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1 **Life history evolution, species differences and phenotypic plasticity in hemiparasitic eyebrights**
2 **(*Euphrasia*)**

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11 Running head: Life history of hemiparasitic eyebrights

12 **ABSTRACT**

13 Premise of the study: Species delimitation in parasitic organisms is challenging as traits used in the
14 identification of species are often plastic and vary depending on the host. Here, we use species from a
15 recent radiation of generalist hemiparasitic *Euphrasia* to investigate trait variation and trait plasticity.

16 We test whether *Euphrasia* species show reliable trait differences, investigate whether these
17 differences correspond to life history trade-offs between growth and reproduction, and quantify
18 plasticity in response to host species.

19 Methods: We perform common garden experiments to evaluate trait differences between eleven
20 *Euphrasia* taxa grown on a common host, document phenotypic plasticity when a single *Euphrasia*
21 species is grown on eight different hosts, and relate our observations to trait differences recorded in
22 the wild.

23 Key results: *Euphrasia* exhibit variation in life history strategies; some individuals transition rapidly
24 to flower at the expense of early season growth, while others invest in vegetative growth and delay
25 flowering. Life history differences are present between some species, though many related taxa lack
26 clear-cut trait differences. Species differences are further blurred by phenotypic plasticity—many
27 traits are plastic and change with host type or between environments.

28 Conclusions: Phenotypic plasticity in response to host and environment confounds species
29 delimitation in *Euphrasia*. When grown in a common garden environment it is possible to identify
30 some morphologically distinct taxa, though others represent morphologically similar shallow
31 segregates. Trait differences present between some species and populations demonstrates the rapid
32 evolution of distinct life history strategies in response to local ecological conditions.

33

34 Keywords: flowering time; host range; life history evolution; parasitic plants; phenotypic plasticity,
35 Orobanchaceae

36 INTRODUCTION

37 Parasitism is a ubiquitous feature of the natural world, with parasitic organisms present in every
38 ecosystem and found to exploit all free-living organisms (Price, 1980; Windsor, 1998). Parasitic
39 plants are a group of c. 4500 species of 12 separate evolutionary origins that have evolved a modified
40 feeding organ, the haustorium, which allows them to attach to a host plant and extract nutrients and
41 other compounds (Westwood, Yoder, and Timko, 2010; Nickrent and Musselman, 2017; Twyford,
42 2018). Parasitic plants are morphologically diverse and present a broad range of life history strategies
43 and host interactions (Schneeweiss, 2006; Těšitel, Plavcová, and Cameron, 2010). Hemiparasitic
44 plants, i.e. taxa that are parasitic but also photosynthesize, can often attach to a broad range of hosts,
45 with the well-studied grassland parasite *Rhinanthus* found to attach to over 50 co-occurring grass and
46 herbaceous species (Cameron, Coats, and Seel, 2006). All hemiparasitic plants are exoparasites whose
47 leaves, stems, roots and flowers grow outside the host and only the haustorium invades and grows
48 within the host (Twyford, 2017).

49 Research to date has largely focused on three aspects of life history variation in parasitic plants.
50 Firstly, a body of work has looked to understand variation for specific traits between populations and
51 related species. For example, work on the hemiparasite *Pedicularis* has shown how investment in
52 male reproductive organs primarily depends on extrinsic environmental conditions (Guo, Mazer, and
53 Du, 2010a), while seed mass is primarily determined by intrinsic factors such as plant size rather than
54 extrinsic factors such as elevation (Guo, Mazer, and Du, 2010b). Secondly, researchers have
55 investigated how parasite life history traits are affected by interactions with their host. In the
56 widespread and weedy obligate holoparasite *Phelipanche ramosa*, the duration of the lifecycle differs
57 between 14 weeks and 40 weeks depending on the host (Gibot-Leclerc et al., 2013), with evidence of
58 local host adaptation. In hemiparasitic *Rhinanthus minor*, biomass depends on the host species and the
59 number of haustorial connections (Rowntree et al., 2014). Finally, a number of studies have looked at
60 life history variation between species studied in a phylogenetic context (Schneeweiss, 2006; Těšitel et
61 al., 2010). For example, broad-scale analyses of the Rhinanthae clade in the Orobanchaceae has

62 shown a shift from a perennial ancestor to annuality, with correlated shifts to a reduced seed size
63 (Těšitel et al., 2010). Despite the diversity of this research, there are still considerable gaps in our
64 knowledge as to how life history trait variation is maintained (e.g. how common trade-offs are
65 between life history traits), how much of this variation is genetic and how much is plastic, and which
66 traits are the targets of natural selection.

67 In this study, we explore trait variation in generalist hemiparasitic eyebrights (*Euphrasia*,
68 Orobanchaceae). *Euphrasia* is one of the largest genera of parasitic plants, and is characterized by
69 recent transoceanic dispersal and rapid species radiations (Gussarova et al., 2008). In the United
70 Kingdom there are 21 *Euphrasia* species, which are mostly indistinguishable at DNA barcoding loci
71 (Wang et al., 2018), show complex morphological variation (Yeo, 1968; Metherell and Rumsey,
72 2018), and readily hybridize (Liebst, 2008; Stace, Preston, and Pearman, 2015). Despite shallow
73 species differences due to postglacial divergence, *Euphrasia* species demonstrate substantial
74 ecological divergence, with many taxa restricted to specific habitats such as coastal turf, mountain
75 scree, heathland or open grassland. Habitat differences would be expected to exert strong selection on
76 life history traits, and this may include selection on growth to match seasonal water availability and to
77 exploit local hosts, or selection on flowering time in response to local competition from surrounding
78 plants, or in response to mowing or grazing (Hellström et al., 2004).

79 Our research builds on a large body of experimental work, with *Euphrasia* used in common garden
80 studies for over 125 years (Koch, 1891). The first experimental work on *Euphrasia* revealed that
81 phenotypic differences between two related species, *E. rostkoviana* and *E. montana*, are maintained in
82 a common garden environment (Wettstein, 1895). Experimental work in the 1960s showed the growth
83 of various *Euphrasia* species differs depending on the host species (Wilkins, 1963; Yeo, 1964). More
84 recent experiments using large sample sizes in common garden (Matthies, 1998; Zopfi, 1998; Lammi,
85 Siikamäki, and Salonen, 1999; Svensson and Carlsson, 2004) or in experimental field sites (Seel and
86 Press, 1993; Hellström et al., 2004), have shown the effect of commonly encountered hosts such as
87 grasses and legumes on hemiparasite biomass, mineral accumulation, plant architecture and

88 reproductive output. Despite this extensive experimental work, studies in *Euphrasia* have yet to
89 compare life history strategies of different species, and the extent of phenotypic plasticity in life
90 history traits. This work is critical for improving our knowledge of hemiparasite evolution, and for
91 understanding the nature of species differences in a taxonomically complex group. It is also unclear
92 whether *Euphrasia* are restricted to growing on hosts such as grasses and herbaceous species, or can
93 parasitize a broad range of taxa including novel hosts rarely encountered in the wild. To address these
94 questions requires simultaneously investigating the growth of multiple *Euphrasia* species and multiple
95 host species with sufficient replication to enable suitable statistical comparisons.

