POLLINATOR DECEPTION AND PLANT REPRODUCTIVE SUCCESS IN

JACK-IN-THE-PULPIT

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ABSTRACT

I conducted a study of the deceptive pollination system of Jack-in-the-pulpit (*Arisama triphyllum*, Araceae) in forests of west-central Indiana. I focused on (a) determining the identities and abundances of insect visitors to spathes, (b) evaluating the success of female spathes in setting fruit, (c) determining the relative importance of pollinator visitation and plant size for fruit number, and (d) investigating the function of the female spathe's lack of an exit hole, which has been hypothesized to improve pollination success. I found that (a) Jack-in-the-pulpit receives visits from both flies and thrips. Counts of fly corpses from spathes showed the most prevalent families to be Mycetophilidae and Sciaridae with other nematoceran families and a few brachyceran families present as well. Visitation by thrips, determined by visual inspection of spathes, was low, involving only 30% of plants. (b) Fifty-seven percent of female plants set fruit, with much variation among sites. (c) Mushroom flies, especially the families Mycetophilidae and Sciaridae, were found to be the primary pollinators of Jack-in-the-pulpit based on pollen loads, visit rates, and an exclusion experiment. Pollination by thrips, though possible, probably had only a minor effect. (d) An experiment that created an exit hole in female spathes yielded no support for the hypothesis that lack of an exit hole (the natural condition) improves fruit set.

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CHAPTER 1

POLLINATOR DECEPTION IN JACK-IN-THE-PULPIT: INSECT VISITATION

Introduction

In flowering plants, a common strategy of sexual reproduction is to attract animals with colorful petals or odors, provide food rewards of nectar or pollen, and transfer pollen to and from the bodies of visitors. This makes sex possible when plants are dispersed, enables them to avoid inbreeding, and can greatly enhance fitness when a plant succeeds in exporting pollen to many potential mates. Natural selection has favored the elements of such a strategy in countless plant lineages, altering attractants, rewards, and dimensions of stamens and pistils to suit a variety of animal visitors. Typically, despite conflict of interest and costs to both parties, the outcome is mutually beneficial (i.e., a mutualism) for both the plant species and its most effective pollinator(s).

However, selection has also favored specialized nectar-robbing and other cheating mechanisms in certain animal species (Inouye, 1980), and traits that deceive, exploit, and even kill pollinators in some plant lineages (Renner, 2005). One such lineage is subfamily Aroideae of the monocot family Araceae, notably species of *Arisaema* (ca. 150 species; Gusman and Gusman, 2006) and *Arum* (15 species). The reproductive shoot

consists of a tubular, leaf-like spathe surrounding and hiding from direct view a pole-like inflorescence (spadix) embedded with numerous tiny unisexual flowers, either all of one sex or a mixture (monoecious). The tip of the spadix bears an elongated osmophore that emits odors attractive to small mushroom flies and fungus gnats (families Mycetophilidae and Sciaridae, Diptera; Vogel and Martens, 2000). Flies are attracted into the spathe opening near the osmophore and are usually trapped, temporarily or permanently, depending on genus or species and the plant's sexual phase.

Three common deceptive strategies of aroid flowers are as follows:

1. *Trap briefly and release*: There is an exit hole at the bottom of the spathe, through which an insect departs after entering at the top of the spathe and tumbling down. The exit is reached with difficulty after contact with anthers and a pool of fallen pollen beneath the spadix. The visitor departs loaded with pollen. (Genus *Arisaema*: male spathes of dioecious species, bisexual spathes of monoecious species)

2. *Trap for a day and release*: In *Arum* species of Europe and Asia, the bisexual inflorescence is protogynous (female-first), exposing receptive female florets before the anthers of male florets dehisce. Upon receiving a visitor (who may carry a pollen load), the inflorescence closes for a day, encouraging deposition of pollen by the struggling insect. Male florets then dehisce, depositing pollen on the live insect, and the spathe opens to release the fly (e.g., Mendez and Diaz, 2001).

3. *Trap and kill*: In all-female inflorescences of many *Arisaema* species, flies are permanently trapped because the spathe lacks an exit hole at the base. If they enter

bearing outcross pollen from a male-phase *Arisaema*, their struggles probably improve pollen transfer to stigmas (Vogel and Martens, 2000; Nishizawa et al., 2005).

Sexual reproduction in Jack-in-the-pulpit (*Arisaema triphyllum*), one of two *Arisaema* species in eastern North America, combines strategies 1 and 3. Jack-in-the-pulpit is sexually dimorphic for presence of an exit hole: male plants have one, females and rare hermaphrodites do not. Sex expression is fixed only for a given year and size-dependent, females being larger (Bierzychudek, 1982). Mushroom flies, a critical set of pollinators for the genus worldwide (Vogel and Martens, 2000), but not the only ones (see below), are exploited for pollen movement by both sex-phases of Jack-in-the-pulpit and targeted by the osmophore's odor, which apparently mimics the mushroom substrate on which they feed and breed. Successful pollination requires that an individual fly visit at least one male and then a female plant. No food or other reward for this behavior seems to exist, and thus, since death is the outcome for the fly, the system is the ultimate in deceptive pollination.

A very different type of insect, thrips (order Thysanoptera), may also pollinate as a result of their pollen- and leaf-eating habits, at least when plants grow densely (Rust, 1980). *Heterothrips arisaemae* Hood was described from "twelve females and two males, taken at Urbana, Illinois, in flowers of Jack-in-the-pulpit" (Hood, 1908). Females are about 1.25 mm in length, males 0.72 – 0.80 mm. Later work (Rust, 1980; Feller et al., 2002) has shown the species to be a widespread specialist herbivore on *A. triphyllum*, present wherever it has been looked for (e.g., Illinois, Indiana, New Jersey, Maryland). The life cycle involves a phase of pollen-eating and some movement between plants, and

in and out of spathes (Feller et al., 2002). The tiny flat-bodied thrips can move between

the overlapping folds of a spathe, and thus are neither trapped nor deceived (since they

eat pollen and leaf tissue). Although male jack-in-the-pulpit plants are preferred, female

plants are also visited (Feller et al., 2002), and pollination mainly by thrips has been

demonstrated in one study (Rust, 1980). There is no indication that jack-in-the-pulpit has

evolved features to encourage or discourage pollinating behavior by thrips, but few

authors have considered it. Thrips are common pollen- and nectar-eating inhabitants of

flowers (Kirk, 1996), but because of their tiny size and presumably limited mobility are

rarely considered as potential pollinators. However, they are important pollinators of

some species (Terry, 2001; García-Fayos and Goldarazena, 2008).

How effective is the separate-sex, lethal-trap system of jack-in-the-pulpit in

achieving sexual reproductive success for individual plants and populations? On the one

hand, one can observe that the species is widespread and fairly common in upland

deciduous forests of eastern North America, occurring from southern Canada (Nova

Scotia to Manitoba) south to the Gulf Coast and southern Florida, and from the Atlantic

seaboard to the eastern parts of Great Plains states. In most states except the western

border, it is found in most counties (PLANTS profile for *Arisaema triphyllum*,

http://plants.usda.gov). Its conservation status is "globally secure", and 8 states and 6

provinces have ranked it as "secure" or "apparently secure", except Manitoba where it is

"imperiled" (Nature Serve, 2009). Thus, populations are maintaining themselves.

However, like many herbaceous forest perennials, *A. triphyllum* reproduces vegetatively

(by offsets of the underground corm) as well as by seed (Bierzychudek, 1982); relative importance of the two modes is little studied.

My focus here is on jack-in-the-pulpit's ability to produce seeds using a deceptive and lethal pollination system. The evolution of deceptive pollination may be favored by various selective forces, including energy savings from not offering food rewards (Renner, 2005); but a cost is likely to be low rates of visitation, since individuals of the targeted vector will not tend to enthusiastically re-visit conspecific flowers. Bierzychudek (1981, 1982, 1984) used spectacular results from *A. triphyllum* to develop the concept of "pollinator limitation of reproductive effort", i.e., seed set being limited by inadequate pollinator service rather than plant resources. In a New York population, naturally-visited female plants averaged only 1.0 seeds per spathe, whereas hand-pollinated ones averaged 74.3 seeds (Table 1). Parker (1987) obtained a similar result in Illinois, where naturally pollinated plants had 12 seeds per plant and hand-pollinated ones 73. These are the only two studies to use hand-pollination.

Overall, studies of 9 populations in several states and provinces show a wide range in measures of seed or fruit set by female plants, both among and within populations (Table 1). In some studies, pollinators have been collected or quantified in some way, but more often they have not been investigated. Further complications include the fact that fruit set is influenced by the size of female plants (Bierzychudek 1982), infestation by an endemic rust (Parker, 1987), and the population sex ratio (Richardson and Clay 2001).

A number of unresolved issues related to *A. triphyllum* pollination need clarification, in light of the highly variable reports of fruit set and pollinator presence. Do mushroom flies and thrips both typically contribute to pollination, or are thrips important only in unusually dense populations of jack-in-the-pulpit? When mushroom flies are involved, how many victims does a female spathe usually attract, and how many are needed to achieve good levels of fruit set? I addressed these issues by studying jack-in-the-pulpit pollination in several forest sites in west-central Indiana near Terre Haute, in Vigo and Clay counties. Case studies of populations throughout the species range seem like a productive approach. This is the second area in the Midwestern states where jack-in-the-pulpit reproductive success has been studied, the other one being Cook County, Illinois (Parker, 1987).

