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Research

Spatial and temporal heterogeneity in pollinator communities maintains within-species floral odour variation

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Flowering plants emit complex bouquets of volatile organic compounds (VOCs) to mediate interactions with their pollinators. These bouquets are undoubtedly influenced by pollinator-mediated selection, particularly in deceptively-pollinated species that rely on chemical mimicry. However, many uncertainties remain regarding how spatially and temporally heterogeneous pollinators affect the diversity and distribution of floral odour variation. Here, we characterized and compared the floral odours of ten populations of deceptively-pollinated *Arum maculatum* (Araceae), and inter-annual and decadal variation in pollinator attraction within these populations. Additionally, we transplanted individuals from all sampled populations to two common garden sites dominated by different pollinator species (*Psychoda phalaenoides* or *Psycha grisescens*), and compared pollinator attraction rates to investigate whether populations maintained odour blends adapted to a specific pollinator. We identified high within- and among-population variation in a common blend of VOCs found across the range of *A. maculatum*. We also observed shifts in pollinator community composition within several populations over 1–2 years, as well as over the past decade. Common garden experiments further revealed that transplanted inflorescences generally attracted the dominant local pollinator species in both transplant sites. However, one population (Forêt du Gâvre, France) appears to exclusively attract *P. grisescens*, even when transplanted to a *P. phalaenoides*-dominated site. Together, our results suggest that maintaining diverse floral odour bouquets within populations may be advantageous when pollinator communities vary over short timescales. We propose that temporally-replicated ecological data are one potential key to understanding variation in complex traits such as floral odour, and in some cases may reveal resiliency to shifting pollinator communities.

Keywords: balancing selection, evolutionary ecology, floral volatiles, plant–pollinator interactions, reciprocal transplant, temporal variation

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Introduction

Widely distributed species are subject to an array of eco-evolutionary settings across their range, due to variation in abiotic conditions and biotic interactions through space and time. These mosaics of selection are known to influence the traits that underpin diverse plant–herbivore and plant–pollinator interactions, affecting the evolutionary trajectories of all species involved (Levin 2000, Hendry 2017). Plant–pollinator interactions appear to be a particularly important mechanism underlying the comparatively rapid rate of speciation in flowering plants (van der Niet and Johnson 2012, Hernández-Hernández and Wiens 2020); this macroevolutionary pattern is the result of microevolutionary processes acting on floral traits at the intraspecific level (Herrera et al. 2006). Understanding the evolutionary and functional ecology of complex floral traits therefore requires data on how plant–pollinator interactions vary through both space and time (Waser and Ollerton 2006). However, characterizing complex trait variation and biotic interactions across wide geographic ranges (Friberg et al. 2019) and through time (Fishbein and Venable 1996) requires substantial effort. As a result, few studies have simultaneously investigated the impact of spatial and temporal variation in pollinators on intraspecific floral trait variation.

As the dominant pollinators across all terrestrial ecosystems, insects exert key selective pressures on many floral traits. They may be attracted or repelled by odour (Whitehead and Peakall 2009), colour (Goyret et al. 2007, du Plessis et al. 2018), morphology (Ibanez et al. 2010, Murúa and Espíndola 2015), nectar (Parachnowitsch et al. 2019), pollen (Dobson and Bergström 2000) or the multimodal expression of several of these traits (reviewed in Junker and Parachnowitsch 2015). Here, we focus on floral odour bouquets, which are often complex blends of many volatile organic compounds (VOCs) that mediate diverse interactions between plants, insects and microbes (Holopainen 2004, Raguso 2008).

Recent studies on plant–pollinator interactions have identified many cases of pollinator-driven evolution of floral odour (Chess et al. 2008, Klahre et al. 2011, Breitkopf et al. 2013, Peter and Johnson 2014, Gross et al. 2016). However, a review by Delle-Vedove et al. (2017) found that floral odour variation often cannot be explained by pollinator-mediated selection alone. Several factors may explain this discrepancy, including tradeoffs between pollinator attraction and chemical defence against herbivory (Schiestl et al. 2014), the nature of the interaction (i.e. deceptive pollination; Renner 2006), biochemical and energetic limitations (Delle-Vedove et al. 2011), phylogenetic constraints in the biosynthetic pathways for VOC production (Raguso et al. 2006), gene flow (Svensson et al. 2005) or genetic drift (Suinyuy et al. 2012). Another factor that may maintain floral odour variation within populations is temporal heterogeneity in pollinator community composition, as a result of pollinator declines (Yuan et al. 2009, Thomann et al. 2013, IPBES 2016, Zattara and Aizen 2021) or shifts in pollinator phenology (Burkle and Runyon 2019). However, the impact of temporally heterogeneous pollinators on floral odour is not yet clearly understood, because most of the aforementioned studies relied on VOC and pollinator data collected at a single timepoint. Here, we aim to address this gap in our knowledge, by examining how spatio–temporal variation in pollination interactions influences floral odour variation in *Arum maculatum* (Araceae).

Arum maculatum inflorescences (Fig. 1) are pollinated deceptively, by emitting complex VOC blends which are known to vary within and among populations in England (Kite 1995, Kite et al. 1998, Diaz and Kite 2002), France (Chartier et al. 2011, 2013) and across the Alps (Gfrerer et al. 2021). The floral odour of *A. maculatum* is similar to dung or decomposing organic matter (Lack and Diaz 1991), mimicking the natural brood sites of their main pollinators, the moth flies *Psychoda phalaenoides* and *Psycha grisescens* (Diptera: Psychodidae). Since pollinators are temporarily trapped in a specialized floral chamber (Bröderbauer et al. 2013) until the day after anthesis (Gibernau et al. 2004), it is possible to collect accurate quantitative data on pollinators attracted by individual inflorescences. Furthermore, pollinators appear to be attracted by VOCs alone (Dormer 1960, Urru et al. 2011), meaning that an inflorescence's reproductive success should be closely tied to its unique floral odour. Since *A. maculatum* floral odour is known to vary, and VOCs such as indole, 2-heptanone and p-cresol are attractive to *Psychoda* species (Kite et al. 1998), we chose to focus on floral odour in this study. Specifically, we investigated whether VOC variation is maintained within populations due to temporally variable pollinator communities, or across the species distribution due to spatial divergence in pollinator communities.

Floral odour variation may be maintained within populations through balancing selection (Delph and Kelly 2013) and/or phenotypic plasticity (Callaway et al. 2003, Majetic et al. 2009, Campbell et al. 2019). In the case of *A. maculatum*, balancing selection may occur if relative abundances of *P. phalaenoides* and *P. grisescens* vary temporally within populations (Schemske and Horvitz 1989), or if pollinator learning or avoidance to escape deception (Ayasse et al. 2000, Baguette et al. 2020) leads to frequency-dependent selection. In either case, we would expect to observe high floral odour variation within populations of *A. maculatum* across Europe. Phenotypic plasticity under variable abiotic and biotic conditions may also promote the maintenance of trait variation (Gulisija et al. 2016). If we observe shifts in VOC emissions when *A. maculatum* inflorescences are transplanted to non-native conditions, we may conclude that plasticity also contributes to the maintenance of floral odour variation.

Alternatively, if pollinators only vary spatially, they may exert divergent selective pressures on floral VOCs, leading to locally-adapted bouquets in populations with different pollinators (Leimu and Fischer 2008, Gervasi and Schiestl 2017, Suinyuy and Johnson 2018, Sayers et al. 2020). Previous research has shown that *P. phalaenoides* and *P. grisescens* are respectively trapped by inflorescences in the northern/central and southern/eastern portions of *A. maculatum*'s distribution

