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THE POLLINATION BIOLOGY OF TUCKAHOE, *PELTANDRA VIRGINICA* (ARACEAE)

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This study describes the specialized brood-site-based pollination system of *Peltandra virginica* Kunth (Araceae) with the chloropid fly *Elachiptera formosa* Loew and provides experimental evidence that gender-related changes in floral odor composition synchronize pollinator behavior with the blooming sequence. *P. virginica* is protogynous and does not self-pollinate because of a strong temporal separation in sexual function, and it is dependent upon insects for pollination because the spathe completely surrounds the spadix during the pistillate stage. Field observations conducted in central New Jersey and southeastern Pennsylvania showed that *E. formosa* is closely associated with *P. virginica* inflorescences. Within the floral chamber, *E. formosa* adults feed on pollen, mate, and find oviposition sites while the larvae complete their development. Although drosophilid and syrphid flies were collected and reared from inflorescences of both sexual stages, only *E. formosa* emerged from pistillate-stage inflorescences and adult *E. formosa* rapidly transferred fluorescent dye particles between inflorescences of both sexual stages in laboratory enclosures. These findings indicate that this fly species is the primary pollinator at our study locations. Field censuses demonstrated that although *E. formosa* visited *P. virginica* inflorescences of both sexual stages with equal frequency, the female flies preferentially oviposited within pistillate-stage inflorescences. Analysis of floral volatiles with gas chromatography–mass spectrometry showed that only the spathes emit the floral fragrance and that the composition of the floral volatiles changed during the transition from pistillate to staminate stage. A particularly noticeable change occurred in the emission ratio of the two primary floral odor components, 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane and an unidentified chemical analog with molecular weight of 142. The relationship between floral volatile composition and fly oviposition behavior was evaluated using sham inflorescences placed in the *P. virginica* study population. The sham inflorescences were constructed by covering spadices dissected from mature inflorescence buds with spathes excised from different-age inflorescences. After a 2-hr-long exposure period, sham inflorescences fitted with pistillate-stage spathes contained more *E. formosa* eggs than the sham inflorescences with staminate-stage spathes. However, the highest number of ovipositions occurred in sham inflorescences fitted with spathes excised from mature inflorescence buds. The fragrance emitted by these spathes was composed almost entirely of the two principal odor components. The decline in ovipositions observed in both real and sham inflorescences corresponded to an increase in the emission ratio of 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane to the molecular weight 142 analog. This suggests that gravid flies searching for oviposition sites used the ratio of the two primary floral volatile components as an inflorescence gender or age recognition cue.

Plants emit visual and olfactory floral signals to attract and influence the behavior of their pollinators (Vogel, 1962; von Frisch, 1967; Proctor and Yeo, 1972; Faegri and van der Pijl, 1979). Floral signal production is a dynamic process and changes in floral signals are adaptive with respect to conserving floral resources and maximizing pollinator efficacy (Gori, 1983; Weiss, 1991). Circadian, ontogenetic, and postpollination changes in floral odor are thought to affect strongly pollinator behavior (Nilsson, 1978; Matile and Altenburger, 1988; Tollsten and Bergström, 1989; Ware et al., 1993). However, because floral odors are complex mixtures of volatile compounds (Williams, 1983; Bicchi and Joulain, 1990; Dobson, 1991, 1994; Knudsen, Tollsten, and Bergström, 1993), it is difficult to determine how alterations in the composition, proportion, and concentration of floral odor components influence pollinator behavior; and experimental evidence for the role of floral fragrance in mediating plant-insect interactions are lacking (Williams, 1983; Dobson, 1994).

Studies of honeybee-pollinated flowers (Pham-Delegue et al., 1986, 1989; Henning et al., 1992) and of orchids pollinated by male solitary bees (Dodson et al., 1969; Williams and Whitten, 1983; Borg-Karlson, Bergström, and Groth, 1985; Borg-Karlson, 1990) have shown a strong correlation between bee attraction and floral volatile composition and emission ratio of individual components. Plants that are specifically pollinated by insects that utilize the flowers as a mating arena or brood site may also provide suitable study systems for examining the rela-

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tionship between floral odor composition and biological activity. This is because these plants are often dichogamous, and stereotypical pollinator behaviors, such as aggregation, mating, and oviposition, are displayed at discrete floral sexual stages (Gottsberger and Amaral, 1984; Young, 1986; Bronstein, 1992). In these flowers, specific pollinator behaviors may be induced by particular volatiles emitted during each floral stage, and specific changes in floral volatile composition may promote the synchronization of pollinator behavior with the sequence of anthesis (Galil, Zeroni, and Bar Shalom, 1973).

Goldberg (1941) found chloropid and syrphid fly eggs and larvae within the inflorescences of *Peltandra virginica* Kunth (tuckahoe or arrow arum) (Araceae), a widespread and abundant emergent aquatic plant found in upper tidal and freshwater wetlands throughout eastern and central North America (Goldberg, 1941; Whigham, Simpson, and Leck, 1979). We conducted preliminary observations in central New Jersey and southeastern Pennsylvania in 1987, which indicated that *P. virginica* has a specialized brood-site-based pollination system with the chloropid fly *Elachiptera formosa* Loew. Preliminary tests conducted in a laboratory wind tunnel indicated that the floral odor of *P. virginica* was highly attractive to *E. formosa* and that exposure to the floral odor could induce the fly to oviposit on artificial spadices constructed from plastic pipettes.

Like all aroids, *P. virginica* is protogynous (Goldberg, 1941) and the floral odor changes during gender transition: to the human nose pistillate-stage inflorescences have a pleasant flowery odor while staminate-stage inflorescences have a strong musty odor, reminiscent of decaying vegetation. Whereas the floral odor of other aroid species that are pollinated by dung-visiting flies and beetles contain many aminoid compounds (Smith and Meeuse 1966), the floral odor of *P. virginica* is composed primarily of isomers of novel trimethyl-6,8-dioxabicyclo[3.2.1.]octanes (correctly identified but incorrectly named as trimethyl-2,5-dioxabicyclo[3.2.1.]nonanes by Patt et al. [1992]). Two components, 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1]octane and an unidentified trimethyl-6,8-dioxabicyclo[3.2.1.]octane chemical analog with a molecular weight of 142 amu quantitatively dominate the floral volatile mixture.

