

1 17 **Abstract**

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5 18 *Arisaema* sect. *Pistillata* (Araceae) is a rapidly diversifying taxon in the Japanese
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9 19 archipelago. Several sympatric *Arisaema* species selectively attract different fungus
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13 20 gnats (Mycetophilidae and Sciaridae), suggesting that frequent pollinator shifts induce
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17 21 adaptive radiation. Since no study has compared pollinator species between sister
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21 22 *Arisaema* species, we examined the floral visitor assemblages of two closely related
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25 23 species (*Ar. nagiense* and *Ar. ovale*) in sympatric, parapatric, and allopatric
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29 24 populations. In all six sites, at the level of genus, male *Anatella* spp. (Mycetophilidae)
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33 25 dominated the floral visitors in both *Arisaema* species, but *Ar. nagiense* and *Ar. ovale*
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37 26 selectively attracted different *Anatella* species. The pollinator shift during the
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41 27 allopatric speciation of the two sister *Arisaema* species suggests the contribution of a
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45 28 species-specific pollination system to the rapid diversification of Japanese *Arisaema*
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49 29 species.

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52 30 **Keywords:** aroids; deceptive pollination; Japanese archipelago; myophily; sexual
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56 31 mimicry; speciation.
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25 39 **Availability of data and material:** The list of all floral visitors is provided in Table
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33 41 **Code availability:** No applicable.
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37 42 **Authors' contributions:** TKM: Study concept and design, field survey, insect
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41 43 identification, manuscript drafting. MS: Insect identification and manuscript
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45 44 drafting. SS: Field survey. YM and MH: Supervision and manuscript drafting.
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1 46 **Introduction**

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5 47 Most flowering plants (~85.7%) depend on animals for pollen exchange between
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9 48 conspecific mates (Ollerton et al. 2011). Their high species diversity appears to be
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13 49 mediated by plant–pollinator interactions because differences in pollinator
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17 50 assemblages can prevent gene flow and/or reproductive interference among diverging
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21 51 lineages, effectively inducing reproductive isolation (Ramsey et al. 2003; Dell’Olivo
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25 52 et al. 2011; Whitehead and Peakall 2014). In particular, species-specific relationships
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29 53 between plants and pollinators may enhance diversification in several plant taxa
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33 54 (Peakall et al. 2010; Moe and Weiblen 2012).

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36 55 The genus *Arisaema* Mart. (Araceae) is mainly distributed across the
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41 56 Northern Hemisphere (Northeast Africa, southern Arabian Peninsula, South India,
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45 57 Himalayas, Southeast and East Asia, and North America) and consists of
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49 58 approximately 180 species, which are subdivided into 15 sections (Murata et al. 2018).
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53 59 Of them, the *Arisaema* section *Pistillata* (Engl.) Nakai is highly diversified in the
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57 60 Japanese archipelago (Murata et al. 2018). Several Japanese *Arisaema* species
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61 61 selectively attract different fungus gnat pollinators (Mycetophilidae and Sciaridae) in

1 62 sympatric situations (Kakishima et al. 2020; Matsumoto et al. 2021; Suetsugu et al.
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5 63 2021), suggesting that frequent pollinator shifts have contributed to rapid
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9 64 diversification and **species** maintenance after speciation. Unfortunately, the
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13 65 **phylogenetic** relationships of most Japanese *Arisaema* species are not clear because
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17 66 of the extremely small genetic distances among them (Ohi-Toma et al. 2016; Murata
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21 67 et al. 2018). Therefore, pollinator **shifts** between sister species **have** not yet been
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25 68 examined.

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29 69 As an exceptional case, the *Ar. ovale* group, which consists of *Ar. inaense*
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33 70 (Seriz.) Seriz. ex K. Sasam. et J. Murata, *Ar. nagiense* Tom. Kobay., K. Sasam. et J.
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37 71 Murata, and *Ar. ovale* Nakai (Fig. 1, Kobayashi et al. 2008), appears to be a
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41 72 monophyletic species complex based on molecular analyses (Ohi-Toma et al. 2016;
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45 73 Murata et al. 2018), the basic chromosome number (Sasamura et al. 2021), and
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49 74 morphological similarity (Kobayashi et al. 2008). Considering the diversity of ploidy
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53 75 levels and their distribution (Fig. 2), Sasamura et al. (2021) estimated the
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57 76 diversification history of this group as follows: (1) The distribution area of the
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60 77 common diploid ancestor fragmented during the glacial period. (2) These scattered
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1 78 populations were allopatrically diversified into three species (*Ar. inaense*, *Ar. nagiense*,
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5 79 and *Ar. ovale*) in each refugium. (3) A tetraploid race with triplicated productivity of
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9 80 cormllets (Murata 1993) was differentiated from diploid *Ar. ovale*. (4) Intensive clonal
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13 81 reproduction allowed tetraploid *Ar. ovale* to expand its distribution across the Japanese
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17 82 archipelago. Consequently, the distribution area overlapped between *Ar. nagiense* ($2n$
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21 83 = 26) and tetraploid *Ar. ovale* ($2n = 52$) in the eastern Chugoku Mountains (Figs 1G,
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25 84 2B, and 2D, Kobayashi 2021).

