

# Appendices

## Appendix 1: Chapter 2

Figure 1: The distribution of ploidy levels across the British and Irish angiosperms in the four families with the highest number of species. Shown are Rosaceae, Poaceae, Asteraceae and Fabaceae. Each family has distinct distributions of ploidy levels.

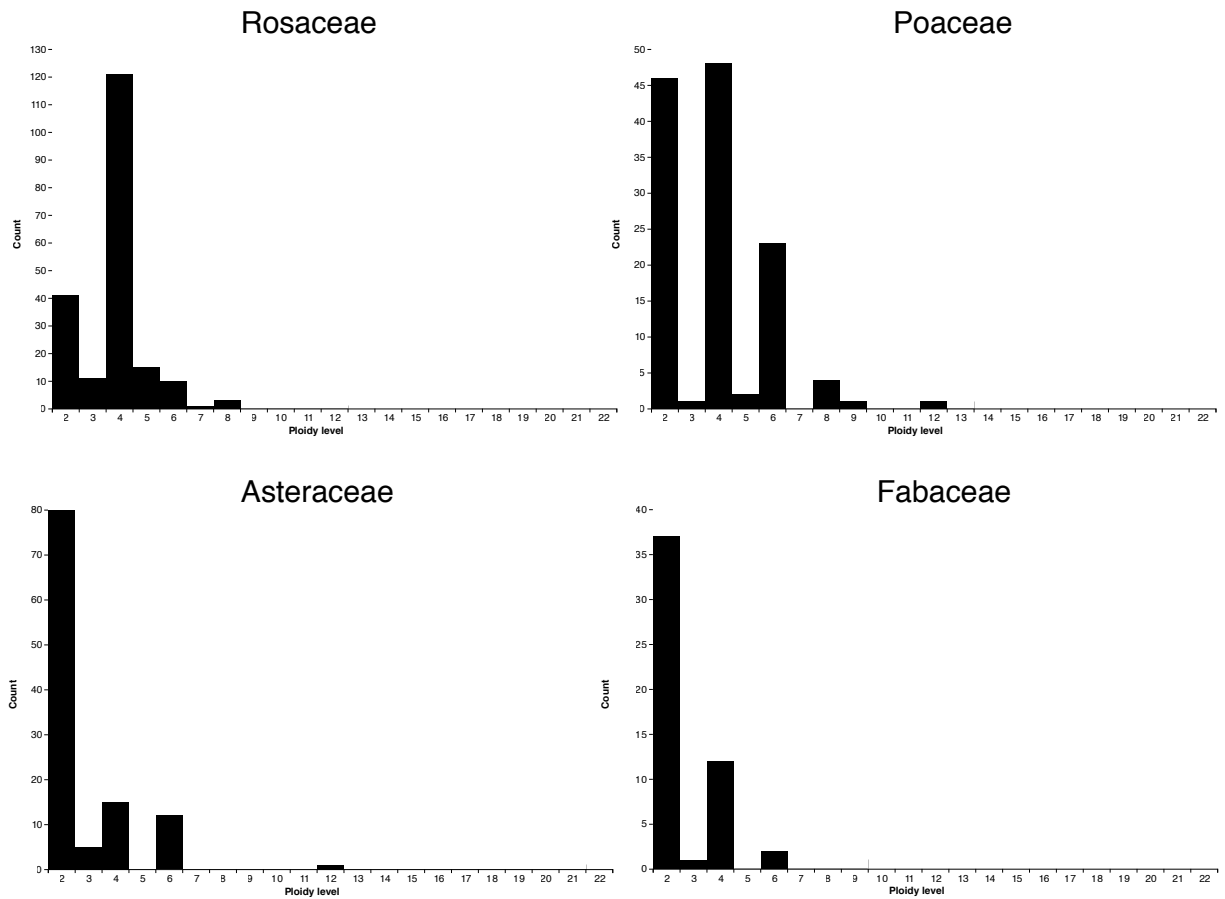


Table 1: Search strings for Google Scholar searches used to generate the list of examples of cross-ploidy hybrids in Chapter 2. Note that other examples were added if they were deemed to be important and/or well known.

Journal	Search string
Molecular Ecology	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"Molecular Ecology"
Evolution	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid site:onlinelibrary.wiley.com source:"Evolution" -source:"and Evolution" -source:"Organic Evolution"
Heredity	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"Heredity"
Annals of Botany	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"Annals of Botany"
American Journal of Botany	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"American Journal of Botany"
New Phytologist	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"New Phytologist"
PNAS	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"PNAS"
Biological Journal of the Linnean Society	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"Biological Journal of the Linnean Society"
Botanical Journal of the Linnean Society	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"Botanical Journal of the Linnean Society"
Journal of Evolutionary Biology	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"Journal of Evolutionary Biology"
PLoS One	Ploidy hybrid genetic introgression diploid OR tetraploid OR hexaploidy OR octoploid source:"PLoS One"

## Appendix 2: Chapter 3

Figure 2: Trees with root nodes containing the highest and lowest posterior mean probability of hybridisation from Model 1 (BLUP's of nodes in the phylogeny). A is the top tree (subset of Orchidaceae) whilst B is the tree with lowest probability of hybridisation (Fabaceae and Polygalaceae).

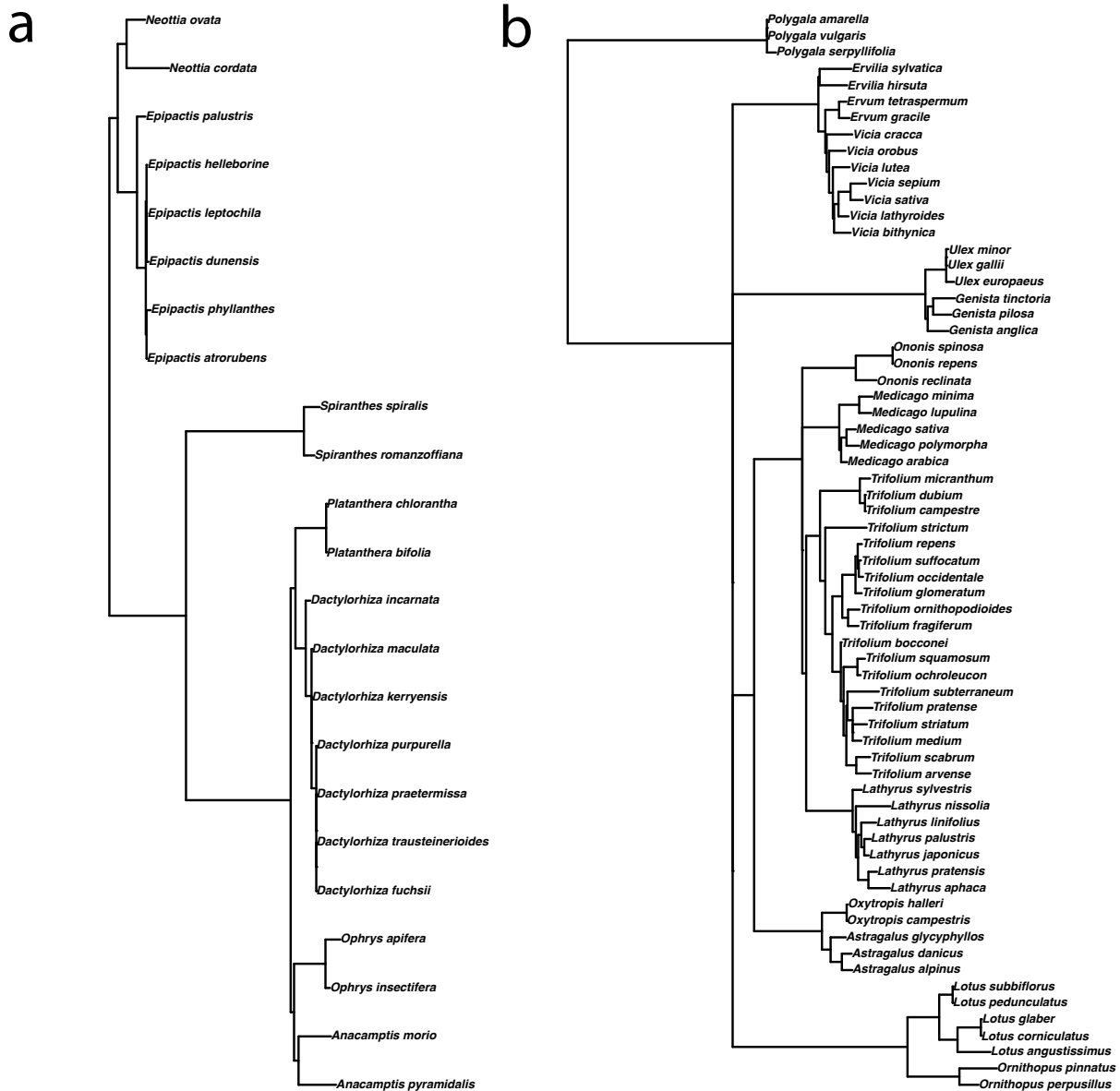


Figure 3: The joint probability of hybridisation between two parental species give both branch length between species (tree based genetic distance) and geographical overlap between parental species (measured as overlap in occupancy of 10x10km grid squares in the UK). The degree of shading in the scale bar and tiles represent the posterior probability of hybridisation from Model 1 given parameter values for each variable. Estimates are visualised at mean genus size, for annual-perennial parental combinations and accounting for phylogenetic relationships between species.

