**Diverse parasite-host interactions underlie the performance of hemiparasitic Eyebrights**

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**Summary/Abstract**

Generalist hemiparasitic plants attach and feed to a diverse range of plant host species, however the host attributes that determine parasite performance have not been well characterised. A general expectation is that host functional group determines parasite performance, with legumes being the best hosts. In this study we test host-dependent parasite performance using common garden experiments that follow survival and reproduction of nearly 1900 hemiparasitic eyebrights individuals (*Euphrasia*) on a range of hosts. We show that extensive variation in hemiparasite performance can be attributed to host life history and that host phylogenetic relationships predict this pattern of variation, but not host functional group. We then show that while host-dependent parasite performance is conserved between divergent eyebright species, some eyebrights have more specialised parasite-host interactions. Our results show that a generalist hemiparasitic plant only performs well with a subset of cooccurring hosts, with performance determined by a complex interaction of host attributes.

**Main**

Parasitic plants are a diverse group of 4,500 species of 12 separate origins that obtain water and nutrients from other plants using a specialised feeding organ called a haustorium [1]. The majority of parasitic plant species are hemiparasites, which feed directly from other plants, but maintain their green habit and photosynthetic competency. Hemiparasitic plants include ecosystem engineers that reduce the growth of competitively dominant taxa in grassland communities [2], and species that threaten food security and cause billions of dollars’ worth of crop losses in agricultural systems every year [3]. Studies of hemiparasitic plants have provided extraordinary insights into diverse topics such as horizontal gene transfer, plastid genome degradation, growth and development of a novel organ, and the role of genome duplication in evolutionary novelty [4-7]. Despite the breadth of this research, there are still major knowledge gaps in our understanding of the complex interactions that occur between hemiparasitic plants and their hosts.

Generalist hemiparasites usually grow in mixed plant communities and have the opportunity to parasitize a diverse range of plant hosts. A century of experiments and dozens of publications have documented hemiparasite growth in response to host in the largest clade of parasitic plants, the Orobanchaceae [8-12]. These studies have shown that hemiparasites benefit most from hosts that are large, have a high nitrogen content, and lack or have weak mechanisms of defence to parasite attack [13, 14]. These benefits have been shown for multiple aspects of parasite performance including survival [15], biomass [9], and reproductive output [16]. The dominant paradigm is that such traits are associated with particular plant functional groups such as legumes, grasses, or forbs, with legumes often the best hosts [9, 17]. However, substantial variation in host quality within functional groups suggests functional group alone is not be a good predictor of host quality [17]. From an evolutionary perspective, it would be surprising if a single functional group uniformly represented the best hosts for the thousands of hemiparasites in the ~32 million year old radiation of the Orobanchaceae [18], when these species vary considerably in the hosts with which they co-occur, due to their very diverse geographic ranges and habitats they frequent.

In addition, or alternatively, to host functional group, there are a number of non-mutually exclusive host attributes that may affect the performance of hemiparasitic plants. First, functional groups include some monophyletic clades such as legumes and grasses, and some unnatural groupings such as forbs, which are paraphyletic with legumes and woody taxa nested within. As such, the performance of hemiparasites may be better predicted by host phylogeny rather than functional group, with some clades possessing attributes that confer good parasite growth. Second, we may expect different host life history strategies to affect parasite performance, due to differences in resource accessibility and allocation [19] or relative carbon and nitrogen content of annuals and perennials [20]. Finally, we would expect that if the performance of generalist parasites is governed by specific interactions between parasite and host, host quality would vary on a species-by-species basis. If specific parasite-host interactions underlie parasite fitness, this would raise the prospect of congeneric parasite species becoming adapted to different host species, as widely predicted in theoretical models of parasitism [21]. Determining how host type affects parasite performance requires experimentation with a range of host and parasite species, detailed characterisation of parasite fitness, and appropriate analyses accounting for the phylogenetic relationships of hosts.

Here we use the facultative generalist parasite *Euphrasia* to investigate multiple determinants of host-dependent parasite performance. This genus is an ideal model for studying parasite-host interactions as it is experimentally tractable, with plants being small in size and easy to cultivate with a rapid annual lifecycle [22]. *Euphrasia* species also co-occur with diverse hosts due to its wide distribution range and prevalence in different habitats [23]. We test the host-attributes that determine parasite performance using two common garden experiments with a range of hosts that co-occur with *Euphrasia* in natural populations, and some novel hosts. In Experiment 1, we (1) characterise host-dependent survival and variation in host-dependent reproductive output throughout the lifecycle of hemiparasitic *Euphrasia* and (2) identify the general host attributes such as functional group or life history that affect hemiparasite growth, accounting for phylogenetic relationships of the host species. In Experiment 2, we (3) investigate how parasite-host interactions determine performance of diverse *Euphrasia* species grown with different hosts.

