Re-examining Plant on Plant Parasitism

Parasitic relationships capture our imagination with the specificity and creativity of their interactions, fascinating us even as they often creep us out. More than that, they also provide a fairly straightforward example of two organisms directly impacting one another, which has made them appealing models for scientific study. Amongst plants that parasitize other plants, these relationships have been investigated mainly to gain insight into mechanisms of parasite movement and attachment, often with an eye towards developing pest-control strategies. However, a number of studies have been published that imply these relationships may be more complex than previously supposed. In light of studies regarding host fitness and, more subtly, the inter-plant exchange of mRNA, evidence strongly supports that the "host-parasite" relationship has the potential both for contextual commensalism and to influence evolutionary trajectories.

Firstly, it's important to explore the method of interaction between host and parasite. A prominent example of plant parasitism is found in the genus *Cuscuta*, also called the dodder. *Cuscata* plants have extremely limited amounts of chlorophyll. Instead of photosynthesizing for themselves, they put out stalks called haustoria. These stalks respond to chemical cues put out by host plants, growing towards the host until contact is made, whereupon they connect and puncture the surface of the host plant. Vascular connections are then established that allow the dodder to siphon off nutrients, growing at the host's expense (see figure 1) (Vaughn, 2006).

Studies have shown that strength and frequency of parasitic interactions can vary with internal and external environments (Wolinsaka et. al., 2009)[2]. Host factors such as age and reproductive status can affect strength and frequency of interactions. Temperature, nutrient availability, and other such environmental factors that are often in flux can also have a profound effect. Due to this heterogeneity, within one population, the exact nature of the parasitic interaction can vary across both time and space. Varied special and temporal presence of parasite within populations helps to explain the lack of greater selection for parasite resistance. Koskela et. al. measured that body mass in plants resistant to parasite infection was, on average, smaller than in non-resistant plants, indicating an energetic cost to resistance unfavorable in situations where parasite presence isn't constant. If parasitism is relatively infrequent within a population, then even if being parasitized has negative consequences for an individual plant, selection may not be great enough to warrant development of resistance in the larger population (see figure 2.a).

Furthermore, the notion that being parasitized always negatively impacts the individual has been called into question. One researcher examined fitness consequences of being parasitized in a natural as opposed to laboratory context (Gomez, 1994) and found that the relationship has indirect benefits for the parasitized plant. Following a Spanish dodder population over the course of six years, Gomez saw that parasitized plants did have their fertility curtailed by factors that affected non-parasitized less heavily. However, plants parasitized by the dodder were ultimately about as fertile as non-parasitized plants due to reduced impact of other factors (see figure 2.b). Strikingly, it was found that herbivores preferentially graze on plants that aren't parasitized, thus demonstrating an indirect benefit of parasitism. Whereas non-parasitized plants had 45.2% of their fruits eaten, parasitized only had 6% lost to herbivores. The study concludes that the parasitized plants are about as fit as the non-

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[2] Note, the study referenced reviewed factors affecting parasitism in general, not just plant parasites. It's not unreasonable to assume its results hold true for plants, though.

parasitized, ultimately dispersing similar numbers of seeds. Other studies have since corroborated the benefits of parasites in combating herbivores (Puustinen & Mutikainen, 2000). Taken in conjunction with the population studies, these experiments are significant. If parasitism has no substantial effect on the fitness of the host plant, then the relationship might better be described as one of commensalism.

Another underappreciated element of this relationship is its potential for horizontal gene transfer (HGT), a phenomenon capable of dramatically influencing evolutionary trajectory. Horizontal gene transfer involves one organism taking in and incorporating the genes of another. It's an interesting phenomenon that differs from the better-known vertical transfer, wherein genes are passed from an organism to its descendants (Keeling and Palmer, 2008). HGT can involve one organism engulfing another and integrating parts of the genome, as is hypothesized to have occurred with eukaryotic mitochondria, or via direct transfer of nucleic acids. The transfer of adaptive genes via HGT is a significant event because it increases evolutionary potential. As the new trait spreads through a population, the transferred genes might enable its members to explore new niches or weather environmental changes.

While not yet observed in parasite systems, adaptive HGT *has* been observed between plants. One 2013 paper found evidence of extremely adaptive plant-plant gene transfer between hornworts and ferns (Li et. al., 2014). Though the neochrome gene is integral for the survival of ferns in low-light conditions, its origin in certain fern clades had been unclear. Comparing homologous sequences from other land plants indicated to the team that the gene likely originated in the hornwort clade and was transferred via HGT to the ferns. The researchers hypothesize that the hornwort genetic material somehow entered into a fern seed (which, compared to seeds from other clades, is relatively exposed). This example provides an important precedent for adaptive HGT of nuclear genes between plants. While examples of adaptive HGT are intriguing, they're infrequent, and so it's difficult to argue that selection would act as a result of this possibility. Nevertheless, the possibility of HGT does represent a benefit of host association with parasites.

