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RESEARCH PAPER

# Pollination ecology and reproductive success in Jack-in-the-pulpit (*Arisaema triphyllum*) in Québec (Canada)

I. Barriault[1], D. Barabé[1], L. Cloutier[2] & M. Gibernau[3]

1 I.R.B.V., Jardin botanique de Montréal, Université de Montréal, Montréal, Canada

2 Collection Entomologique Ouellet-Robert, Département de Sciences Biologiques, Université de Montréal, Montréal, Canada

3 Laboratoire Évolution et Diversité Biologique, Université Paul Sabatier, UMR-CNRS, Toulouse, France

**Keywords**

Araceae; bisexual inflorescence; deceptive pollination; Mycetophilidae; pollen load; Thysanoptera; visitation rates.

**Correspondence**

M. Gibernau, Université Paul Sabatier, Laboratoire Évolution & Diversité Biologique (UMR 5174), Bât 4R3-B2, 31062 Toulouse cedex 9, France.

E-mail: gibernau@cict.fr

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## ABSTRACT

**Pollination ecology and reproductive success of Jack-in-the-pulpit (*Arisaema triphyllum*) were studied in two natural populations in Québec, Canada. Individual *A. triphyllum* plants can be of three types: male, female or bisexual. In both populations studied, the presence of bisexual inflorescences was not negligible (13%), where 'female' and 'male' bisexual plants were categorised according to the relative number of stamens and ovaries. 'Male bisexual' plants produce only pollen and 'female bisexual' plants produce only fruit. Hence, *A. triphyllum* is a true dioecious plant, as each plant only reproduces through either the male or the female function. 'Female bisexual' plants were equivalent to female plants in terms of visitation rate by insects, fructification rate and production of berries and seeds. Neither agamospermy in female plants nor self-pollination in 'female bisexual' plants was found, thus *A. triphyllum* relies on insects for cross-pollination. Despite the long flowering cycle, a low visitation rate was documented: only 20–40% of inflorescences were visited, according to gender, by a mean of 1.5 insects. In this study, Mycetophilidae represented the most generically diversified and abundant family, as well as the most efficient insect pollinator, especially the genera *Docosia* and *Mycetophila*.**

## INTRODUCTION

Araceae are an entomophilous monocot family (Grayum 1990), in which Diptera are frequent pollinators (Gibernau 2003). The Araceae/Diptera interaction can be classified into two types, regardless of the taxonomical group of fly involved in pollination. The interaction can be mutualistic, the inflorescence providing a mating and/or ovipositing site for flies (Sultana *et al.* 2006; Takenaka *et al.* 2006), or antagonistic, the inflorescence mimicking the ovipositing site of the lured flies (Gibernau *et al.* 2004). Such a deceptive pollination system has only been observed in the tribe Areae within the subfamily Aroideae in genera such as *Arisaema*, *Arisarum*, *Arum*, *Biarum*, *Helicodiceros*, *Eminium*, *Sauromatum* and *Theriophonum* (Koach & Galil 1986; Dakwale & Bhatnagar 1997; Diaz & Kite 2002; Stensmyr *et al.* 2002; Gibernau 2003; Gibernau *et al.* 2004).

The deceptive genus *Arisaema* Mart. contains about 200 species, most native to Asia, but three of which are present in North America: *A. triphyllum* (L.) Torr., *A. dracontium* (L.) Schott and *A. macrospathum* Benth., which is endemic to Mexico (Dieringer & Cabrera 2000). *Arisaema* species possess unisexual flowers and are unique among Araceae in being dioecious, with male, female and sometimes bisexual individuals. Male plants produce inflorescences bearing only male flowers (*i.e.* with stamens), female plants have only female flowers with ovaries on their inflorescences, while bisexual plants have inflorescences with both male and female flowers.

*Arisaema triphyllum*, the species studied here, is a perennial herb and typically grows in the understorey of forests in southern Canada and the eastern United States (Bierzychudek 1982). Among mature plants (*i.e.* those producing inflorescences), which are larger than immature plants, smaller individuals are male, larger plants female (Bierzychudek 1982) and those of intermediate size are often bisexual (Ewing & Klein 1982).

In the genus *Arisaema*, insects attracted by the odour fall into the spathe, a modified bract surrounding the fertile central axis, the spadix (Vogel & Marten 2000). After their capture in male inflorescences, the insects can exit through a small opening present in the basal portion of the spathe, and may achieve cross-pollination if captured by a receptive female inflorescence. In female inflorescences, which do not possess a basal exit, some insects die of exhaustion within the spathe, thus giving this trap mechanism the name 'lethal kettle trap' (for more details see Vogel & Marten 2000: p. 86, Fig. 6).

*Arisaema triphyllum*, like many *Arisaema* species, depends on insects for pollination as there is no agamospermy (Treiber 1980; Bierzychudek 1982). Several authors have mentioned that fungus gnats (Mycetophilidae and Sciaridae) are pollinators of *A. triphyllum* (Treiber 1980; Bierzychudek 1982; Lovett Doust *et al.* 1986; Barriault *et al.* 2009). Fungus gnats are also described as pollinators of many *Arisaema* species in Japan and Nepal (Vogel & Marten 2000; Nishizawa *et al.* 2005). These insects are known pollinators of several other families, such as Aristolochiaceae, Liliaceae, Saxifragaceae and Orchidaceae (Mesler *et al.* 1980; Goldblatt *et al.* 2004; Okuyama *et al.* 2004). In addition to fungus gnats, a thrip, *Heterothrips arisaema*, is cited as an efficient pollinator in two other studies (Rust 1980; Feller *et al.* 2002), and Thysanoptera are involved in pollination of several other families (Momose *et al.* 1998; Mound & Terry 2001; Sakai 2001; Solomon & Ezradanam 2002; Mizuki *et al.* 2005). Hence, there is no consensus about the principal pollinator of *A. triphyllum* since data from the literature indicate different pollinators among different populations.