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29 85 These populations across the eastern Chugoku Mountains may provide us
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33 86 **with** a unique opportunity to understand the role of pollinator shift during the
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37 87 speciation process of Japanese *Arisaema* for the following two reasons. First, the
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41 88 distributional overlap of the two *Arisaema* species enables **a meaningful comparison**
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45 89 **of** pollinator **assemblages** between species. Differences in pollinator use between
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49 90 allopatric species can be derived from selective pollinator attraction by plants and/or
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53 91 regional heterogeneity in entomofauna (Matsumoto et al. 2021). **In contrast,**
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57 92 interspecific pollinator differences in **sympatry** strongly **support** the former hypothesis.
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61 93 **Second,** intraspecific pollinator comparison between sympatric and allopatric
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1 94 populations can clarify the effect of polyploidy on the pollinator shift. Interspecific
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5 95 differences in chromosome number will lead to hybrid sterility in Japanese *Arisaema*
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9 96 (Kobayashi et al. 2005; Murata et al. 2018). This post-zygotic barrier often strengthens
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13 97 pollinator isolation (van der Niet et al. 2006). The fitness reduction due to
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17 98 reproductive interference can be a strong selective pressure that changes floral traits
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21 99 to avoid interspecific pollination (reinforcement, Weber and Strauss 2016). If the
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25 100 reproductive interference due to polyploidy promotes the pollinator shift, pollinator
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29 101 assemblage should differ between species in sympatric situations but could be less
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33 102 distinct in allopatric situations. In contrast, the pollinator isolation may have evolved
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37 103 before secondary contact if interspecific difference in pollinator species is observed
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41 104 in both sympatric and allopatric situations.

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44 105 In this study, we compared the floral visitor assemblages between *Ar.*
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48 106 *nagiense* and *Ar. ovale* in sympatric/parapatric and allopatric populations (Table 1) to
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52 107 estimate the role of pollinator shifts and reinforcement due to polyploidization in the
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56 108 diversification of Japanese *Arisaema*. Then, we constructed two working hypotheses:
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60 109 (1) different fungus gnats selectively visit the two sister *Arisaema* species, and (2)

1 110 differences in pollinator use between them **become** clearer in a sympatric situation
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5 111 than in an allopatric situation. Of fungus gnat pollinators, we focused on the species
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9 112 of *Anatella* Winnertz, 1863 (Mycetophilidae), because some unidentified *Anatella*
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13 113 species frequently visited *Ar. ovale* (Matsumoto et al. 2021). Based on these results,
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17 114 we discuss the speciation process of the *Ar. ovale* group.
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21 115 22 23 24 25 116 **Materials and methods**

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32 118 The genus *Arisaema* consists of paradioecious perennial herbs that grow in the forest
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36 119 understory (Murata et al. 2018). In most Japanese *Arisaema* species, each plant
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41 120 produces only one male or female inflorescence **per** year. Their inflorescences **lack**
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44 121 **any** floral rewards **and** attract fungus gnat pollinators using odor emissions (Suetsugu
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48 122 et al. 2021). Fungus gnats are imprisoned by pitcher-like spathe tubes (Vogel and
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52 123 Martens 2000). The captured pollinators with pollen grains can leave the male spathe
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56 124 tube through an exit hole. Female inflorescences lack this hole and the captured
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60 125 pollinators die within the female spathe tube after they deposit pollen grains on the
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1 126 stigma (Vogel and Martens 2000).
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5 127 In the Japanese archipelago, 53 *Arisaema* species are found, and 48 of them
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9 128 belong to *Arisaema* sect. *Pistillata* (Murata et al. 2018). These are subdivided into
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13 129 several groups (Murata 1995; Kobayashi 2016; Matsumoto 2021). The *Ar. ovale* group
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17 130 includes three species (Kobayashi et al. 2008), which are morphologically
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21 131 characterized by raised longitudinal veins on the spathe tube (Fig. 1E and 1F) and the
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25 132 absence of leaf rachis and second foliage leaf (Fig. 1C and 1D) (Kobayashi et al. 2008;
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29 133 Matsumoto 2021). Of them, *Ar. nagiense* is endemic to the eastern Chugoku Mountains
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33 134 (Hyogo, Okayama, and Tottori Prefectures), western Japan (Fig. 2B, Kobayashi 2016).
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37 135 This species usually displays one digitate leaf with five narrowly elliptical blades (Fig.
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41 136 1C). The narrowly triangular spathe blade is dark purple (Fig. 1E). The chromosome
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45 137 number is $2n = 26$ (Kobayashi et al. 2008; Sasamura et al. 2021). *Arisaema ovale* is
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49 138 widely distributed across the Japanese archipelago (Fig. 2C, Sasamura et al. 2021). Its
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53 139 digitate leaves are subdivided into five to seven elliptic blades (Fig. 1D and 1G). The
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57 140 widely triangular spathe blade is usually pale green (Fig. 1F). This species shows high
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61 141 diversity in chromosome number ($2n = 26, 39, 52, 65, 78$), but tetraploid populations
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1 142 (2n = 52) dominate the species' wide range (Fig. 2D, Sasamura et al. 2021). In the
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5 143 eastern Chugoku Mountains, the **distribution ranges of diploid** *Ar. nagiense* and
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9 144 tetraploid *Ar. ovale* **overlap**, although the former is often distributed in areas with
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13 145 **higher** altitudes than the latter (Kobayashi 2021). Both species usually bloom from
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17 146 May to June and flowering season continues for approximately one month (Matsumoto
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21 147 et al. 2021; T. K. Matsumoto, personal observation).

25 148 26 27 28 29 149 Study sites

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33 150 We conducted field surveys at six sites (**A–F**; Table 1). The distance between **sites** was
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37 151 greater than 3 km. Because the two *Arisaema* species examined are endangered
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41 152 (Okayama Prefecture 2020), detailed information about the study sites **is** not shown
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45 153 to protect these habitats. At site A, *Ar. nagiense* and *Ar. ovale* were parapatrically
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49 154 distributed in high and low altitudinal areas, respectively, but sometimes grew
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53 155 sympatrically at an intermediate elevation. At site D, some *Ar. ovale* plants were found
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57 156 in **a** *Ar. nagiense* **population** (Table 1, Fig. 1G). At other sites, **only one** of the two
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61 157 *Arisaema* species was present (hereafter, allopatric **populations**; Table 1). Ideally, the

