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Title: **Floral scents of a deceptive plant are hyperdiverse and under population-specific phenotypic selection**

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ABSTRACT

Floral scent is a key mediator in plant-pollinator interactions; however, little is known to what extent intraspecific scent variation is shaped by phenotypic selection, with no information yet in deceptive plants. We collected inflorescence scent and fruit set of the deceptive fly-pollinated *Arum maculatum* from various populations north vs. south of the Alps. We recorded 291 scent compounds and found that scent and fruit set differed north vs. south of the Alps. Seven and two compounds were under phenotypic selection in the largest northern and southern population respectively, among them none of the four presently known pollinator attractants of *A. maculatum*. Our study provides the highest number of scent compounds so far reported in a single plant species and provides evidence for the first time that floral scents of a deceptive species are under phenotypic selection. It suggests that regional differences in scent are only partly due to divergent selection.

Key words: *Arum maculatum*, brood-site deception, chemical ecology, geographic variation, hyperdiverse floral scent, phenotypic selection, Psychodidae

1 INTRODUCTION

2 About 88% of angiosperms are cross-pollinated by animals¹ that are attracted to flowers by
3 multifaceted cues². Together with visual cues, the main attractant for pollinators is floral
4 scent^{3,4}. Therefore, scent has strong effects on pollinator visitation and frequency, and hence
5 the plant's reproductive success^{3,5}. With more than 2,000 floral volatile organic compounds
6 (VOCs) described^{4,6}, and an average of 20–60 VOCs per species⁷, floral scent blends can
7 tremendously vary among species in composition and quantity. Consequently, they facilitate
8 discrimination by pollinators among species and contribute to reproductive isolation of (closely
9 related) species^{8–11}.

10 In addition to interspecific variation, floral scent is also known to vary intraspecifically,
11 both within and among populations^{5,12–16}. Such intraspecific variability might result directly
12 from abiotic (e.g., temperature¹⁷, soil chemistry^{18,19}) and/or biotic factors (e.g., herbivores^{20,21},
13 microbes²²). Given that scent is heritable^{23–25}, intraspecific differences can also result from
14 varying evolutionary forces, such as natural selection and genetic drift^{4,26,27}.

15 Although not explicitly demonstrated, genetic drift was suggested to be responsible for
16 strong inter-population differences in floral scents⁵, or to counteract pollinator-mediated
17 selection in two *Yucca* species²⁸). Natural selection on floral scent emission has been shown,
18 both on total scent amount and on individual scent components, by analyses of phenotypic
19 selection by correlating scent phenotypes and fitness measures^{29–36}. Phenotypic selection on
20 floral scent can also vary intraspecifically, potentially leading to variable adaptive responses to
21 spatially different pollinators^{32,36,37}. Until now, studies examining phenotypic selection on floral
22 scent have tested rewarding, but not deceptive species, although the latter also often rely on
23 luring and deceiving their pollinators with scents^{38–40}. Compared to their rewarding relatives,
24 non-rewarding species often display higher variation in scent and other traits attractive to
25 pollinators^{41–43}, and are frequently more pollen-limited^{44–46}. In consequence, they might
26 experience stronger selection on floral scent than rewarding species, as shown for phenotypic
27 floral traits other than scent⁴⁷. An ideal target for studying phenotypic selection on scent is the
28 fly-pollinated and brood-site deceptive *Arum maculatum* L. (Araceae), which attracts their
29 pollinators by olfactory deception.

30 This perennial herb is widespread in Europe and shows high variation in fruit and seed
31 sets within and among populations^{48,49}. The main pollinators are two psychodid flies (*Psychoda*
32 *phalaenoides* L. and *P. grisescens* TÖNN., Psychodidae) that are attracted by the strong, dung-
33 like inflorescence scent of *A. maculatum* while looking for oviposition sites and/or mating
34 partners^{50–52}. Previous analyses have shown that the scent profile of *A. maculatum* consists of

35 up to 60 components, also differing among populations^{53–58}. At least in part, this scent variation
36 appears to reflect variation in the pollinator community of *A. maculatum* across its distribution
37 range^{55,58}. In Central and much of Western Europe, high abundances of a single *Psychoda*
38 species and sex (females of *P. phalaenoides*) were found⁵². In other regions (Mediterranean
39 Europe and Western France), insects occurred in lower abundances but with a higher diversity
40 (mostly both sexes of *P. grisescens* and females of *P. phalaenoides*, plus a few other psychodid
41 species), also with some variation among populations⁵². This geographic pollinator pattern is
42 particularly pronounced north vs. south of the Alps^{52, D. Laina et al., unpublished} and matches a weak
43 genetic (AFLP) subdivision of *A. maculatum* across this geographic barrier⁵⁹. Presently, it is
44 unclear whether those regional pollinator and genetic patterns between populations of *A.*
45 *maculatum* are also reflected in their scent patterns. It is known, however, that the two main
46 pollinating psychodid species have dissimilar floral scent preferences^{55,58}. Hence, we assume
47 that the dissimilar scent preferences of the two fly species, together with their geographic north-
48 south distribution, could have led to differing selection pressures on scent of *A. maculatum* from
49 north vs. south of the Alps.

50 In this study, we investigated the floral scent characteristics and fruit set (as an indicator
51 for female fitness) of *A. maculatum* in six populations north of the Alps vs. five populations
52 south of the Alps and tested for phenotypic selection on scent in the two most extensively
53 sampled populations, one per region. Specifically, we asked: (1) Do scent and fruit set differ
54 between populations north vs. south of the Alps, and among populations within regions? (2) Is
55 there phenotypic selection on floral scent? (tested in the most extensively sampled populations
56 north and south of the Alps) And if so, (3) do compounds under selection differ between these
57 populations? Considering the differences in pollinator compositions between regions and also
58 among populations south of the Alps, but not north⁵², we expect to find pronounced differences
59 in scent between the two studied regions but also among southern populations. Considering
60 further regional differences in pollinator spectra and abundance, as well as the different
61 olfactory preferences of pollinator species, we additionally expect lower fruit set and stronger
62 selection on more scent components in the most extensively sampled population south of the
63 Alps, compared to the northern conspecific.

64 RESULTS

65 Floral scent

66 The total absolute amount of scent was highly variable among the 234 sampled individuals
67 (range: 1–2,052 ng inflorescence⁻¹ h⁻¹; Table 1). Across plants from north of the Alps, we
68 overall detected a three-fold lower amount than from those in the South, along with differences
69 among populations within regions (permANOVA: *region*: pseudo- $F_{1,223} = 25.70$, *population*
70 nested within *region*: pseudo- $F_{9,223} = 5.36$, both $P < 0.001$). From plants of most southern
71 populations, we detected a median of *c.* 200 ng inflorescence⁻¹ h⁻¹, except for DAO and UDI,
72 where we detected 1.5-fold higher and five-fold lower amounts, respectively. In the North, we
73 detected between 40 and 81 ng inflorescence⁻¹ h⁻¹ in half of the populations, while in the other
74 half amounts were manifold higher (JOS and HOH) or lower (BUR) (Table 1).

75 Across all scent samples, we detected a total of 291 floral volatiles (283 north vs. 265
76 south), and 92 of those could be (tentatively) identified (Table 1, and Supporting Information
77 Table S2). A median of 102 compounds per individual was recorded (Fig. 2), and the number
78 of compounds was independent of the region (permANOVA: pseudo- $F_{1,223} = 1.68$, $P = 0.21$),
79 but varied among populations within regions (pseudo- $F_{9,223} = 4.47$, $P < 0.001$). At the
80 population level, between 166 (BUR) and 266 (JOS) compounds were recorded in the North,
81 and between 88 (MON) and 254 (DAO) in the South (Fig. 2). The two most extensively sampled
82 northern (JOS) vs. southern (DAO) populations covered 92% vs. 87% of their respective
83 regional diversity (Fig. 2), and together 97% (283/291) of the total number of compounds
84 (Table 1, and Supporting Information Table S2). The five most frequent compounds, found in
85 more than 99% of the samples, were the nitrogen-bearing compound indole, the
86 monoterpenoids 3,7-dimethyloct-1-ene and β -citronellene, the sesquiterpenoid β -
87 caryophyllene, and the unidentified UNK1492 (Table 1).

