POLLINATION BIOLOGY OF *ASARUM HARTWEGII* (ARISTOLOCHIACEAE): AN EVALUATION OF VOGEL'S MUSHROOM-FLY HYPOTHESIS

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ABSTRACT

Stefan Vogel proposed that flowers of *Asarum* s.l. mimic the fruiting bodies of fungi and are pollinated by flies whose larvae feed on mushrooms. Contrary to this view, the flowers of *A. hartwegii* are predominantly autogamous in the Klamath Mountains of northern California. Seed set of bagged flowers in one large population was equivalent to that of unmanipulated controls while emasculated flowers set only about 3% as many seeds as controls. Circumstantial evidence suggests, however, that the vectors responsible for the limited amount of allogamy are mycophagous flies lured by deception. We found fly eggs in 38% of more than 1100 flowers inspected over a four year period. The eggs belonged to 8 species in at least 4 families. The most abundant were laid by *Suillia thompsoni* (Heleomyzidae), whose larvae are obligately mycophagous. Two of the other three flies we identified, *Docosia* sp. (Mycetophylidae) and *Scaptomyza pallida* (Drosophilidae), also have mycophagous larvae while the larvae of the third species, *Hylemya fugax* (Anthomyiidae), normally feed on decaying plant material. Hatched eggs were common in the flowers, but we rarely saw larvae, implying that floral tissue is not a suitable larval substrate and that ovipositing females are attracted by deception. Evidence that the flies are pollinators comes from studies of emasculated flowers: those with eggs were more than three times as likely to set fruit as those without eggs.

Vogel (1973, 1978) described a class of flowers that appear to chemically and structurally mimic the fruiting bodies of fungi and thereby attract flies, especially fungus gnats, who normally oviposit on mushrooms. He proposed that the flies mate and lay eggs in the flowers, and in the process pollinate them. Since the larvae that emerge cannot feed on floral tissue, pollination is acquired by deception (Dafni 1984). Vogel identified several potential examples of such "pilzmüchenblumen", including members of the Araceae and Orchidaceae (see Meeuse and Morris 1984, p. 53, for a striking example), but most of the empirical evidence for the hypothesis came from his studies of *Asarum* and related genera (Aristolochiaceae). Some wild gingers, especially Asian species in the genus *Heterotropa,* have elaborate lamellar or reticulate sculpturing on their sepals that Vogel likened to gill and pore hymenophores, places where fungus gnats ordinarily lay their eggs.

Little is known about the pollination of *Asarum* and its close relatives. Fewer than 10 of the 100 or more species in the complex have been studied in the field, but it appears that at least some are

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pollinated by ovipositing fungus gnats, in accord with Vogel's hypothesis (Sugawara 1988). Other species, however, are exclusively or predominantly autogamous (Wildman 1950; Werth 1951; Daumann 1972; Lu 1982; Tanaka and Yahara 1987). In this paper, we present the results of a four-year study of the pollination biology of *Asarum hartwegii* Wats. in California. Although its flowers are mostly self-pollinating, we argue that autogamy has been superimposed on a system fundamentally adapted for pollination by mycophagous flies.

Methods

Species and study sites. Asarum hartwegii is restricted to the understories of relatively open, mixed conifer-hardwood forests in the Klamath and Sierra Nevada Mountains of California (Mesler and Lu 1990). Unlike some other North American species in the genus (e.g., *A. caudatum, A. canadense*), which form extensive mat-like clones, individual plants of *A. hartwegii* occur as distinct clumps. Each plant bears from 1 to several concurrently blooming flowers at ground level. The flowers have reddish-brown sepals and produce a faint, musty fragrance. The bases of the sepals stand close together to form a false calyx tube. On the inner surface of the tube, each sepal bears several dense parallel bands of white hairs. The flowers are protogynous; stigmas are receptive for about seven days before anthers dehisce. At the close of the female stage, the filaments change orientation, so that first 6 (early-male stage) and then all 12 anthers (late-male stage) come into contact with stigmas. Lu (1982) and Tanaka and Yahara (1987) illustrate an essentially identical autopollination mechanism in two other species of *Asarum*. Ovule number ranges from 21 to 95 per flower (average = 53.2, SE = 0.9, n = 383).