1 158 parapatric (i.e., most parts of site A) and sympatric situations (some parts of site A
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5 159 and site D) should be distinguished in the interspecific comparison of pollinator
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9 160 assemblages, while the objective delimitation is difficult due to the distributional
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13 161 continuity of the two *Arisaema* species in these sites. Moreover, in a previous study,
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17 162 five Japanese *Arisaema* species constantly attracted species-specific pollinators along
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21 163 wide altitudinal gradient (650–900 m asl, Matsumoto et al. 2021), suggesting that the
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25 164 spatial heterogeneity in pollinator resource is not so large within each site. Therefore,
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29 165 we treated these parapatric and sympatric populations as same site type (hereafter,
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33 166 sympatric/parapatric **populations**) in this study. Consequently, we conducted field
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37 167 survey at two sympatric/parapatric (sites A and D) and four allopatric populations
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41 168 (sites B, C, E, and F) in this study. **Although we did not directly examine the ploidy**
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45 169 **level of *Ar. ovale* populations in our study sites, they seem to be tetraploids based on**
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49 170 **the distribution map shown in a previous study (Fig. 2D, Sasamura et al. 2021).**
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52 171 53 54 55 56 172 Floral visitor assemblage

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60 173 Since female inflorescences can capture all flower visitors in *Arisaema* (Vogel and
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1 174 Martens, 2000), we can easily collect them without artificial manipulation before the
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5 175 survey. In addition, pollinator collection from male inflorescences may negatively
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9 176 affect successful pollen movement from male to female inflorescences. Therefore, we
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13 177 examined floral visitor assemblages for only female plants in this study, except for
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17 178 site E in 2018 (Table 1). In 2019 and 2020, we captured all floral visitors from female
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21 179 *Ar. nagiense* plants ($n = 91$) in 50 ml falcon tubes using a handmade aspirator. These
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25 180 falcon tubes were kept in a freezer overnight to fix the arthropods. We used an existing
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29 181 pollinator collection from *Ar. ovale* plants ($n = 102$) in a subset of our study sites
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33 182 (Matsumoto et al. 2021) to identify the pollinator assemblage in parapatric (site A)
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37 183 and allopatric *Ar. ovale* populations (sites C, E, and F). In addition, we captured flower
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41 184 visitors from female *Ar. ovale* plants ($n = 3$) grown in *Ar. nagiense* populations (sites
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45 185 A and D, Fig. 1G). All field surveys were conducted after most *Arisaema* individuals
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49 186 in each population began flowering (Table 1). However, we collected floral visitors
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53 187 daily throughout anthesis at site E (see Matsumoto et al. 2021). Arthropods captured
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57 188 were preserved as dry specimens and observed under a stereomicroscope (Leica S
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60 189 APO; Leica Microsystems, Heerbrugg, Switzerland). They were identified to the

1 190 genus level for mycetophilids, the family level for other dipterans, and the order level
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5 191 for other arthropods.
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9 192 **Specimens of the** mycetophilid genus *Anatella*, the main pollinator of *Ar.*
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13 193 *ovale* (Matsumoto et al. 2021), were classified into morphospecies based on gross
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17 194 morphology. We especially observed postabdomens to distinguish the sex of each
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21 195 *Anatella* specimen because Suetsugu et al. (2021) reported an extremely male-biased
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25 196 sex ratio of fungus gnat pollinators in certain Japanese *Arisaema* species. The male
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29 197 genitalia of some specimens were dissected for detailed observations. The genitalia
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33 198 were detached from the abdomen using sharpened tweezers and macerated with heated
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37 199 10% KOH. Thereafter, the remaining chitinous parts were washed with distilled water
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41 200 and observed in pure glycerin under a stereomicroscope (Olympus SZ60, Olympus
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45 201 Corp., Tokyo, Japan). The dissected parts were preserved in pure glycerin within
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49 202 plastic microvials pinned under the body. Based on gross morphology, including the
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53 203 male genitalia, we identified the *Anatella* species using taxonomic keys (Chandler
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57 204 1977; Sasakawa and Ishizaki 2003; Zaitzev 2003). Voucher insect specimens were
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61 205 deposited at the Forestry and Forest Products Research Institute, Tsukuba, and TKM's
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1 206 private collection in Okayama University, Okayama, Japan.

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9 208 **Statistical analysis**

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13 209 Interspecific differences in the floral visitor assemblage were examined using
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17 210 permutational multivariate analysis of variance (PERMANOVA) with 10,000
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21 211 permutations. To clarify whether species-specific flower visitation by *Anatella* species
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25 212 contributes to the interspecific differences in pollinator use, we conducted two
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29 213 PERMANOVAs using floral visitor data with and without the subdivision of *Anatella*
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33 214 species. The distance matrix was obtained using the Chao index. We considered
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37 215 species (*Ar. nagiense* or *Ar. ovale*), site type (sympatric/parapatric or allopatric) and
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41 216 their interaction as factors.

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44 217 Statistical significance was set at $P < 0.05$. PERMANOVAs were performed
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48 218 with the free software R (version 3.5.1.; R Development Core Team 2018) and the
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52 219 adonis2 function in the vegan package (Oksanen et al. 2018).