88 The absolute amounts of single compounds significantly differed both between regions
89 (permANOVA: pseudo- $F_{1,223} = 22.52$, $P < 0.001$) and among populations within regions
90 (pseudo- $F_{9,223} = 6.44$, $P < 0.001$), but differences were more pronounced between regions
91 (*north* vs. *south* OOB error: 9.9%) than among populations (within *north* OOB error: 28.3%;
92 within *south* OOB error: 25.2%). Only a few abundant compounds dominated the scent bouquet
93 of *A. maculatum*, including indole, β -citronellene, the unknown UNK1415, and 3,7-
94 dimethyloct-2-ene (in both regions), *p*-cresol (only north), and 2-heptanone (only south) (Table
95 1). The absolute scent patterns among populations could not be explained by their geographic
96 distances (Mantel's *Rho*: 0.108, $P = 0.25$).

97 We also detected differences in the relative amounts of scent components between
98 regions (permANOVA: pseudo- $F_{1,223} = 30.34$, $P < 0.001$) and among populations (pseudo-
99 $F_{9,223} = 4.87$, $P < 0.001$; Fig. 3). This scent pattern was also more distinct between regions than
100 among populations within regions (*north* vs. *south* OOB error: 8.2%; among populations within
101 *north* OOB error: 33.0%; among populations within *south* OOB error: 25.2%; see also
102 Supporting Information Figure S1). These relative patterns among populations were also not
103 related to population geographic distances (Mantel's Rho : -0.154 , $P = 0.85$).

104 Based on the *randomForest* analyses, from the 25 compounds each that were most
105 responsible for regional differences in the absolute and relative data, respectively, 20 were
106 common to both datasets (Supporting Information Table S3). Among the latter were 2-
107 heptanone, 2-heptanol, and α - and β -citronellene, all of which were more abundant (in relative
108 and absolute amounts) south of the Alps. Others, such as 1-pentadecanol, the unknown
109 UNK1503, *p*-cresol, and indole occurred in higher amounts north of the Alps (Table 1, and
110 Supporting Information Tables S2, S3). Many of these compounds, but also some non-
111 overlapping compounds (absolute: α -copaene, β -caryophyllene; relative: UNK1409,
112 bicyclogermacrene) generally explained most variation in scent among all the samples (for
113 relative data, see Fig. 3; for absolute data, see Table S4).

114 Despite the differences in scent between regions and among populations, we also
115 observed considerably high variation in scent within populations, most prominently in the most
116 extensively sampled northern (JOS) and southern (DAO) populations, which harboured almost
117 all of the absolute and relative scent variation of their respective region (for relative data, see
118 Fig. 3).

119

120 **Fruit set**

121 Of the 234 individuals surveyed for inflorescence scent, 113 set fruit in summer. Percentages
122 of fruit set were significantly higher north (mean \pm sd: $42 \pm \%$, 0–100% Min–Max) than south
123 (mean \pm sd: $26 \pm \%$, 0–100% Min–Max) of the Alps (Fig. 4; *region*: $F_{1,209} = 10.11$, $P = 0.002$),
124 and differed significantly among populations within regions (Fig. 4; *population* nested within
125 *region*: $F_{8,209} = 2.23$, $P = 0.03$).

126

127 **Phenotypic selection on scent**

128 Of the 19 and three volatile compounds that were tested for β - and γ -selection in the two most
129 extensively sampled populations north (JOS) vs. south (DAO) of the Alps, respectively, seven
130 showed signals of linear β -selection (two of which as a significant interaction), all in the north,

131 and two of nonlinear γ -selection, all in the south (Fig. 5). Six volatiles correlated positively
132 (positive β -gradient) with relative fruit set (i.e., 2-nonanol, 2-heptanol, α -terpinene, UNK681,
133 and the interaction of UNK1496 with UNK1503), and one (UNK960) negatively, and of the
134 two volatiles with significant γ -gradients one had a positive (sabinene) and the other one (4-
135 terpinenol) a negative gradient (Fig. 5 and Supporting Information Table S5).

136 Only three compounds that strongly contributed to the absolute (and relative)
137 differences in scent between the regions were under selection (north: 2-heptanol, 2-nonanol,
138 UNK681; Supporting Information Table S3), but not others (e.g., 2-heptanone, α - and β -
139 citronellene). Differences in absolute and relative scent traits between the northern JOS and the
140 southern DAO remained significant, regardless of performing the analyses exclusively with the
141 22 volatiles that correlated with fitness in the pre-selective analyses (absolute: pseudo- $F_{1,109} =$
142 12.8, relative: pseudo- $F_{1,109} = 21.9$, both $P < 0.001$), or with the 80 compounds that did not
143 (absolute: pseudo- $F_{1,109} = 10.0$, relative: pseudo- $F_{1,109} = 25.2$, $P < 0.001$ each) (see Supporting
144 Information Fig. S2).

145 **DISCUSSION**

146 Our study shows that *Arum maculatum* has hyperdiverse inflorescence scents that differ in their
147 composition between populations north vs. south of the Alps. In contrast to our expectations,
148 scent differed not only among populations within the southern but also within the northern
149 region. As expected, individuals from southern populations had lower fruit set than northern
150 ones, yet more volatiles were under phenotypic selection in the most extensively sampled
151 northern population than in the southern one.

152

153 **Hyperdiversity of floral scent**

154 With 291 floral volatiles recorded, the inflorescence scent diversity of *A. maculatum* is
155 extraordinarily high and, to the best of our knowledge, not matched by any other plant species.
156 In fact, we are not aware of any species from which more than 200 floral compounds are
157 reported, a number that a single *A. maculatum* individual can reach by three quarters (max. =
158 147 VOCs). This difference in the number of scent compounds between *A. maculatum* and other
159 species cannot just be explained by differences in techniques used for scent analyses, given that
160 scents of a high number of species were analysed using a similar approach as we did (dynamic
161 headspace and thermal desorption of samples). Species closest to the high number in *A.*
162 *maculatum* include the sapromyiophilous *Sauromatum guttatum* (Araceae, with altogether 196

163 different VOCs^{60,61}), as well as the insect-pollinated and rewarding *Geonoma macrostachys*
164 (Arecaceae, 176 VOCs⁶²), and *Echinopsis ancistrophora* Cactaceae, 145 VOCs⁶³). Other
165 species for which *c.* 100 VOCs are described likewise include insect-pollinated rewarding
166 species (e.g., *Acleisanthes wrightii*, Nyctaginaceae⁶⁴; *Saraca asoca*, Fabaceae⁶⁵; *Philodendron*
167 *bipinnatifidum*, Araceae⁶⁶; *Pyrus communis*, Rosaceae⁶⁷), but also the sexually deceptive orchid
168 *Ophrys sphegodes*⁶⁸. Thus, high numbers of compounds are found across a wide range of plant
169 families and are apparently not restricted to a specific pollination system.

170 One explanation for the high diversity of scent compounds in *A. maculatum* is that this
171 species likely imitates various breeding substrates of its pollinators, all potentially differently
172 scented. The two main pollinators, *Psychoda phalaenoides* and *P. grisescens*, breed in rotting
173 manure from cattle and horse, fungi (*P. grisescens*), waste pits, mud-flats, plant litter in
174 drainages and ditches (*P. phalaenoides*), and in the hygropetric zones of river banks and
175 ponds^{69–76}. *Arum maculatum* emits compounds described from quite a number of such
176 substrates, e.g., cattle and horse manure (e.g., indole, *p*-cresol, skatole), fungi (1-octen-3-ol,
177 (*E*)-2-octen-1-ol, 3-octanone), and general degrading and fermenting plant or animal material
178 (e.g., 2,3-heptanedione, acetoin, butanoic acid)^{40,53,77,78}. Highly specialised deceptive plant
179 systems frequently rely on only a few volatiles to attract pollinators – they seem to imitate a
180 more specific model, and thus, release less complex scent blends^{e.g. 40,79–82}.