Our main study site was Steelbridge campground, located 7 km SE of Weaverville, CA. (Trinity Co.). Here a large population of more than 1000 plants of *A. hartwegii* occurs in a relatively mesic Douglas Fir forest on a north-facing slope at 700 m. Additional observations were made at four other sites in northern California, the most distant approximately 80 km NE of Steelbridge (Trinity Co.: Cal Trans rest stop, 12.8 km E of Willow Creek; Gray's Falls Campground, 19.5 km E of Willow Creek; Denny Rd, 2.4 km N of CA 299; Shasta Co.: Ah-di-na Campground, 16 km S of McCloud).

Pollination experiments. Plants selected from throughout the Steelbridge site were used to assess the relative importance of autogamy, allogamy, and apomixis. A single flower per plant was assigned to one of the following pollination treatments. (1) *Controls*—Unmanipulated, open-pollinated flowers provided an estimate of

natural levels of fruit and seed production in 1984 and 1985. (2) *Emasculated*—To estimate the potential for allogamy during the female stage, we emasculated newly opened flowers in 1984, and then bagged them after 7 additional days of exposure. The bags were left on for 2–3 weeks, until the stigmas turned brown and were apparently no longer receptive. In 1985, flowers were emasculated in the same way but not bagged, to allow the maximum opportunity for detection of cross-pollination. In both 1984 and 1985, the average distance between emasculated flowers and the nearest male flower was less than 1 m, insuring a source of pollen for cross-pollination. Because the closest pollen source was often another flower on the same plant, seeds produced by emasculated flowers could result from either geitonogamous or xenogamous pollinations. (3) *Bagged*—To test for self-compatibility and the effectiveness of autopollination, we bagged a set of flowers in 1984 prior to anthesis. The bags were removed after the stigmas were no longer receptive. (4) *Emasculated and bagged*—To check for apomixis, we emasculated and bagged 13 flowers in 1984. The average number of ovules per flower did not differ significantly across treatments in either year (P's > 0.10).

Experimental flowers were collected prior to fruit maturation at the end of the season, after allowing enough time for maturing seeds to be unambiguously distinguished from unfertilized ovules and abortive seeds. We expressed pollination success on two levels, the percentage of flowers that set fruit and the average number of seeds per fruit. We calculated G and t statistics to test for differences in fruit set and seed number, respectively (Sokal and Rohlf 1981). In 1984, a priori pairwise tests were used to compare the performance of control vs. emasculated flowers and control vs. bagged flowers. Seed number was square-root transformed, as necessary, to equalize variances prior to statistical analysis. Emasculated flowers were checked for fly eggs and larvae, as described below, to determine the relationship between oviposition and pollination.

Surveys for fly eggs and larvae. To determine the frequency of flower visitation by ovipositing flies, additional flowers were checked for eggs and larvae at Steelbridge over a four-year period. In 1984–1986, sets of female, early male, and late male flowers were collected weekly throughout the flowering season. In 1987, collections were made at the end of the season when male-phase flowers predominated. Flowers from the other four study sites, most of which were in late male phase, were inspected for eggs and larvae in 1985 or 1986. In all cases, sepals were removed from flowers and examined at 30× as eggs are easily overlooked, especially the translucent ones of fungus gnats. Basidiocarps were uncommon at Steelbridge during the flowering season, but we checked those we found for fly eggs.

TABLE 1. COMPARISON OF FRUIT AND SEED PRODUCTION BY CONTROL, EMASCULATED, AND BAGGED FLOWERS. G-tests and t-tests were used to compare fruit set and seed number, respectively. [a] Control vs. emasculated, $P < 0.001$; control vs. bagged, $P > 0.10$. [b] Control vs. emasculated, $P < 0.001$. [c] Control vs. emasculated, $P < 0.001$; control vs. bagged, $P > 0.90$. [d] Control vs. emasculated, $P < 0.001$.

	Fruit set		Number of seeds per fruit	
	1984[a]	1985[b]	1984[c]	1985[d]
	% (N)	% (N)	$\bar{x} \pm$ SE (N)	$\bar{x} \pm$ SE (N)
Control	98.2 (111)	100 (58)	35.8 ± 1.5 (109)	34.3 ± 1.6 (58)
Emasculated	20.9 (115)	65.6 (99)	5.0 ± 1.1 (24)	17.3 ± 1.4 (65)
Bagged	100 (51)	—	35.1 ± 1.6 (51)	—

Flies were reared at room temperature in glass culture dishes with a 2–3 cm layer of slightly moistened vermiculite at the bottom, covered with a piece of filter paper. Eggs or larvae from the flowers were transferred to a piece of moistened substrate placed on top of the filter paper. We used pieces of unflavored rice cake or commercial mushroom as the substrate. Mushrooms used in the cultures were checked carefully in advance for eggs and larvae.