The components of reproductive success of a flowering plant are the floral traits that are generally adapted to increase the probability of pollination and the context in which the plants reproduce, *i.e.* ecological factors (Proctor *et al.* 1996; Méndez & Diaz 2001). The flowering context is influenced by population size, abundance and quality of pollen donors, as well as plant isolation. With regard to the pollination context of *A. triphyllum*, the variables to consider are floral sex ratio and pollinator activity (Ollerton & Diaz 1999; Méndez & Diaz 2001; Diaz & Kite 2002; Barriault *et al.* 2009).

Through our study, we seek a better understanding of the pollination ecology of *A. triphyllum.* The objectives of the study of the pollination ecology of *A. triphyllum* were to:

1 Study the pollination ecology of two natural populations of *A. triphyllum* in Québec (Canada) over 2 years (2005–2006);
2 Compare the pollination context of two different habitats/populations;
3 Determine insect diversity and the efficiency of insect visitors in these populations in relation to the sex of the plant (male, female and bisexual); and
4 Quantify the mode of reproduction and reproductive success in female and bisexual plants of *A. triphyllum*.

## MATERIALS AND METHODS

### Study sites

The main study was conducted between May 6 and August 30 2005 and between April 30 and August 23 2006 on one natural population of *A. triphyllum* situated in Angell Woods in Beaconsfield (45°26′69″ N, 73°53′51″ W) on Montreal Island (Québec, Canada). A preliminary study of reproductive success was also conducted between May 6 and August 23 2004. The study area (200 $m^2$) is a sugar maple grove and *A. triphyllum* covers about 15% of the area. Voucher specimens of the population were collected and deposited at the Marie Victorin Herbarium (MT): *A. triphyllum* (*Barriault* 25). The second natural population of *A. triphyllum* was studied between May 3 and August 28 2006. This population is at the Morgan Arboretum (45°25′57″ N, 73°56′33″ W), a 245-ha forested reserve, situated on the MacDonald Campus of McGill University in Ste. Anne de Bellevue on the western tip Montreal Island (Québec, Canada). The area studied (300 $m^2$) is also a sugar maple grove and *A. triphyllum* covers about 32%.

### Population descriptions

In both studied populations, all mature plants had a single inflorescence that was male, female or bisexual. We classified the bisexual inflorescences into two types, bisexual female (BF) and bisexual male (BM), according to the ratio of male/female flowers. In order to compare the four types of inflorescences, the number of flowers was counted on 30 male, 30 female and 20 bisexual inflorescences (14 BF and 6 BM) in the Angell Woods population. Moreover, the frequencies of these four types of inflorescence were estimated by sexing 496 plants in Angell Woods and 515 plants at the Morgan Arboretum. Differences in frequencies of plant gender between populations were analysed with Chi-square tests (Systat 8 2004).

### Insect visitation patterns

*2005 survey*

In the Angell Woods population, the frequency of insect visits was quantified by daily observation of inflorescences of 185 plants (79 male, 80 female and 26 bisexual) between May 6 and 27 2005. The bisexual inflorescences were not divided by type, because we were interested in comparing insect visitation patterns only according to plant gender, *i.e.* between male, female and bisexual plants. Each inflorescence was checked three times a day – in the morning (between 09:00 and 10:00), at midday (12:00–13:00) and at the end of the afternoon (17:30–18:30) to determine the insect visitation pattern. A total of 8112 inflorescence surveys were conducted in 2005, 3472 on male, 3511 on female, and 1129 on bisexual inflorescences.

*2006 survey*

In the following year, the frequency of insect visits was observed in both populations. The Angell Woods population was sampled between May 7 and 18 for 118 plants (49 male, 44 female and 25 bisexual) and the Morgan Arboretum population between May 9 and 18 for 134 plants (60 male, 38 female and 36 bisexual). Each population was surveyed once or twice a day. Inflorescences checked once a day for insect presence were examined in the morning and those checked twice a day were surveyed in the morning and early afternoon. During this survey, a total of 2109 inflorescence checks (883 male, 783 female and 443 bisexual) were performed in Angell Woods and 1865 checks (836 male, 525 female and 504 bisexual) at the Morgan Arboretum.

*Insect collection and identification*

During the 2005 survey, insects present in the spathe were collected with a brush, preserved in 70% alcohol and later identified. Frequencies of insect visits at different times of the day (morning, noon, afternoon) or to different types of inflorescence (male, female or bisexual) were compared with Chi-square tests (Systat 8 2004). In the 2006 survey, insects were collected with an aspirator and identified. This aspiration technique allowed determination of the efficiency of each species as pollinator.

During sampling, the small hole at the base of the male inflorescence was closed with duct tape to prevent insects from escaping. Specimens collected over the 2 years were identified to family and, for Diptera, to genus (McAlpine *et al.* 1981). All insects identified in 2005 and 2006 were deposited at the Collection entomologique Ouellet-Robert at the Université de Montréal.

