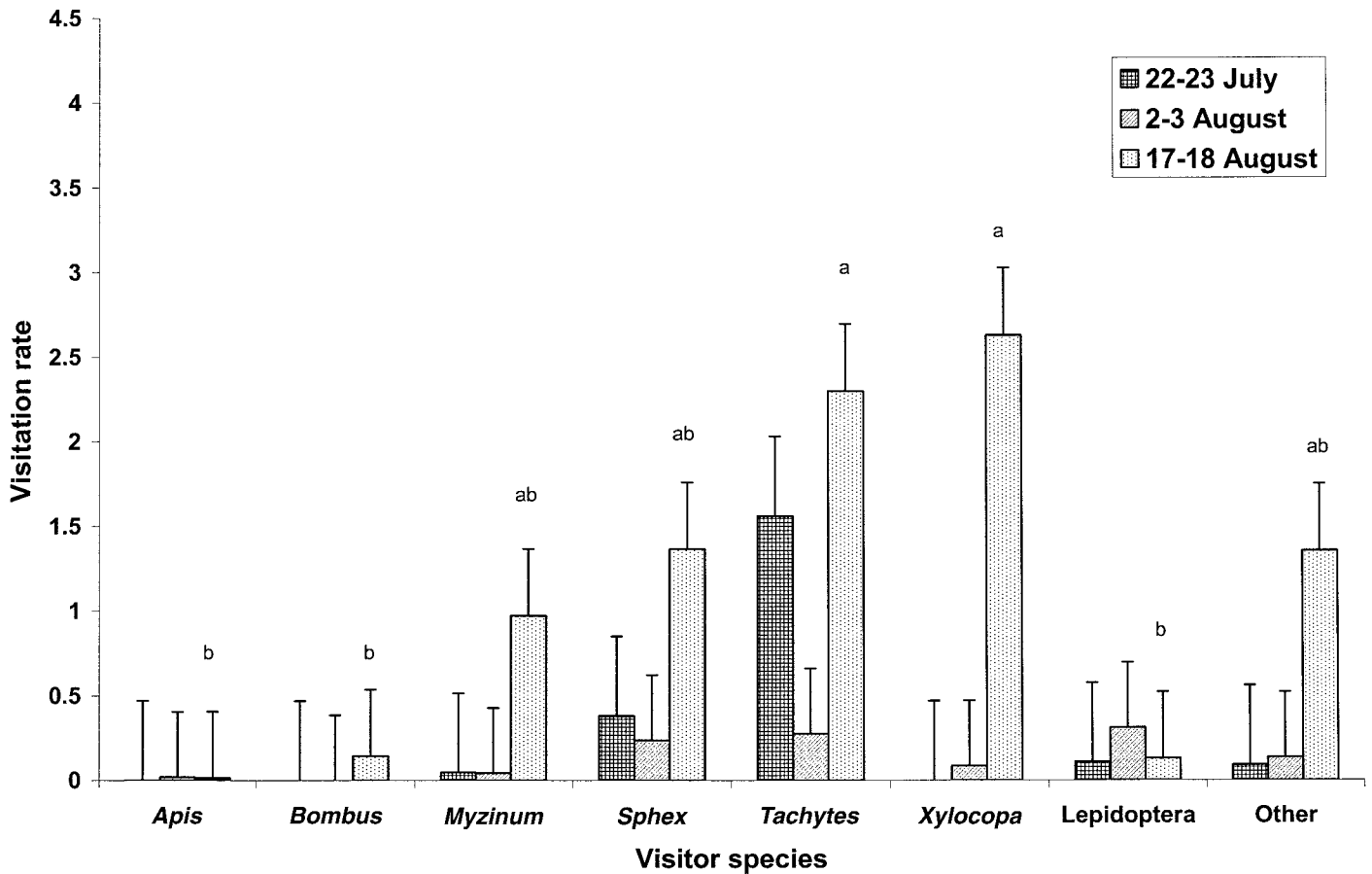


Fig. 1. Least-squares means of the number of visits per flower per 10-min observation period $\times 10^2$ (+1 SE) by insects to *Asclepias incarnata* flowers during 1995 in northern Virginia. Sample sizes of 10-min observations: 22–23 July = 35; 2–3 August = 52; 17–18 August = 50. Columns of the final sample period headed by the same letter indicate taxa that did not significantly differ within sample times at $\alpha \geq 0.05$ by Bonferroni-corrected *t* tests. Visitation rate did not significantly differ among taxa within the first two sample times. Visitation rate was significantly higher during the third sample time for *Xylocopa*; otherwise, there were no differences among sample times within taxa. Overall mean visitation rates per sample time increased significantly during the last sample time.

