



HAL
open science

Local Insect Availability Partly Explains Geographical Differences in Floral Visitor Assemblages of *Arum maculatum* L. (Araceae)

Danae Laina, Eva Gfrerer, Valerie Scheurecker, Roman Fuchs, Marielle Schleifer, Carina Zित्रा, Rüdiger Wagner, Marc Gibernau, Hans Peter Comes, Anja Hörger, et al.

► **To cite this version:**

Danae Laina, Eva Gfrerer, Valerie Scheurecker, Roman Fuchs, Marielle Schleifer, et al.. Local Insect Availability Partly Explains Geographical Differences in Floral Visitor Assemblages of *Arum maculatum* L. (Araceae). *Frontiers in Plant Science*, 2022, 13, <10.3389/fpls.2022.838391>. <hal-03781294>

HAL Id: hal-03781294

<https://hal.science/hal-03781294v1>

Submitted on 20 Sep 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Local insect availability partly explains geographical differences in floral visitor assemblages of *Arum maculatum* L. (Araceae)

1 Danae Laina¹, Eva Gfrerer¹, Valerie Scheurecker¹, Roman Fuchs¹, Marielle Schleifer¹, Carina
2 Zित्रा², Rüdiger Wagner³, Marc Gibernau⁴, Hans Peter Comes¹, Anja C. Hörger^{1†*}, Stefan
3 Dötterl^{1†*}
4

5 ¹ Department of Biosciences, Paris Lodron University of Salzburg, Salzburg, Austria.

6 ² Department of Functional and Evolutionary Ecology, University of Vienna, Vienna, Austria.

7 ³ Department of Limnology, University of Kassel, Kassel, Germany.

8 ⁴ Laboratory of Sciences for the Environment, CNRS – University of Corsica, Ajaccio, France.

9

10 * **Correspondence:** Stefan Dötterl, stefan.doetterl@plus.ac.at, Anja C. Hörger,
11 anja.hoerger@plus.ac.at

12 † These authors share senior authorship

13

14 **Keywords:** local pollinator availability ¹, pollinator limitation ², specialization ³, generalization ⁴,
15 reproductive success ⁵, *Arum maculatum* ⁶, Psychodidae ⁷, Sphaeroceridae⁸.

16

17 ORCID:

18 Danae Laina 0000-0002-1058-5095

19 Eva Gfrerer 0000-0003-0837-1327

20 Roman Fuchs 0000-0001-9423-5588

21 Carina Zित्रा 0000-0002-8963-6421

22 Marc Gibernau 0000-0003-3866-3099

23 Hans Peter Comes 0000-0002-2659-8069

24 Anja Hörger 0000-0001-6816-195X

25 Stefan Dötterl 0000-0001-5228-1332

26

27 **Abstract**

28 Geographical variation in abundance and composition of pollinator assemblages may result in
29 variable selection pressures among plant populations and drive plant diversification. However, there
30 is limited knowledge on whether differences in local visitor and pollinator assemblages are the result
31 of site-specific strategies of plants to interact with their pollinators and/or merely reflect the
32 pollinator availability at a given locality. To address this question, we compared locally available
33 insect communities obtained by light trapping with assemblages of floral visitors in populations of
34 *Arum maculatum* (Araceae) from north vs. south of the Alps. We further investigated whether and
35 how the abundance of different visitors affects female reproductive success, and examined the pollen
36 loads of abundant visitors. Local insect availability explained inter-regional differences in total
37 visitor abundance, but only partly the composition of visitor assemblages. Northern populations
38 predominantly attracted females of *Psychoda phalaenoides* (Psychodidae, Diptera), reflecting the
39 high availability of this moth fly in this region. More generalized visitor assemblages, including other
40 psychodid and non-psychodid groups, were observed in the south, where the availability of *P.*
41 *phalaenoides*/Psychodidae was limited. Fruit set was higher in the north than in the south but
42 correlated positively in both regions with the abundance of total visitors and psychodids; in the north,
43 however, this relationship disappeared when visitor abundances were too high. High pollen loads
44 were recorded on both psychodids and other Diptera. We demonstrate, for the first time, that
45 recording both quantitative data of floral visitor assemblages and the availability of these visitors in
46 the respective localities is helpful to understand the underpinnings of geographical variation in plant–
47 pollinator interactions. This approach revealed that geographical differences in floral visitors of *A.*
48 *maculatum* are only partly shaped by the local insect availability while other factors, such as the
49 plant’s region-specific attractiveness to flower visitors or population-specific pollinator behaviours,
50 also contribute to the geographical pattern of visitor assemblages.

51

52 **1 Introduction**

53 It is widely recognized that geographical variation in abundance and composition of pollinator
 54 assemblages can impose variable selective pressures on floral phenotypes within and among plant
 55 species (Dodd et al., 1999; Hernández-Hernández & Wiens, 2020; Johnson, 2010; Van der Niet &
 56 Johnson, 2012; Waser et al., 1996). Indeed, geographical differences in pollinator assemblages are
 57 often associated with variation in floral traits related to pollination (e.g. advertisement, rewards,
 58 morphology) (Cosacov et al., 2014; Ishii et al., 2019; Newman et al., 2015; Parker et al., 2018),
 59 frequently resulting in different degrees of pollinator specialization (Bustamante et al., 2010),
 60 pollinator effectiveness, pollination success and plant fitness (Campbell, 1987; Gómez et al., 2009a;
 61 Johnson & Bond, 1992; Moeller et al., 2012).

62 Variation in pollinator assemblages among populations is generally thought to correspond to local
 63 pollinator availability (Grant & Grant, 1965; Phillips et al., 2020), but may also result from site-
 64 specific differences in pollinator attraction (Majetic et al., 2009; Peakall et al., 2010). However, there
 65 is limited knowledge about the relative importance of local pollinator availability vs. site-specific
 66 pollination strategies in shaping the local abundance and composition of pollinator assemblages. This
 67 is mainly because local pollinator availability is usually estimated either from presence/absence data
 68 of particular pollinators (Diaz & Kite, 2002; Johnson & Raguso, 2016; Moeller, 2006 but see also
 69 Sayers et al., 2020) or by comparing pollinator visitation rates among populations, assuming that
 70 visitation rates positively correlate with local pollinator abundance (e.g. Gómez et al., 2009b, 2014;
 71 Rech et al., 2018).

72 However, without further quantification of local insect availability, a high visitation rate observed for
 73 a particular pollinator species could reflect either local predominance of that species, or its specific
 74 attraction from a diverse pool of potential pollinators (Johnson, 2010; Primack & Inouye, 1993).
 75 Similarly, a low visitation rate at a given locality could indicate that pollinators are indeed locally
 76 scarce (Herrera, 1989) and plants are pollen-limited; if so, such a plant population would be expected
 77 to evolve towards less reliance on pollinators (e.g. spontaneous selfing) (Fausto et al., 2001; Kalisz et
 78 al., 2004; Knight et al., 2005; Moeller, 2006) or switch to novel pollinators (Pattamore & Wilcove,
 79 2012). Low visitation rates could also indicate that pollinators (even if abundant in the habitat) are
 80 not effectively attracted by the observed plant individuals (Herrera, 1989), and evolution towards
 81 increased attraction of these particular pollinators might be expected (Ashman, 2000; Knight et al.,
 82 2005; Trunschke et al., 2017). In either instance, low visitation rates result in plant populations that
 83 are pollinator-limited, but the evolutionary implications would be different. Nevertheless, up to now,
 84 studies that quantify local insect availability in relation to visitor assemblages are scarce (Sayers et
 85 al., 2020).

86 Here, we use the brood-site deceptive *Arum maculatum* (Araceae) to investigate the role of local
 87 insect availability (in terms of composition and abundance) in shaping visitor assemblages and
 88 female reproductive success (fruit set and number of seeds per fruit) of individual plants. This
 89 widespread perennial herb is ideal for such a study as it is one of the few European plant species from
 90 which all floral visitors can be easily collected, because attracted insects are temporarily trapped in a
 91 floral chamber (Bröderbauer et al., 2012; Kite, 1995; Lack & Diaz, 1991; Szenteczki et al., 2021).
 92 *Arum maculatum* is mainly pollinated by psychodid moth flies (*Psychoda* spp. *sensu lato*,
 93 Psychodidae, Diptera; Chartier et al., 2011, 2013; Diaz & Kite, 2002; Espíndola et al., 2011; Lack &
 94 Diaz, 1991), while pollinator visitation patterns vary across the species' range, especially between
 95 populations north vs. south of the Alps (Espíndola & Alvarez, 2011; Espíndola et al., 2011). More
 96 specifically, Espíndola et al. (2011) reported high floral visitor abundances consisting predominantly

97 of female *Psychoda phalaenoides* in North–Central Europe but low visitor abundances composed
98 mainly of male and female *P. grisescens* in the Mediterranean region. Little is known about the
99 pollinator effectiveness of other insect taxa found in the floral chambers, especially of Mediterranean
100 *A. maculatum* (e.g. Chironomidae; Chartier et al., 2011; Espíndola et al., 2011; Gibernau, 2016 but
101 see Diaz & Kite, 2002). Regardless, there are two main hypotheses to explain geographical
102 differences in pollinator assemblages among populations of *A. maculatum*. The first states that local
103 insect availability affects the composition and abundance of visiting insects (Chartier et al., 2013;
104 Szenteczki et al., 2021). The second hypothesis posits that the observed geographical pattern of floral
105 visitors can be explained by the differential attraction of the two psychodid species. Specifically, it is
106 assumed that *P. phalaenoides* is more attracted by *A. maculatum* populations located in North–
107 Central Europe while *P. grisescens* is more attracted by *A. maculatum* populations in the
108 Mediterranean region (Espíndola et al., 2011; Gibernau, 2016). However, it is still unclear how the
109 abundance and composition of pollinators are related to local insect availability and/or the plant’s
110 site-specific strategies to attract pollinators.

111 We determined patterns of visitor assemblages in 11 populations of *A. maculatum* (six from north
112 and five from south of the Alps), recorded and quantified the insect communities at each site using
113 light trap catches, and determined the effect of floral visitors on individual fruit set and on the
114 number of seeds per fruit. In addition, we quantified and compared the pollen loads among the main
115 visiting insects (e.g. Psychodidae and other dipteran families) to determine whether non-psychodids
116 can also carry pollen and act as potential pollinators. Specifically, we asked: (1) How do floral visitor
117 assemblages of *A. maculatum* differ in their abundance and composition between northern and
118 southern populations? (2) Is there a difference in local insect availability between northern and
119 southern populations and if so, can this explain inter-regional differences in visitor assemblages? (3)
120 Is there a positive correlation, in each region, between the reproductive success of *A. maculatum* and
121 the abundance of all or certain visitors that are trapped by this plant species? And (4) do non-
122 psychodids carry pollen, and if so, are they potentially as efficient pollinators as psychodids, when
123 their pollen load is compared to that of psychodids? Based on previous studies (Espíndola et al.,
124 2011; see above), we hypothesize that visitor assemblages of northern *A. maculatum* populations are
125 dominated by female *P. phalaenoides*, while those of the southern populations are more diverse but
126 less numerous in individuals. Such regional differences in visitor assemblages could reflect
127 respective differences in insect availability and/or plant attractiveness. Finally, we expect a positive
128 correlation of fruit set and seed number per fruit with the number of trapped psychodids, but not of
129 other visitors as their pollen loads are supposedly lower.

