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**Citation for published version:**

Twyford, AD 2017, 'New insights into the population biology of endoparasitic Rafflesiaceae' American Journal of Botany, vol. 104, no. 10, pp. 1433-1436. DOI: 10.3732/ajb.1700317

**Digital Object Identifier (DOI):**

[10.3732/ajb.1700317](https://doi.org/10.3732/ajb.1700317)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

American Journal of Botany

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## COMMENTARY

# New insights into the population biology of endoparasitic Rafflesiaceae<sup>1</sup>

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Parasitic plants demonstrate a diversity of growth strategies, life histories, and developmental and physiological characteristics. Most research to date has focused on a narrow range of parasitic taxa, particularly in the Orobanchaceae, while the other independent origins of parasitism have largely gone unstudied. One type of parasite that has received relatively little attention are the endophytic parasites, which have a fascinating growth strategy where the parasite is embedded within the host tissue, with the flower the only externally visibly plant part. Endophytic growth makes it challenging to understand basic aspects of species biology, such as the size of a given parasite, the number of parasites per host, and the genetic diversity of populations. Recent studies by Barkman et al. (2017) and Pelsner et al. (2017) have used microsatellite genotyping to investigate the population biology of endoparasitic Rafflesiaceae species in Asia. They show the potential for extensive parasite spread within a host vine and the strong partitioning of genetic diversity by host. These species are also shown to have an outcrossing mating system. However, these studies suggest different reproductive strategies, one supporting monoecy and one suggesting dioecy. Overall, these studies partly “lift the lid” on the cryptic biology of *Rafflesia* and the Rafflesiaceae and open the door for future comparative studies between endophytic and free-living parasitic plants.

**KEY WORDS** endoparasitism; genetic diversity; parasitic plants; population biology; *Rafflesia*; Rafflesiaceae

## POPULATION BIOLOGY OF PARASITIC PLANTS

Parasitic plants have long inspired interest from botanists, horticulturalists, and evolutionary biologists alike. These fascinating species form a direct connection with a suitable host plant using a specialized feeding structure called a haustorium, which allows them to extract nutrients and water (Westwood et al., 2010). The parasitic strategy is hugely successful and has evolved independently on at least a dozen occasions in the angiosperms (Barkman et al., 2007). The ca. 4000 species of parasitic plants have adopted a range of trophic strategies, from full reliance on a host (holoparasites) to those that can survive independently without host attachment (facultative hemiparasites). Parasitic plants also differ in whether they make a haustorial connection to the host stem or roots and whether the vascular connection is limited to the xylem or extends into the xylem and phloem. They may also show other notable developmental and physiological characteristics, such as loss of chlorophyll or reduction in organ size (Těšitel, 2016).

Given the diversity of parasitic plant species and their diversity of parasitic growth strategies, it seems surprising that research to date has focused on a narrow taxonomic range that represents only a subset of parasite evolutionary origins and morphological variation. The most widely studied parasitic plant genus is *Striga* (Orobanchaceae), which includes the devastating agricultural pest species *Striga hermonthica* and *S. asiatica*. Research on this genus has been instrumental for improving our understanding of parasite genetic diversity (Yoshida et al., 2010b), horizontal gene transfer (Yoshida et al., 2010a), germination cues (Jamil et al., 2011), and host resistance (e.g., Gurney et al., 2006; Li and Timko, 2009). Other widely studied parasitic plant taxa include other members of the Orobanchaceae, particularly *Orobanche* and *Phelipanche*, as well as species of *Cuscuta* (Convolvulaceae) and parasites in the Santalales. The other independent radiations of parasitic plants remain largely unstudied, especially those groups that are found in remote locations, those that have complex or cryptic life histories, and those which do not affect agricultural crops.

Studies across the full diversity of parasitic plants will reveal how novel parasitic traits, trophic strategies, and host interactions impact on parasite genetic diversity. In many animal parasites, such as parasitic tapeworms and flatworms, related individuals coinfect a host and subsequently experience inbreeding and kin-mating (Detwiler and Criscione, 2017). This inbreeding may lead

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<https://doi.org/10.3732/ajb.1700317>

to genetically distinct populations with low within population genetic diversity (Charlesworth, 2003), and in turn, population divergence may drive local adaptation, host–parasite coevolution, and parasite speciation (Vienne et al., 2013). Inbreeding may also be hypothesized to play an important role in the genetic structuring of parasitic plant populations. This could arise as a by-product of dispersal limitation, particularly in an environment where host availability is patchy. Alternatively, inbreeding may occur if related parasites share alleles for host interactions (e.g., to overcome host defenses) and thus have the highest fitness when infecting the same host.

