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ECOLOGY AND EVOLUTION OF REPRODUCTION IN MILKWEEDS

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Abstract

Asclepiadaceae are the dicot counterparts to the Orchidaceae, which also transmit their pollen grains in large groups within pollinia. Unlike many terrestrial, nectar-producing orchids, however, milkweeds are characterized by low fruit-set, typically averaging 1–5%. Transfer of hundreds of pollen grains as a unit makes it possible to quantify pollinator activity and male and female reproductive success more directly and more easily in milkweeds than in plants with loose pollen grains. It also leads to the production of fruits whose seeds all share a single father, thus simplifying paternity analysis. Recent anatomical work has demonstrated that three of the five stigmatic chambers of milkweed flowers transmit pollen tubes to one of the two separate ovaries, whereas the other two chambers transmit only to the second ovary. Milkweed flowers are long-lived and produce copious nectar, which flows from nectaries within the stigmatic chambers to fill the hoods, which serve as reservoirs. Nectar also serves as the germination fluid for pollen grains, but concentrations above 30% inhibit germination. Most milkweeds are genetically self-incompatible and express an unusual late-acting form of ovarian rejection. Some weedy milkweeds, however, are self-compatible, and levels of self-insertion of pollinia are apparently high in these, as well as in self-incompatible, species. Early

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attempts to explain the evolution of inflorescence size in milkweeds were hampered by failure to consider the genetic basis of the variation observed and by failure to determine the unit on which selection should act. Direct tests of the "pollen donation" hypothesis have cast doubt on the validity of the view that flower number and other floral traits evolved primarily to enhance male reproductive success. Milkweeds are pollinated by a diverse array of large bees, wasps, and butterflies, and these generalist pollinators effect extensive gene flow within and between populations, augmented by wind dispersal of comose seeds. Morphological and biochemical evidence support the view that limited, localized hybridization occurs between sympatric species of milkweeds.

INTRODUCTION TO MILKWEEDS AS A MODEL SYSTEM

The remarkably complex flowers of milkweeds (Asclepiadaceae) provide a number of unusual advantages to students of plant reproductive ecology. In many respects the Asclepiadaceae are the dicot equivalents of the Orchidaceae. Both families are unique within their respective classes in that their pollen grains cohere and transport as a unit, termed a "pollinium." Delivery of pollen in discrete packets has numerous reproductive consequences. It makes it possible to quantify the results of pollinator activity easily and directly: One need only count the numbers of pollinia removed from a flower or inserted into a flower to estimate the success of a hermaphroditic plant as a pollen donor (male function) or as a pollen receiver (female function). It also simplifies the counting of pollen units carried by the pollinators themselves. Coherence of the pollen into discrete packages makes it possible to use genetic markers to genotype individual pollinia following dispersal events. Moreover, delivery of hundreds of pollen grains to the stigma at one time ensures that more than enough grains are available to fertilize all of the ovules within an ovary. This results in consistently high seed numbers. Finally, this form of pollen delivery also leads to the production of fruits whose seeds all share a single father. This greatly simplifies paternity analysis, making the milkweed system ideal for studies focusing on sexual selection or gene flow.

Because milkweed pollinia must conform in size and shape to the stigmatic chambers into which they are inserted, there is the potential for mechanical barriers to interspecific hybridization. Such barriers to crossing are otherwise almost unknown in flowering plants. In *Asclepias* another unusual feature is the presence of a cucullate corona including hoods, which function as nectar reservoirs. This makes species of *Asclepias* ideal subjects for observations and experiments relating to nectar production, as the nectar can be easily removed and measured repeatedly. Moreover, the nectar is almost pure sucrose and represents the only reward for pollinating insects (the pollen being protected by the tough durable wall of the pollinium). Flowers of Asclepiadaceae also are unusual in consisting of two separate ovaries. This enables the study of resource competition not only between flowers but also within flowers. A final characteristic of the milkweed system is that most species appear to have an unusual form of late-acting self-incompatibility. Such systems appear to be rare in the angiosperms as a whole, and their functioning and genetic consequences are poorly understood.

Of course, there are also characteristics of milkweeds that make them undesirable subjects for some kinds of studies in reproductive ecology. One of these is the very low rate of fruit-set in natural populations (typically about 1% to 5%). Although fruit-set is higher from hand cross-pollinations, it never approaches 100%. This makes it difficult and time-consuming to carry out experiments designed to assess breeding systems, ovary competition, factors limiting fruit-set, etc. Another characteristic of milkweeds that might prove disadvantageous in some contexts is the fact that, unlike the case in many orchids, there is little specialization of different species for pollination by specific insects. For example, virtually any insect of appropriate size and behavior can effectively remove and insert pollinia of any species of *Asclepias*. Thus, the apparent high degree of coadaptation between some orchids and their species-specific pollinators does not exist in *Asclepias*.