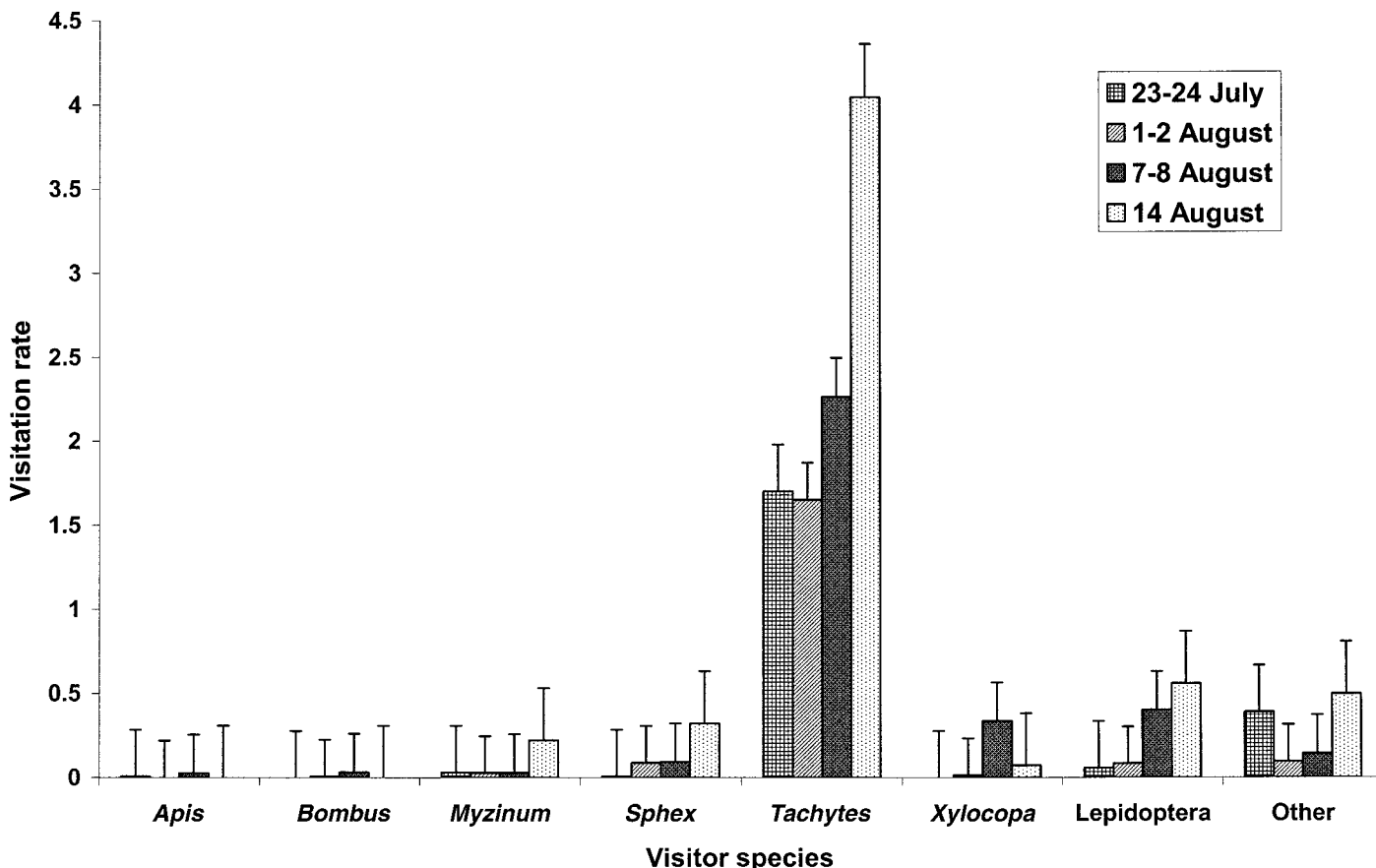


Fig. 2. Least-squares means of the number of visits per flower per 10-min observation period × 10² (+1 SE) by insects to *Asclepias incarnata* flowers during 1996 in northern Virginia. Sample sizes of 10-min observations: 23–24 July = 30; 1–2 August = 47; 7–8 August = 44; 14 August = 24. Visitation rates by *Tachytes* were significantly higher than other taxa during all sample times at $\alpha \geq 0.05$ by Bonferroni-corrected *t* tests. Visitation rate was significantly higher during the fourth sample time for *Tachytes*; otherwise, there were no differences among sample times within taxa. Overall mean visitation rates per sample time increased significantly during the last sample time.

lated flowers, the rank of foraging time per flower across taxa remained almost the same (Table 5).

