

Chapter 7 - general discussion

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The interaction and importance of ploidy and hybridisation

The first part of my thesis investigated the interaction between ploidy variation and hybridisation at different phylogenetic and geographical scales. While it is generally thought that ploidy level acts as a significant barrier to hybridisation (Husband and Sabara 2004), a literature review (Chapter 2) showed strong evidence that hybridisation between ploidy levels was common, particularly in many groups of plants. Such interactions between ploidy level of the parental species and hybridisation can have long lasting evolutionary consequences disproportional in effect to the frequency of their occurrence, such as new lineage or species formation, and may be being amplified by global change. At a regional floristic scale, the British flora provided an excellent framework for studying hybridisation and polyploidy due to the wealth of morphological, ecological, and genetic data. At this flora wide level, I found (phylo)genetic factors and ploidy level to be important in predicting whether a species pair will successfully form a hybrid (Chapter 3). Species of differing ploidy level were less likely to form hybrids, but my model indicated that the barrier is porous. At a finer scale still, I used the genus *Euphrasia* to investigate a contact zone between two species of differing ploidy level (Chapter 4). Although I found a lack of evidence for hybridisation and introgression using conventional population genetic tools, demographic modelling indicated there may have been limited historical or ongoing gene flow between these two species. Combining all present evidence in the thesis, hybridisation between ploidy levels was common globally, but may be rare in particular study systems. In the TCG *Euphrasia*, even rare hybridisation between ploidy levels may be sufficient to generate new species. My thesis adds to the emerging view that polyploidisation, in conjunction with hybridisation plays an important role in creating and maintaining diversity across different taxonomic groups both historically (e.g. *Galium* Kolar et al. (2015)) and presently (e.g. *Senecio* Abbott and Lowe (2004)).

Evidence of parasitism playing a role in taxonomic complexity

My results using common garden experiments with *Euphrasia* showed that the hemiparasitic habit can contribute to taxonomic complexity. In the first experiment (Chapter 5), the results suggested that although some species were consistently morphologically distinct (e.g. *Euphrasia micrantha*), other closely related species overlapped in many traits and sometimes could not be reliably told apart in a common garden setting. I suggested that there may be differential natural selection for local ecological conditions in the wild that drive

life history differences between species. There was also considerable phenotypic plasticity in relation to the host species being parasitised, with only a few traits (e.g. node to first flower) that showed consistency when a *Euphrasia* species was grown on a particular host species. Next, I investigated host-parasite interactions across a range of host-*Euphrasia* species combinations to see if there was evidence of host specificity (Chapter 6). I found evidence of host parasite interactions, which could be responsible for local host adaptation, and in turn may drive the evolution of cryptic specialisation. Although this hypothesis is consistent with my results, more work would need to prove local host adaptation in the wild. In sum, host species influenced both the morphology and fitness of *Euphrasia* individuals, creating the potential for species confusion through phenotypic plasticity, and cryptic specialisation of *Euphrasia* on certain host species. Little work has been done on taxonomic complexity through the lens of parasitism in other hemiparasitic plant genera. A good candidate for investigation would be *Pedicularis*, which is a large genus, containing species complexes (Garg 2010). Due to the relatedness to *Euphrasia* both phylogenetically and in terms of the presence of much recent speciation, I would postulate that *Pedicularis* has similar processes that may be driving taxonomic confusion and diversity in the genus.

Critique and further study

Given the broad aims of the thesis, there are areas which could benefit from further study. In particular, an increased taxonomic scope would help to generalise more across plants. Good data is available for the British flora (Chapter 3), but this is not so for many other floras, especially in tropical regions of the world. Regions with data available (i.e. temperate regions) may also not be representative of the processes operating elsewhere, as these same regions are for the most part highly degraded habitats with disturbed ecological processes and altered evolutionary trajectories. Degradation and disturbance would affect findings, as we expect hybrids to be more common, and more alien species to be present in these situations. Another area which would benefit from increased scope is the GBS study (Chapter 4). Here, I investigated only one contact zone and a limited number of individuals, where hybrids may have been missed due to the small sample sizes. A single contact zone does not represent the whole spectrum of possibilities, for example some contact zones may be younger than others, or present asymmetrical numbers of parental plants and hybrid genotypes (Twyford, Kidner, and Ennos 2015). Therefore sampling multiple contact zones across multiple species which do and do not differ in their ploidy level would have been ideal. On the other hand, Yeo (1954) found only a single triploid in his large cytogenetic survey of *Euphrasia*, indicating hybridisation between ploidy levels is rare. In the latter growth experiment (Chapter 6), relating host preferences to *Euphrasia* growing in the wild would give a clearer indication of host preferences. This could be either done by using quadrats to relate nearby hosts, or more accurately by looking for haustorial connections to host roots. This technique of looking at haustorial connections by uprooting *Euphrasia* individuals would give a better proxy for fitness and host suitability in a common garden setting.