*Pollen loads*

Pollen loads were counted under a binocular stereomicroscope on insects collected by aspiration in 2006 from both sites. Four classes of pollen load were distinguished: (i) no pollen, (ii) low (1–24 pollen grains), (iii) medium (25–35 pollen grains), and (iv) large (45 or more pollen grains).

Differences in pollen loads between taxa and sites in 2006 were analysed using a generalised linear model (GLIM 1986) with a Poisson error (count data, Chi-square statistics). Over-dispersion of data was corrected using Pearson's Chi-square (Crawley 1993). First, a model with all 20 insect taxa collected in 2006, the two-level factor 'site' (Angell and Morgan Woods) and their interaction was fitted to the data (full model). Then, the interaction and effect were removed in a backward way if non-significant. Finally, models grouping certain insect taxa were adjusted to the data and only those that were not significantly different from the full model (Chi-square test) were retained (simplified models). The simplified models allowed taxa to be grouped into pollen load classes, whereas in the full model, each taxon was considered different and characterised by its own pollen load class.

Fungus gnats of the genus *Docosia* (Mycetophilidae) were collected in Angell Woods in 2006 in order to identify pollen grains carried. Insects were stored in 70% ethanol, mounted on metal stubs and grounded with conductive silver paint. Specimens were sputter coated to approximately 50 nm using a HUMMER II sputter coater and viewed with a JEOL JSM-35 scanning electron microscope (SEM) with Kodak Tmax 100 profilm at the Université de Montréal.

**Reproductive success**

*Natural fructification rates*

The fructification rate of non-manipulated (*e.g.* control) inflorescences was determined in both populations. In Angell Woods, the survey was conducted in 2004 on 40 female and 36 bisexual female (BF) inflorescences; in 2005 on 119 female and 76 BF inflorescences; and in 2006 on 106 female and 33 bisexual inflorescences (24 BF and nine BM). At the Morgan Arboretum, the survey was carried out in 2006 on 62 female and 61 bisexual inflorescences (45 BF and 16 BM). Reproductive success was recorded in Angell Woods (2004–2006) and at the Morgan Arboretum (2006) only when an infructescence reached complete maturation, with developed red berries (*i.e.* fruits). All the successful infructescences were collected in autumn for berry and seed counts. Differences in fructification rates between female and bisexual infructescences and among years were analysed by Chi-square tests (Systat 8 2004). Differences in fruit and seed number between female and bisexual infructescences (*i.e.* count data) and among years were analysed by ANOVAS after square-root transformation of the data (Systat 8 2004).

*Self-pollination and/or agamospermy*

To verify spontaneous self-pollination and/or agamospermy, we bagged 14 female and 20 bisexual female (BF) inflorescences before spathe unfolding in Angell Woods in 2005, thus excluding the possibility of any insect visits during the flowering cycle.

*Hand-pollination*

In comparison with the controls, hand-pollinations were performed in 2006 to test whether reproduction may be limited by pollinator activity or by resource levels. In Angell Woods, 21 female and 6 bisexual inflorescences (5 BF and 1 BM) were hand-pollinated, and at the Morgan Arboretum, 20 female and 9 bisexual inflorescences (8 BF and 1 BM). Hand-pollinations were performed by pollinating receptive female and bisexual inflorescences with a small brush covered with a mix of fresh pollen harvested from male inflorescences located at least 3 m away from the plants to be fertilised.

Fruit and seed set were counted on these pollinated infructescences. Differences in fructification rates in female and bisexual infructescences and in naturally and hand-pollinated infructescences were analysed by Chi-square tests (Systat 8 2004). Differences in fruit and seed number (*i.e.* count data) in female and bisexual infructescences, and in natural and hand-pollinated infructescences were analysed by ANOVA after square-root transformation of the data (Systat 8 2004).

## RESULTS

### Population descriptions

The frequencies of plant gender (male, female and bisexual) were significantly different between the two populations studied ($\chi^2_2 = 41.67$; $P < 10^{-5}$) and the sex ratio of plants was more or less male-biased (54.6% in Angell Woods and 70.5% at Morgan Arboretum). The Angell Woods population had proportionally twice as many female plants and less male plants than the Morgan Arboretum population. Moreover, the average number of male flowers on male inflorescences (41.9 ± 9.2) was lower than the average number of female flowers on female inflorescences (72.2 ± 16.9).

Bisexual inflorescences were common (about 13%) in both populations and at comparable frequencies ($\chi^2_1 = 0.5$; P = 0.48). Two different types of bisexual inflorescence were observed. The 'female bisexual' plants (BF) exhibited far more female flowers (57.5 ± 12.8) than male flowers (3.9 ± 2.1) and never released pollen. In comparison to BF, 'male bisexual' plants (BM) presented more male flowers (19.8 ± 12.5) that released pollen, and fewer female flowers (38.5 ± 14.2). The BF type was most frequent in both populations, representing about 80% of bisexual inflorescences. The frequencies of the two types of bisexual inflorescence were not statistically different between the two populations ($\chi^2_1 = 0.68$; P = 0.41).

### Insect visitation patterns

*Diversity of insect visitors*

The 355 insect visitors collected from both sites represented nine orders. The most diverse orders in terms of families were Diptera (eight) and Coleoptera (six). The most diverse order, Diptera, was represented by 15 genera. In Angell Woods, in both 2005 and 2006, Mycetophilidae, Cecidomyiidae, Chironomidae and Thysanoptera (Heterothripidae) were most frequent. Together they represented 72% of insect visitors in 2005 and 82% in 2006 (Table 1). At the Morgan Arboretum, in 2006, Mycetophilidae, Sciaridae and Chironomidae were most common and represented 76% of the total number of insect visitors (Table 1).