Plant pollination success and fruit-set—The best-fitting models of pollinium removal in plants had deviance values that suggested a poor fit (Table 1), which reflected over-dis-

persed data. The tests of main effects that we report (Table 1) were therefore adjusted to account for over-dispersion following the methods described by Littell et al. (1996). These tests, as well as tests from models of pollinium insertion in plants, indicate significant variation among sample times and between years in pollinium transfer (Tables 1 and 6). Removal and insertion of pollinia per flower in unmanipulated flowers was nearly twice as high in 1995 than 1996 (Tables 1 and 6). The efficiency of plants in recapturing pollinia that had dispersed (insertion/removal) dropped by 4% in 1996. During both years, rates of pollination increased significantly as the flowering season progressed (Tables 1 and 6). Pollinium capture efficiency of plants nearly doubled by the end of the 1995 season, but during 1996 it remained relatively stable after an early-season low (Table 6). Plants that had higher pollinium removal rates also had higher insertion rates, both in 1995 ($r_s = 0.85, P = 0.001$) and 1996 ($r_s = 0.81, P = 0.001$). Positive correlations were observed between removal and insertion rates of plants within sample times as well ($r_s > 0.7, P = 0.0001$). Plants that had high pollination success in 1995, however, were not any more likely to have high success in 1996, either in terms of removal ($r_s = 0.28, P = 0.2$) or insertion ($r_s = 0.13, P = 0.5$) of pollinia per flower. Similarly, we observed no significant relationship in pollination success of individual plants across sample times within years ($r_s < 0.3$,

TABLE 3. Comparison of the behavior of focal visitors while foraging on unmanipulated *Asclepias incarnata* plants in northern Virginia. Numbers in parentheses following focal visitor species are the minimum numbers of individuals observed. *N* = number of times species observed, flowers visited = least squares mean (1 SE) number of flowers visited per plant at the mean number of open flowers on plant, time per flower = least squares mean (1 SE) time (in seconds) spent foraging per flower. Means followed by the same letter were not significantly different at $\alpha \geq 0.05$ by Bonferroni-corrected *t* tests.

Focal visitor	<i>N</i>	Flowers visited	Time per flower
<i>Apis mellifera</i> (2)	6	21.2 (27.0) ^A	3.7 (0.9) ^{AC}
<i>Bombus</i> spp. (1)	7	13.3 (26.3) ^A	1.6 (0.6) ^{ACD}
<i>Myzinum carolinianum</i> (15)	47	50.0 (15.9) ^A	3.5 (0.3) ^A
<i>Sphex ichneumoneus</i> (4)	31	32.6 (19.6) ^A	1.7 (0.3) ^{BC}
<i>Tachytes crassus</i> (8)	21	33.9 (15.0) ^A	1.5 (0.2) ^{BD}
<i>Xylocopa virginica</i> (22)	78	43.8 (11.1) ^A	2.3 (0.2) ^{BC}

TABLE 6. Reproductive success of unmanipulated *Asclepias incarnata* plants over a 2-yr period in a northern Virginia population. Removal (insertion) = least-squares mean (1 SE) number of pollinia removed (inserted) per-flower, capture efficiency = least-squares mean (1 SE) number of pollinia inserted/number of pollinia removed, and fruit-set = least-squares mean (1 SE) percent fruit-set. Means followed by the same letter were not significantly different within years at $\alpha \geq 0.05$ by Bonferroni-corrected t tests.