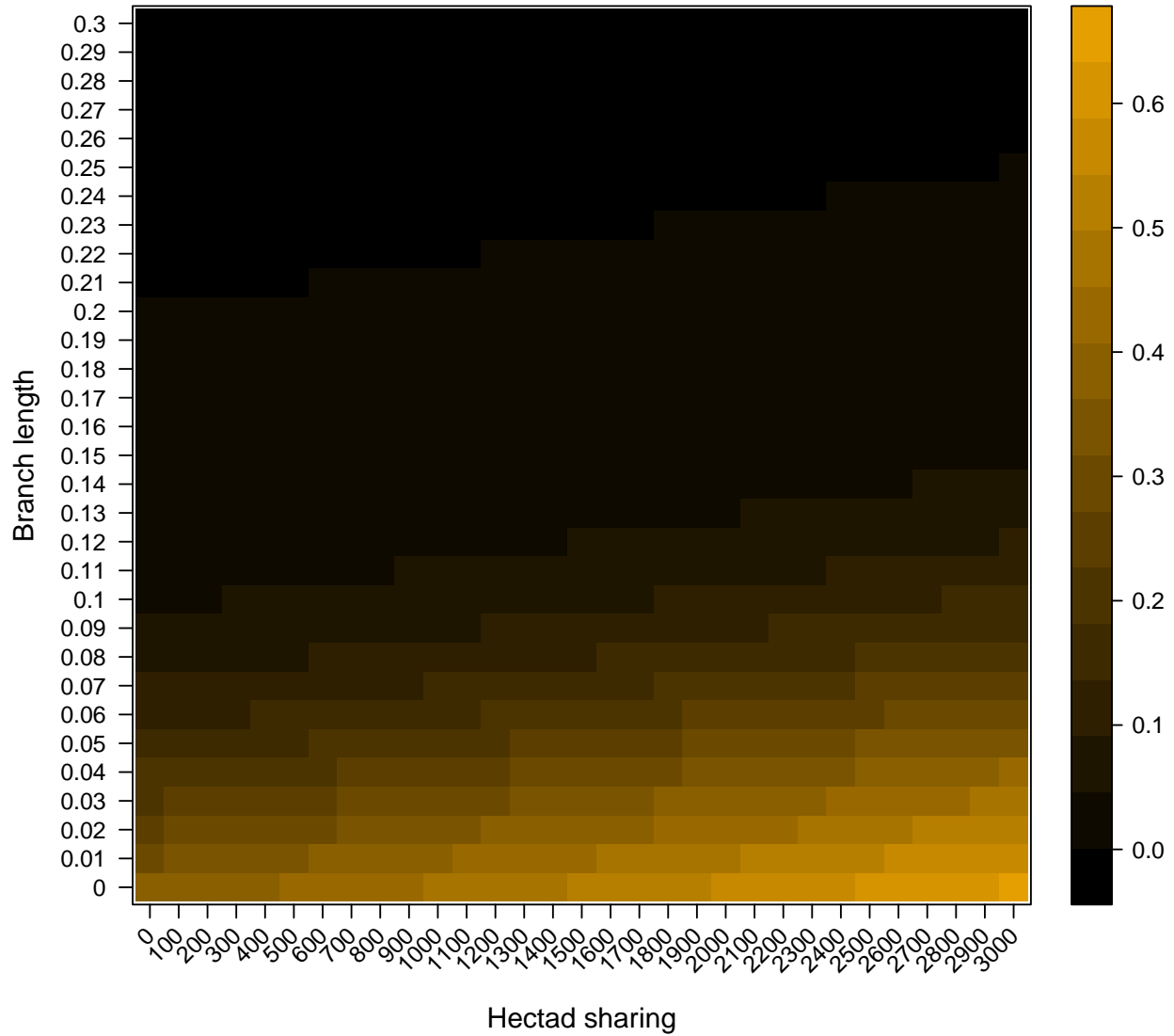


Figure 4: Predicted fit of probability of hybridisation given hectad sharing and ploidy difference of parental species from Model 2. Dashed lines indicate the 95% Credible Intervals, and the bold lines represent the posterior mode of the coefficients of congeneric pairs of species hybridising as a function of pairwise overlap in distribution, conditional on parental ploidy status. The effect is visualised at mean genetic distance for annual-perennial parent combinations and accounting for phylogenetic effects. The bold red dashed line indicates mean pairwise overlap in distribution ( $10 \times 10 \text{ km}^2$ ).

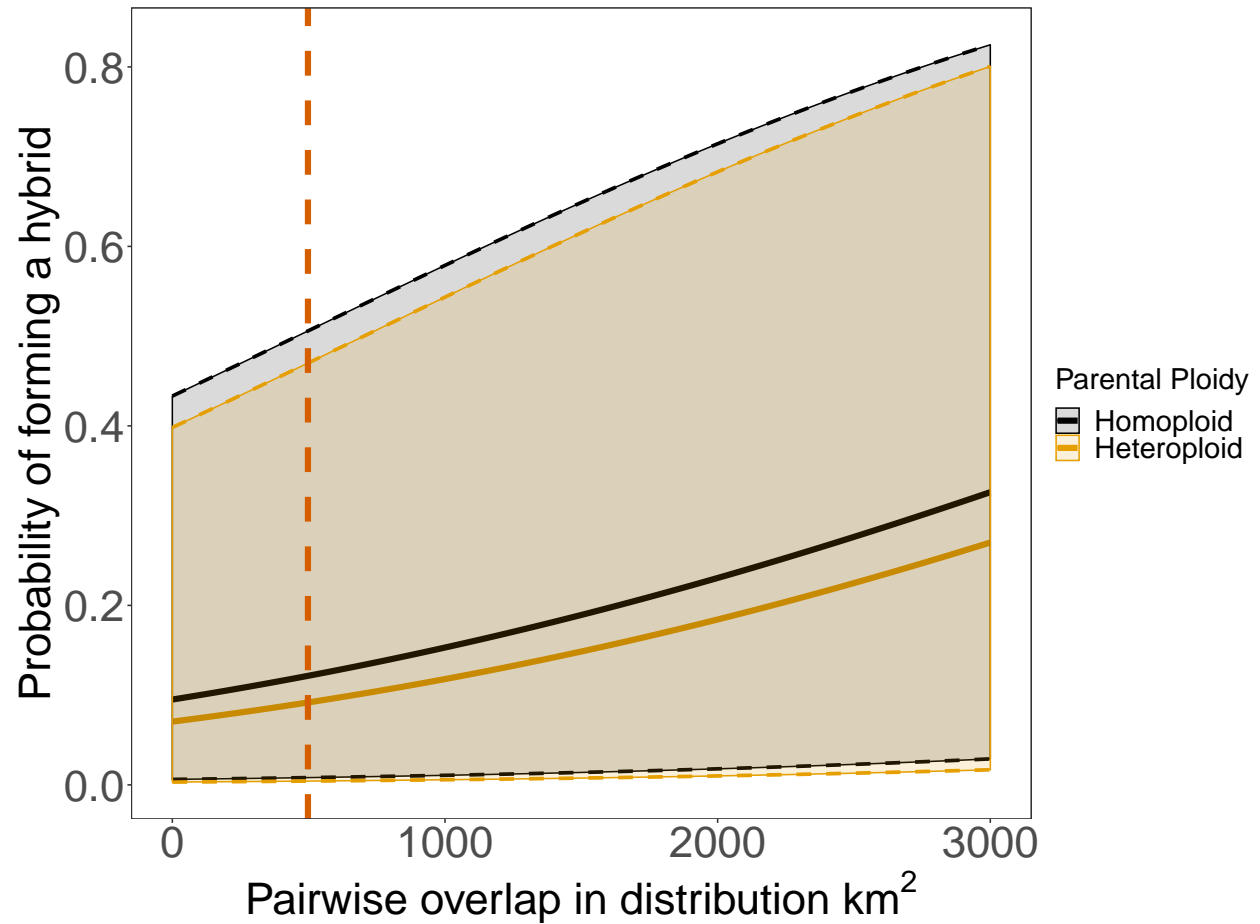


Figure 5: Predicted fit of probability of hybridisation given branch length between parental species and ploidy difference of parental species from Model 2. Homoploid indicates parental species of the same ploidy level, and heteroploidy indicates parental species of different ploidy levels. Dashed lines indicate the 95% Credible Intervals, and the bold lines represent the posterior mode of the coefficients of congeneric pairs of species hybridising as a function of pairwise branch length, conditional on parental ploidy status. The effect is visualised at mean hectad sharing for annual-perennial parent combinations and accounting for phylogenetic effects. The red dashed line indicates mean pairwise branch length between all pairs of species.

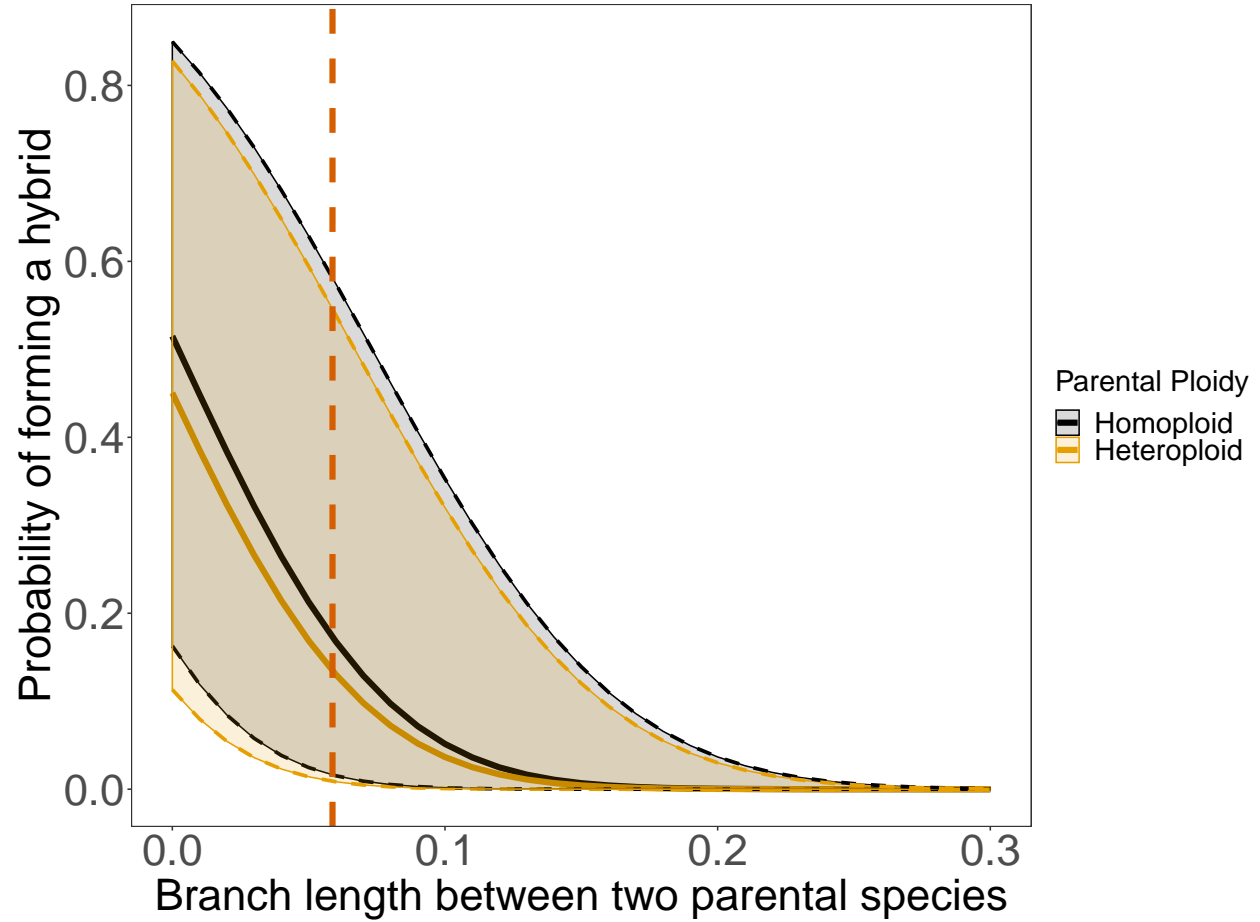


Figure 6: Predicted fit of probability of hybridisation given branch length between parental species from Model 1. Black dashed lines are the 95% Credible Intervals, bold line is the posterior mean of the coefficient for the probability of congeneric pairs of species hybridising as a function of branch length. This effect is visualised at mean hectad sharing, for annual-perennial parent combinations and accounting for phylogenetic effects. The bold red dashed line indicates mean genus level genetic distance between pairs of species.

