It's not easy being not green: the making of a parasitic plant – Teaching Guide

Overview

Parasitism is a successful life strategy that has evolved in all known kingdoms of life. Parasites are often thought of as bacteria, fungi, or metazoans that cause disease in humans and other animals. The word "parasite" may not often bring plants to mind, but parasitic plants are diverse, destructive, and distributed all over the world. While some parasitic plants play important ecological roles as keystone species, parasitic weeds – which attack cultivated plants – are a serious threat to food security. Parasitic weeds attack many important crops, causing annual losses that affect millions of smallholder farmers and cost billions of dollars. Parasitic plants provide a valuable teaching tool topic because of their importance in agriculture and in natural ecosystems.

This teaching tool will have four major sections and learning objectives (LO's). First, we will introduce parasitism in plants and other life forms. We will explore convergent evolution and identify examples of it among parasitic plants with independent origins of parasitism. Second, we will bring host plants into the picture. We will discuss the ways in which hosts are impacted by their parasites, and we will explore antagonistic host-parasite coevolution. Third, we will investigate current control methods for parasitic plant infestations and identify aspects of parasitic weed biology that make them difficult to manage. Fourth, we will analyze in detail an evolutionary adaptation that has enabled some parasitic plants to become virulent weeds.

Learning objectives

Four sets of learning objectives correspond to the four sections of this teaching tool.

Part one: Introducing parasitism in plants

- Explore parasitic plants within the broad context of parasitism
- Identify common adaptations among parasitic plants
- Discuss the range of host dependence among parasitic plants

Part two: Bringing hosts into the picture

- Examine the coevolution of parasitic plants and their hosts.
- Describe adaptations that have arisen on both sides of this coevolutionary arms race.

Part three: Discussing the fight against parasitic weeds

- Understand why parasitic weeds are difficult to control
- Evaluate the practicality of various control methods

Part four: Exploring an adaptation that allows parasites to become virulent weeds

- Explore the convergent evolution of strigolactone perception in non-parasitic and parasitic plants.

Study/exam questions

Part one:

- What are three types of symbiotic relationships? What is a real-life example of each?
- Describe two important adaptations that parasitic plants need in order to utilize a host.
- Explain the various levels of host dependence that are observed among parasitic plants.
- Describe morphological and physiological characteristics that have evolved convergently among parasitic plants.
- What are some examples of convergent evolution that have not been discussed in class?
- What is trait reduction, and why is it commonly observed in parasites?
- Provide a hypothesis for why parasitism has evolved so many times in plants.

Part two:

- What are some common effects of parasitism on host plants?
- What is the difference between non-weedy parasites and weedy parasites? Do they differ in how they impact their hosts? (Extra challenge: Why might weedy parasites be more damaging to their hosts than non-weedy parasites?)
- Explain the Red Queen Hypothesis.
- What are the three main mechanisms of host/parasite compatibility in parasitic plants and their hosts?
- Explain the genetic basis of resistance to *Orobanche cumana* that has been observed in sunflower. Has antagonistic coevolution occurred in this system?

Part three:

- Describe the agricultural problems caused by parasitic plants. Why are parasitic weeds difficult to control?
- What are some current parasitic weed control strategies? What characteristics must they have in order to be effective?
- How can our understanding of strigolactone signaling help us develop more effective parasitic weed control strategies?

Part four:

- What are strigolactones? What do they do in parasitic and in nonparasitic plants?
- What are karrikins? How are they similar to strigolactones, and how are they different?
- Why is it difficult to study strigolactone signaling in parasitic plants? How has the model nonparasitic plant (Arabidopsis) contributed to our understanding of strigolactone signaling in parasites?
- Describe the common fates of duplicated genes.
- How do parasitic plants (likely) perceive strigolactones? Briefly describe experimental evidence supporting this mechanism of strigolactone perception.
- How has strigolactone perception evolved convergently in parasitic plants and in nonparasites?

Discussion questions

Part one:

- What environmental conditions might favor the evolution of parasitism in different kingdoms?
- Besides the characteristics already discussed, what might parasites need (physiologically, environmentally, etc.) in order to succeed?
- Let's imagine we've accidentally introduced two unrelated facultative parasitic plant species to an island where the only other plants are large trees. What convergent evolution might you expect these two parasites to undergo?