To characterise host attributes that affect hemiparasite survival, in Experiment 1 we followed 1252 individuals of *E. arctica* grown with 45 diverse host species in a common garden experiment (see Methods; Supplementary Table 1). The average probability of *Euphrasia* survival was not significantly affected by host life history (χ2 = 0.40, df=1, P=0.53; Supplementary Table 2) or host functional group in our event history analysis (χ2 = 3.38, df=4, P=0.50; Figure 1 shows legumes and grasses as examples). Between-host effects explained 24.6% of variation in survival when accounting for phylogeny (13.4-55.4% CI, 95% Credible Intervals), with the probability of survival ranging from 0.31 when grown on heather (*Erica tetralix*) to 0.75 on cleavers (*Galium aparine*). The standard deviation of the host effects (0.57, 0.39-1.11 CI) being larger than the fixed effects of life history (0.24) and functional group (0.19 to 0.51) shows that there is considerable variability in the effect of host species on *Euphrasia* survival. Taken together these results indicate host species impact hemiparasite survival even in a benign common garden environment.

We then tracked reproductive output of *Euphrasia* individuals through the growing season. Days to first flower differed 3.5-fold across *Euphrasia* plants, with *Euphrasia* on good hosts flowering earlier (e.g. Bird’s foot trefoil, *Lotus corniculatus* = 78 days ± 3.5 SE, Standard Error) than those on poor hosts (e.g. maize, *Zea mays* = 129.2 days ± 5.1 SE). The difference in days to flower cannot be explained by host functional group (χ2 =2, df=4, P=0.73) and instead between-host effects explained 35.1% (20.0 - 83.5% CI) of the variation when accounting for phylogeny. Life history was marginally significant (χ2 =3.88, df=1, P=0.05; Supplementary Table 3) due to high variability in its effect (77.4-101.9 days to flower CI), with annual hosts on average decreasing *Euphrasia* time to flower. We then observed a significant increase in reproductive output by August and September (pMCMC < 0.001, Supplementary Table 4). Over time, the effect of host functional group was non-significant (χ2 = 7.37, df=4, P=0.12), however host life history significantly interacted with the September census point, with 4.7 times fewer reproductive nodes in *E. arctica* on annual hosts than perennial hosts (0.14-127.0 times, CI; χ2 = 103.16, df=2, P<0.001), Supplementary Table 4). *Euphrasia* individuals were able to reproduce throughout the season and end with high reproductive success on the hosts such as *Lotus corniculatus* (61.5 reproductive nodes ± 15.3 SE in August, raw data) and the clover *Trifolium pratense* (17 ± 7.3 in August). Other hosts such as the grass *Cynosurus cristatus* conferred high reproduction on *Euphrasia* earlier in the season (26.7 reproductive nodes ± 4.9, in July) with reproduction plateauing (25.8 ± 4.3, in August) and then declining to zero. Overall, this shows the trajectories of reproductive success in *Euphrasia* depend on host type (Supplementary Figure 1).

By the end of the season, *Euphrasia* produced on average more than one reproductive node on 28 out of the 45 hosts. Total reproductive output could not be explained by host functional group (χ2 = 6.83, df=4, P=0.14, Supplementary Table 5) or host life history (χ2 = 0.076, df = 1, P=0.78). However, host species explained 81.8% (65.9-95.6% CI) of the variability in end of season reproductive nodes accounting for phylogeny, with high phylogenetic signal for this trait (0.88, 0.17-0.98 CI). *Euphrasia* produced a large numbers of reproductive nodes only with few host species such *Lotus corniculatus* (104.5 ± 19.1 SE reproductive nodes), *Cynosurus cristatus* (53.6 ± 8.4) and the plantain *Plantago lanceolata* (35.5 ± 3.7) (Figure 2). We accounted for variance in early development of *Euphrasia*, a known factor influencing lifetime fitness [24], by adding transplant date to all models. Although significant in each case (pMCMC < 0.001), phylogenetic signal of reproductive output remained high (Supplementary Figure 2). These results highlight the importance of phylogenetic relatedness of host plant species in predicting *Euphrasia* fitness above host species functional group.

In Experiment 2 we tested for specific hemiparasite-host interactions by measuring reproductive success of six populations from four divergent species of *Euphrasia* in a common garden using a suite of hosts from different habitats (see Methods; Supplementary Tables 6,7). After taking into account differences between *Euphrasia* species and populations in their reproductive output (χ2 = 4.4, df=6, P=<0.001; Supplementary Table 8), there was evidence for both consistent host driven differences in parasite performance, and specific parasite-host interactions (Figure 3). Host species accounted for most of the variation in reproductive nodes at the end of the season (26%; χ2 = 15.6, df=1, P <0.001), followed by host interacting with *Euphrasia* species (12.3%; χ2 = 27.1, df=1, P <0.001; Supplementary Figure 3). *Euphrasia* species tended to react similarly to a given host, with a 0.76 (0.37-0.93 CI) correlation in reproductive output when two hosts are picked at random. By investigating model best linear unbiased predictors (BLUPs), differences in host effect are driven by *L. corniculatus*, the speedwell *Veronica chamaedrys*, and sea plantain *Plantago maritima,* each of which have antagonistic interactions with different *Euphrasia* species. Moreover, two divergent species of *Euphrasia* from the same geographic location, diploid *E. vigursii* and tetraploid *E. tetraquetra*, show similar responses to the same set of hosts, with no significant interactions detected in these two species (Supplementary Figure 4; χ2 = 0.22, df=1, P=0.64). Although the dominant signal is that of conservatism of performance across *Euphrasia* species on the same host, parasite-host interactions explain a significant proportion of the variation in *Euphrasia* performance.