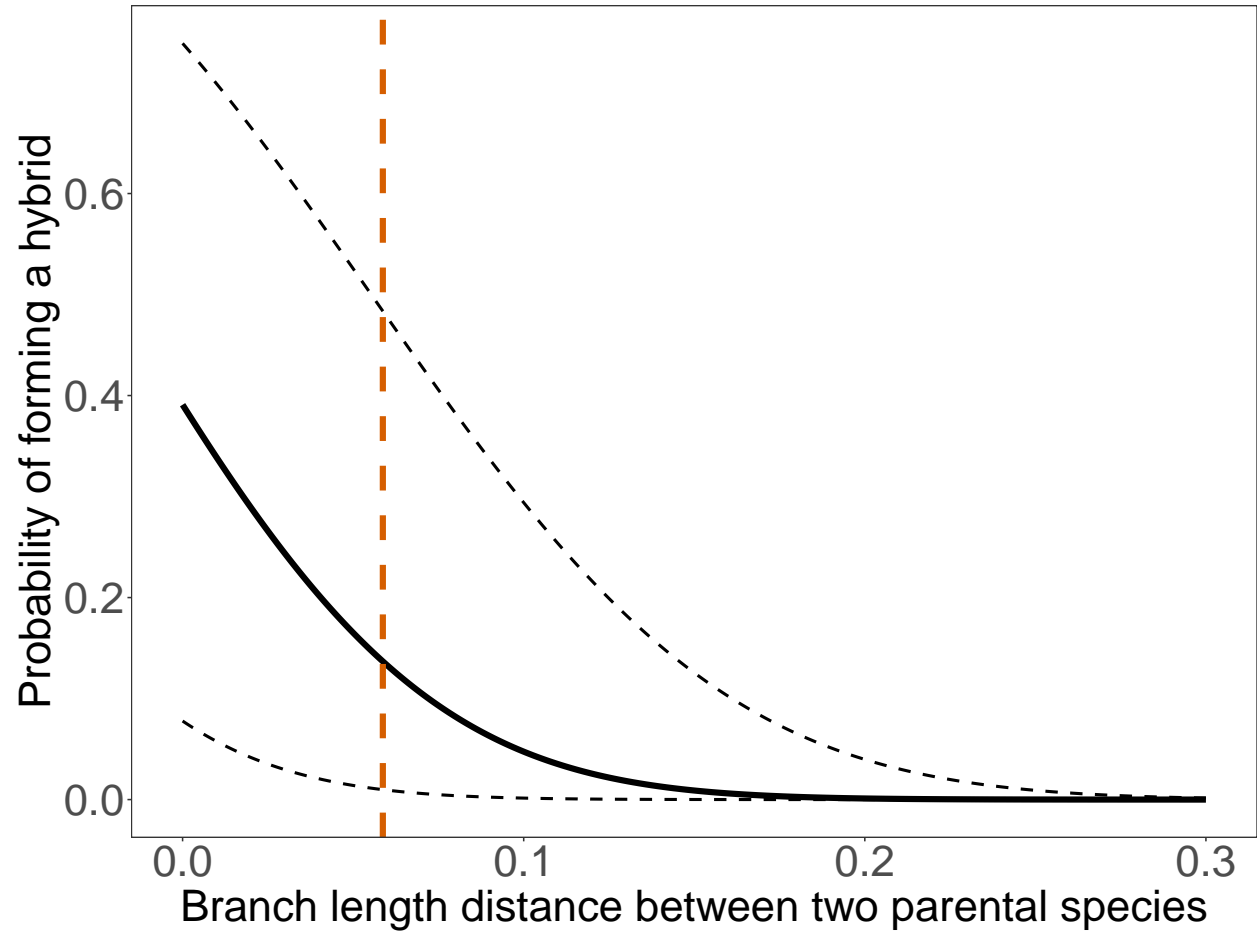


Table 2: Model 1: Probability of hybridisation on the probit scale with genetic distance, hectads shared and life history of parental species as fixed covariates. The posterior mean of the distribution of each coefficient is given, along with lower and upper 95% Credible Intervals. The p-value (pMCMC) is also reported and given in bold where significant. Annual-perennial and perennial-perennial levels are jointly tested using a Wald test in the main text.

Covariate	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	-1.31	-3.76	0.60	1000	0.22
Branch length between species pairs	-59.75	-66.69	-51.98	185.41	0.0010
Hectads shared between species pairs	0.001	0.0007	0.0012	1000	0.0010
Annual-perennial parent pair	-0.12	-0.97	0.66	1000	0.76
Perennial-perennial parent pair	0.64	-0.25	1.58	1000	0.16
Genus size	-0.0014	-0.041	0.031	1107	0.92

Table 3: Phylogenetic signal of probability of hybridisation and the species variance independent of phylogenetic effects on the probit scale. 95% Credible Intervals of the variances are also presented. See Methods in Chapter 3 for calculation.

Variance Component	Posterior Mode	Lower Credible Interval	Upper Credible Interval
Model 1 Phylogenetic Variance	0.62	0.32	0.77
Model 1 Species Variance	0.33	0.18	0.58
Model 2 Phylogenetic Variance	0.61	0.30	0.82
Model 2 Species Variance	0.34	0.084	0.44



Table 4: Model 2: Probability of hybridisation on the probit scale with ploidy, genetic distance, hectads shared and life history of parental species as covariates. The posterior mean of the distribution of each coefficient is given, along with lower and upper 95% Credible Intervals. The p-value (pMCMC) is also reported and given in bold where significant.

Covariate	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	-0.11	-2.41	2.12	813	0.93
Branch length between species pairs	-74.93	-88.38	-63.46	319	0.0010
Crosss ploidy effect	-0.73	-1.02	-0.40	1000	0.0010
Hectads shared between species pairs	0.0013	0.0009	0.0016	883	0.0010
Annual-perennial parent pair	0.093	-1.15	1.23	836	0.89
Perennial-perennial parent pair	0.82	-0.40	1.96	836	0.16
Genus size	-0.029	-0.084	0.031	621	0.32

### Appendix 3: Chapter 4

Figure 7: Distributions of parameters fitted to the models with constant gene flow (blue), secondary contact (green), and without gene flow (grey) in the demographic simulation software,  $\delta a \delta I$ . The model with historic gene flow (orange) had poor AIC values, and so is omitted here.

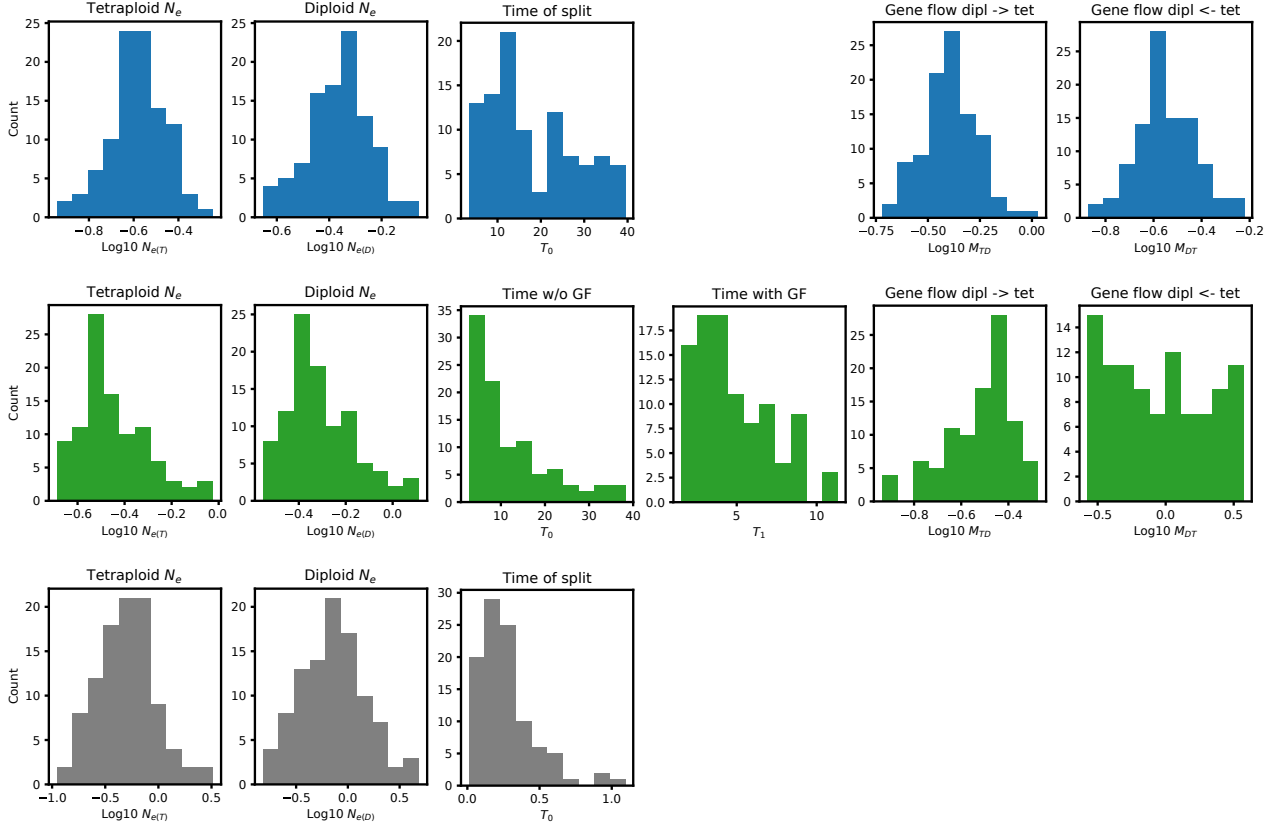


Table 5: Primers and PCR conditions used to amplify the *rpL32-trnL<sub>UAG</sub>* plastid marker in *Euphrasia* species.

Primer	Orientation	Sequence (5'-3')	Reagents (1 reaction)	PCR conditions	References
<i>rpL32</i> -F	Forward	CAGTTCCAAAAAACGTACTTC	12.5μM Taq 2X Master Mix, 0.5μL Bovine Serum Albumen, 0.5μL forward and reverse primers at 10μM, 10.5μL water, 1μL sample DNA	5 min at 94°C, 35× (30 s at 94°C, 45 s at 50°C, 40 s at 72°C), 5 min at 72°C	(Wang et al., 2018)
<i>trnL<sub>UAG</sub></i>	Reverse	CTGCTTCCTAAGAGCAGCGT			

Table 6: Primers and PCR conditions used to amplify the ITS1 nuclear marker in *Euphrasia* species.

Primer	Orientation	Sequence (5'-3')	Reagents (1 reaction)	PCR conditions	References
ITS4	Forward	TCCTCCGCTTATTGATATGC	12.5 $\mu$ M Taq 2X Master Mix, 0.5 $\mu$ L Bovine Serum Albumen, 0.5 $\mu$ L forward and reverse primers at 10 $\mu$ M, 10.5 $\mu$ L water, 1 $\mu$ L sample DNA	5min at 94°C, 30 x (30s at 94°C, 30s at 54°C, 2min at 72°C), 10 min at 72°C.	(Wang et al., 2018)
ITS5	Reverse	GGAAGTAAAAGTCGTAACAAGG			

## Appendix 4: Chapter 6

Table 7: Host species used in the common garden experiment in Chapter 6. The species along with the taxonomic family they belong to, their ecological functional group and the source of the seeds are also given. Commercial seed stocks list the original collection where known.

Common name	Species name	Family	Functional group (informal)	Seed source
Thale cress	<i>Arabidopsis thaliana</i>	Brassicaceae	Herb	Laboratory stock
Field horsetail	<i>Equisetum arvense</i>	Equisetaceae	Fern	Wild collected in Edinburgh (GPS coordinates: 55.9679, -3.2129)
Red fescue	<i>Festuca rubra</i>	Poaceae	Grass	Commerical: Emorsgate seeds (Yorkshire + Dorset)
Yorkshire fog	<i>Holcus lanatus</i>	Poaceae	Grass	Commerical: Emorsgate seeds
Common liverwort	<i>Marchantia polymorpha</i>	Marchantiaceae	Bryophyte	Wild collected in Edinburgh (GPS coordinates: 55.9679, -3.2129)
Ribwort plantain	<i>Plantago lanceolata</i>	Plantaginaceae	Herb	Commerical: Emorsgate seeds (Somerset + Wiltshire)
Scots pine	<i>Pinus sylvestris</i>	Pinaceae	Tree	Commerical: Scotia Seeds
White clover	<i>Trifolium repens</i>	Fabaceae	Herb	Commerical: Emorsgate seeds (Yorkshire + Wiltshire)

Table 8: Collection details for *Euphrasia* species used in the common garden experiment. \*Population also used in the multiple host phenotypic plasticity experiment.