130

131 **2 Materials and Methods**

132 **2.1 Study plant species**

133 The distributional range of *A. maculatum* covers most of Europe, extending eastward to northern
134 Turkey and the western Caucasus (Boyce, 2006; Govaerts et al., 2020). Its preferential habitats
135 include deciduous woodlands, hedgerows and other shaded areas, on various soils (Sowter, 1949).
136 The spike-like inflorescence consists of a central elongated structure (spadix), whose upper part is
137 sterile (appendix), while its lower part bears the flowers (Gibernau et al., 2004). Fertile female
138 flowers (14–36 per inflorescence, Albre et al., 2003; Chartier & Gibernau, 2009; number of ovules
139 per flower: 1–7, M. Gibernau, unpubl. res.) are found at the base, followed by hair-like sterile female
140 flowers, fertile male flowers (85–155 per inflorescence, Chartier & Gibernau, 2009), and another set

141 of hair-like sterile male flowers (Chouteau et al., 2008; Gibernau et al., 2004; Lack & Diaz, 1991).
 142 The spadix is surrounded by a modified bract (spathe), which forms a floral chamber at the level of
 143 the flowers (Gibernau et al., 2004; Lack & Diaz, 1991; Sowter, 1949). During the first day of
 144 anthesis, the stigmas become receptive and the spathe unfolds revealing the appendix.
 145 Concomitantly, the appendix heats up and emits a dung-like scent, as typical for the oviposition sites
 146 of putative main pollinators (moth flies, Psychodidae, Diptera) (Bermadinger-Stabentheiner &
 147 Stabentheiner, 1995; Chartier et al., 2013; Kite, 1995; Kite et al., 1998). Pollinators are thus lured by
 148 chemical deception, and trapped in the floral chamber for at least one night. On the next day, pollen
 149 is released and shed onto the insects while the spathe and sterile flowers wither, allowing the insects
 150 to escape (Gibernau et al., 2004; Lack & Diaz, 1991). A few months after pollination, the red berry-
 151 like fruits are retained in an infructescence with usually up to five seeds per fruit (Lack & Diaz, 1991;
 152 Sowter, 1949).

153 2.2 Study populations and sampling of floral visitors

154 During three consecutive flowering seasons (April/May 2017–2019), we sampled floral visitors in 11
 155 populations of *A. maculatum*, including six located north of the Alps (Austria, Germany,
 156 Switzerland: BUR, HOH, JOS, MUR, NEC, RUM) and five south of the Alps (North Italy: BER,
 157 DAO, LIM, MON, UDI; see Fig. 1, Supporting Information Table S1). These populations were
 158 located in shady habitats in the vicinity of (small) rivers, and selected given their size and number of
 159 flowering individuals, with a minimum number of 30 to 40 inflorescences per population at the time
 160 of sampling (i.e. typically mid-flowering season). At most sites, we randomly selected 14 to 16
 161 individuals (inflorescences), with a minimum distance of 1 m among each other to avoid sampling
 162 clones. Sample sizes were smaller in a northern population (HOH; $N = 8$) and higher in the largest
 163 northern (JOS, $N = 71$) and southern (DAO, $N = 73$) populations (Fig. 1, Supporting Information
 164 Tables S1, S2).

165 All insects trapped in the floral chambers were collected in the morning (0800 h – 1000 h) of the
 166 second day of anthesis (i.e. before the release of the trapped insects), using exhausters. After
 167 application of this non-invasive method, insects were placed in a preservative solution (96% ethanol,
 168 4% glycerol) for later identification. We use the term ‘visitors’ to describe all insects trapped in a
 169 floral chamber, regardless of their importance to pollination (see Discussion). Sampled plants were
 170 individually marked with metal tags, and infructescences were collected in summer, just before fruit
 171 ripening, to determine fruit set (see also Gfrerer et al., 2021) and the number of seeds per fruit.

172 2.3 Assessment of insect availability at the study sites by light trap catches (LT)

173 To determine insect abundance and community composition at each site, we carried out UV light
 174 trapping (‘Big Outback’, 12V, Bioform, Nuremberg, Germany). The UV lamp was attached to a
 175 metal rod and surrounded by a net, from where attracted insects were collected using exhausters.
 176 Light-trapping has been documented as an efficient method for the attraction of Psychodidae and
 177 other dipteran families (Borkent et al., 2018; Withers, 1989). The catches took place on the day(s)
 178 immediately after visitor sampling, between 2000 h and 2300 h. This time period corresponds to the
 179 flowering time (female phase) of *A. maculatum* and the time of high activity of many (moth) fly
 180 species (Withers, 1989). At sites with a high insect availability (e.g. BUR, MUR, NEC, RUM), *c.*
 181 100 insects could be collected within 15 to 45 min. At sites with lower insect availability (e.g. MON,
 182 LIM), light trap catches were performed for up to 90 min in order to collect a similarly high number
 183 of insects. Light trap catches were carried out either once (most sites), twice (LIM) or three times
 184 (BER, DAO, JOS; see Results and Supporting Information Tables S1, S2). Since we were only

185 interested in potential visitors of *A. maculatum*, we only caught light-attracted insects that were
186 similar in size to those recorded in the floral chambers (c. 2–8 mm).

187 **2.4 Insect identification**

188 All insects sampled from the floral chambers and light traps were first sorted into Psychodidae vs.
189 non-Psychodidae (Kvifte & Wagner, 2017; Oosterbroek, 2006; Vaillant, 1971), followed by sex
190 determination of specimens belonging to Psychodidae. Whenever abdomens for sex determination
191 were missing due to mechanical damage, specimens were categorized as ‘unknown sex
192 (Psychodidae)’. Female psychodids were identified to species level according to morphological keys
193 (Faucheux & Gibernau, 2011; Ježek, 1990; Svensson, 2009; Vaillant, 1971; Withers, 1989). In the
194 present study, *Psychoda zetterstedti* refers to *P. albipennis*, which was also previously found in *A.*
195 *maculatum* inflorescences (Espíndola et al., 2011), following the synonymy of these two taxa (Ježek,
196 1983). When species identification was not possible, individuals were categorized as ‘unidentified
197 females (Psychodidae)’. All males of the same family were grouped under the category ‘males
198 (Psychodidae)’, as further identification to species level was not possible. The remaining Diptera
199 were identified to family level (Oosterbroek, 2006) and other insects to a higher taxonomic rank
200 (Chinery, 1976). Unidentifiable specimens were grouped as ‘unidentified Diptera’ or ‘unidentified
201 Insecta’, respectively. In the following, insect categories (Supporting Information Table S2) will be
202 referred to as ‘insect groups’.

203 For our analyses of floral visitors and light trap catches, we only considered insect groups that
204 occurred at least three times in floral chambers. Insects or other arthropods that occurred only once or
205 twice (i.e. *Psychoda gemina*, *Philosepedon* spp., Phoridae, Hymenoptera, Lepidoptera, Arachnida,
206 Acarina), or were only recorded in light trap catches, were excluded from subsequent analyses. The
207 same applied to rare visitors, which are very unlikely pollinators of *A. maculatum*, such as
208 Collembola.

209 **2.5 Assessment of pollen loads**

210 To determine the pollen load of visiting insects, inflorescences of 45 individuals from JOS were
211 enclosed in fine-mesh gauze bags, early in the morning after the first day of anthesis. After escaping
212 from the plants, all trapped insects were collected carefully from the bags, placed in glass vials and
213 frozen. Pollen morphology (e.g., Weber et al., 1999) and the absence of other *Arum* species at this
214 northern site allowed us to assign the pollen grains to *A. maculatum*. Pollen grain counting was
215 carried out using a Leica DVM6 z-stacking digital microscope (Leica Microsystems, Wetzlar,
216 Germany).

217 **2.6 Statistical analyses**

218 **2.6.1 Insect composition and abundance in floral chambers (FC) and light trap catches (LT)**

219 Differences in the overall abundance of insect groups between northern and southern populations
220 were determined separately for floral chambers (FC) and light trap catches (LT), using asymptotic
221 Wilcoxon–Mann–Whitney (WMW) tests. For the JOS population, no LT data were available in 2018.
222 To allow for comparison, the respective FC data were excluded from subsequent visitation analyses
223 (Supporting Information Table S1). Differences in insect composition between the two regions were
224 again separately assessed for FC and LT, using permutational analysis of variance (PERMANOVA;
225 9,999 permutations) in PRIMER ver. 6.0 (Clarke & Gorley, 2005). For this analysis, pairwise Bray–
226 Curtis similarities were calculated on the relative abundance of the insect groups (i.e. absolute
227 abundance of each insect group divided by the total number of insects in a floral chamber or light

228 trap). The term *region* was used as fixed factor and we additionally included *population* nested in
 229 *region* as a random factor for stratification by population. As most of the southern populations were
 230 sampled in 2017 and most of the northern ones in 2018 (Supporting Information Table S1), the term
 231 *year of sampling* was not included as a random factor due to the unbalanced sample sizes among
 232 years. Unidentified taxa [i.e. ‘unidentified females (Psychodidae)’, ‘unknown sex (Psychodidae)’,
 233 ‘unidentified Diptera’ and ‘unidentified Insecta’; see Supporting Information Table S2] were not
 234 considered for PERMANOVA.

235 The Bray–Curtis similarities were also used to visualize similarities and dissimilarities in the relative
 236 abundances of the insect groups among plant samples and among light trap catches, by employing
 237 non-metric multidimensional scaling (NMDS) in PRIMER. Vectors, representing Spearman’s rank
 238 correlations of individual insect groups with the ordination axes, were calculated. Only vectors with a
 239 correlation coefficient of at least 0.2 are reported. Insect groups that were strongly correlated with the
 240 ordination axes in the FC plot, and explained most of the variation observed between northern and
 241 southern FC-samples (see Results, Fig. 2A), were retained for further analyses. Specifically, we
 242 tested whether the total abundance of each of these specific insect groups differed between the
 243 regions for FC and LT separately, using asymptotic WMW tests in the R package COIN (Hothorn et
 244 al., 2008; R Core Team, 2020).

245 For each data set (FC, LT), we also tested for differences in abundance among the different insect
 246 groups within each region, first using Kruskal–Wallis tests, followed by exact WMW tests for *post-*
 247 *hoc* analyses in COIN. For the FC tests, both analyses were stratified by population. For the LT data,
 248 such stratification was not meaningful as for most populations only a single light trap catch was
 249 performed (Supporting Information Tables S1, S2). The α -levels obtained from *post-hoc* analyses
 250 were adjusted using the Benjamini–Hochberg correction in R ver. 3.6.3 (R Core Team, 2020).

251 For most analyses described above, LT data were standardized to the number of insects per hour of
 252 trapping (except for PERMANOVA and NMDS that were calculated on relative insect data; see
 253 above). For those populations where repeated light trap catches were performed (i.e. BER, DAO,
 254 JOS, LIM), mean insect numbers (per hour of trapping) of the replicate samples were used for
 255 statistical analyses.

256 2.6.2 Relationships between visitor abundances and fruit set

257 Fruit set data were available for 260 out of the 273 plant individuals examined. First, we performed
 258 logistic regressions for the two regions to test whether the presence of an infructescence is dependent
 259 on the *total number of all floral visitors (sum)*, using generalized linear models (GLMs) in R (R Core
 260 Team, 2020). Fruit set was then quantified as the percentage of fruits with seeds divided by the total
 261 number of female flowers. If no infructescence was available ($N = 144$ out of 260 plant individuals),
 262 fruit set was coded as zero. To assess whether there is a relationship between fruit set and the
 263 abundance of floral visitors north and south of the Alps, we constructed linear models (LMs) in R (R
 264 Core Team, 2020), including *fruit set* as response variable. In the full (across-region) model, the *total*
 265 *number of all floral visitors (sum)* (Supporting Information Table S2) and *region* were incorporated
 266 as explanatory variables, testing also for their interaction. In addition, LMs were constructed
 267 separately for each region. In all three models, *population* was not included as random factor. This is
 268 because a significant random effect was only detected in the linear mixed effect model for the north
 269 due to the observations after the breakpoint (see below) (R package LME4, Bates et al., 2015; R
 270 package RLRsim, Scheipl et al., 2008; Supporting Information Table S4). Hence, the simpler model
 271 (i.e. without random effect) was preferred. The term *year of sampling* was not included as random
 272 factor due to the unbalanced sample sizes among years (see also above; Supporting Information

273 Table **S1**). For all models presented above (GLMs, LMs), insect counts were *log*-transformed (\log_{10}
 274 + 1) to reduce the skewness in the data set. As various northern plants had higher visitor abundances
 275 than any from south of the Alps (see Results, Fig. 4), we also examined the relationship between
 276 visitor abundance and fruit set by focusing only on those observations where the visitor-abundance
 277 values for the two regions overlapped. Therefore, we set a breakpoint at the maximum value of
 278 abundance observed in the south (i.e. maximum of 71 insects recorded in a floral chamber) and the
 279 analyses described above were repeated. As the interaction of the two variables (*sum* and *region*)
 280 proved non-significant (data not shown), no such term was employed in the models. For the north,
 281 observations with more than 71 insects were analysed in a separate linear model.