181 The number of volatiles detected across the 235 individuals (11 populations) of *A.*
182 *maculatum* (291) is five to ten times higher than previously reported for this species (18–61,
183 and 143 VOCs in total^{53–58,83}). This discrepancy cannot be explained by differences in sample
184 size, as a similar number of individuals were surveyed in previous studies (*n* = 222 in total,
185 representing 23 populations). Interestingly, we found in some single individuals a similar
186 number of compounds (up to 147 VOCs; median of 102) as overall detected previously. With
187 the exception of two studies^{56,58} who each share one of our sampled populations (JOS and
188 MON, respectively) all previous studies sampled scents in other populations. Thus, some of the
189 differences in the number of compounds detected across studies might reflect region-specific
190 population characteristics (see Fig. 2). More importantly, however, we believe that the
191 discrepancy in the number of compounds recorded largely reflects differences in methodology
192 between the present and previous studies. These are, for example, higher sensitivity of modern
193 GC/MS systems; usage of more selective adsorbent agents (Carbotrap/Tenax-TA vs. solid-
194 phase micro-extraction^{55,57} vs. Twister⁵⁸); *in situ* vs. *ex situ* sampling^{56,83}; and including all vs.
195 only compounds above a specific threshold in relative amounts^{55,58}. Of the 92 compounds
196 (tentatively) identified in this study, more than half (50) were previously unknown to be

197 released by *A. maculatum*. Some of these newly described compounds for *A. maculatum* are
198 known from other species of Araceae (e.g., nerol, (*E,E*)- α -farnesene, 6-methyl-5-heptene-2-ol,
199 γ -terpinene; *Sauromatum guttatum*⁶⁰, *Anthurium* spp.^{84,85}) or other plant families (e.g., methyl
200 anthranilate, isobutyl butyrate, citronellal^{4,6}). However, this study is, to the best of our
201 knowledge, the first to identify *p*-cresyl butyrate as a floral scent compound.

202

203 **Geographic patterns of floral scent**

204 The qualitative, absolute and relative differences in scent detected herein among populations of
205 *A. maculatum* south of the Alps may be explained by the fact that pollinator spectra are more
206 diverse in abundance, species and sex composition south of the Alps^{52, D. Laina et al., unpublished}. North
207 of the Alps, however, where females of *P. phalaenoides* are the principal pollinators in all
208 populations^{52, D. Laina et al., unpublished}, even though the other psychodid species also occur in small
209 numbers^{55,86}, the variation in scent among populations is not reflected by variations in pollinator
210 spectra. In our study, all multivariate scent patterns were more pronounced between regions
211 compared to variation among populations within each region. These differences in scent across
212 the Alps are in agreement with strong differences in pollinator spectra^{52, D. Laina et al., unpublished} and
213 a genetic (AFLP) differentiation of *A. maculatum* across this geographic barrier⁵⁹, possibly
214 reflecting different colonisation histories during the Pleistocene⁸⁷. Previous studies in *A.*
215 *maculatum* also found population effects in scent composition^{55,57,58}; however, our study
216 demonstrates for the first time such population differentiation in scent across the Alps.
217 Interestingly, such regional differences in scent were not recorded in Szenteczki *et al.*⁵⁸,
218 although the authors analysed inflorescence scents in *A. maculatum* across Europe, including
219 some populations north and south of the Alps. Intraspecific scent differences between regions
220 and among populations are also known for other plant species^{14,15,37,63,88}. For some of those
221 species it has been shown that observed scent patterns, as in our study, reflect pollinator and/or
222 genetic patterns^{15,37,88}, but not for others^{14,63}.

223

224 **Phenotypic selection on floral scents**

225 In the present study, the absolute and relative scent differences found between the two most
226 extensively sampled populations of their respective region were evident, independent of
227 whether all compounds, only compounds that correlated with fruit set in the pre-selective
228 analyses, or only compounds that did not, were included in the analyses (Supporting
229 Information Fig. S2). Hence, this regional scent pattern appears to be a result of both divergent
230 selection and of other reasons, such as phenotypic plasticity^{but see⁵⁸} or genetic drift^{89,90}. In

231 support of divergent selection, we detected population-specific signatures of direct selection on
232 scent, potentially as a result of the different olfactory preferences of *P. phalaenoides* and *P.*
233 *griseascens*^{55,58}, the two species most responsible for the regionally different pollinator spectra⁵²,
234 *D. Laina et al., unpublished*. These different selection regimes, however, cannot explain the most
235 obvious differences in scent between *A. maculatum* from north vs. south of the Alps (see also
236 Supporting Information Fig. **S1**). This is because most compounds identified as being mainly
237 responsible for those regional differences (e.g., 2-heptanone, 3,7-dimethyloct-1-ene, UNK966;
238 Supporting Information Table **S3**) did not show signals of direct selection (see Fig. **5**). Some
239 other compounds, however, which also differed in their absolute amounts between regions (2-
240 heptanol, 2-nonanol, UNK681, sabinene; Supporting Information Table **S3, S5**) differed in their
241 selection in JOS and DAO (Fig. **5**), and some of the differences between regions might thus be
242 due to divergent selection. Interestingly, β -selection on scent was only detected in the
243 population north of the Alps, the region which attracts higher numbers of pollinators and
244 predominantly a single species, i.e, females of *P. phalaenoides*^{52, D. Laina et al., unpublished}, and has a
245 higher fruit set than south of the Alps (Fig. **4**). The preferences by a more consistent single
246 pollinator species might exert strong selection pressures and result in stronger (and thus better
247 detectable) phenotypic selection on specific compounds in the most extensively sampled
248 population north of the Alps, compared to south. There, a more diverse composition of
249 pollinator species^{52, D. Laina et al., unpublished} as it occurs in South of the Alps and their putative
250 different scent preferences^{55,58} might lead to conflicting selection pressures^{33,34,36}.

251 For the five compounds under phenotypic selection that could be identified by authentic
252 standards (Supporting Information Table **S2**), i.e., 2-nonanol, 2-heptanol, sabinene, 4-
253 terpinenol and α -terpinene, we lack information on their attractiveness to psychodid pollinators
254 of *A. maculatum*. However, the aliphatic components 2-heptanol and 2-nonanol together are
255 found as (sex-)pheromones of female Diptera (Cecidomyiidae⁹¹) and female non-Diptera
256 (Trichoptera⁹²), and as an attractant for bees (Meliponini⁹³). The volatile 2-nonanol alone also
257 attracts kleptoparasitic flies⁹⁴ and is attractive for other Meliponini⁹⁵, where it is used for host-
258 finding and as a pheromone. The monoterpenoids α -terpinene, 4-terpinenol, and sabinene are
259 defence substances of some insects (Coleoptera^{96,97}, Lepidoptera⁹⁸) that repel Coleoptera⁹⁹, but
260 are used by Hymenoptera¹⁰⁰ and Lepidoptera¹⁰¹ for host-finding (beetles) and as an oviposition
261 stimulant. Also, α -terpinene and 4-terpinenol are pheromones in fruit flies¹⁰². It would be very
262 interesting to know whether these five compounds, which are all widespread among floral
263 scents^{4,6}, are attractive to pollinators of *A. maculatum*, especially because they elicit responses
264 in other insects, are known from other sapromyiophilous species^{60,61,77,80,103,104}, and some of

265 them (α -terpinene and 4-terpinenol) are also known from cattle dung¹⁰⁵, E. Gfrerer, unpublished, i.e.,
266 one of the oviposition substrates of psychodid flies.

267 Somewhat unexpectedly, we did not find positive β -selection gradients for the most
268 abundant compounds in the scent of *A. maculatum* (e.g., β -citronellene, β -caryophyllene,
269 unknown UNK1415), with the expectation of 2-heptanol (Fig. 5). Even more surprisingly, we
270 also did not find phenotypic selection for those known to attract *P. phalaenoides* (i.e. indole, 2-
271 heptanone, *p*-cresol, α -humulene^{50,83}), one of the two main pollinators of this plant species,
272 occurring both north and south of the Alps⁵². This is in contrast to most other studies, where
273 main compounds and/or pollinator attractants showed signals of β -selection and had positive
274 gradients^{but see 31,34}. Possible explanations for these findings are that the main compounds may
275 generally be released in amounts high enough to result in maximum pollinator attractiveness^{see}
276 also³⁴, that the relationship between the compound and different selection agents is conflicting,
277 i.e., opposing selection by different pollinator species or herbivores^{33,34}, or that different
278 amounts of the compounds have different effects on flower visitors, i.e., the relationship is
279 nonlinear^{106–108}. Support for the latter hypothesis comes from our nonlinear quadratic analyses,
280 where the amounts of sabinene and 4-terpinenol were correlated with relative fruit set in the
281 southern population DAO (Fig. 5). Although we detected direct nonlinear selection by including
282 quadratic terms in our multivariate models, these quadratic analyses do not uncover all potential
283 nonlinear relationships^{e.g.109}. Hence, we cannot exclude the possibility that such compounds are
284 still under phenotypic selection, which in turn calls for future approaches that allow testing for
285 any kind of nonlinear multivariate relationships.

286 Deceptive plant species might experience stronger selection than rewarding ones⁴⁷. In
287 deceptive *A. maculatum*, we did not find a higher number of volatiles with signatures of
288 selection (direct: 7% vs. 3–42%), but slightly stronger positive β -selection (-0.3–0.5 Min–Max
289 vs. -0.3–0.4) compared to other, rewarding, species^{30–35}, and a stronger γ -selection (-0.9–9.0
290 Min–Max vs. -0.5–-0.3³⁶, Fig. 5). Future studies on other deceptive plant species that also
291 attract specific pollinators by chemical cues, but have lower levels of fruit set, might reveal
292 even stronger signatures of direct selection.

