Life history evolution and phenotypic plasticity in parasitic eyebrights (*Euphrasia*, Orobanchaceae)

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Running head: Life history of parasitic eyebrights

Abstract

Parasite lifetime reproductive success is determined by both genetic variation and phenotypically plastic life history traits that respond to host quality and external environment. In this study we use the generalist parasitic plant genus *Euphrasia* to test aspects of life history theory. We evaluate species life history strategies when grown on a common host, document phenotypic plasticity when grown on many different hosts, and relate our observations to trait differences recorded in the wild. We find *Euphrasia* in a common garden and in the field exhibit a range of life history strategies that differ between species that transition rapidly to flower at the expense of early season growth, and those that invest in vegetative growth and delay flowering. However, aspects of life history show extensive phenotypic plasticity in response to host quality, and demonstrate the costs of attaching to a low-quality host. Our experiments suggest life history strategies in a generalist parasitic plant are the product of natural selection on traits related to growth and flowering, while host quality may be a primary determinant of lifetime reproductive success.

Keywords: flowering time; host range; life history evolution; parasitic plants; phenotypic plasticity

Background

Parasitism is a ubiquitous feature of the natural world, with parasitic organisms present in every ecosystem and found to exploit all free-living organisms [1, 2]. The life history of parasitic organisms must be synchronised with the host to maximise a parasite's individual growth, survival and fecundity. However, the life history of external parasites (exoparasites) must not only be timed to their host, but to variable and often stochastic external environmental conditions [3]. Such responses will primarily be plastic when responding to local conditions or fluctuating environments, or genetic in response to more predictable or spatially structured variation [4]. Understanding how genetic and plastic differences contribute to life history trait variation in parasite species and populations is essential for understanding parasite speciation, host-parasite interactions, and transmission dynamics [5].

Parasitic plants are a group of c. 4500 species of at least 12 separate evolutionary origins that have evolved a modified feeding organ, the haustorium, which allows them to attach to a host plant and extract nutrients and water [6, 7]. Parasitic plants are incredibly morphologically diverse and present a broad range of life history strategies [8], with the host species range a critical trait that determines parasite interactions and performance in the wild. Generalist parasitic plants can attach to a broad range of hosts with the grassland parasite *Rhinanthus* found to attach to over 50 co-occurring grass and herbaceous species [9]. All generalist parasitic plants are exoparasites where the leaves, stems, roots and flowers grow outside the host and only the haustorium invades and grows within the host [10]. The range of hosts and their visible external growth make them ideal for investigating host-parasite interactions. Generalist parasitic plants have been used in experimental studies for over 125 years [11], with these studies revealing that parasite growth, reproduction and metabolism are determined by host functional group (herb, grass, legume etc.) and host species [12]. Despite this large body of experimental work manipulating host type, most studies have not simultaneously used the replication of parasite species, host species and individuals, to identify the different life history

strategies of parasite species and how phenotypic plasticity in life history traits determines parasite performance.

In this study, we explore life history trait evolution in parasitic eyebrights (*Euphrasia*, Orobanchaceae). *Euphrasia* represents an ideal study system for investigating the recent evolution of parasite life history strategies. These taxa are hemiparasites that are photosynthetically competent and can complete their lifecycle without a host, but grow much better after attachment to one of many suitable hosts. In the United Kingdom, the 21 *Euphrasia* species demonstrate recent postglacial divergence with many species indistinguishable at DNA barcoding loci [13], presenting similar and overlapping morphological variation [14, 15], and found to readily hybridise [16]. Despite this shallow species divergence, *Euphrasia* demonstrates substantial ecological divergence, with many taxa restricted to specific habitats such as coastal turf, mountain scree or open grassland. This ecological diversity would be expected to exert strong selection on aspects of the life history of a generalist parasitic annual plant species such as *Euphrasia*. This may include selection on growth to match seasonal water availability and to exploit local hosts, or selection on flowering time in response to local competition.

In our experiments, we first assess the extent of life history trait variation among *Euphrasia* species and their hybrids when grown on a single host in standardised growing conditions. This experiment addresses whether there is life history trait divergence among recently diverged parasite species. We then inspect plasticity of a single focal *Euphrasia* population grown on many different hosts. This experiment tests whether they are truly generalists by growing them on a wide range of hosts, and also includes a treatment without a host to observe the phenotype when free-living. Finally, we relate our trait observations made in a common garden to recordings made on herbarium specimens collected in the wild. This comparison will help us understand whether life history trait differences hold in both the common garden and in nature. Overall, our joint observations of phenotypic variation between closely related species, and the extent of host-induced plasticity within species, both in an experiment and in the wild, provides new insights into life history variation in parasitic taxa.