96 Here, we use a series of common garden experiments, in conjunction with field observations, to
97 understand life history trait evolution, species differences, and phenotypic plasticity in hemiparasitic
98 *Euphrasia*. Our first experiment assesses the morphological distinctiveness among several *Euphrasia*
99 species and their hybrids when grown on a single host species in standardized common garden
100 conditions. This experiment also addresses whether there is life history trait divergence among
101 recently diverged hemiparasite species and whether these trait differences correspond to life history
102 trade-offs. We then inspect the plasticity of a single focal *Euphrasia* population grown on many
103 different hosts. This experiment quantifies the magnitude of trait change when *Euphrasia* are grown
104 on different hosts. It also tests whether they are truly generalist parasites by observing their growth on
105 a wide range of hosts as well as growing them without a host. Finally, we relate our trait observations
106 made in a common garden to recordings made on herbarium specimens collected in the wild. This
107 comparison will help us understand whether life history traits and species' morphological differences
108 are consistent between the common garden and the wild. Overall, our joint observations of phenotypic
109 variation between closely related taxa, and the extent of host-induced plasticity within a species, both
110 in an experiment and in the wild, provide new insights into variation in life history strategies in
111 hemiparasitic taxa.

112

113 **MATERIALS AND METHODS**

114

115 **Experimental design and plant cultivation**—We performed two common garden experiments to
116 investigate phenotypic variation in *Euphrasia*. Both common garden experiments took place in
117 parallel in 2016. The experiments used wild-collected open-pollinated *Euphrasia* seeds that were
118 pooled across individuals in a population. Seeds were contributed by plant recorders as part of the
119 ‘Eye for Eyebrights’ (E4E) public engagement project and as such included a scattered geographic
120 sample across Great Britain (Appendix S1 (see the Supplementary Data with this article)). All
121 *Euphrasia* species were identified from the herbarium specimens of field collections, and from living
122 material grown in the glasshouse, by *Euphrasia* referee Chris Metherell. Host seeds were sourced
123 from commercial suppliers and from field collections (Appendix S2).

124 *Species differences experiment*—We observed trait differences of twenty four populations from five
125 *Euphrasia* species and six natural *Euphrasia* hybrids when grown on clover (*Trifolium repens*). This
126 experiment included multiple populations of three widespread and closely related grassland species,
127 *E. arctica*, *E. confusa* and *E. nemorosa*, and sparse population sampling of the moorland specialist *E.*
128 *micrantha* (one population) and calcareous grassland specialist *E. pseudokernerii* (two populations).
129 We chose clover as a host because it usually supports vigorous hemiparasite growth and confers high
130 survival (Zopfi, 1998).

131 *Phenotypic plasticity experiment*—We measured traits of a focal *Euphrasia* taxon, *E. arctica*, when
132 grown with eight potential hosts (*Arabidopsis thaliana*, *Equisetum arvense*, *Festuca rubra*, *Holcus*
133 *lanatus*, *Marchantia polymorpha*, *Pinus sylvestris*, *Plantago lanceolata*, and *Trifolium repens*), and
134 without a host. These hosts were chosen to include a broad representation of functional groups and
135 phylogenetic diversity, with species encountered in the wild as well as novel hosts (full details in
136 Appendix S2). The novel hosts were included to see the limits to which parasitic *Euphrasia* can
137 associate, namely with a tree (*Pinus*), a pteridophyte that produces adventitious roots (*Equisetum*),
138 and a liverwort that produces rhizoids (*Marchantia*).

139 *Cultivation protocol*—Reliable cultivation of *Euphrasia* can be challenging due to low seed
140 germination, variation in time to establishment, the requirement of seed stratification, and high
141 seedling mortality when transplanted (Yeo, 1961; Zopfi, 1998). We developed cultivation protocols
142 that combine winter germination cues that improve germination and mimic nature, but also used
143 highly standardized and replicated pot conditions that avoid transplanting *Euphrasia* and thus
144 maximize survivorship. We filled 9 cm plastic pots with Melcourt Sylvamix Special growing media
145 (Tetbury, Gloucestershire, UK) in December, placed one *Euphrasia* seed per pot, and left pots outside
146 over winter at the Royal Botanic Garden Edinburgh (RBGE) for seeds to experience natural seed
147 stratification. Hosts were planted in seed trays in April. *Euphrasia* plants were moved to an unheated
148 and well-ventilated greenhouse in the spring once the cotyledons were fully expanded, and a single
149 seedling from each host (or a 1cm² clump of *Marchantia*) transplanted into the pot containing
150 *Euphrasia*. Hosts that died within ten days of planting were replaced. Twenty or more replicates were
151 grown for each host-parasite combination. Plants were subsequently grown to flowering with regular
152 watering, the locations of pots randomized at weekly intervals, and foreign weed seedlings removed.

153 **Common garden trait measurements and statistical analyses**—We measured seven morphological
154 traits at first flowering related to life history variation, indicators of plant vigor, or characters used in
155 taxonomy. In addition to date of first flowering, we measured: corolla length, the ratio of cauline leaf
156 length to internode length below the measured leaf ('internode ratio'), number of leaf teeth on the
157 lower floral leaf (bract), number of nodes to flower, number of branches and plant height. All length
158 measurements were made to the nearest millimetre, and followed Metherell and Rumsey (2018). For
159 the phenotypic plasticity experiment, we also recorded early season growth (height six weeks after
160 transplantation of potential host) and height at the end of season after senescence. We did not make
161 direct observations of host attachment, as preliminary investigations revealed a fine root structure
162 where haustoria were difficult to observe. Instead, we inferred that attachment is likely to have taken
163 place based on observations of height, following Yeo (1964). By the end of season, *Euphrasia* that
164 have attached to a 'good' host tended to grow tall and have elongated internodes, while *Euphrasia*

165 that have not attached or have attached to a ‘bad’ host are much smaller (see discussion for more
166 details).

167 We used a combination of fixed effect and mixed models to gain insights into the differences in means and the
168 magnitude of variability in our data. In all models, response variables were analyzed as either Gaussian (and
169 log transformed if necessary) or Poisson. If the response variable was analyzed as Poisson, the model was
170 checked for overdispersion and if it was overdispersed, an observation level random effect was fitted. All
171 correlations between variables were Pearson’s Correlations. Multiple correlation comparisons were corrected
172 using Holm’s correction method. Phenotypic clustering was inspected using Principal Component Analysis
173 (PCA). All analyses were done in R version 3.4.3, with the packages lme4 (Bates et al., 2014) and
174 MCMCglmm (Hadfield, 2010) for generalized linear mixed effects models, base R for linear models,
175 RemdrMisc for correlations (Fox, 2018) and ggplot2 for data visualisation (Wickham, 2016). MCMCglmm
176 models were run for a minimum of 70,000 iterations using either inverse Wishart or parameter expanded
177 priors with a minimum burn in period of 30,000 iterations. Model convergence was assessed visually by
178 plotting the posterior distributions and Markov chains.