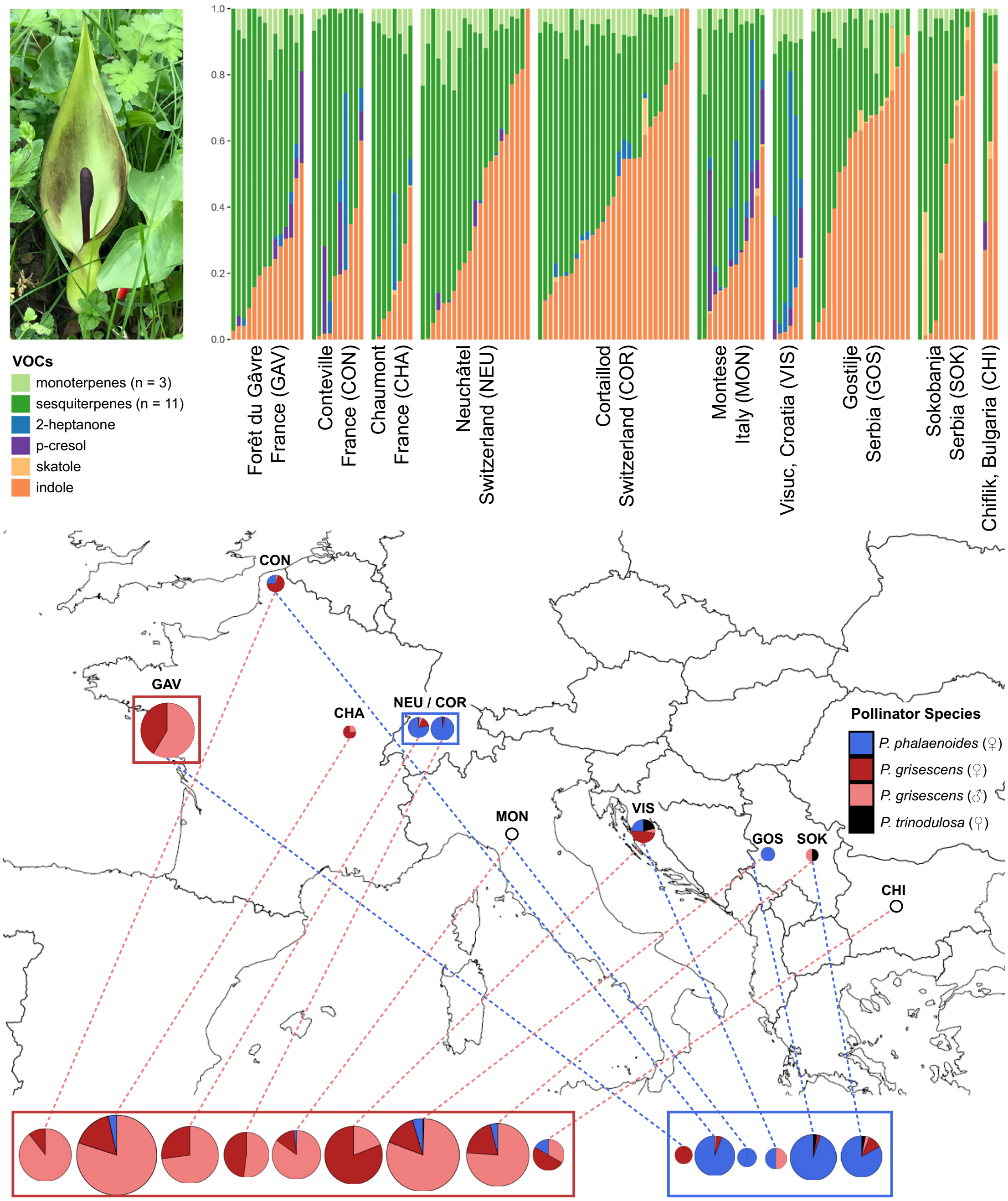

Figure 1. Europe-wide variation in floral volatile organic compounds (VOCs) and pollinator attraction of *Arum maculatum*. Stacked barplots present the relative quantities of VOCs in two terpene classes (mono- and sesquiterpenes) and four dung-mimicking VOCs emitted by individual inflorescences. Pie charts indicate the composition of Psychodidae trapped by *A. maculatum* inflorescences during field surveys (on map), and following transplants to two common garden sites (below map), scaled to represent mean quantities of Psychodidae per plant. Dotted lines link each field result with corresponding transplant results. Note: empty chart = no Psychodidae attracted during our surveys.

(Espíndola et al. 2010) – except in the Atlantic fringe of France, where inflorescences trap *P. grisescens*. Furthermore, the population genetic structure of *A. maculatum* roughly aligns with this pattern (Espíndola and Alvarez 2011). Under spatially varying selection, we may predict that distinct *A. maculatum* floral VOCs or phenological adaptations will be maintained in these two pollinator backgrounds. If these variations are local adaptations, we would expect to observe decreased pollinator attraction efficiency when inflorescences are transplanted to non-native pollinator backgrounds.

In this study, we investigated whether variation in *A. maculatum* floral odour was consistent with the patterns predicted under either of the two scenarios described above. To this aim, we surveyed variation in floral odour and pollinator attraction across Europe, and transplanted individuals from all sampled populations to two common garden sites dominated by either *P. phalaenoides* or *P. grisescens*. By selecting populations where pollinator communities were first surveyed a decade ago (Espíndola et al. 2010), we were further able to assess temporal heterogeneity in pollinators at both the inter-annual (i.e. our surveys between 2017 and 2019), and decadal scales, with the aim of understanding how temporally heterogeneous pollinators affect intraspecific floral odour variation. Our specific objectives were to 1) characterize and compare floral odour variation within and among populations of *A. maculatum*, 2) determine whether the pollinators trapped by *A. maculatum* vary spatially and/or temporally and 3) use these results to infer potential drivers of floral VOC variation, namely pollinator-driven balancing selection and local adaptation.

Material and methods

Natural history and pollination ecology of *Arum maculatum*

Arum maculatum is a common European flowering plant, which is usually found in shaded areas of deciduous woodlands, particularly beech woods (Bown 2000). Its pollination cycle takes place over two days (reviewed in Gibernau et al. 2004). On the evening of the first day, the appendix is thermogenic and emits a dung-like odour to attract dipteran pollinators; heat alone is not sufficient to attract pollinators (Prime 1960), but likely aids in the dispersal of VOCs such as indole, p-cresol and 2-heptanone, which are attractive to *Psychoda* species when used in scented traps (Kite et al. 1998). Notably, the widely distributed *Psychoda phalaenoides* and *Psycha grisescens* are both trapped by *A. maculatum* across most of Europe, together representing 87.49% of all insects found within floral chambers (Espíndola et al. 2010). *Psychoda setigera*, *P. albipennis* and *P. trinodulosa* are also infrequently observed within inflorescences, together representing 0.46% of trapped insects in Espíndola et al. (2010). Other infrequently attracted dipterans include Chironomidae (*Smittia pratorum*), Ceratopogonidae and Sphaeroceridae (Brachycera) (Rohacek et al. 1990, Diaz and Kite 2002, Espíndola et al. 2010).

Psychodidae are also known to be efficient carriers of *Arum* pollen (Diaz and Kite 2002, Albre et al. 2003); one individual can carry 50–150 grains of pollen, and a significant fruit set can develop from a single trapped pollinator (Lack and Diaz 1991). Based on their similar behaviours and morphologies, the above-mentioned *Psychoda* species are all presumably efficient pollinators of *A. maculatum*. However, antennal sensilla are known to vary among Psychodidae (Faucheux and Gibernau 2011), and species may therefore vary in their responses to VOCs. Although *A. maculatum* do not provide any nutritive rewards, this should not be detrimental to trapped *P. phalaenoides* and *P. grisescens*, as they do not feed during their short adult lifespan (P. Withers, cited in Lack and Diaz 1991). The conservation status of *P. phalaenoides* and *P. grisescens* has not been assessed; however, *Psychoda* populations are known to be large and dense (up to thousands of individuals per m^2; Arshad and Moh Leng 1991) due to their reliance on modern agriculture and human activities (e.g. manure and decomposing organic matter) for reproduction (Satchell 1947, Vaillant 1971). Peak *P. phalaenoides* abundances also appear to correspond with the peak flowering period of *A. maculatum* (Ollerton and Diaz 1999).

Field sampling sites

We sampled ten populations of *A. maculatum* (Fig. 1, Supporting information), including three in France (Forêt du Gâvre, Conteville and Chaumont), two in Switzerland (Neuchâtel and Cortaillod), one in Italy (Montese), one in Croatia (Visuć), two in Serbia (Gostilje and Sokobanja) and one in Bulgaria (Chiflik). This sampling covers the majority of the *A. maculatum* species distribution range (Supporting information). During the typical flowering period of *A. maculatum* (April–May), we conducted field sampling each year between 2017 and 2019, visiting each site in at least two years during this timeframe.

Common garden experiment sites

We selected the Swiss population of Neuchâtel and the French population Forêt du Gâvre (on the Atlantic fringe) as our common garden sites, since they were respectively dominated by *Psychoda phalaenoides* and *Psycha grisescens*. We conducted common garden experiments in Neuchâtel in 2018 and 2019, and in Forêt du Gâvre in 2019. These two sites experienced a relatively similar climate; over the course of our sampling in 2019, the mean temperature and humidity in Neuchâtel at the time of sampling were 15.8°C and 61% respectively, and in Forêt du Gâvre, the mean temperature and humidity were 17.2°C and 50% respectively. Five to ten *A. maculatum* individuals with unopened inflorescences were potted in soil from their native habitat, and then transplanted from all ten populations listed above to the two common garden sites. Inflorescences from Neuchâtel and Forêt du Gâvre were also reciprocally transplanted as part of this experiment. The total numbers of inflorescences sampled in situ and in both common garden sites are given in Supporting information.

Floral odour and pollinator sampling methods

During both the field surveys and common garden experiments, we collected dynamic headspace VOCs using identical methods, and analysed them using gas chromatography coupled to mass spectrometry (GC–MS; full details in the Supporting information). Briefly, we collected VOCs from *A. maculatum* inflorescences undergoing anthesis in the early evening on polydimethylsiloxane (PDMS) coated Twister stir bars (Gerstel: Mülheim an der Ruhr, Germany), at an air flow rate of 200 ml min^{-1} for 30 min. Twisters were kept on ice in sealed glass containers until GC–MS analyses, where volatiles were thermally desorbed and separated on a HP-5MS column. During the morning following VOC sampling, we collected all insects trapped within inflorescences and preserved them in 70% ethanol until identification (full identification methods detailed in Supporting information).