This is the first comprehensive study of hemiparasitic plant performance throughout their life, across many different host species, analysed in a rigorous phylogenetic context of host relationships*.* Our results support the emerging view that host functional group is not a good predictor of parasitic plant performance [9, 17]. The importance of life history is most notable when investigating the temporal effects of host plants on parasite performance, and this shift in temporal effects initiated by host life history may be significant in natural communities [25]. Host quality also has a phylogenetic signal, with *Euphrasia* similarity in performance being correlated with the closeness of relationship of host species. This phylogenetic signal may be correlated with multiple host attributes contributing to the host phenotype, such as host defences [13], root characteristics [26], and uptake of secondary compounds [27], as well as post-establishment competitive interactions determined by virulence-resistance trade-offs [28]. These factors point to *Euphrasia* being true generalists able to benefit from plants throughout the vascular plant phylogeny, but only gaining major benefit from a minor subset of taxa.

Our finding that hosts beneficial to one *Euphrasia* species are generally beneficial across all *Euphrasia* species studied reveals conserved parasite-host interactions. While conservation of host specificity is expected in related parasite species [29], we do find significant parasite-host interactions and species-specific responses to some hosts, suggesting weak differential host adaptation. Host species are spatially heterogenous in their distribution and vary in abundance by habitat and geographic area, creating conditions that may allow local host adaptation. The low migration rate between *Euphrasia* populations, particularly in small flowered selfing taxa [30], may cause differentiation and promote local adaptation. While the drivers and tempo of local host adaptation are not fully understood, further investigations with dense host sampling will shed light on the nature of these interactions, and reveal if they should be considered in conservation translocations.

**Figures**

Figure 1 - Probability of *Euphrasia arctica* survival in a common garden experiment on 14 host species from two representative families, the Fabaceae (a) and Poaceae (b), using host species binomial regressions. Pale regressions represent individual species and bold regressions represent family level regressions. Pale grey dots are jittered raw values of an individual’s living status (binary) at each time point from earliest census in May to the latest in August.



Figure 2 – Performance of hemiparasitic *Euphrasia arctica* measured as cumulative reproductive nodes in the context of host species and host phylogeny. (a) Maximum likelihood phylogeny of 45 hosts based on rbcL and matK. Bootstrap values are shown for each node on the phylogeny. Monocots, the two largest orders and two superorders are labelled. Host species are coloured by functional group, orange = forbs, grey = woody plants, blue = legumes, green = grasses and yellow = ferns. (b) Values are mean cumulative reproductive nodes of *Euphrasia* per species with colours corresponding to functional group of host ± one standard error. Silhouetted pictures are from phylopic.org.

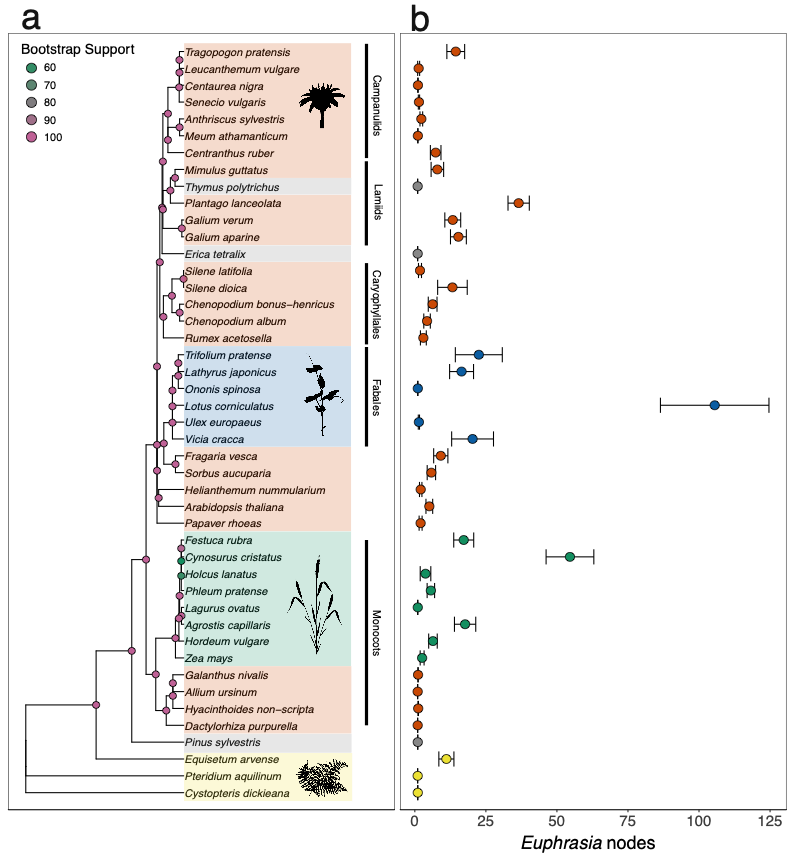


Figure 3 – Performance of four species of *Euphrasia* on thirteen different species of host plants measured as cumulative reproductive nodes at the end of the season. Each panel represents a unique *Euphrasia* species. The x-axis represents the number of reproductive nodes of *Euphrasia* for each host averaged across all *Euphrasia* species, while the y-axis shows reproductive nodes per *Euphrasia* species ± one standard error. Both axes are log transformed. The red dashed line graphs *y*=*x*; points above the line indicate elevated response to a host beyond the average, while points below the line indicate the opposite. Host species are ranked by average performance conferred to a *Euphrasia* species, where HPU = *Hypericum pulchrum*, CVU = *Calluna vulgaris*, HLA = *Holcus lanatus*, OVU = *Origanum vulgare*, UGA = *Ulex gallii*, PMA = *Plantago maritima*, PLA = *Plantago lanceolata*, VCH = *Veronica chamaedrys*, FOV = *Festuca ovina*, DFL = *Deschampsia flexuosa*, ACU = *Agrostis curtisii*, LPE = *Lolium perenne* and LCO = *Lotus corniculatus*.