Materials and Methods

(a) Experimental design and plant cultivation

We performed two common garden experiments to investigate phenotypic variation in parasitic *Euphrasia*. Our 'species differences' (SpD) experiment observed life history trait differences of five species and six natural hybrids when grown on clover (*Trifolium repens*), which is often used as a host in parasitic plant experiments because the parasite grows vigorously and has high survival [17]. This experiment includes multiple populations of three widespread and closely related species, *E. arctica*, *E. confusa* and *E. nemorosa*, and a single population of two more distinct taxa, *E. micrantha* and *E. pseudokerneri*. Our 'phenotypic plasticity' (PP) experiment assessed the impact of eight hosts (*Arabidopsis thaliana*, *Equisetum arvense*, *Festuca rubra*, *Holcus lanatus*, *Marchantia polymorpha*, *Pinus sylvaticus*, *Plantago lanceolata*, and *Trifolium repens*), and growing without a host, on parasite phenotype. These hosts were chosen to represent both typical hosts encountered in the wild, as well as novel hosts, and a broad representation of phylogenetic diversity and functional groups (full details in Table S1). Both common garden experiments took place in parallel in 2016. Wild-collected open-pollinated *Euphrasia* seeds were contributed by plant recorders as part of the 'Eye for Eyebrights' (E4E) public engagement project and as such included a scattered geographic sample across Great Britain (Table S2). Host seeds were sourced from commercial suppliers and from wild-collected material (Table S1).

Reliable cultivation of *Euphrasia* can be challenging due to low seed germination, variation in time to establishment, the requirement of seed stratification, and high seedling mortality when transplanted [17, 18]. Here, we develop cultivation protocols that combine winter germination cues that improve germination and mimic nature, but also use highly standardised and replicated pot conditions that avoid transplanting *Euphrasia* and thus maximise survivorship. We planted a single *Euphrasia* seed into 9cm plastic pots filled with Melcourt Sylvamix® Special growing media in December, with

plants left outside overwinter to experience natural seed stratification. Hosts were planted in seed trays in April. *Euphrasia* plants were moved to an unheated and well-ventilated greenhouse at the Royal Botanic Garden Edinburgh (RBGE) in the spring once the cotyledons were fully expanded, and a single seedling from each host introduced. Hosts that died within ten days of planting were replaced. Twenty or more replicates were grown for each host-parasite combination. Plants were subsequently grown to flowering with regular watering, randomised at weekly intervals, and foreign weed seedlings removed.

(b) Trait measurements and statistical analyses

We measured a suite of seven morphological traits at first flowering. These traits are characters related to life history variation, indicators of plant vigour, or characters used in taxonomy. In addition to date of first flowering, we measured: corolla length, cauline leaf length:internode length ratio (‘internode ratio’), leaf teeth on the lower floral leaf, nodes to flower, number of branches and plant height. All length measurements were made to the nearest millimetre, and followed protocols from reference [14]. For the many hosts experiment, we also recorded early season growth (height six weeks after transplantation) and height at the end of season after senescence. We did not make direct observations of below-ground attachment due to the fine root structure of *Euphrasia*, and instead used height at first flowering to infer likely attachment [19].

We analysed data for the two experiments separately but with similar statistical approaches. For the SpD experiment we used generalised linear mixed effects models to fit species as a fixed effect and population as a random effect. For the PP experiment we used generalised linear mixed effects models with presence or absence of host as a fixed effect and host species identity as a random effect. We used a Gaussian distribution for all models except where the response variable were positive integer counts, where we used a Poisson distribution. To calculate the significance of a fixed or random effect we used Likelihood Ratio Tests where the full model was compared with a nested model where one effect had been dropped. When a random effect was dropped, we implemented generalised linear

models using R base functions. For the trait number of branches the generalised linear mixed effects models failed to converge so we used MCMCglmm [20], with the best fitting of either a full or nested model chosen based on a lower Deviance Information Criterion (DIC) value. For the many hosts, models for number of branches failed to converge in every model run, so this model was omitted. Using the flexible variance structures accommodated by MCMCglmm we were able to calculate "variance explained" by random effects in each model. In the case of a Gaussian model, variance explained by a variable is the fraction of the total variance accounted for by the variable. In the case of a Poisson model the process is more complex due to the mean-variance relationship [21]. The models were run for 91,000 iterations with a burn-in of 21,000 and a thinning interval of 70. The priors were standard inverse-Wishart for the (co)variances and parameter expanded where there was poor mixing. We calculated r^2 values from Pearson Correlation Coefficients between all traits and used Principal Component Analyses to quantify phenotypic clustering of individuals. All analyses were done in R version 3.4.3, with the packages lme4 [22] and MCMCglmm for generalised linear mixed effects models and ggplot2 for data visualisation [23].