Characterizing and comparing floral odour variation across the range of *A. maculatum*

We compared total VOC emissions within and among populations of field-sampled inflorescences (i.e. VOCs sampled in their native habitats) using Bray–Curtis distance matrices, and visualized inter-individual variation using nonmetric multidimensional scaling (NMDS). Then, to test for a significant effect of population (fixed effect factor) on the entire VOC matrix, we performed pairwise permutational multivariate analysis of variance (PERMANOVA, Bray–Curtis distance, n = 999 permutations) with Bonferroni correction using the *adonis* function in the R ver. 3.6.1 (<www.r-project.org>) package *vegan* (Oksanen et al. 2019).

Characterizing temporal variation in pollinator community composition

We investigated inter-annual variation in pollinator community composition during our study (i.e. 2017–2019) by calculating Bray–Curtis distance matrices, comparing the mean quantities of pollinators trapped per inflorescence for all populations with in situ pollinator data collected in two or more years. We then visualized the result using nonmetric multidimensional scaling (NMDS) ordinations. Next, we repeated this process to investigate shifts in the dominant pollinators trapped by *A. maculatum* over the past decade. Using Bray–Curtis distance matrices and NMDS ordinations, we compared the mean quantities of 1) Psychodidae species only, and 2) all insect families trapped per inflorescence in our study, with those collected approximately a decade ago (2006–2008; Espíndola et al. 2010).

Identifying VOCs associated with species-specific pollinator attraction

Since we collected paired VOCs and pollinator data for each sampled inflorescence, we were able to correlate these two matrices and identify candidate compounds associated with the attraction of either *P. phalaenoides* or *P. grisescens*. To this aim, we used the Random forest implementation in the R package *randomForest* (Liaw and Wiener 2002), with permutation importance enabled (*ntree* = 500, *mtry* = 8; optimized using the *tuneRF* function). We then calculated conditional feature contributions and identified combinations of compounds which had the greatest influence on the predictive strength of the classifier, using the Python package *TreeInterpreter* (Saabas 2019). As a measure of plasticity in these candidate compounds when flowering in different habitats (i.e. transplant effects), we calculated and plotted population standard scores of these compounds for both in situ and common garden samples. We then used Mann–Whitney U-tests to test whether populations with sufficient sample sizes ($n \geq 8$) emitted different quantities of candidate compounds when transplanted to common garden sites.

Testing for local adaptation to pollinators using common garden experiments

We began by visualizing geographic patterns in mean pollinator attraction for each population in situ, and following transplants to both common garden sites. Then, following the 'local versus foreign' definition of local adaptation (Kawecki and Ebert 2004), we tested the hypotheses that: in the Neuchâtel common garden, transplanted *A. maculatum* inflorescences which attract *P. phalaenoides* in their native population should catch 1) more *P. phalaenoides* and/or 2) more pollinators in total than inflorescences which attract *P. grisescens* in their native population. We expected to observe the inverse result in the Forêt du Gâvre common garden (i.e. transplanted inflorescences that attract *P. grisescens* in their native population should perform better on average in Forêt du Gâvre). These expectations were tested using a two-way ANOVA on log + 1 transformed pollinator counts, including 'native pollinator' (i.e. *P. phalaenoides*- or *P. grisescens*-dominated origin) and 'common garden location' (i.e. whether the common garden site was dominated by *P. phalaenoides* or *P. grisescens*) and their interaction as fixed factors. Here, a significant interaction would indicate local adaptation, which could be confirmed using contrasts comparing trait values between native and transplant sites. Finally, we analysed 'deme × habitat' interactions (Kawecki and Ebert 2004) for mean attraction rates of *P. phalaenoides*, *P. grisescens* and all insects. Here, deme and habitat respectively referred to an *A. maculatum* population and its local pollinator community conditions. In this analysis, data were subset based on each inflorescence's native pollinator and common garden location, as described above.

Results

Floral odour is highly variable within and among *A. maculatum* populations

After filtering out compounds present in blank samples, we retained 18 *A. maculatum* floral VOCs present in relative abundances above 1% (Table 1). All of the major compounds we identified (e.g. indole, p-cresol, 2-heptanone, β-citronellene and three unnamed sesquiterpenes) had been previously reported in studies of *A. maculatum* floral odour (Diaz and Kite 2002, Chartier et al. 2013, Marotz-Clausen et al. 2018). We observed substantial variation in

Table 1. Average proportional emissions (M) of volatile organic compounds (± SD) emitted by (A) north/central and (B) southern (Italy) and eastern (Balkan) populations of *Arum maculatum*. Compounds representing more than 5% of the average total blend are highlighted in bold.

(A)		Forêt du Gâvre, France (n=14)			Conteville, France (n=10)			Chaumont, France (n=8)			Neuchâtel, Switzerland (n=21)			Cortaillod, Switzerland (n=29)		
VOC	RI	M	SD	Freq	M	SD	Freq	M	SD	Freq	M	SD	Freq	M	SD	Freq
2-heptanone	891	1.09	1.69	50.0	**7.74**	**16.54**	**40.0**	4.68	10.37	25.0	0.14	0.29	28.6	1.12	2.01	41.4
β-citronellene	943	3.58	6.02	64.3	0.02	0.07	10.0	2.25	2.28	75.0	**6.32**	**7.17**	**81.0**	3.39	3.74	82.8
cis β-ocimene	1037	0.10	0.37	7.1	0.00	0.00	0.0	0.02	0.05	12.5	0.32	0.75	33.3	0.51	1.02	27.6
p-cresol	1076	4.04	7.54	42.9	**5.71**	**10.15**	**30.0**	0.04	0.12	12.5	0.79	2.11	14.3	0.00	0.00	0.0
indole	1289	**22.50**	**15.45**	**100**	**19.92**	**20.11**	**90.0**	**15.23**	**15.61**	**87.5**	**39.02**	**30.26**	**95.2**	**46.26**	**26.81**	**96.6**
skatole	1383	1.89	2.84	50.0	1.44	2.13	50.0	4.21	2.28	87.5	1.59	1.84	57.1	1.62	1.41	69.0
α-copaene	1374	0.02	0.04	28.6	0.01	0.02	20.0	0.25	0.53	25.0	0.02	0.07	14.3	0.48	2.06	20.7
Z-caryophyllene	1405	**9.26**	**11.07**	**71.4**	1.87	4.44	30.0	2.11	2.00	62.5	**8.39**	**7.52**	**85.7**	3.94	4.74	75.9
β-caryophyllene	1416	1.48	2.14	50.0	**7.87**	**9.84**	**70.0**	1.92	2.30	62.5	1.37	1.93	47.6	2.82	5.68	72.4
α-humulene	1452	**5.15**	**4.43**	**71.4**	**17.55**	**22.93**	**70.0**	**11.32**	**2.66**	**100**	**5.82**	**5.44**	**76.2**	3.87	3.10	72.4
alloaromadendrene	1459	4.66	3.81	85.7	3.96	4.53	60.0	**7.02**	**4.26**	**87.5**	**5.62**	**4.41**	**85.7**	**8.09**	**9.94**	**82.8**
β-humulene	1473	**5.46**	**7.02**	**64.3**	1.24	1.95	40.0	**10.13**	**5.87**	**87.5**	4.97	6.45	85.7	2.72	1.91	82.8
α-selinene	1491	2.06	3.68	35.7	0.35	1.12	10.0	3.97	4.52	62.5	1.66	3.43	28.6	0.43	1.18	17.2
bicyclogermacrene	1493	0.58	1.02	42.9	0.87	2.75	10.0	1.18	1.70	50.0	2.64	4.07	57.1	**6.46**	**9.51**	**75.9**
d-cadinene	1520	0.48	1.09	35.7	1.63	4.66	20.0	**9.59**	**12.96**	**100**	3.75	6.47	57.1	1.55	2.14	58.6
Unnamed sesqui.	1404	0.63	1.21	42.9	0.04	0.14	10.0	0.20	0.37	37.5	4.94	10.48	57.1	2.49	6.65	37.9
Unnamed sesqui.	1470	4.62	6.00	71.4	2.17	3.98	30.0	**11.17**	**5.59**	**100**	3.48	3.27	76.2	3.96	2.82	79.3
Unnamed sesqui.	1681^	**32.39**	**20.75**	**100**	**27.61**	**17.68**	**80.0**	**14.71**	**8.88**	**100**	**9.15**	**14.36**	**81.0**	**10.29**	**10.45**	**75.9**