I asked the following questions about interactions of Jack-in-the-pulpit and its potential pollinators, using observational and experimental approaches. They fall in two categories.

Questions regarding insect visitation

1. Which insect types visit *A. triphyllum* in forests near Terre Haute, Indiana, and what are their relative abundances? Does the visitor assemblage in this region match that predicted by previous studies elsewhere in the eastern US? Does the visitor assemblage match, in proportional abundance, samples of insects trapped in flight near spathes?

2. Do male and female spathes attract the same insects, in terms of species composition and relative abundance? Do insects discriminate between male and female spathes?

3. Which visitor types (fly families, thrips, etc.) carry pollen?

Questions regarding fruit set

4. What is the probability that a female spathe sets one or more fruits? How effective are different visitor sets: thrips alone, versus thrips and flies?

5. How does plant size affect (a) sexual phase expressed by a plant in a given year, and (b) probability of fruit set by female spathes?

6. Does the "lethal trap" feature (no exit) of female spathes improve fruit set, relative to having an exit hole?

The questions regarding insect visitation are addressed in the remainder of Chapter 1, and those related to fruit set in Chapter 2. The Literature Cited, tables, and figures for both chapters are placed at the end of Chapter 2.

INSECT VISITATION TO JACK-IN-THE-PULPIT

Which insect types visit *A. triphyllum* in forests near Terre Haute, Indiana, and what are their relative abundances? From a global perspective on the genus *Arisaema* (Vogel and Martens, 2000), flies in the families Mycetophilidae and Sciaridae are expected to be the main visitors of most species and populations. This expectation is based on (a) identification of insect corpses from spathes in wild and cultivated populations, and (b) the suite of features in male and female spathes that appear to target flies, which attract (osmophore), trap (slick inner walls of spathe), allow for escape (exit hole of males), or permanently trap them (lack of exit in females). Furthermore, virtually all 150 *Arisaema* species occur in mesic forest understories where mycetophilids and

sciarids are common members of the leaf-litter, decomposer food web (Renner et al., 2004).

Studies of *A. triphyllum* itself are mostly in accord with the global perspective, but relatively few studies of visitors have been made. When contents of female spathes are examined and identified, Mycetophilidae and Sciaridae are usually found, and usually outnumber other fly taxa and other insects. Robertson (1928), near Carlinville, Illinois, recorded "twenty specimens belonging to about seven species of Nematocera" [the suborder to which Mycetophilidae, Sciaridae, and many other families belong], plus one Hemipteran. Rust (1980) recorded Mycetophilidae (genera unknown) as the second most common group of pollinators after thrips; they averaged 0.3 flies per male and 0.9 per female flower. He also found Collembolans in the pollen. Feller et al. (2002) recorded an average of 1.6 flies per female spathe, and listed 10 fly families captured, including Mycetophilidae and Sciaridae. Finally, Barriault et al. (2009) recorded four main groups of insects in spathes: Mycetophilidae, Cecidomyiidae (Diptera), thrips (*H. arisaemae*), and Coleoptera. Mycetophilidae were the most common visitors during the early phase of female spathe receptivity.

Although *Arisaema*'s adaptations to mushroom flies seem obvious, the neat story of deceptive-and-lethal pollination (in which a plant genus exploits an abundant insect which has not counter-evolved) is complicated by the discovery that thrips may be important, overlooked pollinators. Although *Heterothrips arisaemae* was scientifically described long ago (Hood 1908), it was not until 1980 that its role as a pollinator was hypothesized. Rust (1980), in Delaware, found more thrips in spathes than flies. His

population of flowering jack-in-the-pulpit was dense and fruit set was observed mainly in females that were very close to (within 2 meters of) a male spathe. He reasoned that thrips might pollinate effectively at such a distance, and attributed most fruit set in this population to thrips. Feller et al. (2002), in Maryland, clarified the thrips's life cycle and concluded, based on its ubiquity, pollen loads, and movement among male and female spathes, that it was likely an important pollinator. In fact, Levine and Feller (2004) refer to thrips as "the pollinator" at the Maryland site. However, both Rust (1980) and Feller et al. (2002) recorded Mycetophilidae in spathes; and though they emphasize their relative rarity (e.g., 0.3 to 0.5 flies/collected spathe, Rust 1980), low rates of visitation may be typical, yet fairly effective.

Vogel and Martens (2000) also report the frequent occurrence of thrips in spathes of various *Arisaema* species, where they were typically observed "swimming in pollen". However, they consider thrips of little or no importance as pollinators. After thoroughly reviewing the literature on visitors to *Arisaema* spathes, they make the following pronouncement (p. 81): "From the above data we conclude that mycetophilids and sciarids are the regular pollinators of *Arisaema* over its whole geographic range. There is no local specificity in terms of gnat species, and the plants are able to lure a vicariant assembly of this pollinator type even when they are growing far from their home country."

In summary, although it is possible that thrips are important only where jack-in-the-pulpit populations are dense, any study of jack-in-the-pulpit pollination should

quantify thrips abundance in spathes and attempt to evaluate their contribution to seed set.

Three approaches have been used to identify both the actual insect visitors to *Arisaema* spathes, and the flying insects available in the understory near the plants. First, since the spathes collect visitors temporarily or permanently, spathes themselves can be collected (Rust, 1980). Destructive sampling allows for complete dissection of the spathe and enumeration of all visitors. Insects can also be removed from spathes in the field by carefully unrolling the spathe-leaves of females, or by removing cotton plugs placed in the exit holes of males. Such collections are likely to be less complete. Secondly, insect traps (e.g., water-filled bowls, or tanglefoot sticky traps) may be placed near *Arisaema* spathes at the same height as spathe openings, to sample "available" flying insects in the habitat (Hickman et al., 2001; Feller et al., 2003). Finally, thrips can be checked for by raising the hood of a spathe and inspecting visually (Barriault et al., 2009), although this may miss thrips buried in pollen at the base of a male spathe, especially (Rust, 1980).

Do male and female spathes attract the same insects, in terms of species composition and relative abundance? Because jack-in-the-pulpit populations are dioecious, with separate male and female individuals, movement of pollinators between plants is essential. Flies are deceived by jack-in-the-pulpit, since no reward is obtained from males and death is the usual fate for entering a female. Thrips, on the other hand, feed on pollen and leaf tissue, and are not trapped when they visit either sex; however, they probably benefit more from visiting and occupying male plants.

For flies, the ability to discriminate against either morph, but especially against females, would be favored by natural selection as long as it did not impair the fly's normal use of fungi. Jack-in-the-pulpit is a common understory plant that takes the lives of a fraction of fungus gnat populations every flowering season. On the one hand, if a fly visited a male inflorescence first, it might learn from the experience and be less likely to visit another male or female, although some obviously do since jack-in-the-pulpit gets pollinated. I did not address the question of learning. Alternatively, an innate discrimination might evolve that led to greater avoidance of females. Yet another possibility is that the larger females produce stronger odors which make flies more likely to visit them than males.

Almost no data exist quantifying visit rates by flies to male spathes. Visit rates are probably too low for direct observation to be productive. To obtain data from males for comparison with females, I plugged the exit holes of males for a number of days, collected these spathes and a set of female spathes open during the same period, and compared composition of the fly (and other insect) visitor assemblages.

Which visitor types (fly families, thrips, etc.) carry pollen? Flower visitors will only be effective pollinators if they transfer pollen from anthers to stigmas of a plant species. In most plant species, the structure of the flower or inflorescence results in some visitors picking up and depositing pollen at higher rates than others, and some visitors may avoid contact with sexual parts almost completely. I looked at one basic measure of visitor effectiveness in *A. triphyllum* — the number of pollen grains on bodies of trapped visitors — and compared the major groups of visitors.

Methods

Collections of insects. I collected insect visitors from spathes of both sexes at multiple sites in 2008 and 2009, and also set out water and tanglefoot traps near plants to sample available insects, including potential visitors.

Collections from spathes. Corpses were collected from female spathes in two years, with some differences in methods and timing. In 2008, samples of spathes were collected on three dates during the flowering period, cut, bagged, and examined in the laboratory. The dates were early, middle, or late in the flowering period (30 April, 5 and 16 May). In 2009, dead insects were collected only at the end of the flowering season (11 May) by gently peeling the spathe from the spadix so visitors could be collected with forceps. Flies and fly parts were stored in alcohol for later examination. Fly counts were determined by finding the maximum number of flies that a sample could contain (i.e. four wings from one species was said to be two flies unless other evidence was available).

Male spathes naturally trap only a small proportion of visitors. However, when the exit hole at the bottom of a male spathe is plugged with cotton, the same size range of visitors trapped by female spathes can be collected. Male spathes plugged with cotton were used to trap visitors at one site in 2008 and three sites in 2009, with differences between years. In 2008 males were plugged for as long as plants were receptive, and collections happened three times during anthesis (April 30, May 5, and May 16). Cotton was temporarily removed, corpses were collected, and cotton replaced. In 2009 only five males were plugged at a time to allow more pollen flow during trapping efforts. At the end of a one week trapping period, the five spathes were cut and contents were stored in

alcohol. Trapping periods for male spathes happened at three different sites on April 24, May 2, 7, and 11, 2009; a total of 28 male spathes was collected.