*Diversity of Diptera*

In terms of genera, the most diverse family of Diptera was the Mycetophilidae, with seven genera in Angell Woods and three genera at the Morgan Arboretum (Table 2). The other families were generally represented by only one genus and rarely by two (Table 2).

*Diptera abundance*

In terms of specimen numbers, *Docosia* (Mycetophilidae) was the most abundant genus in both years and both populations (Table 2). The genera *Exechia*, *Mycetophila* (Mycetophilidae), *Parallelodiplosis* (Cecidomyiidae) as well as the thrip, *Heterothrips arisaema* were also abundant in Angell Woods in both years, whereas the genus *Bradysia* (Sciaridae) with an unidentified Chironomini were the second and third most abundant Diptera at Morgan Arboretum in 2006 (Table 2).

*Insect abundance*

Insect visits were rare overall. In Angell Woods, the 147 insects collected in 2005 were the result of 8112 inflorescence surveys, and in 2006, the 125 insects collected were from 2109 surveys. At the Morgan Arboretum in 2006, 83 insects were collected after 1865 surveys.

When only considering the visited inflorescences, the mean number of insects for the flowering period (10–12 days) in 2005 was similarly low for male (1.6 ± 0.8), female (1.5 ± 0.8) and bisexual (1.4 ± 0.7) inflorescences. In fact, in Angell Woods in 2005, most inflorescences (63% male, 72% female and 80% bisexual) were not visited during their entire flowering cycle. Male inflorescences had a greater chance (38%) of being visited than female or bisexual inflorescences (25%) ($\chi^2_1 = 4.96$; P = 0.026). Whatever the gender, most visited inflorescences (60–70%) were visited only once during their flowering cycle, some twice (20–30%) and very few three times (10%).

In Angell Woods in 2005, the visitation pattern of inflorescences, in terms of numbers of insects collected at different times of the day, varied according to the time of day. Inflorescences tended to be visited more in the morning (45%) or around noon (34%) than at the end of the day (21%), and these differences were significant ($\chi^2_2 = 11.84$; P = 0.003). This visitation pattern did not differ for the three types of inflorescence gender ($\chi^2_4 = 5.18$; P = 0.27). In 2006, the visitation pattern of inflorescences did not differ between the two populations ($\chi^2_1 = 1.01$; P = 0.31).

*Pollen loads*

It is important to note that all insects collected for pollen load counts in 2006 only carried pollen of *A. triphyllum* (Fig. 1A and B). Pollen loads varied significantly among different insects visiting *A. triphyllum* ($\chi^2_{19} = 77.9$; $P < 10^{-7}$) but not between sites ($\chi^2_1 = 0.14$; P = 0.71) or for the interaction sites * taxa ($\chi^2_{14} = 13.7$; P = 0.47). We therefore retained the simplified model, with species grouped into four distinct classes of pollen loads ($\chi^2_3 = 55.7$; $P < 10^{-7}$). These classes were arbitrarily referred as: no, low, medium and large

**Table 1.** Insect visitor abundances.

| | | Angell Woods | | | | Morgan Arboretum | |
|---|---|---|---|---|---|---|---|
| | | 2005 | | 2006 | | 2006 | |
| order | family | total | frequency | total | frequency | total | frequency |
| Diptera | | | | | | | |
| | Mycetophilidae | **42** | **28.57** | **64** | **51.2** | **33** | **39.76** |
| | Sciaridae | 3 | 2.04 | 3 | 2.4 | **17** | **20.48** |
| | Cecidomyiidae | **36** | **24.49** | **14** | **11.2** | 2 | 2.41 |
| | Chironomidae | **9** | **6.12** | **14** | **11.2** | **13** | **15.66** |
| | Empididae | 1 | 0.68 | 0 | 0 | 0 | 0 |
| | Tipulidae | 0 | 0 | 1 | 0.8 | 2 | 2.41 |
| | Culicidae | 0 | 0 | 5 | 4 | 1 | 1.21 |
| | Chaoboridae | 2 | 1.36 | 4 | 3.2 | 0 | 0 |
| (Brachycera) | | 1 | 0.68 | 0 | 0 | 0 | 0 |
| Collembola | | 2 | 1.36 | 1 | 0.8 | 1 | 1.21 |
| Hemiptera | | 5 | 3.4 | 1 | 0.8 | 0 | 0 |
| Hemiptera (larvae) | | 3 | 2.04 | 2 | 1.6 | 7 | 8.43 |
| Thysanoptera | | | | | | | |
| | Heterothripidae | **19** | **12.93** | **10** | **8** | 1 | 1.21 |
| Coleoptera | | | | | | | |
| | Lathridiidae | 15 | 10.2 | 3 | 2.4 | 0 | 0 |
| | Staphylinidae | 0 | 0 | 1 | 0.8 | 0 | 0 |
| | Chrysomelidae | 0 | 0 | 0 | 0 | 1 | 1.21 |
| | Coccinellidae | 3 | 2.04 | 0 | 0 | 1 | 1.21 |
| | Tenebrionidae | 0 | 0 | 0 | 0 | 2 | 2.41 |
| | Erotylidae | 1 | 0.68 | 0 | 0 | 0 | 0 |
| Homoptera | Aphididae | 1 | 0.68 | 0 | 0 | 0 | 0 |
| Psocoptera | | 1 | 0.68 | 0 | 0 | 0 | 0 |
| Neuroptera | | 1 | 0.68 | 0 | 0 | 0 | 0 |
| Hymenoptera | | | | | | | |
| | unidentified | 2 | 1.36 | 1 | 0.8 | 1 | 1.21 |
| | Ichneumonidae | 0 | 0 | 0 | 0 | 1 | 1.21 |
| | Apoidea | 0 | 0 | 1 | 0.8 | 0 | 0 |
| | total | 147 | 100 | 125 | 100 | 83 | 100 |