STRUCTURAL AND FUNCTIONAL ASPECTS OF FLOWERS

Floral Morphology and Anatomy

Flowers of *Asclepias* consist of five showy, reflexed petals covering five smaller, usually green sepals (Figure 1A). Two separate, superior ovaries are united by their styles to form a gynostegium with five lateral stigmatic surfaces (Figure 1B, C). These surfaces are enclosed by the tightly abutting wings of adjacent anthers to produce five stigmatic chambers. From the bases of the five stamens extend the hoods, each of which usually contains an arching horn and which serve as reservoirs for the nectar secreted by nectaries located within the stigmatic chambers (25a). Together the hoods and horns comprise the corona (Figure 1A, B). There are five pollinaria (36), each of which consists of paired pollinia from adjacent anthers joined by translator arms to a corpusculum that sits just above the alar fissure, a narrow opening into the stigmatic chamber (Figure 1A, B, D).

Milkweed pollination is a two-stage process: (i) removal of a pollinarium occurs when a groove in the corpusculum catches on a bristle or other appendage of an insect and is forcibly pulled from the flower, and (ii) insertion is

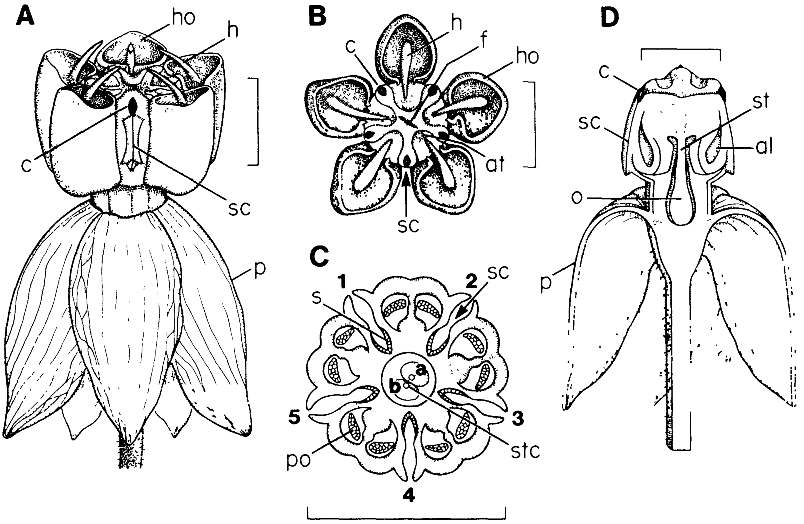

Figure 1 Flower morphology of *Asclepias amplexicaulis*. A. Whole flower, showing reflexed petals, corona of hoods and horns, and one surface of the gynostegium. B. Top view, showing location of the hoods and horns and entrances to the stigmatic chambers relative to the furrow in the gynostegium. C. Transverse section, showing the location of the stigmatic chambers relative to the styles. Chambers 1, 2, and 3 transmit pollen tubes to style a, whereas chambers 4 and 5 transmit pollen tubes to style b. D. Longitudinal section along the axis of the furrow, showing the location of the gynostegium relative to the style and ovary. In all figures, the scale delimits 3 mm. Abbreviations are: al, anther locule; at, anther tip; c, corpusculum; f, furrow; h, horn; ho, hood; o, ovary; p, petal; po, pollinium; s, stigmatic surface; sc, stigmatic chamber; st, style; and stc, stylar canal.

effected when a pollinium lodges in a stigmatic chamber (79). Following successful insertion, pollen tubes emerge from a thin-walled area along the convex surface of the pollinium (26, 66). Pollen tubes subsequently grow down the stylar canal and finally enter the ovary (17, 25).

Until recently, the relationship between the five stigmatic chambers and two subtending ovaries with respect to the pathway of pollen tube transmission was a mystery. It is now known that three adjacent stigmatic chambers transmit pollen tubes to one of the two separate ovaries, whereas the other two chambers transmit to the second ovary (58). These observations confirm Woodson's (77) expectations. Despite the obvious potential for pollen tubes to cross over at the point of fusion of the two styles, this was never observed in *A. amplexicaulis*. Out of hundreds of observations, Sparrow & Pearson (65) detected only one case of such "crossing-over" in *A. syriaca*. Morse (43), however, observed the phenomenon more commonly in natural populations of *A. syriaca* from Maine. He reported that nearly 2% of all successful hand pollinations with a single pollinium produced twin follicles, indicating fertilization of both ovaries by pollen tubes from a single stigmatic chamber.