Collection number	Taxon	Locality	Latitude	Longitude	Collector
E4E0138	<i>E. arctica</i>	Fintallick, Glen Ledock, Comrie, Perthshire	56.41318	−4.03085	Dot Hall
E4E0144	<i>E. arctica</i>	Balachuirn, Isle of Raasay	57.38996	−6.06877	S.J. Bungard
E4E0032	<i>E. arctica</i>	South Links, Burray, Orkney	58.85275	−2.88701	John Crossley
E4E0139	<i>E. arctica</i>	Dalreoch Farm, Enochdhu	56.74199	−3.53350	Martin Robinson
E4E0049	<i>E. arctica</i>	Ouaisne, Jersey	49.17707	−2.18293	Anne Haden
E4E0247	<i>E. arctica</i>	Elsdon. Newcastle upon Tyne	55.22770	−2.10234	Stephanie Miles
NBer001*	<i>E. arctica</i>	North Berwick Glenn, East Lothian	56.05696	−2.70456	Alex Twyford
E4E0038	<i>E. confusa</i>	Oldbury, near Hartshill, Warwickshire	52.55285	−1.53980	John and Monika Walton
E4E0114	<i>E. confusa</i>	Trethew Mill, Bodmin, Cornwall	−4.709558 50.39585	Rosemary Parslow	
E4E0095	<i>E. confusa</i>	North Anston Grassland, South Yorkshire	53.34738	−1.20803	Graeme Coles
E4E0009	<i>E. confusa</i>	Devil's Hole Blowout, Ravenmeols Local Nature Reserve, Merseyside	53.54062	−3.09041	Philip H. Smith
E4E0188	<i>E. micrantha</i>	Meall a Bathaich, Glen Garry, East Perthshire	56.82082	−4.182812	Alistair Godfrey
E4E0064	<i>E. nemorosa</i>	Castle Hill Local Nature Reserve, East Sussex	50.7842	0.052719	David Harris

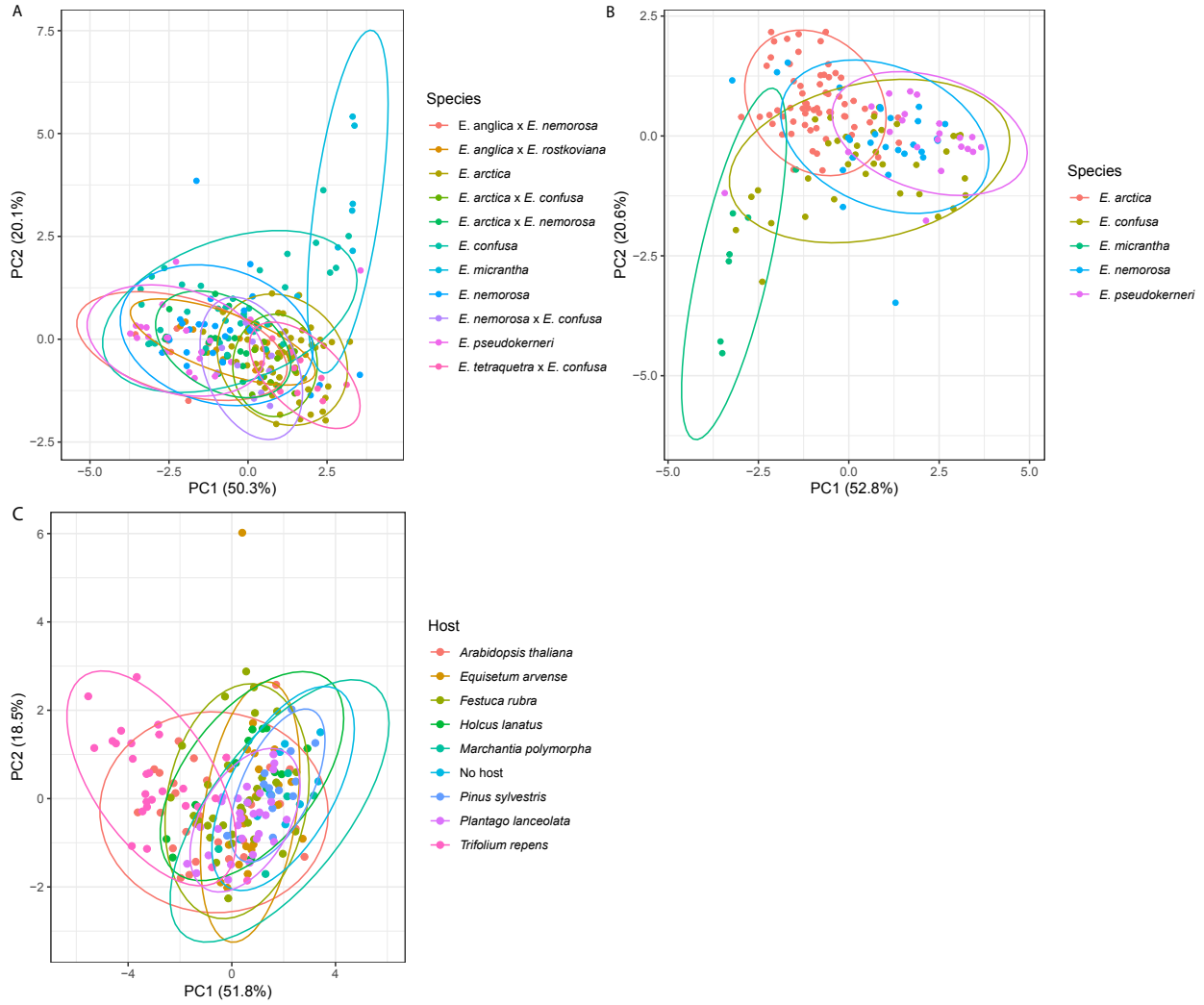
Collection number	Taxon	Locality	Latitude	Longitude	Collector
E4E0069	<i>E. nemorosa</i>	Meridian Business Park, Leicester	52.60857	-1.19809	Geoffrey Hall
E4E0123	<i>E. nemorosa</i>	Bloody Oaks Triangle, Tickercote, Rutland	52.68950	-0.56263	Geoffrey Hall
E4E0029	<i>E. pseudokernerii</i>	Levin Down, Sussex	50.91346	-0.74150	Elizabeth Sturt
E4E0112	<i>E. pseudokernerii</i>	Beeston Common, Norfolk	52.93442	1.220071	Francis Farrow
E4E0027	<i>E. anglica</i> x <i>E. nemorosa</i>	West Dean Woods, Sussex	50.93212	-0.79735	Elizabeth Sturt
E4E0016	<i>E. anglica</i> x <i>E. rostkoviana</i>	Straduff Rathcabbin, Co. Tipperary	53.11902	-8.02454	David Nash
E4E0033	<i>E. arctica</i> x <i>E. confusa</i>	Nr Quoyorally, South Ronaldsay, Orkney	58.75897	-2.93473	John Crossley
E4E0145	<i>E. arctica</i> x <i>E. nemorosa</i>	Kylfakin, Wof, Skye	57.26685	-5.76042	S.J. Bungard
E4E0021	<i>E. arctica</i> x <i>E. nemorosa</i>	Dunamase, Co. Laois	53.03153	-7.21015	David Nash
E4E0031	<i>E. nemorosa</i> x <i>E. confusa</i>	Dolebury Fort, Somerset	51.32605	-2.79432	C.W. Hurfurt
E4E0143	<i>E. tetraquetra</i> x <i>E. confusa</i>	Ballyteige Burrow, Co Wexford, Ireland	52.20268	-6.64325	Jim Hurley

Table 9: Summary of trait values for many *Euphrasia* species and hybrids grown on a clover host (i.e. the species differences experiment). Values are means  $\pm 1$  SE. Length measurements are in millimeters. Note: Date of first flower not recorded.

Taxon	Corolla length	Height	Internode ratio	Julian days to flower	Lower floral leaf teeth	Nodes to flower	Number of branches
<i>E. arctica</i>	8.0 $\pm$ 0.2	82.9 $\pm$ 4.4	1.1 $\pm$ 0.1	195.2 $\pm$ 1.5	4.4 $\pm$ 0.1	8.6 $\pm$ 0.2	*4.56 $\pm$ 0.2
<i>E. confusa</i>	6.9 $\pm$ 0.2	134.4 $\pm$ 7.2	1.6 $\pm$ 0.1	200.2 $\pm$ 2.4	5.3 $\pm$ 0.2	11.1 $\pm$ 0.4	7.26 $\pm$ 0.5
<i>E. micrantha</i>	5.6 $\pm$ 0.2	70.6 $\pm$ 8.1	3.0 $\pm$ 0.4	—	2.4 $\pm$ 0.3	8.3 $\pm$ 0.2	0.57 $\pm$ 0.4
<i>E. nemorosa</i>	7.7 $\pm$ 0.1	127.4 $\pm$ 8.1	1.4 $\pm$ 0.1	206.6 $\pm$ 1.7	5.1 $\pm$ 0.2	11.9 $\pm$ 0.5	7.67 $\pm$ 0.5
<i>E. pseudok- ernerii</i>	8.8 $\pm$ 0.4	176.4 $\pm$ 15.6	1.4 $\pm$ 0.1	205.1 $\pm$ 2.0	5.5 $\pm$ 0.2	13.2 $\pm$ 0.4	8.67 $\pm$ 0.6
<i>E. anglica</i> x <i>E. nemorosa</i>	9.1 $\pm$ 0.5	148.1 $\pm$ 11.8	1.4 $\pm$ 0.1	195.7 $\pm$ 1.9	6.0 $\pm$ 0.3	12.0 $\pm$ 0.6	10.00 $\pm$ 1.0
<i>E. anglica</i> x <i>E. rostkoviana</i>	7.9 $\pm$ 0.2	122.6 $\pm$ 8.3	1.3 $\pm$ 0.1	192.3 $\pm$ 12.3	5.9 $\pm$ 0.3	10.6 $\pm$ 0.5	7.44 $\pm$ 0.7
<i>E. arctica</i> x <i>E. confusa</i>	9.5 $\pm$ 0.2	100.3 $\pm$ 4.3	1.4 $\pm$ 0.1	193.4 $\pm$ 3.2	3.8 $\pm$ 0.1	7.8 $\pm$ 0.3	5.70 $\pm$ 0.4
<i>E. arctica</i> x <i>E. nemorosa</i>	8.0 $\pm$ 0.2	132.2 $\pm$ 14.5	1.3 $\pm$ 0.1	205.3 $\pm$ 2.4	6.0 $\pm$ 0.3	11.3 $\pm$ 0.4	6.50 $\pm$ 0.4
<i>E. arctica</i> x <i>E. nemorosa</i>	7.9 $\pm$ 0.2	92.5 $\pm$ 5.9	1.0 $\pm$ 0.1	199.3 $\pm$ 2.8	5.1 $\pm$ 0.2	9.8 $\pm$ 0.3	7.00 $\pm$ 0.5
<i>E. confusa</i> x <i>E. tetraquetra</i>	7.2 $\pm$ 0.2	57.4 $\pm$ 5.8	0.7 $\pm$ 0.1	194.1 $\pm$ 2.7	4.2 $\pm$ 0.2	7.6 $\pm$ 0.4	4.00 $\pm$ 0.3



Figure 8: Principal component analysis of morphological variation of *Euphrasia* in a common garden. Panels show (A) five species and six hybrids grown with a single clover host, (B) five species grown with a clover host omitting hybrids, and (C) *E. arctica* with nine host treatments. Points represent individuals, and ellipses represent the standard error of the (weighted) average of scores.



Table(s) 10: The first five principal components extracted from the principal component analysis, with the contribution of variance of each trait to each principal component. The last two rows of each table show the standard deviation and the proportion of variance explained by the principal component.

Species differences (including hybrids)	PC1	PC2	PC3	PC4	PC5
Branches	0.229	0.053	0.071	0.252	0.094
Corolla length	0.089	0.262	0.369	0.032	0.136
Height	0.211	0.115	0.149	0.047	0.379
Internode ratio	0.005	0.441	0.186	0.030	0.190
Leaf teeth	0.213	0.056	0.097	0.428	0.128
Nodes to flower	0.224	0.093	0.126	0.181	0.081
Standard deviation	1.738	1.099	0.964	0.616	0.533
Proportion of variance	0.503	0.201	0.155	0.063	0.047

Species differences (excluding hybrids)	PC1	PC2	PC3	PC4	PC5
Branches	0.226	0.024	0.096	0.233	0.017
Corolla length	0.100	0.269	0.361	0.082	0.141
Height	0.214	0.128	0.151	0.063	0.367
Internode ratio	0.029	0.434	0.202	0.000	0.171
Leaf teeth	0.214	0.032	0.064	0.424	0.159
Nodes to flower	0.217	0.113	0.125	0.198	0.145
Standard deviation	1.780	1.111	0.932	0.612	0.433
Proportion of variance	0.528	0.206	0.145	0.062	0.031

Phenotypic plasticity	PC1	PC2	PC3	PC4	PC5
Branches	0.183	0.065	0.032	0.098	0.220
Corolla length	0.139	0.001	0.252	0.340	0.030
Height	0.179	0.150	0.016	0.065	0.128
Internode ratio	0.070	0.301	0.274	0.119	0.146
Julian days to flower	0.158	0.198	0.056	0.077	0.191
Leaf teeth	0.178	0.024	0.090	0.153	0.166
Nodes to flower	0.093	0.262	0.280	0.147	0.119
Standard deviation	1.904	1.137	0.924	0.725	0.586
Proportion of variance	0.518	0.185	0.122	0.075	0.049

Figure 9: Pairwise differences in trait value of *Euphrasia* species grown with clover in a common garden experiment. Tukey comparisons are presented between each pair of species, with significant comparisons shown in bold. Point estimates are the mean difference of the comparison, and error bars are  $\pm$  one standard error, calculated from the species differences model using the emmeans R package. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ .

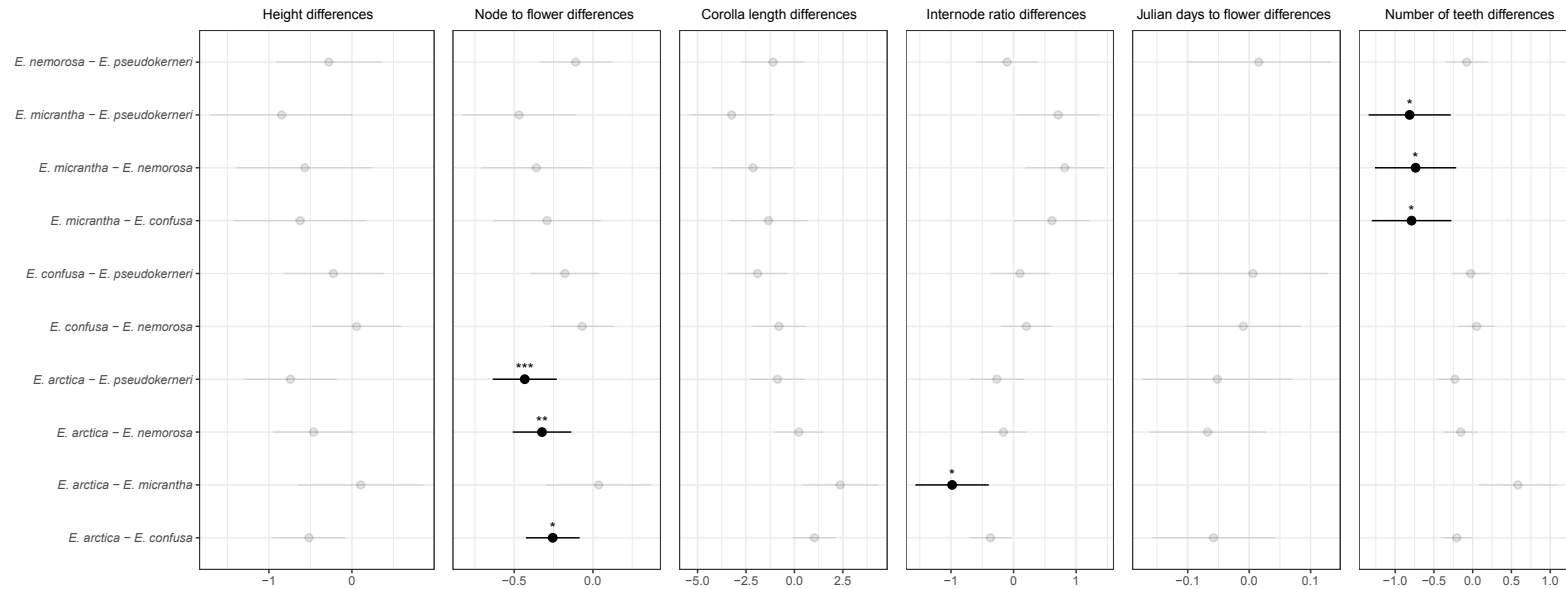


Table 11: Summary of trait values for *Euphrasia arctica* grown on many different hosts. Values are mean  $\pm$  one standard error. Length and height measurements are in millimeters.

Early season	At first flowering							End of season	
Host	Height	Corolla length	Height	Internode ratio	Julian days to flower	Lower floral leaf teeth	Nodes to flower	Number of branches	Height
<i>A. thaliana</i>	12.8 $\pm$ 1.1	6.1 $\pm$ 0.3	19.2 $\pm$ 1.6	2.4 $\pm$ 0.1	201.6 $\pm$ 4.3	3.2 $\pm$ 0.1	8.8 $\pm$ 0.3	2.1 $\pm$ 0.4	30.0 $\pm$ 3.2
<i>E. arvense</i>	6.1 $\pm$ 0.4	5.9 $\pm$ 0.3	15.1 $\pm$ 1.1	2.6 $\pm$ 0.2	215.3 $\pm$ 4.6	2.4 $\pm$ 0.1	9.3 $\pm$ 0.3	0.4 $\pm$ 0.1	35.6 $\pm$ 4.8
<i>F. rubra</i>	6.7 $\pm$ 0.4	6.3 $\pm$ 0.1	19.5 $\pm$ 1.4	2.6 $\pm$ 0.2	216.5 $\pm$ 4.4	2.8 $\pm$ 0.2	9.6 $\pm$ 0.3	0.8 $\pm$ 0.3	39.6 $\pm$ 4.1
<i>H. lanatus</i>	7.1 $\pm$ 1.3	6.3 $\pm$ 0.1	16.0 $\pm$ 1.6	2.4 $\pm$ 0.2	224.5 $\pm$ 7.0	2.5 $\pm$ 0.2	9.8 $\pm$ 0.4	0.8 $\pm$ 0.4	33.8 $\pm$ 6.8
<i>M. polymorpha</i>	6.3 $\pm$ 0.7	5.5 $\pm$ 0.4	9.6 $\pm$ 1.3	2.9 $\pm$ 0.4	222.6 $\pm$ 17.0	1.7 $\pm$ 0.3	9.7 $\pm$ 0.5	0	11.3 $\pm$ 2.5
No host	5.9 $\pm$ 0.3	5.3 $\pm$ 0.2	11.2 $\pm$ 1.1	2.8 $\pm$ 0.2	241.3 $\pm$ 7.9	1.9 $\pm$ 0.3	9.9 $\pm$ 0.5	0	9.7 $\pm$ 2.0
<i>P. lanceolata</i>	7.5 $\pm$ 0.5	6.1 $\pm$ 0.1	14.1 $\pm$ 0.8	2.8 $\pm$ 0.1	211.2 $\pm$ 3.7	2.9 $\pm$ 0.1	10.4 $\pm$ 0.3	0.4 $\pm$ 0.1	28.3 $\pm$ 3.4
<i>P. sylvestris</i>	6.2 $\pm$ 0.6	5.7 $\pm$ 0.3	12.2 $\pm$ 1.3	2.9 $\pm$ 0.2	233.8 $\pm$ 6.1	1.9 $\pm$ 0.2	9.2 $\pm$ 0.3	0	17.2 $\pm$ 2.6
<i>T. repens</i>	12.9 $\pm$ 1.4	7.4 $\pm$ 0.2	39.4 $\pm$ 2.6	2.1 $\pm$ 0.2	189.8 $\pm$ 2.0	3.9 $\pm$ 0.1	8.7 $\pm$ 0.3	4.7 $\pm$ 0.4	143.2 $\pm$ 8.6

Table 12: Comparison of *E. arctica* traits in the phenotypic plasticity common garden experiment. Tukey comparisons are presented between *E. arctica* traits with two different host treatments. Point estimates are the mean difference of the comparison, calculated from the phenotypic plasticity model using the emmeans R package. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ .

Host 1	Host 2	Corolla length	Height	Internode ratio	Julian days to flower	Nodes to flower	Number of leaf teeth
<i>Arabidopsis thaliana</i>	No host	1.065*	0.49*	0.102	-0.178***	-0.116	0.508
<i>Equisetum arvense</i>	No host	0.946*	0.304	0.041	-0.112***	-0.066	0.212
<i>Festuca rubra</i>	No host	1.04*	0.529**	0.073	-0.112***	-0.034	0.397
<i>Holcus lanatus</i>	No host	1.05*	0.332	0.077	-0.063	-0.009	0.258
<i>Marchantia polymorpha</i>	No host	0.25	-0.181	0.07	-0.031	-0.025	-0.136
<i>Pinus sylvestris</i>	No host	0.481	0.067	0.015	-0.03	0.051	0.01
<i>Plantago lanceolata</i>	No host	0.879	0.246	0.016	-0.137***	-0.071	0.419
<i>Trifolium repens</i>	No host	2.102***	1.241***	0.180*	-0.244***	-0.134	0.711
<i>Equisetum arvense</i>	<i>Arabidopsis thaliana</i>	-0.119	-0.186	-0.061	0.066*	0.05	-0.296
<i>Festuca rubra</i>	<i>Arabidopsis thaliana</i>	-0.024	0.039	-0.029	0.065**	0.082	-0.111
<i>Holcus lanatus</i>	<i>Arabidopsis thaliana</i>	-0.015	-0.158	-0.025	0.114***	0.107	-0.25
<i>Marchantia polymorpha</i>	<i>Arabidopsis thaliana</i>	-0.815	-0.671***	-0.032	0.147***	0.091	-0.644
<i>Pinus sylvestris</i>	<i>Arabidopsis thaliana</i>	-0.584	-0.423*	-0.087	0.148***	0.167	-0.498
<i>Plantago lanceolata</i>	<i>Arabidopsis thaliana</i>	-0.186	-0.244	-0.086	0.041	0.044	-0.089
<i>Trifolium repens</i>	<i>Arabidopsis thaliana</i>	1.037***	0.751***	0.077	-0.066	-0.018	0.204
<i>Festuca rubra</i>	<i>Equisetum arvense</i>	0.095	0.225	0.031	0	0.032	0.185
<i>Holcus lanatus</i>	<i>Equisetum arvense</i>	0.104	0.028	0.035	0.049	0.057	0.046
<i>Marchantia polymorpha</i>	<i>Equisetum arvense</i>	-0.696	-0.486*	0.029	0.081	0.041	-0.348
<i>Pinus sylvestris</i>	<i>Equisetum arvense</i>	-0.465	-0.237	-0.025	0.082**	0.117	-0.202
<i>Plantago lanceolata</i>	<i>Equisetum arvense</i>	-0.067	-0.059	-0.024	-0.025	-0.006	0.207
<i>Trifolium repens</i>	<i>Equisetum arvense</i>	1.156***	0.937***	0.138*	-0.132***	-0.068	0.499*
<i>Holcus lanatus</i>	<i>Festuca rubra</i>	0.01	-0.197	0.003	0.049	0.025	-0.139
<i>Marchantia polymorpha</i>	<i>Festuca rubra</i>	-0.79	-0.71***	-0.002	0.081	0.009	-0.533
<i>Pinus sylvestris</i>	<i>Festuca rubra</i>	-0.56	-0.462**	-0.057	0.083**	0.085	-0.387
<i>Plantago lanceolata</i>	<i>Festuca rubra</i>	-0.161	-0.283	-0.056	-0.025	-0.038	0.022
<i>Trifolium repens</i>	<i>Festuca rubra</i>	1.062***	0.712***	0.106	-0.132***	-0.1	0.315
<i>Marchantia polymorpha</i>	<i>Holcus lanatus</i>	-0.8	-0.513*	-0.006	0.033	-0.016	-0.394

Host 1	Host 2	Corolla length	Height	Internode ratio	Julian days to flower	Nodes to flower	Number of leaf teeth
<i>Pinus sylvestris</i>	<i>Holcus lanatus</i>	-0.569	-0.265	-0.061	0.034	0.06	-0.248
<i>Plantago lanceolata</i>	<i>Holcus lanatus</i>	-0.171	-0.086	-0.06	-0.074**	-0.063	0.161
<i>Trifolium repens</i>	<i>Holcus lanatus</i>	1.052**	0.909***	0.102	-0.18***	-0.125	0.454
<i>Pinus sylvestris</i>	<i>Marchantia</i> <i>polymorpha</i>	0.231	0.248	-0.055	0.001	0.076	0.146
<i>Plantago lanceolata</i>	<i>Marchantia</i> <i>polymorpha</i>	0.629	0.427	-0.054	-0.106***	-0.047	0.555
<i>Trifolium repens</i>	<i>Marchantia</i> <i>polymorpha</i>	1.852***	1.423***	0.109	-0.213***	-0.109	0.847*
<i>Plantago lanceolata</i>	<i>Pinus sylvestris</i>	0.398	0.178	0.001	-0.107***	-0.123	0.409
<i>Trifolium repens</i>	<i>Pinus sylvestris</i>	1.621***	1.174***	0.164*	-0.214***	-0.185	0.701*
<i>Trifolium repens</i>	<i>Plantago lanceolata</i>	1.223***	0.996***	0.163*	-0.107***	-0.063	0.292

Table 13: Analysis of deviance for each trait in the phenotypic plasticity experiment with *E. arctica* grown with many different hosts, assuming a Poisson distribution. For each model, we report the change in degrees of freedom (df), deviance, residual degrees of freedom, residual deviance, and p-value generated from the  $\chi^2$  distribution. Factor host, where the model includes all host species, is compared to the intercept model where no hosts are fitted.