(B)		Montese, Italy (n=13)			Visuć, Croatia (n=6)			Gostilje, Serbia (n=19)			Sokobanja, Serbia (n=11)			Chiflik, Bulgaria (n=3)		
VOC	RI	M	SD	Freq	M	SD	Freq	M	SD	Freq	M	SD	Freq	M	SD	Freq
2-heptanone	891	**8.28**	**14.17**	**61.5**	**29.36**	**27.79**	**100**	0.00	0.00	0.0	0.00	0.00	0.0	0.00	0.00	0.0
β-citronellene	943	3.26	5.49	69.2	**8.77**	**2.18**	**100**	0.65	2.12	21.1	0.05	0.15	9.1	0.00	0.00	0.0
cis β-ocimene	1037	0.21	0.40	30.8	0.32	0.79	16.7	0.01	0.04	5.3	0.00	0.00	0.0	0.00	0.00	0.0
p-cresol	1076	**7.58**	**11.99**	**61.5**	4.34	5.81	66.7	0.00	0.00	0.0	0.00	0.00	0.0	2.85	4.94	33.3
indole	1289	**22.38**	**16.81**	**92.3**	**7.99**	**9.73**	**83.3**	**55.41**	**26.79**	**100**	**43.41**	**37.98**	**90.9**	**54.24**	**27.05**	**100**
skatole	1383	1.46	1.49	61.5	1.07	1.10	66.7	4.34	5.80	73.7	2.92	3.19	63.6	1.84	0.45	100
α-copaene	1374	0.32	0.64	46.2	0.10	0.20	50.0	1.61	4.51	68.4	4.46	10.94	72.7	2.52	2.65	66.7
Z-caryophyllene	1405	**12.78**	**14.31**	**69.2**	**22.25**	**20.66**	**83.3**	4.65	6.29	63.2	**9.88**	**19.53**	**45.5**	**6.90**	**3.86**	**100**
β-caryophyllene	1416	0.88	1.20	38.5	1.96	2.50	50.0	0.54	2.06	15.8	0.73	2.34	18.2	0.00	0.00	0.0
α-humulene	1452	6.25	8.75	76.9	**6.11**	**5.24**	**100**	**6.91**	**10.62**	**57.9**	1.81	3.34	27.3	0.70	1.21	33.3
alloaromadendrene	1459	2.89	2.70	69.2	2.04	1.65	83.3	1.86	3.22	36.8	4.71	5.97	54.5	0.73	1.26	33.3
β-humulene	1473	**8.07**	**13.22**	**100**	0.75	1.09	50.0	1.54	2.77	42.1	2.64	4.09	36.4	0.67	1.16	33.3
α-selinene	1491	0.88	1.55	30.8	0.00	0.00	0.0	1.38	3.12	31.6	0.47	1.11	18.2	0.00	0.00	0.0
bicyclogermacrene	1493	0.43	0.69	38.5	0.00	0.00	0.0	1.02	1.60	47.4	0.97	2.18	27.3	**6.95**	**12.04**	**33.3**
d-cadinene	1520	1.36	2.55	46.2	0.62	0.96	33.3	2.36	8.28	21.1	0.28	0.76	18.2	0.00	0.00	0.0
Unnamed sesqui.	1404	0.47	0.87	30.8	0.00	0.00	0.0	0.99	2.61	42.1	0.74	1.43	27.3	0.00	0.00	0.0
Unnamed sesqui.	1470	3.35	3.61	69.2	4.06	2.17	100	2.99	5.27	52.6	4.97	7.83	36.4	4.00	1.71	100
Unnamed sesqui.	1681^	**19.15**	**18.28**	**84.6**	**10.23**	**12.36**	**100**	**13.73**	**13.14**	**84.2**	**21.96**	**20.18**	**81.8**	**18.60**	**15.25**	**100**

RI=Kovats retention index (^=RI 1716 in Diaz and Kite 2002).
Freq=proportion of chromatograms (%) in which the compound was identified in a given population.

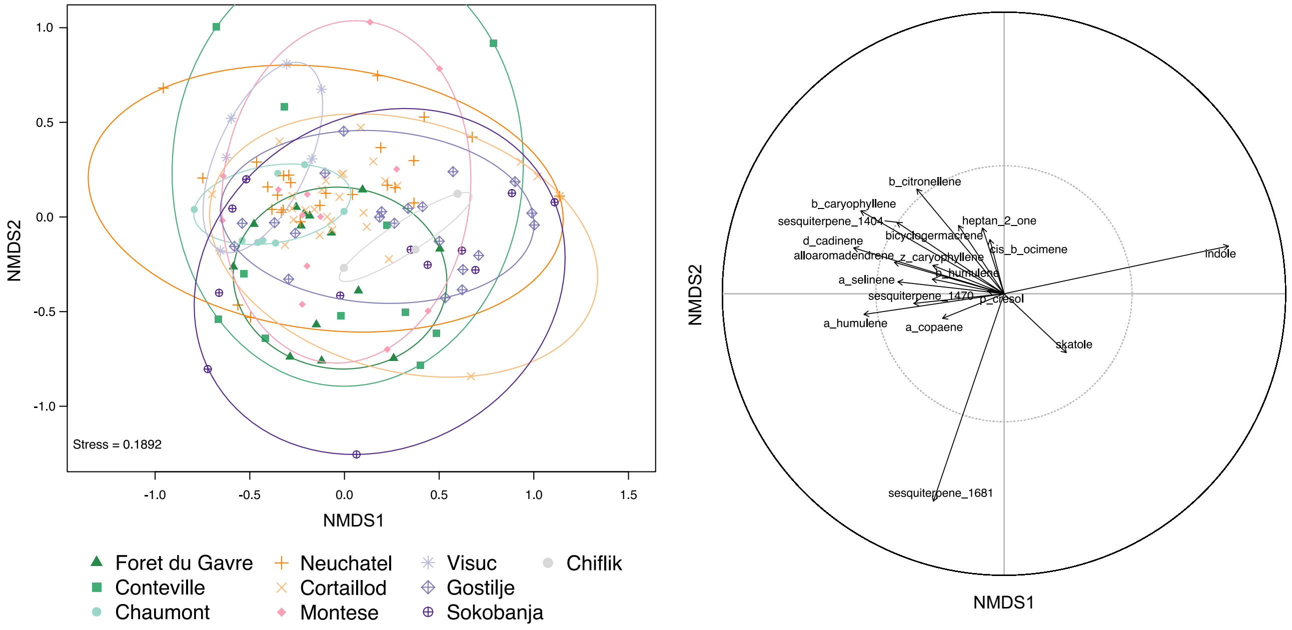

Figure 2. (A) Multivariate representation of *Arum maculatum* floral volatile organic compound (VOC) emissions, using nonmetric multidimensional scaling (NMDS) of Bray–Curtis distances between individuals. Points and ellipses are coloured according to individuals' population of origin. (B) Circle plot visualizing the correlations between all VOCs and the NMDS axes.

floral odour both within (Fig. 1) and among populations (Fig. 2).

Pollinator communities are variable at both annual and decadal scales

Our sampling allowed us to compare inter-annual (i.e. 2017/2018 versus 2019) variation in Psychodidae trapped by *A. maculatum* in six populations. The dominant Psychodidae species trapped by inflorescences appear to have shifted in three of these populations (Conteville, FR, Neuchâtel, CH and Visuć, HRV) over this time period (Fig. 3).

We also observed temporal shifts in the average abundances of trapped Psychodidae in several populations over the past decade (i.e. compared to Espíndola et al. 2010). The relative compositions of the complete pollinator communities trapped by *A. maculatum* appears to have shifted in five out of six populations where comparisons could be made; only Forêt du Gâvre remained consistent over the past decade (Supporting information). We also observed apparent shifts in the dominant Psychodidae pollinator trapped within inflorescences in four out of six populations over the past decade, in Chaumont FR, Conteville FR, Gostilje SRB and Visuć, HRV (Table 2, Supporting information).

Low population-level VOC divergence coincides with temporally variable pollinators

PERMANOVA pairwise contrasts did not identify any pairs of populations with significant divergence in their proportional emissions of VOCs sampled in their native habitats, after correction for multiple testing. This lack of population-level differentiation in VOC blends is evident when visualizing Bray–Curtis similarities between field-sampled individuals (Fig. 2), and appears to coincide with the temporal heterogeneity in pollinator communities identified in the previous section (Fig. 3, Supporting information).

Arum maculatum populations typically are not locally adapted to specific pollinators

Through Random forest analyses (Supporting information), we identified one compound positively associated with *P. phalaenoides* attraction (β-humulene), and three compounds positively associated with *P. grisescens* attraction (unnamed sesquiterpenes RI 1470 and 1681, and α-selinene). After evaluating combined feature contributions within the Random forest classifier, we found that its predictive strength was most strongly influenced by unnamed sesquiterpene (RI 1681) alone. Other strong combinations included unnamed sesquiterpene RI 1681 paired with unnamed sesquiterpene RI 1470 or α-selinene, as well as β-humulene alone, mirroring our initial results (full *Treeinterpreter* results in the Supporting information).