282 To test for a relationship between the abundance of individual insect groups and fruit set, we
 283 constructed LMs for each region separately, including only the data up to the previously defined
 284 breakpoint. To assess whether there are differences in fruit set between the regions, we further
 285 constructed LMs for each insect group incorporating the term *region*. The analyses were performed
 286 for each major insect group (pooled psychodids, pooled non-psychodids; Supporting Information
 287 Table **S2**) and other groups that contributed most to the floral visitation (see Results, Fig. 2A). Insect
 288 species and groups with less than five observations per region (i.e. *Psychoda zetterstedti* and
 289 Hemiptera) were excluded.

290 2.6.3 Relationship between visitor abundances and number of seeds per fruit

291 The number of seeds produced per fruit was calculated for each plant by dividing the total number of
 292 seeds by the number of fruits with seeds. Therefore, only individuals exhibiting an infructescence in
 293 summer ($N = 116$ out of 260 plant individuals) were included in this analysis. To test for a
 294 relationship between the number of seeds produced per fruit and the abundance of floral visitors,
 295 LMs were constructed as for the fruit set analyses (R Core Team, 2020), i.e. including a full (across
 296 region) model and one for each region separately. For the northern region, two separate models were
 297 additionally constructed with respect to the maximum visitor abundance observed in the south
 298 (breakpoint at 71 insects; see above). The number of seeds per fruit was used as response variable
 299 and the overall visitor abundance (*sum*) or the abundances of each individual insect group separately
 300 (see also above) as explanatory variables. The individual insect groups tested for the number of seeds
 301 per fruit were the same as in the fruit set analyses (listed in Supporting Information Table **S3**).

302 2.6.4 Analysis of pollen loads

303 A Kruskal–Wallis test (R package COIN; Hothorn et al., 2008) was employed to determine
 304 differences in the number of pollen grains among insect groups, considering only those groups with
 305 more than five individuals.

306 3 Results

307 3.1 Total absolute abundances of insects in floral chambers and light trap catches north and 308 south of the Alps

309 Across the 11 *A. maculatum* populations, a total of 19,306 insects were collected, of which 16,296
 310 were found in the floral chambers ($N = 273$) and 3,010 derived from light trap catches ($N = 18$). The
 311 abundance of floral visitors was quite variable in both regions, but significantly higher in northern
 312 plants (median = 16, range = 0–2,097) than southern ones (median = 6, range = 0–71; asymptotic
 313 WMW, $Z = 5.41$, $P < 0.001$, $N_{\text{North}} = 139$, $N_{\text{South}} = 134$) (Fig. 1, Supporting Information Table **S2** and
 314 Fig. **S1**). Similarly, the abundance of insects trapped by light per hour was significantly higher in the
 315 northern region (median = 490, range = 55–701) compared to the south (median = 54, range = 6–158;

316 asymptotic WMW, $Z = 2.73$, $P = 0.006$, $N_{\text{North}} = 6$, $N_{\text{South}} = 5$) (Fig. 1, Supporting Information Table
317 **S2** and Fig. **S1**).

318 **3.2 Relative composition of floral visitors and locally available insect communities north and** 319 **south of the Alps**

320 3.2.1 Floral chambers (FC)

321 In the northern populations, insect visitors collected from the floral chambers were mainly
322 Psychodidae, while besides the latter, other dipteran families (e.g. Sphaeroceridae, Chironomidae)
323 were the predominant visitors in the south (see below; Supporting Information Table **S2** and Fig. **S1**).
324 Within Psychodidae, females were the strongly dominating sex in the northern floral chambers (Fig.
325 1; Supporting Information Table **S2**). In the southern floral chambers, males were relatively more
326 abundant compared to the north (Fig. 1; Supporting Information Table **S2**).

327 There were significant within- (PERMANOVA, $pseudo-F_{9, 226} = 6.26$, $P < 0.001$) and among-region
328 differences in the relative abundances of the different insect groups found in the floral chambers
329 (PERMANOVA, $pseudo-F_{1, 226} = 21.36$, $P = 0.002$). The latter difference was mainly due to the
330 higher relative abundances of (1) female *Psychoda phalaenoides*, followed by *P. zetterstedti* and
331 Ceratopogonidae in the north; and (2) Sphaeroceridae, followed by various other groups (i.e. female
332 *Psychoda grisescens*, Chironomidae, Hemiptera, male psychodids) in the south (see length and
333 direction of vectors in Fig. 2A).

334 3.2.2 Light trap catches (LT)

335 Light trap catches north of the Alps consisted mostly of Psychodidae and few other dipteran families
336 (e.g. Ceratopogonidae, Chironomidae), while the latter dominated the light trap catches in the south
337 (Supporting Information Table **S2** and Fig. **S1**). In one southern population (UDI) no psychodids
338 were trapped. Within Psychodidae, females were dominant in most catches of both regions, with the
339 exception of one population in the north (NEC; mostly males) (Fig. 1; Supporting Information Table
340 **S2**).

341 Similarly to the floral chambers, there were significant among-region differences in the relative
342 abundances of the different insect groups found in the light trap catches (PERMANOVA, $pseudo-F_{1, 7} = 3.54$, $P = 0.017$) whereas within-region differences were not detected (PERMANOVA, $pseudo-F_{9, 7} = 1.21$, $P = 0.29$). Northern light trap catches exhibited higher relative abundances of psychodid groups (e.g. females of *P. phalaenoides*, *P. grisescens* and *P. trinodulosa*) compared to the southern catches (Fig. 2B). In contrast, southern catches had higher relative abundances of non-psychodids, such as Sphaeroceridae, Sciaridae, Chironomidae and Cecidomyiidae (Fig. 2B).

348 **3.3 Absolute abundances of single insect groups in floral chambers and light trap catches**

349 3.3.1 North of the Alps

350 In the northern region, insect groups that were most responsible for similarities and dissimilarities in
351 relative visitor assemblages among floral chambers (Fig. 2A; see above) also showed significantly
352 different absolute abundances across both floral chambers (Kruskal–Wallis, $\chi^2 = 387.13$, $df = 7$; $N =$
353 992 , $P < 0.001$) and light traps (Kruskal–Wallis, $\chi^2 = 30.19$, $df = 7$, $N = 48$, $P < 0.001$). Floral
354 chambers were dominated by female *P. phalaenoides* followed by female *P. grisescens* (Fig. 3A;
355 FC). The median of absolute abundance of male psychodids, female *P. zetterstedti*, Ceratopogonidae,

356 Chironomidae, Sphaeroceridae and Hemiptera in the floral chambers was zero while some
 357 representatives thereof (e.g. female *P. zetterstedti*) were found at high numbers in a few chambers
 358 (Fig. 3A; FC). Male psychodids, female *P. phalaenoides*, female *P. grisescens*, Chironomidae and
 359 Ceratopogonidae were attracted by the light traps in significantly higher numbers than
 360 Sphaeroceridae and Hemiptera, while the numbers of female *P. zetterstedti* were in between these
 361 two groups (Fig. 3A; LT).

362 3.3.2 South of the Alps

363 In the southern region, the respective insect groups likewise differed from each other in their absolute
 364 abundances in both floral chambers (Kruskal–Wallis, $\chi^2 = 222.46$, $df = 7$, $N = 1072$, $P < 0.001$) and
 365 light traps (Kruskal–Wallis, $\chi^2 = 20.34$, $df = 7$, $N = 40$, $P = 0.005$). In the southern floral chambers,
 366 however, Sphaeroceridae were the dominating taxon, followed by male psychodids, female *P.*
 367 *grisescens*, female *P. phalaenoides* and Chironomidae (Fig. 3B; FC). The remaining taxa, female *P.*
 368 *zetterstedti*, Ceratopogonidae and Hemiptera, occurred in very few chambers (Fig. 3B; FC). In
 369 contrast to the northern region, almost all insect groups found in the southern light traps exhibited
 370 similarly low abundances (Fig. 3B; LT). As the only exception, Chironomidae were more abundant
 371 than all other insect groups, followed by Ceratopogonidae and the remaining insect groups (Fig. 3B;
 372 LT).

373 3.3.3 Comparison between northern and southern regions

374 There was no significant difference between northern and southern floral chambers with regard to the
 375 total abundances of male psychodids and female *P. grisescens*, although higher numbers of these
 376 groups were attracted by the light at northern sites (see Fig. 3). Female *P. phalaenoides* and
 377 Ceratopogonidae were significantly more abundant in the north than in the south, both in floral
 378 chambers and light traps; the opposite was true for Sphaeroceridae, which were significantly more
 379 abundant in the south compared to the north, both in floral chambers and light traps (Fig. 3). Females
 380 of *P. zetterstedti* were more abundant in northern than southern chambers, but their numbers were
 381 similar in the light traps of each region (Fig. 3). Although Hemiptera were generally low in
 382 abundance, they were more frequent in the southern chambers, but similarly abundant in the light
 383 traps of the two regions. Finally, Chironomidae exhibited similar numbers in the two regions, both in
 384 floral chambers and light traps with high abundances in the light traps and low abundances in floral
 385 chambers (Fig. 3). Overall, these results indicate that differences in visitor assemblages within and
 386 between the two regions can only partly be explained by the light trap catches (see Discussion).

387 3.4 Effects of total visitor abundance on fruit set

388 Floral visitor abundance was a significant predictor of the presence of an infructescence in both north
 389 ($P = 0.004$, $df = 125$; performance of the GLM, hereinafter referred to as GLM fit: $\chi^2 = 9.26$, $P =$
 390 0.002) and south ($P < 0.001$, $df = 131$; GLM fit: $\chi^2 = 19.35$, $P < 0.001$) of the Alps (Supporting
 391 Information Fig. S2). Fruit set was highly variable (Fig. 4) and the mean fruit set among the
 392 populations ranged between 1% and 86.1% in both of the regions (Supporting Information Table S2).
 393 When pooled across regions, the overall visitor abundance had a significant effect (slope = 11.06, $P =$
 394 0.005) on fruit set (performance of the LM, hereinafter referred to as LM fit: adj. $R^2 = 0.13$, $df = 256$,
 395 $P < 0.001$). Fruit set was significantly lower in the south than in the north [$\Delta(\text{South–North}) = -27.40$,
 396 $P = 0.002$], whereby the slopes for the two regions also differed significantly, indicating a stronger
 397 effect of visitor abundance on fruit set in the south ($\Delta\text{slopes} = 21.19$, $P = 0.009$; Fig. 4A). The LMs
 398 constructed separately for each region revealed a significant positive effect of the number of floral
 399 visitors on fruit set (higher insect numbers resulted in higher fruit set) in both the south

400 (slope = 32.25, $P < 0.001$; LM fit: adj. $R^2 = 0.18$, $df = 131$, $P < 0.001$; Fig. 4A, Supporting
 401 Information Table S3) and the north (slope = 11.06, $P = 0.01$; LM fit: adj. $R^2 = 0.04$, $df = 125$, $P =$
 402 0.01; Fig. 4A).

403 When only considering floral chambers with a maximum of 71 insects (i.e. the highest number
 404 observed in the south; LM fit: adj. $R^2 = 0.18$, $df = 231$, $P < 0.001$), fruit set was significantly lower,
 405 again, in the south [$\Delta(\text{South-North}) = -9.83$, $P = 0.03$; Fig. 4B], and the effect of visitor abundance
 406 (pooled across regions) on fruit set was also significant (slope = 30.51, $P < 0.001$). Interestingly,
 407 based on this analysis, although the total number of insects correlated positively with fruit set in the
 408 north (Fig. 4B, Supporting Information Table S3), no such correlation was detected in this region
 409 when only chambers with more than 71 insects were considered (LM fit: adj. $R^2 = -0.04$, $df = 24$, $P =$
 410 0.78; Fig. 4B).

411 3.5 Effects of individual insect groups on fruit set

412 When testing for relationships between the number of single insect groups and fruit set, significant
 413 effects (slopes) were detected in each of the two regions for (1) pooled Psychodidae (i.e. male
 414 psychodids, females of *Psychoda brevicornis*, *P. grisescens*, *P. phalaenoides*, *P. sp. 1*, *P.*
 415 *trinodulosa*, and *P. zetterstedti*, unidentified females, unknown sex; Supporting Information Table
 416 S2); and (2) *P. phalaenoides* females (Fig. 5, Supporting Information Table S3). In the south,
 417 significant effects were additionally detected for (1) pooled non-psychodids (i.e. Cecidomyiidae,
 418 Ceratopogonidae, Chironomidae, Sciaridae, Sphaeroceridae, unidentified Diptera, Coleoptera,
 419 Hemiptera and unidentified Insecta; Supporting Information Table S2); (2) male psychodids; (3) *P.*
 420 *grisescens* females; (4) Chironomidae; and (5) Sphaeroceridae (Fig. 5, Supporting Information Table
 421 S3). Interestingly, pooled Psychodidae and female *P. phalaenoides* had the same effects on fruit set
 422 north and south of the Alps (no differences in the intercept), while for all other insect groups tested,
 423 the intercept of the model was higher in the north than in the south, i.e. for a given number of
 424 individuals, the fruit set was higher in the north (Fig. 5, Supporting Information Table S3).