Observations of insects within spathes. Thrips can crawl in and out of spathes, so collections from spathes typically yield few. Direct observations into receptive spathes in the field are more productive. These visitors can be seen when the hood of a female spathe is lifted. The two groups of visitors can be distinguished by size and movement (flies are large and frantic movers; thrips are small and usually walk slowly on the plant surface). Naked eye examinations of visitor contents by this method took place once at each of four sites during the first two weeks of May. A total of 136 male and 52 female spathes were checked for thrips.

Traps. Water-pan traps are a common sampling method for flying insects such as flies, wasps, and bees (Hickman et al., 2001). Colored bowls (bright yellow and green) were filled with a mixture of detergent and water (1 tsp. Dawn detergent per gallon of water). The detergent reduces surface tension causing the insects to drown.   Water pan traps placed along transects where jack-in-the-pulpit was found were used to collect and document the relative abundance of flies. These traps were set up in three woods in 2008 where they were collected after a 24 hour exposure. The 24-hour period allowed for capture of crepuscular flies, thought to be visitors of *Arisaema* spp. (Vogel and Martens, 2000). Corpses were collected from bowls by first straining bowl contents through a coffee filter, then placing insects or parts thereof into alcohol, and later rinsed, dried, and mounted on pins for identification.

In 2009, sticky traps were placed 10 cm away from spathes in woods for 24 hour periods. Sticky traps consist of a colored (green) piece of card stock with a thin layer of tanglefoot®, a sticky spreadable substance good for collecting flies and other flying insects, applied to the surface. Traps were oriented vertically at spathe height by attaching the cardstock to a thin piece of wood (lath) imbedded in the ground. After 24 hour exposure cardstock was removed from lath and stored for later insect identification.

Identification. Fly specimens were examined with a dissecting microscope at 45X power, and identified to family (Mycetophilidae, Sciaridae) or suborder (Nematocera, Brachycera) using keys in McAlpine et al. (1981). Specimens are presently housed in cabinets in the laboratory of Dr. Peter Scott, and will be preserved in the Indiana State University Insect Collection.

Thrips observations: In 2009 I checked for the presence of thrips by lifting the hoods of male and female spathes at four sites during the first two weeks of May. Because naked eye observations are not sufficient to identify fly taxa, these observations are only used for thrips visitation analysis. Observation of thrips is more a more accurate measure of thrips presence than trapping because thrips are not affected by the pitfall trap mechanism of jack-in-the-pulpit in the same way that flies are (i.e. they can freely walk on the surface of the spathe).

Pollen loads. I examined pollen loads of visitors found in 17 male spathes at Kieweg Woods in 2008, between 30 April and 16 May (part of the data set described in the previous section). I extracted the visitors with forceps from the bottom of male spathes, where they were wading in pollen. I stored them in alcohol and later rinsed them

(in a group, from each plant), dried them, and mounted them individually on a paper point. Each specimen was identified as fly or thrips, and flies were further identified to family (Mycetophilidae, Sciaridae) or suborder. I counted the number of *Arisaema* pollen grains on each specimen, using pollen from male *Arisaema* florets as a reference. Despite the rinsing, some pollen remained on the body bristles of visitors. Four taxonomic categories of visitors were relatively common in the collections. I used one-way ANOVA to compare mean pollen loads of these groups.

I also obtained a partial pollen count from one live mycetophilid fly, which I captured in an empty vial on 3 May 2009 as it pushed its way through the exit hole of a male spathe. A picture (40X magnification) of the live fly was taken through a dissecting microscope and a partial pollen load was counted from the picture.

Results

Flies caught in spathes. The insects captured in collected spathes of both sexes included various fly taxa (Table 2) and an occasional thrips, spider, or beetle (not shown). The two classic *Arisaema* fly families, Mycetophilidae and Sciaridae, were present in males and females; other nematoceran flies were also present, but were never the most abundant category. Brachyceran flies were rare.

Relative abundances of the three most common fly categories (Mycetophilidae, Sciaridae, other Nematocera) differed significantly between male and female spathes (χ^2 = 8.23, df = 2, P < 0.05). The greatest difference was in the proportion of flies that were sciarids, which was 0.38 in males and 0.19 in females.

The total numbers of flies captured in spathes were not large. Summing the means for each visitor type in Table 2 yields totals of 2.3 - 2.4 flies (all types) per male spathe, and 1.1 – 3.4 flies per female spathe. Looking at the frequency distribution of flies in spathes, 28% of males and 41% of females contained no flies. Only five male (11%) and one female spathe (2%) had more than five fly corpses, the maximum numbers being 17 flies in a male and 13 in a female.

Flies caught in traps. Water and tanglefoot traps, which sampled insects flying near *Arisaema* spathes, both caught approximately the same spectrum of flies as were found in spathes, namely Mycetophilidae, Sciaridae, other Nematocera, and (for tanglefoot traps) Brachycera (Table 2). The traps differed from *Arisaema* spathes in having "other Nematocera" as the most abundant category and Mycetophilidae as least abundant. A test comparing proportional abundance of the four taxa of flies in *Arisaema* spathes (both sexes combined, including Brachycera as a fourth category) and traps (both types combined) was highly significant ($\chi^2 = 132.1$, df = 3, P < 0.0001). Thus, *Arisaema* spathes captured a distinctive subset of the available flies in the understory.

Thrips observations. Most spathes contained no thrips when checked visually by lifting the spathe hood (83% of male spathes, N = 136; 87% of female spathes, N = 52). Among spathes containing thrips, the median number was 2 (males) or 1 (females), with maxima of 5 (males) or 7 (females). The mean number of thrips per spathe was 0.30 for males, and 0.37 for females.

Pollen loads. All four categories of visitor (three fly taxa and thrips), despite preservation in alcohol and rinsing, carried at least some pollen grains on their bodies, in

all individuals. Mycetophilids had the most leftover pollen attached to their body,
sciarids the second highest amount, followed by thrips, and finally other fly visitors
(Figure 1). A one-way ANOVA was significant, indicating that at least one pollen load
mean differed from others (F = 11.19, df = 3, P < 0.05). In a post-hoc contrast of means,
mycetophilids carried significantly more pollen than other visitor groups. Sciarids and
thrips did not differ significantly in pollen loads. Sciarids did carry more pollen than
"other flies", while thrips did not.

The photograph of a live mycetophilid showed 126 grains attached to hairs on the
dorsal thorax. This partial count was higher than the mean value for the rinsed
mycetophilids. The live fly probably carried more pollen on other parts of its body.
Although only a single observation, it shows that a fly leaving a male spathe carried a
good quantity of pollen.

Discussion

Visitor assemblages of A. triphyllum *spathes: species composition*. Flowering
Jack-in-the-pulpit populations near Terre Haute, Indiana, are primarily visited by flies of
suborder Nematocera, and especially by Mycetophilidae and Sciaridae, the classic
Arisaema visitors. The flies were found as corpses in female spathes in the field at the
end of the flowering season, as well as in female and male spathes collected at various
time points during flowering. This is the typical pattern for *Arisaema* species worldwide,
and supports the hypothesis of a deceptive, lethal-trap pollination mechanism targeting
such flies (Vogel and Martens, 2000).

Although male and female spathes differed in the relative abundances of mycetophilid, sciarid, and other nematoceran flies, there was broad overlap between the sexes. Thus, the system of separate sexes has not led to a serious discordance in visitor assemblages. Co-flowering, occurrence in the same habitat, and similar attractive features (odor production) are likely responsible for the broad overlap.

Thrips were also found in a minority of spathes (13-17%). Their presence in both male and female spathes supports the hypothesis that they may contribute to pollination (Rust, 1980; Feller et al., 2002), but their low frequency does not strongly support it. However, my observations probably underestimate thrips abundance, since thrips buried in pollen at the base of the spathe would not be seen on a visual check.

Comparison of visitor assemblage to available flying insects. Water pans and tanglefoot traps yielded a similar set of fly taxa, but proportions differed significantly from spathe captures. Mycetophilid flies were proportionally much more common in spathes (45% of flies) than in traps (5%), perhaps indicating that they go out of their way to visit jack-in-the-pulpit. Sciarid flies, in contrast, occurred in similar proportions in spathes (29% of flies) and traps (35%).

Although water and tanglefoot traps were relatively small and were exposed for only 24 hours, they caught approximately as many flies as the spathes, which were exposed for one to three weeks. (Water traps caught an average of 2.1 flies, tanglefoot traps 3.7 flies, male spathes 2.3-2.4 flies, and female spathes 1.4-3.4 flies.) This perhaps indicates that attraction and capture of flies by jack-in-the-pulpit spathes is a rare event relative to the abundance of nematoceran flies in the habitat.

Quantities of flies and thrips in spathes – comparison to other studies. Only three previous studies have quantified the number of flies and/or thrips found per spathe in *A. triphyllum.* Rust (1980) found an average of 0.3 flies per male spathe (with open escape hole) and 0.5 flies per female. Feller et al. (2002) found no dipterans in male spathes (with open escape hole) and 1.6 flies per female spathe. Barriault et al. (2009) found an average of 0.4 flies per spathe (male and female spathes pooled, male spathes plugged). Compared with these values, our average fly totals in 2008-2009 of 2.3 – 2.4 per male spathe (plugged) and 1.4 – 3.4 per female spathe are high.