The total number (total) and frequency (%) of insect specimens per family collected in the spathes of *Arisaema triphyllum* in Angell Woods (2005–2006) and at the Morgan Arboretum (2006) (Montréal, Canada). The most abundant families are in bold type.

pollen loads relative to the mean number of pollen grains carried (Fig. 2). This simplified model was not significantly different from the full model ($\Delta\chi^2_{16} = 22.2$; P = 0.13).

Among the most frequent visitors in 2006, the genus *Docosia* (Fig. 1A) (Mycetophilidae), with 61 specimens, was most abundant in both populations and fell into the 'large pollen load' class (61 ± 68 pollen grains; Fig. 2). The second most common genus, *Mycetophila* (Mycetophilidae), with 11 specimens, also fell into the 'large pollen load' class (90 ± 91 pollen grains; Fig. 2). These two genera can be considered as efficient pollinators. The other species may be considered as occasional pollinators because their abundance and/or pollen load was low (Table 1; Fig. 2). For example, *Heterothrips arisaema* was frequent in Angell Woods (10 specimens), but rare at the Morgan Arboretum (only one specimen) and fell into the 'low pollen load' class (9 ± 11 pollen grains; Fig. 2). Other species included in the 'large pollen load' class (*Rymosia*, Tipulidae and Hymenoptera) were rare in our sampling (one, three and four insects, respectively).

## Reproductive success

### *Natural pollination*

In Angell Woods, the fructification rate did not differ in female and bisexual female (BF) infructescences in 2004 and 2005 (respectively: $\chi^2_1 = 0.34$; P = 0.56 and $\chi^2_1 = 2.7$; P = 0.10). However, in 2006, the fructification rate for BF plants was abnormally high (67%) compared to other years and to female plants (28%). Therefore, in Angell Woods, a difference in fructification rate in BF plants among years (2004–2006) was found ($\chi^2_2 = 43.98$; $P < 10^{-6}$). At the Morgan Arboretum in 2006, fructification rate in female and BF plants did not differ ($\chi^2_1 = 0.89$; P = 0.35). In 2006, the fructification rate in female plants did not differ between the two populations

**Table 2.** Diptera and Thysanoptera visitor abundances.

| order | family | subfamily/tribe/genera/species | Angell Woods 2005 | Angell Woods 2006 | Morgan Arboretum 2006 | total |
|---|---|---|---|---|---|---|
| Diptera | | | | | | |
| | Mycetophilidae | | | | | |
| | | *Brevicornu* | 1 | 7 | 2 | 10 |
| | | *Docosia* | **26** | **34** | **27** | **87** |
| | | *Exechia* | **7** | **8** | **0** | **15** |
| | | *Exechiopsis* | 1 | 0 | 0 | 1 |
| | | *Mycetophila* | **5** | **7** | **4** | **16** |
| | | *Phronia* | 1 | 2 | 0 | 3 |
| | | *Rymosia* | 0 | 1 | 0 | 1 |
| | | *Trichonta* | 1 | 1 | 0 | 2 |
| | Sciaridae | | | | | |
| | | *Bradysia* | **3** | **2** | **16** | **21** |
| | | *Scatopsciara* | 0 | 1 | 0 | 1 |
| | Cecidomyiidae | | | | | |
| | | *Caryomyia* | 3 | 0 | 0 | 3 |
| | | *Parallelodiplosis* | **33** | **11** | **1** | **45** |
| | Chironomidae | | | | | |
| | | Chironomini (tribe) | **6** | **5** | **9** | **20** |
| | | Orthocladiinae (subfamily) | 3 | 3 | 0 | 6 |
| | Chaoboridae | | | | | |
| | | *Chaoborus* | 2 | 3 | 0 | 5 |
| Thysanoptera | | | | | | |
| | Heterothripidae | | | | | |
| | | *Heterothrips arisaema* | **19** | **10** | **1** | **30** |
| | | total | 111 | 95 | 60 | 266 |

The total number of specimens in each genus of Diptera and in the Thysanoptera collected in the spathes of *Arisaema triphyllum* in Angell Woods (2005–2006) and at the Morgan Arboretum (2006) (Montréal, Canada). The most abundant genera are in bold type.