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60 221 **Results**

1 222 In total, 246 and 783 visitors were collected from *Ar. nagiense* and *Ar. ovale*,
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5 223 respectively (Table 1). At all sites, *Anatella* spp. dominated the floral visitor
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9 224 assemblages in both *Arisaema* species (987 of total 1,029 visitors; Table 1, Fig. 3).
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13 225 Although we could not count pollen grains attached to all visitors due to poor sample
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17 226 conditions, some pollen grains often adhered to the *Anatella* spp. (Fig. 4).
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21 227 Of the 987 specimens of *Anatella* spp., 970 and three were male and female,
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25 228 respectively (Table S1, Fig. 5). We could not determine the sex for the remaining 14
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29 229 specimens (Table S1, Fig. 5) because of the poor sample conditions. Based on the
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33 230 gross morphology (Fig. 4), male *Anatella* specimens were classified as *An. prominens*
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37 231 Sasakawa, 2003 ($n = 199$), *An. flavicauda* Winnertz, 1863 ($n = 630$), and *An. altaica*
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41 232 Zaitzev, 1987 ($n = 16$). *Anatella prominens* and *An. flavicauda* have a long flagellum
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45 233 of the antenna (Fig. 4A and 4C), while that of *An. altaica* is short (Fig. 4E). *Anatella*
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49 234 *prominens*, *An. flavicauda*, and *An. altaica* had broad (Fig. 4B), short (Fig. 4D), and
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53 235 slender (Fig. 4F) dorsal branches of the gonostylus, respectively. All male *Anatella*
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57 236 specimens ($n = 198$) collected from 91 inflorescences of *Ar. nagiense* were *An.*
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60 237 *prominens* (Table S1, Fig. 5). *Anatella flavicauda* ($n = 630$) dominated male *Anatella*
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1 238 specimens collected from 103 inflorescences of *Ar. ovale*, but *An. prominens* ($n = 1$)
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5 239 and *An. altaica* ($n = 16$) were occasionally collected (Table S1, Fig. 5). Male *Anatella*
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9 240 specimens ($n = 4$) collected from three *Ar. ovale* plants that grew sympatrically with
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13 241 *Ar. nagiense* (Fig. 1G) were *An. flavicauda* ($n = 3$) and *An. altaica* ($n = 1$). The three
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17 242 females were identified as *An. unguigera* Edwards, 1921, based on their general
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21 243 appearance (antennae, mid femur, mid tibia, hind coxa, and body color). Two and one
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25 244 specimens of female *An. unguigera* were collected from an inflorescence of *Ar.*
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29 245 *nagiense* and two inflorescences of *Ar. ovale*, respectively (Table S1, Fig. 5).
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33 246 When the genus *Anatella* was not subdivided, the floral visitor assemblage
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37 247 did not differ between the two *Arisaema* species (PERMANOVA, $r^2 = 0.009$, $P = 0.25$)
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41 248 nor the site type ($r^2 = 0.01$, $P = 0.15$). Their interaction was not significant ($r^2 =$
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45 249 0.0007 , $P = 0.99$). A significant difference in floral visitor assemblage with the
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49 250 subdivision of *Anatella* species was detected between the two *Arisaema* species ($r^2 =$
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53 251 0.44 , $P < 0.001$), but not between site types ($r^2 = 0.004$, $P = 0.34$), and their interaction
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57 252 was not significant ($r^2 = 0.002$, $P = 0.71$).
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1 254 **Discussion**

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5 255 Based on the selective attraction of fungus gnats in sympatric species pairs (Kakishima
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9 256 et al. 2020; Matsumoto et al. 2021; Suetsugu et al. 2021), Matsumoto et al. (2021)
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13 257 speculated that frequent pollinator shifts have contributed to the rapid diversification
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17 258 of Japanese *Arisaema* species. Our results partly supported this hypothesis because
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21 259 the species-specific fungus gnat visitation in the two closely related *Arisaema* species
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25 260 (Fig. 5) suggests that the pollinator shift occurred during their speciation process.

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29 261 Although the sample size was not very large in sympatric situations (Table 1), the
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33 262 clear separation of *Anatella* species in sympatric/parapatric situations (sites A and D)
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37 263 suggests that the pollinator specificity was mainly derived from selective attraction
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41 264 by *Arisaema* plants (Matsumoto et al. 2021; Suetsugu et al. 2021) but not from
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45 265 geographic heterogeneity in entomofauna (Økland 1996; Toft et al. 2001). Although
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49 266 the species composition of *Anatella* pollinators partly differed among *Ar. ovale*
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53 267 populations (Fig. 5), it might reflect the seasonal transition of fungus gnat
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57 268 assemblages (Kurina and Grootaert 2006). This is because *An. altaica* was collected
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61 269 from sites A, C, and E (2019), in which we conducted field survey in the later stage

1 270 of anthesis (from June to July, Table 1).

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5 271 Such pollinator shift can drive initial speciation because **pollinator**
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9 272 **specificity** functions as an effective reproductive barrier (Lowry et al. 2008); however,
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13 273 **the** actual contribution **of pollinators** on speciation is still **debated** question in plant
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17 274 speciation study because pollinator isolation **can** also **evolve** after speciation
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21 275 theoretically (van der Niet and Johnson 2012). In our study system, the difference in
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25 276 ploidy level between *Ar. nagiense* and *Ar. ovale* (Sasamura et al. 2021) may result in
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29 277 hybrid sterility (Kobayashi et al. 2005; Murata et al. 2018). Since the formation of
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33 278 maladaptive progenies through interspecific pollination (i.e., reproductive
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37 279 interference) critically **reduces** the population growth of parent lineages (Wolf et al.
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41 280 2001; Buggs and Pannell 2006), natural selection can reinforce the pollinator shift
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45 281 when the two allopatrically diversified species **encounter** due to the expansion of their
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49 282 distribution **ranges**. If so, pollinator segregation should be **stronger** in sympatric
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53 283 situations (van der Niet et al. 2006), whereas **in our study** the pollinator specificity
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57 284 was consistent between sympatric/parapatric and allopatric situations (Fig. 5).
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60 285 Therefore, species-specific pollinator attraction seems to have developed before
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1 286 secondary contact since reproductive interference did not contribute to pollinator shift
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5 287 in this study system. Our results imply that pollinator shift is one of initial drivers of
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9 288 the allopatric speciation in Japanese *Arisaema* species, although more comprehensive
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13 289 study is needed because other lineages also show polyploidy in this group (Murata et
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17 290 al. 2018).