Is it likely that such low visit rates would result in effective pollination, especially since some of the flies captured by females may not have visited a male spathe? Until well-replicated tests of single-visit effectiveness have been done, it is an open question.

Regarding thrips, our values of 0.30 thrips per male spathe and 0.37 per female spathe are intermediate compared with other reports. Rust (1980) found 10 thrips per male spathe, but only 0.4 per female spathe. Feller et al. (2002) averaged 4.4 thrips per male spathe, 0.8 thrips per female. Barriault et al. (2009) averaged only 0.1 thrips per spathe. The main difference is that Rust (1980) and Feller et al. (2002) found much higher thrips activity in male spathes.

Pollen loads. The fact that all fly and thrips specimens taken from male spathes had pollen adhering to them, even after rinsing, indicates that male spathes succeed in transferring pollen to all four categories of insect visitors. This is not surprising, given the internal structure of spathes: any insect which descends to the level of florets on the spadix can hardly avoid contacting them, since the space between spadix and spathe wall

is slim. Assuming that the number of pollen grains on rinsed insect specimens was proportional to the number they carried prior to rinsing, the data suggest that the two classic *Arisaema* pollinators, mycetophilid and sciarid flies, carry more pollen than thrips or "other flies". The difference between mycetophilids and sciarids is not surprising, because mycetophilids are larger than sciarids, on average (mean thorax length, mycetophilids: $0.73 \pm$ SD 0.11 mm, N = 42; sciarids, $0.52 \pm$ SD 0.08 mm, N =20). Both fly taxa were larger than thrips (mean thorax length, $0.33 \pm$ SD 0.04 mm, N =8). The "other flies" had a smaller mean thorax length than sciarids (0.45 mm $\pm$ SD 0.14 mm, N=18).

Feller et al. (2002) examined 236 arthropods caught on sticky traps placed near jack-in-the-pulpit spathes, and counted a total of 252 pollen grains on them. The largest percentage of pollen was carried by thrips (53%), the next largest by dipterans of ten families (38%). They do not report the number of grains per individual, so pollen-carrying ability cannot be evaluated independently of insect abundance.

Would the modest pollen loads I recorded (for example, the mycetophilid mean of 74 pollen grains) be sufficient to fertilize female flowers? Since most individual fruits contain only 1, 2, or 3 seeds, only a few pollen grains might be needed to fertilize a single flower; however, in flowering plants it is rare for fertilization to occur unless the amount of deposited pollen is several times greater than the number of ovules. Furthermore, a female spadix contains many (ca. 50) individual flowers, each with several ovules. The relationship between pollen quantity deposited on stigmas and seed set needs study.

CHAPTER 2

FRUIT SET AND THE EFFECTS OF PLANT SIZE, DIFFERENT VISITORS, AND

SPATHE MORPHOLOGY

Introduction

In the previous chapter, I addressed questions concerning "who visits" *A.*
triphyllum spathes in west-central Indiana. Here I examine fruit set in natural and
experimental situations.

Natural pollination success and plant size. Levels of fruit and seed set in natural
populations of *A. triphyllum* varied widely in previous studies, from very low to moderate
to high (Table 1). Inadequate pollinator service has been demonstrated dramatically in
the two instances where hand-pollination tests were made (Bierzychudek, 1982; Parker,
1987). An important part of the present study was to evaluate naturally occurring
pollination success (fruit set) in my study area, which I did by monitoring approximately
50 female plants at five sites in 2009.

Plant size is an important factor to consider in evaluating pollination success of
females. Jack-in-the-pulpit is a long-lived perennial which photosynthesizes with one or
two large leaves for 3 to 6 months per year (barring major herbivory), and has a below-
ground corm or tuber as a perennating organ. This corm grows over the years, and
produces offset buds for vegetative propagation as well as the leaf(s) and (when mature) a

reproductive shoot. The energy and nutrient reserves of the corm strongly influence its annual sex expression. Given the size of the flowering unit (the spathe) and the large mass of fruits and seeds (compared to pollen), it is no surprise that small, medium, and large plants tend to be non-flowering, male, and female, respectively (Bierzychudek 1982, Ewing and Klein 1982, Lovett Doust and Cavers 1982, Clay 1993, Vitt et al., 2003; Figure 2).

Although there is a threshold corm size for female plants, members of a given cohort of females vary in size. Such variation might affect attractiveness to pollinators or resources for fruit development, either of which could affect fruit set. In a highly productive population, larger females set more fruit than smaller ones (Lovett Doust et al., 1986). In a population with low natural fruit set and inadequate pollinator service, no relationship was found between size and fruit set, but among hand-pollinated plants a positive relationship was seen (Bierzychudek, 1982). An easily measured trait, the diameter of the main stem at ground level (basal stem diameter), is an excellent index of corm size (Clay, 1993). I measured the basal stem diameter of female plants and related it to fruit set measures. I also measured size of male plants, and re-examined the question of plant size and sex expression.

Experiment 1: Effectiveness of different visitors as pollinators. There is disagreement in the literature as to the relative importance of flies and thrips as pollinators, as discussed in section I. Although mycetophilid and sciarid flies are usually recognized as the principle pollinators of *A. triphyllum* and *Arisaema* species (Vogel and Martens, 2000), two authors have made a case for thrips being more important (Rust,

1980; Feller et al., 2002). Both insect types visit spathes of both sexes and carry

Arisaema pollen. Experiments to evaluate their effectiveness on a per-visit basis have not

been done. Of course, such information would need to be combined with good measures

of visitation rate to evaluate relative importance in a given locality.

To shed light on the thrips-versus-flies question, I designed a caging experiment.

One cage type (in theory) excluded flies but not thrips from a female spathe, while the

second was large enough for both insects and controlled for the other effects of caging.

In practice, some flies infiltrated the thrips-only cages, but in low numbers. A third

treatment was an uncaged spathe, which would help indicate whether presence of any

cage had a large effect. I predicted that fruit set would differ among treatments, and that

the order of fruit set would be: uncaged > flies-and-thrips admitted > thrips-only.

Experiment 2: Lack of an escape hole in female spathes.   Vogel and Martens

(2000) detailed many of the spectacular contrivances of different *Arisaema* species to

pollination by fungus gnats. One such contrivance is the exit hole at the bottom of male

spathes, which is lacking at the bottom of female spathes. They favor the hypothesis that

the hole is lacking from female spathe in order to increase the time that a fly is around the

florets. If flies have visited a male plant previous to their visit to a female, the increased

time should increase pollen deposition. The circumstantial evidence for the adaptive

lethal trap includes: the regular occurrence of a well-shaped, functional hole in males

(and rare hermaphrodites), and its absence in females; the build-up of insect corpses in

female, but not male spathes; and the fact that females have no pollen to disperse.

Alternatively, the closed exit may be a neutral feature, or one that protects ovaries from

herbivory, rather than improving pollination success. I devised an experiment to test whether the lethal trap increases fruit set.

Methods

Natural pollination success and plant size. I used basal stem diameter as a proxy for plant size, following Ewing and Klein's (1982) work on *A. triphyllum* and Clay's (1993) work on *A. dracontium*. Other workers have used different measures of plant size, such as leaf area (Bierzychudek, 1982; Feller et al., 2002; Vitt et al., 2003). Corm size is probably the best predictor of sex in the present or subsequent year, but can only be sampled somewhat destructively. However, Vitt et al. (2003) showed basal stem diameter is an excellent proxy for corm mass.

Five woods were searched for flowering plants from April 7- May 18, 2009. Although most inflorescences emerged in April, monitoring continued because of a second, delayed period of emergence at two of the sites. Plants were identified with a colored flag placed in the ground nearby, to which a numbered aluminum tag was attached; a colored plastic twist-tie was placed around the plant's main stem. Once the spathe was sufficiently developed, I determined sex by two methods: checking for presence of an open exit hole for small insect pollinators, located externally at the base of the spadix; and peering into the open top of the spathe to examine the florets, with aid of a flashlight when necessary. Florets of females have large green ovaries; those of males are usually reddish or yellow with noticeable pollen. At the same time, basal stem diameter was measured with a digital caliper at ground level, where the main stem

emerged from the soil. Overall sample sizes for the size-gender study were 75 female and 132 male plants.

Of 75 female plants initially found, 47 were left uncaged and used to measure natural fruit set and its relationship to size. The others were assigned to caging treatments that affected insect entry (see Experiment 1 below). Most female spathes flowered between April 7 and May 12. Afterwards, fruit set initiation was determined by feeling the collapsed spathe enveloping the fruiting spadix and scoring it as (a) having fruit expansion, (b) contracting, or (c) no change from its size during flowering. In most cases this was an easy distinction because not all ovaries expanded equally (probably because of pollen deposition), so a spadix with an uneven surface, beneath the dried collapsed spathe hiding it, was said to have expansion occurring. Spathes going through contraction were usually characterized by a weak stem and often chlorosis of the leaf or peduncle along with a decrease in size of the fruiting spadix.

Plants maturing one or more seed-containing fruits were designated as setting fruit, although some were lost to herbivory in late stages of ripening or to accidents such as a tree fall. Plants that withered before maturing one or more fruits were scored as no-fruit-set; this included plants that showed no signs at all of fruit set and others that initially were scored as maturing fruit, but subsequently quickly senesced, perhaps aborting some fruits. By mid-July all females could be scored as belonging to one of these categories. Those with developing fruits had large, green hard fruits at this stage.