Methods:

Plant material, cultivation and trait measurements

For Experiment 1, which looked at a single species of *Euphrasia*, forty-five diverse species were used as hosts. These were grown separately in trays and introduced after *Euphrasia* germination in spring 2017 (Supplementary Table 1). For Experiment 2, to detect parasite-host interactions, thirteen species of hosts were grown and introduced after *Euphrasia* germination in spring 2018 (Supplementary Table 2). For both experiments, wild-collected open-pollinated seeds of *Euphrasia* species were collected (Supplementary Table 3). *Euphrasia* seeds were sown in individual pots filled with Sylvamix 1 compost and placed in an outside array at the Royal Botanical Gardens in Edinburgh (RBGE). Details of soil mixes and sowing protocols followed [22]. In Experiment 1, a total of 3000 *Euphrasia* seeds from a single population of *Euphrasia arctica* (Supplementary Table 3) were sown in winter 2016 of which 1252 *Euphrasia* plants survived to be measured at the first time point. In Experiment 2, a total of 2880 *Euphrasia* seeds from six populations (Supplementary Table 3) were sown in winter 2017, of which 635 *Euphrasia* individuals survived to the end of season and were measured. In both experiments after germination, all plants were grown in an unheated, well ventilated greenhouse. Host plants were then transplanted into *Euphrasia* pots, with the protocol following [22]. Host plants were replaced if mortality occurred within two weeks of transplant. All pots containing *Euphrasia*-host combinations were randomised weekly.

For Experiment 1 we measured date of first flowering and then both the number of reproductive nodes and whether an individual *Euphrasia* was alive or dead every 30 days beginning on the 30.05.17. For Experiment 2, we measured reproductive nodes at the end of the season. In both experiments, germination date and date a host was transplanted to a *Euphrasia* individual were recorded.

Statistical analyses

The phylogeny used in the analysis of Experiment 1 was based on the two gene alignment of rbcL and matK from [31]. Six sequences from three species (*Zea mays, Hordeum vulgare* and *Lagurus ovatus*) were added from NCBI database [32], as they were not present in the original dataset. The maximum likelihood phylogeny was generated using IQ-TREE with 1000 ultrafast bootstrap and using the TESTNEWMERGE flag for model selection. A constraint tree was created using the phylomatic function in the R package brranching [33] and used to topologically constrain the phylogeny in IQ-TREE based on the APG IV phylogeny. The tree was then forced to be ultrametric prior to analyses.

All statistical analyses were conducted in R version 3.6.1 [34] with all data manipulation in base R or data.table [35]. Survival, number of days to flower and reproductive output of *Euphrasia* were estimated using a Bayesian generalized linear mixed effect model approach in the MCMCglmm package [36]. Models were run for a minimum of 130000 iterations, a burn-in of 30000, and a thinning interval of 100. Parameter expanded priors were used to improve convergence and effective sample sizes of focal parameters were in excess of 500 and mostly approaching 1000. Significance of categorical covariates with more than one level were determined using Wald Tests [37], otherwise the *p*MCMC value of the covariates were reported. Variance explained by random effects including phylogeny (phylogenetic signal) were calculated as ratios of the variance of the parameter of interest to the residual variance in the model. For joint phylogenetic estimates, the posterior distributions of the phylogenetic and host species effects were summed. Significance of random effects were determined using likelihood ratio tests in the package lme4 where appropriate [38]. Convergence and autocorrelation of models was assessed visually by plotting the posterior distributions of the estimated parameters.

For Experiment 1, functional group and life history of host, as well as normalized transplant date (time lag between germination and receiving a host, scaled to difference in first transplant date), were added as fixed effects, whilst host species and phylogenetic effects were treated as random effects. In the event history analysis (EHA; survival) time point was also added as a fixed effect and was modelled using the binomial distribution with a probit link function, otherwise response variables were modelled using the Poisson distribution. Time point five was removed from the EHA, as all but two individuals were dead at this time. We parameterised the reproductive output over time model differently. Time point and its interaction with life history were additional fixed effects and time points one and five were removed due to lack of reproduction at these time points. We included a random effect variance structure of an interaction of time point and host species using the us() variance function in MCMCglmm which allows covariance between host and time point:

Where VHE is the variance in host effect and T is the time point. The residual (V*e*) variance-covariance matrix allowed no covariance between time points using the MCMCglmm function idh():

In Experiment 2, we modelled end of season reproductive output as a function of *Euphrasia* species, population of *Euphrasia* species and normalized transplant date. Host species and the host species interaction with *Euphrasia* species were added as single parameter random effects. The correlation in hosts effects was calculated as the ratio of host effects to the host effects and host species interaction with *Euphrasia* species combined.

