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# Back from the dead: A fungus gnat pollinator turns *Arisaema* lethal trap into nursery

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## Societal Impact Statement

The research explores the complex interaction between flowering plants and their pollinators, specifically focusing on the genus *Arisaema*. Unlike most plants, *Arisaema* has a distinctive trait in which killing pollinators can be beneficial. Traditionally, this interaction has been viewed as highly antagonistic because it appears to favor the plants at the expense of the pollinators. However, new evidence reveals that a pollinator uses the lethal floral trap of *Arisaema thunbergii* as a nursery. Remarkably, some individuals probably even escape from the trap after laying eggs. This finding challenges the prevailing notion that deceptive pollination is the sole outcome in *Arisaema*, a genus known for its intricate lethal pollination mechanisms.

## Summary

- While many flowering plants engage in mutualistic relationships with their pollinators, flower-insect interactions are not always mutually beneficial. Some plants exploit their pollinators by deceptively promising rewards without delivering them. Among such plants, the genus *Arisaema* (Araceae) is notable for its antagonistic behavior towards pollinators, as female plants invariably entrap them with lethal effects.
- Here we tested the hypothesis that the succulent appendix of some *Arisaema* species, such as *Arisaema thunbergii*, may serve as a food source for pollinator larvae, leading to the possibility of nursery pollination. To investigate this, we retrieved adult insect corpses trapped within the spathes of *A. thunbergii*. Subsequently, we incubated the spathes after collecting the corpses to determine if conspecific insects emerged from them.
- We present novel evidence that one of the main pollinating fungus gnats, *Leia ishitanii*, uses the lethal floral trap of *A. thunbergii* inflorescences as a nursery. Astonishingly, some *L. ishitanii* individuals may even succeed in escaping from the female spathe after depositing their eggs.
- These findings challenge the conventional understanding that deceptive pollination is the norm within the genus *Arisaema*. The interaction between *A. thunbergii* and *L. ishitanii*, in which a significant portion of female adults die inside but still

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reproduction seems successful, suggests an intermediate stage between brood-site deception and nursery pollination mutualism.

#### KEYWORDS

brood pollination, deceptive pollination, lethal kettle trap, mutualism–parasitism continuum, nursery pollination, trap flower

## 1 | INTRODUCTION

Most flowering plants depend on animal pollinators for reproduction, and many animal pollinators rely on floral resources (Ollerton et al., 2011). Despite this, flower–insect interactions are not always mutualistic, including those exclusively benefitting floral visitors (e.g., via reward-theft) and those exclusively benefitting plants (e.g., via deception), these two extremes being separated by a range of balanced interactions (mutualism) (Johnson & Schiestl, 2016; van der Kooi et al., 2021). Approximately 4%–6% of flowering plants employ deception in their pollination strategies by advertising rewards without actually providing them (Jersáková et al., 2006). Deceptive pollination systems encompass various mechanisms, including mimicking food sources, potential mates, or brood sites (Johnson & Schiestl, 2016). This mimicry involves structural, visual, and chemical signals imitating rewarding flowers, pheromones, fermented fruit, carrion, and even fungi (Brodmann et al., 2008; Goodrich & Jürgens, 2018; Martos et al., 2015; Oelschlägel et al., 2015; Policha et al., 2016).

*Arisaema* (Araceae), a genus comprising approximately 200 species, is renowned for its unique pollination system that permanently ensnares pollinators, primarily fungus gnats belonging to the family Mycetophilidae and Sciaridae (Matsumoto et al., 2021; Suetsugu, 2022; Suetsugu et al., 2021; Vogel & Martens, 2000). Historically, mushroom mimicry, or brood-site mimicry, has been perceived as the main pollination strategy for *Arisaema*, because the majority of species are pollinated by fungus gnats that are typically mycophagous during their larval stages (Vogel & Martens, 2000). Yet the lack of mushroom-like scents and structures in many *Arisaema* species has challenged the prevalence of this strategy (Suetsugu, 2022; Suetsugu et al., 2021). Suetsugu et al. (2021) suggested that sexual deception could be more widespread, especially because some *Arisaema* species predominantly attract male fungus gnats, which may indicate the emission of pheromones that mimic female gnats. Nevertheless, there are notable exceptions, such as *Arisaema sikokianum*, in which the sterile appendix exhibits remarkable fungal mimicry, closely resembling both the appearance and scent of mushrooms (Kakishima et al., 2019; Matsumoto et al., 2019).