Date	Removal	Insertion	Capture efficiency	Fruit-set
1995				
24 July	3.74 (0.21) ^A	0.61 (0.06) ^A	0.16 (0.01) ^A	
4 August	3.44 (0.18) ^A	0.70 (0.06) ^A	0.20 (0.01) ^B	
19 August	7.34 (0.42) ^B	1.90 (0.17) ^B	0.28 (0.03) ^C	
Yearly mean	3.98 (0.25)	0.76 (0.07)	0.19 (0.01)	1.87 (0.25)
1996				
25 July	1.20 (0.18) ^A	0.07 (0.03) ^A	0.05 (0.02) ^A	
3 August	1.61 (0.18) ^A	0.22 (0.04) ^B	0.13 (0.02) ^B	
9 August	2.48 (0.25) ^B	0.39 (0.06) ^C	0.16 (0.02) ^B	
15 August	3.18 (0.32) ^C	0.50 (0.08) ^D	0.17 (0.02) ^B	
Yearly mean	2.40 (0.16)	0.39 (0.04)	0.15 (0.01)	2.13 (0.30)

swamp milkweed. Some of the components, however, were strongly correlated, which may provide insight into the mechanisms that influence them. In particular, the rankings of taxa with respect to removal and insertion of pollinia were identical. This suggests that no focal visitor had a strong bias in its ability to remove rather than insert pollinia and reinforces the idea that pollination in milkweeds is incidental to nectar foraging by insects (Wyatt, 1976; Wyatt and Broyles, 1994). Transfer efficiency (insertion/removal) of focal visitors, however, was not significantly correlated with pollinium removal or insertion. This could be interpreted as evidence for slight biases in removal vs. insertion of pollinia among species, which may reflect differences among pollinators in morphologies or behaviors that affect efficiency. Interestingly, a positive relationship between insertion and removal of pollinia was not apparent for pollinators of *A. tuberosa* (Fishbein and Venable, 1996), although very few pollinium insertions were observed in that study. For plants frequented by pollen foragers, of course, this relationship is not as likely to hold (Wilson and Thomson, 1991; Thomson and Thomson, 1992).

Several components of effectiveness were correlated with foraging time per flower. Variation in flower-handling time probably reflects differences among pollinators in some time-dependent behavior such as nectar extraction rate (Harder, 1986; Mitchell and Waser, 1992) or efficiency at flower manipulation. For example, foraging time per flower increased on experimental flowers relative to unmanipulated flowers for all pollinator taxa (Tables 3 and 4), which probably reflected the increased time required to extract nectar that had pooled in experimentally bagged flowers. Nonetheless, this increase was not proportionate across all taxa, which may indicate that pollinators vary in extraction efficiency or thoroughness. Our observations in the field suggest that some visitors (e.g., *Apis*, *Bombus*, *Xylocopa*) methodically probe all hoods of a flower and visit most of the flowers in an umbel, whereas others (*Myzium*, *Sphex*, *Tachytes*) appear to forage more haphazardly. Flower-handling time is also likely to be influenced by efficiency at flower manipulation. For example, while moving between flowers on an umbel, *Myzium carolinianum* often rock their bodies and wave their legs repeatedly before anchoring onto a flower, which makes them appear "clumsy." In con-

trast, other taxa (*Sphex*, *Bombus*) move among flowers with quick, precise movements. Although we do not know which behaviors are reflected in flower-handling time, we can examine this parameter to assess the consequences of variation in time-dependent behaviors on components of effectiveness.

Patterns revealed by correlations with flower-handling time were intriguing. When we examined relationships across focal visitor means, we found that the taxa that were most effective at pollen transfer spent a longer time foraging at each flower. This suggests that plants attractive to slow-foraging pollinators should have increased pollination success. This result stands in contrast to other studies, in which the pollinator taxa that were more effective with respect to pollen deposition (Gomez and Zamora, 1999) or seed yield (Richards, 1987) spent less time foraging per flower. At the level of individual visits, however, we found that flower-handling time did not explain pollen transfer success, either overall or within pollinator species, after differences among pollinators had been controlled. This suggests that *A. incarnata* plants with traits that increased flower-handling time had no advantage in terms of pollination success. Similar findings have been reported for other plants (Pettersson, 1991; Mitchell and Waser, 1992; Cresswell, 1999). In contrast, flower-handling time was found to have a positive relationship to pollination success in other plant species (Thomson, 1986; Galen and Stanton, 1989; Conner, Davis, and Rush, 1995; Hurlbert et al., 1996). Selection to increase flower-handling time (and thereby pollination success) has been implicated in the evolution of flexible pedicels (Hurlbert et al., 1996) and corolla morphology (Smith et al., 1996). Across focal visitors, we found no evidence for a trade-off between the frequency and duration of visits as has been observed in other plants (Herrera, 1989; Jones, Reithel, and Irwin, 1998; Utelli and Roy, 2000).