All code and data is available at <https://github.com/Euphrasiologist/euphrasia_host_parasite>.

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Contributions

A.D.T. designed the research; M.R.B, P.M. and N.F. carried out the experiment and collected the data; M.R.B. analysed the data; M.R.B and A.D.T wrote the manuscript.

Competing interests

The authors declare no competing interests.

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**Supplementary Information:**

Table 1: Plant names, attributes and collection sources for host species used in Experiment 1.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Host species** | **Authority** | **Functional group** | **Life History** | **Seed source** |
| No host | - | - | - | - |
| *Agrostis capillaris* | L. | Grass | Perennial | Emorsgate |
| *Allium ursinum* | L. | Forb | Perennial | RBGE |
| *Anthriscus sylvestris* | [(L.) Hoffm.](http://www.theplantlist.org/tpl1.1/record/kew-2641868) | Forb | Perennial | Emorsgate |
| *Arabidopsis thaliana* | [(L.) Heynh.](http://www.theplantlist.org/tpl1.1/record/kew-2645262) | Forb | Annual | Inbred lines University of Edinburgh |
| *Centaurea nigra* | L. | Forb | Perennial | Emorsgate |
| *Centranthus ruber* | [(L.) DC.](http://www.theplantlist.org/tpl1.1/record/kew-2709046) | Forb | Perennial | Chiltern Seeds |
| *Chenopodium album* | L. | Forb | Annual | Author collections |
| *Chenopodium bonus-henricus* | L. | Forb | Perennial | Surplus seed RBGE |
| *Cynosurus cristatus* | L. | Grass | Perennial | Emorsgate |
| *Cystopteris dickeniana* | [R. Sim](http://www.theplantlist.org/tpl1.1/record/tro-26604225) | Fern | Perennial | RBGE |
| *Dactylorhiza purpurella* | [(T.Stephenson & T.A.Stephenson) Soó](http://www.theplantlist.org/tpl1.1/record/kew-55540) | Forb | Perennial | RBGE |
| *Equisetum arvense* | L. | Fern | Perennial | RBGE |
| *Erica tetralix* | L. | Woody | Perennial | RBGE |
| *Festuca rubra* | L. | Grass | Perennial | Emorsgate |
| *Fragaria vesca* | L. | Forb | Perennial | Scotia seeds |
| *Galanthus nivalis* | L. | Forb | Perennial | RBGE |
| *Galium aparine* | L. | Forb | Annual | Author collection, Upper Halliford, Surrey, Engalnd, 11/16 |
| *Galium verum* | L. | Forb | Perennial | Emorsgate |
| *Helianthemum nummularium* | [(L.) Mill.](http://www.theplantlist.org/tpl1.1/record/kew-2842625) | Forb | Perennial | Scotia seeds |
| *Holcus lanatus* | L. | Grass | Perennial | Emorsgate |
| *Hordeum vulgare* | L. | Grass | Annual | Wiggly Wigglers |
| *Hyacinthoides non-scripta* | [(L.) Chouard ex Rothm.](http://www.theplantlist.org/tpl1.1/record/kew-278557) | Forb | Perennial | RBGE |
| *Lagurus ovatus* | L. | Grass | Annual | www.wildflowershop.co.uk |
| *Lathyrus japonicus* | [Willd.](http://www.theplantlist.org/tpl1.1/record/ild-8875) | Legume | Perennial | RBGE |
| *Leucanthemum vulgare* | [(Vaill.) Lam.](http://www.theplantlist.org/tpl1.1/record/gcc-135712) | Forb | Perennial | Emorsgate |
| *Lotus corniculatus* | L. | Legume | Perennial | Emorsgate |
| *Meum athamanticum* | [Jacq.](http://www.theplantlist.org/tpl1.1/record/kew-2365193) | Forb |  | RBGE |
| *Mimulus guttatus* | [DC.](http://www.theplantlist.org/tpl1.1/record/kew-2506223) | Forb | Perennial | Author collections |
| *Ononis spinosa* | L. | Legume | Perennial | Emorsgate & Wild Flower Shop |
| *Papaver rhoeas* | L. | Forb | Annual | Emorsgate |
| *Phleum pratense* | L. | Grass | Perennial | Wild Flower Shop |
| *Pinus sylvestris* | L. | Woody | Perennial | Scotia seeds |
| *Plantago lanceolata* | L. | Forb | Perennial | Emorsgate |
| *Pteridium aquilinum* | L. (Kuhn) | Fern | Perennial | British Pteridological Society spore exchange |
| *Rumex acetosella* | L. | Forb | Perennial | Scotia seeds |
| *Senecio vulgaris* | L. | Forb | Annual | RBGE |
| *Silene dioica* | [(L.) Clairv.](http://www.theplantlist.org/tpl1.1/record/kew-2488209) | Forb | Perennial | D. Charlseworth, Univ. Edinburgh |
| *Silene latifolia* | [Poir.](http://www.theplantlist.org/tpl1.1/record/kew-2488689) | Forb | Perennial | D. Charlseworth, Univ. Edinburgh |
| *Thymus polytrichus* | [A.Kern. ex Borbás](http://www.theplantlist.org/tpl1.1/record/kew-205257) | Woody | Perennial | Emorsgate |
| *Sorbus aucuparia* | L. | Woody | Perennial | RBGE |
| *Tragopogon pratensis* | L. | Forb | Perennial | Scotia seeds |
| *Trifolium pratense* | L. | Legume | Perennial | Chiltern Seeds & Wild Flower Shop |
| *Ulex europaeus* | L. | Legume/Woody | Perennial | Tree Seed Online Ltd |
| *Vicia cracca* | L. | Legume | Perennial | Emorsgate |
| *Zea mays* | L. | Grass | Annual | Chiltern Seeds |