425 3.6 Effects of the abundance of all visitors and of individual insect groups on the number of 426 seeds per fruit

427 The number of seeds per fruit varied in both regions (north: median = 1.12, range = 1–3.46; south:
 428 median = 1.18, range = 1–2.5), with no significant differences between the regions [$\Delta(\text{South-North})$
 429 = 0.03, $P = 0.64$; LM fit: adj. $R^2 = -0.01$, $df = 113$, $P = 0.82$]. Moreover, no correlation was detected
 430 between the abundance of floral visitors and the number of seeds per fruit both in the global LM
 431 (pooled across regions; slope = 0.03, $P = 0.58$) and in the LMs constructed separately for each region
 432 (north: slope = 0.08, $P = 0.23$; LM fit: $R^2 = 0.007$, $df = 63$, $P = 0.23$; south: slope = -0.16 , $P = 0.15$;
 433 LM fit: $R^2 = 0.02$, $df = 49$, $P = 0.15$) (Supporting Information Fig. S3). Similar results were obtained
 434 when the same analysis was performed for the northern region, considering only chambers with
 435 either a maximum of 71 insects (slope = 0.18, $P = 0.09$; LM fit: $R^2 = 0.04$, $df = 47$, $P = 0.09$) or more
 436 insects (slope = 0.21, $P = 0.60$; LM fit: $R^2 = -0.05$, $df = 14$, $P = 0.60$) (Supporting Information Fig.
 437 S3). Furthermore, no correlation was detected between the abundance of individual insect groups and
 438 the number of seeds per fruit for both regions before the breakpoint (adj. $R^2 < 0.01$, $P > 0.05$).

439 3.7 Pollen loads

440 In total, 188 insect specimens were examined for pollen load (i.e. 154 females of *P. phalaenoides*, 17
 441 females of *P. grisescens*, eight Chironomidae and nine Sphaeroceridae) (Fig. 6, Supporting
 442 Information Fig. S1, S4). Of those, 23 specimens carried no pollen grains (i.e. 15 females of *P.*

443 *phalaenoides*, five females of *P. grisescens*, one Chironomidae and two Sphaeroceridae). Overall, the
 444 amount of pollen grains per individual was highly variable (range = 0–542). The median number of
 445 pollen grains was higher in Chironomidae (median = 187, range = 0–542) than in Sphaeroceridae
 446 (48, 0–385), *P. phalaenoides* (30, 0–475) and *P. grisescens* (27, 0–223); however, there were no
 447 significant differences in the number of pollen grains across these insect groups (Kruskal–Wallis, χ^2
 448 = 7.03, $df = 3$, $N = 188$, $P = 0.07$) (Fig. 6). Pollen grains were also detected on male psychodids
 449 (median = 18, range = 0–72, $N = 4$) (Supporting Information Fig. S4), Ceratopogonidae (11, 0–47, N
 450 = 4), Sciaridae (8, 0–380, $N = 3$) and Coleoptera (10, 1–27, $N = 3$), but these groups were excluded
 451 from statistical analysis due to small sample sizes.

452 4 Discussion

453 Based on our insect collections from floral chambers (Fig. 1, Supporting Information Fig. S1),
 454 individuals of *Arum maculatum* from north of the Alps attracted more visitors than southern ones.
 455 Moreover, the visitor assemblages of northern plants were strongly dominated by females of
 456 *Psychoda phalaenoides* (Figs. 2A and 3A). By contrast, in the south, Sphaeroceridae were the most
 457 abundant insect visitors, followed by female *P. grisescens*, male psychodids and Chironomidae (Figs.
 458 2A and 3B). When combined with our light trap results (Figs. 2B and 3), these differences in visitor
 459 assemblages across the Alps could only be partially explained by the insects available in the different
 460 regions. Moreover, in each region, fruit set was quite variable, but significantly higher in the north
 461 than in the south, and positively correlated with the total number of trapped insects as well as some,
 462 but not all specific visitor groups (Fig. 5, Supporting Information Table S3). In the north, however,
 463 this positive relationship disappeared when numbers of trapped insects were too high (Fig. 4B). The
 464 number of seeds per fruit did not differ between the regions and did not correlate with the overall
 465 abundance of floral visitors or with the abundance of individual insect groups (Supporting
 466 Information Fig. S3). Not only Psychodidae but also other fly taxa were found to carry high pollen
 467 loads, with amounts similar to those of psychodids (Fig. 6).

468 4.1 Does local insect availability shape the visitor assemblages of *A. maculatum* populations 469 from north vs. south of the Alps?

470 The visitor assemblages we observed in *A. maculatum* populations from north vs. south of the Alps
 471 broadly concur with those reported by Espíndola et al. (2011), especially in terms of total and relative
 472 abundances of single psychodid species and the total abundance of other Diptera. For the southern
 473 region, however, we newly identified Sphaeroceridae as abundant visitors (Figs. 2A and 3). This
 474 dipteran family has so far only been reported for a single northern (southwest German) population of
 475 *A. maculatum* (Roháček et al., 1990). That said, previous visitor collections from south of the Alps
 476 might well include specimens of Sphaeroceridae, yet classified as suborder Brachycera (Espíndola et
 477 al., 2011; Szenteckzki et al., 2021). Regardless, the overall floral visitation patterns reported by
 478 Espíndola et al. (2011) are remarkably similar to those of the present study, suggesting spatial and
 479 temporal consistency in the regionally differing visitor assemblages across the Alps. It is noteworthy,
 480 however, that beyond our study region, Szenteckzki et al. (2021) reported some temporal variation in
 481 floral visitors of *A. maculatum* populations in France, Croatia and Serbia, after having re-surveyed
 482 those studied by Espíndola et al. (2011).

483 As evidenced by our light trap results, all psychodid groups, apart from female *P. zetterstedti*,
 484 exhibited similarly high and low abundances in the northern and southern regions, respectively (Fig.
 485 3; LT). Possible factors driving those inter-regional differences may include climatic conditions
 486 (Espíndola et al., 2011) and/or the availability of suitable habitats, which might be limited for these

487 psychodid taxa in the south (R. Wagner, unpubl. res.). Further ecological investigations are needed to
 488 identify the underlying causes of the high and low abundances of Psychodidae north and south of the
 489 Alps, respectively.

490 Based on our comparative analyses of floral visitor and light trap catches (Figs. 2 and 3), local insect
 491 availability can only partly explain the different visitor assemblages of *A. maculatum* from north vs.
 492 south of the Alps. Specifically, both the higher overall abundance of floral visitors and the higher
 493 absolute abundance of female *P. phalaenoides* north than south of the Alps can be explained by the
 494 locally available insects (Figs. 2B and 3A). Similarly, the higher relative and absolute floral
 495 abundances of Sphaeroceridae in the south can be explained by their higher availability in this region
 496 (Figs. 2B and 3B). These findings confirm a previous hypothesis by Chartier et al. (2013), proposing
 497 that local insect availability plays an important role in shaping the composition and abundance of
 498 floral visitors in *A. maculatum*.

499 However, the high relative abundances of female *P. phalaenoides* in the northern floral chambers
 500 (Fig. 2A), and the similar absolute floral abundances of female *P. grisescens* and male psychodids in
 501 northern and southern populations (Fig. 3), do not reflect the availability of these three insect groups
 502 in the respective regions. Instead, northern individuals of *A. maculatum* appear to be relatively more
 503 attractive to female *P. phalaenoides* than southern individuals, which preferentially trapped female *P.*
 504 *grisescens* and male psychodids (Fig. 3). Our results therefore do not support the hypothesis that the
 505 relatively higher visitation of southern *A. maculatum* by female *P. grisescens* is due to the higher
 506 availability of this species (compared to *P. phalaenoides*) in the Mediterranean region (Espíndola et
 507 al., 2011). On the other hand, the hypothesis of high attractiveness of northern *A. maculatum* to
 508 female *P. phalaenoides* inferred herein could also explain why this specific interaction has been
 509 repeatedly observed in other northern locations of the plant's distribution (Chartier et al., 2013; Diaz
 510 & Kite, 2002; Espíndola et al., 2011; Gibernau 2016; Lack & Diaz, 1991). The recently reported
 511 differences in scent bouquets between *A. maculatum* populations from north vs. south of the Alps
 512 (Gfrerer et al., 2021) might play a key role in the differential attraction of the psychodid moth flies.
 513 To test this hypothesis, behavioural assays assessing whether these psychodid species respond
 514 differently to northern vs. southern scent bouquets would be needed. Furthermore, it would be of
 515 interest to test whether the behaviour of specific pollinator taxa differs among regions.

516 **4.2 Are regional differences in plant reproductive success caused by variations in pollinator**
 517 **abundance, effectiveness and/or resource limitation?**

518 The number of seeds per fruit was similar between the regions, but we observed lower levels of fruit
 519 set in southern than in northern populations of *A. maculatum* (Fig. 4, Supporting Information Table
 520 **S2** and Fig. **S3**). Taken together, these findings show that northern plants have a higher seed
 521 production than southern ones. The lower fruit set in the south is in agreement with the lower
 522 abundances of floral visitors in this region (Figs. 1, 4 and 3, Supporting Information Table **S2**).
 523 However, this inter-regional difference in fruit set is not due to differences in the numbers of insects
 524 trapped, as the same visitor abundance yielded higher fruit set in the north (Fig. 4B). Possible
 525 explanations for this difference involve higher rates of fruit abortion in southern populations, which
 526 could be caused by stronger resource limitation (Albre et al., 2003; Ollerton & Diaz, 1999; Suzuki,
 527 2000; Uemura et al., 1993) and/or bad weather conditions during flowering in this region (Kite, 1995;
 528 Wilcock & Neiland, 2002). Maternal effects might also differently affect the percentage of flowers
 529 setting fruit (Richardson & Stephenson, 1991) in the two regions. Furthermore, a relatively higher
 530 pollination effectiveness of northern, psychodid dominated, visitor assemblages could also explain

531 this inter-regional difference in fruit set (Ne'eman et al., 2010; Ollerton et al., 2007; Willcox et al.,
532 2017).

533 The latter hypothesis gains some support from the significant positive correlation observed between
534 the abundance of pooled Psychodidae and fruit set in both regions (Fig. 5, Supporting Information
535 Table S3) and the disappearance of significant inter-regional differences in fruit set, when the
536 numbers of trapped Psychodidae and female *P. phalaenoides* were similar between the regions (Fig.
537 5, Supporting Information Table S3). Both findings confirm the pollination effectiveness of
538 Psychodidae in *A. maculatum* (Diaz & Kite, 2002; Lack & Diaz, 1991); however, they also suggest
539 that reduced fruit set in the south could be due to the lower floral abundance of psychodids there
540 (Fig. 5, Supporting Information Table S3). Diaz and Kite (2002) reported that the high pollination
541 effectiveness of *P. phalaenoides* could be related to the high amount of pollen carried, also by
542 comparison with non-psychodid flies (e.g. Chironomidae: *Smittia pratorum*). Although we found no
543 higher pollen loads on psychodids than non-psychodids (Fig. 6), the former still might be the most
544 efficient pollinators of *A. maculatum* due to certain behavioural characteristics (e.g. frequent
545 movements around stigmas, less efficient cleaning behaviour: Borkent & Schlinger, 2008; Ne'eman
546 et al., 2010) or morphological traits (dense hairiness: Stavert *et al.*, 2016).

547 Nonetheless, even if less effective, our results suggest that non-psychodids (e.g. Chironomidae,
548 Sphaeroceridae) also contribute to the pollination of *A. maculatum* (Fig. 5, Supporting Information
549 Table S3), as already demonstrated for Sphaeroceridae in Mediterranean *A. pictum* (Quilichini et al.,
550 2010) and in an Australian aroid, *Typhonium eliosurum* (Sayers et al., 2020). This hypothesis is also
551 supported by preliminary tests, where Sphaeroceridae were transferred from male to female stage
552 inflorescences of *A. maculatum*, resulting in fruit set (M. Schleifer et al., unpubl. res.). Given the
553 scarcity of Psychodidae in areas south of the Alps (Figs. 1 and 3, Supporting Information Table S2),
554 it is feasible that southern populations of *A. maculatum* evolved a more generalized pollination
555 strategy by additionally attracting other, locally more abundant insect groups to counterbalance
556 negative effects on fruit set (see also Bustamante et al., 2010; Hallett et al., 2017).