293

294 **Conclusions**

295 Our study on sapromyiophilous *Arum maculatum* reports the highest number of floral volatiles
296 ever found in a single plant species to date. This plant species likely imitates the odours of a
297 multitude of differently scented breeding substrates of its psychodid fly pollinators (e.g., dung,
298 fungi, rotting plant material), which might result in this chemical hyperdiversity. We recorded

299 pronounced scent differences between populations across the Alps, and this geographic pattern
300 in scent agrees with previously described pollinator and genetic patterns across this geographic
301 barrier. Our results provide for the first time evidence that floral scents of a deceptive species
302 are under phenotypic selection and suggest that populational/regional differences in scent are
303 partly due to divergent selection, but also due to other reasons, such as phenotypic plasticity
304 and genetic drift. In *A. maculatum* and other plants where phenotypic selection on scent was
305 demonstrated^{29–36} the biological role of most compounds under selection is unknown and awaits
306 determination in future studies.

307 **MATERIALS AND METHODS**

308 **Study species and populations**

309 Brood-site deceptive *Arum maculatum* L. (Araceae) is a rhizomatous perennial woodland herb
310 ($2n = 4x = 56$) that is widespread throughout Western and Central Europe, including the British
311 Isles, and reaches as far south as Italy, Northern Spain, and the Balkans^{52,110,111}. It exhibits a
312 sapromyophilous pollination strategy, is thermogenically active, and emits a strong dung-like
313 scent for attracting fly pollinators during the evening on the first day of anthesis^{53,56,112,113}. The
314 inflorescence of *Arum maculatum* consists of a spadix (fleshy spike) and a spathe (bract), is
315 protogynous, and the anthesis lasts less than two days^{51,56,112}. The spathe, which completely
316 encloses the spadix during floral development, partially opens during anthesis to reveal the
317 sterile appendix of the apical part of the spadix. This appendix produces and releases the scent
318 for pollinator attraction^{50,51,53,83}. At the base of the spadix, female (fertile and sterile) flowers
319 are situated lowest, followed upwards by male flowers and staminodes (sterile male flowers).
320 The sequential maturation of female (first) and male flowers (later) prevents selfing¹¹⁴. All
321 flowers remain enveloped by the spathe during anthesis, forming a chamber that is closed by
322 the staminodes throughout the female stage to prevent trapped insects from leaving. Pollinators
323 are attracted in the evening on the first day of anthesis, during the female stage, slip and fall
324 into the floral chamber, and are trapped overnight^{51,52,113,115}. On the next morning, during the
325 male stage, they are dusted with pollen, before being released at around noon when the
326 staminodes wither^{51,52,112}. After pollination in spring, red berry-like fruits develop as an
327 infructescence until summer¹¹⁶.

328 In 2017–2019, during springtime, we collected scent from randomly chosen *A.*
329 *maculatum* individuals of six populations located north of the Alps ($n = 107$; Northwestern
330 Austria: JOS; Central/Southern Germany: BUR, HOH, MUR, NEC; Northern Switzerland:

331 RŪM) and from five populations south of the Alps ($n = 127$; Northern Italy: DAO, LIM, MAH,
332 MON, UDI) (Fig. 1). We kept a minimum distance of one metre between sampled individuals
333 to avoid sampling potential clones, as *Arum maculatum* can reproduce vegetatively. In summer,
334 we harvested fruits of the same plant individuals. At most sites, we surveyed 15 individuals,
335 except for each of the largest population per region (JOS and DAO; $n = 70$ each), and a northern
336 population (HOH; $n = 7$) where only a few individuals flowered at the time of sampling (Fig. 1
337 and Supporting Information Table S1).

338

339 **Plant volatile collection and analysis**

340 Scent sampling took place on the first day of anthesis during the female stage between 18:00
341 and 19:30, the period of maximum scent emission⁵⁶, employing a non-invasive dynamic
342 headspace technique. We enclosed each inflorescence *in situ* using an odourless plastic oven
343 bag (c. 30×12 cm; Toppits®, Melitta, Germany) and immediately collected scent for five
344 minutes at 200 ml min⁻¹ on adsorbent tubes (inner diameter: 2 mm) filled with a mixture of
345 Tenax-TA (mesh 60–80) and Carbotrap B (mesh 20–40; 1.5 mg each; both Supelco, Germany),
346 using a battery-operated vacuum pump (rotary vane pump G12/01 EB, Gardner Denver Austria
347 GmbH, Vienna, Austria)⁵⁶. In the same way, we collected scent samples from leaves and
348 ambient air as negative controls in each population.

349 The dynamic headspace samples were analysed by thermal desorption-gas
350 chromatography/mass spectrometry (TD-GC/MS)⁵⁶, and obtained data were handled using
351 *GCMSolution* v.4.41 (Shimadzu Corporation, Kyoto, Japan) (for details see Supporting
352 Information Method S1). Components were tentatively identified by comparison of Kováts'
353 retention indices (KRIs¹¹⁷), based on commercially available *n*-alkanes (C₇–C₂₀), and mass
354 spectra to data available in the libraries of Adams¹¹⁸, FFNSC 2, Wiley9, NIST11, and
355 ESSENTIAL OILS (available in *MassFinder 3*, Hochmuth Scientific Consulting, Hamburg,
356 Germany). We established an own library of mass-spectral and KRIs for semi-automatic
357 analysis (Supporting Information Method S1). Whenever possible, components were verified
358 by comparison to authentic reference standards available in the collection of the Plant Ecology
359 Lab of Salzburg University, or to chemically synthesised reference components (Supporting
360 Information Method S2). Of the 267 collected scent samples, 234 yielded a sufficiently
361 informative chromatogram and were included in the analysis (Fig. 1). Ultimately, a compound
362 was only considered if it occurred in more than three scent samples and did not occur in leaf
363 and air controls.

364

365 **Fruit set**

366 Percentage fruit set (i.e., number of fruits/total number of flowers per individual \times 100) was
367 determined as a measure of female reproductive success. For selection analyses we further
368 estimated relative fruit set (i.e. number of fruits per individual/ mean number of fruits per given
369 population) as a measurement for female reproductive success, standardised per population for
370 the most extensively sampled populations JOS and DAO^{32,35}. ~~Around 50% of the individuals~~
371 ~~used for scent sampling failed to develop an infructescence, due to unknown reasons such as~~
372 ~~herbivory, lack of pollination, or other causes (e.g. resource limitation, shallow landslide). In~~
373 ~~consequence, we only included those plants in subsequent fruit set and selection analyses for~~
374 ~~which fruit (and scent) data were available ($n_{\text{north}} = 61$, $n_{\text{south}} = 52$).~~ In one southern population
375 (MON), a shallow landslide destroyed all plants, with the exception of one (Fig. 1); hence, this
376 population was excluded from fruit set analyses.

377

378 **Statistical analyses**

379 **Geographic patterns in scent and fruit set data** In order to test for geographic differences in
380 floral scent, we performed permutational multivariate analyses of variance (permANOVAs;
381 Anderson, 2001) as implemented in the R package *vegan* v.2.6-6¹¹⁹. We did this on (1) pairwise
382 Bray–Curtis dissimilarities of either absolute or relative scent data (i.e., absolute amount of
383 single compounds or relative amount of single compounds in relation to the total amount of
384 scent in a sample, respectively); and on (2) Euclidean distances of total absolute emission of
385 scent and of total number of floral volatiles per individual. In all these analyses, we used *region*
386 (north vs. south of the Alps) and *population* nested within *region* as explanatory variables
387 (9,999 permutations). To test for geographic patterns (north vs. south Alps) in relation to
388 selection in absolute and relative scent, we focussed on the (reduced) dataset of the two most
389 extensively sampled populations using only compounds that correlated with fruit set in the pre-
390 selective analyses (see below), or only compounds that did not, with a permANOVA
391 (*population* as explanatory variable, 9,999 permutations). The Bray–Curtis dissimilarity
392 matrices (based on absolute and relative scent data) were further used to conduct constrained
393 analyses of principal coordinates (CAP¹²⁰) with *population* as factor, using the *capscale*
394 function in *vegan*, in order to visualize similarities and dissimilarities in scent among the
395 samples^{following 121,122}. For each ordination, we also calculated vectors, representing compounds
396 most correlating with the axes (Pearson correlations with *capscale scores*, $r > |0.5|$, corrected
397 for false-discovery rate¹²³). Additionally, we subjected the absolute and relative data to random
398 forest analyses (Breiman, 2001) by the R package *randomForest* v.4.6-14¹²⁴ (*ntree* = 9,999

399 bootstrap samples with $mtry = 17$) to evaluate the distinctness in scent of northern and southern
400 samples (factor *region*) and among populations within each region (factor *population* each)¹²⁵.
401 Distinctness was quantified as the average out-of-bag (OOB) error estimate (in %), i.e., the
402 more distinct, the lower the OOB error. From the resulting *randomForest* objects, we further
403 extracted the *importance* measurements to determine volatiles crucial for regional distinction.