Experiment 1: Effectiveness of different visitors as pollinators. Cages were cylindrical and constructed of PVC material, measuring 36 cm long x 36cm wide x 49 cm

tall. The thrips-only cages were covered with small diameter (1 X 1 mm) nylon mesh. A second cage type with large diameter nylon mesh (4 x 4 mm) had holes large enough for flies (and thrips) to enter (flies-and-thrips treatment). The third treatment was lack of a cage. I expected presence of a cage to deter both flies and thrips to some extent, which this last treatment would help assess.

Cages were set up over a female plant between 14 and 24 April 2009 as plants emerged from the ground. Since flies are known to walk along the ground, the bottoms of cages were buried with dirt to ensure that the only entrance to cages was through the mesh. During flowering, cages were removed briefly to measure plant size and observe visitors; during fruit development, they were checked once every three weeks. Cages remained on plants up to the point where a fruit stalk fell prostrate (27 July – 1 September), at which point spathes were collected to record fruit set. I first determined whether spathes matured at least one fruit, or failed to. I also collected what were apparently fruits, dissected them to determine if they contained seeds, and estimated the number of flowers produced to determine fruit to flower ratios. Though ovaries shrank they could still be counted after collection in August and September, allowing an estimate of flower number in April.

To obtain information on insect visits to spathes in the caging treatments and control, I did two things. First, I checked each spathe once during flowering by removing the cage briefly, then lifting the spathe hood to see if flies were present and count them with the naked eye aided by a flashlight if necessary (2- 11 May). This level of observation was not sufficient to differentiate between the families of flies, but thrips

could be seen and differentiated readily with the naked eye. Secondly, after flowering

ended and initial fruit set was determined, I unraveled the spathe and collected any

corpses of flies that I could find (19 May). I calculated the total flies detected per spathe

by taking the maximum value of observed or corpse counts. I then related fly counts to

fruit set data.

     Experiment 2: Lack of escape hole in female spathes. In 2008 I tested the

effectiveness of the lethal trap by comparing fruit set in three experimental treatments of

female spathes. Plants were either surgically altered or left to naturally trap fly visitors

(Figure 3). All treatments were set up on 27 April, by which date only 3 females had

become receptive and no flies had yet been seen inside spathes. The three females that

were receptive prior to 27 April were placed in the natural pollination treatment

(treatment 3). *Treatment 1* was a manipulation that allowed flies to exit female spathes

by way of a surgical cut, 1-2 mm diameter, in the bottom of the spathe like the escape

hole of males (exit-hole treatment). *Treatment 2* was manipulated in the same way as

treatment 1 (i.e. an escape hole was cut); however the hole was then plugged with cotton

(control treatment). This served as a control for damage possibly caused by surgery.

Treatment 3 consisted of no manipulation (natural treatment). Each treatment was

applied to 6 or 7 plants at Hews-Scott woods (original N=20). On 14 July one plant from

the hole-cut treatment was excluded from analysis because of deer herbivory. By this

time other females in this study had senesced naturally and were left in analysis.

     Fruit set was measured weekly beginning (14 July) with a final count on 27

August when infructescences were collected. Counts of flowers were obtained in July.

The number of fruits was obtained by dissecting fruit in the lab; a fruit was counted as such if it contained one or more seeds greater than 2 mm diameter was scored as "setting fruit". In the analysis, fruit set values were compared in two ways. First, fruits per flower were compared between treatments, omitting the plant eaten by deer. Finally, the three treatments were compared with a chi-square test using two outcome categories: "fruit set" (at least one fruit matured), and "no fruit set".

Results

   Natural fruit set and plant size.   Overall, across five study sites, 57.4% of female plants set at least one fruit, as determined by mid-July counts when fruits (if present) were large and green (Table 3). This basic measure was quite variable across sites, ranging from 14.3% to 100% at four sites with $\geq$ 5 plants. The fruiting plants were fairly productive, on average; site means ranged from 17.0 to 35.2 fruits per female, with a grand mean of 22.6 per spathe (Table 3). When zero values were included in calculations, mean productivity dropped to 11.3 fruits per female.

   Between mid-July and fruit maturity and dispersal in late August, 11 of 27 infructescences were lost, primarily to herbivory (Table 3). The herbivore was not identified, but was likely a mammal (presumably rodent or deer). Typical evidence for this was that, prior to the general ripening period (mid-August onward) an infructescence with numerous exposed green fruits would, on next inspection, be missing all but a few fruits, along with the axis of the infructescence that supported them. These losses are not

reflected in the calculations of fruit set, but they illustrate the problems associated with a lengthy fruit maturation period.

Female plant size and probability of fruit set: Pooling data from five populations, female plants setting fruit were not significantly larger, on average, than those that failed to set fruit ($t = 1.34$, df = 45, P = 0.094; Figure 4). The two fruit set categories showed much overlap in size, and plants as small as 9.0 mm BSD set fruit. Female plants ranged in size from 8.2 -19.0 mm BSD, a two-fold difference. A plot of the relationship between basal stem diameter and fruit set (Figure 5) shows, for the pooled sample, that the smallest plants ($\leq$ 9 mm) rarely set fruit and that most fruit set occurred on plants with spathes of 10-14 mm diameter. Curiously, only two of four plants larger than 14 mm set fruit. In contrast to the overall pattern, at one site (Whitaker) there was a dramatic positive relationship between size and fruit set: the four smallest plants set no fruit, and the five largest ones did (Figure 6).

Plant size and gender: At four sites, good numbers of both sexes were measured. Female plants were significantly larger than males at these sites; *t*-tests were highly significant in each case (Table 4). In a two-way ANOVA with sex as the main factor and site as the blocking factor, sex was highly significant ($F_{1, 3.4} = 81.8$, P = 0.002), site was not ($F_{3, 3} = 0.59$), nor was there a significant site*sex interaction ($F_{3, 199} = 1.42$, P = 0.24). Thus, female-phase plants were consistently larger than males (by 2.7 – 4.1 mm), and mean sizes of each sex varied little among sites.

Experiment 1, testing effectiveness of different visitors. Caging treatments did have an effect on the proportion of spathes setting one or more fruits. Pooling data from

four sites, the null hypothesis that the three treatments were from the same population

was rejected ($X^2 = 6.22$, df = 2, P < 0.05). Fruit set in the thrips-only treatment was much

lower than in the flies-and-thrips or uncaged treatments (Figure 7a). However, the fruit

set of the thrips-only treatment was not zero (3 of 17 spathes set fruit). Surprisingly, fruit

set of uncaged plants was lower than in the caged, "flies-and-thrips admitted" treatment.

Cages were not completely effective in creating the desired conditions.   During

brief checks, approximately one-third of the "flies excluded" cages did have a fly present.

This proportion was lower than in the other treatments, so fly visitation was significantly

reduced in the "flies excluded" cages ($X^2 = 5.98$, df= 2, P= 0.05, Figure 7b).   Also, I

could not confirm that thrips entered the cages designed to be "flies excluded, thrips

only". On the other hand, cages with mesh allowing "flies and thrips" to enter had flies

present at about the same rate as did the open treatment spathes; so cages did not seem to

deter flies.

Experiment 2: Lack of escape hole in female spathes. The three treatments

showed similar distributions of fruit set, namely a mixture of low and high values (Figure

8), and did not differ significantly (Kruskal-Wallis test $X^2 = 0.496$, df = 2, P > 0.05; chi-

squared test $X^2 = 2.14$, df = 2, P > 0.05). Surprisingly, plants with artificial exit holes had

high fruit set in 3 of 6 cases, similar to unmanipulated females with no exits, and controls

with exits plugged with cotton. However, sample sizes were low. Variation in fruit set

was extensive, with five plants setting close to zero fruits and eight plants with at least

70% fruit set, and few in the middle range.

Discussion

    Natural fruit set and size relationships. Natural fruit set of *A. triphyllum* in west-central Indiana was moderately successful, compared with previous studies (Table 1). Slightly more than half of female spathes matured at least one fruit, and those that did produced an average of 23 fruits. The overall average of 11.3 fruits/female is similar to that recorded by Rust (1980) and Parker (1987), greater than the lowest values observed for the species (Bierzychudek 1982, 1984), and substantially lower than records by Sakamoto (1961), Treiber (1980), Policansky (1981), and Lovett Doust et al. (1986).

    Factors likely to affect fruit set include pollinator abundance and effectiveness, availability of resources to mature fruits, density of conspecifics (especially distance to male plants), and genetic quality of received pollen. Here I focus on the first two factors, pollinator abundance and resource availability (plant size). Potential pollinators were not abundant: flies averaged between 1.1 and 3.4 per female spathe (Table 2); thrips were observed in only 13% of female spathes. However, these low numbers were typical of Rust's (1980) study, which had similar fruit set to mine. Higher values have been observed in other *Arisaema* species (Vogel and Martens, 2000). Unfortunately, there are few quantitative data relating fruit set to visitor number in *A. triphyllum*.

    Plant size did not differ significantly between females that set fruit and those that failed to do so, except at one site. The overall mean was (non-significantly) higher for fruit-set plants, as expected. From these mixed results, I conclude that there was probably some effect of resource availability on fruit set, but also a substantial (probably greater) effect of inadequate pollinator service. This could be clarified by hand-

pollination experiments, which in two instances have led to dramatic improvement of fruit set (Bierzychudek, 1982; Parker, 1987). Careful association of corpse collections (by unraveling spathes without damage at the proper time) with fruit set, using a large sample size, would also be instructive. Finally, comparing fruit set among local populations that differ in conditions favorable for mushroom flies would be helpful. For instance, the flies are supposedly most common in dark, damp locations; jack-in-the-pulpit are present there, and also in drier upland situations.