In parasitic plant systems, the transfer of mRNA may facilitate HGT.
In the host plant, mRNA transcripts are sent via the vasculature as a means of internal communication. Once the haustorium has been established, parasitic plants will take up these mRNA transcripts along with host water, sugars, amino acids, and other nutrients. For the parasite, these transcripts are thought to provide information on host life cycle, allowing the parasite to coordinate its development. The potential mechanism of action for these transcripts is still an open question. Alonkoya et. al. engineered transgenic host plants to express RNAi targeted against parasite genes involved in haustoria formation (2012). Following initial connection, the dodders attached to transgenic hosts were overall less fit than controls, showing "poor establishment and decreased growth," along with earlier flowering time, decreased seed weight and number, and defects in haustoria vascularization. This demonstrates that uptaken transcripts can interact with those transcribed from the parasite's own genome, in this case by interrupting normal function. Scientists are unsure whether host mRNA affects parasitic function via an interference mechanism, as a transcription factor, or perhaps by being transcribed itself into protein. Whatever the mechanism, this study shows the potential for the interaction of interspecies genetic material.

Among plant parasites specifically there have been recorded instances of HGT via an mRNA mechanism, albeit for non-adaptive genes. One group of researchers performed an expansive analyses of genome tags in the plant *Striga hermonthica*, a parasitic eudicot (Yoshida et. al., 2010). Via a haustorium method very similar to the dodder's, *S. hermonthica* removes water, nutrients, and mRNA from its host plants, which are exclusively monocots. Yoshida's team was able to identify one gene that had no counterparts in closely related eudicots but which strongly resembled a gene found in the monocots on which the parasite preys. For this gene to develop independently within the *Striga* line would be improbable so the researchers put forth the hypothesis that this gene was likely transferred from a host and incorporated into the parasite genome. This proof-of-principle study shows that parasitic relationships have the potential to facilitate HGT events. Also important to note is the presence of a stretch of adenines found at the 3' end of the transferred gene that once might have been the poly-A tail of the host mRNA. In light also of studies showing that transcripts are able persist in parasites after being transferred from the host (David-Schwartz et. al. 2008), the Yoshida study indicates that transcripts are sometimes retrotranscribed and incorporated into genomes.

Importantly, there's potential for HGT to benefit both parties. While it's been observed with parasites as the beneficiaries, one team has shown that mRNA can be transferred from parasite to host in addition to the opposite direction. Examining a system where one parasite was attached to multiple host plants, Birschwilks et. al. demonstrated that labeled amino acids and metabolites in one host plant could be found in other host plants connected to the same dodder (2006). This result shows that mRNA not only enter the parasite, but can also enter into the host. The possibility for the host to benefit from HGT is therefore not excluded.

Evidence suggesting that the transfer mechanism has been refined over time further supports the presence of host benefits. One team of researchers has found that only certain mRNA transcripts are transferred, implying the presence of a filtering mechanism in either the host or the parasite (Roney et. al., 2007). This study surveyed transcripts found in the dodder, finding that the foreign mRNA in the parasite didn't represent the full diversity of transcripts of the host. Instead, only fully processed transcripts, many related to regulatory activities, were discovered. Since a filtering mechanism has been observed to keep certain transcripts and viruses localized within plants, these researchers posit that there may exist a similar mechanism selectively allowing certain transcripts through the phloem, (Foster, 2002). Alternately, it could be that selective symplastic transport occurs via plasmodesmata connecting host and parasite cells (Roney et. al., 2007). While it is possible that non-specific transfer likely occurs via the phloem at levels small enough to have not yet been detected (David-Schwartz, 2008), all current evidence points towards selective transfer of mRNA.

In order to better characterize this system, more research needs to be done on the mechanisms involved in the relationship and their fitness consequences. Does the transcript selective mechanism originate in the host or parasite or both? Is this selectivity even really there- prior investigations of transferred transcripts admit to using sequencing technology that may have missed smaller transcripts, so doubt remains as to the specificity of mRNA transfer. Studies using high-resolution sequencing to look for and characterize non-native transcripts inside hosts would help to clarify the strictness of gene flow direction as well its selectivity. Beyond that, studies examining the anti-herbivory effects of parasitism should be replicated with an eye towards determining density dependence- at what level of parasitism is this benefit outbalanced by the costs and how does this level correspond to those naturally occurring?