*Arisaema* plants nearly always produce unisexual inflorescences, and their sexual expression is contingent upon size: Smaller plants produce male inflorescences while larger ones produce female inflorescences (Takasu, 1987; Vogel & Martens, 2000). An *Arisaema* inflorescence consists of a spathe, a spadix appendix, and flowers (Vogel & Martens, 2000). Floral visitors, attracted by the scent emanating from

the spadix appendix, enter the spathe (Suetsugu, 2022; Vogel & Martens, 2000). Once inside, the trapped insects are treated differently by the male and female plant morphs. Within male inflorescences, pollinators accumulate pollen grains while attempting to break free and eventually exit through a small opening at the base of the spathe. However, female inflorescences lack the exit hole in the spathe, causing the trapped pollinators to remain confined until their demise (Figure S1) (Suetsugu, 2022; Vogel & Martens, 2000). The lack of the exit in the female morph, extending pollinator retention time, likely enhances the likelihood of pollen deposition on the stigma (Vogel & Martens, 2000).

Several deceptive plants, including *Helicodiceros*, *Arum* (Araceae), *Aristolochia* (Aristolochiaceae), and *Ceropegia* (Apocynaceae), possess hermaphroditic flowers that are protogynous (the female stigmas mature before the male anthers) and temporarily trap fly pollinators. However, once their pollen grains are shed, they release pollinators so that pollen grains are carried among plants (Oelschlägel et al., 2015; Ollerton et al., 2009; Vogel & Martens, 2000). Some species in the family Araceae, such as *Helicodiceros muscivorus*, possess trap chambers so overcrowded that flies may suffocate; but this is detrimental to the plants as it reduces male reproductive success (Vogel & Martens, 2000). Therefore, *Arisaema* is unique in increasing reproductive success by permanently retaining its pollinators to maximize the chances of pollen deposition within female-morph inflorescence (Vogel & Martens, 2000). However, the succulent appendix of some *Arisaema* species, such as *Amaranthus thunbergii*, may serve as a food source for pollinator larvae, leading to the possibility of nursery pollination (i.e., brood-site pollination). This idea is supported by observations in other plant species, such as *Aspidistra longipetala* (Asparagaceae) and *Gastrodia foetida* (Orchidaceae), in which decaying succulent floral tissues provide nourishment for larvae of their brood pollinators (Lin et al., 2022; Suetsugu, 2023).

In nursery pollination, plants offer brood sites as a reward for pollination (Hembry & Althoff, 2016; Sakai, 2002a). While fungus gnat larvae primarily feed on fungi, nursery pollination has been confirmed in *Rheum nobile* (Polygonaceae), in which larvae of a *Bradysia* species (Sciaridae) consume seeds (Song et al., 2014), and in *A. longipetala* (Asparagaceae), in which larvae of another *Bradysia* species feed on decaying floral tissues (Lin et al., 2022). Although mutualisms in which plants sacrifice a subset of ovules or developing seeds in exchange for pollination are classic examples of nursery pollination (Hembry & Althoff, 2016; Herre et al., 2008; Kato et al., 2003), several plant groups are pollinated by insects that breed on decaying floral tissues (Kawakita & Kato, 2002; Lin et al., 2022; Sakai, 2002b, 2002a). This

form of mutualism is likely to be more prevalent because it imposes fewer costs on the plants than mutualisms in which plants sacrifice ovules or developing seeds (Sakai, 2002a). Here we focus on *Arisaema thunbergii*, aiming to investigate the potential for nursery pollination facilitated by rewards in the form of decaying floral tissues. This exploration contributes to our understanding of the spectrum of plant-pollinator interactions, spanning from brood-site mimicry to nursery pollination mutualism.

## 2 | MATERIAL AND METHODS

### 2.1 | Study species and sites

*Arisaema thunbergii* is commonly found in the dark, shaded understory of evergreen broad-leaved forests in western Japan (Murata et al., 2018). Similar to other *Arisaema* species, *A. thunbergii* employs a pollination system that effectively traps pollinators within the female spathe (Figure S1) (Suetsugu, 2022; Vogel & Martens, 2000). The sterile appendix of *A. thunbergii* is characterized by its thick, stout base that gradually tapers above the helmet, forming a flaccid thread that curves downward, resembling a fishing rod (Takasu, 1987). Notably, the swollen base of the appendix emits a subtle, musty scent detectable by humans. This unique structure, combined with its mushroom-like appearance and succulent nature, makes it a potential food source for larval development. Consequently, *A. thunbergii* presents an ideal opportunity to explore the concept of brood site pollination within the genus *Arisaema*.