RESULTS

Mating system. None of the emasculated and bagged flowers set fruit, indicating that apomixis (agamospermy) does not occur. Very little allogamy occurred in 1984, as shown by the low fecundity of emasculated flowers, which produced only about 3% as many seeds as controls (Table 1). The difference was the result of both a higher percentage of fruit set on the part of control flowers and a greater average number of seeds per fruit. In contrast, fruit and seed sets of controls and bagged flowers were equivalent, indicating that autogamy was responsible for most seed production.

The stigmas of the flowers we emasculated and left unbagged in 1985 remained turgid and apparently receptive for several weeks. Not surprisingly, these flowers experienced a higher level of pollination success than the ones we emasculated in 1984 (Table 1). Nevertheless, fruit and seed sets of controls still greatly exceeded those of emasculated flowers.

Frequency of flower visitation and oviposition. During our four-year study at Steelbridge, we checked hundreds of flowers, from early morning to dusk. Adult insect visitors, which included fungus gnats, other flies (Heleomyzidae, Anthomyiidae), and a staphylinid beetle (*Anthobium* sp.), were present in far less than 1% of the flowers. In contrast, we consistently found fly eggs and larvae in them during the same four year period. The fraction of flowers with at least one

TABLE 2. COMPARISON OF FRUIT AND SEED PRODUCTION BY EMASCULATED FLOWERS WITH AND WITHOUT FLY EGGS. G-tests and Mann-Whitney U-tests were used to compare fruit set and seed number, respectively.

	Fruit set		Number of seeds per fruit	
	1984	1985	1984	1985
	% (N)	% (N)	$\bar{x} \pm$ SE (N)	$\bar{x} \pm$ SE (N)
Eggs present	46.2 (26)	76.7 (73)	7.0 ± 2.1 (12)	18.3 ± 1.5 (56)
Eggs absent	13.5 (89)	34.6 (26)	3.1 ± 0.7 (12)	10.8 ± 3.5 (9)
	$P < 0.001$	$P < 0.001$	$P = 0.08$	$P = 0.03$

egg ranged from about 21% in 1984 to 59% in 1987 (Table 3). On average, flowers contained 2.6 to 4.0 eggs, depending on year, but the distribution was strongly skewed, with a single egg present in more than 35% of the cases. Ovipositing flies visited female, early male, and late male phase flowers, as shown by the increase in the percentage of flowers with eggs in successively older flowers (Fig. 1). More than 90% of the eggs were found on the inside of the calyx tube, between rows of hairs or beneath the hairs. Larvae were much less common than eggs (Table 3), even though collapsed (and presumably hatched) eggs were frequently seen. Larvae were usually found at the base of the flower, where they appeared to be eating pollen, not floral tissue.

We found the eggs of eight different species of flies in flowers at Steelbridge, four of which we were able to rear and identify (Table 3). The most common eggs are fusiform, 0.75–0.80 mm long, and have a reticulate, opaque white chorion. These belong to *Suillia thompsoni* (Heleomyzidae). Another higher fly, *Hylemya fugax* (An-

TABLE 3. PERCENTAGE OF FLOWERS WITH FLY EGGS AND LARVAE. With few exceptions, larvae were those of higher flies (= lacked a head capsule).

	1984	1985	1986	1987
	Percentage			
Eggs				
Suillia thompsoni Gill (Heliomyzidae)	12.6	33.8	24.2	28.5
Hylemya fugax (Meigen) (Anthomyiidae)	5.1	5.4	9.0	29.2
Scaptomyza pallida Zetterstedt (Drosophilidae)	0.2	0.2	0.3	0.0
Docosia sp. (Mycetophilidae)	0.2	0.0	2.0	1.5
Unidentified fungus gnats	0.2	0.0	1.2	0.1
Other unidentified eggs	5.1	4.5	13.5	16.9
All egg types	20.9	40.4	45.8	58.5
Larvae	1.8	2.9	7.4	8.5
Number of flowers inspected	334	314	244	130