These observations prompted the present study where we describe the aroid's association with *E. formosa,* provide details on the compositional changes that occur in the floral volatiles during the blooming sequence, and present experimental evidence suggesting that specific changes in floral volatile composition have a direct effect on *E. formosa* oviposition behavior. A series of observations and experiments was conducted in the field, laboratory, and greenhouse to determine the flowering phenology, breeding system, sequential pattern of floral odor emission, and mode of pollination of *P. virginica.* The biological significance of changes that occur in the floral odor during the sequence of anthesis was demonstrated by behavioral bioassays performed in the field.

MATERIALS AND METHODS

Floral morphology—The 10–40 cm long inflorescence (Fig. 1) is composed of a yellow spadix enclosed by a green elongated spathe (Goldberg, 1941). The basal portion of the spadix has several whorls of globose pistillate flowers,

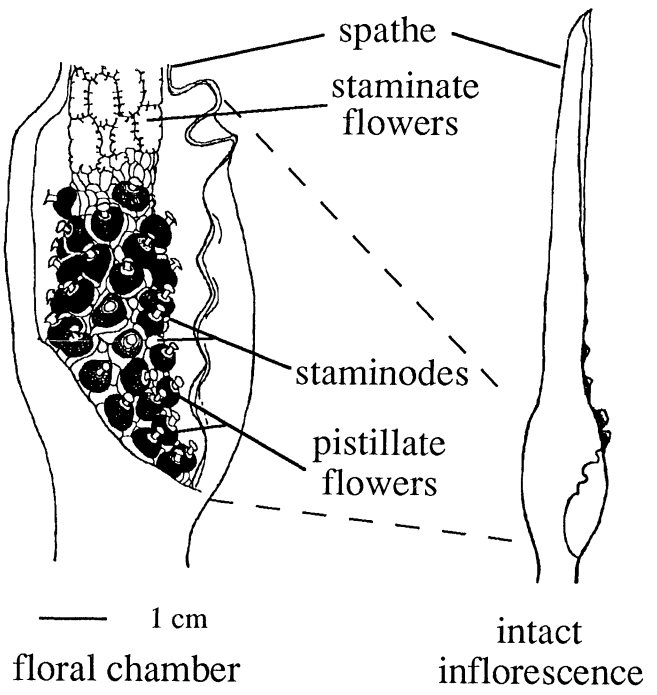

Fig. 1. Intact *P. virginica* inflorescence with cut-away view showing floral chamber formed by the enveloping spathe, the location of pistillate flowers with surrounding staminodes, and staminate flowers (synandria) on the upper portion of the spadix.

each surrounded by sterile plate-like staminodes. The staminate flowers, in the form of synandria, comprise the bulk of the spadix. Wind pollination is precluded because the spathe encloses the spadix during the initial phase of anthesis.

On the 1st d of anthesis, the stigmas are receptive and the basal portion of the spathe margins separates slightly and forms apertures that permit entry only by small insects. As the flowering sequence progresses, the spathe margins continue to separate and begin to curl at the edges. By the 2d d of flowering, the stigmas have withered, the basal synandria begin to dehisce, and the upper portion of the spathe margins separates until it is fully apart and develops wavelike undulations. On succeeding days, the staminate flowers continue to dehisce acropetally until the uppermost synadria open (Goldberg, 1941).

Study sites—This study was conducted at four sites in central New Jersey and southeastern Pennsylvania: 1) East Millstone, a freshwater swamp on the Millstone River floodplain in the Delaware and Raritan Canal State Park (Somerset County, NJ); 2) New Brunswick, an extensive *P. virginica* population growing in a wet depression near the banks of the Raritan River (Middlesex County, NJ); 3) Weston Mills Pond, a large *P. virginica* population growing on a delta at the confluence of a small creek and Weston Mills Pond in North Brunswick (Middlesex County, NJ); and, 4) Tinicum, where *P. virginica* grew along the inner littoral fringe of a tidal pond connected to Darby Creek in the John Heinz National Environmental Center (Delaware County, PA). A greenhouse colony of plants was established for breeding system and floral odor stud-

ies. Thirty mature plants from the East Millstone population were individually transplanted into 20-liter plastic buckets filled with water. The plants were grown under a 16-hr light : 8-hr dark photoperiod and fertilized weekly. After the plants completed their growth cycle and senesced, they were transferred from the greenhouse to an unlighted coldroom and kept at 3 C for 6 mo for vernalization. The vernalized plants flowered ≈3 wk after transfer from coldroom to greenhouse.

Flowering phenology—Plastic identification markers were placed on 25 plants at the New Brunswick field population immediately prior to the flowering season in May 1990. The number of unopened inflorescence buds and pistillate- and staminate-stage inflorescences were counted on each tagged individual once per week from late May through mid-July.

Breeding system—To investigate the effectiveness of outcrossing, interspadix pollination within the same plant and intraspadix pollination in *P. virginica,* hand pollinations were performed in the greenhouse. To demonstrate outcrossing, 12 inflorescences from six plants were emasculated on the 1st d of anthesis and hand pollinated with pollen from another plant. To test for interspadix geitonogamy on the same plant, nine pistillate-stage inflorescences from nine plants were hand pollinated with pollen collected from the same plant. Intraspadix pollination was tested with a group of 18 inflorescences from nine different plants. These inflorescences were not emasculated but left intact and allowed to self-pollinate. To clarify the temporal separation of sexual stages, the duration of stigma receptivity was determined by emasculating nine inflorescences on the 1st d of anthesis then hand pollinating them with outcrossed pollen either in the morning ($N = 4$) or afternoon ($N = 5$) of the next day.

For all treatments, hand pollinations were performed in the morning of the 1st d of anthesis and pollen was transferred with an artist's paintbrush containing fresh pollen. Following hand pollination, the inflorescences were covered with fine nylon mesh bags to exclude greenhouse insects, e.g., fungus gnats. Fruit growth and development were recorded for all pollination treatments. After the fruit matured and was harvested, the pericarp was removed and the seeds were planted ≈2 cm deep in moistened vermiculite to test for seed germination.