Self-Incompatibility Systems

Early workers believed that all species of *Asclepias* are self-incompatible (17, 18, 28). Experimental crosses in *A. syriaca* by Moore (38) and Sparrow & Pearson (65) supported this view, and Woodson (77) arbitrarily discounted reports of successful self-pollination in this species (52, 67) and in *A. incarnata* (24). More recently, however, Kephart (30) and Kahn & Morse (29) reported low levels ($< 5\%$) of self-compatibility in *A. syriaca.* Wyatt (78) reported that 2% of self-pollinations of *A. tuberosa* resulted in fruit-set, and Kephart (30) reported 29% success for self-pollinations of *A. incarnata.* Our recent work has uncovered two additional species that appear to be fully self-compatible: *A. curassavica,* in which 23.3% of self-pollinations were successful versus 25.9% of cross-pollinations; and *A. fruticosa,* in which 12.7% of self-pollinations set fruit versus 17.5% of cross-pollinations (R Wyatt, SB Broyles, unpublished data).

Experimental crosses in *A. perennis* and *A. texana* have revealed that these two species are completely self-incompatible (R Wyatt, AL Edwards, SR Lipow, CT Ivey, unpublished data), as is *A. verticillata* (30). *Asclepias subulata* also is largely self-incompatible: Only 1 of 99 self-pollinations resulted in fruit-set (R Wyatt, CT Ivey, SR Lipow, unpublished data). It appears, therefore, that those milkweeds investigated to date fall clearly into two distinct categories: (i) those that are largely or entirely self-incompatible (*A. exaltata, A. perennis, A. subulata, A. syriaca, A. texana, A. tuberosa,* and *A. verticillata*), and (ii) those that are largely or entirely self-compatible (*A. curassavica, A. fruticosa,* and *A. incarnata*).

It appears from the limited information available that self-incompatible species of *Asclepias* possess an unusual form of genetic self-incompatibility. Traditionally, two basic forms of self-incompatibility (SI) have been recognized: (i) sporophytic (SSI), in which pollen fails to germinate on an incompatible stigma; and (ii) gametophytic (GSI), in which incompatible pollen germinates, but pollen tube growth is arrested in the style. Recent reviews have argued, however, that other self-incompatibility systems exist that do not conform to the classically defined GSI and SSI models (57, 59). In many plants, it appears that SI may act very late, so that the incompatibility reaction occurs in the ovary. These ovarian self-incompatibility systems (OSI) can be categorized on the basis of whether pollen tube rejection occurs before or after the ovules are penetrated, whether syngamy occurs, and whether ovular inhibition is involved. Most of the details of OSI have not been worked out for any species of flowering plant, but it appears that OSI is typical of milkweeds (12, 29, 30, 65). It is intriguing that so inefficient a system exists in the morphologically derived genus *Asclepias*.

CAUSES OF LOW FRUIT-SET

Fruit-set is generally very low in natural populations of milkweeds, with averages ranging from 0.33% to 5.0% (84). Attempts to explain these low values have centered on two hypotheses: (i) resources to mature fruits are limiting, and (ii) insufficient numbers of compatible pollinia are reaching stigmatic chambers. Those favoring resource limitation have pointed out that pollinator activity is frequently high in milkweed populations and that abortion of apparently fertilized fruits is common (54, 55, 73, 75). Moreover, addition of inorganic fertilizer increased, whereas shading and leaf removal decreased, fruit-set in *A. syriaca* and *A. verticillata* (74). Application of fertilizer also increased fruit-set in *A. exaltata* (55). Chaplin & Walker (14) concluded that resources stored in the taproot of *A. quadrifolia* controlled flower and fruit production. All of these studies, however, are complicated to interpret because of possible side effects of the experimental treatments (especially addition of water) on nectar production (86), which, in turn, could have increased pollinator visitation. On the other hand, shading could have had a direct negative effect on pollinator activity.

Many populations of milkweeds undoubtedly receive high levels of pollination (13, 36, 60, 71). There is some question, however, as to how many of these apparently successful pollinium insertions represent self-pollinations or otherwise incompatible crosses. Hand pollinations increased fruit-set from natural levels of 0.33% to 14.8% in *A. tuberosa* (78, 82) and from 2.5% to 19.7% in *A. exaltata* (55). Morse & Fritz (44) argued that *A. syriaca* is pollen-limited, because they were able to double fruit-set by supplementing natural levels of pollination. Unfortunately, as with tests of the resource limitation hypothesis, all of these tests for pollen limitation are flawed in design (89). Wyatt (78, 80, 82, 83) has argued for a compromise position, noting that under different sets of circumstances, either pollination or resources can limit fruit-set in milkweeds.