(c) Trait variation in the wild

We tested how phenotypes in the experiments relate to phenotypes in nature, by comparing results from experiment SpD to phenotypic measurements made on herbarium specimens of the same populations sampled in the wild. Pressed plants submitted by field collectors varied in quality, and these sometimes missed the base of the plant, therefore we were unable to measure height. We measured traits on three individuals from each collection sheet for a given population. The results were then averaged for graphical representation, but pooled for statistical analysis. We used generalised linear mixed effects models to fit variables as a function of where individuals were measured (i.e. common garden or measured on herbarium specimens). This two levelled categorical factor was implemented as a fixed effect. Adding species as a random effect shows how much of the variability of the trait was explained by species given where individuals were measured. Count data was analysed with a Poisson distribution, in all other cases a Gaussian distribution was used. Analyses were performed in MCMCglmm.

Results

Species differences

Our first common garden experiment reveals extensive morphological trait variation across *Euphrasia* species. From the 222 individuals surviving to flowering on their clover host, we see a 3-fold difference in mean height, while other traits also proved variable (Figure 1a; Table S3). A large degree of this variation is separated by species and by population (Table 1). The species with the most distinct lift-history strategy is *E. micrantha*, which flowers early in the season from a low node on the plant, and flowers while it is short. It also forms a distinct cluster in the PCA analysis (Figure S1). *E. pseudokerneri* is relatively distinct, flowering once it has grown tall and from a high node on the plant, but shows little separation in the PCA analysis. The morphologically similar *E. arctica*, *E. confusa* and *E. nemorosa* are partly distinct, with *E. nemorosa* initiating flowering 14 days later and from 3.3 nodes higher than *E. arctica*, but overlaps in other traits and in overall multi-trait phenotype (Figure S1). Hybrids have intermediate morphology that is consistent with the phenotype of their parental progenitors, for example hybrids involving *E. nemorosa* flower later in the season and from a later node than *E. arctica* hybrids (Figure 1a).

Correlation analyses reveal clear suites of traits that are related, with strong correlations present in 6 of 21 pairwise comparisons ($R^2 > 0.4$, Table 2a). Plants flowering at a late node are more likely to be tall, more highly branched, as well as having many teeth on the lower floral leaf. The relationship of traits is also supported in the PCA analysis, with many traits contributing to multiple principal components (Table S4). These height and flowering node related traits are largely uncorrelated with cauline internode-leaf ratio and corolla length.

Phenotypic plasticity

Our phenotypic plasticity experiment shows substantial morphological variation across 194 *E. arctica* plants grown with 8 different host species, and the 22 plants grown without a host. Plants growing on clover transitioned to flower quickly, grew tall by the time of first flowering, and produced large flowers (Figure 1b, Table S5). This contrasts with *Euphrasia* with no host, which flowered on average 53 days later, were extremely short at first flowering, and produced small flowers. Despite this, the presence or absence of host was not significant (Table 1), due to *Euphrasia* grown with *Marchantia* and *Pinus* growing similarly to the no host treatment (Figure 1b). The other hosts, *Arabidopsis*, *Equisetum*, *Festuca*, *Holcus* and *Plantago* conferred some benefit for *Euphrasia* and fell between clover and no host. Our comparison of growth across host treatments measured through the year showed that height at the end of the season is poorly predicted from early season growth ($r^2 = 0.22$), but strongly correlated with height at first flowering ($r^2 = 0.69$). Plants that flowered early were more likely to grow larger by the end of season ($r^2 = -0.30$) and become more highly branched ($r^2 = 0.62$).

Across host treatments, there was a significant negative correlation between Julian days to flower and most other traits (Table 2b). We find that late flowering individuals are likely to be smaller at first flowering, are less highly branched, have less highly toothed leaves, and have smaller flowers (Table S4). While strongly correlated, the magnitude of change for different traits varied substantially. Days to flower differed considerable depending on host, with a 3.8x greater difference than seen between means of different *Euphrasia* species (Fig. 1aiii and 1biii). In contrast corolla length and node to flower proved less variable depending on host, with a 1.4x and 1.2x change between means, respectively.