Table 2: Model output from MCMCglmm for the event history analysis (survival) model in Experiment 1. The intercept represents the latent probit estimate of mean *Euphrasia* survival on a perennial grass transplanted at the earliest date, measured at the first time point. The posterior means are reported along with the lower and upper 95% credible intervals as well as the effective sample size and *p*-value for the effect (*p*MCMC).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Covariates** | **Posterior mean** | **l-95% CI** | **u-95% CI** | **Effective sample size** | **pMCMC** |
| **(Intercept)** | 3.0348 | 1.8630 | 4.1519 | 1000.0000 | **<0.001** |
| **Time** | -1.0533 | -1.1164 | -0.9912 | 1000.0000 | **<0.001** |
| **AnnPerAnn** | 0.1390 | -0.2489 | 0.6076 | 1000.0000 | 0.5300 |
| **Normalized transplant date** | -0.0164 | -0.0213 | -0.0117 | 1000.0000 | **<0.001** |
| **Functional\_groupFern** | -0.2583 | -1.5117 | 1.0171 | 1000.0000 | 0.6520 |
| **Functional\_groupForb** | -0.3076 | -0.9687 | 0.3844 | 1000.0000 | 0.3700 |
| **Functional\_groupLegume** | -0.0828 | -1.0457 | 0.7646 | 1000.0000 | 0.8500 |
| **Functional\_groupWoody** | -0.6675 | -1.4986 | 0.1819 | 1000.0000 | 0.0980 |

Table 3: Model output from MCMCglmm for the days to flower model in Experiment 1. The intercept represents the log of the mean days to flower since germination of *Euphrasia* on a perennial grass transplanted at the earliest date. The posterior means are reported along with the lower and upper 95% credible intervals as well as the effective sample size and *p*-value for the effect (*p*MCMC).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Covariates** | **Posterior mean** | **l-95% CI** | **u-95% CI** | **Effective sample size** | **pMCMC** |
| **(Intercept)** | 4.6197 | 4.1765 | 5.0536 | 1000.0000 | **<0.001** |
| **AnnPerAnn** | -0.1380 | -0.2703 | 0.0043 | 1188.3374 | 0.0560 |
| **Functional\_groupFern** | -0.1127 | -0.5410 | 0.3556 | 1000.0000 | 0.6000 |
| **Functional\_groupForb** | -0.0879 | -0.3087 | 0.1793 | 1106.4845 | 0.3780 |
| **Functional\_groupLegume** | -0.0650 | -0.3307 | 0.3032 | 860.8955 | 0.6160 |
| **Functional\_groupWoody** | 0.0991 | -0.2964 | 0.4466 | 1000.0000 | 0.5520 |
| **Normalized transplant date** | 0.0034 | 0.0008 | 0.0060 | 1000.0000 | **0.0160** |

Table 4: Model output from MCMCglmm for the number of reproductive nodes over time model in Experiment 1. The intercept represents log of the mean number of reproductive nodes of *Euphrasia* on a perennial grass transplanted at the earliest date, measured at the first time point. The posterior means are reported along with the lower and upper 95% credible intervals as well as the effective sample size and *p*-value for the effect (*p*MCMC).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Covariates** | **Posterior mean** | **l-95% CI** | **u-95% CI** | **Effective sample size** | **pMCMC** |
| **(Intercept)** | -4.1298 | -17.0773 | 5.4805 | 550.1727 | 0.3420 |
| **Time3** | 2.3713 | 1.5862 | 3.2031 | 773.1872 | **<0.001** |
| **Time4** | 3.0630 | 2.1378 | 3.9166 | 1000.0000 | **<0.001** |
| **AnnPerAnn** | 0.7872 | -1.2385 | 2.8500 | 1000.0000 | 0.4460 |
| **Functional\_groupFern** | -4.3612 | -16.8977 | 6.6709 | 789.8238 | 0.3960 |
| **Functional\_groupForb** | -2.3178 | -9.4309 | 3.7584 | 793.8151 | 0.4420 |
| **Functional\_groupLegume** | -2.3657 | -10.7235 | 5.1473 | 756.9460 | 0.5760 |
| **Functional\_groupWoody** | -7.6673 | -15.5032 | -1.0839 | 549.4358 | **0.0180** |
| **Normalized transplant date** | -0.0760 | -0.0919 | -0.0625 | 1000.0000 | **<0.001** |
| **Time3:AnnPerAnn** | -0.9448 | -2.0965 | 0.1002 | 1000.0000 | 0.0920 |
| **Time4:AnnPerAnn** | -2.3383 | -3.6057 | -0.8897 | 1000.0000 | **0.0040** |