NECTAR PRODUCTION

Compared to other plants, milkweeds have long-lived flowers that produce copious quantities of nectar. On average, individual flowers of *A. tuberosa* are reproductive for 7.4 + 0.34 (mean + standard deviation) days (82); of *A. exaltata,* 6.2 + 0.85 days (88); of *A. incarnata,* 4.87 + 1.66 or 3.87 + 1.04 days (early- versus late-season: Ref. 32); of *A. syriaca,* 5.18 + 1.24 or 5.30 + 0.82 days (32); and of *A. verticillata,* 6.30 + 2.02 or 5.14 + 1.18 days (32). Flowers that last 4 to 8 days are long-lived compared to most other flowering plants (53).

Cross-comparisons of nectar production data are complicated because different workers use different sampling methods (86). In *A. exaltata,* an average flower produced 63.5 μl of nectar over its 6-day life span, a net production considerably higher than that for *A. verticillata* (72), *A. quadrifolia* (49), *A. curassavica* (47, 81), or *A. syriaca* (40, 63, 71). In Southwick's (63) population of *A. syriaca,* for example, total nectar production ranged from 3.8 to 17.8 μl of nectar per flower. Similarly, the 23.6 mg of sucrose produced over 6 days by *A. exaltata* (88) greatly exceeds sugar production by *A. syriaca* (63, 64, 71), *A. quadrifolia* (49), or *A. verticillata* (72).

In *A. exaltata* (86, 88), *A. verticillata* (3, 71), and *A. syriaca* (63, 72), most nectar is secreted overnight. In *A. quadrifolia,* however, nectar production peaked in the morning and was very low at night. Nectar concentrations in *A. exaltata* and *A. syriaca* are typically low in the morning, averaging < 30%, but they increase steadily to 40–60% late in the afternoon (86, 88). These diurnal changes are strongly associated with temperature and relative humidity and are apparently caused by passive evaporation of the nectar within the open hoods (88). Similar increases in nectar concentration have been reported in *A. syriaca* (63, 71) and *A. verticillata* (72).

In *A. exaltata,* plants that produced more concentrated nectar had greater reproductive success (88). Nectar concentration was positively correlated with both the number of pollinia inserted per flower and the number of pollinaria removed per flower. Moreover, plants that produced concentrated nectar matured more fruits and had higher levels of fruit-set than plants that produced dilute nectar.

POLLEN VIABILITY AND GERMINATION

An unusual feature of milkweed reproduction is germination of the pollen in a nectar solution secreted within the stigmatic chamber. Among others, Shannon & Wyatt (61) reported that a 30% sucrose solution yielded highest germination of pollinia of *A. exaltata.* In *A. syriaca,* germination is inhibited by sucrose concentrations above 30% (20), and many failures of hand-pollinations may be due to inhibition of pollen germination by concentrated nectar within the stigmatic chamber (58, 84). Recently it has been shown that contamination of the germination fluid (nectar) by growth of microorganisms can inhibit germination (21, 22). Although these observations were made under laboratory conditions, these yeasts are natural contaminants of milkweed nectar and may be transmitted between flowers by insect pollinators.

Morse (40) speculated that the durable covering of milkweed pollinia should allow a long residence time on pollinators, thus enhancing pollen dispersal distances. This assumes that pollinia are resistant to desiccation and lose viability only slowly following their removal from flowers. In *A. syriaca,* pollinia appear to retain high germinability for at least 4 days under natural conditions (20). In vitro pollen germination experiments using *A. exaltata,* however, showed a loss of viability after 24 hr to about 50% of the original value (61). Pollinia of milkweed species, such as *A. syriaca,* that inhabit open sites may be more resistant to desiccation than are those of species such as *A. exaltata* that grow in moist forests and meadows.

It appears that pollen viability does not decline significantly merely as a function of flower age (42, 61). Stigma receptivity, however, decreased more than threefold over the five-day life span of flowers of *A. syriaca* (42). The possible repercussions of these effects are complicated because of the high degree of variation among plants in pollen germination and apparent fertilization in both *A. exaltata* (61) and *A. syriaca* (29).

LEVELS OF SELF-POLLINATION

Because most milkweeds are self-incompatible (see above), self-pollination (i.e. insertion of a pollinium into a stigmatic chamber of the same genetic individual) can be another cause of low fruit-set. This is an especially serious problem for milkweeds, as their flowers contain only five stigmatic chambers into which compatible pollinia can be inserted. Thus, "stigma clogging" by incompatible pollinia is very likely. Moreover, the late-acting incompatibility system of milkweeds opens up the possibility of ovules being preempted by fertilizations involving incompatible pollen tubes.