The field sampling of *A. thunbergii* was conducted at a location on Yakushima Island, which is part of Kagoshima Prefecture in southern Japan. The specific coordinates were 30°26' N latitude and 30°30' E longitude. This sampling was carried out annually from late February to mid-March, covering the years 2021 through 2023. This extended sampling period is invaluable for gaining a comprehensive understanding of *A. thunbergii* pollination ecology.

### 2.2 | Pollinator assemblages

Deceptive plants often experience extremely low pollinator visitation, making direct observation of pollinator behavior challenging (Widmer et al., 2000). Therefore, we adopted an approach to assess the pollinator status, drawing from previous research on *Arisaema* pollination ecology (Kakishima et al., 2019; Matsumoto et al., 2019, 2021; Suetsugu et al., 2021, 2022; Vogel & Martens, 2000). Our methodology involved examining the pollinator assemblages of *A. thunbergii* by collecting floral visitors trapped inside 62 spathes, comprising 39 male and 23 female ones.

For the 39 male inflorescences, we sealed the exit hole of the spathes with cotton to prevent attracted insects from escaping. In contrast, the 23 female inflorescences, which inherently lacked the exit hole in the spathe, were left intact. Inflorescences were collected at the end of the flowering season. We note that while insect

samples trapped in female spathes were examined for pollen grain attachment (see later), the presence or absence of pollen grains might not necessarily yield absolute results on their pollinator status. This uncertainty arises from factors such as effective pollinators shedding a large portion of pollen grains while roaming within spathes until their demise. Additionally, the potential for pollen transfer among insect individuals within the trap complicates interpretations (Kubo et al., 2023). Therefore, to establish a clear definition of pollinators, we applied the following criteria to insect species, established from prior research: (i) they exhibited entrapment in both sealed male and female spathes, and (ii) they possessed a size conducive to passing through the exit hole of the male spathe, thereby facilitating pollen transport (Kakishima et al., 2019; Matsumoto et al., 2019, 2021; Suetsugu et al., 2021, 2022; Vogel & Martens, 2000).

The adult insect corpses trapped inside the inflorescences were carefully retrieved, and samples trapped in female spathes were checked for pollen grain attachment on their body using a stereomicroscope (M165C; Leica Microsystems, Cambridge, UK). Subsequently, they were preserved in 99.5% ethanol and identified to the genus level (for Mycetophilidae), family level (for other dipterans), or order level (for other visitors) using the same stereomicroscope based on relevant taxonomic sources (McAlpine et al., 1981; Sasakawa, 1994; Sølvi et al., 2000). Mycetophilid adults, assigned to the genus *Leia* emerged from the decaying *A. thunbergii* inflorescences, were identified to the species level using a stereomicroscope (SZ60; Olympus, Tokyo, Japan) to determine if any trapped and emerged mycetophilid adults of the same species. All voucher specimens were deposited at the Department of Biology, Graduate School of Science, Kobe University, Kobe, Japan.

If necessary, to identify male specimens to the species level, their terminal region of the abdomen, including the genitalia, was detached from the abdomen using sharpened tweezers. Subsequently, they were macerated in heated 10% KOH, neutralized with 3% acetic acid, washed with distilled water, and observed in pure glycerin under a microscope. The genitalia were preserved in microvials with pure glycerin, while the remaining body parts were stored in pure ethanol.

### 2.3 | Insect rearing

The 62 *A. thunbergii* inflorescences (comprising 39 males and 23 females), gathered to assess pollinator assemblages, were preserved in plastic containers with moistened vermiculite to explore the potential for nursery pollination. Additionally, 44 intact male *A. thunbergii* inflorescences, devoid of exit hole sealing treatments, were also gathered and stored under similar conditions in plastic containers. By investigating whether adult insects emerged from these intact male *A. thunbergii* inflorescences, we were able to assess whether potential nursery pollinators utilized male inflorescences as brood sites, even in their normal state without exit hole sealing.