Variation in the wild

The comparison between common garden and wild-collected herbarium specimens revealed a single trait, nodes to flower, is strongly correlated (r^2=0.78) and not significantly different (p_{MCMC}=0.71) between environments (Figure 2). All other traits did differ significantly between environments ($p_{MCMC} < 0.05$), with *Euphrasia* plants in the common garden having corollas on average 1.4mm

longer, with 0.2 more teeth on the lower floral leaves, an increase in cauline:internode ratio of 1.0mm, and 4 more pairs of branches. Despite differing between environments, trait values were highly correlated for corolla length ($r^2 = 0.97$, $p_{MCMC} < 0.001$) and cauline internode:leaf length ratio ($r^2 = 0.63$, $p_{MCMC} < 0.001$), but not for number of leaf teeth ($r^2 = 0.13$, $p_{MCMC} = 0.034$) and number of branches ($r^2 = 0.047$, $p_{MCMC} < 0.001$).

Discussion

Our study sheds new light on life history evolution and phenotypic plasticity in generalist parasitic plants. We find different life history strategies between recently divergent parasitic *Euphrasia* species, with some species rapidly transitioning to flower at the expense of growth-related traits, while others delay flowering and invest in early-season vegetative growth. However, many traits related to life history differences are phenotypically plastic and show major changes in response to host quality. In addition to our experimental observations, the consistency between life history traits in a common garden and in the wild suggest our experimental observations generalise to patterns observed in nature. Overall our study highlights the value in integrating multiple common garden experiments and field collections to study life history strategies in parasitic plants, and demonstrates the rapid evolution of life history differences in a postglacial radiation of parasites.

Life history variation in a parasitic plant

Our study finds evidence for different life history strategies between *Euphrasia* species in Britain. *E. arctica*, *E. micrantha* and hybrids such as *E. arctica* x *E. confusa*, transition rapidly to flower, flower while they are short, and produce their first flower from a low node on the plant. This contrasts with *E. pseduokerneri*, *E. nemorosa* and hybrids involving *E. nemorosa* that delay flowering until later in the season, grow tall before flowering, and produce their first flower from a late node on the main axis. These different life history strategies correspond to the known of ecology of these species, with *E. nemorosa* flowering late in tall mixed grassland, while *E. micrantha* flowers early in patchy

heathland where competition is less intense [14]. Our observations are consistent with the classic life history trade-off between growth and reproduction [24, 25]. For *Euphrasia* growing in the wild, early reproduction allows the plants to reliably complete their lifecycle before summer competition, herbivory, mowing, summer drought and other seasonal abiotic and biotic stresses. However early flowering involves reproducing at the expense of early season growth and at a time when the resource budget is constrained by relatively few haustorial connections. These trait trade-offs pose an interesting comparison to the well-studied *Mimulus guttatus* (*syn. Erythranthe guttata*), a non-parasitic relative in the Lamiales that shares the same basic plant architecture. In *M. guttatus* multiple traits related to growth and reproduction are correlated both within and between populations, due to genetic trade-offs between time to flower and fecundity [26, 27]. In *Euphrasia* the genetics underpinning this life history trade-off have yet to be characterised, and may be a consequence of multiple independent loci or trade-offs at individual loci [28].

While much life history variation is captured by differences in time to flower and growth-related traits, we also see evidence for flower size representing a separate axis of variation across *Euphrasia* species. In our common garden *E. micrantha* has small corollas, while *E. arctica* and *E. nemorosa* have larger corollas, and corolla size is not correlated with other traits. *Euphrasia* species are well-known to have flower size variation, with a continuum between small flowered species that are highly selfing (e.g. *E. micrantha*, corolla size = 4.5 - 6.5mm, inbreeding coefficient $F_{IS} > 0.88$, [29]), and large flowered species that are highly outcrossing (e.g. *E. rostkoviana* flower size 8 - 12mm, $F_{IS} =$ 0.17 - 0.25 [30]). Such continuous variation in outcrossing rate has been documented in species of *Datura* [31], *Mimulus* [32] and *Nicotiana* [33]. Small flowers have shorter anther-stigma separation and thus increased potential for autogamous selfing [32], while also having reduced attractiveness to pollinators and thus receiving less outcross pollen [34]. In addition to differences in corolla size between *Euphrasia* species, corolla size also shows a change of up to two millimetres in response to host quality, with this change potentially affect the mating system [35]. This suggests host quality represents a previously unaccounted factor affecting the mating system of parasitic plants.