404 To test for relationships between the dissimilarity of median absolute and relative scent
405 properties of populations and their geographical distances (in km), we performed Mantel tests
406 with the function *mantel* in *vegan* (9,999 permutations, Spearman's rank correlation). To assess
407 whether absolute amounts of single compounds under selection (see below) differ between the
408 two regions, we performed Mann–Whitney U tests. Differences in percentage of fruit set across
409 regions and among populations within regions were assessed by a generalised linear model
410 (*regions* and *populations* nested within *regions* as factors).

411

412 **Analyses of phenotypic selection** To estimate the direction and strength of phenotypic
413 selection on individual scent compounds and on total amount of scent, we tested for direct
414 phenotypic selection in the two most extensively sampled populations north (JOS) and south
415 (DAO) of the Alps¹²⁶. These two populations cover a large part of their corresponding regional
416 scent variation (see Fig. 3). As many compounds were quite rare (*c.* 70% of VOCs in <50% of
417 samples), the scent matrix contained many zeros (non-detects). This zero-inflation can cause
418 severe problems when fitting linear models (see below), as estimates will be biased¹²⁷. That is,
419 the influence of an individual scent compound on fruit set can be either over- or underestimated,
420 leading to potentially wrong conclusions. Additionally, our data has a considerable higher
421 number of factors (VOCs) than samples. Previous studies solved such issues by pre-selecting
422 variables^{30,31,34} to reduce high dimensionality, and/or performed selection analyses only on
423 most abundant compounds^{29,33}, on PC-scores^{29,32,35}, or on physiologically active volatiles³⁴. As
424 we only have very limited knowledge of attractive compounds (four volatiles^{50,83}), and also as
425 minor volatiles can be under selection³⁴, we pre-selected volatiles that correlate with relative
426 fruit set via elastic net, a penalised multivariate linear regression³¹. By adding a penalty to the
427 regression (regularisation), coefficients that are not important for the model are shrunk towards
428 zero (L_1 penalisation). An elastic net additionally selects groups of correlated variables (L_2
429 penalisation) instead of only one random variable from a multicollinear group of variables, as
430 e.g. LASSO does¹²⁸. By combining L_1 with L_2 penalisation, it is well suited for datasets that
431 have a larger number of predictor variables (in our case VOCs) than number of observations,
432 and copes well with multicollinearity^{128,129}, which is advantageous as volatiles that share the same

433 biosynthetic pathway can be highly multicollinear. Zero-inflation can still influence these
434 analyses; thus, to quantify the impact of non-detects on estimates, we performed a simulation
435 study based on our scent data for JOS and DAO separately (see Supplementary Information
436 Notes **S3**). This approach resulted in 93 and 81 scent compounds included in the consecutive
437 elastic net regression analyses for JOS and DAO, respectively. For the simulation and
438 consecutive elastic net analyses¹²⁸, we used *glmnet* v.4.1¹³⁰ in R.

439 For calculating the elastic net regression between relative fruit set and the z -transformed
440 variables (individual VOCs and total absolute scent emission) of the reduced data sets, we first
441 cross-validated for α (L_1/L_2 penalty ratio, range: 0–1, 0.1 increment) and λ (penalty strength
442 parameter; range: 100–0.001, -0.1 increment), for JOS and DAO separately. This resulted in an
443 elastic net for the northern population JOS with $\alpha_{(JOS)}=0.9$ and for the southern population DAO
444 with $\alpha_{(DAO)}=0.2$, and regression coefficients $\hat{\beta}$ were extracted at $\lambda_{min(JOS)}=0.05$ and
445 $\lambda_{min(DAO)}=1.99$. Pollinators might respond to different amounts of compounds in a nonlinear
446 manner^{106–108}, potentially resulting in a nonlinear relationship of fitness and scent traits. To
447 account for this, we ran a *boruta* algorithm (R package *Boruta* v.7.0.0¹³¹; *ntree* = 9,999
448 bootstrap samples with *mtry* = 9, Bonferroni-adjusted for multiple comparisons) to also identify
449 nonlinear relationships between individual volatiles, total absolute scent emission, and relative
450 fruit set. *Boruta* is a feature selection method that has shown high stability in variable selection
451 of high-dimensional data sets¹³², and classifies whether a feature (in our case VOCs) is
452 important or not¹³¹. In the northern population JOS, 19 volatiles emerged from the elastic net
453 and four from the *boruta* analysis; all four of which were already among the 19 linear ones (Fig.
454 **5**). In the southern DAO population, no common volatile correlated with fruit set in the elastic
455 net, but three in the *boruta* analysis. No volatile appeared in the analyses of both populations
456 (Fig. **5**). Total absolute scent amount did not correlate with fruit set in any of the analyses.

457 To ultimately determine β -gradients for phenotypic selection on scent (directional
458 selection¹²⁶), we performed with those variables selected by the elastic net model (i.e. $\hat{\beta}$
459 coefficient > 0) a multivariate linear regression. For volatiles selected by the *boruta* we
460 estimated multivariate quadratic regressions coefficients (γ -gradients, stabilizing and disruptive
461 selection¹²⁶) by squaring the terms and doubling resulting estimates¹⁰⁹, to also test for nonlinear
462 signs of phenotypic selection. For the southern model, we excluded the plant individual
463 “DAO076”, as it was determined as an outlier influencing the model ($D_{DAO076}=235.4$) by
464 Cook’s distance¹³³. Although elastic net handles multicollinearity well, resulting individual
465 volatiles might still correlate with another (L_2 penalty, see above). We tested for
466 multicollinearity within the multivariate models by calculating the variance inflation factor

467 (VIF) (R package *car* v.3.0.8¹³⁴) for each scent compound in each multivariate model. In the
468 northern model, the VIF value of various compounds was high (> 5) and for the unknowns
469 UNK1496 and UNK1503 even exceeded 10, a threshold that indicates strong
470 multicollinearity¹³⁵. After including these two compounds as an interaction, the VIF of most
471 compounds was below 5 with the exception of 3-octanol and UNK1279 (VIF > 6). Once also
472 the interaction of the latter two volatiles was included in the model, the VIF of all volatiles
473 dropped below 4, and the final northern model had an adjusted R^2 of 0.71. The southern model
474 had only VIFs below 2, and an adjusted R^2 of 0.26. All statistical analyses were performed in
475 R v.4.0.2¹³⁶.

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Author contributions

SD, MG, ACH and HPC designed the research; EG and DL conducted the fieldwork; RF executed the scent sample laboratory work; EG and SD built the scent library, and EG analysed all scent and fruit set data; TT identified and synthesised unknown components; MH, WT, RF, SD and EG discussed statistical approaches for selection analyses; MH performed the simulations, and EG all selection analyses; EG wrote the first draft of the manuscript and all authors contributed to the final version.

Data availability statement

The dataset that supports the findings of this study and the R code for the simulation are available at the Dryad digital repository at <https://doi.org/10.5061/dryad.pnvx0k6kn>

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Relative amounts of the most abundant compounds (see Table 1) in the inflorescence scents of *A. maculatum*, and whether or not they are under phenotypic selection.

Fig. S2 Non-metric multidimensional scaling of relative (a, b) and absolute (c, d) *Arum maculatum* scent patterns (a, c) with VOCs correlating with fitness in the pre-selective analyses and (b, d) only with VOCs that did not.

Table S1 Locality information on the sampled populations.

Table S2 Complete inflorescence scent list of *Arum maculatum* (median relative amount per population and area).

Table S3 Volatiles important for regional distinction in relative and absolute scent bouquets.

Table S4 Volatiles most correlating with the *capscale* scores in absolute scent samples.

Table S5 Direct selection gradient β and γ for volatiles of *Arum maculatum*.

Methods S1 Scent analysis (TD-GC/MS), quantification, and set-up of scent library.