Each inflorescence was covered with an air-permeable polyester mesh to minimize the risk of emerged adult insects escaping. They were incubated at an ambient temperature of 25°C. The emerged adult insects were then identified to the species level, as described above. By wrapping each inflorescence in a mesh and subsequently identifying the species of emerged adult insects, we were able to determine the species of the eggs and larvae found on the spadix and whether the adult insects emerged from inflorescences from which conspecific corpses had been collected.

### 3 | RESULTS AND DISCUSSION

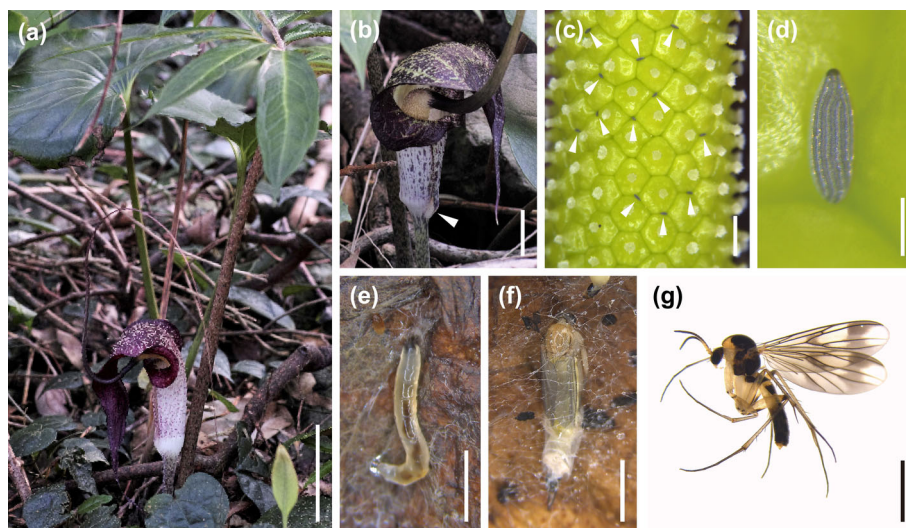
Here, we revealed that *Leia ishitanii*, one of the principal pollinators, employs the lethal floral trap of *Arisaema thunbergii* as a nursery for its offspring (Figure 1). Intriguingly, some individuals of *L. ishitanii* may even manage to escape from the female spathe after laying their eggs. These findings refute the dogma that deceptive pollination is ubiquitous in the genus *Arisaema*.

Upon retrieving adult insects trapped in 62 (39 males and 23 females) spathes, we found that the most numerous trapped visitors were the families Mycetophilidae and Sciaridae, comprising 61.5% and 19.2% of all visitors, respectively (Table S1). Mycetophilidae and Sciaridae are small enough to escape from male inflorescences through the exit hole. Meanwhile, larger insects such as lepidopterans and brachycerans cannot escape and, therefore, cannot pollinate this species. Most *Arisaema* species primarily depend on mycetophilids and sciarids for pollination (Matsumoto et al., 2021; Suetsugu, 2022; Vogel & Martens, 2000); therefore, it is reasonable to assume that this is also true for *A. thunbergii*. Although other fungus gnats in the Mycetophilidae and Sciaridae may also play a role in the pollination process, our observation suggests that *L. ishitanii* is the primary pollinator of *A. thunbergii*. This conclusion is supported by the following evidence: (i) *L. ishitanii* individuals were the most commonly trapped mycetophilids in both sealed male and female spathes, accounting for 37.5% of all mycetophilids (Figure 2), and (ii) *L. ishitanii*