21 291 Considering the extremely male-biased sex ratio of *Anatella* pollinators
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25 292 (Fig. 5), the pollinator specificity appears to be derived from chemical mimicry for
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29 293 the sexual pheromone of female insects by the floral odor of the two *Arisaema* species,
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33 294 as in deceptive orchids (Dafni 1984; Schiestl 2005; Bohman et al. 2016). Interestingly,
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37 295 the male-biased sex ratio of other fungus gnat pollinators was also found in several
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41 296 *Arisaema* species (Suetsugu et al. 2021; T. K. Matsumoto, unpublished data). In
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45 297 addition, sexual pheromone mimicry has been verified in some fungus gnat-pollinated
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49 298 orchids based on the observation of pollinator behavior (Blanco and Barboza 2005;
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53 299 Phillips et al. 2013; Reiter et al. 2019) and floral odor analyses (Hayashi et al. 2021).
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56 300 Since subtle odor changes allow sexually deceptive orchids to shift from species-
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60 301 specific wasp pollinators to other allied wasps (Peakall et al. 2010), the shift among
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1 302 congeneric fungus gnat pollinators (Fig. 5) may partly explain the rapid diversification
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5 303 of Japanese *Arisaema* species if they attract fungus gnats using sexual mimicry.
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9 304 It is, however, important to note that the male-biased pollinator visitation
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13 305 is not sufficient to prove sexual deception (Phillips et al. 2013). Similar results are
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17 306 expected under other situations. First, male pollinators selectively visit flowers in the
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21 307 shelter mimicry pollination systems, in which the tube-shaped dark-red flowers attract
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25 308 solitary male bees by providing protective shelter (Vereecken et al. 2012, 2013).
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29 309 However, since the same pollinator species is widely shared among plant taxa with
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33 310 shelter mimicking flowers (Vereecken et al. 2013), **this mechanism cannot** be applied
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37 311 to the species-specific pollination system **of our study species** (Fig. 5). Second, the
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41 312 temporal fluctuation in the sex ratio of insect populations (Lounibos and Escher 2008;
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45 313 Tabadkani et al. 2013) can result in male-biased pollinator visitation. In contrast, no
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49 314 female pollinators visited inflorescences (Fig. 5) during the entire flowering season
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53 315 of *A. ovale* (more than one month) in two successive years at the site E (Table 1), even
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57 316 though the sex ratio of emerged flies can change daily (Tabadkani et al. 2013) or yearly
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61 317 (Lounibos and Escher 2008). Third, the reduced flight ability of fungus gnat females
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1 318 due to brachypterism (Matile 1975, 1976), possible autotomy of wings (Sutou et al.
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5 319 2012), and inactive behavior (Hu et al. 2019; Ishihara and Tagami 2020) can decrease
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9 320 the visitation frequency of female pollinators. We cannot reject this hypothesis
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13 321 because no female has been described in the three *Anatella* pollinators (Sasakawa and
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17 322 Ishizaki 2003; Zaitzev 2003). However, in other sexual deception systems, orchid
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21 323 flowers **use** male wasps by mimicry for wingless females (e.g., Wong and Schiestl
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25 324 2002; Hooper and Brown 2007). Thus, the sexual dimorphism of flight ability might
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29 325 not always contradict the sexual mimicry by flowers. More direct evidence for sexual
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33 326 mimicry is needed to reject these alternative possibilities completely.
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40 328 **Conclusion**

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44 329 In this study, we demonstrated that a pollinator shift occurred between the two sister
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48 330 *Arisaema* species during the allopatric speciation process but not after the secondary
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52 331 contact in the Chugoku Mountains, and that sexual mimicry might be potentially
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56 332 responsible for the high pollinator specificity. To verify this hypothesis, future studies
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60 333 should examine (1) flower visitation behavior of *Anatella* pollinators, (2) the chemical
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1 334 similarity between the floral odor of *Arisaema* species and the sexual pheromone of
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5 335 *Anatella* pollinators, and (3) the pollinator assemblage in the other ploidy races of *Ar.*
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9 336 *ovale* ($2n = 26, 39, 65$) and the diploid species *Ar. inaense*. These missing pieces will
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13 337 contribute to the understanding of the allopatric speciation process of the *Arisaema*
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17 338 sect. *Pistillata* in the Japanese archipelago.
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29 341 We thank Shungo Kariyama and Tomiki Kobayashi for providing information on *Ar.*
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33 342 *nagiense* populations and three anonymous reviewers and Associate Editor for helpful
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37 343 comments on our manuscript.
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44 345 **References**

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501 **TABLES**

502 **Table 1.** Information about study sites. Detailed locations are not shown to protect habitats of the two *Arisaema* species.

Examined species	Site	Sampling date	No. of examined plants¹	No. of collected arthropods	No. of <i>Anatella</i> spp.
<i>Ar. nagiense</i>	Site A ²	30 May 2019	38	80	66
	Site B	31 May and 1 June 2019	32	136	114
		23 and 27 May 2020			
	Site D ²	2 June 2019	21	30	24
24 May 2020					
<i>Ar. ovale</i>	Site A ²	30 May, 5 and 6 June 2019	12	22	14
	Site C	8 and 13 June 2019	5	40	36
	Site D ²	24 May 2020	2	4	3
	Site E (2018)	From 29 April to 15 June 2018	16 females and 51 males	630	545
	Site E (2019)	From 3 June to 9 July 2019	9	33	20
	Site F	25 May and 10 June 2019	10	54	40

503 ¹ In all sites but the site E (2018), we examined floral visitor assemblages for only female plants.

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504 ²The two *Arisaema* species were distributed sympatrically and/or parapatrically.