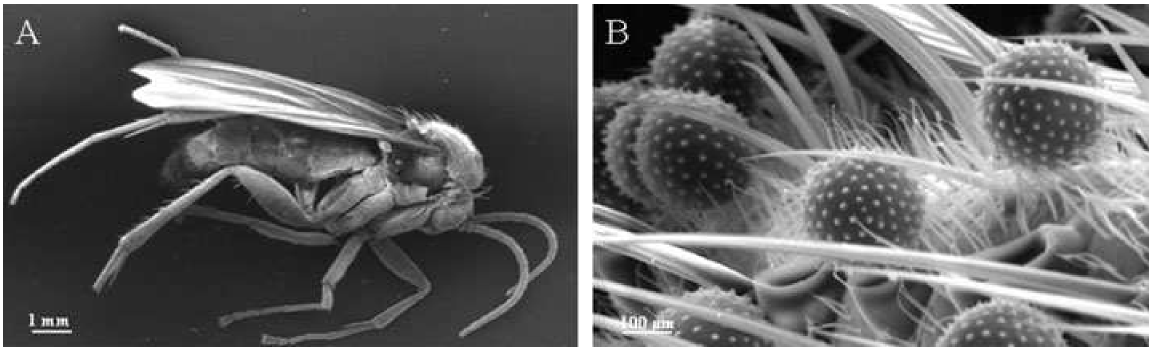


**Fig. 1.** A: General view of *Docosia* sp. (Mycetophilidae). B: Pollen grains of *Arisaema triphyllum* between thoracic hairs of *Docosia* sp. (Mycetophilidae).

studied ($\chi^2_1 = 0.29$; $P = 0.59$), while the higher fructification rate in BF plants in Angell Woods caused a significant difference between the two populations ($\chi^2_1 = 15.88$; $P < 10^{-4}$).

In Angell Woods in 2004–2006, the number of berries varied statistically in female infructescences among years ($F_{2,\ 52} = 3.26$; $P = 0.046$). Berry production in 2004 and 2005 was not statistically different ($P = 1.00$) or in 2004 and 2006 ($P = 0.51$). However, there was a difference between 2005 and 2006 ($P = 0.048$), with a higher mean of 28.7 (±19.8) berries per inflorescence in 2006 relative to 15.8 (±9.0) berries in 2005 (Table 3).

Similar results were found for seed production in Angell Woods in 2004–2006; the number of seeds varied statistically in female infructescences among years

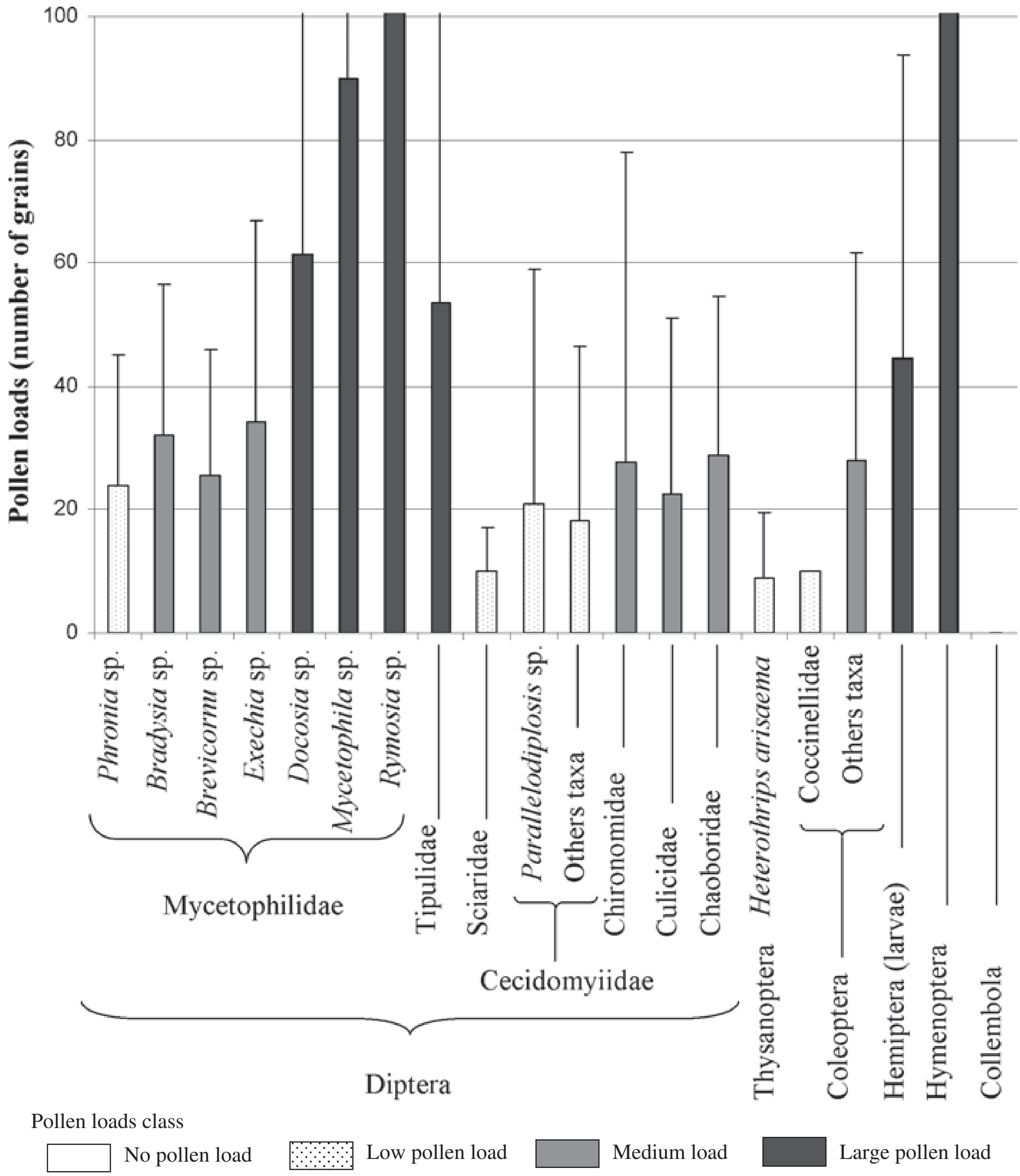


**Fig. 2.** Pollen loads (number of grains) counted on insects collected in spathes of *Arisaema triphyllum* in 2006 from both sites. Four classes of pollen load were distinguished and are referred to as: (i) no pollen, (ii) low (1–24 pollen grains), (iii) medium (25–35 pollen grains), and (iv) large (45 or more pollen grains). Bars represent standard deviations. Non-pollinators were only represented by Collembola.