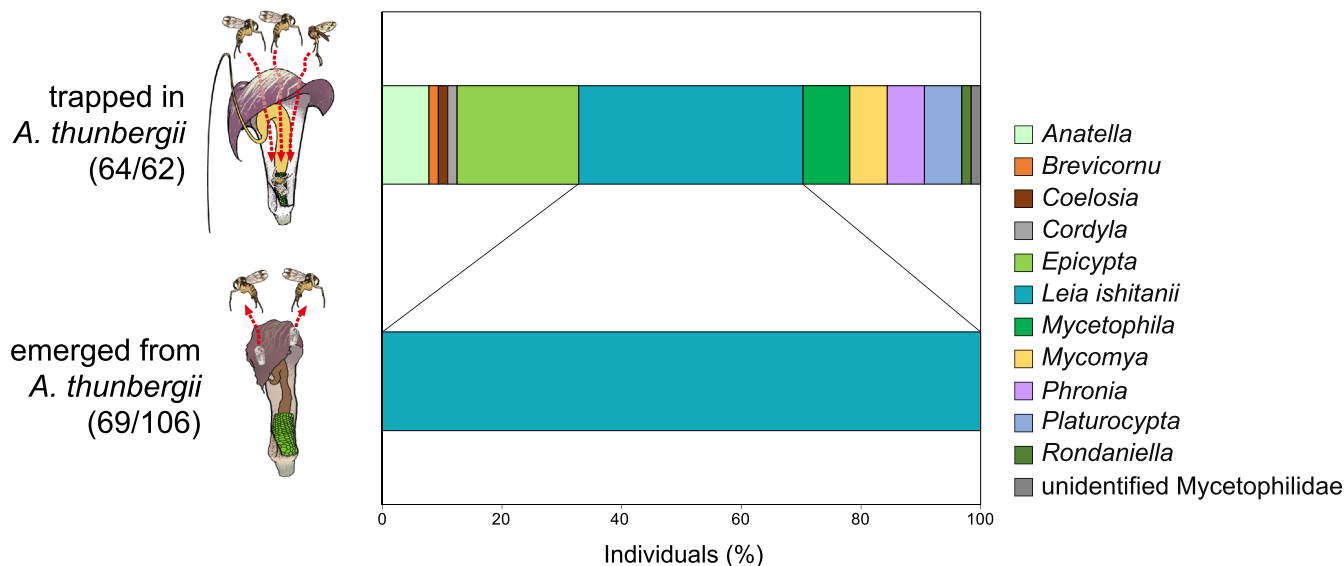
possessed a body size that allowed them to pass through the exit hole of intact male spathes. Notably, *Arisaema* pollen grains were found attached to *L. ishitanii* corpses trapped not only in male but also in female spathes (5 out of 20; Figure S2).

During the retrieval of insect adult corpses from *A. thunbergii*, mycetophilid eggs and larvae were discovered on the spadices of both male and female inflorescences (Figure 2). Although the decomposition rate may not be consistent between indoor and outdoor conditions, the presence of developing larvae of *L. ishitanii* at the time of collection (at the end of the flowering season) suggests that the fungus gnat naturally uses a floral trap as a nursery. Therefore, we further investigated the potential for nursery pollination by incubating *A. thunbergii* inflorescences. Subsequently, we found that the fungus gnat larvae actively feed on the decaying spadix or the mycelia growing on it without destroying the ovules, with their functional mandibles (Movie S1). At the pupal stage, the larvae made cocoons on the decaying spathe. During a 4-week incubation period, a total of 69 adult *L. ishitanii* individuals emerged from at least 15 *A. thunbergii* inflorescences, consisting of 7 sealed male, 3 non-sealed male, and 5 female inflorescences. Notably, up to 23 *L. ishitanii* individuals originated from one sealed male inflorescence. The emergence of *L. ishitanii* from male *A. thunbergii* inflorescences is not solely due to the prolonged entrapment of fungus gnats within the spathes caused by exit hole sealing because *L. ishitanii* used male *A. thunbergii* inflorescences as brood sites even under normal, non-sealed conditions.

Our observations also revealed that certain individuals managed to escape through the upper opening of the spathe after laying eggs (Figure S1). This phenomenon is substantiated by the emergence of 44 offspring of *L. ishitanii* from five male inflorescences with the sealed exit hole and no conspecific corpses (Figure 2; Table 1). Considering that male inflorescences with the sealed exit hole are structurally analogous to female inflorescences, the emergence of conspecific adults in sealed male inflorescences without *L. ishitanii* corpses indirectly suggests that some *L. ishitanii* individuals can escape the female spathe following egg deposition (Figure 3).



