

Abstract

Terrestrial plants are generally characterized by their green color, and are assumed to have an autotrophic lifestyle. However, several plant lineages have evolved dependence upon other organisms for their carbon demands, and consequently can be categorized as heterotrophs. Heterotrophic plants can be divided into parasitic plants, which directly parasitize other autotrophic plants, and mycoheterotrophic plants, which rely upon associations with fungi for their supply of organic nutrients. Both groups contain examples of fully heterotrophic plants, where the ability to produce chlorophyll has been lost, and partially heterotrophic plants, where chlorophyll is retained. In the case of parasitic plants, the obligate and partial states are referred to as being holoparasitic and hemiparasitic, respectively, while the corresponding terms for plants that parasitize fungi are (fully) mycoheterotrophic and partially mycoheterotrophic. Botanists have long been fascinated by heterotrophic plants; however, the strategies that heterotrophic plants adopt to sustain their complex biological interactions are still poorly understood.

The research detailed in this thesis was aimed to clarify our understanding of several important aspects of the life history of heterotrophic plants by using a multi-faceted approach, including conducting field observations and field experiments, as well as anatomical and molecular investigations. The different studies were presented as a series of loosely connected, self-contained chapters grouped under four main categories: host associations (Chapters 2-3), life-history traits constrained by a heterotrophic lifestyle (Chapters 4-7), adaptive aspects of partial heterotrophy (Chapter 8), and the impact of heterotrophic plants on their surrounding communities (Chapter 9).

In Chapter 1, I briefly introduced the history of the study of heterotrophic plants as a means of identifying aspects of their life history that have not been thoroughly studied, and consequently remain poorly understood.

In Chapter 2, I described the host range and host specificity of *Thesium chinense* (Santalaceae), which is the first quantitative investigation of host range and selectivity in the family Santalaceae under field conditions. It was found that of the 38 co-occurring species, only 2 occurred more frequently than expected. However, direct examination revealed that *T. chinense* formed haustorial connections with 23 species in 11 different families. These results suggested that examination of the aboveground spatial relationships of co-occurring plants is not enough to identify the host range of root parasites.

In Chapter 3, I examined the host associations of two arbuscular mycorrhizal

(AM) forming mycoheterotrophic species of the family Burmanniaceae (i.e. *Burmannia championii* and *B. cryptopetala*). While previous studies have shown that mycoheterotrophic plants tend to harbor a narrow group of fungi, with closely related mycoheterotrophs specialized to different fungal taxa, the current study found that the two species from the *Burmannia* exhibited extensive host sharing. These results indicated that fungal host sharing may occur in mycoheterotroph-fungal associations more commonly than previously thought.

In the next two chapters (Chapters 4-5), I investigated the breeding systems of mycoheterotrophic orchids. Several previous reviews had suggested that the limitations associated with such a highly specialized life history could be a strong determinant factor of the reproductive biology of heterotrophic species that results in a propensity for a selfing strategy. In Chapter 4, I investigated the breeding system of the mycoheterotrophic species *Cyrtosia septentrionalis* (Orchidaceae) in comparison with closely related photosynthetic genus *Vanilla*. Despite the showy appearance of *Cyrtosia* flowers, they failed to attract pollinators, and instead reproduced by self-pollination. In contrast, the majority of the *Vanilla* species lacked the capacity for self-pollination as a consequence of their effective rostellum. These results provided further evidence supporting the view that a heterotrophic lifestyle favours a selfing strategy.

In Chapter 5, I targeted multiple species of *Cymbidium* (Orchidaceae), which have a different nutritional mode with a robust phylogenetic framework to precisely determine the correlations between mycoheterotrophy and the breeding system. In the genus *Cymbidium*, a shift to autogamy has preceded the adoption of full mycoheterotrophy because not only mycoheterotrophic *C. macrorhizon* and *C. aberrans*, but also mixotrophic *C. lancifolium*, have an autogamous ability. While many studies have reported a shift in fungal host associations in accordance with the evolution of mycoheterotrophy, the results of Chapter 5 suggested that the shift to autogamy could be another important factor in mycoheterotrophy evolution.

The next two chapters (Chapters 6-7) investigated seed dispersal systems in heterotrophic plants. Although the seeds of most heterotrophic plants are readily dispersed in the air like dust particles, dependence on the wind in closed canopies, where wind speeds are significantly reduced, could reduce the success of this dispersal mechanism. In Chapter 6, I reported an avian seed dispersal system in the mycoheterotrophic species *Cyrtosia septentrionalis*, which is the first demonstration of animal-mediated seed dispersal in the Orchidaceae family. Unlike other orchid species, *C. septentrionalis* possessed several adaptations to ornithochory, including the production of red indehiscent fruits and a lignified seed testa. The occurrence of similar

morphological characteristics in other members of *Cyrtosia* suggested that avian seed dispersal systems were likely to be widespread in the genus, and indicated that shifts in seed dispersal mechanisms may have played a significant role in promoting the ecological and morphological diversity of orchid species.

In Chapter 7, I described the convergent recruitment of camel crickets as seed dispersal agents in the parasitic plant *Phacellanthus tubiflorus* (Orobanchaceae) and the mycoheterotrophic plant *Monotropastrum humile* (Ericaceae). Although orthopterans are rarely associated with seed dispersal, the notable exception is the large flightless Orthoptera known as weta, which are considered ecological replacements for the small mammals that are absent from their native New Zealand. However, the results presented in Chapter 7 challenged the view that orthopteran seed dispersal resulted from the absence of small mammals, and indicated that relatively large orthopteran visitors can independently occupy similar ecological niches as small mammals, in terms of acting as seed dispersal agents.

In Chapter 8, I investigated the demography of a partially mycoheterotrophic perennial *Cephalanthera falcata* (Orchidaceae) to assess the adaptive significance of vegetative dormancy. In a natural population of the plant, resource depletion and stress were the most likely factors inducing dormancy. However, unlike most autotrophic species, dormancy did not lower the survival rate, which suggested that the mixotrophic potential of this species could alleviate the costs of dormancy. In the absence of negative consequences, it is possible that dormancy is an adaptive response that allows mixotrophic species to recover from the resource depletion experienced after fruiting and herbivory, and/or to escape from temporal severe above-ground herbivory pressure.

In Chapter 9, I examined the influence of the hemiparasitic plant *Parentucellia viscosa* (Orobanchaceae) on its surrounding plant community. The removal of *P. viscosa* significantly increased the above-ground biomass of grasses and legumes, but the biomass of forbs was not significantly altered, which indicated that parasite removal resulted in a decline in the relative abundance of forb species. Root excavations showed that grasses and legumes were the preferred hosts of *P. viscosa*. Therefore, it is likely that the alternation in community structure by *P. viscosa* was, at least in part, the result of this differential host preference, which resulted in the decreased competitiveness of the dominant legumes and grasses.

In Chapter 10, I represented a brief summary of Chapters 2-9, placing them in a broader ecological and/or evolutionary context, as well as highlighting unexplored avenues of research and providing direction for future research of heterotrophic plants.

Overall, the studies detailed in this thesis have provided great insight into several poorly understood aspects of heterotrophic plant ecology. Taken together, the results provided a synergistic overview of how these plants sustained their intriguing life histories, emphasized the importance of life-history traits other than host associations such as breeding and seed dispersal system, and indicated the benefit of a multi-faceted research approach to expand our understanding of the ecology and evolutionary biology of heterotrophs.