Experiment 1: Effectiveness of different visitors as pollinators. Data in Figure 7a, taken at face value, support the conclusion that mushroom flies are the primary pollinator of jack-in-the-pulpit, with thrips delivering a small percentage of pollen.   The cage designed to admit flies and thrips had a three-times greater proportion of plants setting fruit than the one designed to exclude flies and admit thrips. However, some flies got into the "thrips-only" cages, although I used a mesh size which has been successful in experiments with other thrips-visited plants (Terry, 2001; Garcia-Fayoís and Goldarazena, 2008). I also do not know whether or not thrips entered either cage type on a regular basis. Thus, this experiment is inconclusive, except in demonstrating that fruit set is accomplished by small insects.

Experiment 2: Lack of escape hole in female spathes. Opening an exit hole in female spathes was not detrimental to fruit set. This treatment showed a similar wide range of values as the two alternatives, the natural condition of no exit and a surgically opened hole plugged with cotton. Each treatment had > 0.5 fruits/flower in three females. Assuming that flies exited quickly from the spathes with holes, the data

suggests that pollen deposition was often sufficient for good fruit set. However, sample size was low, owing partly to plant losses to deer. Although the results are intriguing, the experiment needs to be repeated with larger sample sizes.

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Table 1. Fruit and seed production by female *Arisaema triphyllum* in various populations, incorporating data from Table 4 of Lovett Doust et al. (1986).

Author(s)	State	N, spathes	Seeds per plant (SE)	Range, seeds per plant	Fruits per plant	Seeds per fruit (SE)
Sakamoto (1961)[a]	Minnesota	12	52.4		65.2	1.6 (0.1)
Rust (1980)	Delaware	100	14.4	0-109	9.6	1.5 (0.1)
Treiber (1980)[a]						
subspecies *triphyllum*	N. Carolina	78	51.9		33.2	1.6
subspecies *stewardsonii*	?	21	72.6		39.5	1.8
subspecies *pusillum*	?	32	28.7		22.4	1.3
Policansky (1981)	Mass.	80	51.9	2-230		
Bierzychudek (1982)	New York					
Hand-pollinated		29	74.3 (13.4)	7-300		
Naturally pollinated		24	1.0			
Bierzychudek (1984)	New York	240	5.2			
Lovett Doust et al. (1986)	Ontario	24	75.1 (5.4)	34-148	60.2	1.2 (0.1)
Parker (1987)	Illinois					
Hand-pollinated[b]		14	73.0 (12.7)			
Naturally pollinated[b]		72	12.3 (3.0)			

[a] Original not seen; values from Lovett Doust et al. (1986).

[b] Parker 1987: values are for healthy female plants, uninfected by rust.

Table 2. Types of flies collected in (a) male and female spathes of jack-in-the-pulpit, or (b) in colored water traps or tanglefoot® traps placed near flowering plants.

Capture method	Year (# sites)	N	Total visitors[a] (mean per spathe or trap)			
			Mycetophilidae	Sciaridae	Other fly, Nematocera	Other fly, Brachycera[b]
Male spathe, plugged [c]	2008 (1)	17[e]	18 (1.06)	14 (0.82)	7 (0.41)	-
	2009 (3)	29	23 (0.79)	26 (0.90)	18 (0.62)	2 (0.07)
Female spathe[d]	2008 (1)	14[e]	26 (1.86)	8 (0.57)	14 (1.00)	-
	2009 (4)	35	20 (0.57)	8 (0.23)	7 (0.20)	2 (0.06)
Water trap	2008 (3)	66	4 (0.06)	42 (0.64)	89 (1.35)	-
Tanglefoot trap	2009 (3)	54	13 (0.24)	75 (1.39)	88 (1.63)	25 (0.46)

[a] Mycetophilidae and Sciaridae are families within the dipteran suborder Nematocera. "Other fly, Nematocera" refers to different families within the same suborder which are less important pollinators of jack-in-the-pulpit. "Other fly, Brachycera" refers to phylogenetically advanced flies in the suborder Brachycera, which are not mushroom-feeders. Thrips were likely all one species, *Heterothrips arisaemae*.

Table 3. Natural fruit set and spathe survival of female jack-in-the-pulpits at various sites in west-central Indiana in 2009.

Site	Female plants, N	Basal stem diam., mean $\pm$ SD	% plants with fruit[a]	Spathe survival to at least mid-July, stage of large green fruits — Fruits/plant, mean $\pm$ SD (N) All plants[b]	Fruiting plants only[c]	Spathe survival to late Aug. Eaten	Lost	Survived
Ford	7	11.4 $\pm$ 2.7	14.3	2.4 (7)	17.0 (1)	0	0	1
Hews-Scott	25	11.3 $\pm$ 2.5	64.0	10.7 $\pm$ 13.9 (22)	18.2 $\pm$ 13.8 (13)	5	2	9
Kieweg	5	12.0 $\pm$ 3.4	100.0	--	--	4	0	1
Lima	1	9	0.0	--	--	--	--	--
Whitaker	9	11.6 $\pm$ 1.6	55.5	9.4 $\pm$ 11.4 (9)	35.2 $\pm$ 14.4 (5)	0	0	5
All sites	47	11.4 $\pm$ 2.4	57.4	11.3 $\pm$ 15.6 (38)	22.6 $\pm$ 15.3 (19)	9	2	16

[a] Includes some plants in which fruit set was confirmed but fruits were not counted prior to disappearance or dispersal.

[b] Includes only plants for which fruits were counted, including zeros; eight additional plants matured fruits that disappeared before counts were made.

[c] Includes only plants for which fruits were counted.

Table 4. Mean ($\pm$ 1 SE) basal stem diameter (in mm) of male and female Jack-in-the-pulpit plants at five sites in Vigo and Clay counties, Indiana, in 2009.

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	Basal stem diameter (mm)				
Site	Male	Female	Student's t	Prob.[a]	Ratio, F:M[b]
Ford	7.7 $\pm$ 0.25 (41)	10.4 $\pm$ 0.65 (16)	4.74	< 0.001	1.35
Kieweg	7.3 $\pm$ 0.20 (40)	10.2 $\pm$ 0.85 (15)	4.88	< 0.001	1.41
Lima	6.2 $\pm$ 0.29 (47)	9.0 (1)	-- [c]	--	--
Hews-S.	6.9 $\pm$ 0.26 (29)	11.3 $\pm$ 0.45 (35)	7.96	< 0.001	1.63
Whitaker	7.5 $\pm$ 0.33 (25)	11.6 $\pm$ 0.52 (9)	6.56	< 0.001	1.55
All sites	7.1 $\pm$ 0.13 (182)	10.9 $\pm$ 0.31 (76)			1.54

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[a] Probability that male and female plants were sampled from the same population of basal stem diameters.

[b] (Mean basal stem diameter of female plants)/(mean basal stem diameter of male plants)

[c] No test because only one female plant was measured.

