Chapter 6 - Conserved and host-specific interactions in a multi-host parasite system

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Abstract

Generalist parasites may infect multiple host species and experience complex host-parasite interactions. However, understanding the dynamics of host-parasite interactions remains a major challenge in generalist parasite systems. In this study, we use an experimentally tractable generalist parasitic plant group to understand host-parasite interactions in a multi-host system. Using common garden experiments, we show that extensive variation in the performance of hemiparasitic eyebrights (*Euphrasia*) across its host range can be attributed to both host life history and host phylogenetic relationships, but not host functional group as widely expected. While host-dependent parasite performance is generally conserved between eyebright species, with a restricted and phylogenetically divergent subset of hosts being highly beneficial, some eyebrights have more specialised host-parasite interactions. These host-parasite interactions show that a generalist parasite can respond to individual host selection pressures and may adapt to local host communities.

Introduction

The fitness of parasites depend on many aspects of the host to which they attach and feed, including their condition (Tschirren et al. 2007), defences (including immunity; Cameron, Coats, and Seel (2006); Bize et al. (2008)), growth rates (Barber 2005), biomass (Matthies 2017) and genotype (Vale and Little 2009). These host attributes have widely been investigated using single-parasite single-host experiments in economically important pathogens and evolutionary model systems, for example malaria in humans (Becker et al. 2004; Mackinnon and Marsh 2010) and nematodes in *Apodemus* wood mice (Meyer-Lucht and Sommer 2005). However, increasing evidence shows that most natural systems have complex parasite dynamics where parasites affect multiple host species (Ameloot, Verheyen, and Hermy 2005), hosts are infected by multiple parasite species (Pedersen and Fenton 2007), and parasite and host abundance differ in space and time (Mudrak and Leps 2010). As such, the single-host single-parasite species doctrine is increasingly seen as a poor fit to natural systems (Poulin and Forbes 2012), and studies of parasitism should seek to understand the more complex parasite-host dynamics present in nature.

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 (Westwood et al. 2010; Twyford 2018). The majority of parasitic plant species are hemiparasites, which feed directly from other plants, but maintain their green habit and photosynthetic competency (Twyford 2018). These hemiparasitic plants are ideal for investigating host-parasite interactions as they are experimentally tractable and can be grown in different combinations and with a wide host range (Brown et al. 2020). Moreover, hemiparasitic plants include ecosystem engineers that reduce the growth of competitively dominant taxa in grassland communities (Pywell et al. 2004), and species that threaten food security and cause billions of dollars' worth of crop losses in agricultural systems every year (Spallek, Mutuku, and Shirasu 2013), making the study of host range of crucial importance.

Generalist hemiparasitic plants that grow in mixed grassland communities may attach to any of the diverse range of co-occurring plant species. The dominant paradigm is that generalist hemiparasite performance is associated with host plant functional groups such as legumes, grasses, or forbs, with legumes often the best hosts (Matthies 2017; Rowntree et al. 2014). However, substantial variation in host quality within functional groups suggests functional group alone may not be a good predictor of host quality (Rowntree et al. 2014). Moreover, some functional groups are monophyletic clades such as grasses (Poaceae), while some are paraphyletic groups such as forbs. As such, the performance of hemiparasites may be better predicted by host phylogeny rather than functional group, with some host clades possessing attributes such as defence against parasites (Cameron, Coats, and Seel 2006) or root architecture (Roumet, Urcelay, and Diaz 2006) that confer higher growth to the parasite. Hemiparasite performance may also be expected to be affected by other aspects of the host, for example different host life history strategies (annual or perennial) that may have different resource accessibility (Garnier 1992) or relative carbon and nitrogen content (Garnier and Vancaeyzeele 1994). Finally, theoretical models of parasitism predict more complex host-parasite interactions will arise in heterogeneous environments with variable host abundance and/or a mix of different host genotypes (Gandon 2002). Such host-parasite interactions are not known in facultative generalist hemiparasitic plants, but have been observed in the obligate hemiparasitic plant *Striga*, where specific host-genotype parasite-population interactions underlie the success of *Striga* parasite development (Huang et al. 2012).

Here, we use facultative generalist hemiparasitic eyebrights (*Euphrasia*, Orobanchaceae) to investigate the host attributes that determine parasite performance. This genus is an ideal model for studying host-parasite interactions as they are small in size and easy to cultivate with a rapid annual lifecycle (Brown et al. 2020), and species co-occur with diverse hosts in different habitats (Metherell and Rumsey 2018). We consider multiple aspects of *Euphrasia* performance, including survival and reproduction through the year, and aim to quantify how hemiparasite performance responds to many host species. Specifically, we ask: (1) how does *Euphrasia* perform across its host range and on non-hosts? (2) Do common host characteristics such as functional group, life history, or relatedness (phylogeny) impact on the survival and performance of hemiparasitic *Euphrasia*? (3) Do different *Euphrasia* species perform similarly with a given host species, or is reproductive success determined by host-parasite interactions? Our aim is to understand the potentially complex responses of a generalist parasite to diverse host attributes.