It has proved technically very difficult to estimate levels of self-pollination in milkweed populations. Conventional techniques for marking and following pollen dispersal (e.g. pollen-analogue dyes) do not work for milkweed pollinia. Pleasants & Ng (51) estimated levels of self-pollination in *A. syriaca* by comparing numbers of insertions in emasculated umbels to those in umbels with intact corpuscula. Over a range of umbel sizes, they calculated that 36% of inserted pollinia were self-insertions. There are problems, however, with the assumption that emasculation has only the effect of removing a source of self-pollen. Wyatt (79) has shown in similiar emasculation experiments on *A. tuberosa* that the presence of an intact corpusculum increases the likelihood of successful insertion. Thus, an alternative explanation of Pleasants & Ng's (51) result is simply that removal of pollinaria decreased the overall level of successful insertions. Using Pleasants & Ng's (51) interpretation, Wyatt's (79) data for *A. tuberosa* yield an estimate of 27.1% self-insertion.

After developing a technique for radioactively labelling pollinia (50), Pleasants (48) measured levels of self-insertion in a field plot of *A. syriaca.* Of 38 insertions into an umbel labelled with ^{14}C, 14 (37%) were from the labelled umbel itself. Moreover, most pollen dispersal occurred over short distances, suggesting that self-pollinations between stems of the same genetic individual might be very common for this species, which produces large clonal patches from gemmiferous roots. Effective levels of self-pollination are likely to be extremely high and could be a major factor limiting fruit-set.

Some recent studies suggest that self-inserted pollinia interfere with outcross-pollinia and prevent them from entering the stigmatic chambers and penetrating the style. It is well-established that self-pollen germinates and penetrates ovaries as quickly as outcross-pollen in milkweed flowers (29). Competition among self- and outcross-pollen tubes reduces the number of ovules effectively fertilized by compatible sperm. Pollen competition studies in *A. exaltata* have demonstrated that self-pollen reduces the ability of cross-pollen to mature fruits by 49% when the self-pollination is performed simultaneously with the cross-pollination, and by 81% when the self-pollination occurred 24 hr before the cross-pollination (12). In addition, fruit-set decreased 29% even when the self-pollination occurred 24 hr after the cross-pollination.

The loss in potential female reproductive success due to self-pollination may be very high. Seed-set in fruits maturing from flowers that were self-pollinated 24 hr prior to cross-pollination produced 37% fewer seeds. If we assume that self- and outcross-pollen compete in 30% of the ovaries and that self-pollination within plants is approximately 66% (as determined for a self-incompatible milkweed like *A. syriaca:* 62), then self-pollination will reduce fruit production in about 20% of all ovaries that also receive compatible cross-pollinations. Thus, the impact of self-pollination on seed and fruit production is potentially great on flowers that may have also received compatible pollinia.

POLLEN DISPERSAL

Milkweed pollinia are dispersed by a diverse array of Hymenoptera and Lepidoptera (31, 44, 71, 72, 77, 88). The distributions of interplant flight distances of insect pollinators are usually leptokurtic and skewed right, with most flights occurring over short distances (35). Interplant flight distances for bumblebees and large fritillary butterflies foraging on *A. exaltata* are similiar to observed patterns on other flowering plants. For example, nearly 80% of the interplant flight distances of large butterflies and bumblebees occurred over distances < 2 m, in natural and experimental populations of *A. exaltata* (6, 10). If pollen dispersal is correlated with pollinator flight distances, then effective pollen dispersal distances should also be leptokurtic and skewed toward longer distances for milkweeds.

Pollinator flight distances may not reflect effective pollen dispersal distances within populations of milkweeds because of several unique features of milkweed pollination. Following extraction of pollinaria from flowers, approximately 90 sec are required for the pollinarium to dry and reorient into a position that permits insertion. Insertion into stigmatic chambers is not possible during this time; thus, the opportunity for pollination on or near the pollen donor flower is decreased (55). In addition, slow turnover of pollinia on pollinators increases the probability of outcrossing and long-distance dispersal of pollen. Bumblebees foraging on *A. syriaca* picked up one pollinarium every 2–5 hr (40). Pollinia transported on bumblebee tarsi were retained approximately 6 hr, whereas pollinia deposited on mouthparts were retained longer than 24 hr (40). Pollinia typically are carried by insects on tarsi, rather than on mouthparts (37). Morse (39, 41) also suggested that carrying large numbers of pollinia reduces the foraging efficiency of bees and increases the probability of long-distance dispersal of pollen. These data suggest that pollinator flight distances are unlikely to provide realistic estimates of pollen dispersal.

Radioactive labelling of pollinia and paternity analysis of seeds have been used to measure effective pollen dispersal distances. Realized pollen dispersal distance determined from paternity exclusion analysis of seeds was three times greater than the mean pollen dispersal distance predicted from pollinator flight distances in populations of *A. exaltata* (6, 10). Mating was random with respect to interplant distances, and matings between neighboring plants were not significantly more common than matings between widely separated individuals. In contrast, by introducing a plant with radioactively labelled pollinia into a population of *A. syriaca,* Pleasants (48) found that 71% of the removed pollinia were inserted within 1 m of the labelled plant. Because *A. syriaca* forms extensive clones, many insertions will result in self-pollination of flowers on other ramets of the clone. It is unclear why pollen dispersal patterns should differ for these two milkweeds.