Pollen tube germination and growth among the different pollination treatments was determined by examining the stigmas of seven intraspadix-pollinated, two intraplant-pollinated and 11 outcrossed inflorescences which were collected 24 hr following hand pollination and preserved in FAA (formalin acetic acid, 9:1:1 of 95% ethanol : formalin : glacial acetic acid). Individual pistillate flowers were then removed from the spadix and softened by immersion in saturated KOH (10 M) for 60 min. The flowers were stained with aniline blue and gently squashed on glass microscope slides prior to examination with an epifluorescence microscope at 375 nm (Martin, 1959).

Pollinator observations—Pollinator observations were conducted at all four study populations throughout the flowering seasons from 1988 to 1991. Insect arrival and behavior were recorded at designated plants bearing inflorescences of both sexual stages. Plastic bags were placed over inflorescences to collect insects that had entered the floral chamber and the insects were removed from the bag with an aspirator. Fly adults and larvae were examined with a dissecting microscope for the presence of pollen on the exoskeleton and within the digestive tract. Observations were made of *E. formosa* larvae presented with *P. virginica* pollen on microscope slides.

The species composition, number, and size of fly larvae within *P. virginica* inflorescences were determined by rearing insects from 25 inflorescences collected from the New Brunswick population on 26 June 1989 and from the East Millstone population on 3 July 1989. The inflorescences were placed on moistened vermiculite within 25-cm-long glass tubes provided with foam stoppers (Grimaldi and Jaenike, 1983). The tubes were kept in a controlled temperature room at 25 C under 16-hr light : 8 hr dark photoperiod and were kept moistened with distilled water. All emerging insects were collected daily, counted, and identified.

Pollination effectiveness and behavior of E. formosa—The ability of *E. formosa* to transfer pollen between inflorescences and its attraction to inflorescences of different sexual stages was evaluated in a controlled temperature room at 25 C using fluorescent powder (Day-Glo Color Corp., Cleveland, OH). Different-colored fluorescent powders were dusted onto the synadria of a pistillate-stage and a staminate-stage inflorescence. The two marked inflorescences were then placed in a circular array containing four other unmarked inflorescences, two of each sexual stage. The inflorescences were arranged inside a ventilated plexiglas box (150 cm long × 50 cm wide × 50 cm high) containing five female and five male *E. formosa.* The inflorescences were exposed to the flies for 2 hr between 1200 hr and 1400 hr and afterwards were removed and examined with a dissecting microscope for the presence of dye particles. This procedure was replicated 5 times with different sets of flies and inflorescences.

To determine if *E. formosa* visitation resulted in pollen transfer in the field, 44 mature inflorescence buds from the Weston Mills Pond population were covered with fine nylon mesh bags between 13 May and 21 May 1991. On succeeding days, the bags were removed from newly opened inflorescences between 0800 hr and 0900 hr and left undisturbed until they were collected at 1400 hr. The inflorescences were immediately taken to the lab and examined with a dissecting scope for the presence of pollen on the stigmas and fly eggs in the floral chamber.

A series of three field censuses was conducted to determine if *E. formosa* preferred to visit or oviposit within inflorescences of a particular sexual stage. These studies were conducted from 1000 to 1800 hr at Weston Mills Pond on 1 June and 22 June 1990 and at Tinicum National Environmental Center on 28 May 1991. Prior to each census, 18–35 inflorescences of both sexual stages were covered with nylon mesh bags to prevent insect entry. At the beginning of each census, the bags were removed and the inflorescences were divided into two groups, one for determining fly visitation preference and the other for fly oviposition preference. The visitation group inflorescences were inspected once every 2 hr, and all insects were collected from them. The oviposition group inflorescences

were left undisturbed after bag removal and were collected at 1800 hr and brought to the lab for microscopic examination. The number and distribution of fly eggs within the floral chamber were determined for each inflorescence.

Floral odor analysis—Only spathes were used for floral volatile sample collections, since preliminary analyses had demonstrated that the floral volatiles in *P. virginica* were emitted exclusively by the spathe and not by the spadix. Spathes from 12 inflorescences of each sexual stage were collected from the Tinicum population and were immediately placed in individual 3-liter three-necked glass flasks. These floral odor collections were made concurrently with the oviposition choice tests (described below) from 1100 hr to 1300 hr on 29 June and 2 July 1991. Each flask contained enough 5% sucrose solution to immerse the cut ends of the spathes. Odor collections were also made from spathes excised from mature inflorescence buds, since these were used as part of the control treatment in the oviposition choice field test.

A purge and trap headspace system was used to collect the floral odor compounds (Patt et al., 1992). Odor samples were collected for 2 hr, from 1100 to 1300 hr; preliminary collections had demonstrated that this was the daily peak period of floral odor emission. Air was drawn from the flasks through 10 cm long × 6 mm outer diameter silanized borosilicate glass trap tubes, which were filled with conditioned Tenax TA (30 mg, 60/80 mesh) and Carbotrap (100 mg) (Supelco) adsorbents with a portable air sampling pump (SKC, Inc., Eighty Four, PA) at a flow rate of ≈30 ml/min. A dual adsorbent trap tube was used to ensure that a wide range of floral volatiles was collected (Dobson, 1991; Hartman et al., 1993).

The trap tubes were desorbed using a short path thermal desorption system prototype (Scientific Instrument Services, Ringoes, NJ) interfaced to the GC injection port. All analyses were performed with a GC directly interfaced to a Finnegan MAT 8230 Mass Spectrometer using the parameters described in Patt et al. (1992). The ratios of emitted compounds were calculated by comparing the peak areas of the compounds in the total ion chromatographs with that of an internal standard (D-toluene) added to each sample immediately prior to analysis. Identification of the compounds was achieved by comparison of the sample mass spectra with reference mass spectra (Heller and Milne, 1978; Patt et al., 1992).