There are many avenues for potential future research and I will highlight a few here. For further investigation into the genetics of *Euphrasia*, a complete and contiguous (potentially phased) whole genome assembly of both a diploid and a tetraploid species is essential. Using these complete genomes it will be possible to accurately characterise regions underlying adaptive introgression, and detect structural changes between the hybrid species and their parental progenitors (Chapman and Abbott 2010; Jay et al. 2018). In Chapter 4, I used genotyping by sequencing to generate SNPs across the genome, however it would be useful to use whole genome data to resolve fine level introgression. For example, this could show recent or historic introgression between the diploid species and homologous regions of the tetraploids. This is difficult, as it requires the identification of ploidy-specific diagnostic sites that must be shown in putative hybrids. Whole genome data across a wider range of species would also yield powerful comparative insights of the extent of genomic introgression across *Euphrasia* in the UK. I did generate whole genome sequence data and draft assemblies for two species of particular interest, *Euphrasia micrantha* and *E. vigursii*. A previously sequenced

E. anglica genome meant that now both putative parental species for *E. vigursii* were available for analysis. With postdoc Hannes Becher, we mapped putatively disomic scaffolds of *E. anglica* and *E. vigursii* to the *E. micrantha* reference and found slightly more sequence of *E. vigursii* mapped (342Mb) than *E. anglica* (327Mb). This result is consistent with *E. vigursii* being a hybrid species, however the results are far from conclusive and work is ongoing to resolve the relationships of these three taxa. Better assemblies, and more individuals of each species along with the parental progenitors would allow us to understand whether these are hybrid species (and if so, what are the parental contributions to the hybrid genome), or simply derived diploid populations diverged in allopatry. Indeed, finding and sequencing hybrid *Euphrasia* individuals or populations would allow major insight into the nature of hybridisation in *Euphrasia*.

In Chapters 5 and 6 I used common garden experiments to understand how host species impact the morphology and fitness of *Euphrasia* individuals. It would be ideal to characterise the hemiparasitic habit of *Euphrasia* further by using field experiments in the wild. Many different populations of *Euphrasia* could be studied to yield information about host association in the wild to understand the correlation between host species and morphology. In an attempt to begin this process, I measured the number of potential host species present around 20 randomly sampled *Euphrasia* individuals at each population that was sampled for use in the growth experiment in Chapter 6. I related these host species occurrences in the wild to the growth of *Euphrasia* in cultivation to see if host species that were more frequently encountered in the wild led to higher *Euphrasia* fitness. Although I found no significant association, there were obvious limitations - I used few populations and my statistical power was low. Another potential field experiment includes excavating *Euphrasia* plants to relate the number of haustorial connections to neighbouring host plant species, to the morphology and fitness of *Euphrasia* plants. This is a more realistic and rigorous approach to the problem, but made difficult due to the fine haustorial connections which can be broken easily. Lastly, to place my results in a more comparative context, it would be good to understand host preferences in other genera in the Orobanchaceae/Rhinanthaceae - are they the same as in *Euphrasia*? Do host shifts between genera occur? If so, why? Is there phylogenetic signal in host preference? I would expect to find differences between genera, as there appears to be some host specialisation at this level (e.g. *Melampyrum* on *Medicago sativa*/*Achillea millefolium* Matthies (2017); *Rhinanthus* on *Festuca ovina*/*Cynosurus cristatus* Cameron, Coats, and Seel (2007)), but more comprehensive datasets are needed to address this rigorously.

Thesis conclusions

The main aims of this thesis were to understand both the interaction between hybridisation and polyploidy in both *Euphrasia* and the British flora, and the role of hemiparasitism in driving taxonomic complexity in *Euphrasia*. In Chapter 2, I found that cross ploidy hybridisation is a common phenomenon across plants, which had previously been little explored. Chapter 3 revealed that (phylo)genetic factors and ploidy level were critical in explaining hybridisation across the British flora. Chapter 4 used GBS data to show that hybridisation in a cross ploidy *Euphrasia* contact zone is rare. In Chapter 5, I showed that *Euphrasia* species can overlap in morphology on a single clover host species, and different host species drive phenotypic plasticity in a common garden. Lastly, in Chapter 6 I used another common garden experiment which revealed that *Euphrasia* responses to host species were mainly conserved across species, however host-parasite interactions were also present. Ploidy variation in conjunction with hybridisation, and parasitism, continue to shape the evolution of plants in profound ways, and warrant further study to understand the mechanisms underlying these phenomena.

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