Differences in pollinators may account for part of the difference in pollen shadows for *A. exaltata* and *A. syriaca.* Pleasants's (48) study population of *A. syriaca* was pollinated by honeybees, whereas study populations of *A. exaltata* were pollinated by butterflies and bumblebees. Naturalists have observed that some native bumblebees (e.g. *Bombus griseocollis*) remove pollinaria and insert pollinia in other flowers far less frequently than do naturalized honeybees (*Apis mellifera*) (6, 37). More rapid turnover of pollinia on honeybees could have resulted in shorter pollen dispersal distances in Pleasants's (48) population of *A. syriaca.* Furthermore, apparent differences exist between lepidopterans and bumblebees in terms of pollination quality and efficiency (10, 44, 45). Thus, pollinators may differ in pollinium removal and deposition schedules on milkweeds, as they do in other flowering plants (69). Further investigations of the effects of pollinators on pollen dispersal in milkweed populations are warranted.

Slow pollinium turnover on large, strong-flying bees and butterflies may contribute to high levels of long-distance pollen dispersal between populations of milkweeds. Paternity exclusion analysis of seeds collected from natural populations of *A. exaltata* in Virginia showed that 11% to 50% of all seeds were sired by plants located outside the six populations that were examined (7, 10). These populations were isolated from other populations by 0.05–1.0 km. The correlation between levels of interpopulation pollen dispersal and isolation distance was statistically significant (Kendall's $t = -0.78$; $N = 7$; $P < 0.05$). Pollen-mediated gene dispersal reported for *A. exaltata* is among the highest reported for any insect-pollinated plant (23).

High levels of gene flow are likely to homogenize the gene pool among populations of milkweeds. Levels of genetic differentiation among populations of milkweeds are much lower than would be expected from studies of other outcrossing perennial herbs. For example, < 10% of total gene diversity ($G_{ST} = 0.093$) is found among populations of *A. exaltata* (11). Similarly, among-population diversity is low for the widespread *A. perennis* ($G_{ST} = 0.082$) and its rare sister species, *A. texana* ($G_{ST} = 0.068$: 19). In other outcrossing, animal-pollinated species, more of their genetic variation is typically partitioned among populations ($\overline{G}_{ST} = 0.197$: 27). Clearly, insect dispersal of large pollinia and wind dispersal of comose seeds (46, 56) contribute to exceptionally high levels of gene flow and low levels of genetic differentiation among populations of milkweeds.

INTERSPECIFIC HYBRIDIZATION

Hybridization in milkweeds is rare, despite many opportunities for interspecific pollination in species that overlap in flowering phenology (31, 34) and habitat (34) and share many of the same generalist pollinators (30, 31, 32, 34, 37, 82). Many authors have attributed the paucity of hybridization in milkweeds to mechanical isolation brought about by a poor fit between pollinia and stigmatic chambers of different species. Recently, the effectiveness of this lock-and-key mechanism has been questioned (33, 34). High levels of interspecific insertions have been reported between several sympatric milkweeds that are not known to hybridize in nature. Mechanical isolation did, however, keep the large pollinia of *A. syriaca* from being inserted into stigmatic chambers of *A. incarnata* and *A. verticillata,* even though insertions of the small pollinia of *A. incarnata* and *A. verticillata* into *A. syriaca* were common (33).

Strong physiological barriers appear to be more important than mechanical barriers in preventing hybridization in milkweeds (30, 77). Foreign pollen germinates and penetrates ovules within the ovary, but seeds fail to develop (30). Even when some hybrid seeds develop, it has been speculated that these fruits may abort because they contain less than a full complement of seeds (85). This phenomenon might reinforce the mechanical isolation between two species that differ greatly in pollinium size. For example, pollinia of *A.*

incarnata would deliver approximately 99 pollen grains to stigmas of *A. syriaca* (R Wyatt, SB Broyles, unpublished data). Ovaries of *A. syriaca*, however, contain more than 200 ovules, and pollen of *A. incarnata* would at best fertilize only half of the ovules. These hybrid fruits with comparatively few seeds would therefore be more likely to abort than fruits with a full complement of seeds.