Table 5: Model output from MCMCglmm for the cumulative reproductive nodes at the end of the season model in Experiment 1. The intercept represents the log of the mean cumulative reproductive nodes at the end of the season of *Euphrasia* on a perennial grass transplanted at the earliest date. The posterior means are reported along with the lower and upper 95% credible intervals as well as the effective sample size and *p*-value for the effect (*p*MCMC).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Covariates** | **Posterior mean** | **l-95% CI** | **u-95% CI** | **Effective sample size** | **pMCMC** |
| **(Intercept)** | -0.4637 | -9.8823 | 9.4058 | 1093.7327 | 0.9240 |
| **AnnPerAnn** | -0.3610 | -2.9028 | 2.1730 | 886.5257 | 0.7720 |
| **Functional\_groupFern** | -3.6600 | -15.1134 | 6.8501 | 1000.0000 | 0.4660 |
| **Functional\_groupForb** | -2.9965 | -8.8016 | 2.1653 | 1097.9740 | 0.2340 |
| **Functional\_groupLegume** | -2.0488 | -9.1675 | 4.6899 | 1000.0000 | 0.5500 |
| **Functional\_groupWoody** | -7.5786 | -14.1020 | -1.0165 | 633.3565 | **0.0100** |
| **Normalized transplant date** | -0.0762 | -0.0945 | -0.0570 | 1000.0000 | **<0.001** |

Table 6: *Euphrasia* species collections across both experiments.

|  |  |  |  |
| --- | --- | --- | --- |
| Experiment | *Euphrasia* species | Location | Grid Reference |
| 1 | *E.arctica* | Inverkeithing, Scotland | NT 1389 82312 |
| 2 | *E.anglica* (A1766) | Cheddar, Somerset | ST 47731 54156 |
| 2 | *E.vigursii* (V1761) | St Agnes Head, Cornwall | SW 5899 4328 |
| 2 | *E.tetraquetra* (T1761) | St Agnes Head, Cornwall | SW 5899 4328 |
| 2 | *E.micrantha* (M1767) | Borrowdale, Cumbria | NY 2468 1631 |
| 2 | *E.micrantha* (M1768) | Alness, Scotland | NH 5521 7126 |
| 2 | *E.micrantha* (M1769) | Orkney, Scotland | HY 321 055 |

Table 7: Plant names, attributes and collection sources for host species used in Experiment 2.

|  |  |  |  |
| --- | --- | --- | --- |
| **Host species** | **Authority** | **Source/Location** | **Plant status** |
| *Agrostis curtisii* | Kerguélen | Millenium Seed Bank, Kew Gardens | Seed |
| *Calluna vulgaris* | (L.) Hull | RBGE | Seed, but small plants from cuttings |
| *Deschampsia (Avenella) flexuosa* | (L.) Trin. | Chiltern Seeds | Seed |
| *Festuca ovina* | L. | Emorsgate | Seed |
| *Holcus lanatus* | L. | Emorsgate | Seed |
| *Hypericum pulchrum* | L. | Scotia Seeds | Seed |
| *Lotus corniculatus* | L. | Emorsgate | Seed |
| *Lolium perenne* | L. | Emorsgate | Seed |
| *Origanum vulgare* | L. | Emorsgate | Seed |
| *Plantago lanceolata* | L. | Emorsgate | Seed |
| *Plantago maritima* | L. | Scotia Seeds | Seed |
| *Ulex gallii* | Planch. | Millenium Seed Bank, Kew Gardens | Seed |
| *Veronica chamaedrys* | L. | Scotia Seeds | Seed |

Table 8: Model output from MCMCglmm for the number of cumulative reproductive nodes of *Euphrasia* individuals at the end of the season from Experiment 2. The intercept represents log of the mean cumulative number of reproductive nodes of *Euphrasia anglica*, population A1766, on a host that was transplanted at the earliest date. The posterior means are reported along with the lower and upper 95% credible intervals as well as the effective sample size and *p*-value for the effect (*p*MCMC).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Posterior mean** | **l-95% CI** | **u-95% CI** | **Effective sample size** | **pMCMC** |
| **(Intercept)** | 1.7842 | 1.2210 | 2.2714 | 787.7612 | **0.0010** |
| **Euphrasia micrantha** | -1.2795 | -1.7479 | -0.8284 | 1000.0000 | **0.0010** |
| **Euphrasia tetraquetra** | -0.3702 | -0.8160 | -0.0076 | 873.2641 | 0.0620 |
| **Euphrasia vigursii** | -0.2457 | -0.7758 | 0.2138 | 1000.0000 | 0.3340 |
| **Population: M1767** | 0.3269 | -0.2098 | 0.9299 | 846.7382 | 0.2760 |
| **Population: M1768** | 0.7931 | 0.4788 | 1.0699 | 1000.0000 | **0.0010** |
| **Normalized transplant date** | 0.0059 | -0.0084 | 0.0237 | 1207.7299 | 0.4820 |

Figure 1: *Euphrasia* reproductive output over time showing differences in reproductive trajectories, data from Experiment 1. Values represent mean reproductive nodes at a particular time point ± one standard error. Eleven species of host are shown, along with the average host where points are the mean of all hosts in the experiment.