179 In the species differences experiment, species of *Euphrasia* was fitted as a fixed effect and population
180 of *Euphrasia* was treated as a random effect. We excluded hybrids from these analyses because we
181 were interested in testing differences between species. In the case of height and cauline:internode ratio
182 the traits were log transformed. Likelihood Ratio Tests calculated the overall significance of species,
183 where this was not possible, Deviance Information Criteria were used to test better model fit. We
184 calculated proportion variance explained by population of *Euphrasia* (after accounting for fixed
185 effects) by dividing the population random effect variance by the total variance in the model. Tukey
186 post hoc tests were performed on each pairwise comparison of *Euphrasia* species and adjusted p-
187 values calculated, using Tukey HSD (Honest Significant Difference) correcting for family-wise error
188 rate in the emmeans R package (Lenth, 2019). For the phenotypic plasticity experiment, host species
189 was fitted as a fixed effect. The models were re-levelled so that ‘no host’ was the baseline. Analysis
190 of variance was used to determine overall significance of host species. Tukey post hoc tests were then

191 performed on each pairwise comparison of host species, with adjusted p-values calculated in base R
192 and the multcomp package (Hothorn, Bretz and Westfall, 2008).

193 **Trait variation in the wild**—We tested how phenotypes in the experiments related to those in nature
194 by comparing results from the species differences experiment to phenotypic measurements made on
195 herbarium specimens of the same population sampled in the wild. Three individuals were measured
196 from each collection sheet for a given population for each trait. Pressed plants submitted by collectors
197 varied in quality and therefore we were unable to measure the height of these plants, nor was it
198 possible to infer date of first flowering. We analyzed the data using generalized linear mixed effect
199 models with where individuals were grown (i.e. common garden or wild-collected) as a fixed effect,
200 with each of five traits as the response variable. We treated species and population of *Euphrasia* as
201 random effects to understand the relative contributions of each to the overall variability in a given
202 trait. Response variables that were considered count data were analysed with a Poisson distribution, in
203 all other cases a Gaussian distribution was used. R-values were calculated using Pearson's
204 correlations of the population level means between the common garden and the wild samples.

205

206 **RESULTS**

207

208 **Species differences**—Our species differences experiment revealed extensive morphological trait
209 variation across *Euphrasia* species when compared at first flowering. From the 222 *Euphrasia*
210 individuals that survived to flower on their clover host, the greatest variation was seen in number of
211 branches (9-fold difference between species), internode ratio (2.7-fold) and height (2.5-fold), while
212 traits such as node to flower (1.6-fold) and corolla length (1.6-fold) proved less variable (Fig. 1 a-d;
213 Appendix S3). A large degree of this variation was separated by species and by population (Table 1).
214 The species with the most distinct life-history strategy was *E. micrantha*, which flowered from a low
215 node on the plant (8.3 ± 0.2 nodes) while it was short (70 ± 8 mm; Appendix S3). It also formed a
216 partly distinct cluster in the PCA analysis (Appendix S4). *E. pseudokernerii* was relatively distinct,
217 flowered once it has grown tall (176 ± 16 mm) and from a high node on the plant (13.2 ± 0.4 nodes),

218 but showed little separation in the PCA analysis. The morphologically similar *E. arctica*, *E. confusa*
219 and *E. nemorosa* differed for some traits, with *E. nemorosa* initiating flowering 14 days later and from
220 3.3 nodes higher than *E. arctica*, but overlapped in many other traits and in overall multi-trait
221 phenotype (Appendix S4, S5). Despite species being a significant factor in the models, and some
222 notable differences in specific traits, there were few significant pairwise Tukey comparisons due to
223 substantial within species variation (Appendix S6). Of the seven significant pairwise trait differences
224 three were for node to flower and three for number of leaf teeth, with four of the seven significant
225 comparisons involving *E. micrantha*. In most cases hybrids combined morphological characters of
226 their parental progenitors, for example hybrids involving *E. nemorosa* flowered later in the season and
227 initiated flowering from a higher node than *E. arctica* hybrids (Fig. 1a-d).

228 Correlation analyses across species revealed clear suites of traits that are related. Significant
229 correlations were found between 12 of the 21 pairwise comparisons after correcting for multiple tests,
230 with 5 of these correlations with an $R > 0.6$ (Table 2a). Plants flowering at a late node are more likely
231 to be tall, more highly branched, as well as having many teeth on the lower floral leaf. The
232 relationship of traits is also supported in the PCA analysis, with many traits contributing to multiple
233 principal components (Appendix S5). Height and flowering node related traits are largely uncorrelated
234 with cauline internode-leaf ratio and corolla length.

235 **Phenotypic plasticity**—Our phenotypic plasticity experiment showed substantial morphological
236 variation across 194 *E. arctica* plants grown with 8 different potential host species, and the 22 plants
237 grown without a host. Plants growing on clover transitioned to flower quickly (189.8 ± 2.0 Julian
238 days), grew tall by the time of first flowering (39 ± 3 mm), and produced large flowers (7.4 ± 0.2 mm;
239 Fig. 1e-h, Appendix S7). This contrasts with *Euphrasia* with no host, which flowered on average 52
240 days later (241.3 ± 7.9 Julian days), were extremely short at first flowering (11 ± 1 mm), and
241 produced small flowers (5.3 ± 0.2 mm). *E. arctica* grown on *Arabidopsis*, *Equisetum*, *Festuca*, *Holcus*
242 or *Plantago* were all statistically significantly different to no host for at least one trait (Tukey
243 comparisons, $P < 0.05$), while *E. arctica* on *Marchantia* or *Pinus* were not significantly different from
244 no host for any trait ($P > 0.05$; Appendix S8). While the overall effect of host was significant for all

245 traits except nodes to flower (Appendix S9 – S11), three traits showed relatively little plasticity, with
246 few statistically significant pairwise Tukey comparisons for nodes to flower (0 significant
247 comparisons), number of leaf teeth (3) and internode length (4), while the other three traits showed
248 many pairwise differences (days to flower, 21 significant comparisons; height, 16; corolla length, 12;
249 Appendix S8). Our comparison of growth across host treatments measured through the year showed
250 that height at the end of the season is weakly predicted from height 6-weeks after introducing a host
251 ($R = 0.47$), but strongly correlated with height at first flowering ($R = 0.82$, Appendix S12). Plants that
252 flowered early were more likely to grow larger by the end of season ($R = -0.55$) and become more
253 highly branched ($R = -0.57$, Appendix S12).

254 Across host treatments, there was a significant negative correlation between Julian days to flower and
255 most other traits (Table 2b). We find that late flowering individuals are likely to be smaller at first
256 flowering, have fewer branches, have leaves with fewer teeth, and have smaller flowers. While these
257 traits were strongly correlated, there were substantial differences in the magnitude of response. For
258 example, days to flower differed considerably depending on host, with a 3.8-fold greater difference
259 than seen between means of different *Euphrasia* species grown on the same host (Fig. 1d & 1h). In
260 contrast corolla length and node to flower proved less variable depending on host, with a 1.4-fold and
261 1.2-fold change between means, respectively.