Methods S2 Synthesis of reference samples: 2,6-dimethylocta-2,6-diene, 3,7-dimethyloct-2-ene and 2,6-dimethylocta-1,7-diene (α -citronellene)

Methods S3 Simulation to quantify impact of non-detects on selection estimates.

Notes S1 Mass spectrometry of unknown volatiles with significant phenotypic selection gradients.

Figures

Fig 1

Localities of the six populations north (blue) and five populations south (red) of the Alps of *Arum maculatum* sampled for the present study. Numbers in brackets give the number of individuals used for scent and selection analyses. The two most extensively sampled populations are indicated by larger circles. *North*: JOS, Josefiu; BUR, Burg Hohenstein; HOH, Hohendilching; MUR, Murnau; NEC, Horb am Neckar; RÜM, Rümikon; *South*: DAO, Daone; LIM, Limone-Piemonte; MAH, Santa Maria Hoè; MON, Montese; UDI, Udine. The map was prepared using the ETOPO1 Global Relief Model¹³⁷ and ArcGIS v.10.4 (ESRI, Redland, CA).

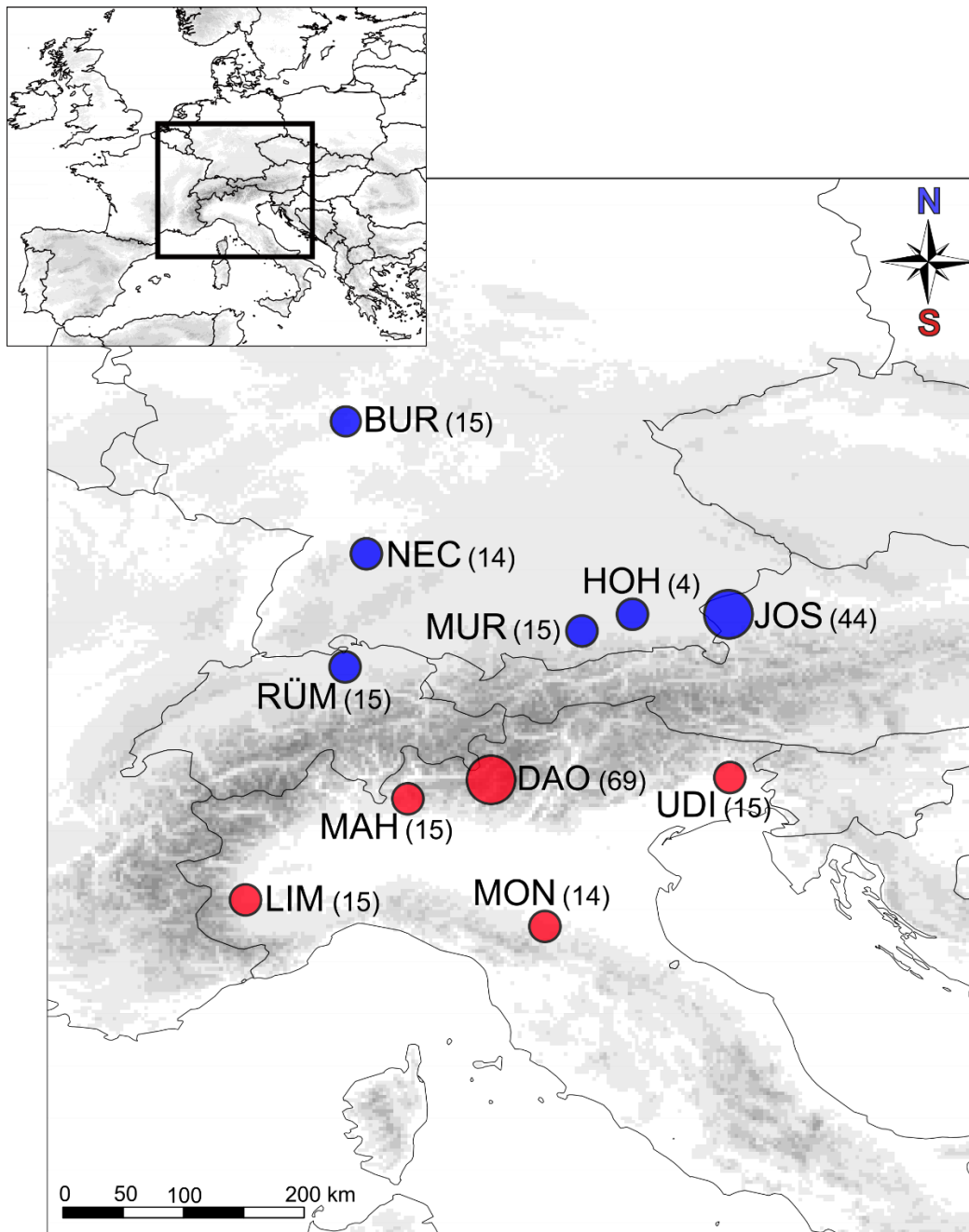


Fig 2

Number of floral scent compounds recorded in individual samples in the six and five populations of *Arum maculatum* from north and south of the Alps, respectively. Filled circles denote the population median of number of volatiles per individual; the vertical lines indicate the distance to the region median (horizontal line); and open circles mark the number of volatiles detected in the individual samples. The sample sizes and the percentage of volatiles detected in a population compared to the number of compounds detected across all samples (291 compounds) are shown as pie charts. See Fig. 1 and Table S1 for identification of population codes.

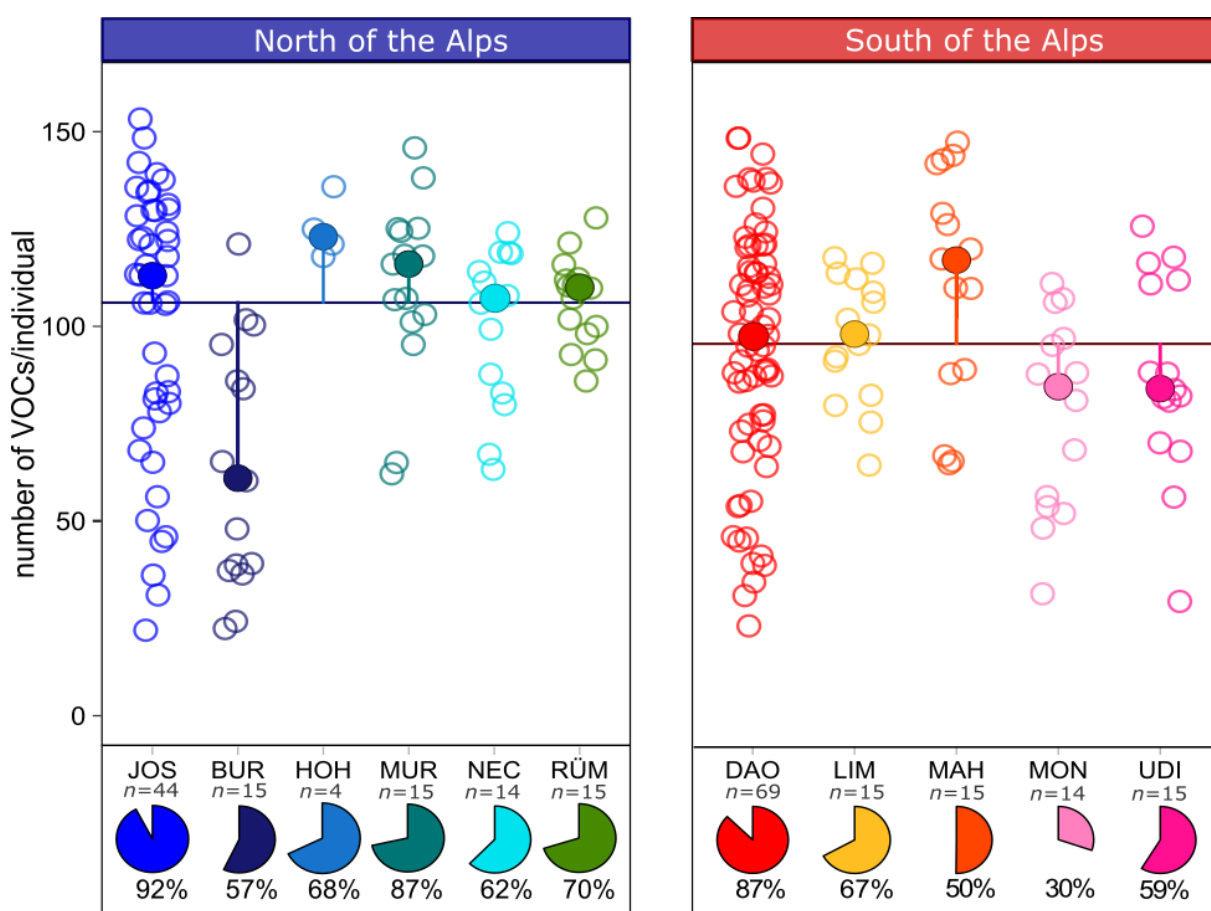


Fig 3

Canonical analysis of principal coordinates (CAP) based on a Bray–Curtis dissimilarity matrix of relative floral scent patterns in *Arum maculatum*. Between four and 69 individual samples were available across the six populations north and five south of the Alps. The vectors depict the volatiles most correlating with the *capscale* scores. See Fig. 1 and Table S1 for identification of population codes.