Oviposition choice test—Field tests with live sham inflorescences were conducted in a *P. virginica* study population to determine if the floral odor emitted by each sexual stage had a differential effect on fly oviposition behavior. Sham inflorescences were constructed by removing a spadix from a freshly collected mature inflorescence bud and inserting it into a fresh spathe from either a pistillate- or staminate-stage inflorescence. Both sexual stages thus afforded a similar oviposition substrate to the flies. Since we could not detect any odor emitted by mature inflorescence buds, control sham inflorescences were constructed by inserting a spadix into the spathe excised from a mature inflorescence bud. Plastic funnels filled with 5% sucrose solution and attached to 1-m-long wooden dowel rods were used to hold the bases of the sham inflorescences.

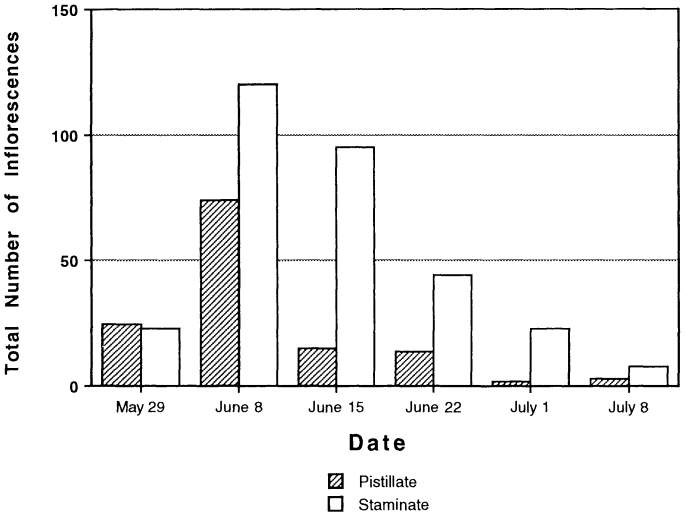

Fig. 2. Blooming phenology of the New Brunswick *P. virginica* study population showing the total number of pistillate-stage and staminate-stage inflorescences from May to July 1990.

Two oviposition choice tests were conducted at Tinicum on 29 June and 2 July 1991 from 1100 to 1300 hr. Immediately prior to the start of each test, ten sham inflorescence groups were positioned in ten different *P. virginica* plants. Each group consisted of three sham inflorescences, one from each sexual stage plus the bud stage control, placed ≈25 cm apart from each other in a large flowering *P. virginica* plant. The 30 sham inflorescences were visually inspected but not handled during the 2-hr test period to prevent disturbance of any *E. formosa* that may have entered them. At the end of the test period the sham inflorescences were collected, and the number and distribution of fly eggs within each one were determined by microscopic examination.

RESULTS

Flowering phenology—From 1988 to 1990, *Peltandra virginica* bloomed at the central New Jersey populations from late May to late July or early August. In 1991, the year with the warmest average spring and summer temperatures on record in New Jersey, blooming commenced in mid-May and finished in late June. In 1991, only plants growing in tidal waters, such as at Tinicum, bloomed after the last week in June. During the 1990 field season, the main flowering period lasted 5 wk (29 May–23 June), but small numbers of open inflorescences were present during a 9-wk period (Fig. 2). Similar numbers of inflorescences at both sexual stages were open during the 1st wk of the flowering period, but, afterwards, staminate-stage inflorescences predominated. These differences in the sexual-stage ratio can be attributed to the duration of each sexual stage: anthesis takes place over a 3-5 d period, but the pistillate-stage lasts only 1 d while the staminate-stage lasts 2-4 d. Individual plants usually had one or two pistillate-stage and two to five staminate-stage inflorescences in bloom at any one time.

Breeding system—Pistillate flowers were receptive primarily on the 1st d of anthesis (Table 1). Fruit-set occurred

TABLE 1. Summary of greenhouse breeding system experiments by pollination treatment showing percentage of pollinated inflorescences that produced mature fruit.

Pollination treatment	Infructescence development		Seed germination	
	%	N	%	N
Self (Intraspadix)	0	18	—	—
Self (Interspadix)	55	9	95	53
Cross, pollinated day 1 of anthesis	83	12	96	74
Cross, pollinated day 2 of anthesis	11	9	95	12

in all flowers outcrossed on the 1st d of anthesis, although two plants subsequently aborted their fruit. Five of the nine intraplant-pollinated inflorescences set viable fruit, demonstrating that interspadix geitonogamy is possible. However, no fruit development was observed in any of the flowers from the self-pollination group. More than 50 pollen tubes were observed in each of the stigmas and styles of flowers hand-pollinated on the 1st d of anthesis, while no pollen tubes were observed in flowers from the self-pollination group or in those pollinated on the 2d d of anthesis. Microscopic examination showed that the stigmatic cells had shriveled by the 2d d of anthesis and were no longer receptive by the time the lowermost synandria dehisced. The short duration of stigma receptivity may be the reason why fruit development occurred in only two of nine inflorescences outcrossed on the 2d d of anthesis.

Pollinator observations—Throughout the study, large numbers of *E. formosa* adults and larvae were observed in *P. virginica* inflorescences of both sexual stages. The adults and larvae of two other fly species, *Drosophila subpalustris* Spencer (Drosophilidae) and an unidentified species of *Helophilus* (Syrphidae), were frequently observed in staminate-stage and senescing *P. virginica* inflorescences (Table 2) but were only rarely observed in the pistillate-stage inflorescences. *D. subpalustris* and *Heliophilus* comprised only a small percentage of the total number of adults flies that emerged from the *P. virginica* inflorescences collected for insect rearing and emerged only from staminate-stage or senescing inflorescences (Table 2). These observations suggest that although the larvae of all three fly species' larvae co-occurred in staminate-stage and senesced inflorescences, *E. formosa* was the primary visitor to the pistillate-stage inflorescences.

Each *P. virginica* inflorescence was capable of supporting a large number of *E. formosa* larvae (Table 2), with as many as 56 adult *E. formosa* observed eclosing from a single collected inflorescence. Field-collected inflorescences contained a mixture of both early- and late-instar larvae, which were distributed throughout the floral chamber. These larvae were observed feeding on pollen, had pollen-filled digestive tracts, and readily ate *P. virginica* pollen when it was presented to them in the laboratory. Throughout the course of the study, *E. formosa* larvae were never observed burrowing into the pistillate flowers or feeding upon the ovules.