### GENETIC DIVERSITY OF ENDOPARASITIC RAFFLESIIACEAE

Two recent studies fill an important gap in our understanding of the population biology of parasitic plants by studying genetic diversity of representative species from the Rafflesiaceae. This Asian plant family is best known for including *Rafflesia arnoldii*, a species that produces one of the largest flowers in the world (up to 107 cm diameter and 7 kg mass, Meijer, 1984; Nais, 2001). In addition to the genus *Rafflesia* (Fig. 1), the family also contains two other genera of obligate parasites, *Rhizanthus* and *Sapria*. Together, these endophytic species share a common growth strategy where they grow embedded within vines of *Tetrastigma* (Vitaceae), with the flowers of the parasite the only externally visible plant structure. This cryptic growth strategy has impeded our understanding of the basic biology and natural history of these taxa. The endophytic lifestyle has made it unclear whether the flowers emerging from a single host are from different individuals or whether a single individual can widely infect a vine. The source of parasite genetic diversity within a single host was also unknown, be it from many seeds from a single seed capsule, or multiple independent infections from different seed sources. Similarly, the extent of genetic differentiation between populations is unknown. Finally, while the flowers are known to be unisexual (Beaman et al., 1988), the lack of knowledge

of spread within a host vine made it impossible to know whether these species are monoecious or dioecious.

A study by Barkman et al. (2017) addressed these knowledge gaps by studying the population biology of *Rafflesia cantleyi*, *Rafflesia tuan-mudae*, and *Sapria himalayana* and their *Tetrastigma* hosts. They genotyped all parasite flowers emerging along a host vine with a suite of microsatellite markers and repeated this genotyping on many vines within a sampling site and across multiple sites in Southeast Asia. They used these genotypic data to trace the growth of individual parasites within their host, a task that would be near impossible by other means. They also used these data to infer parasite genetic diversity within and between host vines. They used host genotypes to confirm that the *Tetrastigma* samples are unique individuals of these large sprawling vines. A second study, by Pelsner et al. (2017), investigated genetic diversity in the *Rafflesia lagascae* species complex in the Philippines using a similar microsatellite genotyping approach. While their sampling did not include spatial information of parasite infection, nor host genotypes, it did include representatives from multiple populations of two rare species and provides valuable information to compare with the study of Barkman et al. (2017).

The first main finding comes from the spatially explicit microsatellite genotyping of Barkman et al. (2017), which revealed the potential for extensive parasite spread within a host vine. The most exceptional case was an individual of *R. tuan-mudae* shown to invade at least 14.7 m of its *Tetrastigma* host. There was also evidence that these parasites can produce a large floral display, with an individual *R. cantleyi* plant producing up to 25 flowers. While the extensive spread and large floral display of some individuals is impressive, most parasites tended to be more localized and produced only a single flower. This new knowledge on the spatial extent of Rafflesiaceae infection also revealed evidence for life history trade-offs, with the most widely spreading parasite individual producing few flowers, while more localized individuals produced many flowers. Moreover, large-flowered *R. tuan-mudae* produced fewer flowers than the smaller-flowered species.

Second, both studies showed that host vines are frequently coinfecting with multiple parasite genotypes. Pelsner et al. (2017) showed that over half the infected *Tetrastigma* vines support multiple *Rafflesia* individuals. The high within-population sampling of Barkman et al. (2017) showed a single *Tetrastigma* host may support up to 13 detectable parasite genotypes, and though individual *Tetrastigma* hosts may have a high carrying capacity of Rafflesiaceae parasites, total parasite population sizes were limited to 10–45 unique genotypes (individuals) in a single population. Moreover, Barkman et al. revealed that parasites within a host vine are closely related, with relatedness coefficients showing individuals may be sibs or half-sibs. However, measures of inbreeding were low ( $F_{is} = -0.178-0.101$ ) and often not significantly different from zero, showing *Rafflesia* and relatives are outcrossers. While no systematic evaluation of inbreeding has been performed across parasitic plants, it seems likely that they encompass the full range of outcrossing



**FIGURE 1** The spectacular flower of *Rafflesia pricei*, photographed on Mt. Kinnabalu. Image by Ding Hou, courtesy of the photographic collections of the Royal Botanic Garden Edinburgh.

rates, with species varying from being fully self-incompatible as observed in *Triphysaria* (Yoder, 1997), to cleistogamous as seen in some species of *Cuscuta* (Rodríguez-Pontes, 2009). In the case of the Rafflesiaceae, the relatedness of individuals within a vine appears to be an outcome of limited seed dispersal rather than selfing, as would be expected given the large flower size and high mobility of pollinating flies. The observed relatedness of individuals within a host is likely to contribute to the differentiation between populations.

While population dynamics appear broadly consistent in each of the studied Rafflesiaceae species, the results of the floral sex survey differ between studies. Barkman et al. (2017) found that a given parasite genotype only produced flowers of a single sex, suggesting these species are dioecious rather than monoecious. In contrast, Pelsner et al. (2017) collected both male and female *Rafflesia* flowers from the same putative individual as identified with 17 microsatellite loci, supporting these species as monoecious. As such, the reproductive strategy of *Rafflesia* should be interpreted with caution. While cogenetic species may have contrasting reproductive strategies of monoecy and dioecy, the confusing nature of working on a parasite that grows within a sprawling mass of vines means that further genetic data, or direct observations of floral sex from cultivated individuals, should be collected to definitively resolve the reproductive strategy of *Rafflesia*.