The four candidate compounds we identified (β-humulene, unnamed sesquiterpenes RI 1470 and 1681, and α-selinene) were widely observed and not restricted to a specific region or pollinator background. Average emissions of these compounds remained relatively consistent between in situ collections and samples transplanted to Neuchâtel, though some appeared to increase after inflorescences were transplanted to

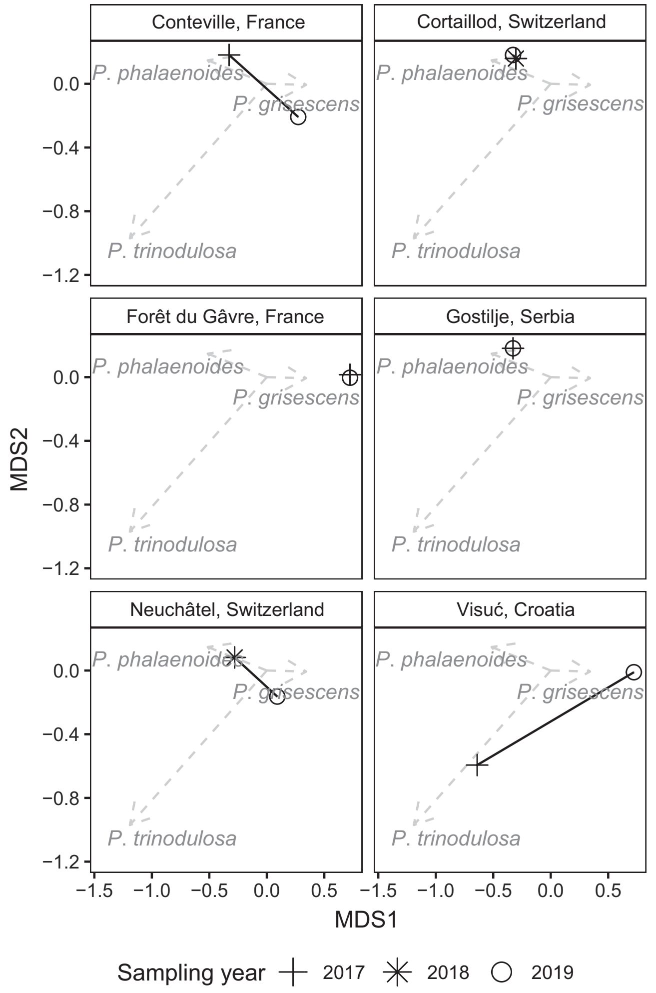

Figure 3. Interannual changes (2017/2018 versus 2019) in the average composition of Psychodidae pollinators trapped by six *Arum maculatum* populations. [NMDS; Bray–Curtis distance; overall stress = 0.005].

Forêt du Gâvre (Supporting information). We only observed a significant transplant effect in one compound (an increase in unnamed sesquiterpene RI 1470) within one population, Neuchâtel (Mann–Whitney tests; full result in the Supporting information).

In both common garden sites, we observed that transplanted inflorescences typically attracted the dominant local pollinator species as efficiently as native inflorescences (Fig. 1). While common garden location had a significant effect on the quantity of *P. phalaenoides* and *P. grisescens* caught by *A. maculatum* (i.e. the two transplant sites were dominated by different Psychodidae species), no native pollinator × common garden location interaction effect was observed (2-way ANOVA, Pr(>F) > 0.05; full results in the Supporting information). Together, these results suggest that *A. maculatum* populations are generally not locally adapted to a single pollinator species (Fig. 4), with notable exceptions in Forêt du Gâvre and the two Serbian populations Gostilje and Sokobanja, which will be discussed below.

While we do not find general indications of populations being locally adapted to specific pollinators, not all inflorescences were equally attractive to all pollinator species. One population (Forêt du Gâvre) continued to exclusively attract their native pollinator *P. grisescens* when transplanted to the Neuchâtel common garden (Fig. 1). Remarkably, this exclusive attraction of *P. grisescens* was maintained even though *P. phalaenoides* was also present (and trapped by inflorescences from other populations) at the time when inflorescences from Forêt du Gâvre opened (Supporting information). Additionally, transplanted inflorescences occasionally attracted the 'non-dominant' Psychodidae species in both common garden sites, and a third *Psychoda* species (*Psychoda trinodulosa*) was also identified within inflorescences in Croatia and Serbia during our field surveys. *Psychoda trinodulosa* was also observed in the Neuchâtel common garden, but not in Forêt du Gâvre; inflorescences from both Serbian populations (Gostilje and Sokobanja) continued to occasionally attract *P. trinodulosa* when transplanted to the Neuchâtel common garden (Fig. 1).

Discussion

In this study, we identified substantial within-and among-population variation in *A. maculatum* floral odour (Fig. 1,

Table 2. Mean in situ abundances of *Psychoda phalaenoides* and *Psycha grisescens* individuals trapped per *Arum maculatum* inflorescence between 2006 and 2008 (data from Espíndola et al. 2010), and in our field surveys between 2017 and 2019. The number of individual inflorescences sampled in situ are indicated in parentheses. NAs indicate that no inflorescences were open yet at the time populations were visited.

	Poll. species/year			
Population	*P. phalaenoides* (2008)	*P. grisescens* (2008)	*P. phalaenoides* (2019)	*P. grisescens* (2019)
Fôret du Gavre (France)	0 (5)	6.4 (5)	0 (16)	25.7 (16)
Conteville (France)	47.6 (8)	2.3 (8)	0.4 (14)	1.3 (14)
Chaumont (France)	1.6 (6)	0.5 (6)	0 (7)	0.6 (7)
Lausanne/Neuchâtel (Switzerland)	3.0 (2)	0 (2)	2.0 (44)	0.6 (44)
Montese (Italy)	0 (5)	2 (5)	NA	NA
Visuć (Croatia)	0.1 (7)	0.6 (7)	1.3 (6)	3.0 (6)
Gostilje (Serbia)	0 (5)	2.4 (5)	0.4 (18)	0 (18)
Sokobanja (Serbia)	0 (5)	0.2 (5)	0 (21)	0.1 (21)
Chiflik (Bulgaria)	0.4 (7)	4.3 (7)	NA	NA

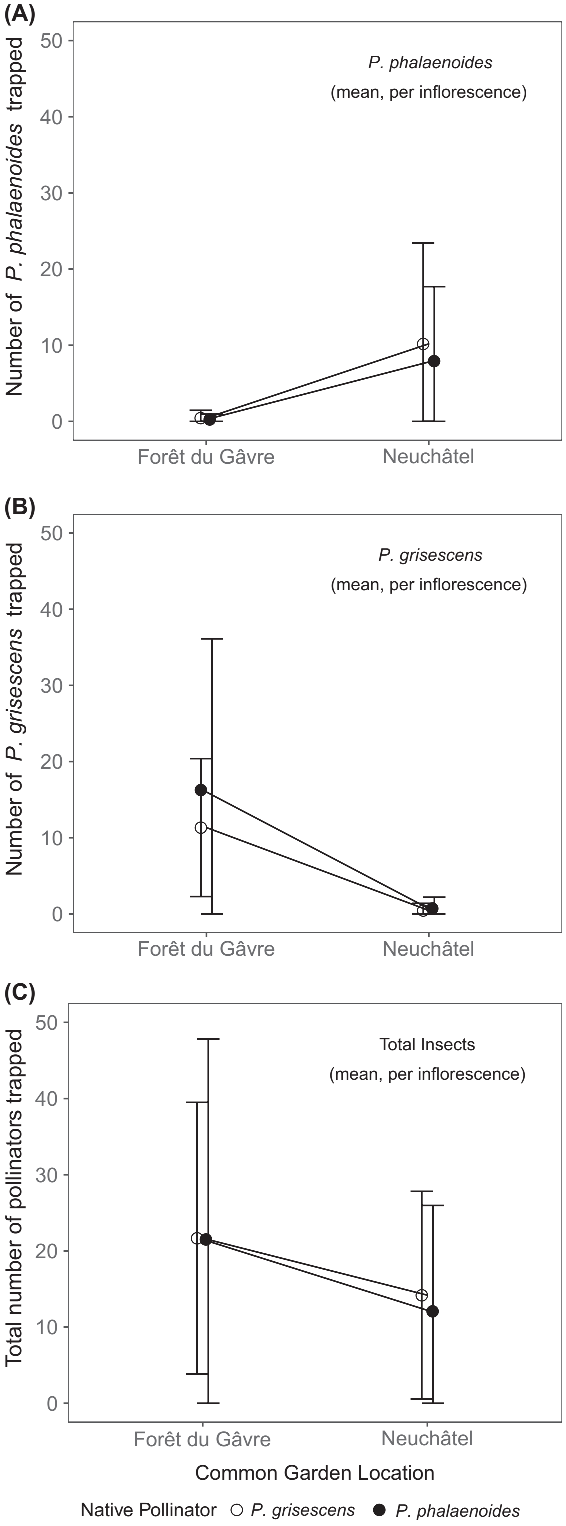

Figure 4. Pollinator attraction in the reciprocal transplant experiment. Plots show the mean (± SE) numbers of (A) *Psychoda phalaenoides*, (B) *Psycha grisescens* and (C) all insects trapped by inflorescences, transplanted from either *P. phalaenoides*-dominated populations (filled circles) or *P. grisescens*-dominated populations (hollow circles). No deme × habitat interactions (indicative of local adaptation) were identified.