($F_{2,52}$ = 3.86; P = 0.027). The years 2004 and 2005 were not statistically different (P = 1.00), nor were 2004 and 2006 (P = 0.40). However, there was a difference between 2005 and 2006 (P = 0.027), with a mean of 30 (±21.1) seeds per inflorescence in 2006 in comparison to 26.9 (±22.4) in 2005 (Table 3).

Unlike female plants, the numbers of berries ($F_{2,33}$ = 0.36; P = 0.7) and seeds ($F_{2,33}$ = 0.12; P = 0.89) were not statistically different in BF infructescences among years (2004–2006) in Angell Woods. When comparing female reproductive success between the two studied populations in 2006, no significant difference was found in the number of berries and seeds on female plants (respectively: $F_{1,44}$ = 2.97; P = 0.09 and $F_{1,44}$ = 3.90; P = 0.06) or on BF plants (berries: $F_{1,33}$ = 0.42; P = 0.53; seeds: $F_{1,33}$ = 0.45; P = 0.51).

**Table 3.** Reproductive success in unmanipulated inflorescences.

| | | number of inflorescence | | mean number of berries per infructescence ± SD | | mean number of seeds per infructescence ± SD | |
|---|---|---|---|---|---|---|---|
| study site | year | F | BF | F | BF | F | BF |
| Angell Woods | 2004 | 7 | 10 | 25.3 ± 18.7 | 20.8 ± 18.1 | 33.7 ± 25.9 | 30.1 ± 30.0 |
| | 2005 | 7 | 19 | 15.6 ± 9.0 | 15.8 ± 14.5 | 26.9 ± 22.4 | 21.6 ± 22.3 |
| | 2006 | 30 | 22 | 30.0 ± 21.1 | 25.3 ± 20.0 | 21.1 ± 39.7 | 36.6 ± 32.7 |
| Morgan Arboretum | 2006 | 20 | 13 | 40.6 ± 20.4 | 31.4 ± 24.9 | 73.5 ± 48.5 | 49.3 ± 48.0 |

Mean number of berries and seeds per female (F) and bisexual female (BF) infructescences in Angell Woods (2004–2006) and Morgan Arboretum (2006). Bisexual male (BM) inflorescences were studied only in 2006, none of which produced fruits.

*Spontaneous self-pollination and/or apomixis*

In Angell Woods in 2005, the 34 inflorescences (14 females and 20 BF) bagged for the entire duration of their flowering cycle did not produce fruits, indicating that there was no self-pollination or agamospermy.

*Hand-pollination*

In hand-pollination experiments, neither of the two bisexual male (BM) plants produced fruit. Fructification rates between female (n = 20) and BF (n = 8) infructescences in hand-pollinated plants were not statistically different at the Morgan Arboretum ($\chi^2_1$ = 1.86; P = 0.17), but were significantly different in Angell Woods ($\chi^2_1$ = 3.86; P = 0.05) because none of the hand-pollinated BF plants (n = 5) produced fruit in comparison to female infructescences (n = 21). Comparisons of fructification rates in 2006 between hand- and natural pollinations gave varied results. At the Morgan Arboretum, fructification rates in female plants were significantly lower after natural pollination (32%) than after hand-pollination (80%) ($\chi^2_1$ = 13.99; $P < 10^{-3}$) but no significant differences were observed in Angell Woods ($\chi^2_1$ = 1.745; P = 0.186). In Angell Woods, all the hand-pollinated BF plants (5) failed to produce berries or seeds, whereas at the Morgan Arboretum, the difference between natural and hand-pollinated BF plants (n = 8) was marginal ($\chi^2_1$ = 3.685; P = 0.055).

The numbers of berries ($F_{1,67}$ = 0.02; P = 0.89) and seeds ($F_{1,67}$ = 0.001; P = 0.98) did not differ significantly between the two populations in 2006, nor between natural and hand-pollinated female inflorescences within each population.

## DISCUSSION

*Arisaema triphyllum* seems to receive few insect visits. From the 12,086 inflorescence surveys made among 437 plants for 2 years and 2 populations, we collected only 355 insect visitors. More than 60% of inflorescences were never visited by insects for the duration of their flowering cycle. The mean number of insects for the entire flowering period was only 1.5 per inflorescence for the plants that were visited. In *Arisaema serratum*, the number of insect visitors was higher, since 42 insects trapped in six female inflorescences were collected by the end of pollination in a study conducted in Japan (Nishizawa *et al.* 2005). Vogel & Marten (2000) collected insects in spathes of *Arisaema* species in Nepal and found up to nine specimens in one spathe of *A. nepenthoides* and up to 20 in *A. jacquemontii*. The same holds true for other genera of tribe Areae with deceptive pollination systems. For example, 455 insects were collected in 142 inflorescences of *Arum italicum* in the south of France (Albre *et al.* 2003) and 301 Diptera were collected from 15 inflorescences of *Helicodiceros muscivorus* in Corsica (Seymour *et al.* 2003). Therefore *A. triphyllum* appears to possess a relatively inefficient trap mechanism.