Methods

Plant material, cultivation and trait measurements

We addressed parasite-dependent host performance in two common garden experiments. Experimental 1 aimed to understand the performance of *Euphrasia* across a range of hosts, and to link performance with host characteristics. For this experiment, we grew a single species, *Euphrasia arctica*, on forty-five diverse host species. *E. arctica* was chosen as the focal species due to its widespread nature in Britain, where it mainly occupies lowland grassland habitats. Experiment 2 was designed to detect parasite-host interactions, and we used four different species of *Euphrasia* and thirteen species of hosts (SI Appendix Table S2). Two diploid species (*E. anglica*, *E. vigursii*) and two tetraploid species (*E. micrantha*, *E. tetraquetra*) of *Euphrasia* were chosen to represent the diversity of the genus in Britain. For both experiments, we used wild-collected open-pollinated seeds of *Euphrasia* (SI Appendix Table S3). *Euphrasia* seeds were sown in individual pots filled with Sylvamix 1 compost and placed in an outside array at the Royal Botanical Garden Edinburgh (RBGE). Details of soil mixes and sowing protocols followed (Brown et al. 2020). In Experiment 1, a total of 3000 *Euphrasia* seeds were sown in winter 2016 of which 1308 germinated. In Experiment 2, a total of 2880 *Euphrasia* seeds were sown in winter 2017 of which 988 germinated. In both experiments after *Euphrasia* germination, plants were grown in an unheated, well ventilated greenhouse. Host plants were then transplanted into *Euphrasia* pots using tweezers which carefully extracted the host plant and a dibber to create space in

the *Euphrasia* pot. Host plants were then placed half way between the central *Euphrasia* germinant and a pot vertex. In Experiment 1, all 45 hosts were transplanted in spring 2017 into *E. arctica* pots (SI Appendix Table S1) and in Experiment 2, all 13 hosts were placed in all four *Euphrasia* host species pots in spring 2018 (SI Appendix Table S7). Host plants were replaced if mortality occurred within two weeks of the transplant date. All pots containing *Euphrasia*-host combinations were randomized weekly.

The traits measured on *Euphrasia* plants were proxies of fitness, to understand how *Euphrasia* fitness is affected by host plant species (Experiment 1) and whether specialised interactions occur between *Euphrasia* and particular host species. 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 until the 30.09.17. These are referred to as time points one (May) to five (September) in the rest of the chapter. For Experiment 2, we measured reproductive nodes at the end of the season. In both experiments, germination date and date of host introduction were recorded. Here, reproductive nodes are defined as whether a node on a *Euphrasia* plant contained either a flower or fruit or not (binary), summed for all nodes on a plant. Cumulative across the season, the number of reproductive nodes is the reproductive output of a *Euphrasia* individual. The traits of host species which were included in Experiment 1 were the functional group of host (woody, fern, forb, grass, or legume), and the life history of the host (whether annual or perennial).

Statistical analyses

Statistical assessment of hemiparasite performance across host species

We integrated a phylogenetic tree into the analysis of Experiment 1 to understand if host plant relatedness impacted reproductive output of *Euphrasia*. The phylogeny used in this analysis was based on the two gene alignment of rbcL and matK from (Lim et al., 2014). Six sequences from three species (*Zea mays*, *Hordeum vulgare* and *Lagurus ovatus*) were added from NCBI, as they were not present in the original dataset. The maximum likelihood phylogeny was generated using IQ-TREE (Nguyen et al. 2015) with branch support estimated using 1000 ultrafast bootstrap replicate, and using the TESTNEWMERGE flag for model selection. A constraint tree was created using the phylomatic function in the R package brranching (Chamberlain 2019) and used to topologically constrain the phylogeny based on the APG IV phylogeny. The tree was then made ultrametric prior to model-based analyses.

The models for Experiment 1 are designed to assess the impact of host species and their attributes on the performance and fitness of *Euphrasia arctica*. All subsequent statistical analyses were conducted in R version 3.6.1 with all data manipulation in base R or data.table (Dowle and Srinivasan 2019). In Experiment 1, the three *Euphrasia* traits of interest – survival, number of days to flower, and reproductive nodes of *Euphrasia* – were estimated using a Bayesian generalized linear mixed effect model approach in the MCMCglmm package (Hadfield 2010). *Euphrasia* survival was modelled using the "threshold" option in MCMCglmm (i.e. the CDF of the Gaussian distribution) which is also known as an event history analysis model (EHA) as it can take into account time-varying covariates. The number of days to flower and reproductive nodes (both at the end of the season, and over time) were modelled using a Poisson distribution.