Hybrid sterility has been observed for artificially produced hybrids between *A. perennis* and *A. texana* (AL Edwards, CT Ivey, R Wyatt, unpublished data). This phenomenon was unexpected, given the relative ease with which interspecific crosses were performed and the vigorous germination and growth of the F_1 interspecific hybrids. Moreover, all species of *Asclepias* are isoploid, with $n = 11$ (77). Hybrids between *A. exaltata* and *A. purpurascens* also show reduced pollen viability relative to parental plants (SB Broyles, R Wyatt, unpublished data). Interestingly, no natural hybrids between these pairs of species have ever been observed. Present-day ranges of *A. perennis* and *A. texana* do not overlap, whereas those of *A. exaltata* and *A. purpurascens* overlap extensively.

Mechanical and physiological isolation is apparently lacking in the few documented cases of natural hybridization in milkweeds. Herbarium records of putative hybrids led Woodson (77) to list nine species pairs that he believed had hybridized in nature. Six of these pairs involved species that Woodson had assigned to different series or even subgenera. More recently, biochemical evidence has been used to document hybridization between *A. exaltata* and *A. quadrifolia* and between *A. purpurascens* and *A. syriaca* (87). Hybridization between *A. syriaca* and *A. speciosa* is supported by the production of artificial hybrids (67, 68), morphological analysis of hybrids (70, 77), and to a lesser extent by biochemical analysis of putative hybrids (1). Hybridization and introgression between *A. exaltata* and *A. syriaca* has been reported from several localities where the two occur sympatrically (34, 85, 87).

Hybridization and introgession between milkweed species appear to be limited. For example, in sympatric populations of *A. exaltata* and *A. syriaca,* fewer than 1% of the seeds produced on *A. exaltata* had been fertilized by pollen of *A. syriaca* (7). Hybridization between these species has been documented from areas associated with human disturbances or elevational gradients (85). These situtations increase the likelihood of finding both *A. exaltata* (a forest species) and *A. syriaca* (a field/meadow species) in close proximity and in flower at the same time. Although hybridization is rare between these species, introgression of genes between these milkweeds may be greater than expected. Alleles diagnostic for *A. syriaca* have been found at low frequencies (<5%) in 22% of *A. exaltata* populations from the southern Appalachian Mountains. Therefore, even low levels of hybridization and introgression may provide a bridge for introducing novel genes into *A. exaltata.*

EVOLUTION OF INFLORESCENCE SIZE

The application of sexual selection theory to explain the evolution of inflorescence size (umbel size) in milkweeds has received considerable attention (2, 6, 8, 9, 13, 15, 51, 54, 55, 71–76, 80, 82). Milkweeds have drawn so much attention because it is easy, compared to plants with loose pollen grains, to estimate male reproductive success by counting the number of pollinaria removed from flowers. According to sexual selection theory, resource-limited fruit and seed production should drive the evolution of reproductive characters, such as inflorescence size, that enhance the probability of siring seeds on other plants (15, 16). Studies supporting this "pollen donation hypothesis" in milkweeds have demonstrated that (i) maximal fruit production is achieved on relatively small umbels, (ii) large umbels have more pollinaria removed than small umbels, and (iii) many more flowers are pollinated on most umbels than can set fruit.

Many of the underlying assumptions of the pollen donation hypothesis have not been critically evaluated in relation to milkweeds. The pollen donation hypothesis assumes that (i) fruit production is not limited by the quantity of pollen that flowers receive, (ii) umbel size is the target of selection, and (iii) male reproductive success is correlated with variation in umbel size. Fruit production may, however, be pollen-limited in many milkweed populations (see above). Simply counting pollinia received by flowers is not an accurate assessment of effective pollination, because pollinia might be improperly inserted (78, 79), pollinia might contain low-quality pollen grains (5), or pollinia might contain incompatible pollen (80). Moreover, a substantial percentage of flowers might not have received any pollinia (13, 88). Second, flower number per umbel is as variable within, as between, plants for many milkweed species (6, 14, 36, 60). In order for the pollen donation hypothesis to work, strong selection would need to target a few specific umbels from a diverse collection of small and large umbels on individual plants. It would, therefore, appear that variation in other inflorescence features, such as umbel number per plant, is more likely to affect reproductive success in milkweeds.

Recent tests have seriously challenged the pollen donation hypothesis in milkweeds. Using paternity exclusion analysis of seeds, Broyles & Wyatt (8, 9) demonstrated that mean umbel size was not significantly correlated with plant-level male or female reproductive success in a natural population of *A. exaltata.* To examine the effect of umbel size on reproductive success more closely, Broyles & Wyatt (13) performed a paternity analysis on seeds in an experimental population of *A. exaltata,* in which umbel size was manipulated by removing flowers. Plants with large umbels (18 flowers) attracted more pollinators and sired more seeds than did plants with small umbels (6 and 12 flowers), but they did not produce significantly more fruits than plants with small umbels. These observations are consistent with the pollen donation hypothesis and would offer strong support for the hypothesis if umbel size were a strong determinant of reproductive success of individuals. In both natural and experimental populations of *A. exaltata,* however, variation in male reproductive success was best explained by flower number per plant, not by flower number per umbel. Furthermore, male reproductive success did not increase at a faster rate than female reproductive success with respect to flower number per plant. Packaging of flowers into umbels and stems appears to be unimportant in determining male reproductive success in *A. exaltata.* On the other hand, umbel and stem number per plant explained most of the variation in female reproductive success. Thus, if natural selection functions to increase total reproductive success, then both male and female reproductive success will be maximized by addition of more inflorescences, rather than through increased investment in flowers on individual umbels.