557

558 **4.3 Disappearance of the positive relationship between visitor abundance and fruit set at too** 559 **high visitor numbers**

560 The common assumption of a positive correlation between visitation rate and fruit set has recently
561 been challenged (Sáez et al., 2014; Willcox et al., 2017). Sáez et al. (2014) found that overall fruit set
562 in *Rubus idaeus* (Rosaceae) decreased with extremely high visitation rates of *Apis mellifera* and
563 *Bombus terrestris*, due to damage of the styles. In Araceae, we are aware of similar results only in
564 *Dieffenbachia longispatha*, where fruit set peaked at intermediate abundances of pollinating beetles
565 and decreased at higher and lower abundances (Young, 1988). This was also explained by cumulative
566 floral damage caused by the beetles' activities (i.e. feeding, mating; Young, 1988). Although adult
567 *Psychoda* flies lack feeding mouth parts (Oosterbroek, 2006), their activity inside the floral chamber
568 of *A. maculatum* might also cause floral damage, e.g., on the stigmatic papillae. Our finding (Fig. 4B)
569 might also be explained by an excess of pollen with a high number of simultaneously growing pollen
570 tubes preventing each other from reaching the ovules (Ashman et al., 2004; Holland & Chamberlain,
571 2007).

572 **4.4 Conclusions**

573 This study highlights how quantitative assessments of floral visitor assemblages in relation to locally
574 available insect communities help understand the basis of intraspecific variation in plant-pollinator
575 interactions. To the best of our knowledge, this combined approach, which independently assesses
576 floral visitation and local insect availability at each site, has never been applied before. We used light
577 traps to quantify local insect availability, but other methods, such as Malaise or pitfall traps (Borkent
578 et al., 2018; Sayers et al., 2020), need to be applied when working with strictly diurnal insects, or
579 more generally, with pollinators that are not attracted by light sources. Consistent with previous
580 studies, we found that psychodids are important pollinators of *A. maculatum*; however, our results
581 also imply other insects, such as Sphaeroceridae and Chironomidae, as pollinators of this species.
582 Future studies are needed to compare the pollination effectiveness of different visitors (psychodids
583 and non-psychodids) and to better understand the high variation in fruit set of *A. maculatum*. Such
584 studies would also reveal whether the lower fruit set observed in southern populations results from
585 lower effectiveness of southern visitor assemblages or other factors, such as resource limitation.

586 **5 Conflict of Interest**

587 The authors declare that the research was conducted in the absence of any commercial or financial
588 relationships that could be construed as a potential conflict of interest.

589 **6 Author Contributions**

590 SD, MG, ACH and HPC designed the research; EG and DL conducted the fieldwork; DL and RW
591 identified the psychodid specimens; VS and CZ identified the non-psychodid specimens; MS
592 assessed the pollen loads; DL and RF performed the statistical analyses; DL wrote the first draft of
593 the manuscript and all authors contributed to the final version.

594 **7 Funding**

595 This study was funded by a grant from FWF (Austrian Science Fund; P30175-B29) to ACH, HPC
596 and SD (PI). All sampling conducted comply with the current laws of the respective countries.

597 **8 Acknowledgments**

598 We thank Karin Moosbrugger, Bernadette Mükisch, S. Sophie Brandauer and Christopher Gorofsky
599 for field assistance, Alexander Gamisch for providing the European map, and Florian Schiestl and
600 Filippo Prosser for their help in finding suitable *A. maculatum* populations.

601 **9 Supplementary Material**

602 Supplementary Material is available for this paper.

603 **10 Data Availability Statement**

604 The raw data supporting the conclusions of this article will be made available by the authors, without
605 undue reservation, to any qualified researcher.

606 **11 References**

607 Aigner PA. 2001. Optimality modeling and fitness trade-offs: when should plants become pollinator
608 specialists? *Oikos* 95: 177–184.

- 609 Albre J, Quilichini A, Gibernau M. 2003. Pollination ecology of *Arum italicum* (Araceae). *Botanical*
610 *Journal of the Linnean Society* 141: 205–214.
- 611 Amante C, Eakins BW. 2009. *ETOPO1 1 Arc-Minute Global Relief Model: Procedures, Data Sources*
612 *and Analysis*. NOAA Technical Memorandum NESDIS NGDC-24. Boulder: National
613 Geophysical Data Center.
- 614 Armbruster WS. 2017. The specialization continuum in pollination systems: diversity of concepts and
615 implications for ecology, evolution and conservation. *Functional Ecology* 31: 88–100.
- 616 Ashman TL. 2000. Pollinator selectivity and its implications for the evolution of dioecy and sexual
617 dimorphism. *Ecology* 81: 2577–2591.
- 618 Ashman TL, Knight TM, Steets JA, *et al.* 2004. Pollen limitation of plant reproduction: ecological and
619 evolutionary causes and consequences. *Ecology* 85: 2408–2421.
- 620 Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4.
621 *Journal of Statistical Software* 67: 1–48.
- 622 Bermadinger-Stabentheiner E, Stabentheiner A. 1995. Dynamics of thermogenesis and structure of
623 epidermal tissues in inflorescences of *Arum maculatum*. *New Phytologist* 131: 41–50.
- 624 Borkent CJ, Schlinger EI. 2008. Pollen loads and pollen diversity on bodies of *Eulonchus tristis*
625 (Diptera: Acroceridae): implications for pollination and flower visitation. *The Canadian*
626 *Entomologist* 140: 257–264.
- 627 Borkent A, Brown BV, Adler PH, *et al.* 2018. Remarkable fly (Diptera) diversity in a patch of Costa
628 Rican cloud forest: why inventory is a vital science. *Zootaxa* 4402: 53–90.
- 629 Boyce PC. 2006. *Arum* – A decade of change. *Aroideana* 29: 132–137.
- 630 Bröderbauer D, Diaz A, Weber A. 2012. Reconstructing the origin and elaboration of insect-trapping
631 inflorescences in the Araceae. *American Journal of Botany* 99: 1666–1679.
- 632 Bustamante E, Casas A, Búrquez A. 2010. Geographic variation in reproductive success of
633 *Stenocereus thurberi* (Cactaceae): effects of pollination timing and pollinator guild. *American*
634 *Journal of Botany* 97: 2020–2030.
- 635 Campbell DR. 1987. Interpopulational variation in fruit production: the role of pollination-limitation in
636 the Olympic Mountains. *American Journal of Botany* 74: 269–273.
- 637 Chartier M, Pélozuelo L, Gibernau M. 2011. Do floral odor profiles geographically vary with the
638 degree of specificity for pollinators? Investigation in two sapromyophilous *Arum* species
639 (Araceae). *Annales de la Société Entomologique de France* 47: 71–77.
- 640 Chartier M, Gibernau M. 2009. Size variations of flowering characters in *Arum maculatum* (Araceae).
641 *Aroideana* 32: 153–158.
- 642 Chartier M, Pélozuelo L, Buatois B, Bessièrè JM, Gibernau M. 2013. Geographical variations of odour
643 and pollinators, and test for local adaptation by reciprocal transplant of two European *Arum*
644 species. *Functional Ecology* 27: 1367–1381.
- 645 Chinery M. 1973. *A field guide to the insects of Britain and Northern Europe*, 1st edn. London: Collins
646 Publishing.
- 647 Chouteau M, Gibernau M, Barabé D. 2008. Relationships between floral characters, pollination
648 mechanisms, life forms, and habitats in Araceae. *Botanical Journal of the Linnean Society* 156:
649 29–42.
- 650 Clarke KR, Gorley R. 2005. *Primer: Getting started with v6*. Plymouth: Primer-E Ltd.
- 651 Cosacov A, Cocucci AA, Sérsic AN. 2014. Geographical differentiation in floral traits across the
652 distribution range of the Patagonian oil-secreting *Calceolaria polyrhiza*: do pollinators matter?
653 *Annals of Botany* 113: 251–266.
- 654 Diaz A, Kite GC. 2002. A comparison of the pollination ecology of *Arum maculatum* and *A. italicum*
655 in England. *Watsonia* 24: 171–181.
- 656 Dodd ME, Silvertown J, Chase MW. 1999. Phylogenetic analysis of trait evolution and species
657 diversity variation among angiosperm families. *Evolution* 53: 732–744.

- 658 Espíndola A, Alvarez N. 2011. Comparative phylogeography in a specific and obligate pollination
659 antagonism. *PloS one* 6: e28662.
- 660 Espíndola A, Pellissier L, Alvarez N. 2011. Variation in the proportion of flower visitors of *Arum*
661 *maculatum* along its distributional range in relation with community-based climatic niche
662 analyses. *Oikos* 120: 728–734.
- 663 Faucheux MJ, Gibernau M. 2011. Antennal sensilla in five Psychodini mothflies (Diptera:
664 Psychodidae: Psychodinae) pollinators of *Arum* spp. (Araceae). *Annales de la Société*
665 *Entomologique de France* 47: 89–100.
- 666 Fausto JA, Eckhart VM, Geber MA. 2001. Reproductive assurance and the evolutionary ecology of
667 self-pollination in *Clarkia xantiana* (Onagraceae). *American Journal of Botany* 88: 1794–1800.
- 668 Gfrerer E, Laina D, Gibernau M, *et al.* 2021. Floral scents of a deceptive plant are hyperdiverse and
669 under population-specific phenotypic selection. *Frontiers in Plant Science* 12: 719092.
- 670 Gibernau M, Macquart D, Przetak G. 2004. Pollination in the genus *Arum* – a review. *Aroideana* 27:
671 148–166.
- 672 Gibernau M. 2016. Pollinators and visitors of Aroid inflorescences III – phylogenetic & chemical
673 insights. *Aroideana* 39: 4–22.
- 674 Gómez JM, Abdelaziz M, Camacho JPM, Muñoz-Pajares AJ, Perfectti F. 2009a. Local adaptation and
675 maladaptation to pollinators in a generalist geographic mosaic. *Ecology Letters* 12: 672–682.
- 676 Gómez JM, Perfectti F, Bosch J, Camacho JPM. 2009b. A geographic selection mosaic in a
677 generalized plant-pollinator-herbivore system. *Ecological Monographs* 79: 245–263.
- 678 Gómez JM, Muñoz-Pajares AJ, Abdelaziz M, Lorite J, Perfectti F. 2014. Evolution of pollination
679 niches and floral divergence in the generalist plant *Erysimum mediohispanicum*. *Annals of*
680 *Botany* 113: 237–249.
- 681 Govaerts R, Bogner J, Boos J, *et al.* 2020. *World Checklist of Araceae (and Acoraceae)*. Kew: Royal
682 Botanic Gardens. <http://wmsp.science.kew.org/> (8 December 2020, date last accessed).
- 683 Grant V, Grant KA. 1965. *Flower pollination in the phlox family*. New York: Columbia University
684 Press.
- 685 Hallett AC, Mitchell RJ, Chamberlain ER, Karron JD. 2017. Pollination success following loss of a
686 frequent pollinator: the role of compensatory visitation by other effective pollinators. *AoB*
687 *PLANTS* 9: 1–11.
- 688 Hernández - Hernández T, Wiens JJ. 2020. Why are there so many flowering plants? A multi-scale
689 analysis of plant diversification. *The American Naturalist* 195: 948–963.
- 690 Herrera CM. 1989. Pollinator abundance, morphology, and flower visitation rate: analysis of the
691 “quantity” component in a plant-pollinator system. *Oecologia* 80: 241–248.
- 692 Holland JN, Chamberlain SA. 2007. Ecological and evolutionary mechanisms for low seed:ovule
693 ratios: need for a pluralistic approach? *Ecology* 88: 706–715.
- 694 Hothorn T, Hornik K, van de Wiel MA, Zeileis A. 2008. Implementing a class of permutation tests:
695 The coin package. *Journal of Statistical Software* 28: 1–23.
- 696 Ishii HS, Kubota MX, Tsujimoto SG, Kudo G. 2019. Association between community assemblage of
697 flower colours and pollinator fauna: a comparison between Japanese and New Zealand alpine
698 plant communities. *Annals of Botany* 123: 533–541.
- 699 Ježek J. 1983. Contribution to the taxonomy of the genus *Logima* Eat. (Diptera, Psychodidae). *Acta*
700 *Entomologica Musei Nationalis Pragae* 41: 213–234.
- 701 Ježek J. 1990. Redescriptions of nine common palaeartic and holarctic species of Psychodini end.
702 (Diptera: Psychodidae). *Acta Entomologica Musei Nationalis Pragae* 43: 33–83.
- 703 Johnson SD, Bond WJ. 1992. Habitat dependent pollination success in a Cape orchid. *Oecologia* 91:
704 455–456.
- 705 Johnson SD. 2010. The pollination niche and its role in the diversification and maintenance of the
706 southern African flora. *Philosophical Transactions of the Royal Society B* 365: 499–516.