262 **Variation in the wild**—The comparison between the species differences common garden experiment
263 and wild-collected herbarium specimens revealed population means of a single trait, nodes to flower,
264 are strongly correlated ($R = 0.79$) and trait values are not significantly different ($p_{\text{MCMC}} = 0.71$)
265 between environments (Fig. 2; Appendix S13; S14). All other traits did differ significantly between
266 environments ($p_{\text{MCMC}} < 0.05$), with *Euphrasia* plants in the common garden having corollas on
267 average 1.4 mm longer, with 0.2 more teeth on the lower floral leaves, an increase in
268 cauline:internode ratio of 1.0 mm, and 4 more pairs of branches. Despite these differences, there were
269 correlations between the common garden and the wild-collected specimens for corolla length ($R =$
270 0.93 , $p_{\text{MCMC}} < 0.001$), cauline internode:leaf length ratio ($R = 0.65$, $p_{\text{MCMC}} < 0.001$) and number of
271 branches ($R = 0.29$, $p_{\text{MCMC}} < 0.001$), but not for number of leaf teeth ($R = 0.07$, $p_{\text{MCMC}} = 0.034$).

272

273 **DISCUSSION**

274

275 Our study sheds light on species differences, life history evolution and phenotypic plasticity of the
276 generalist parasitic plant *Euphrasia*. We find different life history strategies between recently
277 diverged species, with some species rapidly transitioning to flower at the expense of growth-related
278 traits, while others delay flowering and invest in early-season vegetative growth. However, many
279 traits are phenotypically plastic and change in response to the host. While plants in benign common
280 garden conditions grew vigorously, the correlation between life history traits in a common garden and
281 in the wild suggests our experimental observations are indicative of patterns observed in nature.
282 Morphological differences between species in the common garden also suggest that the currently
283 delimited *Euphrasia* species are, at least in part, distinct. Overall our study highlights the value in
284 integrating trait data from multiple common garden experiments and field collections to study life
285 history strategies in parasitic plants, and demonstrates the rapid evolution of life history differences in
286 a postglacial radiation of hemiparasites.

287 **Life history variation in a generalist hemiparasitic plant**—Our study finds evidence for different
288 life history strategies in British *Euphrasia*. *E. arctica*, *E. micrantha* and hybrids such as *E. arctica* x
289 *E. confusa*, transition rapidly to flower, flower while they are short, and produce their first flower
290 from a low node on the plant. This contrasts with *E. pseudokernerii*, *E. nemorosa* and hybrids
291 involving *E. nemorosa* that delay flowering until later in the season, grow tall before flowering, and
292 produce their first flower from a late node on the main axis. These different life history strategies
293 correspond to the known ecology of these species, with *E. nemorosa* flowering late in tall mixed
294 grassland, while *E. micrantha* flowers early in patchy heathland (Metherell and Rumsey, 2018). While
295 species show some general differences in life history strategies, there is also significant variation
296 between populations within species. A relationship between internode number and habitat has
297 previously been observed within *Euphrasia* species, with populations of *E. rostkoviana* in Sweden
298 flowering at a lower node in a common garden if they have been collected from intensely grazed

299 pasture (Zopfi, 1998). Overall, these observations within and between species are consistent with the
300 classic life history trade-off between growth and reproduction (Stearns, 1992; Roff, 2002). For
301 *Euphrasia* growing in the wild, early reproduction allows the plants to reliably complete their
302 lifecycle before summer competition, herbivory, mowing, summer drought and other seasonal abiotic
303 and biotic stresses. However, early flowering involves reproducing at the expense of early season
304 growth and at a time when the resource budget may be constrained by relatively few haustorial
305 connections. These trait trade-offs pose an interesting comparison to the well-studied *Mimulus*
306 *guttatus* (*syn. Erythranthe guttata*), a non-parasitic relative in the Lamiales that shares the same basic
307 plant architecture. In *M. guttatus* multiple traits related to growth and reproduction are correlated both
308 within and between populations, due to genetic trade-offs between time to flower and fecundity
309 (Mojica et al., 2012; Friedman et al., 2015). In *Euphrasia* the genetics underpinning this life history
310 trade-off have yet to be characterized, and may be a consequence of multiple independent loci or
311 trade-offs at individual loci (Hall, Lowry, and Willis, 2010).

312 While much life history variation is captured by differences in time to flower and growth-related
313 traits, we also see evidence for flower size representing a separate axis of variation across *Euphrasia*
314 species. In our common garden *E. micrantha* has small corollas, while *E. arctica* and *E. nemorosa*
315 have larger corollas, and corolla size is not strongly correlated with other traits. *Euphrasia* species are
316 well-known to have flower size variation, with a continuum between small flowered species that are
317 highly selfing (e.g. *E. micrantha*, corolla size = 4.5 - 6.5mm, inbreeding coefficient $F_{IS} > 0.88$, Stone,
318 2013), and large flowered species that are highly outcrossing (e.g. *E. rostkoviana* flower size 8 -
319 12mm, $F_{IS} = 0.17 - 0.25$ French et al., 2005). Such wide variation in outcrossing rate has been
320 documented in species of *Datura* (Motten and Stone, 2000), *Mimulus* (Karron et al., 1997) and
321 *Nicotiana* (Breese, 1959). Small flowers have shorter anther-stigma separation and thus increased
322 potential for autogamous selfing (Karron et al., 1997), while also having reduced attractiveness to
323 pollinators and thus receiving less outcross pollen (Mitchell et al., 2004). In addition to differences in
324 corolla size between *Euphrasia* species, corolla size also shows a change of up to two millimetres in
325 response to host species. This change in flower size is of a magnitude that may potentially affect the

326 mating system (Luo and Widmer, 2013), and suggests host species represents a previously
327 unaccounted factor affecting the mating system of parasitic plants.