Figure 1. Number of pollen grains found on visitors to 17 male spathes of jack-in-the-pulpit. Boxes contain the middle 50% of values and bars extend to 10th and 90th percentiles. Letters indicate which groups had similar means. Sample sizes are given below plots.
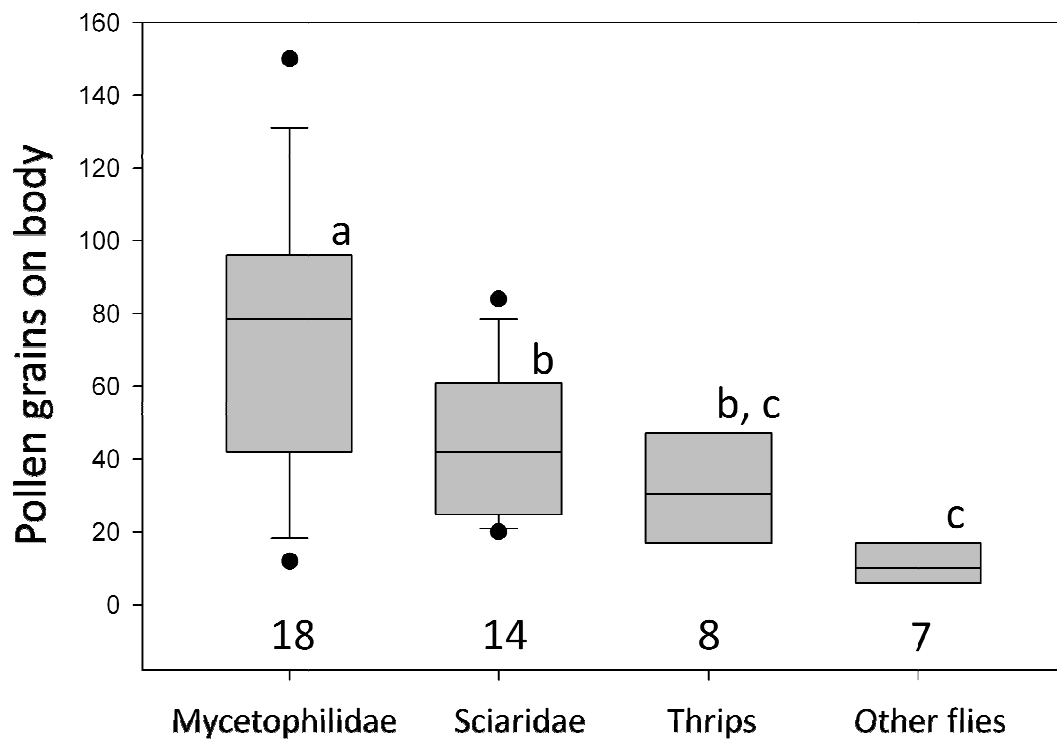
Figure 2. Mean size ($\pm$ 1 SE) of each sex class in five studies of two American *Arisaema* species.
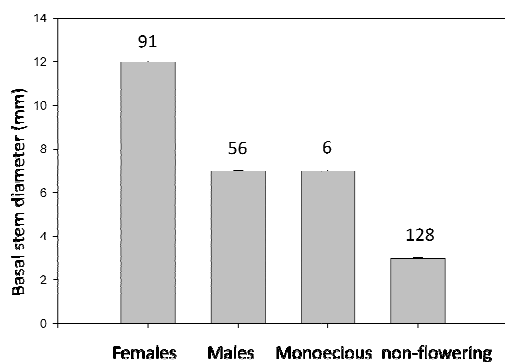
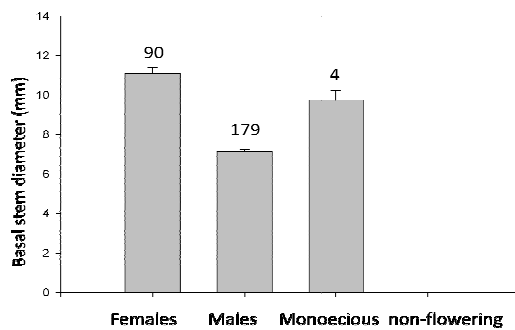a. Ewing and Klein 1982, Massachusetts *A. triphyllum*
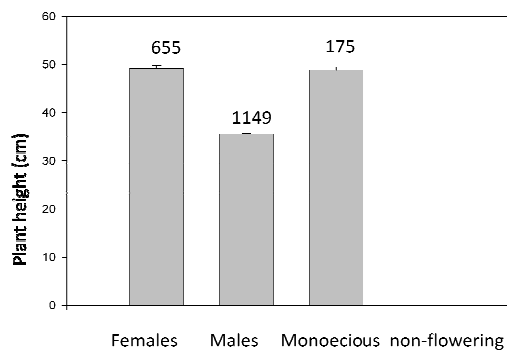
b. Lovett Doust and Cavers 1982, Ontario *A. triphyllum*
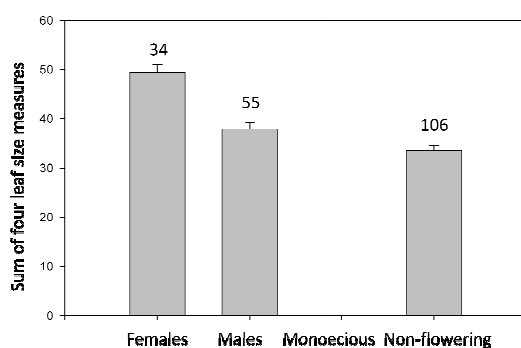
c. Vitt et al. 2003, Connecticut *A. triphyllum*
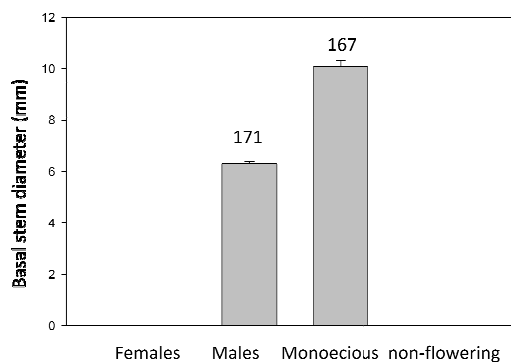
d. Clay 1993, Louisiana *A. dracontium*

e. Pettit 2009, Indiana *A. triphyllum*

Figure 3. One natural and two surgically altered treatments which tested whether the lethal trap feature of female jack-in-the-pulpits increases fruit set.
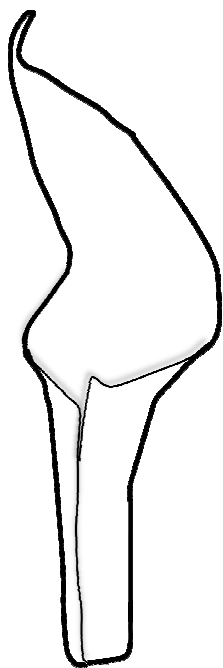
Natural: no manipulation
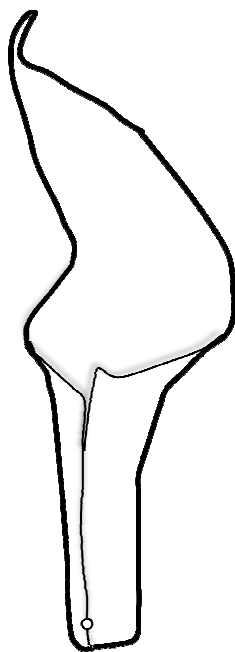
Experimental: cut a hole in the female spathe
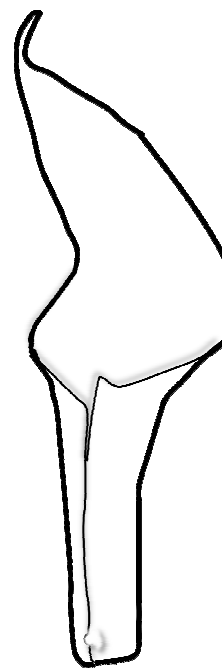
Control: cut a hole then plug it with cotton