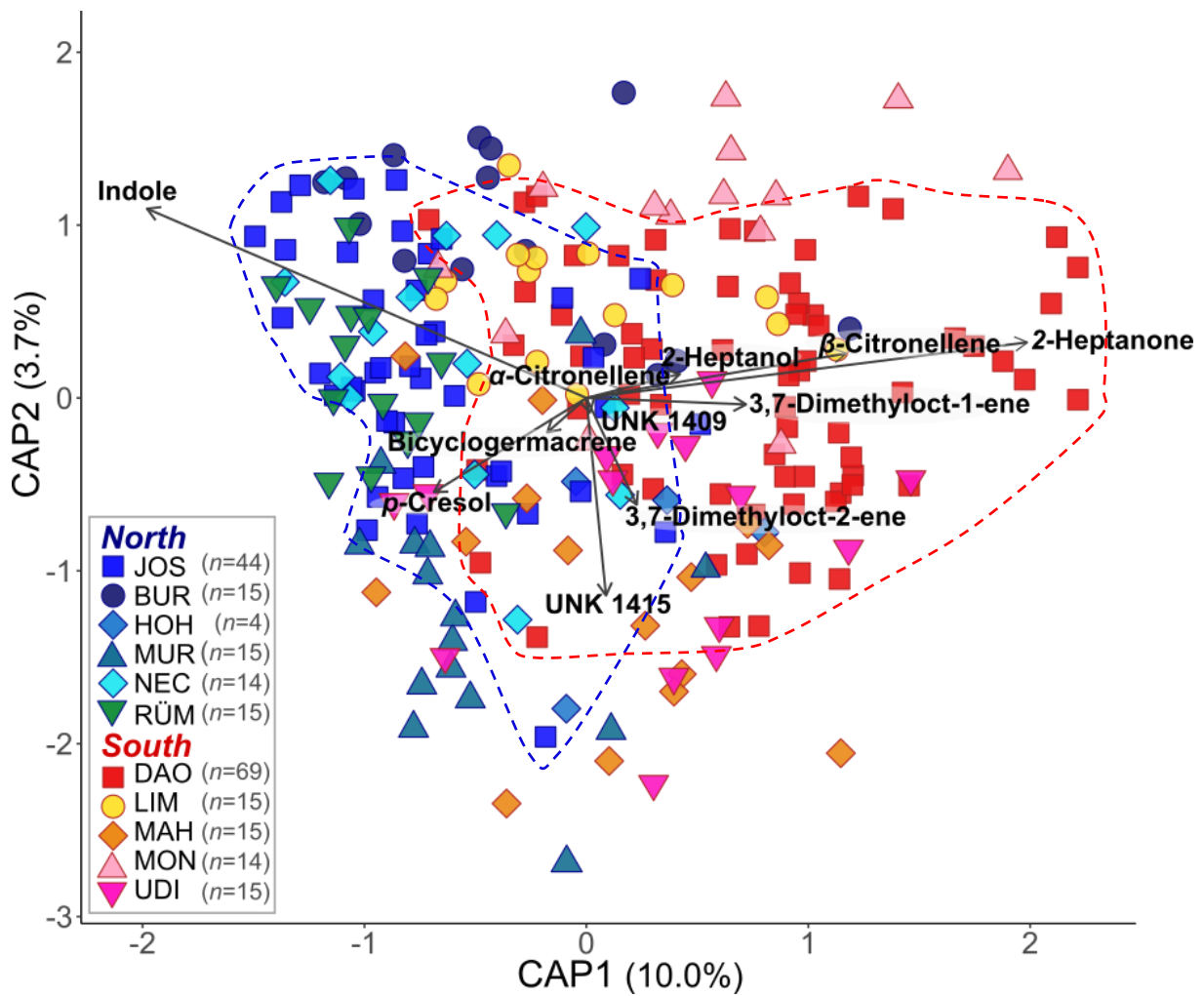
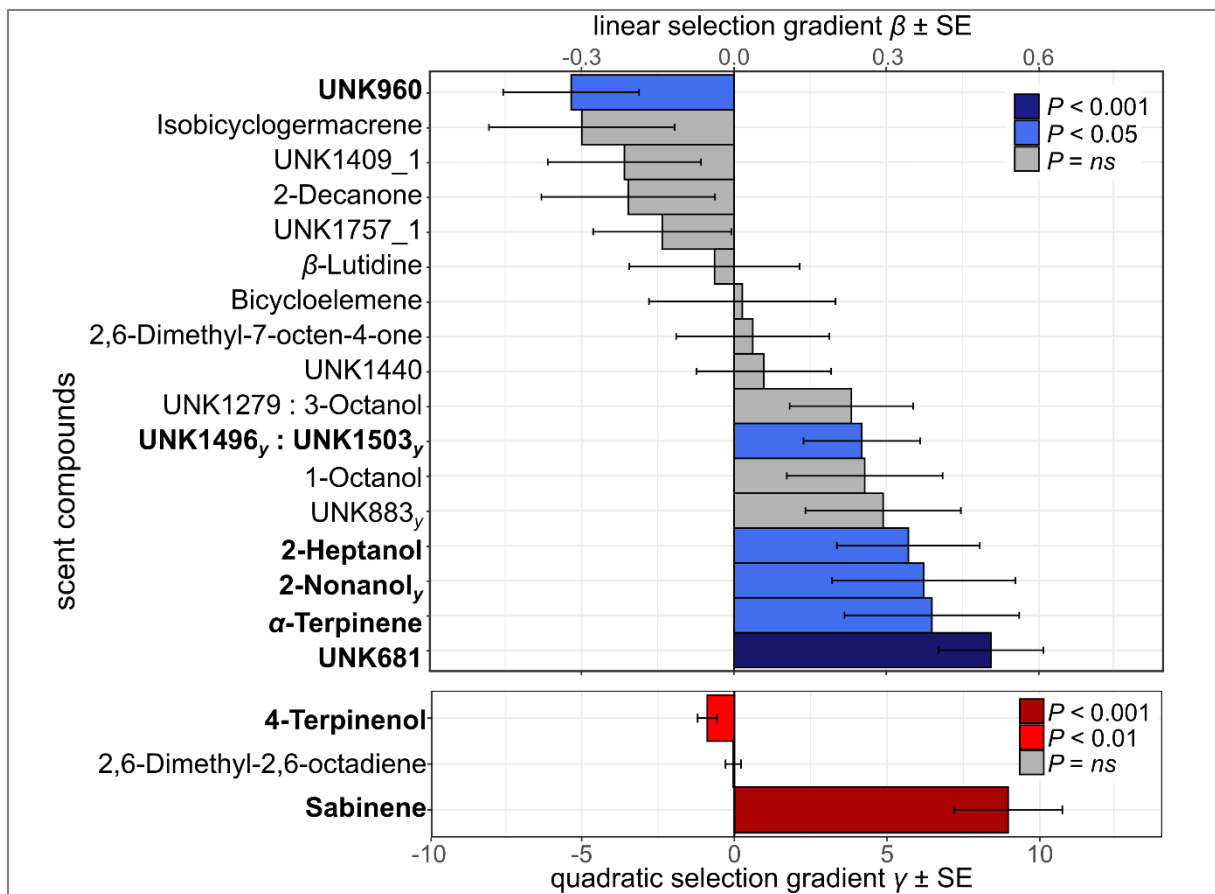


Fig 5

Linear and nonlinear selection gradients $\beta \pm SE$ and $\gamma \pm SE$ for individual floral scent compounds of *Arum maculatum* in the two most extensively sampled populations north (JOS, blue) and south (DAO, red) of the Alps, respectively. Only compounds that resulted from the pre-selective analyses are shown. Scent compounds under significant direct selection ($P < 0.05$) are in bold and their bars are coloured. Note the different scaling for direct β - and γ -selection. For the northern population, compounds that also correlated in the nonlinear pre-selective analyses are indicated (γ). Individual sample sizes are for JOS ($n_{\text{north}} = 43$) and DAO ($n_{\text{south}} = 68$).