**FIGURE 1** Interactions between *Arisaema thunbergii* and its pollinating fungus gnat *Leia ishitanii*. (a) Male *A. thunbergii* plant. (b) Male *A. thunbergii* inflorescence with the spathe and the exit hole (indicated by the arrow). (c) Female flowers with *Leia ishitanii* eggs (indicated by arrows). (d) Close-up of a *L. ishitanii* egg. (e) *L. ishitanii* larva consuming a decaying *A. thunbergii* spadix. (f) *L. ishitanii* adult emerged from its pupa in cocoon on a decaying *A. thunbergii* spathe. (g) *L. ishitanii* adult emerged from *A. thunbergii*. Scale bars: 10 cm (a), 5 cm (b), 2 mm (c,e-g), and 200 μm (d). Photographed by Hiroaki Yamashita (a–b) and Hiroki Nishigaki (c–g).



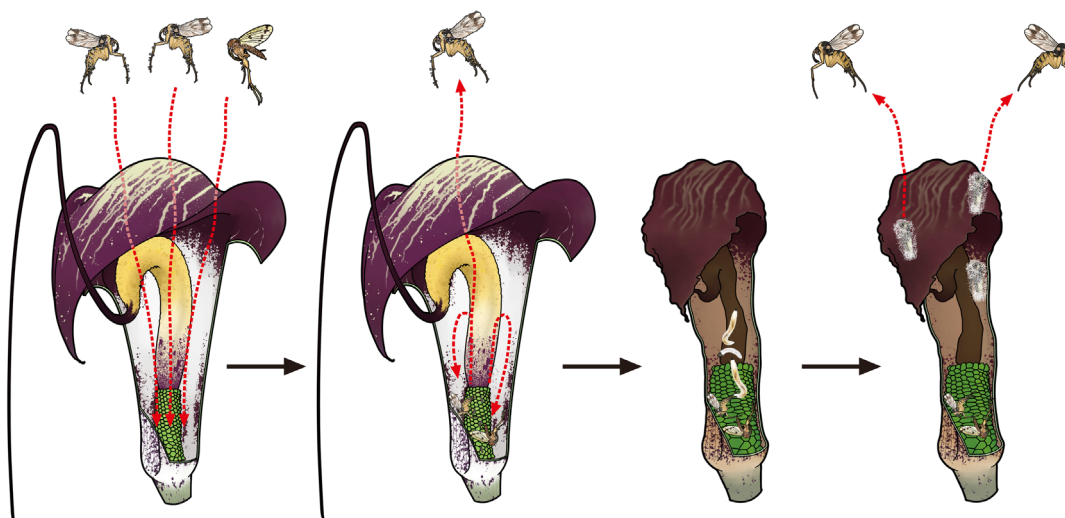
**FIGURE 2** Assemblages of adult mycetophilid fungus gnats trapped by or emerged from *Arisaema thunbergii*. Mycetophilid genera are arranged alphabetically. The number of mycetophilid individuals and inflorescences examined is indicated in parentheses. The red arrows explain insect entrances and exits. Designed by Hiroki Nishigaki & Kenji Suetsugu.

**TABLE 1** The number of *Leia ishitanii* individuals emerging from *Arisaema thunbergii* inflorescences by plant sex and with/without conspecific corpses.

Plant sex	Male				Female	
	Conspecific corpses		No		Yes	No
Exit hole sealing	Yes	No	Yes	No	NA	NA
	3 (2)	0	44 <sup>a</sup> (5)	10 (3)	12 (5)	0

Note: The number of inflorescences *L. ishitanii* emerged from is indicated in parentheses.

<sup>a</sup>Three out of the 44 *L. ishitanii* specimens were retrieved from a container exclusively containing male inflorescences with sealed exit holes, and without any conspecifics, after they had escaped from an air-permeable polyester mesh covering each inflorescence. If these specimens originated from different inflorescences than the five already identified, it would increase the number of inflorescences used as a nursery.



**FIGURE 3** Proposed interaction between female *Arisaema thunbergii* inflorescence and its pollinating fungus gnats. *Leia ishitanii* uses both male and female *Arisaema thunbergii* inflorescences as a nursery, with some individuals possibly escaping even from female inflorescences after laying eggs. By contrast, other fungus gnat species likely do not lay eggs within the inflorescences, ultimately dying without escaping from the females, and without their offspring successfully emerging. The red arrows represent insect entrances and exits, while the black arrows indicate the chronological sequence of events. Designed by Hiroki Nishigaki & Kenji Suetsugu.