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4 505 **FIGURE LEGENDS**

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7 506 **Fig. 1** *Arisaema nagiense* (A, C, E) and *Ar. ovale* (B, D, F) in study sites. (A–B)
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11 507 Growth habit of the two *Arisaema* species. (C–D) Whole plant of female individuals.
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15 508 (E–F) The shape of the spathe blade. (G) Sympatric situation of the two *Arisaema*
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19 509 species in the site D.
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27 511 **Fig. 2** Distributional areas of *Ar. inaense* (a), *Ar. nagiense* (b), and *Ar. ovale* (c, d)
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31 512 in the Japanese archipelago. The **distribution range** of each ploidy types of *Ar. ovale*
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35 513 was separately shown in Fig. 2d. The grey lines indicate the boundaries of each
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39 514 prefecture, and the entire prefecture **is** colored black (grey in Fig. 2d) if one
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43 515 specimen record **exists**. The box on the bottom-right corner indicated the figure
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47 516 legend of Fig. 2d. These diagrams were drawn based on distribution maps in Murata
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51 517 et al. (2018), Sasamura et al. (2020), Seno (2021), and a blank map of the Geospatial
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55 518 Information Authority of Japan
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59 519 (<https://maps.gsi.go.jp/#8/38.272689/141.328125/&base=blank>).
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521 **Fig. 3** Floral visitor assemblage of the two *Arisaema* species. Numbers of examined
522 plants and collected arthropods were shown under the site name and on upper-right
523 corner of each column, respectively. Asterisks on the site names indicate
524 sympatric/parapatric populations.

525

526 **Fig. 4** Fungus gnat pollinators of *Anatella* Winnertz, 1863. (A, C, E) Left lateral
527 view of males and (B, D, F) dorsal view of the apices of male abdomens of (A–B)
528 *An. prominens* Sasakawa, 2003, (C–D) *An. flavicauda* Winnertz, 1863, and (E–F)
529 *An. altaica* Zaitzev, 1987. Antennae (A, C, E) and dorsal branch of gonostylus (B,
530 D, F) were pointed by black arrows.

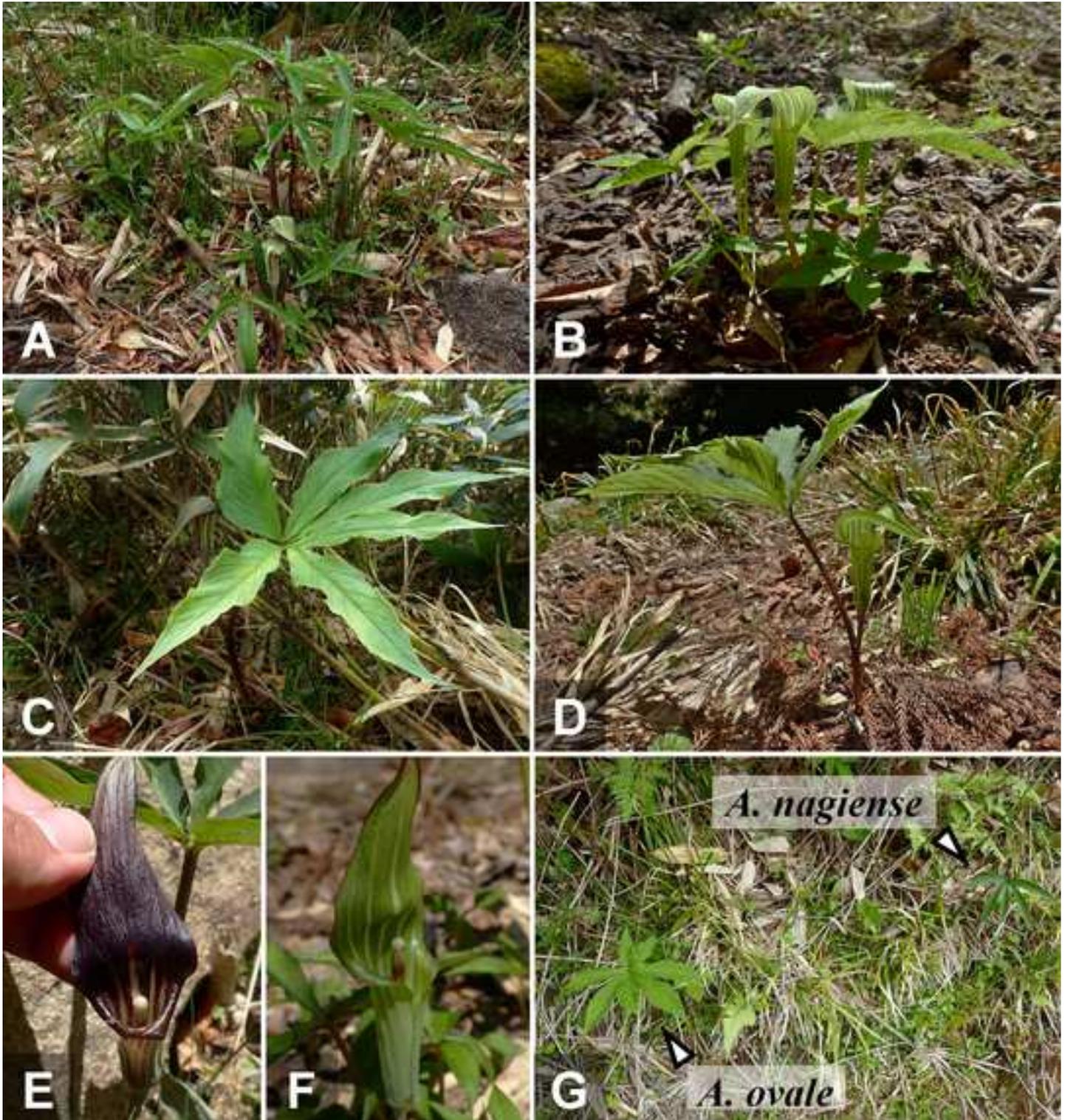
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532 **Fig. 5** The proportion of *Anatella* spp. attracted by the two *Arisaema* species.
533 Numbers of examined plants and collected fungus gnats are shown under the site