Trait	Factor	df	Deviance	Resid. df	Resid. Dev	Pr(> $\chi^2$ )
Julian days to flower	Host	8	192.390	184	419.1153	2.56E-37
	(Intercept)		192	611.5053		
Nodes to flower	Host	8	5.020	185	38.47252	0.755416
	(Intercept)		193	43.49272		
Number of leaf teeth	Host	8	26.793	185	41.37748	0.000767
	(Intercept)		193	68.17096		

Table 14: ANOVAs for traits measured in the phenotypic plasticity experiment with *E. arctica* grown with many different hosts, assuming Gaussian distributed residuals. For each model, we report the degrees of freedom (df), sums of squares (SS), mean squares (MS), F-statistic, and p-value.

Trait	Factor	df	SS	MS	F	p
Corolla length	Host	8	49.469	6.184	9.854565	3.00E-11
	Residuals	173	108.555	0.6275		
Height	Host	8	27.021	3.378	23.139	2.52E-24
	Residuals	185	27.009	0.146		
Internode ratio	Host	8	0.562	0.070	3.362213	0.001275
	Residuals	184	3.845	0.0209		

Table 15: Summary of generalised linear models for the phenotypic plasticity experiment with *Euphrasia arctica* grown on many hosts in a common garden. All models compare *E. arctica* grown with a particular host to the intercept of no host. Generalised linear models assuming Poisson residuals with log link function were used in Julian days to flower, nodes to flower and number of leaf teeth, while all others assumed Gaussian residuals. The model coefficient is reported with standard error in brackets. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ .

Term	Corolla length	Height (log)	Internode ratio	Julian days to flower	Nodes to flower	Number of leaf teeth
(Intercept)	5.250 (0.250)***	2.363 (0.115)***	0.353 (0.043)***	5.489 (0.02)***	2.293 (0.095)***	0.646 (0.218)**
<i>Arabidopsis thaliana</i>	1.064 (0.293)***	0.489 (0.135)***	0.102 (0.051)*	-0.177 (0.024)***	-0.115 (0.114)	0.507 (0.241)*
<i>Equisetum arvense</i>	0.945 (0.300)**	0.304 (0.138)*	0.041 (0.052)	-0.111 (0.024)***	-0.065 (0.116)	0.212 (0.254)
<i>Festuca rubra</i>	1.040 (0.288)***	0.529 (0.134)***	0.073 (0.050)	-0.112 (0.023)***	-0.033 (0.111)	0.396 (0.242)
<i>Holcus lanatus</i>	1.050 (0.323)**	0.331 (0.147)*	0.077 (0.055)	-0.063 (0.025)*	-0.008 (0.123)	0.257 (0.267)
<i>Marchantia polymorpha</i>	0.250 (0.433)	-0.181 (0.171)	0.070 (0.064)	-0.03 (0.029)	-0.024 (0.143)	-0.135 (0.338)
<i>Pinus sylvestris</i>	0.480 (0.333)	0.067 (0.153)	0.015 (0.058)	-0.029 (0.026)	0.051 (0.126)	0.010 (0.290)
<i>Plantago lanceolata</i>	0.879 (0.288)**	0.245 (0.134)	0.016 (0.05)	-0.136 (0.023)***	-0.071 (0.112)	0.419 (0.242)
<i>Trifolium repens</i>	2.101 (0.293)***	1.241 (0.136)***	0.180 (0.051)***	-0.243 (0.024)***	-0.133 (0.115)	0.711 (0.239)**



Figure 10: Relationship between growth-related traits and end of season height for *E. arctica* grown with eight hosts and no host. (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.

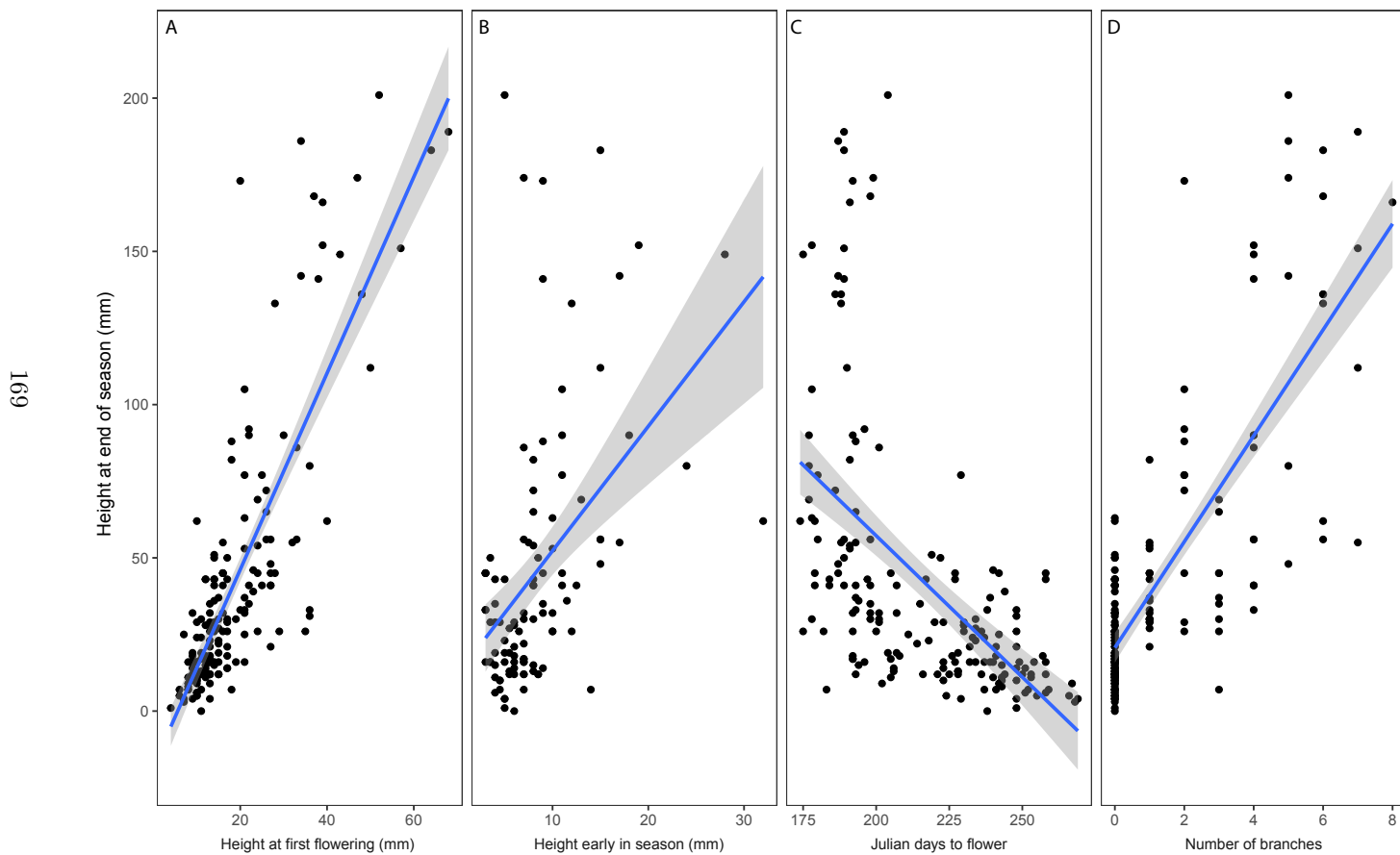


Figure 11: Comparison between trait values for wild-collected herbarium specimens and common garden plants of diverse *Euphrasia* species for (A) nodes to flower, (B) corolla length (mm), (C) number of leaf teeth, (D) internode ratio. Points are for *Euphrasia* population means, with bars representing the standard error of measurements.

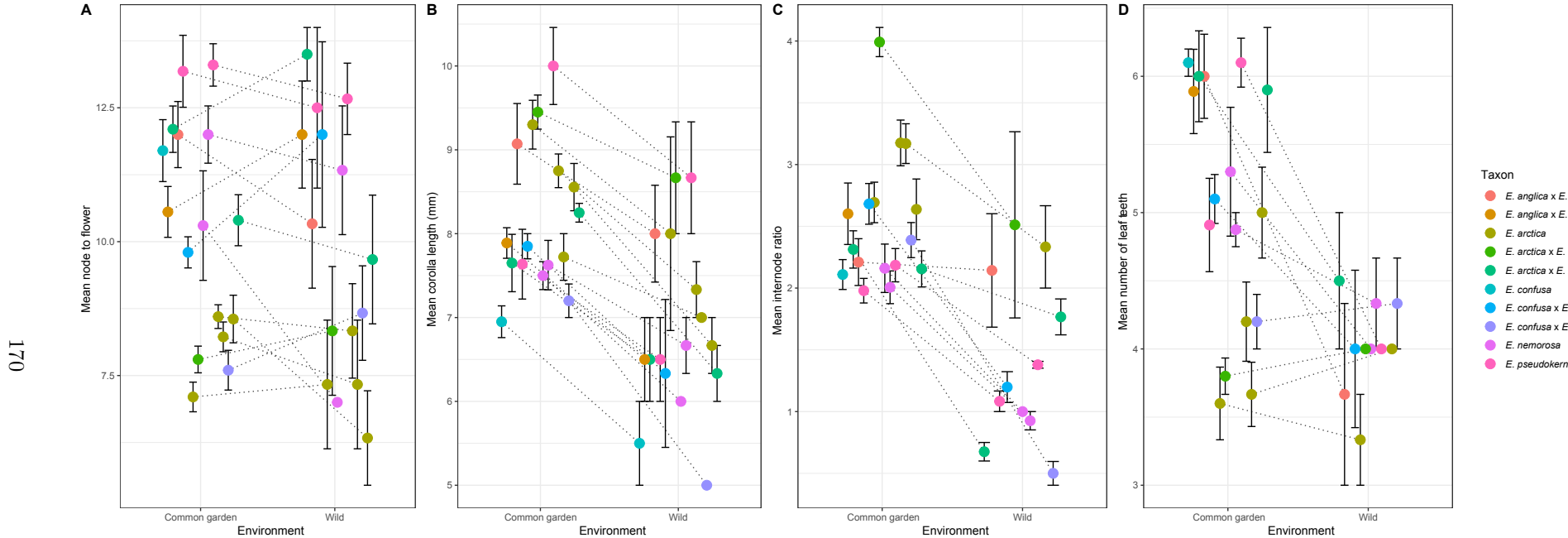


Table 16: Model output from MCMCglmm comparing traits for the wild collected *Euphrasia* specimens to the baseline of the common garden data (Intercept). The posterior means are reported along with the lower and upper 95% credible intervals, as well as the p-value (pMCMC) for the effect.