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 EHA, time point was also added as a fixed effect to model the effect of time itself on Euphrasia survival. Time point five was removed from the EHA, as all but two individuals were dead at this time. We parameterized the reproductive output over time model differently. Time point and its interaction with host 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

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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:

$$V_{HE} = \begin{matrix} T_{2,2} & T_{2,3} & T_{2,4} \\ T_{2,3} & T_{3,3} & T_{3,4} \\ T_{2,4} & T_{3,4} & T_{4,4} \end{matrix} \tag{1}$$

Where V_{HE} 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():

$$V_e = \begin{matrix} T_{2,2} & 0 & 0 \\ 0 & T_{3,3} & 0 \\ 0 & 0 & T_{4,4} \end{matrix} \tag{2}$$

Models were run for a minimum of 130000 iterations, following a burn-in of 30000 iterations, 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 (Brown 2019), otherwise the pMCMC 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 (Bates et al. 2015). Convergence and autocorrelation of models was assessed visually by plotting the posterior distributions of the estimated parameters.

Statistical tests of hemiparasite-host interactions

The model in Experiment 2 differed significantly from the models in Experiment 1, as phylogenetic random effects and host trait fixed effects (functional group and life history) were not included. Only one response variable was modelled, which was the cumulative number of reproductive nodes at the end of the season, using a Poisson distribution. The fixed effects included the *Euphrasia* species themselves, which population a Euphrasia species came from (SI Appendix Table S6), and the normalised transplant date (as above). 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. Models were run as specified for Experiment 1.

All scripts for statistical analysis and figures, as well as the data used, is available at https://github.com/Euphrasiologist/euphrasia_host_parasite.

Results

Hemiparasite performance across host species

We first investigated survival of *Euphrasia arctica* across the 45 different host species, where 1252 *Euphrasia* plants survived to be measured at the first time point. Survival was not significantly affected by host functional group ($\chi^2 = 3.38$, df=4, P=0.50; Figure 1 shows legumes and grasses as examples) or host life history ($\chi^2 = 0.40$, df=1, P=0.53; SI Appendix Table S2) in an event history analysis. Instead, 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 overriding effect of host on *Euphrasia* survival was also evident from the standard deviation of the host effects (0.57, 0.39-1.11 CI) being of a greater magnitude than the fixed effects of life history (0.14, -0.25-0.61 CI) and functional group (-0.19, -1.42-0.67 CI; SI Appendix Table S2). Taken together, these results indicate host species impacts hemiparasite survival in a common garden environment, with survival being species specific rather than being influenced by host plant group (i.e. phylogenetic clade, or life history).
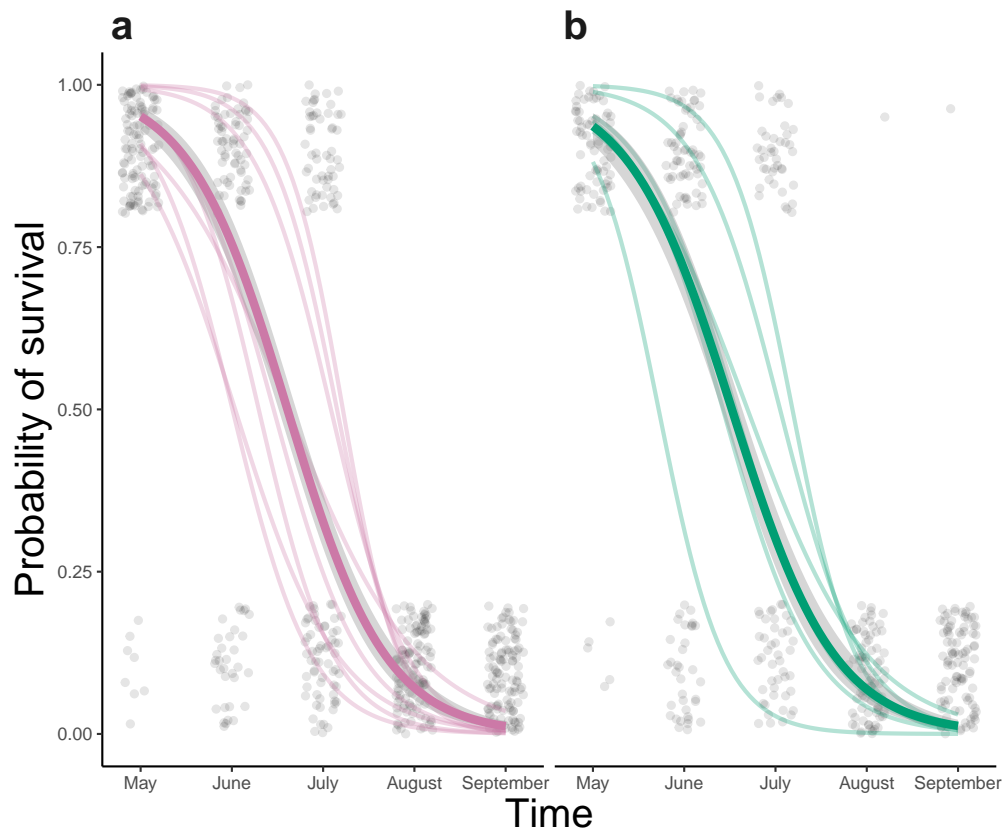

Figure 1: Probability of *Euphrasia arctica* surviving in a common garden experiment on 14 host species from two representative families, the Fabaceae (a) and Poaceae (b), using host species binomial regressions. Pale regressions represent individual species and bold regressions represent family level 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.

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