To date, all pollination studies on *Arisaema* have focused on an asymmetric interaction, in which pollinators are exclusively deceived into visiting the inflorescences (Suetsugu, 2022; Vogel & Martens, 2000). Nonetheless, we have presented the first evidence that a pollinating fungus gnat uses a lethal floral trap found in *Arisaema* inflorescences as a nursery. We suggest that several factors regarding both pollinator and plant may contribute to the evolution of a nursery pollination system in *A. thunbergii*. From the pollinator perspective, the larvae of the genus *Leia*, which thrive on various decomposing organic substrates, including decaying plant matter (Polevoi & Salmela, 2016; Ševčík, 2010), probably facilitate nursery pollination, given that pollinators exclusively reliant on fungal fruiting bodies cannot reproduce on decaying plant material.

Furthermore, several specific adaptations in *A. thunbergii* probably facilitate this novel pollination system. First, its possible mushroom mimicry status distinguishes it from some other *Arisaema* species, which typically attract male pollinators, possibly via sexual deception (Suetsugu, 2022; Suetsugu et al., 2021). Attracting female pollinators is a prerequisite for the evolution of a nursery pollination system. Secondly, the succulent appendix of *A. thunbergii* may have evolved as a food source for its pollinators. Finally, the abrupt thickening at the base of the appendix, a feature that prevents fungus gnats from escaping from their spathes, present in many *Arisaema* species, is absent in *A. thunbergii*. A nursery pollination system, along with the absence of this structure, might have evolved to offset the high costs for pollinators in some *Arisaema* species.

However, the interaction between *A. thunbergii* and *L. ishitanii* probably still differs from other typical examples of nursery mutualism. Specifically, the corpses of *L. ishitanii*, the only species for which offspring emerged in *A. thunbergii*, were the most frequently found inside *A. thunbergii* spathes (Figure 2). The lethal trapping in the female spathes likely diminishes the fitness of *L. ishitanii* by restricting further egg-laying opportunities, considering that *Leia* species can reproduce not only in *A. thunbergii* but also in fungal fruiting bodies and other decomposing substrates (Polevoi & Salmela, 2016; Ševčík, 2010). In other systems, such as the fig–fig wasp nursery pollination mutualism, female wasps are typically trapped indefinitely within the fig after laying their eggs (Dunn, 2020). This observation suggests that the presence of corpses within the spathes of *A. thunbergii* does not necessarily negate a mutualistic relationship. We currently suggest that the interaction between *A. thunbergii* and *L. ishitanii* likely represents an intermediate stage in the evolution of a nursery pollination mutualism. Further research is needed to determine whether a single oviposition opportunity within one inflorescence of *A. thunbergii* is sufficient for the fitness of *L. ishitanii*.

In summary, our results challenge the prevailing assumption that deceptive pollination is the only strategy in the genus *Arisaema*, which typically employs a sophisticated lethal pollination mechanism. Whether this mechanism is unique, or represents a more widespread but undocumented phenomenon, warrants further investigation. However, we speculate that nursery pollination exists in other *Arisaema* species in which the appendix base does not thicken

abruptly (e.g., the sections *Clavata* and *Flagellarisaema* [that include *A. thunbergii*]) (Murata et al., 2018), assuming that such mimicry systems are not reliant on sexual deception. As natural selection tends to favor cost-effectiveness, the replacement of mutualisms by deceptions may be frequent (Bidartondo, 2005; Bronstein, 2001; Perez-Lamarque et al., 2020). On the other hand, given that deceptive pollination is the dominant strategy in *Arisaema* (Chartier et al., 2014; Vogel & Martens, 2000), our observation probably illuminates an unusual evolutionary process, transitioning from deception to mutualism. Further ancestral state reconstruction analysis, incorporating additional pollination data from other *Arisaema* species, will provide deeper insights into the evolution of nursery pollination in the genus *Arisaema*.

## AUTHOR CONTRIBUTIONS

Kenji Suetsugu and Shinji Sugiura designed the project with the input from Satoshi Kakishima. Kenji Suetsugu and Hiroki Nishigaki collected the samples. Hiroki Nishigaki and Kenji Suetsugu conducted the rearing experiments. Masahiro Sueyoshi, Hiroki Nishigaki, and Kenji Suetsugu identified the insects. Kenji Suetsugu wrote the initial draft. All the authors revised the manuscript and approved the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting information of this article.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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