Natural selection could, however, shape the evolution of umbel size if it were determined that developmental and/or architectural constraints limit the number of stems and umbels that plants produce. In this case, large umbels in milkweeds can maximize pollinator attraction and reproduction through both male and female functions. Pollinarium removal and pollinium insertion increase with inflorescence size (71, 78), even though increased insertion is not likely to result in greater fruit production. Female success, however, can increase on large umbels by selective maturation of fruits with many high-quality seeds. For example, in *A. speciosa,* fruits that contain fewer seeds or seeds that are growing more slowly are less likely to mature than fruits with many vigorous, fast-growing seeds (4). Moreover, pollen donors are known to differ in their ability to sire seeds from flowers within the same umbel (5). In *A. exaltata,* umbels that aborted several fruits generally contained more flowers, received more pollinia, and matured fruits with more seeds than did umbels that did not abort any fruits (9). The upper limit to inflorescence size in milkweeds may be set by the deleterious effects (loss of pollen and ovules) of increased self-pollination (80). Nevertheless, the evolution of inflorescence size probably represents a compromise among processes that simultaneously affect both male and female reproductive success.

It is unnecessary to invoke the "pollen donation hypothesis" to explain the existence of large floral displays and low fruit-set in milkweeds. The use of paternity analysis has permitted a more detailed examination of male reproductive success in natural populations than was previously possible. In both milkweeds and other hermaphroditic flowering plants, male success is generally quantified by counting pollinator visits and measuring pollen removal from flowers. Studies in natural populations of milkweeds have shown that the number of pollinaria removed, the usual estimator for male success, is more highly correlated with the number of seeds produced than with the number of seeds sired (8). The richness of paternity data will undoubtedly allow population biologists to examine similar processes in other flowering plants. As we learned from milkweed studies, the widespread application of sexual selection theory to explain the evolution of floral traits (2, 15, 16, 54) may be inappropriate for other hermaphroditic flowering plants as well.

CONCLUSIONS

Many unusual features of the milkweed reproductive system have contributed to the use of *Asclepias* as a model for studying various aspects of the ecology and evolution of plant reproduction. These include the delivery of pollen grains in discrete packets, accumulation of nectar in accessible reservoirs, use of nectar as the germination medium for pollen, production of two separate ovaries per flower, and possession of ovarian self-incompatibility. To some extent, all of these features have been capitalized upon by students of plant reproductive biology. Nevertheless, a great deal of potential has yet to be exploited by innovative and resourceful asclepiadologists.

We expect that paternity analysis in natural populations of milkweeds will enable workers to test various predictions from sexual selection theory regarding the selective forces driving the evolution of reproductive characters. In milkweeds it will be technically feasible to determine male and female reproductive success for all hermaphroditic plants in a population and then to relate these components of fitness to plant traits, such as inflorescence size. Paternity analysis should also permit detailed quantitative analysis of effective gene flow via pollen dispersal in natural populations. Moreover, by assessing pollen movement at several levels (e.g. electrophoresing single inserted pollinia), it will be possible to develop a very complete picture of the dynamics of pollen dispersal. When such analyses are carried out in the context of hybrid zones, the dynamics of interspecific pollen transfer will be revealed. In most plant species, this aspect of hybridization is often dealt with as a "black box."

We also predict a flurry of new studies using milkweeds to study details of nectar production and the foraging behavior of insects on flowers. Aside from plastic models, milkweed flowers appear to be among the most easily manipulated of flowers. It is surprising that no work has been done thus far involving manipulation of nectar rewards in milkweed flowers. Now that the relationship between the five stigmatic chambers and two ovaries of milkweed flowers is known, it is only a matter of time before experiments are carried out to assess the importance of ovary competition within, versus between, flowers. Finally, an area overripe for exploration at the present time is the nature and functioning of ovarian self-incompatibility in milkweeds. It is well-established that most milkweeds express a late-acting form of self-incompatibility, yet virtually nothing is known about how it functions, its phylogenetic distribution in the Apocynaceae/Asclepiadaceae clade, or its evolutionary origin and maintenance.

Clearly much remains to be done with this unusual and, in many ways, unique system for the study of plant reproduction.

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