Trait	Factor	Posterior mean	Lower credible interval	Upper credible interval	pMCMC
Branches	(Intercept)	1.863	1.682	2.086	0.001
	Wild collected	-0.457	-0.619	-0.290	0.001
Internode ratio	(Intercept)	2.533	2.118	2.920	0.001
	Wild collected	-1.008	-1.206	-0.823	0.001
Corolla	(Intercept)	8.182	7.477	8.756	0.001
	Wild collected	-1.363	-1.650	-1.032	0.001
Nodes	(Intercept)	2.322	2.189	2.465	0.001
	Wild collected	-0.016	-0.135	0.086	0.800
Teeth	(Intercept)	1.616	1.485	1.722	0.001
	Wild collected	-0.187	-0.369	-0.004	0.050

## Appendix 5: Chapter 7

Figure 12: *Euphrasia* reproductive output over time showing differences in reproductive trajectories, data from Experiment 1. Values represent mean reproductive nodes at a particular time point  $\pm$  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.

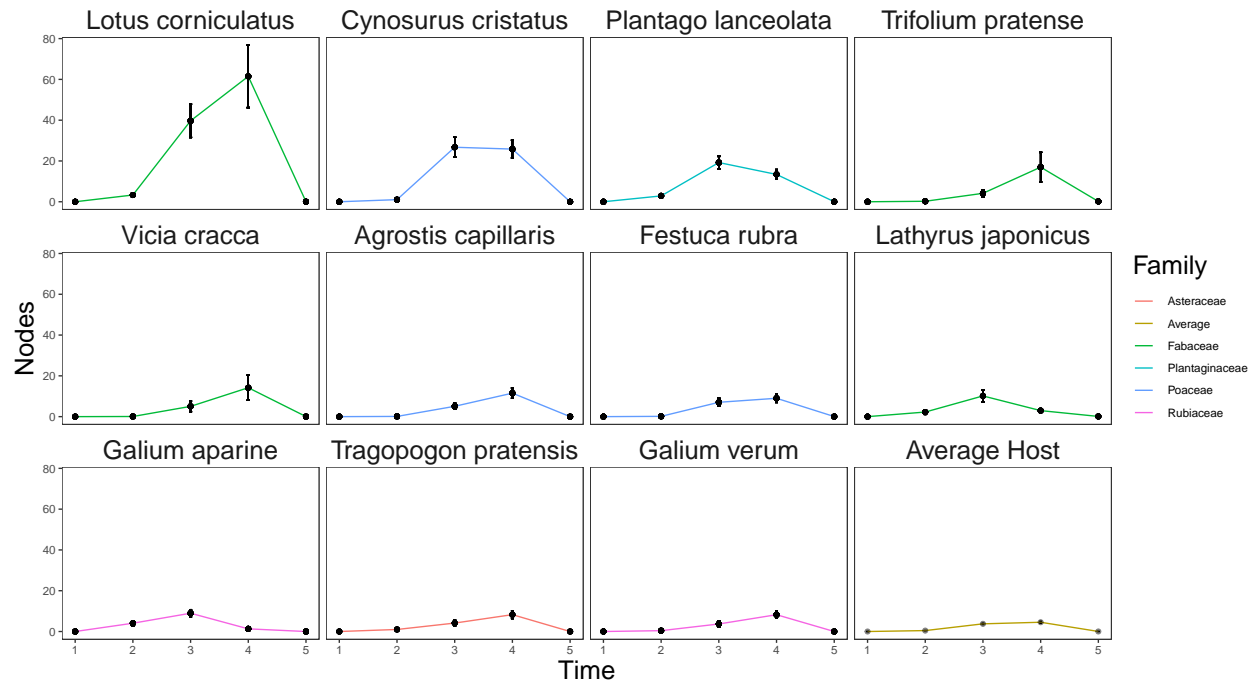


Figure 13: Posterior distributions of the phylogenetic signal for the models from Experiment 1, where 45 different host species were grown with *Euphrasia arctica*. The distributions of phylogenetic signal are shown for three *Euphrasia* traits: survival, total reproductive output at the end of the season, and days to flower. Total reproductive output shows both the highest and least variable estimate of phylogenetic signal, however all are significant as the distributions are not overlapping zero.

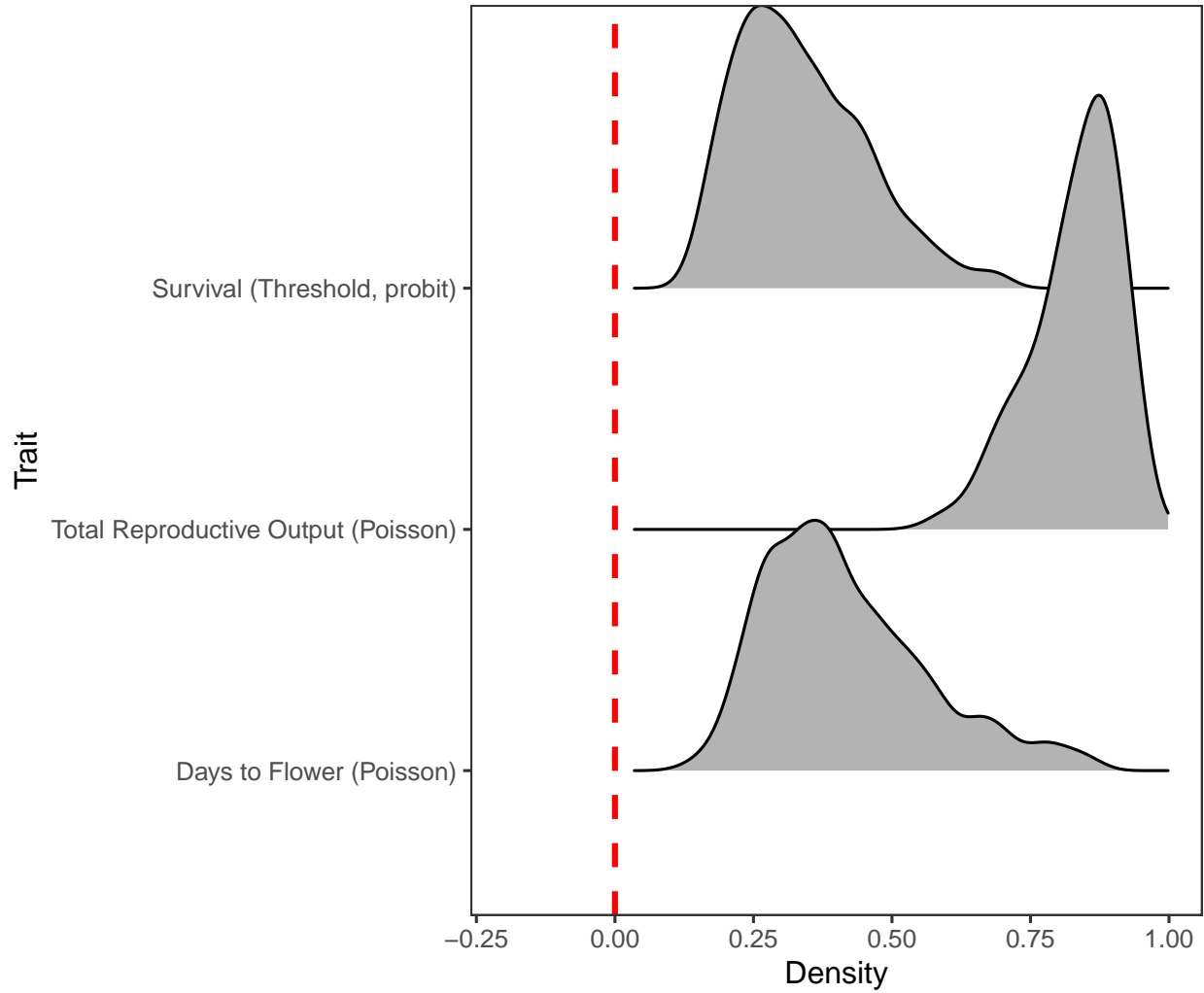


Figure 14. Posterior distribution of the variance for random effects in the model fitted for Experiment 2, where four species of *Euphrasia* were grown on thirteen different species of host. The random effects are the *Euphrasia*-host interaction, the sole effect of host species, and the residual variance. Although the residual variance is the explaining most variation, both the host-parasite interaction and hosts themselves are estimated to be significantly way from zero.

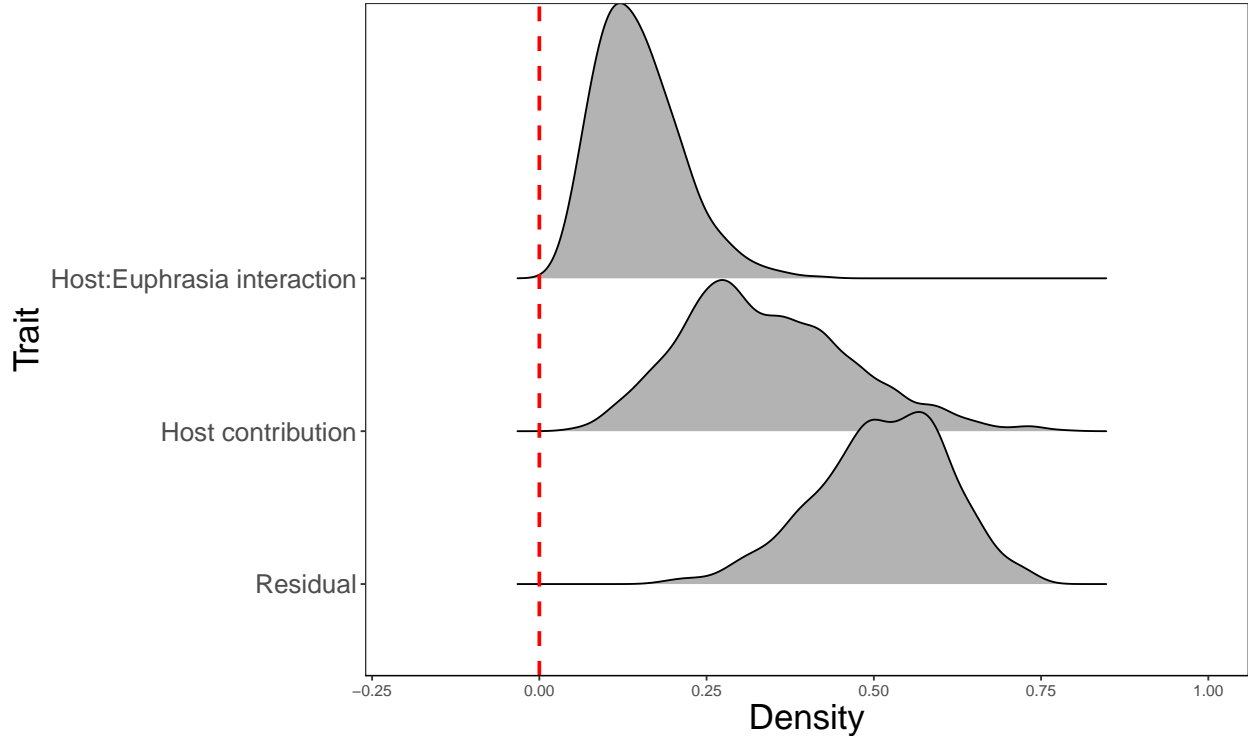


Figure 15. Performance of four species of *Euphrasia* on thirteen different species of host plants measured as cumulative reproductive nodes. Each panel represents a unique *Euphrasia* population (a = A1766, b = T1761, c = V1761, d = M1767, e = M1768, f = M1769), coloured by species. Two populations, (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  $\pm$  one standard error.