Figure 4. Plants setting fruit did not significantly differ in basal stem diameter from those that failed to set fruit (*t* =1.34, df = 45, P = 0.094).
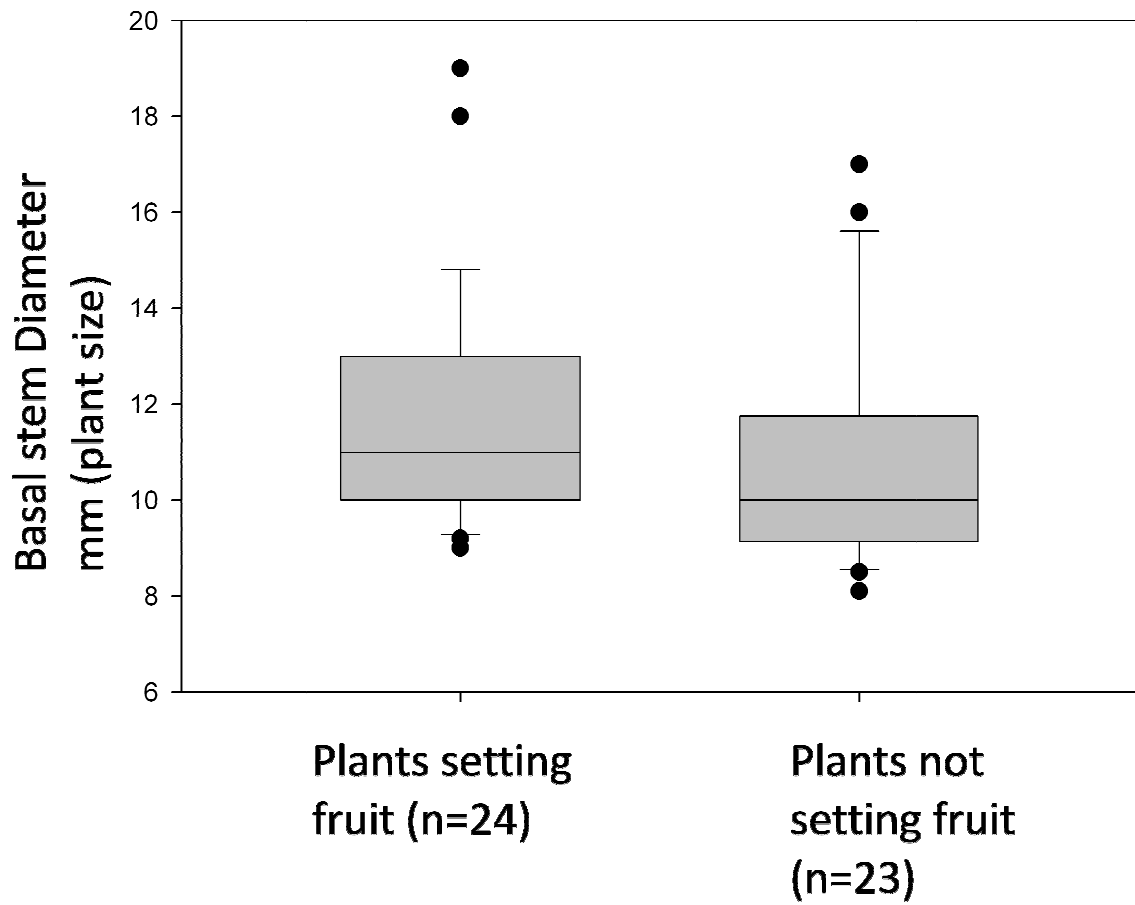
Figure 5. Fruit set per plant compared to basal stem diameter, using data from five sites (N = 47 plants). Open circles represent plants that produced a positive but unknown number of fruits.
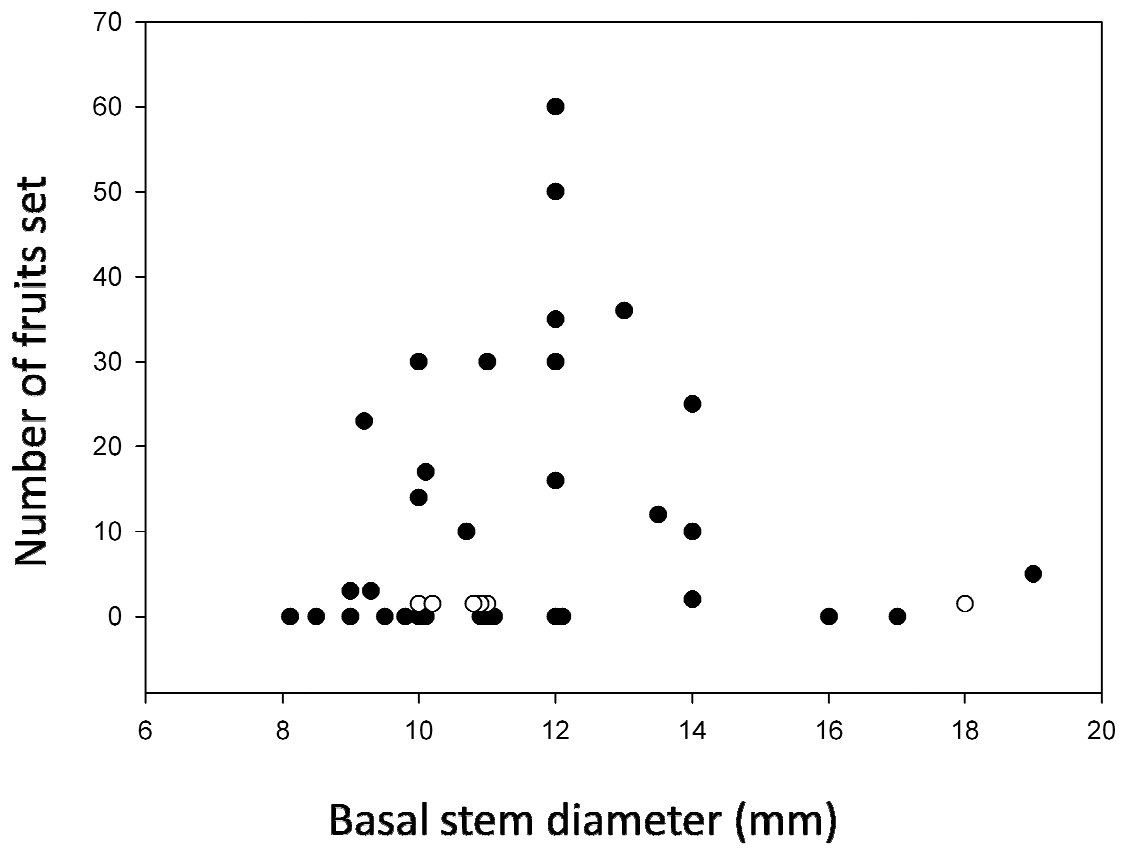
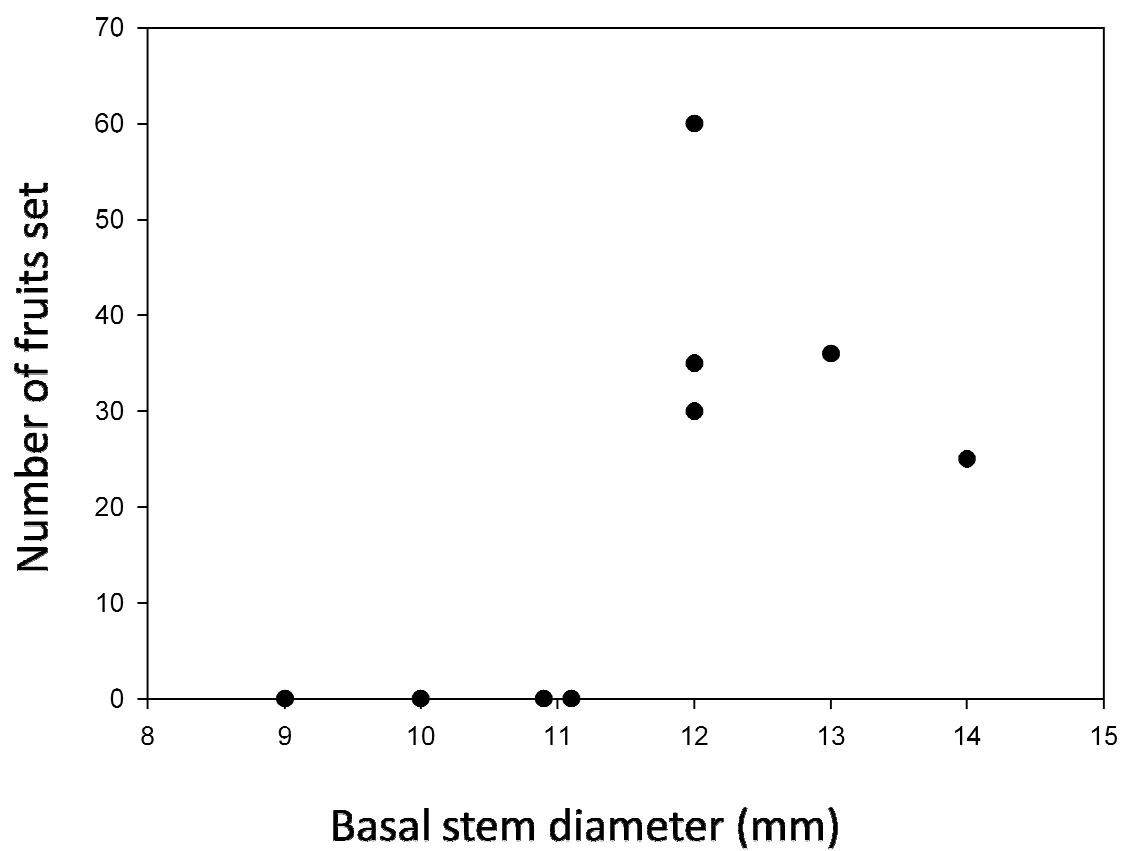Figure 6. Relationship between fruit set per plant and basal stem diameter for 9 plants at Whitaker Woods.
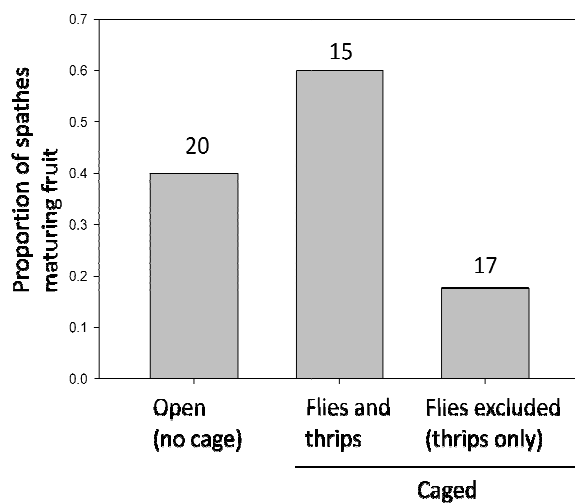
Figure 7 a. The proportion of female plants setting fruit in two caged and one uncaged treatment.

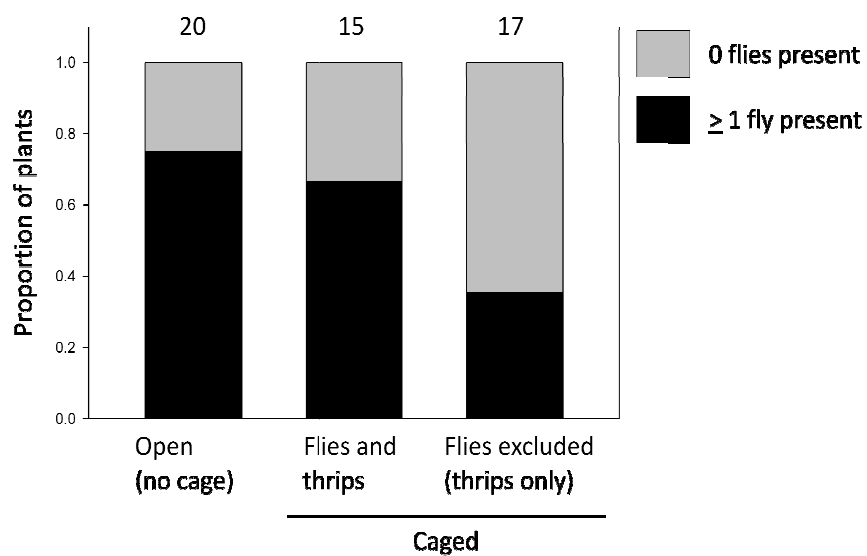b. The proportion of plants per treatment that contained none, or at least one fly.

Figure 8. Fruits per flower for 19 jack-in-the-pulpit spathes placed in three pollination treatments.