328 Our comparisons of *Euphrasia* species in a common garden also sheds light on the distinctiveness of
329 these recently diverged species, and can be used to refine the suite of traits that are reliable in telling
330 *Euphrasia* species apart. *Euphrasia* is a taxonomically complex plant genus, with the 21 currently
331 described British species presenting complex and often overlapping morphological variation (French
332 et al., 2008; Metherell and Rumsey, 2018; Wang et al., 2018). Our study suggests varying degrees of
333 morphological distinctiveness of *Euphrasia* species. We see *E. micrantha* is morphologically distinct
334 in the common garden, and *E. pseudokernerii* somewhat distinct, while the closely related species *E.*
335 *arctica*, *E. confusa* and *E. nemorosa* differ in life history traits such as nodes to flower, but overlap in
336 many other traits and are not clearly separated in the PCA. The morphological trait differences
337 between species observed under standardised conditions are correlated with values from field-
338 collected herbarium specimens where plants have associated with diverse hosts, been exposed to
339 different ecological conditions, and were collected at different life-stages. This suggests that our
340 common garden results generalize to observations in nature. However, our study is likely to
341 overestimate the distinctiveness of taxa by only including a subset of UK species and by choosing
342 populations that could be identified to species-level in the field. We suspect adaptive divergence
343 between closely related *E. arctica*, *E. confusa* and *E. nemorosa* is a consequence of differential
344 natural selection for local ecological conditions such as soil water availability or mowing. Selection
345 appears to be operating at a fine spatial scale, with significant life history trait differences evident
346 between populations within species. *Euphrasia* taxa may be genetically cohesive, either showing
347 genome-wide divergence or divergence in genomic regions underlying life history differences
348 (Twyford and Friedman, 2015), or alternatively these taxa may be polytopic and not genetically
349 cohesive (Hollingsworth, Neaves, and Twyford, 2017). Genomic sequencing of natural populations
350 will help resolve the nature of species differences in *Euphrasia*.

351 **Phenotypic plasticity in response to host**—Our phenotypic plasticity experiment shows *Euphrasia*
352 are affected by growing with a range of different hosts. Specifically, *E. arctica* with a host such as

353 clover rapidly transitions to flowering. At the other extreme, *Euphrasia* grown without a host are
354 small and flower late. These differences in growth are established early in the season, and early
355 flowering plants go on to grow the tallest and are more highly branched. Most other hosts result in a
356 continuum of *Euphrasia* phenotypes between these extremes. Two surprising results were that *E.*
357 *arctica* parasitizing *Arabidopsis* grew relatively tall despite the host senescing early in the growth
358 season, and that *Euphrasia* growing with *Equisetum* performed similarly to when grown on the
359 commonly encountered grass *Holcus lanatus*. This suggests attachment to *Equisetum*, which would
360 need to be confirmed by excavating root systems and observing haustoria, or indirect benefits without
361 attachment through association with *Equisetum* fungal symbionts (Bouwmeester et al., 2007). Less
362 surprising was the poor growth of *E. arctica* with *Pinus*. However, an association between
363 *Melampyrum pratense* and *Pinus sylvestris* suggests at least some hemiparasitic Orobanchaceae
364 benefit from attachment to woody host species, or interactions with their associated ectomycorrhizal
365 fungi (Salonen et al., 2000).

366 The diverse effects of host on parasite growth are complex, but the variation we see in our
367 experiments may be attributed to host root architecture, germination time and resource availability, as
368 well as the presence of mechanisms to defend against parasite attack, such as cell wall thickening,
369 localized host dieback, and chemical defence (Cameron, Coats, and Seel, 2006; Twyford, 2018).
370 While *Euphrasia* is generally thought to have low reliance on host resources, deriving only ~30% of
371 carbon heterotrophically (Těšitel, Plavcová, and Cameron, 2010), at least under our experimental
372 conditions *Euphrasia* only produced multiple flowers on certain hosts. Overall our results point to *E.*
373 *arctica* being a true generalist hemiparasite, but one where vigorous growth is only observed with a
374 subset of potential hosts.

375 In terms of specific traits, only three pairs of trait correlations show consistent correlation coefficients
376 in both *Euphrasia* common garden experiments (between height, number of branches and leaf teeth),
377 with other correlations between species breaking down when *Euphrasia* are grown on different hosts.
378 The most notable plasticity is seen in flowering time, with plants on clover rapidly transitioning to
379 flower within ~100 days of germination, while plants with a more typical host (e.g. *Holcus lanatus*)

380 flower a month later. Phenotypic plasticity in flowering time in response to resource availability is
381 well documented in many plant groups, particularly *Arabidopsis* (e.g. Zhang and Lechowicz, 1994),
382 but has received less attention in studies of parasitic plants, which are more likely to look at growth-
383 related traits such as biomass (Ahonen, Puustinen, and Mutikainen, 2005; Matthies, 2017). However,
384 date of first flowering has been shown to differ by up to 10 weeks in populations of *Rhinanthus*
385 *glacialis* across Switzerland (Zopfi, 1995). Overall, we expect date of first flowering to be critical for
386 the life time reproductive success of parasitic plants in the wild.

387 In contrast to traits showing extensive plasticity, we also see evidence of developmental constraint in
388 number of nodes to flower. For *E. arctica*, this trait showed the least plasticity with different hosts, is
389 consistent between populations within species, and between the common garden and the field. This
390 suggests that the developmental event of transitioning to flower is genetically determined, with
391 changes in flowering time altered by plasticity in internode length and not nodes to flower. This may
392 explain why nodes to flower is such an important diagnostic trait for species identification in
393 *Euphrasia* and related species in the Rhinanthae (Jonstrup, Hedrén, and Andersson, 2016). Despite
394 nodes to flower changing little in response to host species, our overall impression is that *Euphrasia*
395 show considerable plasticity and little developmental constraint in many aspects of growth. In
396 particular, differences between individuals on a given host also suggests other sources of variation,
397 such as genetic background in host and parasite, as well as the timing of attachment, may be crucial in
398 determining performance.

399

400 **CONCLUSIONS**

401

402 Despite over a century of experimental studies in parasitic plants, our understanding of the evolution
403 of life history strategies in these diverse organisms is extremely limited. Our results with *Euphrasia*
404 provide strong support for the rapid evolution of distinct life history strategies in response to local
405 ecological conditions, with phenotypic plasticity further altering plant growth in response to host
406 availability. We anticipate that future studies that test life time reproductive success of many parasitic

407 plant species grown on many different host species will give further insight into the complex nature of
408 host-parasite interactions.

409

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419

420 **Author Contributions**

421 A.D.T conceived and designed the research. A.D.T., N.F. and E. L. Y.W. carried out the experiments.
422 C.M. identified the plants. A.D.T. and M.B. analysed the data. A.D.T. and M.B. wrote the manuscript.
423 All authors read and approved the manuscript.

424

425 **Data Availability Statement**

426 Phenotypic data from both common garden experiments and from herbarium collections, as well as
427 the R scripts used for data analysis, are deposited in Dryad (doi.org/10.5061/dryad.cfxpvnv2d).

428 Source code and data are also deposited on Github:

429 https://github.com/Euphrasiologist/phenotypic_plasticity_euphrasia

430

431 **Supporting information**

432 Additional Supporting Information may be found online in the supporting information section at the
433 end of the article.

434 **Appendix S1.** Collection details for *Euphrasia* species used in the common garden experiments.

435 **Appendix S2.** Species and collection details for hosts used in the phenotypic plasticity common
436 garden experiment.

437 **Appendix S3.** Summary of trait values for many *Euphrasia* species and hybrids grown on a clover
438 host. Values are mean +/- one standard error. Length measurements are in mm.

439 **Appendix S4.** Principal component analysis of morphological variation of *Euphrasia* in a common
440 garden for (a) five species and six hybrids grown with a clover host, (b) five species grown with a
441 clover host, (c) *E. arctica* with nine host treatments. Points represent individuals, and ellipses
442 represent the standard error of the (weighted) average of scores.