Our comparisons of *Euphrasia* species in a common garden also sheds light on the distinctiveness of these recently divergent species. *Euphrasia* is one of the most taxonomically complex plant genera, with the 21 currently described British species presenting complex and often overlapping morphological variation [13, 14, 36]. Our study suggests varying degrees of morphological distinctiveness of *Euphrasia* species. We see *E. micrantha* is clearly morphologically distinct and *E. pseudokerneri* mostly distinct, while the closely related species *E. arctica*, *E. confusa* and *E. nemorosa* differ in life history traits such as nodes to flower, but overlap in many other traits and are not clearly separated in the PCA. Moreover, our study is likely to overestimate the distinctiveness of taxa by only including a subset of UK species. We suspect adaptive divergence between closely related *E. arctica*, *E. confusa* and *E. nemorosa* is a consequence of differential natural selection for local ecological conditions such as soil water availability or mowing. Selection appears to be operating at a fine spatial scale, with significant life history trait differences evident between populations within species. These taxa may be genetically cohesive, either showing genome-wide divergence, or divergence in genomic regions underlying life history differences [37]. Alternatively, these taxa may be polytopic and not genetically cohesive [38]. Genomic sequencing of natural populations is hoped to resolve this issue.

Phenotypic plasticity in response to host quality

Our many hosts experiment shows *Euphrasia* benefit from growing with a range of hosts. *Euphrasia* with a high quality host such as clover rapidly transitions to flowering. Differences in growth are established early in the season, and early flowering plants go on to grow the tallest, are more highly branched and have the potential to produce many extra flowers. At the other extreme, growing with a poor host or without a host results in late-flowering minimally vegetative plants. Most hosts fall between these extremes and offer moderate return to the parasite. *Euphrasia* parasitizing *Arabidopsis* grew relatively tall despite the host senescing early in the growth season, similar to an experiment where hemiparasitic *Melampyrum arvense* grew vigorously on short-lived *Capsella bursa pastoris* [12]. *Euphrasia* on *Equisetum* performed surprisingly well, similar to the commonly encountered

grass *Holcus lanatus*. This could be through direct benefits via haustorial attachment to *Equisetum* roots, or indirect benefits through association with *Equisetum* fungal symbionts [39]. This could be tested by observing below ground attachment, which would also be interesting for *Pinus*, where poor *Euphrasia* performance may be caused by lack of attachment or defence to parasite attack. Our results point to *Euphrasia* being a true generalist parasite in what it can attach to, but much more specialised in what it can benefit from. The effects of hosts on the growth of *Euphrasia* are consistent with *Melampyrum*, where there is a general trend that legumes are the best host, followed by non-leguminous herbaceous species, and grasses, with considerable variation among species within functional groups [12]. Overall host quality may be attributed to root architecture, germination time and resource availability, as well as the presence of mechanisms to defend against parasite attack, such as cell wall thickening, localised host dieback, and chemical defence [7, 9]. Many of these attributes of host quality appear to be shared between related hemiparasites in the Orobanchaceae, though contrasting results from *Rhinanthus* suggest there are not universal 'good' hosts for all parasitic plants [12].

The availability of suitable *Euphrasia* hosts in the wild will vary in space and time, and theory predicts such fluctuating environments should select for trait plasticity [40]. As expected, we see considerable plasticity in response to host quality. Only three pairs of trait correlations are consistent across both *Euphrasia* common garden experiments (between height, number of branches and leaf teeth), with the positive correlation between node to flower, height, and number of branches seen between species breaking down when *Euphrasia* are grown on different hosts. The most notable plasticity is seen in flowering time, with plants on the best hosts rapidly transitioning to flower within ~100 days of germination, while plants with a more typical host flower a month later. Phenotypic plasticity in flowering time in response to resource availability is well documented in many plant groups, particularly *Arabidopsis* (e.g. [41]), but has largely been overlooked in studies of parasitic plants, which are more likely to look at fitness related traits such as number of flowers or biomass [12, 42]. We expect date of first flowering to be critical for the life time reproductive success of parasitic plants in the wild.

In contrast to traits showing extensive plasticity, we also see evidence of developmental constraint in number of nodes to flower. This trait showed the least plasticity with different hosts, is consistent between populations within species, and between the common garden and the field. This suggests that the developmental event of transitioning to flower is genetically determined, with changes in flowering time altered by plasticity in internode length and not nodes to flower. This may explain why nodes to flower is such an important diagnostic trait for species identification in *Euphrasia* and related species in the Rhinantheae [43]. Despite nodes to flower changing little in response to host quality, our overall impression is that *Euphrasia* show considerable plasticity and little developmental constraint in many aspects of growth, or that trait values are affected by complex interactions related to host-parasite attachment. In particular, the variation between individuals on a given host, suggests other variation, such as genetic background in host and parasite, as well as the timing of attachment, may be crucial in determining performance.

Conclusion

Despite over a century of experimental studies in parasitic plants, our understanding of the evolution of life history strategies in these diverse organisms is extremely limited. Our results in *Euphrasia* provide strong support for the rapid evolution of distinct life history strategies in response to local ecological conditions, with phenotypic plasticity further altering plant growth in response to host availability. We anticipate that future studies that test life time reproductive success of many parasitic plant species grown on many different host species will give further insight into the complex nature of host-parasite interactions.