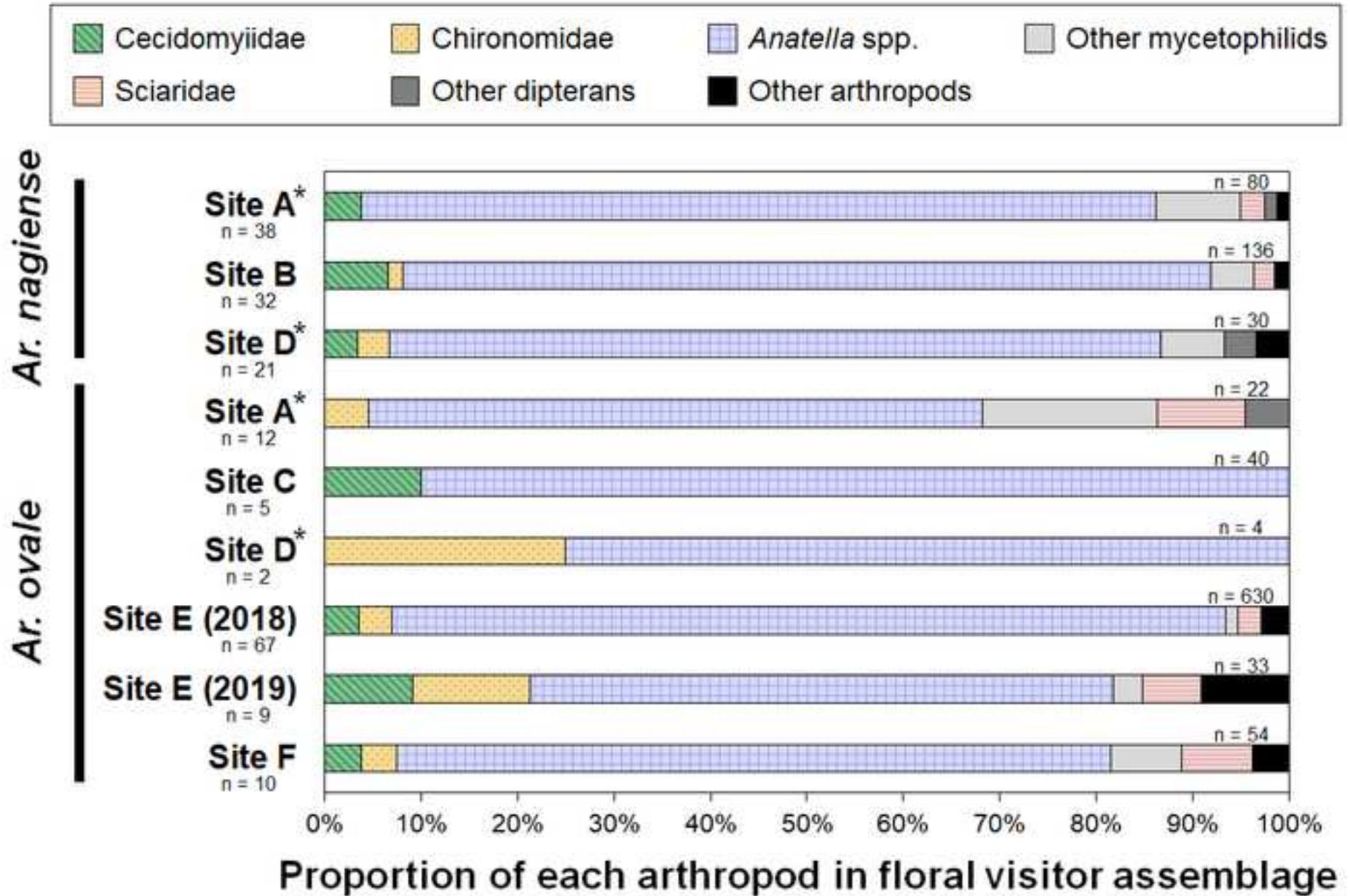
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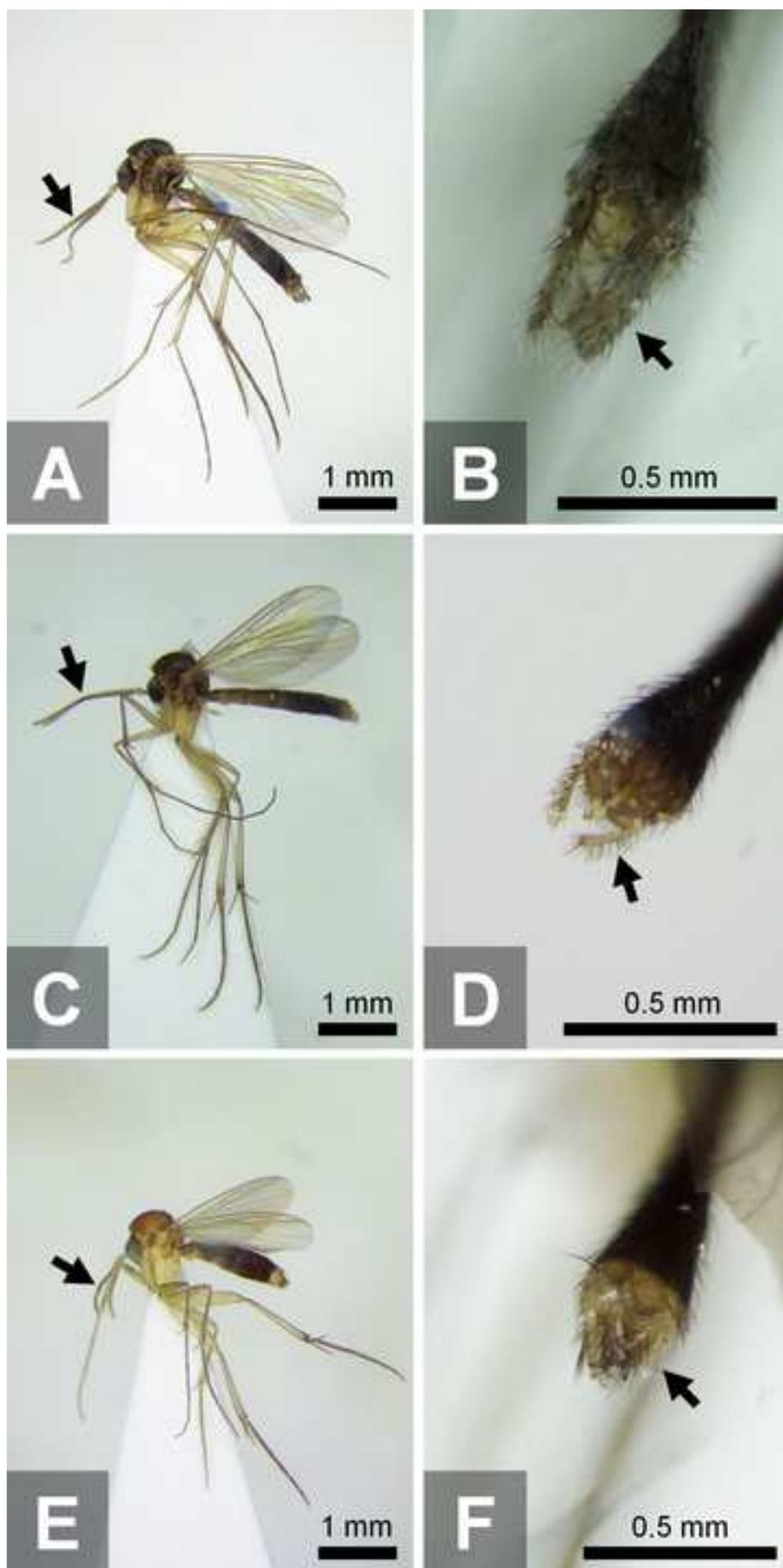
534 name and on upper-right corner of each column, respectively. Asterisks on the site