During field observations, up to 20 *E. formosa* arrivals to a single inflorescence were recorded in a 60-min period. At midday, most inflorescences contained from two to five *E. formosa* adults. The flies were observed to move frequently and rapidly within the floral chamber, and male *E. formosa,* distinguished by their enlarged femurs on the rear legs, frequently perched within the marginal undulations, possibly to await the arrival of female flies. If a perched male encountered another male, the two flies jostled each other for several seconds until they separated. Copulation occurred within both pistillate- and staminate-stage inflorescences. The preferred oviposition sites were located in the sheltered clefts between the staminodes surrounding the pistillate flowers and between the synandria on the lower portion of the male spadix. One or two eggs were usually found in a cleft, but occasionally egg masses containing >35 eggs were found exposed on the inner spathe, either along the margins adjacent to the male spadix or up within the distal tip.

In staminate-stage inflorescences, groups of adult flies, including gravid females, were frequently observed feeding on pollen that had accumulated at the base of the spadix. Microscopic examination of these flies revealed that their bristle patches contained numerous *P. virginica* pollen grains and their digestive tracts were full of *P. virginica* pollen. Flies emerging from staminate-stage inflorescences were often covered with so much pollen that they could not take flight, even when prodded with a dissection needle, until they groomed most of it off of themselves.

Pollination effectiveness and behavior of E. formosa—Field observations and tests in the laboratory demonstrated that *E. formosa* efficiently transferred pollen between *P. virginica* inflorescences. *P. virginica* pollen was found on the stigmas of 86% of the pistillate-stage inflorescences that had been previously covered with fine nylon

TABLE 2. Number (means ± SE) of adult *E. formosa, D. subpalustris,* and *Helophilus* sp. individuals that emerged from 47 inflorescences of different age classes collected from the New Brunswick and East Millstone study populations in 1989.

Fly species	Inflorescence stage			Total flies emerged (% total)
	Pistillate (N = 9)	Staminate (N = 33)	Senesced (N = 5)	
Elachiptera formosa	19.4 ± 5.0	27.5 ± 2.4	18.8 ± 3.6	1,164 (88)
Drosophila subpalustris	0.0 ± 0.0	3.6 ± 0.9	1.4 ± 1.0	112 (9)
Helophilus sp.	0.0 ± 0.0	0.4 ± 0.2	2.4 ± 2.0	20 (3)

TABLE 3. Number (means ± SE) of *E. formosa* caught in pistillate- and staminate-stage *P. virginica* inflorescences during pollinator censuses. Weston Mills Pond (1 June and 22 June 1990) and the Tinicum (28 May 1991) results were analyzed by one-way ANOVA and the least significant difference (LSD) multiple-range test (General Linear Models Procedure, SAS, 1985).[a]

Census date	Pistillate inflorescence	1st-d staminate inflorescence	2d-d staminate inflorescence	*F*
1 June 1990	2.7 ± 0.6 ($N = 7$)	2.7 ± 0.3 ($N = 15$)	na	0.007 (ns)
22 June 1990	5.4 ± 1.2 ($N = 9$)	3.1 ± 0.4 ($N = 16$)	na	4.389 ($P < 0.05$)
28 May 1991	1.3 ± 0.5 ($N = 9$)	1.5 ± 0.2 ($N = 14$)	2.6 ± 0.7 ($N = 11$)	2.220 (ns)

[a] *N* equals the numbers of inflorescences in each group.

mesh bags and then left exposed to pollinators for a 5-hr period and only *E. formosa* was collected from these inflorescences. In addition, *P. virginica* pollen was nearly always present on *E. formosa* collected from pistillate-stage inflorescences during routine observations. In the laboratory, *E. formosa* transferred fluorescent dye among inflorescences of both sexual stages after a 2-hr exposure period to marked and unmarked inflorescences. All of the inflorescences marked with dye received some dye particles from inflorescences of the opposite sexual stage, and the unmarked inflorescences received dye from inflorescences of both sexual stages. Dye deposition occurred throughout the floral chamber, including on the stigmas.

Similar numbers of *E. formosa* visited both floral sexual stages at the Weston Mills Pond and Tinicum study populations during the censuses conducted early in the flowering season, when pistillate- and staminate-stage inflorescences were present in equal numbers (Table 3). During the second Weston Mills Pond census, staminate-stage inflorescences were more prevalent, but *E. formosa* displayed a slightly stronger visitation preference for the pistillate-stage inflorescences. This may be because *E. formosa* had a strong oviposition preference for the pistillate stage in all three censuses (Table 4). Oviposition in pistillate-stage inflorescences was almost 14-fold greater at Weston Mills Pond on June 22 than 3 wk earlier. There was no significant difference in the number of ovipositions between the 1st- and 2d-d staminate-stages at Tinicum.

Floral odor analysis—While a total of 29 different odor compounds was collected during the sequence of anthesis, a specific blend of floral odor components was emitted by each floral stage (Table 5). However, two compounds in particular dominated the floral odor, 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1]octane and the molecular weight 142 analog. A prominent difference between the odor emitted by the pistillate- and staminate-stage inflorescences was a change in the emission ratio of 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane to the molecular weight 142 analog. The pistillate-stage spathes emitted a 2:1 ratio of these two compounds and the staminate-stage emitted a 43:1 ratio, while, interestingly, the bud spathes emitted a 1.0:1.6 ratio (Table 5). In addition, the total amount of floral volatiles emitted by the spathes increased with age, from 8.2 ng in the mature buds to 48.2 ng by the pistillate stage and 145.4 ng by the staminate stage.

A total of 16 compounds was collected from the pistillate-stage spathes, including four isomers of trimethyl-6,8-dioxabicyclo[3.2.1.]octane, alpha-pinene, linalool, 3-carene, limonene, nonanol, nonanal, and dimethyl dodecenone. The staminate-stage odor emitted 18 compounds and was dominated by nine isomers of trimethyl-6,8-dioxabicyclo[3.2.1.]octane. These inflorescences also produced small amounts of a second isomer of the molecular weight 142 analog, alpha-pinene and dimethyl dodecenone. Most of the minor components from the pistillate-stage odor were not present in the staminate-stage odor. Mature inflorescence bud spathes produced a faint odor, detectable only in the collection flasks, that was reminiscent of the pistillate-stage odor. The only major compounds emitted by the mature inflorescence bud spathes were 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1]octane and the molecular weight 142 analog.