In our study, all insect visitors collected in spathes of both sexes carried only *A. triphyllum* pollen and were thus potential pollinators, but Mycetophilidae (fungus gnats) appeared to be the most efficient pollinator, as suggested by Treiber (1980). Among the Mycetophilidae, the best pollinator candidates are the genera *Docosia* and *Mycetophila*, being the most frequently represented in both gender inflorescences of both populations of *A. triphyllum* in all years, carrying an average of 61 and 90 pollen grains, respectively, per individual. The pollinators of the two other North American *Arisaema* species have not been studied, but we identified the same genera of pollinating insects (fungus gnats) for *A. triphyllum* as observed for other European and Asian species (Sasakawa 1994; Vogel & Marten 2000; Nishizawa *et al.* 2005). Despite sharing similar pollinators (fungus gnats), fructification rate and fruit production are higher in female plants in *A. serratum* (Nishizawa *et al.* 2005) than in *A. triphyllum*. This result may be due to the low number of insect visitors to *A. triphyllum*.

The thrip *Heterothrips arisaema* was relatively abundant in Angell Woods (10 specimens) but rare at the Morgan Arboretum (only one specimen); on both sites they carried few pollen grains (*i.e.* nine grains). It seems clear that *H. arisaema* did not act as an efficient insect pollinator in the two studied populations of *A. triphyllum*. These results contradict the findings of Rust (1980) and Feller *et al.* (2002), who found this thrip species to be the main pollinator of *A. triphyllum*. The two populations of our study are located at more northerly latitudes (Canada, Québec, Montréal Island; 45°26′69″ N) compared to the study sites of Rust

(1980) (US, Delaware, University campus; 39°39′56″ N) and Feller *et al.* (2002) [US, Maryland, (SERC) Edgewater; 38°59′ N]. Cold spring temperatures in northern latitudes could decrease the size of thrip populations or slow down their life cycle, explaining the lower number of *H. arisaema* sampled during May in the two Canadian populations (Kirk 1997; Mound 1997). It is interesting to note that Rust (1980) found Mycetophilidae to be the second most abundant insect visitor, while Feller *et al.* (2002) collected unidentified fungus gnats that carried pollen of *A. triphyllum*.

The reproductive success of bisexual plants in *A. triphyllum* has rarely been studied; in fact, only Treiber (1980) has presented data on their natural fruit production. Bisexual plants represented 13% of each population studied. In most other studies on *A. triphyllum*, bisexual plants were absent or rare (1–5%) and were not included (Rust 1980; Bierzychudek 1981; Policansky 1981). We confirmed that female plants of *A. triphyllum* do not reproduce by agamospermy and we showed experimentally that BF plants are unable to self-pollinate (Treiber 1980). Moreover, BF and female plants were shown to have a similar natural fructification rate, except in 2006 in Angell Woods, where BF plants had an exceptionally high fructification rate. However, an increase in fructification rate does not guarantee higher fruit production because the numbers of berries and seeds produced in BF plants did not differ among years in Angell Woods. Similar results were found for fruit production (berries and seeds), suggesting that reproductive success in female and BF plants is comparable. Surprisingly, there was no difference among female plants in terms of the number of berries and seeds, regardless of population location and pollination method. This differs from Bierzychudek's (1982) results, which showed a significant difference between natural and hand-pollinated female inflorescences and between two populations in terms of production of berries and seeds. Lovett Doust *et al.* (1986) also reported that berry and seed production in female plants of *A. triphyllum* varies. The higher mean number of berries and seeds in naturally pollinated inflorescences observed in the two populations we studied could mask the effect of hand-pollination and might explain the difference between our results and those of Bierzychudek (1982). Such inter-annual variability in reproductive success might be due to changes in environmental conditions and/or pollination context, as proposed in previous studies on *A. triphyllum* (Treiber 1980; Bierzychudek 1984) or Asian *Arisaema* species (Kinoshita 1986, 1987; Nishizawa *et al.* 2005). Since the two populations studied here had the same insect pollinators during the 2 years of observation, in particular *Docosia* and *Mycetophila* spp., and were both situated on Montreal Island, inter-annual climatic variations might be responsible for the observed differences in reproductive success among years.

According to our results, bisexual plants show two types of reproduction, *i.e.* 'female reproduction' in BF plants that produce fruit and never release fertile pollen (Treiber 1980; Bierzychudek 1982) and 'male reproduction' in BM plants where viable pollen is shed (Treiber 1980) and no seeds are produced. In fact, *A. triphyllum* is a true dioecious plant, as each plant reproduces only through either the male or the female function.

This study reports pollination ecology and reproductive success in *A. triphyllum*, specifically for the two northernmost populations ever studied. We documented a low number of insect visits and identified Mycetophilidae, especially the genus *Docosia*, as the main pollinating insect of *A. triphyllum*. Bisexual female (BF) plants are functionally similar to female plants as regards visitation rate by insects, natural and hand-pollination fructification rate and production of berries and seeds. Further pollination studies are needed to examine the two other American *Arisaema* species in order to better understand pollination context and reproductive success in North American *Arisaema*.

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