Data accessibility

Phenotypic data from both common garden experiments and from herbarium collections, as well as the R scripts used for data analysis, are deposited in Dryad XXXXXX (accession to be given on acceptance).

Authors' contributions

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

Competing interests

We have no competing interests.

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Legends to figures and tables.

Figure 1. Trait variation in a common garden experiment of (a) diverse *Euphrasia* species and hybrids grown on clover, (b) *Euphrasia arctica* grown on many different hosts. The edges of the boxplots show the first and third quartiles, the solid lines the median, the whiskers the highest and lowest values within 1.5-fold of the inter-quartile range and the dots each individual measurement.

Figure 2. Relationship between morphological trait measurements made in the common garden and on wild-collected herbarium specimens. Bars represent the standard error of measurements.

Table 1. Summary of generalised linear mixed effects models of the influences on life history, plant vigour, and taxonomic traits for *Euphrasia* in a common garden. Table summarises model outputs for the many species and the many hosts experiment. The percentage variance explained by random effects are reported in brackets along with the 95% credibility interval. †Models for many branches were implemented with a different statistical approach in MCMCglmm (see methods). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 2. Correlation coefficients for seven phenotypic traits measured in a common garden experiment for (a) Five *Euphrasia* species and 6 hybrids, (b) *Euphrasia arctica* grown with 8 hosts and without a host. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Supporting information

Figure S1. Principal components analysis of morphological variation of *Euphrasia* in a common garden for (a) five species and six hybrids, (b) five species, (c) *Euphrasia arctica* with nine host treatments.

Figure S2. Relationship between early and midseason growth and flowering related traits and end of season height. (a) Height at first flowering, (b) height 6-weeks after germination, (c) Julian days to flower, (d) number of branches. Length measurements are reported in mm.

Table S1. Species and collection details for hosts used in the common garden experiment.

Table S2. Collection details for *Euphrasia* species used in the common garden experiment.

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

Table S4. Summary statistics for the principal components analyses of (a) five species and six hybrids, (b) five species, (c) *Euphrasia arctica* with nine host treatments.

Table S5. Summary of trait values for *Euphrasia arctica* grown on many different hosts. Values are mean +/- one standard error. Length measurements are in mm.

Table 1

	Many species		Many hosts	
	Species	Population	Host/no host	Host
Branches†	$DIC_{full} = 867.36$; $DIC_{-spp} = 869.11$	$DIC_{full} = 867.36$; $DIC_{-pop} = 905.68$ (27.7%, 16.4 - 61.2%)	n/a	n/a
Corolla length	$\chi^2(1) = 22.38^{*}$	$\chi^2(1) = 42.89^{***}$ (49.1%, 28.8 - 75.5%)	$\chi^2(1) = 3.19$	$\chi^2(1) = 28.79^{***}$ (13.1%, 5.6 - 52.3%)
Height	$\chi^2(1) = 20.10^{*}$	$\chi^2(1) = 67.68^{***}$ (57.2%, 37.3 - 80.2%)	$\chi^2(1) = 0$	$\chi^2(1) = 91.15^{***}$ (54.1%, 35.9 - 85.5%)
Internode ratio	$\chi^2(1) = 24.5^{**}$	$\chi^2(1) = 27.39^{***}$	$\chi^2(1) = 1.38$	$\chi^2(1) = 6.94^{**}$ (4.6%, 0.8 - 23.0%)
Julian days to flower	$\chi^2(1) = 8.34$	$\chi^2(1) = 1.34$ (21.2%, 7.7 - 64.6%)	$\chi^2(1) = 2.52$	$\chi^2(1) = 32.50^{***}$ (37.4%, 13.4 - 66.3%)
Node to flower	$\chi^2(1) = 26.66^{**}$	$\chi^2(1) = 0.71$ (10.1%, 0.4 - 31.6%)	$\chi^2(1) = 0.38$	$\chi^2(1) = 0$ (1%, 0.18 - 9.8%)
Number of leaf teeth	$\chi^2(1) = 23.43^{*}$	$\chi^2(1) = 0$	$\chi^2(1) = 1.49$	$\chi^2(1) = 5.46^{*}$