Though further research is needed, recent research should prompt a reevaluation of the relationship between parasitic plants and their hosts. Fitness studies have shown surprisingly few consequences of being parasitized in a naturalistic context and a large amount of circumstantial evidence points to plant parasites as a means for hosts to increase their genetic diversity, and thus their evolutionary potential. While it's important not to overestimate the benefits of parasitism, in many contexts, it may turn out that these interactions aren't purely parasitic, but instead somewhat commensal.

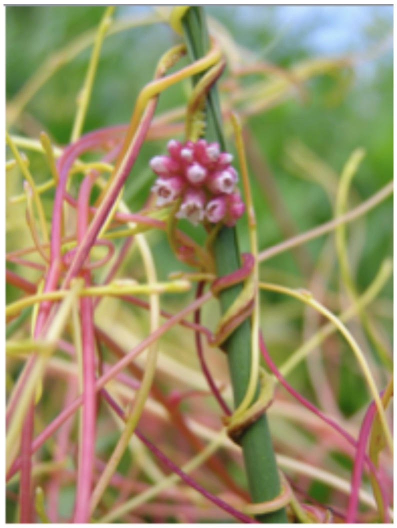

Figure 1: The dodder plant, seen here wrapped around a host.

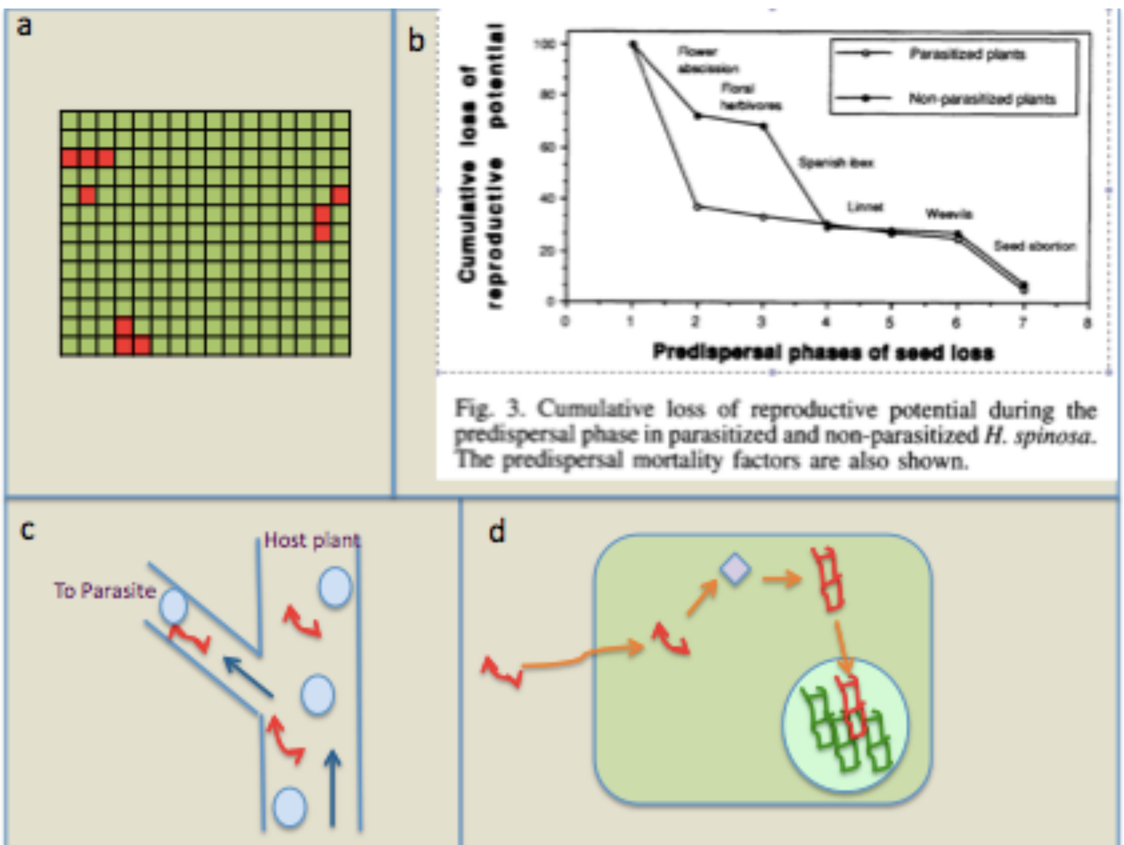

Figure 2: (a) If parasite infection (red squares) into a population is low enough, strong selection for resistance will not occur (b) Figure from Gomez paper showing that reproductive fitness is ultimately about equal in parasitized and non-parasitized plants. (c) mRNA (red) is taken up from host plant along with other nutrients (blue circles) via the parasite's haustorium. (d) Once inside the parasite, host mRNA has the potential to be reverse transcribed and incorporated into the parasite genome (green). This same process could occur in reverse, incorporating into the host.

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