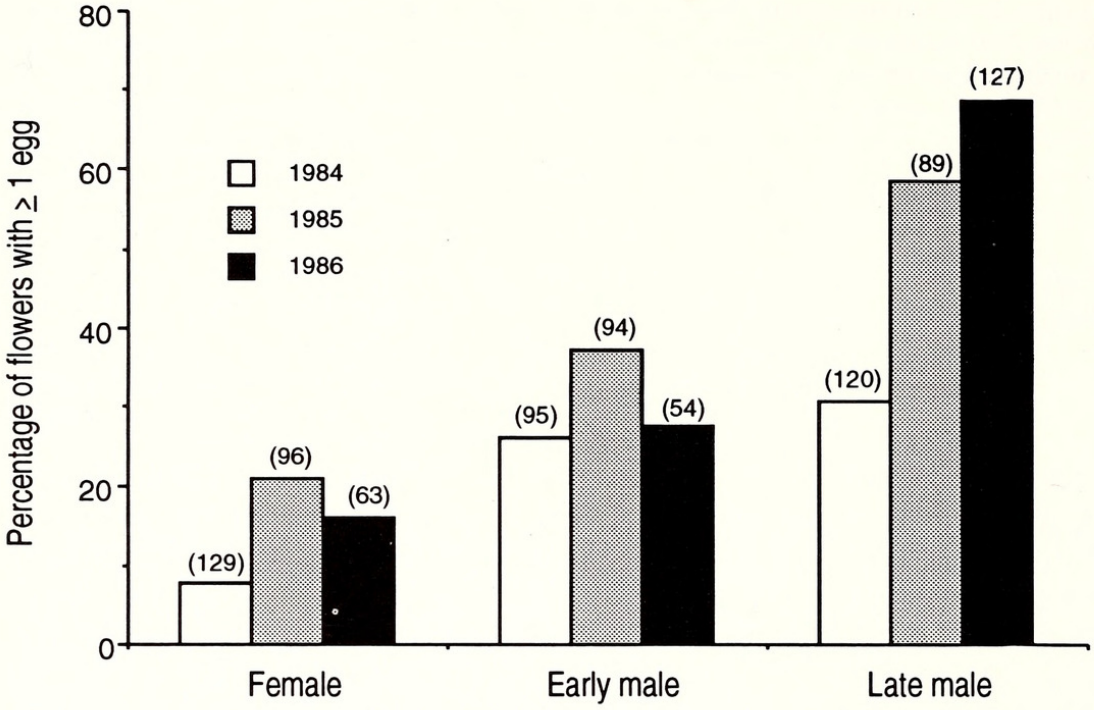

FIG. 1. The percentage of female, early male, and late male flowers with ≥1 egg in 1984–1987 at Steelbridge. Numbers in parentheses are sample sizes.

thomyiidae), laid eggs in fewer flowers, except in 1987. The eggs of this species are similar to those of *S. thompsoni,* except for a pair of ridges that run down one side. Much less common were the eggs of the fungus gnat *Docosia* sp. (Mycetophylidae), which are smaller (0.3 mm long), ovoid, and have a translucent chorion with faint striations. We rarely found eggs of *Scaptomyza pallida* (Drosophilidae), which are 0.5 mm long, with a coarsely reticulate chorion and a pair of prominent wings. Among the unidentified eggs were two other types with the opaque, reticulate or striate chorion characteristic of higher flies, in addition to the elongate, translucent ones of other fungus gnats (Mycetophylidae or Sciaridae). We found eggs of *S. thompsoni* and fungus gnats on fruiting bodies of *Cortinarius* and *Collybia* collected at the site.

Fly eggs were present in 19% (n = 57), 50% (n = 12), 57% (n = 7), and 60% (n = 20) of the flowers inspected at the four other sites, respectively. In general, the same ensemble of eggs was found at these sites as at Steelbridge. *S. thompsoni* accounted for more than half of the ovipositions at all four of the sites. Among the other eggs were those of *H. fugax, Docosia* sp., *S. pallida,* and one of the unidentified higher flies seen at Steelbridge.

Relationship between oviposition and pollination. Although we occasionally saw flies on anthers and stigmas, we could not establish, by direct observation, a link between oviposition and pollination

because flower visits were so infrequent. Correlative evidence suggests, however, that ovipositors are pollinators. In both 1984 and 1985, emasculated flowers with eggs had a much higher probability of setting fruit than those without eggs (Table 2). The number of seeds per fruit was also higher for flowers with eggs in both years, although the difference was not significant in 1984. The number of seeds per flower was correlated with the number of eggs per flower in both years, although much more weakly so in 1984 (1984: Pearson $r = 0.15$, $P = 0.06$, $n = 115$; 1985: $r = 0.49$, $P < 0.001$, $n = 99$).