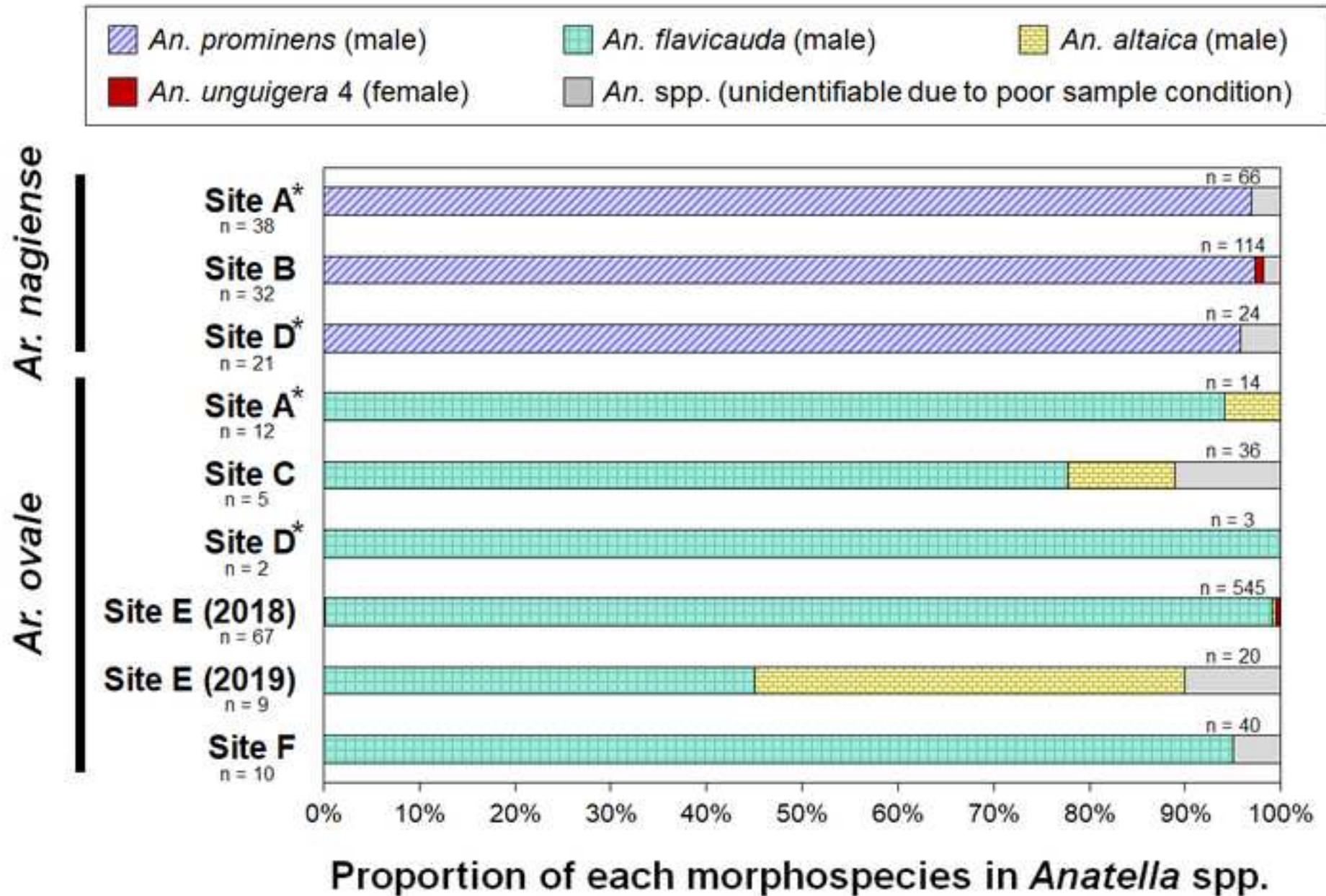
535 names **indicate** sympatric/parapatric populations.



A) *Ar. inaense*B) *Ar. nagiense*C) *Ar. ovale*D) Polyploids of *Ar. ovale*









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