Oviposition choice tests—In both of the two tests, *E. formosa* made significantly more ovipositions in the sham pistillate-stage inflorescences than in the sham staminate-

TABLE 4. Number (means ± SE) of *E. formosa* ovipositions in pistillate- and staminate-stage *P. virginica* inflorescences during pollinator censuses. The Weston Mills Pond (1 and 22 June 1990) and the Tinicum (28 May 1991) results were analyzed by General Linear Models Procedure (SAS, 1985).[a]

Census date	Pistillate inflorescences	1st-d staminate inflorescences	2d-d staminate inflorescence	*F*
1 June 1990	6.4 ± 1.7 ($N = 8$)	1.7 ± 0.9 ($N = 10$)	na	6.579*
22 June 1990	88.7 ± 22.2 ($N = 6$)	12.9 ± 3.0 ($N = 18$)	na	26.214**
28 May 1991	22.0 ± 4.1[a] ($N = 12$)	4.4 ± 1.4[b] ($N = 14$)	5.7 ± 2.4[b] ($N = 9$)	11.310**

[a] *N* equals number of inflorescences in each group.
* $P < 0.025$; ** $P < 0.01$. Means with different superscripts are different at the $P < 0.05$ level.

TABLE 5. *Peltandra virginica* floral odor compounds collected from the spathes of pistillate- and staminate-stage inflorescences and mature inflorescence buds ($N = 12$ for each stage), with total amount (ng) and percentage of total amount collected in 2 hr indicated for each compound.[a]

	Spathe stage					
	Bud		Pistillate		Staminate	
Compound	Amount (ng)	% of total	Amount (ng)	% of total	Amount (ng)	% of total
Alpha-pinene	0	0	1.1	2.1	1.2	0.8
MW 142 isomer	0	0	0	0	0.3	0.2
MW 136 monoterpene	0	0	0	0	tr	<0.1
Beta-pinene	0	0	0	0	tr	<0.1
Linalool	0	0	0.4	0.8	0	0
TDBO isomer 1	0	0	0.6	1.2	6.5	4.5
Octenal	0	0	tr	<0.1	0	0
4,5,7-TDBO	2.9	35.4	25.6	53.1	74.6	51.3
3-Carene	0	0	0.4	0.8	0	0
TDBO isomer 2	tr	<0.1	1.9	3.9	0	0
TDBO isomer 3	0	0	0	0	6.5	4.5
TDBO isomer 4	0	0	0	0	28.6	19.7
Limonene	tr	<0.1	0.9	1.9	0	0
MW 142 analog	4.8	58.5	12.4	25.7	1.8	1.2
TDBO isomer 5	tr	<0.1	1.9	3.9	7.3	5.0
TDBO isomer 6	0	0	0	0	1.6	1.1
MW 140 unknown	tr	<0.1	0	0	0	0
TDBO isomer 7	0	0	0	0	2.8	1.9
Nonanol isomer	tr	<0.1	tr	<0.1	0	0
2-Nonanol	0.5	6.1	1.2	2.5	0	0
TDBO isomer 8	0	0	0	0	0.3	0.2
Nonanal	0	0	0.3	0.6	0	0
Dimethyl dodecenone isomer	0	0	0.6	1.2	0.4	0.3
TDBO isomer 9	0	0	1.1	2.1	0	0
Dimethyl dodecenone isomer	0	0	tr	<0.1	tr	<0.1
TDBO isomer 10	0	0	0	0	3.6	2.5
TDBO isomer 11	0	0	0	0	0.8	0.5
Trimethyl cyclohexane	tr	<0.1	0	0	0	0
TDBO isomer 12	0	0	0	0	8.43	5.8
Total amount	8.2		48.2		145.4	

[a] tr = trace amounts; TDBO = trimethyl-6,8-dioxabicyclo[3.2.1.]octane.

stage inflorescences (Table 6; Fig. 3). This preference for oviposition in pistillate-stage sham inflorescences mirrors the egg distribution found during the field censuses (Table 4). Surprisingly, the control group constructed from mature bud spathes had the most ovipositions (Table 6; Fig. 3).

TABLE 6. Results of the two oviposition choice tests, conducted on 29 June and 2 July 1991, showing the number (means ± SE) of ovipositions made within each of the three different types of sham inflorescences. The lower position of the table shows pairwise *G*-test comparisons of ovipositions made during each bioassay (Sokal and Rohlf, 1981). Significance was determined using the Bonferroni correction for multiple comparisons (Rice, 1990).[a]

	Number of eggs per sham inflorescence			
	29 June		2 July	
Type of sham inflorescence	Total	$\bar{X}$ ± SE	Total	$\bar{X}$ ± SE
Bud	78	15.6 ± 7.0	113	22.6 ± 11.2
Pistillate	54	10.8 ± 4.0	63	12.6 ± 4.9
Staminate	18	3.6 ± 1.6	38	7.6 ± 5.2
	Log-likelihood ratio (*G*-test)			
Pairwise comparisons	29 June		2 July	
Bud v. pistillate	4.61*		14.20**	
Bud v. staminate	40.20**		38.18**	
Pistillate v. staminate	18.90**		6.29**	

[a] $N = 5$ inflorescences for each treatment.
* $P < 0.05$; ** $P < 0.01$ (for all pairwise comparisons).

DISCUSSION

The aroid–fly association — Because of a strong temporal separation in sexual function, *Peltandra virginica* inflorescences do not self-pollinate and the aroid is dependent on insects for pollination. In our study populations, *Elachiptera formosa* is the primary pollinator of *P. virginica,* and the ecological interrelationship between these two organisms appears to be highly specific. Within *P. virginica* inflorescences, adult *E. formosa* feed on pollen, find mates, and seek oviposition sites while the larvae feed on pollen and complete their development.