Contrasting floral visitor assemblages in *Arum maculatum*

- 707 Johnson SD, Raguso RA. 2016. The long-tongued hawkmoth pollinator niche for native and invasive
708 plants in Africa. *Annals of Botany* 117: 25–36.
- 709 Kalisz S, Vogler DW, Hanley KM. 2004. Context-dependent autonomous self-fertilization yields
710 reproductive assurance and mixed mating. *Nature* 430: 884–887.
- 711 Kite GC. 1995. The floral odour of *Arum maculatum*. *Biochemical Systematics and Ecology* 23: 343–
712 354.
- 713 Kite GC, Hettterscheid WLA, Lewis JM, *et al.* 1998. Inflorescence odours and pollinators of *Arum* and
714 *Amorphophallus* (Araceae). In: Owens SJ, Rudall PJ, eds. *Reproductive biology*. Kew: Royal
715 Botanic Gardens, 295–315.
- 716 Knight TM, Steets JA, Vamosi JC, *et al.* 2005. Pollen limitation of plant reproduction: Pattern and
717 process. *Annual Review of Ecology, Evolution, and Systematics* 36: 467–497.
- 718 Kvitte GM, Wagner R. 2017. 24. Psychodidae (sand flies, moth flies or owl flies). In: Kirk-Spriggs
719 AH, Sinclair BJ, eds. *Manual of Afrotropical Diptera*. Pretoria: South African National
720 Biodiversity Institute, 607–632.
- 721 Lack AJ, Diaz A. 1991. The pollination of *Arum maculatum* L. – a historical review and new
722 observations. *Watsonia* 18: 333–342.
- 723 Majetic CJ, Raguso RA, Ashman TL. 2009. The sweet smell of success: floral scent affects pollinator
724 attraction and seed fitness in *Hesperis matronalis*. *Functional Ecology* 23: 480–487.
- 725 Moeller DA. 2006. Geographic structure of pollinator communities, reproductive assurance, and the
726 evolution of self-pollination. *Ecology* 87: 1510–1522.
- 727 Moeller DA, Geber MA, Eckhart VM, Tiffin P. 2012. Reduced pollinator service and elevated pollen
728 limitation at the geographic range limit of an annual plant. *Ecology* 93: 1036–1048.
- 729 Ne’eman G, Jürgens A, Newstrom-Lloyd L, Potts SG, Dafni A. 2010. A framework for comparing
730 pollinator performance: effectiveness and efficiency. *Biological Reviews* 85: 435–451.
- 731 Newman E, Manning J, Anderson B. 2015. Local adaptation: mechanical fit between floral ecotypes of
732 *Nerine humilis* (Amaryllidaceae) and pollinator communities. *Evolution* 69: 2262–2275.
- 733 Ollerton J, Diaz A. 1999. Evidence for stabilising selection acting on flowering time in *Arum*
734 *maculatum* (Araceae): the influence of phylogeny on adaptation. *Oecologia* 119: 340–348.
- 735 Ollerton J, Killick A, Lamborn E, Watts S, Whiston M. 2007. Multiple meanings and modes: on the
736 many ways to be a generalist flower. *Taxon* 56: 717–728.
- 737 Oosterbroek P. 2006. *The European Families of the Diptera: Identification-Diagnosis-Biology*, 2nd
738 edn. Utrecht: KNNV Publishing.
- 739 Parker AJ, Williams NM, Thomson JD. 2018. Geographic patterns and pollination ecotypes in
740 *Claytonia virginica*. *Evolution* 72: 202–210.
- 741 Pattemore DE, Wilcove DS. 2012. Invasive rats and recent colonist birds partially compensate for the
742 loss of endemic New Zealand pollinators. *Proceedings of the Royal Society B: Biological*
743 *Sciences* 279: 1597–1605.
- 744 Peakall R, Ebert D, Poldy J, *et al.* 2010. Pollinator specificity, floral odour chemistry and the
745 phylogeny of Australian sexually deceptive *Chiloglottis* orchids: implications for pollinator-
746 driven speciation. *New Phytologist* 188: 437–450.
- 747 Phillips RD, Peakall R, van der Niet T, Johnson SD. 2020. Niche perspectives on plant–pollinator
748 interactions. *Trends in Plant Science* 25: 779–793.
- 749 Primack RB, Inouye DW. 1993. Factors affecting pollinator visitation rates: a biogeographic
750 comparison. *Current Science* 65: 257–262.
- 751 Quilichini A, Macquart D, Barabé D, Albre J, Gibernau M. 2010. Reproduction of the West
752 Mediterranean endemic *Arum pictum* (Araceae) on Corsica. *Plant Systematics and Evolution*
753 287: 179–187.
- 754 R Core Team. 2020. *R: a language and environment for statistical computing*. Vienna: R Foundation
755 for Statistical Computing. <https://www.R-project.org/> (17 May 2021, date last accessed).

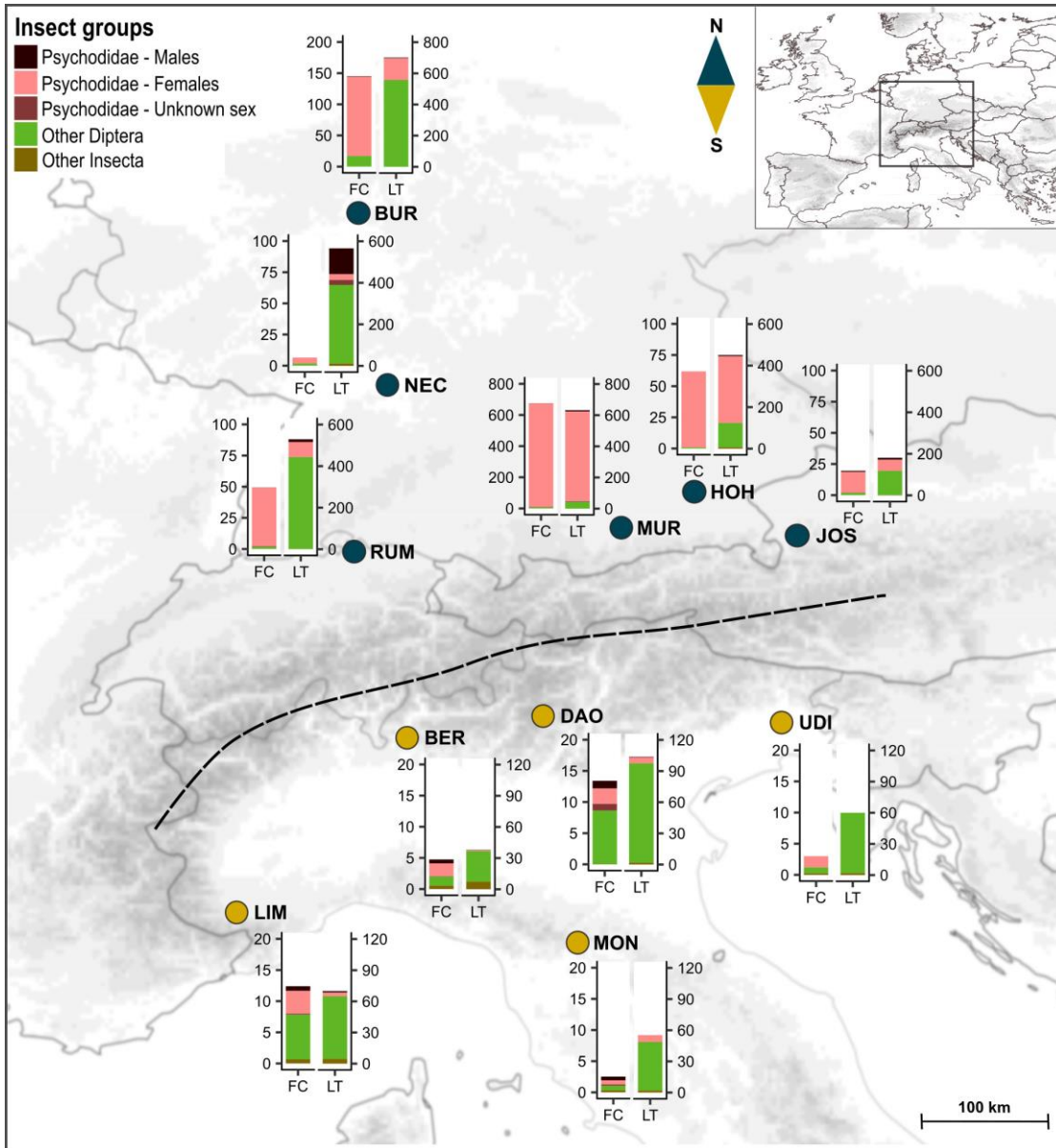
- 756 Rech AR, Jorge LR, Ollerton J, Sazima M. 2018. Pollinator availability, mating system and variation
757 in flower morphology in a tropical savanna tree. *Acta Botanica Brasílica* 32: 462–472.
- 758 Richardson TE, Stephenson AG. 1991. Effects of parentage, prior fruit set and pollen load on fruit and
759 seed production in *Campanula americana* L. *Oecologia* 87: 80–85.
- 760 Roháček J, Beck-Haug I, Dobat K. 1990. Sphaeroceridae associated with flowering *Arum maculatum*
761 (Araceae) in the vicinity of Tübingen, SW-Germany. *Senckenbergiana Biologica* 71: 259–268.
- 762 Sáez A, Morales CL, Ramos LY, Aizen MA. 2014. Extremely frequent bee visits increase pollen
763 deposition but reduce drupelet set in raspberry. *Journal of Applied Ecology* 51: 1603–1612.
- 764 Sayers TDJ, Steinbauer MJ, Farnier K, Miller RE. 2020. Dung mimicry in *Typhonium* (Araceae):
765 explaining floral trait and pollinator divergence in a widespread species complex and a rare sister
766 species. *Botanical Journal of the Linnean Society* 193: 375–401.
- 767 Scheipl F, Greven S, Kuechenhoff H. 2008. Size and power of tests for a zero random effect variance
768 or polynomial regression in additive and linear mixed models. *Computational Statistics & Data*
769 *Analysis* 52: 3283–3299.
- 770 Sowter F. 1949. *Arum maculatum* L. *Journal of Ecology* 37: 207–219.
- 771 Stavert JR, Liñán-Cembrano G, Beggs JR, Howlett BG, Pattermore DE, Bartomeus I. 2016. Hairiness:
772 the missing link between pollinators and pollination. *PeerJ* 4: e2779.
- 773 Suzuki N. 2000. Pollinator limitation and resource limitation of seed production in the Scotch broom,
774 *Cytisus scoparius* (Leguminosae). *Plant Species Biology* 15: 187–193.
- 775 Svensson BW. 2009. Fjärilsmygga-faunan i ett hagmarksområde och en ladugård i östra Blekinges
776 skogsland. Med en översikt av familjen Psychodidae:s morfologi, systematik och utforskande,
777 samt särskilt de svenska *Psychoda* s.l. -arternas biologi. *Entomologisk Tidskrift* 130: 185–208.
- 778 Szenteczki MA, Godschalx AL, Galmán A, *et al.* 2021. Spatial and temporal heterogeneity in
779 pollinator communities maintains within-species floral odour variation. *Oikos* 130: 1487–1499.
- 780 Trunschke J, Sletvold N, Ågren J. 2017. Interaction intensity and pollinator-mediated selection. *New*
781 *Phytologist* 214: 1381–1389.
- 782 Tukey JW. 1977. *Exploratory data analysis*, 1st edn. Reading: Addison-Wesley Publishing.
- 783 Uemura S, Ohkawara K, Kudo G, Wada N, Higashi S. 1993. Heat-production and cross-pollination of
784 the Asian skunk cabbage *Symplocarpus renifolius* (Araceae). *American Journal of Botany* 80:
785 635–640.
- 786 Vaillant F. 1971. 9d. Psychodidae – Psychodinae In: Lindner E, ed. *Die Fliegen der palaearktischen*
787 *Region*. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, 1–48.
- 788 Van der Niet T, Johnson SD. 2012. Phylogenetic evidence for pollinator-driven diversification of
789 angiosperms. *Trends in Ecology and Evolution* 27: 353–361.
- 790 Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996. Generalization in pollination
791 systems, and why it matters. *Ecology* 77: 1043–1060.
- 792 Weber M, Halbritter H, Hesse M. 1999. The basic pollen wall types in Araceae. *International Journal*
793 *of Plant Sciences* 160: 415–423.
- 794 Withers P. 1989. *Moth flies. Diptera: Psychodidae*, 1st edn. Sheffield: Derek Whiteley.
- 795 Wilcock C, Neiland R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends*
796 *in Plant Science* 7: 270–277.
- 797 Willcox BK, Aizen MA, Cunningham SA, Mayfield MM, Rader R. 2017. Deconstructing pollinator
798 community effectiveness. *Current Opinion in Insect Science* 21: 98–104.
- 799 Young HJ. 1988. Differential importance of beetle species pollinating *Dieffenbachia longispatha*
800 (Araceae). *Ecology* 69: 832–844.