2) and shifts in pollinator community composition in several populations at both the inter-annual (Fig. 3) and decadal scales (Supporting information). Although some *A. maculatum* populations continued to attract their native pollinators in transplant sites (e.g. Forêt du Gâvre and Serbian populations), most populations did not exclusively attract *P. phalaenoides* or *P. grisescens* when transplanted to 'foreign' pollinator backgrounds (Fig. 4). Since this result does not appear to be due to transplant effects/phenotypic plasticity (Supporting information), this leaves temporally heterogeneous pollinator communities as a potential mechanism underlying the maintenance of high within-population variation in floral odour.

The influence of temporally variable pollinators on *A. maculatum* floral odour

Overall, it appears that local pollinator availability at the time of anthesis plays a key role in the quantity and composition of pollinators trapped within *A. maculatum* inflorescences. Previous studies on *A. maculatum* pollination in England (Diaz and Kite 2002) and France (Chartier et al. 2013) arrived at a similar conclusion. The difference in sex ratios, particularly that no male *P. phalaenoides* or *P. trinodulosa* are attracted, may be due to differences in copulation timing among *Psychoda* species (i.e. immediately after adult emergence), and parthenogenetic reproduction (F. Vaillant, cited in Albre et al. 2003), or brood-site specific VOCs which are only attractive to females of these two species. While we were unable to reliably collect data on background pollinator communities as part of our sampling design, Diaz and Kite (2002) found that Psychodidae species caught on sticky traps mirrored those found within *A. maculatum* and *A. italicum* inflorescences. Therefore, our data should still allow us to infer whether the patterns in pollinator attraction we observed within each population were a consequence of balancing selection or local adaptation.

Balancing selection may maintain variation within populations through several selective regimes, including relaxed selection, negative frequency-dependent selection due to pollinator learning, or environmental heterogeneity (Delph and Kelly 2013). While relaxed selection on floral odour has been observed in some angiosperms (Salzmann et al. 2007), this is unlikely to be the case for deceptively pollinated *A. maculatum*, given that pollinator attraction is driven by VOC emissions alone (Dormer 1960, Lack and Diaz 1991). Pollinator learning has been shown to maintain polymorphism in floral colour (Gigord et al. 2001) and odour (Ayasse et al. 2000), but this is also less likely to occur in the case of *A. maculatum*. Psychodidae likely experience weak and inconsistent selective pressures caused by deception, due to their large population sizes and short generation times (Prime 1960, Lachmann et al. 2000), and potentially sex-specific attraction in some cases. Araceae also exploit pre-existing VOC detection abilities in their pollinators (Schiestl and Dötterl 2012), which likely contributes to their persistent inability to distinguish deceptive inflorescences (Renner 2006). This leaves temporal heterogeneity in pollinator communities as the mechanism most likely contributing to the maintenance of diverse floral odour bouquets in *A. maculatum*.

In this study, we demonstrated that pollinator communities can vary within populations over relatively short time periods (i.e. over the years- to decade-scale) across most of the range of *A. maculatum*. Temporally heterogeneous pollinators have long been known to influence floral traits (Schemske and Horvitz 1989), but to our knowledge, have not been investigated as a mechanism underlying floral odour variation. Further research on temporal variation in pollinators may provide clarity in other cases where floral trait variation in deceptively pollinated species cannot be explained by pollinator learning (Pellegrino et al. 2005, Jersáková et al. 2006). Temporally variable pollinators may have also contributed to the finding that, contrary to expectations, deceptively pollinated species generally do not maintain more variable floral odours than rewarding species (Ackerman et al. 2011, Delle-Vedove et al. 2017).

Currently, it is not known whether the phenologies of *P. phalaenoides* and *P. grisescens* are influenced by environmental variation; our data indicate that at least in Forêt du Gâvre, *P. grisescens* emerges slightly earlier than *P. phalaenoides* (Supporting information). A recent environmental DNA survey of cow dung (Sigsgaard et al. 2020) also identified *P. phalaenoides*, *P. trinodulosa* and *P. grisescens* all within a single site in Mols Bjerge, Denmark. At the time the sample was taken (June 2019; i.e. later than our field surveys), *P. phalaenoides* overwhelmingly predominated over all other insects in terms of read counts (i.e. abundance). Taken together, these results corroborate two key points: all of the major Psychodidae pollinators of *A. maculatum* are present across the range of *A. maculatum* (Ježek et al. 2018), and their relative abundances may vary temporally over the course of the flowering period.

If environmental variation influences pollinator phenology, then plasticity in floral odour based on the same environmental cues might enhance pollinator attraction. With one exception in a single population (i.e. unnamed sesquiterpene RI 1470 emitted by inflorescences from Neuchâtel), we did not observe significant shifts in the four candidate compounds linked to species-specific pollinator attraction following transplants to common gardens (i.e. phenotypic plasticity). However, as we transplanted mature inflorescences, some environmental effects may have already been in place prior to transplanting (Wund 2012). Growing *A. maculatum* for several generations in common garden sites would give a more accurate result and eliminate maternal effects, but this was not feasible within the scope of our study, as *A. maculatum* requires at least two years (and sometimes longer) from germination to flowering (Bown 2000). Given the central role of VOCs in *A. maculatum* pollination (Kite 1995, Kite et al. 1998), it is unlikely that the high variation in floral odour we observed is the result of plasticity alone. Further research tracking the VOC emissions of the same individual over multiple flowering events would provide greater resolution into environmental influences on floral odour variation.

Since *A. maculatum* are rhizomatous, variation may also persist within populations for longer periods of time compared to annual plants. Gene flow between populations may also contribute to the maintenance of floral odour variation within populations. Pollen dispersal by Psychodidae is likely limited due to their poor flying abilities and short adult lifespans (Lack and Diaz 1991), but seed dispersal by frugivorous birds (Snow and Snow 1988) could lead to gene flow among nearby populations. While there appears to be a strong barrier to gene flow between populations from north/central Europe, and from Italy and the Balkans (Espíndola and Alvarez 2011), our data suggest that floral odour variation is widely maintained across this barrier. This pattern suggests that – at least regionally – balancing selection is at work, possibly in association with phenotypic plasticity and/or local adaptation in populations with consistent pollinator communities.

The influence of spatially variable pollinators on *A. maculatum* floral odour

The results from our Europe-wide transplant and common garden experiment do not support the hypothesis that all *A. maculatum* populations are (locally) adapted to attract exclusively *P. phalaenoides* or *P. grisescens*. This contrasts with the patterns found in other wide-ranging deceptively pollinated species such as the sexually deceptive orchids *Ophrys sphegodes* (Breitkopf et al. 2013) and *Ophrys insectifera* (Triponez et al. 2013), but aligns with the results of a previous reciprocal transplant experiment between two *A. maculatum* populations in France (Chartier et al. 2013). However, we found that the Forêt du Gâvre population in coastal NW France appears to have lost the ability to attract *P. phalaenoides*, possibly due to local adaptation and/or genetic drift; the latter process is known to occur at the limits of species ranges (Geber 2011, Gould et al. 2013). Interestingly, Forêt du Gâvre is also the only population in this study with little recorded inter-annual and decade-scale variation in pollinator communities. It is therefore possible that the pollinator attraction patterns we observed are a result of selective pressures imposed by the likely stable population of *P. grisescens* in this site. Further transplants of Forêt du Gâvre inflorescences to additional populations are needed to test this hypothesis.

Our results also do not exclude the possibility that individual VOCs are differentially attractive to certain Psychodidae species. First, we identified four sesquiterpenes that were able to predict the attraction of *P. phalaenoides* (β-humulene) and *P. grisescens* (unidentified sesquiterpenes RI 1470 and 1681, and α-selinene) through Random Forest analyses. Additionally, both Serbian populations in this study (Gostilje and Sokobanja) attracted a third Psychodidae species (*P. trinodulosa*) in situ and following transplants to the Neuchâtel common garden, suggesting that there may also be VOCs related to species-specific attraction of *P. trinodulosa*. These patterns suggest that specific compounds emitted by inflorescences from Serbia may also be differentially attractive to *P. trinodulosa*. However, this species was too infrequently observed in our study to make any firm conclusions; further studies of these populations are needed to address this question.