Table 2

(a) Many species						
	Corolla length	Height	Internode ratio	Julian days to flower	Leaf teeth	Nodes to flower
Branches	0.068***	0.370***	0.014	0.003	0.433***	0.601***
Corolla length		0.102***	-0.026*	-0.016	-0.039***	-0.002
Height			0.060***	0.085***	0.317***	0.395***
Internode ratio				0.042*	0.014	0.006
Julian days to flower					0.003	0.062***
Leaf teeth						0.424***
(b) Many hosts						
	Corolla length	Height	Internode ratio	Julian days to flower	Leaf teeth	Nodes to flower
Branches	0.275***	0.696***	-0.089***	-0.327***	-0.482***	-0.062***
Corolla length		0.253***	0.010	-0.165***	0.287***	-0.028*
Height			-0.228***	-0.231***	0.480***	-0.035**
Internode ratio				0.001	0.028*	0.000
Julian days to flower					-0.478***	0.281***
Leaf teeth						-0.057***

Literature cited

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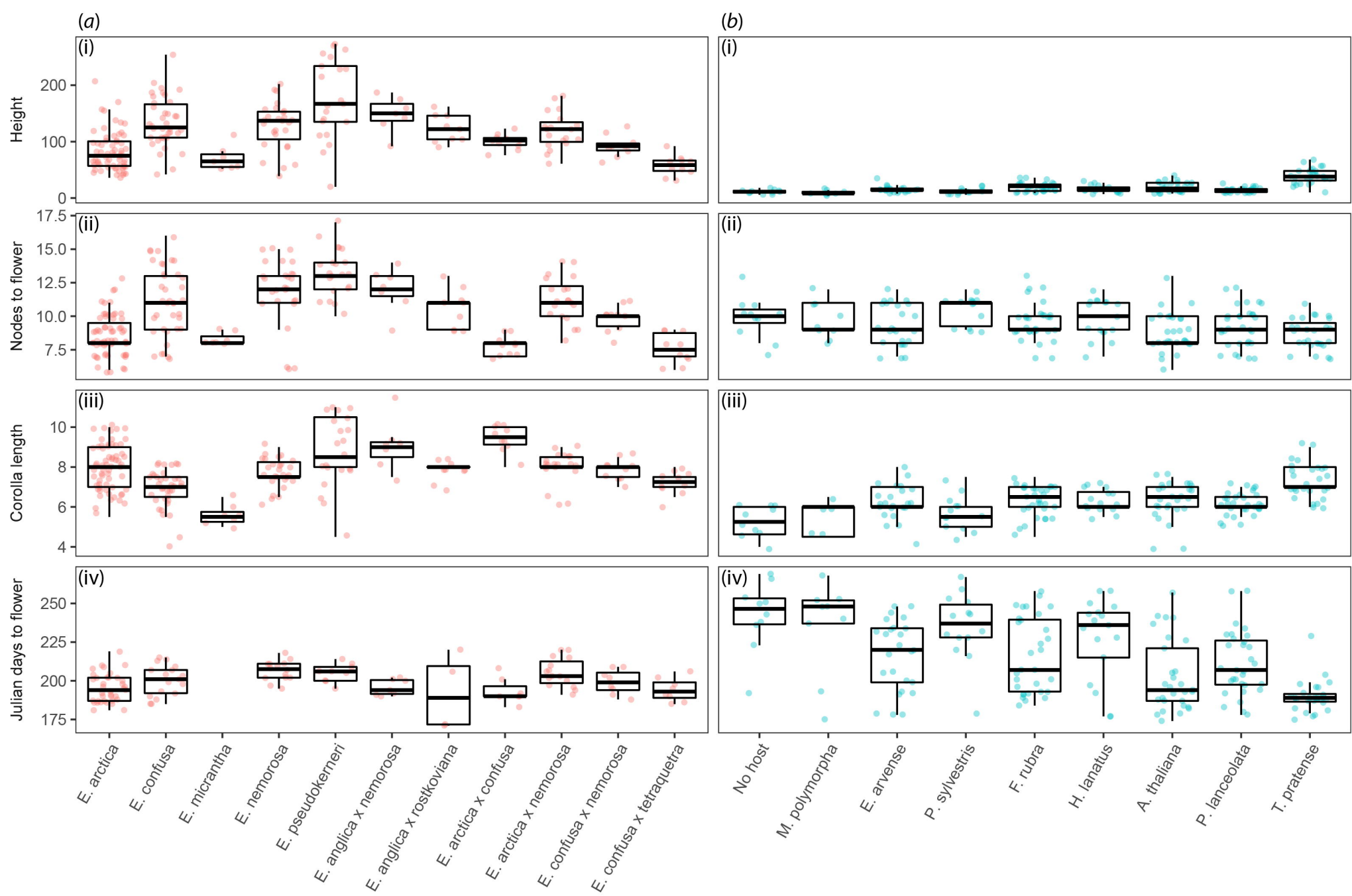
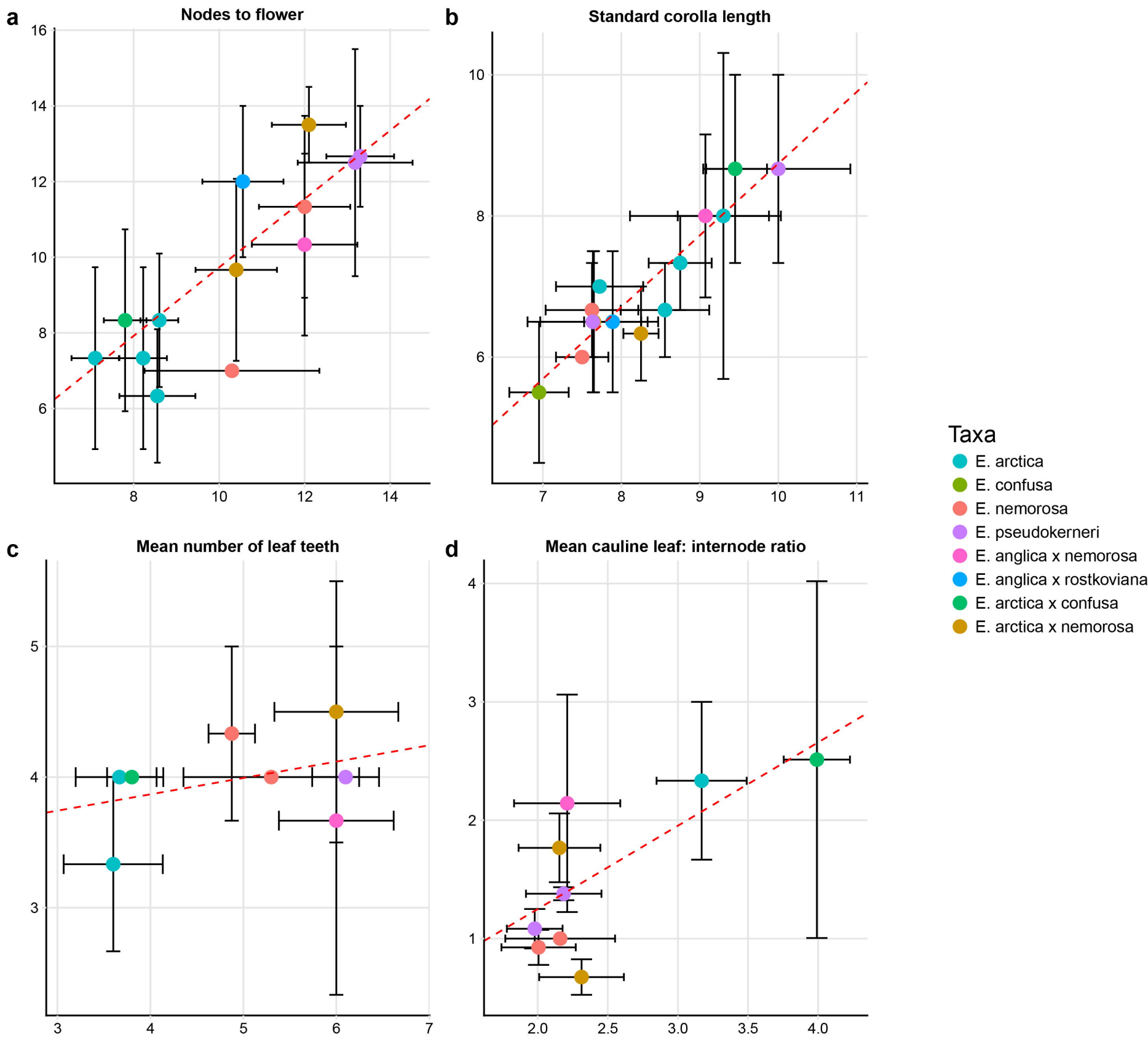

(a)
(b)
(i)
(ii)
(iii)
(iv)
Height
Nodes to flower
Corolla length
Julian days to flower
E. arctica
E. confusa
E. micrantha
E. nemorosa
E. pseudokerneri
E. anglica x nemorosa
E. anglica x rostkoviana
E. arctica x confusa
E. arctica x nemorosa
E. confusa x nemorosa
E. confusa x tetraquetra
No host
M. polymorpha
E. arvense
P. sylvestris
F. rubra
H. lanatus
A. thaliana
P. lanceolata
T. pratense


a
Nodes to flower
b
Standard corolla length
c
Mean number of leaf teeth
d
Mean cauline leaf: internode ratio
Taxa
E. arctica
E. confusa
E. nemorosa
E. pseudokerneri
E. anglica x nemorosa
E. anglica x rostkoviana
E. arctica x confusa
E. arctica x nemorosa