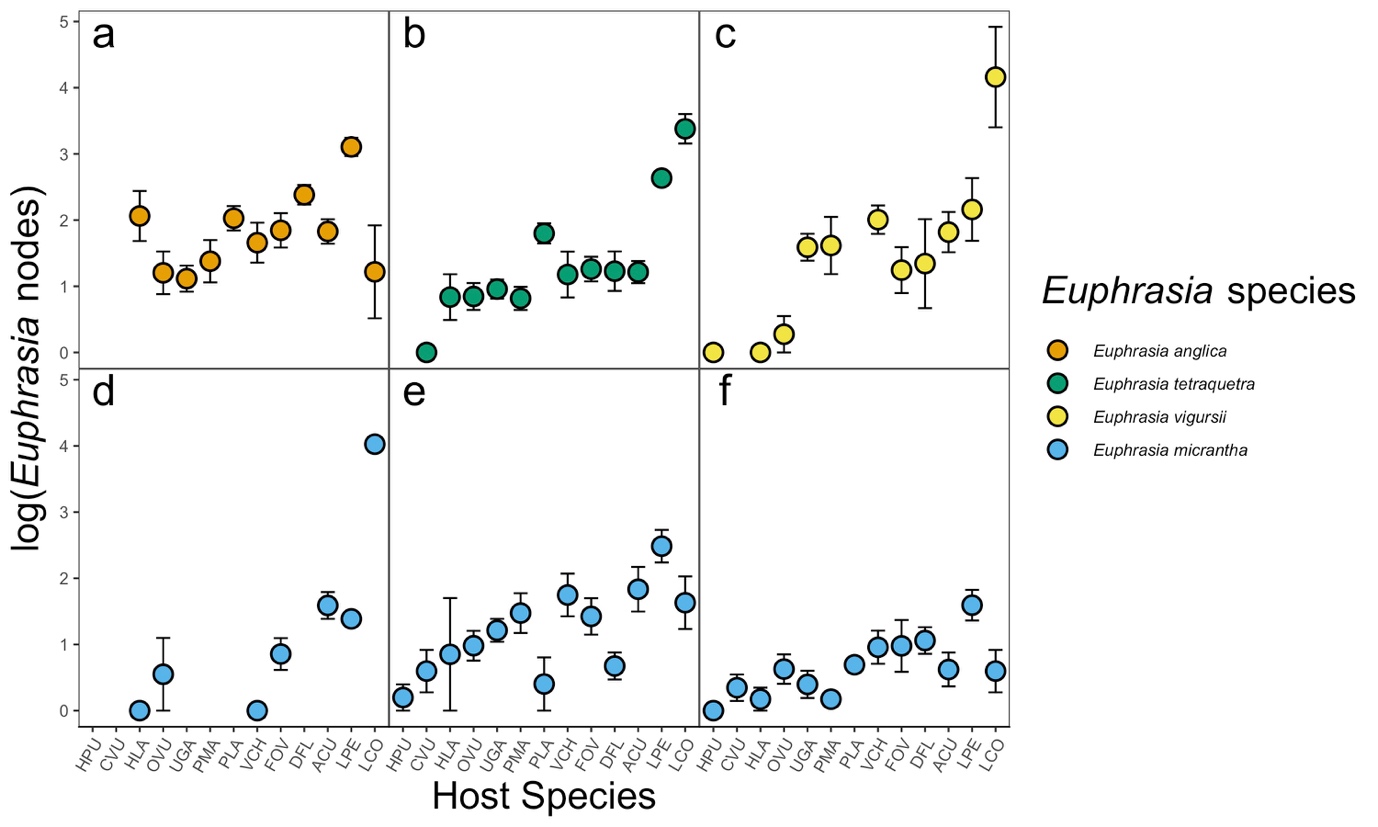
Figure 2: Posterior distributions of the phylogenetic signal for the models from Experiment 1. Total reproductive output shows the highest and least variable estimate of phylogenetic signal, however all are significant as the distributions are not overlapping zero.



Figure 3: Posterior distribution of the variance for random effects in the model fitted for Experiment 2. The random effects are the *Euphrasia*-host interaction, the sole effect of host species, and the residual variance.



Figure 4: Performance of four species of *Euphrasia* on thirteen different species of host plants measured as cumulative reproductive nodes. Each panel represents a unique *Euphrasia* population (a = A1766, b = T1761, c = V1761, d = M1767, e = M1768, f = M1769), coloured by species. Two populations, (e) and (f) co-occur. Host species are ranked by average performance conferred to a *Euphrasia* species, where HPU = *Hypericum pulchrum*, CVU = *Calluna vulgaris*, HLA = *Holcus lanatus*, OVU = *Origanum vulgare*, UGA = *Ulex gallii*, PMA = *Plantago maritima*, PLA = *Plantago lanceolata*, VCH = *Veronica chamaedrys*, FOV = *Festuca ovina*, DFL = *Deschampsia flexuosa*, ACU = *Agrostis curtisii*, LPE = *Lolium perenne* and LCO = *Lotus corniculatus*. Y-axis values are the log of the mean cumulative reproductive nodes ± one standard error.



Data and code for all analyses available at <https://github.com/Euphrasiologist/euphrasia_host_parasite>