**Chapter 7 - general discussion:**

**The interaction and importance of ploidy and hybridisation:**

The first part of my thesis investigates the interaction between ploidy variation and hybridisation at different scales. While it is generally thought that ploidy level acts as a significant barrier to hybridisation in plants (ref), a literature review (Chapter 2) across flowering plants shows strong evidence that hybridisation between ploidy levels is common in many groups of plants. Such interactions 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 smaller scale, the British flora provides an excellent framework for studying hybridisation and polyploidy due to the wealth of morphological, ecological, and genetic data. At this flora wide level, I find (phylo)genetic factors and ploidy level to be important in predicting whether a species pair will form a hybrid (Chapter 3). Species of differing ploidy level are less likely to form hybrids, but my model indicates that the barrier is porous. At a finer scale still, I use the genus *Euphrasia* to investigate a contact zone between two species of differing ploidy level (Chapter 4). Although I found evidence for hybridisation lacking using conventional population genetic tools, demographic modelling indicates there may be limited ongoing gene flow between these two species. Overall, hybridisation between ploidy levels is 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.

**Evidence of parasitism playing a role in taxonomic complexity:**

My results using common garden experiments on *Euphrasia* show that the hemiparasitic habit can contribute to taxonomic complexity. In the first experiment (Chapter 5), the results suggest that although some species were consistently morphologically distinct (e.g. *Euphrasia micrantha*), other closely related species overlap in many traits and sometimes could not be reliably told apart in a common garden setting. It is suggested that there may be differential natural selection for local ecological conditions in the wild, that more clearly separate species or even populations within species. There is also considerable phenotypic plasticity in relation to the host species being parasitised, with only a few *Euphrasia* traits (e.g. node to first flower) showing consistency between host species. Next, we investigate host-parasite interactions across a range of host-*Euphrasia* species combinations to see if there is evidence of host specificity (Chapter 6). I find evidence of host parasite interactions, which could be responsible for local host adaptation, and in turn may drive the evolution of cryptic speciation. Although this hypothesis is consistent with my results, more work would need to be done to prove local adaptation in the wild. In sum, host species influence 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.

**Critique and further study - main problems in the thesis:**

Given the broad aims of the thesis, there are areas which could benefit from further study or include more comprehensive datasets. 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 and evolutionary processes. The GBS study (Chapter 4) 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 possibility, and ideally multiple contact zones across multiple species which do and do not differ in their ploidy level would have been ideal. 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.

**Future directions:**

There are many avenues for 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 of admixture or introgression, and detect structural changes between the hybrid and parental progenitors. 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 genetic structure. Whole genome data across a wider range of species would also yield powerful comparative analyses 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 rivularis* and *Euphrasia* *vigursii*, which are two purportedly hybrid endemic species in the UK. 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 through 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 difficult. Lastly, to place my results in a more comparative context, it would be good to understand host preferences in other genera in the Orobanchaceae/Rhinantheae – are they the same as in *Euphrasia*? Do host shifts between genera occur? If so, why? Is there phylogenetic signal in host preference?

**Thesis conclusions:**

The main aims of this thesis were to understand the both the interaction between hybridisation and polyploidy, and the role of hemiparasitism in driving taxonomic complexity in *Euphrasia* and the British flora. 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, yet ongoing. 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* response 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.