Overall, the results from these two studies greatly add to our understanding of the population biology of this group of endophytic parasites and suggest that population dynamics are driven by a complex interplay between pollen and seed dispersal, host availability, and potentially, host–parasite compatibility. One striking difference between the Rafflesiaceae species studied here and other commonly studied parasites (such as *Orobanchae*) is in their census population sizes. Many of the Rafflesiaceae populations included only a handful of unique individuals, which contrasts with the many weedy species in the Orobanchaceae that can be extremely abundant. However, Bromham et al. (2013) argued that a low parasite census population size may not translate to a low effective population size, due to the potential for long-distance, pollen-mediated gene flow. This hypothesis, also raised by the empirical findings of Barkman et al. (2017) and Pelsner et al. (2017), could be addressed by analyzing genotypic data of exhaustive population samples with parentage and paternity analysis (Jones and Ardren, 2003). This type of parentage assignment test would be particularly interesting with *Rafflesia* species with different flower sizes, to see how this trait affects dispersal dynamics. Complementary information on effective population size could also be generated through genomic sequencing (e.g., Li and Durbin, 2011). Such knowledge could be used to formulate spatial models of colonization, with the view to conserve aspects of *Rafflesia* population dynamics that aid their survival in the wild.

## FUTURE CHALLENGES FOR UNDERSTANDING ENDOPARASITE BIOLOGY

Endophytic parasitism has also evolved in four other land plant families, the Apodanthaceae, Cytinaceae, Mitrastemonaceae, and the Viscaceae. Whether these independent origins of endoparasitism have evolved through the same developmental and genetic pathways is an open question. The stages in the evolution of endoparasitism are also unclear, such as whether endoparasites have evolved

from a nonparasitic relative or via an exoparasitic intermediate (i.e., from a parasitic plant that attaches to a host via haustoria from the outside, or via fungal hyphae; Bellot and Renner, 2015). A limitation to comparative evolutionary analyses of endoparasites is most these groups lack related exoparasitic taxa (Bellot and Renner, 2015). The only exception is in the Viscaceae, where endoparasitic *Arceuthobium* has exoparasitic relatives (Cohen, 1954; Lye, 2006). As such, this group could be a valuable study system for comparative analysis. The only other way to identify the unique evolutionary processes acting in endophytic parasites would be by comparisons with distantly related endoparasites; however, few comparisons have been made. One obvious comparison would be in terms of dispersal distances and gene flow to understand how these types of parasites differ in terms of colonization and establishment. Another area would be in the study of horizontal gene transfer (HGT), which is emerging as an important mechanism for adaptive gene transfer in plants (Davis and Xi, 2015). Evidence for nuclear gene HGT has been found in both endoparasites (Xi et al., 2012) and in exoparasites (Yang et al., 2016), though direct comparisons between these studies is not possible due to the use of different methodologies. As well as acquiring genes via HGT, studies of the plastid genomes of endoparasitic Apodanthaceae showed major gene loss (Bellot and Renner, 2015), and extensive gene loss or indeed entire plastome loss may have occurred in *Rafflesia* (Molina et al., 2014). Again, whether patterns of endoparasite plastome degradation are distinct from those in other holoparasites will only be apparent after detailed comparative studies. Certainly endophytic parasites will experience a different selective environment to exoparasites, which could facilitate HGT and reduce the strength of selection for the photosynthetic function of plastids.

While studies of the Rafflesiaceae and other endoparasites have yielded important insights, there is still much to learn. Understanding more about *Rafflesia* infection is perhaps the most important priority for future endoparasitic plant research. In holoparasitic plants, seed germination occurs in response to host-specific germination cues and must be followed by successful haustorial growth, penetration, and attachment for the parasite to survive. The physiological details and underlying molecular mechanisms are relatively well understood in the Orobanchaceae (Westwood et al., 2010). Detailed studies in *Rafflesia* would reveal the degree of convergence in parasite growth responses between these divergent parasitic lineages and show whether these responses involve the same “core parasitism” genes (Yang et al., 2015). An improved understanding of *Rafflesia*–*Tetrastigma* interactions may also have practical applications in horticulture and conservation. At present, a lack of basic horticultural knowledge has prevented the cultivation of these taxa outside of Asia, and this knowledge gap could be addressed through experimental growth studies (Mursidawati et al., 2015). Knowledge of the ways to germinate and introduce *Rafflesia*, as well as an understanding of how to foster the complex interaction between host and parasite, may enable ex situ conservation of these species and will be essential to preserve the diversity in this remarkable plant group.

## ACKNOWLEDGEMENTS

The author thanks Josh Der, Stéphanie Gibot-Leclerc, Pam Diggle, and an anonymous reviewer for valuable comments on the manuscript. Research by AT is supported by the Natural Environment Research Council (awards NE/L011336/1 and NE/N006739/1).

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