Fractional pollen deposition rates (k_i) were an order of magnitude lower than the lowest rates reported previously in the literature (de Jong, Klinkhamer, and van Staaldunin, 1992; Robertson, 1992), which implies that geitonogamy was uncommon. This was surprising, since direct estimates of self-pollination in other milkweeds have reported that as many as 66% of pollinations are geitonogamous (Shore, 1993). The fraction of geitonogamous pollen deposited on the last flower visited (G_i) in a sequence of average length can be estimated as $G_i = 1 - (1 - k_i)^{i-1}$ (de Jong, Waser, and Klinkhamer, 1993), where i is the mean number of flowers visited by a pollinator on a plant with the population's mean number of flowers (Table 3). Values of G_i were also low for *A. incarnata* and ranged from 0.03% (for *Bombus* spp.) to 1.8% (for *M. carolinianum*). Violations of model assumptions (see Materials and Methods) may have led to inaccurate estimates of k_i . Nonetheless, k_i estimates varied nearly 15-fold, and G_i estimates varied 60-fold, which strongly suggests that visitor taxa vary in their potential for geitonogamous pollination. Much of the variation in k_i could be explained by mean flower-handling time, suggesting that pollinator species with longer handling times may deposit a higher proportion of self-pollinia. The population of *A. incarnata* that we studied was largely outcrossing ($t_m > 0.96$ from 1994 through 1996; Ivey, Lipow, and Wyatt, 1999; Ivey and Wyatt, 1999), although swamp milkweed can be self-fertile (Kephart, 1981; Ivey, Lipow, and Wyatt, 1999; Lipow and Wyatt, 2000). In either case, pollinators that deposit more self-pollen may negatively impact plant fitness (Klinkhamer and de Jong, 1993; Harder and Barrett, 1995), either through interference with fruit-set (Broyles and

Wyatt, 1993; Wyatt and Broyles, 1994) or by increasing inbreeding depression (Husband and Schemske, 1996).

The selective impact of pollinators depends in part on the extent to which fruit-set is limited by resources vs. access to pollen (Zimmerman and Pyke, 1988; Burd, 1994). Although we performed no explicit test for pollen limitation of fruit-set, we found a positive relationship between the frequency of pollinium insertion and the percentage of fruit set by individual plants during 1995, an observation that suggests pollen limitation (Broyles and Wyatt, 1997). This relationship was not observed in 1996, which may indicate temporal variation in pollen limitation (Burd, 1994; Broyles and Wyatt, 1997). Surprisingly, rates of pollination, pollinium capture efficiency, and visitation were all lower in 1996 than in 1995, and visitation was dominated by the least efficient pollinator, *Tachytes crassus*. One might have expected the circumstances observed in 1996 to be more likely to result in pollen limitation, rather than those in 1995. This conflicting observation may indicate a saturation of resources available for fruit-set during 1996, even though pollination rates were lower (Zimmerman and Pyke, 1988; Burd, 1994). Such a circumstance could arise if resources used for reproduction during 1995 caused a “depletion” of resources available during 1996.

In summary, *Asclepias incarnata* provides a striking example of the role of variability in pollination to the ecology and evolution of plant reproduction. The taxonomic diversity of visitors was quite high, and a majority of these visitors were potential pollinators. Temporal variation in the activity of the primary pollinators was considerable, and this activity was closely reflected in the pollination success of unmanipulated plants, both within and between seasons. Nonetheless, substantial variation was also observed in pollinator effectiveness. This variation was linked to variation in flower-handling time, and the influence of time-dependent foraging behaviors on pollination effectiveness seems likely to be a productive line of future inquiry. Variation in pollinator activity and effectiveness, as well as apparent temporal variation in pollen limitation, appears to reinforce, probably through redundancy (Zamora, 2000), a “generalized” pollination system in *A. incarnata* (Waser et al., 1996; Johnson and Steiner, 2000). The ability of *A. incarnata* to exploit a wide diversity of pollinators that vary in abundance and effectiveness may in part explain its success over such a wide geographic range (Woodson, 1954).

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