E. formosa's effectiveness in pollinating *P. virginica* was demonstrated by its ability to transfer rapidly colored-dye particles among inflorescences in the laboratory and by observations of both sexes of the fly actively visiting both pistillate- and staminate-stage inflorescences in study populations. Although *E. formosa* has no specialized morphology for pollen transfer, pollen adheres to various bristle patches on the fly's body. Moreover, the fly's preference for ovipositing at the base of the pistillate flowers increases the likelihood that pollen-bearing flies will come into contact with the stigmas, causing pollination.

Although flies of the genus *Elachiptera* are common in general collections, *E. formosa,* previous to this study, was considered to be extremely rare (Sabrosky, 1948; D. Grimaldi, American Museum of Natural History, NY, personal communication). The abundance of *E. formosa* in *P. virginica* populations during their blooming season,

and its absence from the inflorescences of other native aroid species (Bierzychudek, 1982; Grimaldi and Jaenike, 1983), suggests that it breeds only within *P. virginica* inflorescences. Sabrowsky (1948) reported that the few documented *E. formosa* specimens had been collected within a wide geographical range from Florida, North Carolina, and Virginia to Indiana and Illinois. This is similar to the range of *P. virginica* and indicates an overlap in geographical ranges of the aroid and the fly.

Co-pollinators may make an important contribution to the reproductive success of a plant species (Lindsey, 1984; Schemske and Horvitz, 1984; Horvitz and Schemske, 1990; Thompson and Pellmyr, 1992). However, *Drosophila subpalustris* and *Helophilus* sp. should only infrequently pollinate *P. virginica* flowers as suggested by the field observations and insect rearing data here, which indicate that these flies inhabit primarily the staminate-stage inflorescences and rarely enter the pistillate-stage ones. In addition, these flies do not display oviposition constancy to *P. virginica* inflorescences because they oviposit in the decaying portions of several other aquatic plants, such as *Nymphaea* and *Sagittaria* (Patterson and Stone, 1952; Gilbert, 1986; Hartley, 1961).

Comparison with other brood-site-based pollination systems —Plants that are pollinated by ovule predators, such as figs (Bronstein, 1988, 1992), yuccas (Addicott, 1986), globeflowers (Pellmyr, 1989, 1992), and campions (Brantjes, 1976; Pettersson, 1991) sacrifice a portion of their ovules to their pollinators. However, because *P. virginica* has an androecial brood site, no ovules are lost to *E. formosa*. Rather, the main cost to *P. virginica* appears to be the production of enough pollen to support an entire *E. formosa* population during the blooming season. Pollen was observed here to accumulate at the base of the spadix in staminate-stage inflorescences, suggesting that enough pollen was produced for both pollination and fly consumption. Further studies are needed to determine if pollen consumption by *E. formosa* not only has any effect on the fly's fecundity and survivorship but also on the aroid's male floral function as well.

Other aroids with specialized pollination systems support most or all of the life stages of their pollinators. The adult beetles that mate within and pollinate the inflorescences of aroids such as *Dieffenbachia* (Young, 1986), *Lysichiton* (Pellmyr and Patt, 1986), and *Philodendron* (Gottsberger and Amaral, 1984), may derive a significant proportion of their nutritional requirements from pollen and nutritive secretions or specialized floral parts produced by the inflorescences. This may also be the case for micropterigid moths (Thien et al., 1985) and weevils (Norstog and Fawcett, 1989; Armstrong and Irvine, 1990; Donaldson, 1992) that mate, feed, and oviposit within certain primative angiosperms and cycads.

The aroids *Colocasia* and *Alocasia* have pollination systems that are the most similar to that of *P. virginica*. Polynesian species of these genera have spadices that are used as androecial brood sites by their drosophilid fly pollinators (Faegri and van der Pijl, 1979; Carson and Okada, 1980, 1982; Shaw, Cantrell, and Houston, 1982; Shaw and Cantrell, 1983). Carson and Okada (1982) described the inflorescence of *A. macrorrhiza* as being "a veritable zoological garden" because it is the brood site

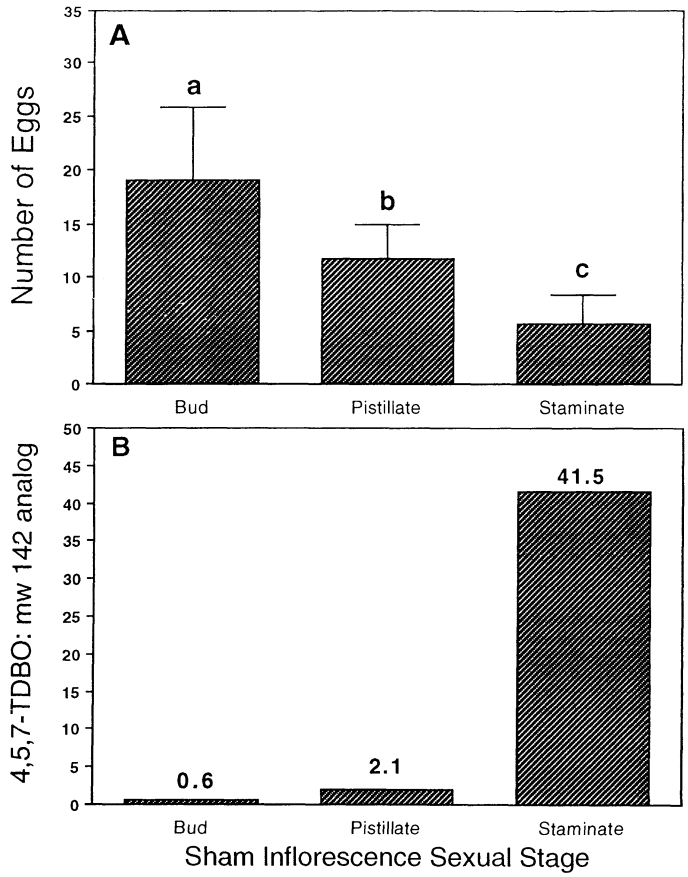

Fig. 3. The number of ovipositions (means ± SE) made during the oviposition choice field tests in sham inflorescences at different stages (mature inflorescence bud, pistillate-, and staminate-stage inflorescence) correlated with the ratio of the two major floral odor compounds. (A) The results of the oviposition choice field tests showing the total number of ovipositions in sham inflorescences. Bars with different letters are significantly different at the $P < 0.05$ level, G-test (df = 1). (B) The ratio of 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane to the molecular weight 142 analog emitted from each inflorescence stage.