443 **Appendix S5.** Factor loadings for the principal component analyses of (a) five species and six
444 hybrids, (b) five species, (c) *Euphrasia arctica* with nine host treatments.

445 **Appendix S6.** Comparison of *Euphrasia* species differences of plants grown with clover in a common
446 garden experiment. Tukey comparisons are presented between each pair of species, with significant
447 comparisons shown in bold. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

448 **Appendix S7.** Summary of trait values for *Euphrasia arctica* grown on many different hosts. Values
449 are mean +/- one standard error. Length measurements are in mm.

450 **Appendix S8.** Comparison of *E. arctica* traits in the phenotypic plasticity common garden
451 experiment. Tukey comparisons are presented between *E. arctica* traits with two different host
452 treatments. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

453 **Appendix S9.** Analysis of deviance for each trait in the phenotypic plasticity experiment with *E.*
454 *arctica* grown with many different hosts, assuming a Poisson distribution. For each model, we report
455 the change in degrees of freedom (Df), deviance, residual degrees of freedom, residual deviance, and
456 p-value generated from the Chi-squared distribution. Factor host is compared to the intercept model
457 where no factors are fitted.

458 **Appendix S10.** ANOVAs for traits measured in the phenotypic plasticity experiment with *E. arctica*
459 grown with many different hosts, assuming Gaussian distributed residuals. For each model, we report
460 the degrees of freedom (Df), sums of squares (SS), mean squares (MS), F-statistic, and P-value.

461 **Appendix S11.** Summary of generalised linear models for the phenotypic plasticity experiment with
462 *Euphrasia arctica* grown on many hosts in a common garden. All models compare *E. arctica* grown
463 with a particular host to the intercept of no host. Generalised linear models assuming Poisson
464 residuals with log link function were used in Julian days to flower, nodes to flower and number of leaf
465 teeth, while all others assumed Gaussian residuals. The model coefficient is reported with standard
466 error in brackets. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

467 **Appendix S12.** Relationship between growth-related traits and end of season height for *E. arctica*
468 grown with nine different host treatments. (a) Height at first flowering, (b) height 6-weeks after
469 germination, (c) Julian days to flower, (d) number of branches. Length measurements are reported in
470 mm.

471 **Appendix S13.** Comparison between trait values for wild-collected herbarium specimens and
472 common garden plants of diverse *Euphrasia* species for (a) nodes to flower, (b) corolla length (mm),
473 (c) number of leaf teeth, (d) internode ratio. Points are for *Euphrasia* population means, with bars
474 representing the standard error of measurements.

475 **Appendix S14.** Model output from MCMCglmm comparing traits for the wild collected *Euphrasia*
476 specimens to the baseline of the common garden data (Intercept). The posterior means are reported
477 along with the lower and upper 95% credible intervals, as well as the p-value (pMCMC) for the effect.

478 **Literature cited**

- 479 AHONEN, R., S. PUUSTINEN, AND P. MUTIKAINEN. 2005. Host use of a hemiparasitic plant: no trade-
480 offs in performance on different hosts. *Journal of Evolutionary Biology* 19: 513-521.
- 481 BATES, D., M. MAECHLER, B. BOLKER, AND S. WALKER. 2015. Fitting Linear Mixed-Effects Models
482 Using lme4. *Journal of Statistical Software* 67(1), 1-48.
- 483 BOUWMEESTER, H. J., C. ROUX, J. A. LOPEZ-RAEZ, AND G. BECARD. 2007. Rhizosphere
484 communication of plants, parasitic plants and AM fungi. *Trends in plant science* 12.
- 485 BREESE, E. 1959. Selection for differing degrees of out-breeding in *Nicotiana rustica*. *Annals of*
486 *Botany* 23: 331-344.
- 487 CAMERON, D. D., A. M. COATS, AND W. E. SEEL. 2006. Differential resistance among host and non-
488 host species underlies the variable success of the hemi-parasitic plant *Rhinanthus minor*.
489 *Annals of Botany* 98: 1289-1299.
- 490 FOX, J. 2018. RcmdrMisc: R Commander Miscellaneous Functions. R package version 2.5-1.
491 <https://CRAN.R-project.org/package=RcmdrMisc>
- 492 FRENCH, G., R. ENNOS, A. SILVERSIDE, AND P. HOLLINGSWORTH. 2005. The relationship between
493 flower size, inbreeding coefficient and inferred selfing rate in British *Euphrasia* species.
494 *Heredity* 94: 44.
- 495 FRENCH, G., P. HOLLINGSWORTH, A. SILVERSIDE, AND R. ENNOS. 2008. Genetics, taxonomy and the
496 conservation of British *Euphrasia*. *Conservation genetics* 9: 1547-1562.
- 497 FRIEDMAN, J., A. D. TWYFORD, J. H. WILLIS, AND B. K. BLACKMAN. 2015. The extent and genetic
498 basis of phenotypic divergence in life history traits in *Mimulus guttatus*. *Molecular Ecology*
499 24: 111-122.
- 500 GIBOT-LECLERC, S., F. DESSAINT, C. REIBEL, AND V. LE CORRE. 2013. *Phelipanche ramosa* (L.)
501 Pomel populations differ in life-history and infection response to hosts. *Flora-Morphology,*
502 *Distribution, Functional Ecology of Plants* 208: 247-252.

503 GUO, H., S. J. MAZER, AND G. DU. 2010a. Geographic variation in primary sex allocation per flower
504 within and among 12 species of *Pedicularis* (Orobanchaceae): Proportional male investment
505 increases with elevation. *American Journal of Botany* 97: 1334-1341.

506 GUO, H., S. J. MAZER, AND G. DU. 2010b. Geographic variation in seed mass within and among nine
507 species of *Pedicularis* (Orobanchaceae): effects of elevation, plant size and seed number per
508 fruit. *Journal of Ecology* 98: 1232-1242.

509 GUSSAROVA, G., M. POPP, E. VITEK, AND C. BROCHMANN. 2008. Molecular phylogeny and
510 biogeography of the bipolar *Euphrasia* (Orobanchaceae): Recent radiations in an old genus.
511 *Molecular Phylogenetics and Evolution* 48: 444-460.

512 HADFIELD, J.D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The
513 MCMCglmm R Package. *Journal of Statistical Software* 33(2), 1-22.

514 HALL, M. C., D. B. LOWRY, AND J. H. WILLIS. 2010. Is local adaptation in *Mimulus guttatus* caused
515 by trade-offs at individual loci? *Molecular Ecology* 19: 2739-2753.

516 HELLSTRÖM, K., P. RAUTIO, A.-P. HUHTA, AND J. TUOMI. 2004. Tolerance of an annual
517 hemiparasite, *Euphrasia stricta* agg., to simulated grazing in relation to the host environment.
518 *Flora-Morphology, Distribution, Functional Ecology of Plants* 199: 247-255.