801
802

803

804

805 **Figure 1.** Localities of the sampled populations of *Arum maculatum* from north vs. south of the Alps
 806 (blue vs. dark yellow dots). The dashed line indicates the main chain of the Alps. Bar plots show the
 807 mean absolute abundances of insect groups recorded in the floral chambers (FC) and light trap
 808 catches per hour (LT) at each location. Note the differences in y-axis scales between the two regions
 809 and among northern populations. For sample sizes of plant individuals and light trap catches, see
 810 Supplementary Information Table S2. Population abbreviations: North: BUR, Burg Hohenstein;
 811 HOH, Hohendilching in Valley; JOS, Josefiu; MUR, Murnau am Staffelsee; NEC, Horb am Neckar;
 812 RUM, Rümikon; South: BER, Santa Maria Hoé; DAO, Daone; LIM, Limone Piemonte; MON,
 813 Montese; UDI, Udine. The map was prepared using the ETOPO1 Global Relief Model (Amante and
 814 Eakins, 2009) and ArcGIS ver. 10.4 (ESRI, Redland, CA).

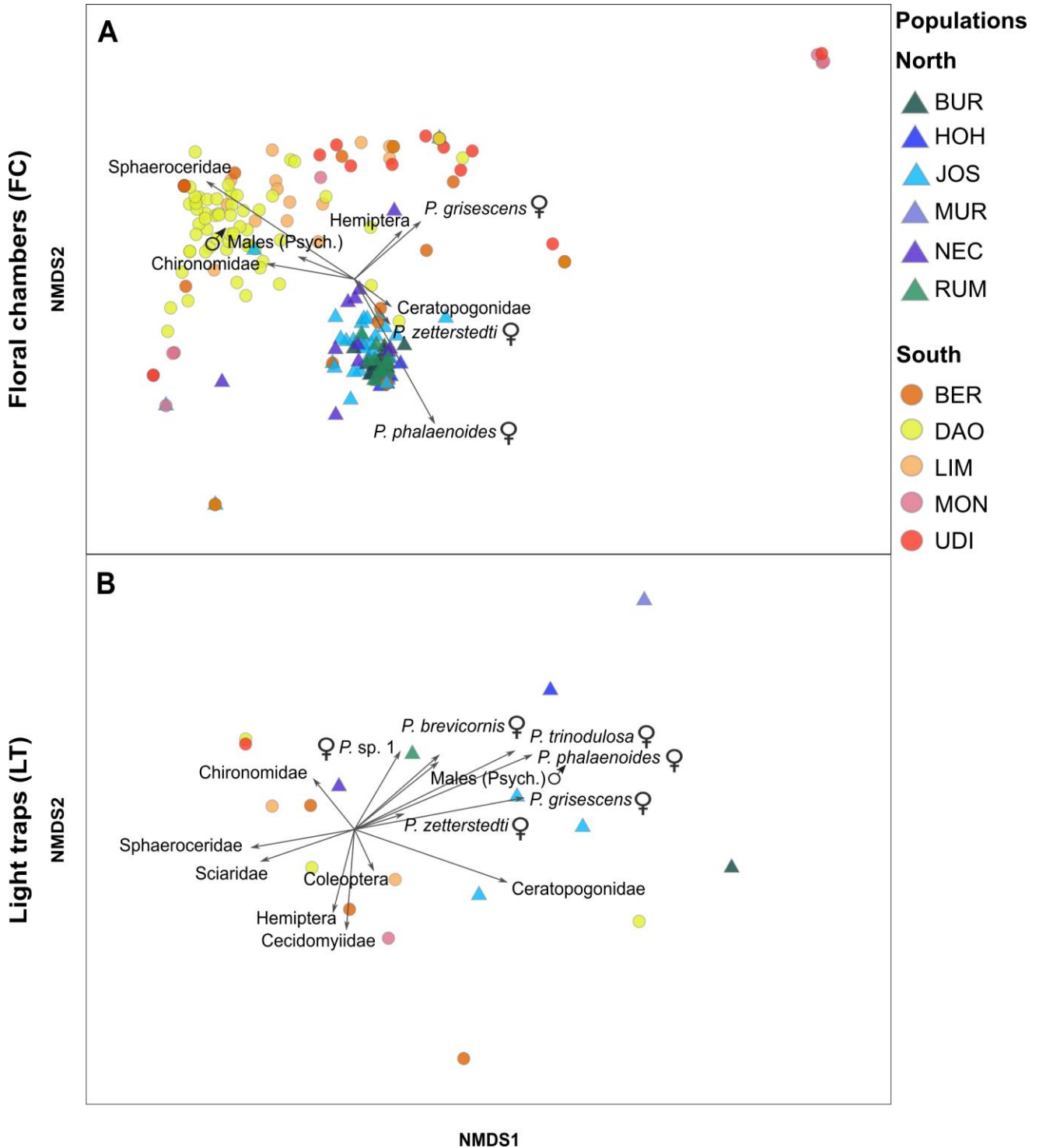


815

816

817

818 **Figure 2.** Non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarities of
 819 relative abundances of insect groups in (A) floral chambers of *Arum maculatum* (stress = 0.11) and
 820 (B) light trap catches (stress = 0.11), as recorded in populations from north (triangles) vs. south
 821 (circles) of the Alps (see Fig. 1 and Supplementary Data Table S1 for population code identification).
 822 Each point (circle or triangle) in ordination space represents either a floral chamber (A) or a light trap
 823 (B). Psych.: Psychodidae, *P.*: *Psychoda*.

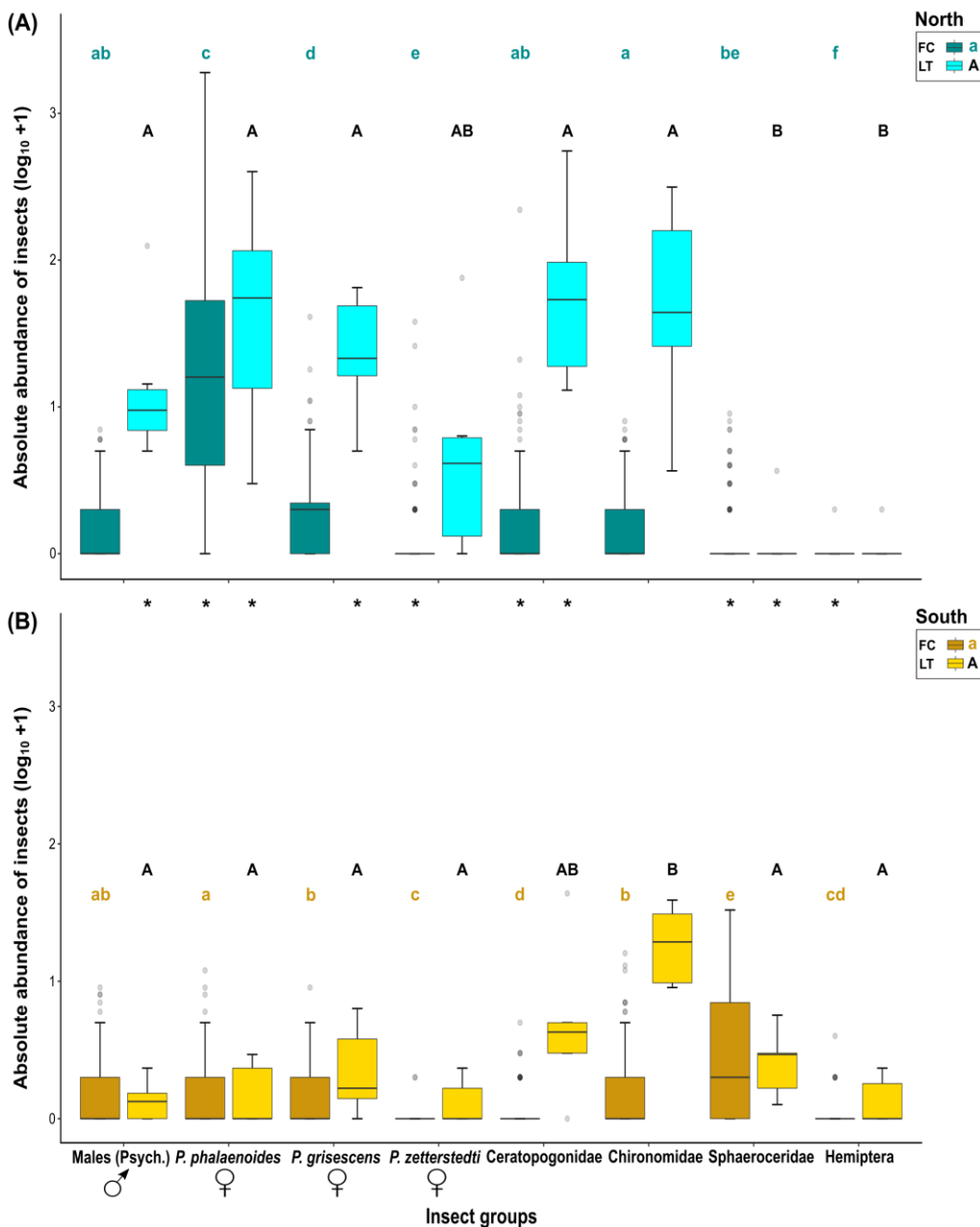


824

825

826

827 **Figure 3.** Absolute abundances ($\log_{10} + 1$) of different insect groups most responsible for similarities
 828 and dissimilarities in relative floral visitor assemblages (see Fig. 2A) in the floral chambers of *Arum*
 829 *maculatum* (FC) and light trap catches per hour (LT), as recorded in populations from (A) north and
 830 (B) south of the Alps, respectively. Different small or capital letters indicate a significant difference
 831 between insect groups within regions for FC or LT respectively, according to exact Wilcoxon–
 832 Mann–Whitney (WMW) post-hoc tests ($P < 0.05$). Asterisks (*) indicate a significant difference
 833 between regions for each insect group, based on asymptotic WMW tests ($P < 0.05$). The bold
 834 horizontal line within each box represents the median of the distribution, and the lower and upper
 835 limits represent the 1st quartile and 3rd quartile. The two whiskers indicate the range of most extreme
 836 values if these are no more than 1.5 times the interquartile distance from the median (Tukey, 1977).
 837 Outliers are depicted as grey circles. Psych.: Psychodidae, P.: *Psychoda*.