In this study, the VOCs typically associated with Psychodidae brood sites (e.g. indole, p-cresol and 2-heptanone) were not correlated with species-specific pollinator attraction. A recent study by Gfrerer et al. (2021) also found that variation in the aforementioned foetid-smelling VOCs were not among those which significantly influenced fruit sets in *A. maculatum*. Given that blends of indole, p-cresol and 2-heptanone are known to be attractive to *Psychoda* species (Kite et al. 1998), these compounds appear to be generally attractive to all species. However, blends of VOCs (e.g. with specific sesquiterpenes) or the ratios at which they are emitted may be differentially attractive. Behavioural assays, where different pollinator species are presented with several *A. maculatum* inflorescences or individual VOCs, would be useful in identifying which compounds or blends elicit general or species-specific responses. Similarly, gas chromatography–electroantennography (GC–EAD; Cork et al. 1990) could be used to identify all VOCs which elicit a physiological response in different pollinator species. The *A. maculatum* populations with unique patterns in pollinator attraction identified in this study may be useful targets for future research testing whether selection is acting on specific compounds (Stensmyr et al. 2002, Urru et al. 2010), or on 'super-attractive mixtures' (e.g. in *A. palaestinum*; Stökl et al. 2010).

Conclusion

To date, almost all studies on floral odour and pollinator variation have been carried out at a single timepoint – possibly contributing to the numerous cases where floral odour diversity appears to exceed pollinator diversity (Delle-Vedove et al. 2017). As evidenced by the extensive literature on local adaptation in plants (Leimu and Fischer 2008), spatially varying selection is an undoubtedly important driver of floral odour divergence, and appears to have influenced a few *A. maculatum* populations (e.g. Forêt du Gâvre) as well. However, the variable floral odour bouquets emitted by *A. maculatum* may also represent an important adaptation to temporally heterogeneous pollinator communities, if the pollinator shifts we observed have been occurring over longer periods of time. In closing, we advocate for increased attention on the temporal dimension of pollinator-mediated selection. By simultaneously studying spatial and temporal variation in selection, we may further our understanding of how and why flowering plants maintain high diversity in key functional traits such as floral odour.

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Author contributions

Mark Szenteczki and **Adrienne Godschalx** participated equally and are considered joint first authors. **Marc Gibernau**, **Nadir Alvarez** and **Sergio Rasmann** participated equally and are considered joint senior authors. **Mark Szenteczki**: Conceptualization (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Writing – original draft (lead); Writing – review and editing (lead). **Adrienne Godschalx**: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (lead); Writing – review and editing (equal). **Andrea Galmán**: Methodology (equal); Writing – review and editing (equal). **Anahí Espíndola**: Investigation (supporting); Resources (supporting); Writing – review and editing (equal). **Marc Gibernau**: Conceptualization (equal); Funding acquisition (supporting); Investigation (equal); Methodology (equal); Resources (supporting); Supervision (equal); Writing – review and editing (equal). **Nadir Alvarez**: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (lead); Investigation (equal); Methodology (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal). **Sergio Rasmann**: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (lead); Investigation (equal); Methodology (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.v15dv41w7> (Szenteczki et al. 2021).

References

Ackerman, J. D. et al. 2011. Are deception-pollinated species more variable than those offering a reward? – Plant Syst. Evol. 293: 91–99.

Albre, J. et al. 2003. Pollination ecology of *Arum italicum* (Araceae). – Bot. J. Linn. Soc. 141: 205–214.

Arshad, A. and Moh Leng, K. Y. 1991. Preliminary population assessment of *Psychoda alternata* (Diptera: Psychodidae) in soil irrigated with wastewater for turf cultivation. – Fl. Entomol. 74: 591–596.

Ayasse, M. et al. 2000. Evolution of reproductive strategies. The sexually deceptive orchid *Ophrys sphegodes*: how does flower-specific variation of odor signals influence reproductive success? – Evolution 54: 1995–2006.

Baguette, M. et al. 2020. Why are there so many bee-orchid species? Adaptive radiation by intraspecific competition for mnemonic pollinators. – Biol. Rev. 95: 1630–1663.

Bown, D. 2000. Aroids: plants of the *Arum* family. – Timber Press.

Breitkopf, H. et al. 2013. Pollinator shifts between *Ophrys sphegodes* populations: might adaptation to different pollinators drive population divergence? – J. Evol. Biol. 26: 2197–2208.

Bröderbauer, D. et al. 2013. The design of trapping devices in pollination traps of the genus *Arum* (Araceae) is related to insect type. – Bot. J. Linn. Soc. 172: 385–397.

Burkle, L. A. and Runyon, J. B. 2019. Floral volatiles structure plant–pollinator interactions in a diverse community across the growing season. – Funct. Ecol. 33: 2116–2129.

Callaway, R. M. et al. 2003. Phenotypic plasticity and interactions among plants. – Ecology 84: 1115–1128.

Campbell, D. R. et al. 2019. Phenotypic plasticity of floral volatiles in response to increasing drought stress. – Ann. Bot. 123: 601–610.

Chartier, M. et al. 2011. Do floral odor profiles geographically vary with the degree of specificity for pollinators? Investigation in two sapromyophilous *Arum* species (Araceae). – Ann. Soc. Entomol. France 47: 71–77.

Chartier, M. et al. 2013. Geographical variations of odour and pollinators, and test for local adaptation by reciprocal transplant of two European *Arum* species. – Funct. Ecol. 27: 1367–1381.

Chess, S. K. R. et al. 2008. Geographic divergence in floral morphology and scent in *Linanthus dichotomus* (Polemoniaceae). – Am. J. Bot. 95: 1652–1659.

Cork, A. et al. 1990. Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. – In: McMaffercy, A. R. and Vilson, I. D. (eds), Chromatography and isolation of insect hormones and pheromones. Plenum Press, pp. 271–279.

Delle-Vedove, R. et al. 2011. Colour–scent associations in a tropical orchid: three colours but two odours. – Phytochemistry 72: 735–742.

Delle-Vedove, R. et al. 2017. Understanding intraspecific variation of floral scent in light of evolutionary ecology. – Ann. Bot. 120: 1–20.

Delph, L. F. and Kelly, J. K. 2013. On the importance of balancing selection in plants. – New Phytol. 201: 45–56.

Diaz, A. and Kite, G. C. 2002. A comparison of pollination ecology of *Arum maculatum* and *A. italicum* in England. – Watsonia 24: 171–181.

Dobson, H. E. M. and Bergström, G. E. M. 2000. The ecology and evolution of pollen odors. – Plant Syst. Evol. 222: 63–87.

Dormer, K. J. 1960. The truth about pollination in *Arum*. – New Phytol. 59: 298–301.

du Plessis, M. et al. 2018. Pollination of the 'carrion flowers' of an African stapeliad (*Ceropegia mixta*: Apocynaceae): the importance of visual and scent traits for the attraction of flies. – Plant Syst. Evol. 304: 357–372.

Espíndola, A. and Alvarez, N. 2011. Comparative phylogeography in a specific and obligate pollination antagonism. – PLoS One 6: e28662.

Espíndola, A. et al. 2010. Variation in the proportion of flower visitors of *Arum maculatum* along its distributional range in relation with community-based climatic niche analyses. – Oikos 120: 728–734.

Faucheux, M. J. and Gibernau, M. 2011. Antennal sensilla in five Psychodini moth flies (Diptera: Psychodidae: Psychodinae) pollinators of *Arum* spp. (Araceae). – Ann. Soc. Entomol. Fr. 47: 89–100.

Fishbein, M. and Venable, D. L. 1996. Diversity and temporal change in the effective pollinators of *Asclepias Tuberosa*. – Ecology 77: 1061–1073.

Friberg, M. et al. 2019. Extreme diversification of floral volatiles within and among species of *Lithophragma* (Saxifragaceae). – Proc. Natl Acad. Sci. USA 116: 4406–4415.

Geber, M. A. 2011. Ecological and evolutionary limits to species geographic ranges. – Am. Nat. 178: S1–S5.

Gervasi, D. D. L. and Schiestl, F. P. 2017. Real-time divergent evolution in plants driven by pollinators. – Nat. Commun. 8: 14691.

Gfrerer, E. et al. 2021. Floral scents of a deceptive plant are hyperdiverse and under population-specific phenotypic selection. – BioRxiv 441155. doi: 10.1101/2021.04.28.441155

Gibernau, M. et al. 2004. Pollination in the genus *Arum* – a review. – Aroideana 27: 148–166.

Gigord, L. D. B. et al. 2001. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soo. – Proc. Natl Acad. Sci. USA 98: 6253–6255.

Gould, B. et al. 2013. Local adaptation and range boundary formation in response to complex environmental gradients across the geographical range of *Clarkia xantiana* ssp. *xantiana*. – J. Ecol. 102: 95–107.

Goyret, J. et al. 2007. The effect of decoupling olfactory and visual stimuli on the foraging behavior of *Manduca sexta*. – J. Exp. Biol. 210: 1398–1405.

Gross, K. et al. 2016. Why do floral perfumes become different? Region-specific selection on floral scent in a terrestrial orchid. – PLoS One 11: e0147975.

Gulisija, D. et al. 2016. Phenotypic plasticity promotes balanced polymorphism in periodic environments by a genomic storage effect. – Genetics 202: 1437–1448.

Hendry, A. P. 2017. Eco-evolutionary dynamics. – Princeton Univ. Press.

Hernández-Hernández, T. and Wiens, J. J. 2020. Why are there so many flowering plants? A multi-scale analysis of plant diversification. – Am. Nat. 195: 948–963.