519 HOLLINGSWORTH, P. M., L. E. NEAVES, AND A. D. TWYFORD. 2017. Using DNA sequence data to
520 enhance understanding and conservation of plant diversity at the species level in S. Oldfield
521 and S. Blackmore [eds.], *Plant conservation science and practice: The role of Botanic
522 Gardens* 23-48. Cambridge University Press, Cambridge.

523 HOTHORN, T., F. BRETZ AND P. WESTFALL. 2008. Simultaneous Inference in General Parametric
524 Models. *Biometrical Journal* 50(3), 346--363.

525 JONSTRUP, A., M. HEDRÉN, AND S. ANDERSSON. 2016. Host environment and local genetic
526 adaptation determine phenotype in parasitic *Rhinanthus angustifolius*. *Botanical Journal of
527 the Linnean Society* 180: 89-103.

528 KARRON, J. D., R. T. JACKSON, N. N. THUMSER, AND S. L. SCHLICHT. 1997. Outcrossing rates of
529 individual *Mimulus ringens* genets are correlated with anther–stigma separation. *Heredity* 79:
530 365.

531 KOCH, L. 1891. Zur Entwicklungsgeschichte der Rhinanthaceen (II *Euphrasia officinalis* L.). *Jahrb.*
532 *Wiss. Bot* 22: 1-34.

533 LAMMI, A., P. SIIKAMÄKI, AND V. SALONEN. 1999. The role of local adaptation in the relationship
534 between an endangered root hemiparasite *Euphrasia rostkoviana*, and its host, *Agrostis*
535 *capillaris*. *Ecography* 22: 145-152.

536 LENTH, R. 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version
537 1.3.5.1. <https://CRAN.R-project.org/package=emmeans>

538 LIEBST, B. 2008. Do they really hybridize? A field study in artificially established mixed populations
539 of *Euphrasia minima* and *E. salisburgensis* (Orobanchaceae) in the Swiss Alps. *Plant*
540 *systematics and evolution* 273: 179-189.

541 LUO, Y., AND A. WIDMER. 2013. Herkogamy and its effects on mating patterns in *Arabidopsis*
542 *thaliana*. *PLoS ONE* 8: e57902.

543 MATTHIES, D. 1998. Influence of the host on growth and biomass allocation in the two facultative
544 root hemiparasites *Odontites vulgaris* and *Euphrasia minima*. *Flora* 193: 187-193.

545 MATTHIES, D. 2017. Interactions between a root hemiparasite and 27 different hosts: Growth, biomass
546 allocation and plant architecture. *Perspectives in Plant Ecology, Evolution and Systematics*
547 24: 118-137.

548 METHERELL, C., AND F. J. RUMSEY. 2018. Eyebrights (*Euphrasia*) of the UK and Ireland. Botanical
549 Society of Britain and Ireland, Bristol.

550 MITCHELL, R., J. KARRON, K. HOLMQUIST, AND J. BELL. 2004. The influence of *Mimulus ringens*
551 floral display size on pollinator visitation patterns. *Functional Ecology* 18: 116-124.

552 MOJICA, J. P., Y. W. LEE, J. H. WILLIS, AND J. K. KELLY. 2012. Spatially and temporally varying
553 selection on intrapopulation quantitative trait loci for a life history trade-off in *Mimulus*
554 *guttatus*. *Molecular Ecology* 21: 3718-3728.

555 MOTTEN, A. F., AND J. L. STONE. 2000. Heritability of stigma position and the effect of stigma-
556 anther separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium*
557 (Solanaceae). *American Journal of Botany* 87: 339-347.

558 NICKRENT, D. L., AND L. J. MUSSELMAN. 2017. Parasitic plants. *In* B. H. Ownley AND R. N.
559 Trigiano [eds.], *Plant Pathology: Concepts and Laboratory Exercises*, 277-288. CRC Press,
560 Boco Raton.

561 PRICE, P. W. 1980. *Evolutionary biology of parasites*. Princeton University Press.

562 ROFF, D. A. 2002. *Life history evolution*, vol. 576.54 R6.

563 ROWNTREE, J. K., D. FISHER BARHAM, A. J. STEWART, AND S. E. HARTLEY. 2014. The effect of
564 multiple host species on a keystone parasitic plant and its aphid herbivores. *Functional*
565 *Ecology* 28: 829-836.

566 SALONEN, V., SETÄLÄ, H., AND S PUUSTINEN. 2000. The interplay between *Pinus sylvestris*, its root
567 hemiparasite, *Melampyrum pratense*, and ectomycorrhizal fungi: Influences on plant growth
568 and reproduction. *Écoscience* 7: 195-200.

569 SCHNEEWEISS, G. M. 2006. Correlated evolution of life history and host range in the
570 nonphotosynthetic parasitic flowering plants *Orobanche* and *Phelipanche* (Orobanchaceae).
571 *Journal of Evolutionary Biology* 20: 471-478.

572 SEEL, W., AND M. PRESS. 1993. Influence of the host on three sub-Arctic annual facultative root
573 hemiparasites. *New Phytologist* 125: 131-138.

574 STACE, C. A., C. D. PRESTON, AND D. A. PEARMAN. 2015. *Hybrid flora of the British Isles*.
575 Botanical Society of Britain and Ireland.

576 STEARNS, S. C. 1992. *The evolution of life histories*, vol. 575 S81.

577 STONE, H. 2013. *Evolution and conservation of tetraploid Euphrasia L. in Britain*. Unpublished
578 Thesis, The University of Edinburgh.

579 SVENSSON, B. M., AND B. Å. CARLSSON. 2004. Significance of time of attachment, host type, and
580 neighbouring hemiparasites in determining fitness in two endangered grassland hemiparasites.
581 *Annales Botanici Fennici*: 63-75.

582 TĚŠITEL, J., L. PLAVCOVÁ, AND D. D. CAMERON. 2010. Heterotrophic carbon gain by the root
583 hemiparasites, *Rhinanthus minor* and *Euphrasia rostkoviana* (Orobanchaceae). *Planta* 231:
584 1137-1144.

585 TĚŠITEL, J., P. ŘÍHA, Š. SVOBODOVÁ, T. MALINOVÁ, AND M. ŠTECH. 2010. Phylogeny, life history
586 evolution and biogeography of the Rhinanthoid Orobanchaceae. *Folia Geobotanica* 45: 347-
587 367.

588 TWYFORD, A. D. 2017. New insights into the population biology of endoparasitic Rafflesiaceae.
589 *American Journal of Botany* 104: 1433-1436.

590 TWYFORD, A. D. 2018. Parasitic plants. *Current Biology* 28: R857-R859.

591 TWYFORD, A. D., AND J. FRIEDMAN. 2015. Adaptive divergence in the monkey flower *Mimulus*
592 *guttatus* is maintained by a chromosomal inversion. *Evolution* 69: 1476-1486.