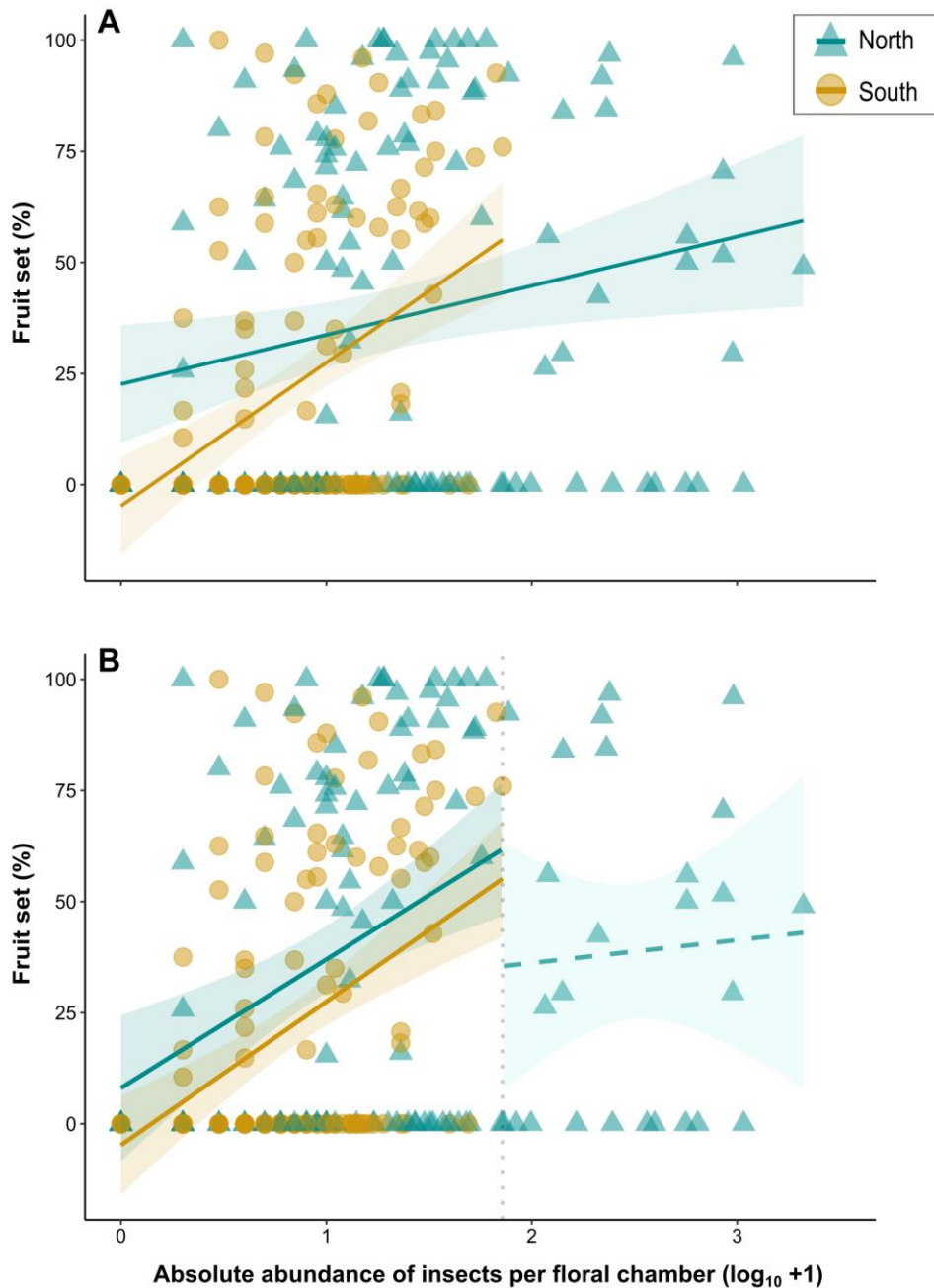


838

839

840

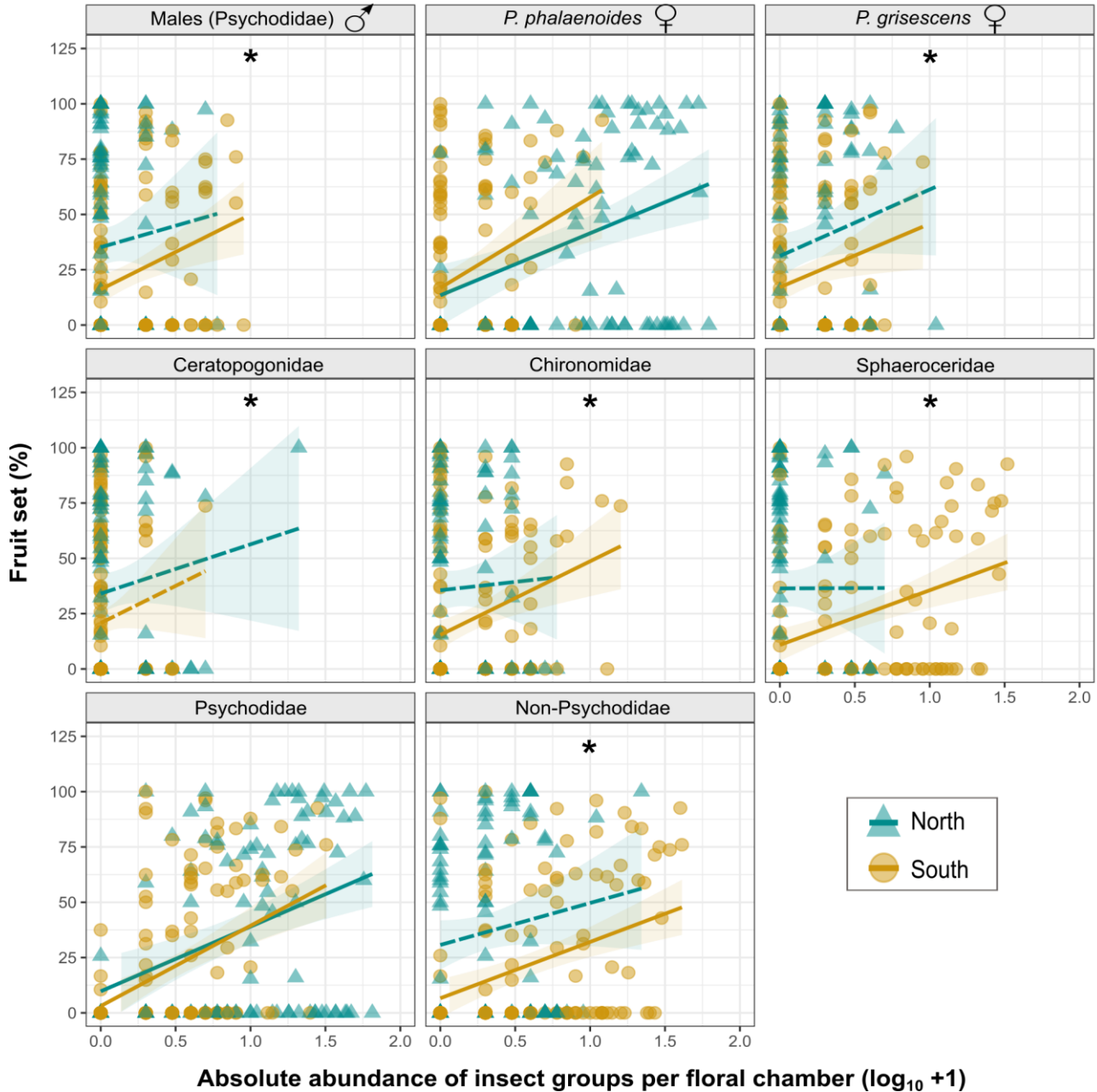
841 **Figure 4.** (A) Linear regressions between fruit set (%) of *Arum maculatum* individuals and absolute
 842 abundance ($\log_{10} + 1$) of visitors per floral chamber for populations from north (blue lines/triangles)
 843 vs. south (dark yellow lines/circles) of the Alps. (B) Linear regressions based on floral chambers with
 844 a maximum of 71 visitors, i.e. the highest number observed in the south (vertical dotted line; see text
 845 for details). The regression line beyond this threshold was calculated separately for the northern
 846 chambers that trapped more than 71 flies. Solid and dashed lines indicate significant and non-
 847 significant model fitting, respectively. Shaded areas indicate 95% confidence intervals (CIs) around
 848 regression lines.



849
850

851

852 **Figure 5.** Linear regressions between fruit set (%) of *Arum maculatum* individuals (see also
 853 Supplementary Information Table S3) and absolute abundance ($\log_{10} + 1$) of different visitor groups
 854 (with a maximum of 71 visitors; see also Fig. 4) per floral chamber for populations from north vs.
 855 south of the Alps (blue lines/triangles vs. dark yellow lines/circles). Psychodidae refer to the pooled
 856 psychodid groups and non-Psychodidae to the pooled non-psychodid groups. Solid and dashed lines
 857 indicate significant and non-significant model fitting, respectively. Shaded areas indicate 95%
 858 confidence intervals (CIs) around regression lines. Asterisks (*) indicate significant differences ($P <$
 859 0.05) in fruit set between the two regions for each insect group.



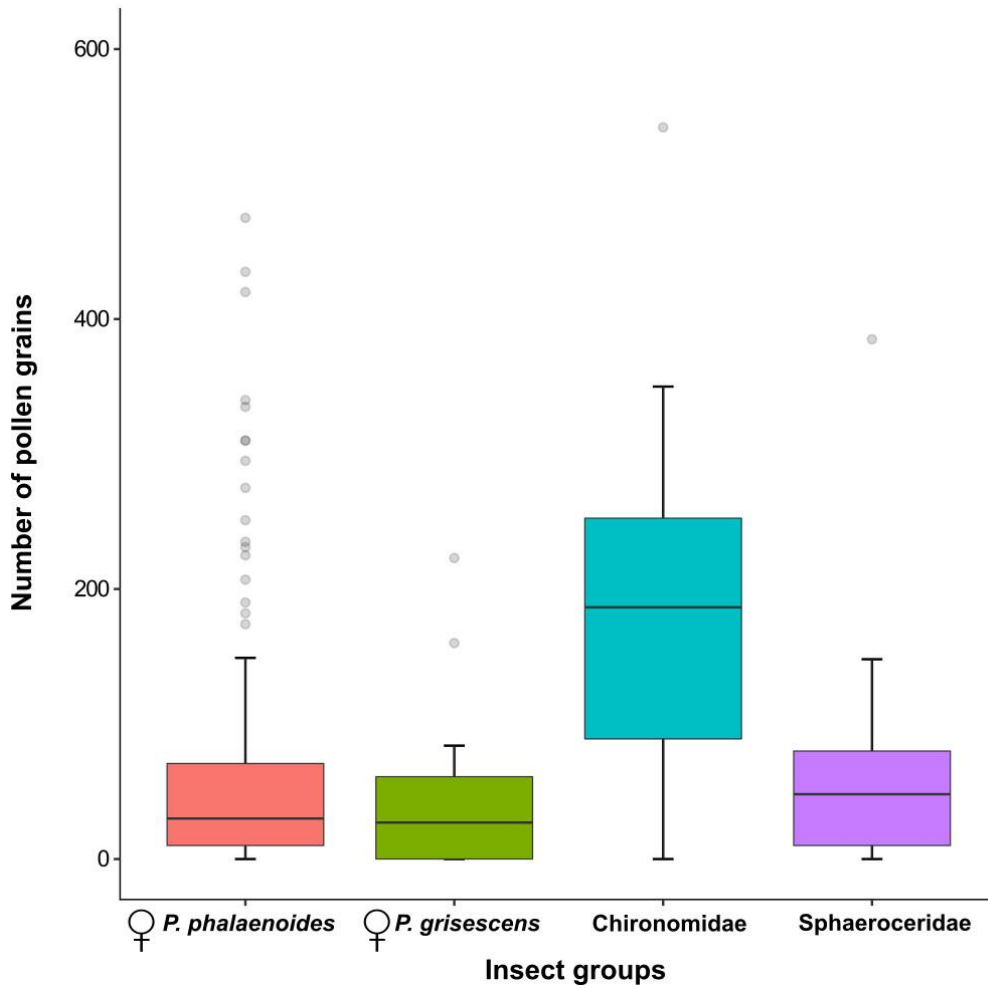
860

861

862

863

864 **Figure 6.** Number of pollen grains per individual for female *Psychoda phalaenoides* ($N = 154$),
 865 female *P. grisescens* ($N = 17$), Chironomidae ($N = 8$) and Sphaeroceridae ($N = 9$). The bold
 866 horizontal line within each box represents the median of the distribution, and the lower and upper
 867 limits represent the 1st quartile and 3rd quartile. The two whiskers indicate the range of most extreme
 868 values if these are no more than 1.5 times the interquartile distance from the median (Tukey, 1977).
 869 Outliers are shown as grey circles. Male psychodids, Ceratopogonidae, Sciaridae and Coleoptera
 870 were not included in this Figure because of their small sample sizes.



871