Discussion

Our observations provide mixed support for Vogel's hypothesis that wild ginger flowers are adapted for pollination by mushroom flies. On the one hand, if our Steelbridge study site is representative, *A. hartwegii* is predominantly autogamous. The initial female floral phase allows for allogamy, but insect visits are very infrequent and automatic self-pollination is responsible for nearly all seed production. Other species of *Asarum* s.s. likewise appear to be mostly or completely autogamous (Wildman 1950; Werth 1951; Daumann 1972; Lu 1982; Tanaka and Yahara 1987). On the other hand, our emasculations revealed a limited amount of pollen transfer between flowers. We did not witness cross-pollination, but strong circumstantial evidence suggests that the pollen vectors are ovipositing flies lured to the flowers by deception: (a) flies commonly visit and lay eggs in both female- and male-phase flowers, (b) their larvae do not eat floral tissue, and (c) emasculated flowers with eggs are much more likely to be pollinated than those without eggs. An analysis of the larval substrates normally used by the flies is consistent with the idea that the deception is based on mimicry of fungi, as Vogel proposed.

Three of the four flies whose eggs we could identify have mycophagous larvae. The most common egg-layer, and presumably the most important pollinator, *S. thompsoni* (Heleomyzidae), is an obligate mycophage which oridinarily oviposits on the fruiting bodies of several genera of fungi (Hayward 1984; R. S. LaChance unpublished data). We found its eggs on *Cortinarius* and *Collybia* at Steelbridge. *Docosia* sp. and other, unidentified Mycetophilidae or Sciaridae also oviposited in the flowers, although much less frequently than *Suillia.* Members of this group are well-known fungivores (Vockeroth 1981). Like many other fruit flies, *S. pallida* is mycophagous, although its larvae are known to use decaying plant material also (Dely-Draskovits and Papp 1973; Courtney et al. 1990). The fourth species, *Hylemya fugax,* apparently has not been collected from mushrooms. Its larvae use a wide variety of substrates, but they prefer decaying plant material (Miles 1950). Other saprophagous flies are known to

use mushrooms as a food source (Bruns 1984), and the same may be true of *H. fugax,* especially since two other species of *Hylemya* are so-called secondary fungivores (Eisfelder 1956; Bruns 1984), ones that specialize on decomposing mushrooms.

Even though *A. hartwegii* is almost exclusively autogamous, its flowers have features, like protogyny, elaborate sepals, and fragrance, that probably evolved as part of an insect-pollinated system. If these traits are, in fact, adaptations for cross-pollination by mycophagous flies, they are largely vestigial at present. The same may be true of the obviously entomophilous features of *A. caudatum,* the species which Vogel (1978) used as the centerpiece of his work on the fungal mimesis hypothesis. Although he observed pollination of this species by ovipositing fungus gnats in his garden in Europe, and found mycetophilid eggs in flowers from several populations in western North America, Lu (1982) showed that *A. caudatum,* like *A. hartwegii,* is primarily autogamous. The apparent loss of the interaction responsible for the evolution of the traits of *A. hartwegii* and *A. caudatum* makes testing Vogel's hypothesis difficult or impossible in these cases. Studies of other members of the *Asarum* s.l. complex, most of which cannot directly self-pollinate and thus must rely on insects for pollination (Sugawara 1987), would provide more convincing tests of the hypothesis. The single species in this group that has been studied, *Heterotropa tamaensis,* is pollinated by ovipositing fungus gnats (Sugawara 1988).

Vogel's hypothesis that certain flowers obtain pollination by masquerading as mushrooms is worthy of further study. Our results indicate, however, that the hypothesis should be extended to include potential pollinators other than Mycetophylidae. Flies in several families, including Anthomyiidae, Drosophilidae, Heleomyzidae, Muscidae, and Phoridae, lay their eggs on mushrooms (see references in Bruns 1984). Moreover, the feeding habits of mycophagous fly larvae are diverse; some prefer fresh, living tissue while others use decomposing mushrooms (Eisfelder 1956; Bruns 1984; Hayward 1984). Given this diversity, we should expect a range of different mushroom-fly blossoms, depending on the larval preferences of the flies involved. Some, in fact, may rely exclusively on fungus gnats (e.g., *Heterotropa tamaensis,* Sugawara 1988) but others, like *A. hartwegii,* may have evolved as more generalized mushroom mimics that attract an array of mycophagous flies.

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