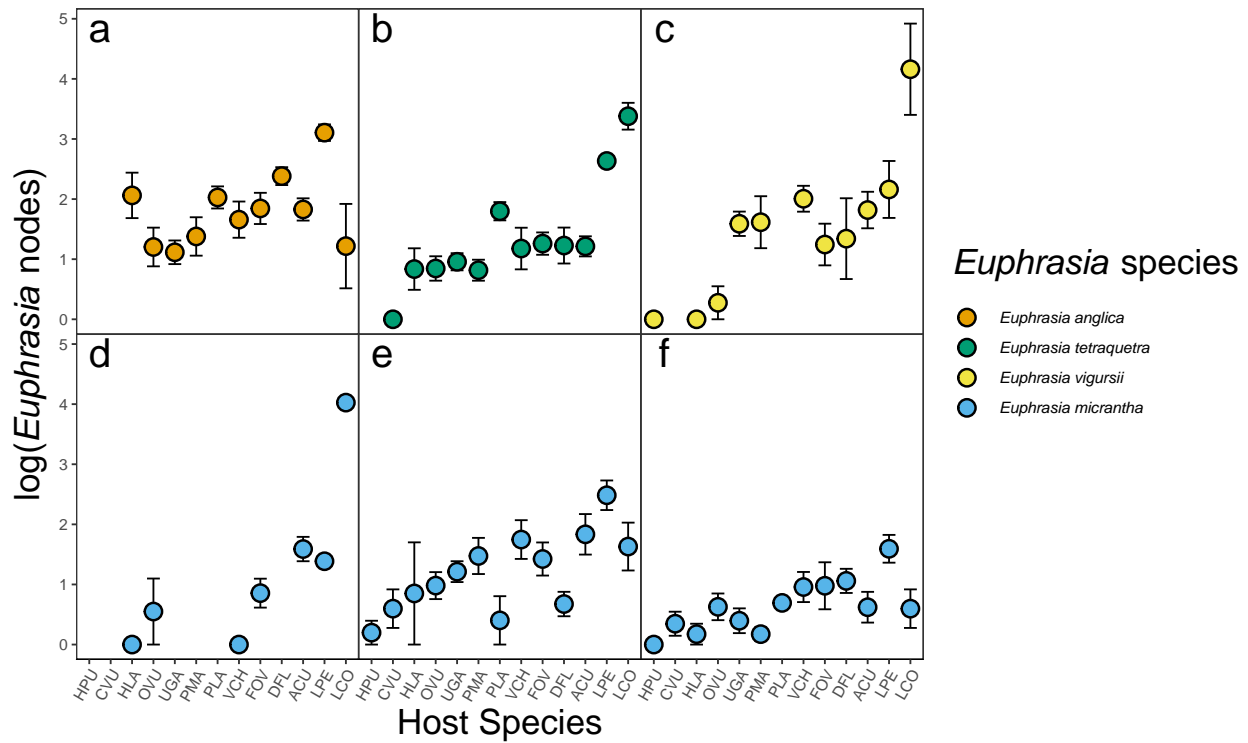


Table 17: Plant names, attributes and collection sources for host species used in Experiment 1.

Host species	Authority	Functional group	Life History	Seed source
No host	-	-	-	-
<i>Agrostis capillaris</i>	L.	Grass	Perennial	Emorsgate
<i>Allium ursinum</i>	L.	Forb	Perennial	RBGE
<i>Anthriscus sylvestris</i>	(L.) Hoffm.	Forb	Perennial	Emorsgate
<i>Arabidopsis thaliana</i>	(L.) Heynh.	Forb	Annual	Inbred lines University of Edinburgh
<i>Centaurea nigra</i>	L.	Forb	Perennial	Emorsgate
<i>Centranthus ruber</i>	(L.) DC.	Forb	Perennial	Chiltern Seeds
<i>Chenopodium album</i>	L.	Forb	Annual	Author collections
<i>Chenopodium bonus-henricus</i>	L.	Forb	Perennial	Surplus seed RBGE
<i>Cynosurus cristatus</i>	L.	Grass	Perennial	Emorsgate
<i>Cystopteris dickeniana</i>	R. Sim	Fern	Perennial	RBGE
<i>Dactylorhiza purpurella</i>	(T.Stephenson & T.A.Stephenson) Soó	Forb	Perennial	RBGE
<i>Equisetum arvense</i>	L.	Fern	Perennial	RBGE
<i>Erica tetralix</i>	L.	Woody	Perennial	RBGE
<i>Festuca rubra</i>	L.	Grass	Perennial	Emorsgate
<i>Fragaria vesca</i>	L.	Forb	Perennial	Scotia seeds
<i>Galanthus nivalis</i>	L.	Forb	Perennial	RBGE
<i>Galium aparine</i>	L.	Forb	Annual	Author collection, Upper Halliford, Surrey, Engalnd, 11/16
<i>Galium verum</i>	L.	Forb	Perennial	Emorsgate
<i>Helianthemum nummularium</i>	(L.) Mill.	Forb	Perennial	Scotia seeds
<i>Holcus lanatus</i>	L.	Grass	Perennial	Emorsgate
<i>Hordeum vulgare</i>	L.	Grass	Annual	Wiggly Wiggles
<i>Hyacinthoides non-scripta</i>	(L.) Chouard ex Rothm.	Forb	Perennial	RBGE
<i>Lagurus ovatus</i>	L.	Grass	Annual	www.wildflowershop.co.uk
<i>Lathyrus japonicus</i>	Willd.	Legume	Perennial	RBGE
<i>Leucanthemum vulgare</i>	(Vaill.) Lam.	Forb	Perennial	Emorsgate
<i>Lotus corniculatus</i>	L.	Legume	Perennial	Emorsgate
<i>Meum athamanticum</i>	Jacq.	Forb		RBGE
<i>Mimulus guttatus</i>	DC.	Forb	Perennial	Author collections



Host species	Authority	Functional group	Life History	Seed source
<i>Ononis spinosa</i>	L.	Legume	Perennial	Emorsgate & Wild Flower Shop
<i>Papaver rhoeas</i>	L.	Forb	Annual	Emorsgate
<i>Phleum pratense</i>	L.	Grass	Perennial	Wild Flower Shop
<i>Pinus sylvestris</i>	L.	Woody	Perennial	Scotia seeds
<i>Plantago lanceolata</i>	L.	Forb	Perennial	Emorsgate
<i>Pteridium aquilinum</i>	L. (Kuhn)	Fern	Perennial	British Pteridological Society spore exchange
<i>Rumex acetosella</i>	L.	Forb	Perennial	Scotia seeds
<i>Senecio vulgaris</i>	L.	Forb	Annual	RBGE
<i>Silene dioica</i>	(L.) Clairv.	Forb	Perennial	D. Charlseworth, Univ. Edinburgh
<i>Silene latifolia</i>	Poir.	Forb	Perennial	D. Charlseworth, Univ. Edinburgh
<i>Thymus polytrichus</i>	A.Kern. ex Borbás	Woody	Perennial	Emorsgate
<i>Sorbus aucuparia</i>	L.	Woody	Perennial	RBGE
<i>Tragopogon pratensis</i>	L.	Forb	Perennial	Scotia seeds
<i>Trifolium pratense</i>	L.	Legume	Perennial	Chiltern Seeds & Wild Flower Shop
<i>Ulex europaeus</i>	L.	Legume/Woody	Perennial	Tree Seed Online Ltd
<i>Vicia cracca</i>	L.	Legume	Perennial	Emorsgate
<i>Zea mays</i>	L.	Grass	Annual	Chiltern Seeds

Table 18: Plant names, attributes and collection sources for host species used in Experiment 2.

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

Table 19: Euphrasia species collections across both experiments in Chapter 7.

Experiment	Euphrasia species	Location	Grid Reference
1	<i>E. arctica</i>	Inverkeithing, Scotland	NT 1389 82312
2	<i>E. anglica</i>	(A1766)	Cheddar, Somerset
2	<i>E. vigursii</i>	(V1761)	St Agnes Head, Cornwall
2	<i>E. tetraquetra</i>	(T1761)	St Agnes Head, Cornwall
2	<i>E. micrantha</i>	(M1767)	Borrowdale, Cumbria
2	<i>E. micrantha</i>	(M1768)	Alness, Scotland
2	<i>E. micrantha</i>	(M1769)	Orkney, Scotland

Table 20: 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 (pMCMC).

Covariates	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	3.0348	1.8630	4.1519	1000	<0.001
Time	-1.0533	-1.1164	-0.9912	1000	<0.001
AnnPerAnn	0.1390	-0.2489	0.6076	1000	0.5300
Normalised transplant date	-0.0164	-0.0213	-0.0117	1000	<0.001
Functional group fern	-0.2583	-1.5117	1.0171	1000	0.6520
Functional group forb	-0.3076	-0.9687	0.3844	1000	0.3700
Functional group legume	-0.0828	-1.0457	0.7646	1000	0.8500
Functional group woody	-0.6675	-1.4986	0.1819	1000	0.0980

Table 21: 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 (pMCMC).

Covariates	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	4.6197	4.1765	5.0536	1000	<0.001
AnnPerAnn	-0.1380	-0.2703	0.0043	1188	0.0560
Functional group fern	-0.1127	-0.5410	0.3556	1000	0.6000
Functional group forb	-0.0879	-0.3087	0.1793	1106	0.3780
Functional group legume	-0.0650	-0.3307	0.3032	860.9	0.6160
Functional group woody	0.0991	-0.2964	0.4466	1000	0.5520
Normalised transplant date	0.0034	0.0008	0.0060	1000	0.0160

Table 22: 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 (pMCMC).

Covariates	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	-4.1298	-17.0773	5.4805	550	0.3420
Time3	2.3713	1.5862	3.2031	773.2	<0.001
Time4	3.0630	2.1378	3.9166	1000	<0.001
AnnPerAnn	0.7872	-1.2385	2.8500	1000	0.4460
Functional group fern	-4.3612	-16.8977	6.6709	789.8	0.3960
Functional group forb	-2.3178	-9.4309	3.7584	793.8	0.4420
Functional group legume	-2.3657	-10.7235	5.1473	756.9	0.5760
Functional group woody	-7.6673	-15.5032	-1.0839	549.4	0.0180
Normalised transplant date	-0.0760	-0.0919	-0.0625	1000	<0.001
Time3:AnnPerAnn	-0.9448	-2.0965	0.1002	1000	0.0920
Time4:AnnPerAnn	-2.3383	-3.6057	-0.8897	1000	0.0040

Table 23: 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 (pMCMC).

Covariates	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	-0.4637	-9.8823	9.4058	1093	0.9240
AnnPerAnn	-0.3610	-2.9028	2.1730	886.5	0.7720
Functional group fern	-3.6600	-15.1134	6.8501	1000	0.4660
Functional group forb	-2.9965	-8.8016	2.1653	1097	0.2340
Functional group legume	-2.0488	-9.1675	4.6899	1000	0.5500
Functional group woody	-7.5786	-14.1020	-1.0165	633.3	0.0100
Normalised transplant date	-0.0762	-0.0945	-0.0570	1000	<0.001

Table 24: 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 (pMCMC).

Covariates	Posterior mean	l-95% CI	u-95% CI	Effective sample size	pMCMC
(Intercept)	1.7842	1.2210	2.2714	787.7	0.0010
<i>Euphrasia micrantha</i>	-1.2795	-1.7479	-0.8284	1000	0.0010
<i>Euphrasia tetraquetra</i>	-0.3702	-0.8160	-0.0076	873.2	0.0620
<i>Euphrasia vigursii</i>	-0.2457	-0.7758	0.2138	1000	0.3340
Population: M1767	0.3269	-0.2098	0.9299	846.7	0.2760
Population: M1768	0.7931	0.4788	1.0699	1000	0.0010
Normalised transplant date	0.0059	-0.0084	0.0237	1208	0.4820