of at least 13 drosophilid species. There are certain similarities between these aroids and their relationships with the flies that inhabit their inflorescences. Just as *E. formosa* oviposits only within *P. virginica* inflorescences, several of the Polynesian drosophilid species oviposit only within the inflorescence of particular species of *Alocasia* or *Colocasia* (Carson and Okada, 1980, 1982; Shaw, Cantrell, and Houston, 1982; Shaw and Cantrell, 1983). Additionally, in a manner similar to *D. subpalustris* and *Helophilus* sp., many of the drosophilid species ovipositing within *Alocasia* and *Colocasia* inflorescences do not function as pollinators because either they enter inflorescences of only a single sexual stage or because they oviposit in parts of the floral chamber away from the pistillate flowers (Carson and Okada, 1980, 1982; Shaw and Cantrell, 1983).

The androecial brood-site pollination syndrome appears to have evolved numerous times in *Alocasia, Colocasia,* and once in *Peltandra.* The larvae of most species of *Elachiptera* (Sabrowsky, 1948) and many *Drosophila* species (Patterson and Stone, 1952) develop in decaying plant tissue, and the secondary chemicals and volatile

compounds present in these substrates are used by the adult flies as oviposition cues (Fogleman, 1982; Starmer and Fogleman, 1986; Fogleman and Abril, 1990). This suggests that anthophily in these aroid-pollinating flies may have evolved from an original habit where the flies visited senescing, staminate-stage, aroid inflorescences to search for mates and oviposition sites, scavenge pollen, or forage on microbes growing in the senesced floral tissue.

Influence of floral odor on fly oviposition behavior—The apparent stimulatory effect of trimethyl-6,8-dioxabicyclo[3.2.1.]octanes on the fly's oviposition behavior, along with the scarcity of other insects in the pistillate-stage inflorescences, suggests that these compounds are specific attractants and behavioral modifiers of *E. formosa.* The sham inflorescences used in the oviposition choice tests presented the flies with the full array of chemical cues present in actual inflorescences. Since the oviposition substrate (spadices excised from mature inflorescence buds) was identical in all three treatments, differences in oviposition can be related directly to differences in the floral odor emitted from spathes placed on the sham inflorescences. The bud spathes, which induced the largest oviposition response, emitted an odor composed mostly (≈94%) of the two principal odor components, the molecular weight 142 analog (58.5%) and 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane (35.4%), together with a very small amount of several other compounds. This suggests that the two principal odor components alone can strongly induce oviposition in *E. formosa.*

Furthermore, *E. formosa* oviposition appears to be stimulated primarily by the emission ratio of the two major compounds rather than by other factors such as total odor concentration or by one of the two principal components acting alone. This is suggested by three events that occur simultaneously as the sequence of anthesis progresses: a decline in the frequency of ovipositions, a change in emission ratio of the two principal floral odor components, and an increase in the quantity of floral volatiles emitted. The mature bud spathes emitted more of the molecular weight 142 analog than 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane, and sham inflorescences constructed from bud spathes had the greatest number of ovipositions. Similar proportions of the two principal components were emitted by the pistillate-stage spathes and sham inflorescences constructed from these spathes had fewer ovipositions than the sham inflorescences constructed from bud spathes. However, the sham inflorescences constructed from the older, staminate-stage spathes received the fewest ovipositions and emitted a far greater proportional amount of 4,5,7-trimethyl-6,8-dioxabicyclo[3.2.1.]octane (Fig. 3). This pattern indicates a correspondence between fly oviposition and emission ratio.

The quantity of floral volatiles emitted was negatively correlated with oviposition behavior because as the total floral odor concentration increased, fly response decreased (Tables 5, 6). Likewise, the absolute amount of the individual principal components was negatively correlated with oviposition response because a higher level of fly response occurred at lower individual component concentrations. However, it is possible that higher concentrations of volatiles may have actually inhibited fly response. Further experimentation is needed to determine if volatile concentration has a positive or negative stimulatory effect on fly oviposition. Likewise, further experimentation is needed to determine the effect of the other floral volatiles on fly behavior. These volatiles probably also help mediate *E. formosa* behavior, as such synergistic effects of plant volatiles have been shown in other fly species that oviposit in plant tissue (Harris, Keller, and Miller, 1987).

The oviposition behavior of other highly specific pollinators that oviposit within their host flowers may also be influenced by floral odor composition (Brantjes, 1976; Thien et al., 1985; Bergstrom et al., 1991; Ware et al., 1993). It has been suggested that the biological activity of floral odors in such systems is based on only a few components (Bergstrom et al., 1991; Ware et al., 1993). Changes in floral odor composition may also play a direct role in mediating pollination in *P. virginica.* Accordingly, by using the odor emitted by each floral sexual stage as a floral resource recognition cue, individual *E. formosa* could efficiently locate inflorescences providing a particular resource, viz., pistillate-stage inflorescences signal oviposition sites whereas staminate-stage inflorescences signal food, namely pollen. This may be the reason why fly visitation was similar to both inflorescence sexual stages during the field censuses. If gravid flies are attracted to pistillate-stage inflorescences and hungry flies are attracted to staminate-stage inflorescences, then the aroid's fitness would be enhanced because flies would move among inflorescences in search of particular floral resources as their physiological needs dictated. This is suggested by findings that the hunger level of parasitoid wasps strongly influences their response to resource related odors (Lewis and Takasu, 1990; Takasu and Lewis, 1993). Furthermore, field observations suggest that female flies compete for oviposition sites in pistillate-stage inflorescences because in many inflorescences nearly all of the gaps and spaces between the staminodes and synadria were observed to contain eggs. If intraspecific competition for floral resources exists, it should further promote fly movement among inflorescences.

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