Herrera, C. M. et al. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. – In: Harder, L. D. and Barrett, S. C. H. (eds), Ecology and evolution of flowers. Oxford Univ. Press, pp. 278–294.

Holopainen, J. 2004. Multiple functions of inducible plant volatiles. – Trends Plant Sci. 9: 529–533.

Ibanez, S. et al. 2010. The role of volatile organic compounds, morphology and pigments of globeflowers in the attraction of their specific pollinating flies. – New Phytol. 188: 451–463.

IPBES 2016. The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. (eds Potts, S. G. et al.). – Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany.

Jersáková, J. et al. 2006. Is the colour dimorphism in *Dactylorhiza sambucina* maintained by differential seed viability instead of frequency-dependent selection? – Folia Geobot. 41: 61–76.

Ježek, J. et al. 2018. Moth flies (Diptera: Psychodidae) of the western Hercynian mountains, Sokolov open-cast coal mines and dumps (Czech Republic). – Acta Musei Silesiae Sci. Nat. 67: 193–292.

Junker, R. R. and Parachnowitsch, A. L. 2015. Working towards a holistic view on flower traits – how floral scents mediate plant–animal interactions in concert with other floral characters. – J. Indian Inst. Sci. 95: 43–67.

Kawecki, T. J. and Ebert, D. 2004. Conceptual issues in local adaptation. – Ecol. Lett. 7: 1225–1241.

Kite, G. C. 1995. The floral odour of *Arum maculatum*. – Biochem. Syst. Ecol. 23: 343–354.

Kite, G. C. et al. 1998. Inflorescence odours and pollinators of *Arum* and *Amorphophallus* (Araceae). – In: Rudall, S. J. and Owens, P. J. (eds), Reproductive biology. R. Bot. Gard. Kew, pp. 295–315.

Klahre, U. et al. 2011. Pollinator choice in petunia depends on two major genetic loci for floral scent production. – Curr. Biol. 21: 730–739.

Lachmann, A. D. et al. 2000. Life cycle of *Psychoda cinerea*, *P. parthenogenetica* and *P. trinodulosa* (Diptera, Psychodidae). 2. Adults. – Studia Dipterol. 7: 533–542.

Lack, A. J. and Diaz, A. J. 1991. The pollination of *Arum maculatum* L. – a historical review and new observations. – Watsonia 18: 333–342.

Leimu, R. and Fischer, M. 2008. A meta-analysis of local adaptation in plants. – PLoS One 3: e4010.

Levin, D. A. 2000. The origin, expansion and demise of plant species. – Oxford Univ. Press.

Liaw, A. and Wiener, M. 2002. Classification and regression by randomForest. – R News 2: 18–22.

Majetic, C. J. et al. 2009. Sources of floral scent variation: can environment define floral scent phenotype? – Plant Signal. Behav. 4: 129–131.

Marotz-Clausen, G. et al. 2018. Incomplete synchrony of inflorescence scent and temperature patterns in *Arum maculatum* L. (Araceae). – Phytochemistry 154: 77–84.

Murúa, M. and Espíndola, A. 2015. Pollination syndromes in a specialized plant–pollinator interaction: does floral morphology predict pollinators in *Calceolaria*? – Plant Biol. 17: 551–557.

Oksanen, J. et al. 2019. vegan: community ecology package. – R package ver. 2.5-6. <https://CRAN.R-project.org/package=vegan>.

Ollerton, J. and Diaz, A. 1999. Evidence for stabilising selection acting on flowering time in *Arum maculatum* (Araceae): the influence of phylogeny on adaptation. – Oecologia 119: 340–348.

Parachnowitsch, A. L. et al. 2019. Evolutionary ecology of nectar. – Ann. Bot. 123: 247–261.

Pellegrino, G. et al. 2005. Effects of local density and flower colour polymorphism on pollination and reproduction in the rewardless orchid *Dactylorhiza sambucina* (L.). – Plant Syst. Evol. 251: 119–129.

Peter, C. I. and Johnson, S. D. 2014. A pollinator shift explains floral divergence in an orchid species complex in South Africa. – Ann. Bot. 113: 277–288.

Prime, C. T. 1960. Lords and ladies: new naturalist monograph no. 17. – Collins.

Raguso, R. A. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. – Annu. Rev. Ecol. Evol. Syst. 39: 549–569.

Raguso, R. A. et al. 2006. Phylogenetic fragrance patterns in *Nicotiana* sections Alatae and Suaveolentes. – Phytochemistry 67: 1931–1942.

Renner, S. S. 2006. Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. – In: Waser, N. M. and Ollerton, J. (eds), Plant–pollinator interactions: from specialization to generalization. Univ. of Chicago Press, pp. 123–144.

Rohacek, J. et al. 1990. Sphaerocidae associated with flowering *Arum maculatum* (Araceae) in the vicinity of Tübingen, SW Germany. – Senckenberg. Biol. 71: 259–268.

Saabas, A. 2019. Treeinterpreter library. – <https://github.com/andosa/treeinterpreter>.

Salzmann, C. C. et al. 2007. Variability in floral scent in rewarding and deceptive orchids: the signature of pollinator-imposed selection? – Ann. Bot. 100: 757–765.

Satchell, G. H. 1947. The ecology of the British species of *Psychoda* (Diptera, Psychodidae). – Ann. Appl. Biol. 34: 611–621.

Sayers, T. D. J. et al. 2020. Dung mimicry in *Typhonium* (Araceae): explaining floral trait and pollinator divergence in a widespread species complex and a rare sister species. – Bot. J. Linn. Soc. 193: 375–401.

Schemske, D. W. and Horvitz, C. C. 1989. Temporal variation in selection on a floral character. – Evolution 43: 461.

Schiestl, F. P. and Dötterl, S. 2012. The evolution of floral scent and olfactory preferences in pollinators: coevolution or pre-existing bias? – Evolution 66: 2042–2055.

Schiestl, F. P. et al. 2014. Herbivory and floral signaling: phenotypic plasticity and tradeoffs between reproduction and indirect defense. – New Phytol. 203: 257–266.

Sigsgaard, E. E. et al. 2020. Environmental DNA metabarcoding of cow dung reveals taxonomic and functional diversity of invertebrate assemblages. – Mol. Ecol. doi: 10.1111/mec.15734.

Snow, B. and Snow, D. 1988. Birds and berries. – Calton Poyser, Staffordshire.

Stensmyr, M. C. et al. 2002. Rotting smell of dead-horse arum florets. – Nature 420: 625–626.

Stökl, J. et al. 2010. A deceptive pollination system targeting drosophilids through olfactory mimicry of yeast. – Curr. Biol. 20: 1846–1852.

Suinyuy, T. N. and Johnson, S. D. 2018. Geographic variation in cone volatiles and pollinators in the thermogenic African cycad *Encephalartos ghellinckii* Lem. – Plant Biol. 20: 579–590.

Suinyuy, T. N. et al. 2012. Geographical variation in cone volatile composition among populations of the African cycad *Encephalartos villosus*. – Biol. J. Linn. Soc. 106: 514–527.

Svensson, G. P. et al. 2005. Chemistry and geographic variation of floral scent in *Yucca filamentosa* (Agavaceae). – Am. J. Bot. 92: 1624–1631.

Szenteczki, M. A. et al. 2021. Data from: Spatial and temporal heterogeneity in pollinator communities maintains within-species floral odour variation. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.v15dv41w7>.

Thomann, M. et al. 2013. Flowering plants under global pollinator decline. – Trends Plant Sci. 18: 353–359.

Triponez, Y. et al. 2013. Morphological, ecological and genetic aspects associated with endemism in the fly orchid group. – Mol. Ecol. 22: 1431–1446.

Urru, I. et al. 2011. Pollination by brood-site deception. – Phytochemistry 72: 1655–1666.

Urru, I. et al. 2010. Pollination strategies in Cretan *Arum* lilies. – Biol. J. Linn. Soc. 101: 991–1001.

Vaillant, F. 1971. 9d. Psychodidae–Psychodinae. – In: Lindner, E. (ed.), Die Fliegen der Palaearktischen Region. E. Schweizerbart'sche Verlagsbuchhandlung.

van der Niet, T. and Johnson, S. D. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. – Trends Ecol. Evol. 27: 353–361.

Waser, N. M. and Ollerton, J. 2006. Plant–pollinator interactions: from specialization to generalization. – Univ. of Chicago Press.

Whitehead, M. R. and Peakall, R. 2009. Integrating floral scent, pollination ecology and population genetics. – Funct. Ecol. 23: 863–874.

Wund, M. A. 2012. Assessing the impacts of phenotypic plasticity on evolution. – Integr. Comp. Biol. 52: 5–15.

Yuan, J. S. et al. 2009. Smelling global climate change: mitigation of function for plant volatile organic compounds. – Trends Ecol. Evol. 24: 323–331.

Zattara, E. E. and Aizen, M. A. 2021. Worldwide occurrence records suggest a global decline in bee species richness. – One Earth 4: 114–123.