593 WANG, X., G. GUSSAROVA, M. RUHSAM, N. DE VERE, C. METHERELL, P. M. HOLLINGSWORTH, AND
594 A. D. TWYFORD. 2018. DNA barcoding a taxonomically complex hemiparasitic genus reveals
595 deep divergence between ploidy levels but lack of species-level resolution. *AoB PLANTS* 10:
596 ply026-ply026.

597 WESTWOOD, J. H., J. I. YODER, AND M. P. TIMKO. 2010. The evolution of parasitism in plants.
598 *Trends in plant science* 15: 227-235.

599 WETTSTEIN, R. 1895. Der Saison-Dimorphismus als Ausgangspunkt für die Bildung neuer Arten im
600 Pflanzenreiche.

601 WICKHAM, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag New York, 2016.

602 WILKINS, D. 1963. Plasticity and establishment in *Euphrasia*. *Annals of Botany* 27: 533-552.

603 WINDSOR, D. A. 1998. Controversies in parasitology, Most of the species on Earth are parasites.
604 *International Journal for Parasitology* 28: 1939-1941.

605 YEO, P. 1961. Germination, seedlings, and the formation of haustoria in *Euphrasia*. *Watsonia* 5: 1-22.

606 YEO, P. 1964. The growth of *Euphrasia* in cultivation. *Watsonia* 6: 1-24.

607 YEO, P. 1968. The evolutionary significance of the speciation of *Euphrasia* in Europe. *Evolution* 22:
608 736-747.

609 ZHANG, J., AND M. J. LECHOWICZ. 1994. Correlation between time of flowering and phenotypic
610 plasticity in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* 81: 1336-1342.

611 ZOPFI, H.-J. 1995. Life history variation and infraspecific heterochrony in *Rhinanthus glacialis*
612 (Scrophulariaceae). *Plant Systematics and Evolution* 198: 209-233.

613 ZOPFI, H. J. 1998. The genetic basis of ecotypic variants of *Euphrasia rostkoviana* Hayne
 614 (Scrophulariaceae) in relation to grassland management. *Flora* 193: 41-58.

615

616 **Tables**

617 **Table 1.** Summary of generalized linear mixed effects models for *Euphrasia* trait values measured in
 618 a common garden environment. Table summarizes model outputs for five *Euphrasia* species grown
 619 with clover in the species differences experiment, and *E. arctica* grown with eight hosts plus no host
 620 treatment in the phenotypic plasticity experiment. For the phenotypic plasticity experiment, we report
 621 model outputs with all potential hosts, as well as models excluding *Pinus* and *Marchantia* where there
 622 was no evidence of attachment or interactions (reported in square brackets). The percentage variance
 623 explained by random effects are reported in brackets along with the 95% credibility interval. *** P <
 624 0.001, ** P < 0.01, * P < 0.05

	Species differences		Phenotypic plasticity
	Species	Population	Host
Branches	DIC _{full} = 676.08; DIC _{-spp} = 679.48	DIC _{full} = 676.08; DIC _{-pop} = 714.87 (25.9%, 13.4 – 57.4%)	NA
Corolla length	$\chi^2(4) = 11.91^*$	$\chi^2(1) = 41.38^{***}$ (54.1%, 24.4 – 69.2%)	$(F_{8,173} = 9.85)^{***}$ [[$F_{6,157} = 11.38$] ^{***}]
Height	$\chi^2(4) = 11.67^*$	$\chi^2(1) = 57.13^{***}$ (61.2%, 35.0 – 79.7%)	$(F_{8,185} = 23.14)^{***}$ [[$F_{6,164} = 24.39$] ^{***}]
Internode ratio	$\chi^2(4) = 13.00^*$	$\chi^2(1) = 34.38^{***}$	$(F_{8,184} = 3.36)^{**}$ [[$F_{6,163} = 4.11$] ^{***}]
Julian days to flower	$\chi^2(3) = 2.26$	$\chi^2(1) = 1.42 \text{ E-}14$ (58.7%, 28.6 – 80.9%)	$\chi^2(8) = 192.39^{***}$ [[$\chi^2(6) = 141.67$] ^{***}]
Node to flower	$\chi^2(4) = 15.42^{**}$	$\chi^2(1) = 2.87$	$\chi^2(8) = 5.02$

		(14.1%, 1.0 – 33.5%)	$[\chi^2(6) = 3.04]$
Number of leaf teeth	$\chi^2(3) = 12.45^{**}$	$\chi^2(1) = 0.0059$ (0.12%, 2.8% - 23%)	$\chi^2(8) = 26.79^{***}$ $[\chi^2(6) = 17.04^{**}]$

625 Note: Models for number of branches were implemented with a different statistical approach in
626 MCMCglmm, with models compared using Deviance Information Criterion (DIC; see methods).

627

628 **Table 2.** Pearson's correlation coefficients for seven phenotypic traits measured in a common garden
629 experiment for (a) Five *Euphrasia* species and 6 hybrids, (b) *Euphrasia arctica* grown with 8 hosts
630 and without a host. *** P < 0.001, ** P < 0.01, * P < 0.05. Asymptotic p-values values are reported
631 from the Hmisc package in R using the rcorr() function.

(a) Species

differences

experiment

	Corolla					
	length	Height	Internode	Julian days	Leaf teeth	Nodes to
	(mm)	(mm)	ratio	to flower		flower
Branches	0.260 **	0.609***	-0.116	0.057	0.658***	0.775***
Corolla length						
(mm)		0.319***	-0.161	-0.127	0.197 *	0.049
Height (mm)			0.246**	0.292*	0.563***	0.628***
Internode ratio				0.204	-0.120	0.076
Julian days to						
flower					0.053	0.249*
Leaf teeth						0.651***

(b) Phenotypic

plasticity

experiment

	Corolla					
	length	Height	Internode	Julian days	Leaf teeth	Nodes to
	(mm)	(mm)	ratio	to flower		flower
Branches	0.524***	0.834***	-0.299***	-0.572***	0.694***	-0.572**
Corolla length						
(mm)		0.503***	0.098	-0.406***	0.536***	-0.166
Height (mm)			0.477***	-0.481***	0.692***	-0.186
Internode ratio				-0.034	0.168	-0.009
Julian days to						
flower					-0.691***	0.530***
Leaf teeth						-0.239**

633

634 **Figure legends**

635 **Figure 1.** Trait variation in a common garden experiment of diverse *Euphrasia* species and hybrids
636 grown on clover (A-D); *Euphrasia arctica* grown on many different hosts (E-H). The edges of the
637 boxplots show the first and third quartiles, the solid lines the median, the whiskers the highest and
638 lowest values within 1.5-fold of the inter-quartile range and the jittered dots each individual
639 measurement. Length measurements were recorded in millimetres.

640 **Figure 2.** Relationship between morphological trait measurements made in the common garden and
641 on wild-collected herbarium specimens for diverse *Euphrasia* species. Points are *Euphrasia*
642 population means, with bars representing the standard error of measurements. The line of best fit was
643 calculated using coefficients from linear regression models on the means of each *Euphrasia*
644 population. Length measurements are reported in mm. For an alternative representation of pairwise
645 comparisons see Appendix S13.