Tables

Table 1 Median amounts of total absolute and relative (contribution of single compounds to total scent) inflorescence scent of *Arum maculatum* surveyed in six and five populations north and south of the Alps, respectively. North and South columns (bold headers) present the average regional median of the corresponding populations (following columns). Volatiles with a median amount of <1% in any population are pooled.

KRI	Compound name	North (n=107)	JOS (n=44)	BUR (n=15)	HOH (n=4)	MUR (n=15)	NEC (n=14)	RUM (n=15)	South (n=128)	DAO (n=69)	LIM (n=15)	MAH (n=15)	MON (n=14)	UDI (n=15)
	Median total absolute amount of scent trapped (ng inflorescence ⁻¹ h ⁻¹)	67.4	167.2	13.0	565.8	80.7	39.4	41.7	214.7	311.4	203.8	196.9	201.4	42.3
	Total number of volatiles	283	269	166	197	204	181	208	265	254	195	146	88	171
	Aliphatic components													
893	2-Heptanone*	1.4	1.4	2.4	0.8	1.3	1.1	1.4	6.9	9.3	0.3	2.9	11.9	4.0
902	2-Heptanol*	0.1	0.1	0.3	0.3	0.2	0.2	0.1	1.2	1.9	tr	0.8	2.5	0.5
982	1-Octen-3-ol*	1.9	2.4	tr	2.3	2.0	1.8	1.3	0.3	0.4	tr	6.4	tr	1.3
1096	2-Nonanone*	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.7	0.9	tr	0.2	1.3	0.4
	23 more aliphatic components <1%	0.5	0.5	0.4	2.0	1.7	1.3	0.5	0.7	0.9	0.9	2.0	1.0	0.7
	Aromatic components													
1076	<i>p</i> -Cresol*	4.2	1.8	0.1	19.4	11.9	9.2	1.5	0.5	0.5	0.3	0.7	0.6	0.9
	4 more aromatic components <1%	tr	tr	tr	0.6	0.3	0.1	0.1	tr	tr	tr	0.1	tr	tr
	C5-branched chain components													
	4 C5-branched chain components <1%	tr	tr	tr	0.1	tr	0.2	0.1	tr	tr	tr	tr	tr	tr
	Nitrogen-bearing components													
965	β -Lutidine	0.2	0.1	0.7	0.2	0.4	0.4	0.3	0.1	tr	0.6	0.1	tr	1.3
1310	Indole*	24.2	22.3	20.8	12.6	24.6	33.4	35.6	11.9	11.9	24.8	8.8	12.3	9.5
	5 more nitrogen-bearing components <1%	0.1	0.1	tr	0.1	tr	tr	0.3	0.1	tr	0.1	0.1	tr	tr
	Irregular terpene													
	3 irregular terpenes <1%	tr	tr	0.4	0.3	tr	0.6	0.1	0.1	0.1	0.2	tr	0.3	0.1
	Monoterpenoids													
914	3,7-Dimethyloct-1-ene*	1.5	1.2	1.1	2.6	2.1	1.8	1.7	4.0	4.3	4.1	2.4	4.6	2.7

935	α -Citronellene* [§]	0.4	0.3	0.5	0.9	0.5	0.5	0.4	1.3	1.3	1.9	1.1	1.3	1.0
949	β -Citronellene* [§]	4.2	3.5	8.0	11.6	3.4	7.1	3.5	9.7	10.8	10.1	6.5	9.9	8.2
972	3,7-Dimethylocta-2-ene*	3.1	1.8	2.8	5.1	9.6	2.6	3.3	4.3	4.5	4.4	5.6	2.1	3.2
982	Sabinene*	0.2	tr	1.0	0.2	tr	0.4	0.4	0.4	0.3	1.4	0.3	1.2	tr
1005	2,6-Dimethylocta-2,6-diene*	1.2	0.9	1.0	3.4	4.1	1.0	1.5	1.7	1.8	1.7	1.9	0.5	1.6
1076	Dihydromyrcenol	tr	tr	tr	tr	tr	tr	tr	0.4	0.4	1.0	tr	0.2	0.5
	21 more monoterpenoids <1%	0.3	0.4	tr	2.2	0.9	0.6	0.9	0.6	0.7	1.9	1.2	0.4	1.1
Sesquiterpenoids														
1357	Bicycloelemene	0.4	0.5	0.1	0.5	1.9	0.1	0.5	0.2	0.1	0.2	0.6	0.1	0.9
1399	α -Copaene*	1.0	1.8	1.3	0.5	0.5	0.7	0.8	0.8	0.6	1.0	1.0	1.6	0.6
1434	Isocaryophyllene	0.9	1.3	0.7	0.4	0.6	0.5	1.1	0.9	0.7	1.2	1.3	1.2	0.8
1450	β -Caryophyllene*	3.0	5.5	3.0	2.3	1.4	2.7	2.7	2.9	2.2	3.0	4.0	5.3	2.8
1484	α -Humulene*	2.8	4.7	2.8	1.7	1.2	2.3	2.7	2.3	1.6	2.5	3.0	4.0	2.6
1501	Germacrene D*	0.9	1.3	1.4	0.3	0.3	0.9	0.7	0.5	0.3	0.5	0.7	1.3	0.7
1520	Bicyclogermacrene	0.9	1.0	tr	0.6	2.1	tr	1.3	0.4	0.2	0.2	1.3	tr	1.7
1547	δ -Cadinene	1.2	1.9	1.4	0.6	0.6	1.5	1.1	0.4	0.3	0.5	0.7	0.4	1.0
	10 more sesquiterpenoids <1%	0.4	0.5	0.3	0.4	0.3	0.5	0.6	0.3	0.1	0.3	0.4	0.7	0.3
Unknown compounds														
829	UNK 829 <i>m/z</i> : 54,67,110,41,81,39	0.3	0.8	tr	0.2	0.2	0.3	0.1	tr	tr	tr	2.0	tr	0.3
1394	UNK 1394 <i>m/z</i> : 69,55,41,82,95	0.2	0.2	tr	0.1	0.6	0.2	0.2	0.1	0.1	0.1	1.1	0.2	0.1
1409	UNK 1409_1 <i>m/z</i> : 81,55,67,95,41	0.2	0.2	tr	0.4	0.4	0.1	0.1	0.2	0.2	0.1	0.6	0.2	1.1
1415	UNK 1415 <i>m/z</i> : 69, 81,41,95,55	3.7	3.9	1.7	2.3	7.3	3.7	3.4	3.8	3.1	2.8	10.4	2.3	11.3
1492	UNK 1492 <i>m/z</i> : 105,161,91,41,93	1.7	2.7	1.4	0.3	0.5	0.5	1.8	1.2	1.0	1.4	1.6	3.1	0.6
1503	UNK 1503 <i>m/z</i> : 81,107,163	0.8	0.9	0.2	0.4	0.8	0.6	1.0	0.2	0.2	0.1	0.4	0.2	0.3
1524	UNK 1524 <i>m/z</i> : 105,161,204,119,93	0.7	1.0	1.3	0.2	0.2	0.8	0.7	0.4	0.2	0.4	0.5	0.9	0.5
1699	UNK 1699 <i>m/z</i> : 81,163,191,95,123	3.6	4.1	3.0	0.5	1.8	3.2	5.2	1.3	1.1	1.6	2.0	0.8	3.2
	192 more unknowns <1%	2.9	3.9	1.6	4.7	4.9	3.8	4.9	2.7	2.2	4.8	4.6	3.4	3.7

Volatiles are ordered according to compound class, and within class by Kováts' retention index (KRI). The total number of volatiles is also given.

Abbreviations: * = identification of compound was verified by authentic standards; tr = trace relative amount (<0.05%); *m/z* = mass-to-charge ratio in decreasing order of abundance. North: JOS, Josefiu; BUR, Burg Hohenstein; HOH, Hohendilching; MUR, Murnau; NEC, Horb am Neckar; RÜM, Rümikon; South: DAO, Daone; LIM, Limone-Piemonte; MAH, Santa Maria Hoè; MON, Montese; UDI, Udine

[§] Synthetic (+)- α - and β -Citronellene coeluted with natural detected α - and β -Citronellene on a chiral column (MEGA-DEX DMT Beta SE, 30m \times 0.25mm ID, 0.23 μ m film) (Gfrerer *et al.* unpublished)