Part two:

- We know a lot about host/parasite coevolution in agricultural systems. Why might we know less about this coevolution in the wild?
- Why might wild hosts and non-weedy parasites have a different coevolutionary trajectory relative to crop hosts and weedy parasites?
- How could we go about looking for coevolution among wild hosts and non-weedy parasites?

Part three:

- Why are parasitic weeds much more devastating in sub-Saharan Africa than in the United States?
- Imagine that you are a scientist who studies parasitic weeds. You are responsible for educating smallholder farmers so that they can minimize their yield losses to parasitic weeds. How would you accomplish this? What are the most important pieces of information that you would need to share with the farmers?
- If you had unlimited money, how would you go about designing a more effective parasite control strategy?

Part four:

- Why do you think host plants produce so many different types and combinations of strigolactones?
- What are the strengths and weaknesses of using cross-species complementation and *in vitro* ligand-binding assays to study strigolactone signaling in parasitic weeds?
- Why would high *KAI2* copy number and high overall genetic variation make parasites difficult to control?

Lecture synopsis

Parasitism as a life strategy (3-16)

Parasitism is a highly successful life strategy that has convergently evolved in all known kingdoms of life. Plants aren't usually the first organisms to come to mind in discussions of parasitism, but parasitic plants are nonetheless important in natural ecosystems and in agriculture. Mycoheterotrophs are plants that indirectly steal nutrients from other plants by tapping into a fungal intermediate. Haustorial parasites form direct connections to hosts with a specialized invasive structure called a haustorium. Haustorial parasitism has convergently evolved 12 times among flowering plants, likely because stealing nutrients gives parasites a competitive advantage. To succeed as parasites, plants need mechanisms for host detection and for nutrient acquisition. Thus, similar traits have convergently evolved in plants with independent origins of parasitism. Host dependence varies among parasitic plants, but complete host dependence – or holoparasitism – has convergently evolved 11 times in plants. Holoparasitic plants often have reduced structure and no photosynthetic capability. These losses contribute to their complete dependence upon a host for survival and reproduction.

Host-parasite coevolution (17-34)

By definition, parasites cause damage to their hosts. This means that parasitic weeds exert selection pressure on their hosts; hosts that are resistant to parasite infestation will survive and reproduce more successfully than hosts that are susceptible. Here, we narrow our focus to parasitic plants in the Orobanchaceae family, which includes diverse parasite species that have been thoroughly studied because of their negative impact on agriculture. The interaction of a parasitic plant with its host can be divided into three main stages: detection of a host, attachment to a host, and post-attachment survival. Hosts might evolve resistance to parasites at any of these stages. According to the Red Queen Hypothesis, hosts and parasites exert selection on one another, and often end up in an "arms race" of antagonistic coevolution. In other words, hosts and parasites must both continually evolve to keep up with one another's adaptations. For example, parasitic weeds have evolved a germination response to strigolactones, which are hormones synthesized by host plants. Some potential hosts have lower strigolactone output, possibly making them less "visible" to parasites. Whether decreased strigolactone output is an evolutionary response to parasitism is unknown. Host plants may also be resistant to invasion by a parasite's haustorium, or they may not support a parasite after it has attached itself. These resistance mechanisms are not completely understood. In one host-parasite system, genes that can confer resistance to the parasite *Orobanche cumana* have been identified in sunflower hosts. Likely as a result of antagonistic coevolution, different "races" of *O. cumana* have evolved higher virulence, such that they can overcome the genetic resistance of sunflower hosts. The relationship between *Orobanche cumana* and sunflower is an example of the Red Queen Hypothesis in action.

The fight against parasitic weeds (35-42)

Parasitic weeds cause billions of dollars of damage each year and present a major threat to agriculture in resource-limited parts of the world. They are difficult to control

because each plant can produce tens or hundreds of thousands of tiny, easily dispersible seeds that can remain viable in the soil for years and only germinate when a host is nearby. Parasitic weeds can often attack a broad range of hosts, and they begin damaging their hosts as tiny seedlings underground. This means that by the time they become visible above the soil surface, it is often too late to prevent crop loss. A variety of control strategies have been used to combat parasitic weeds, but no current methods are sufficient. In the United States, a parasitic weed outbreak was controlled with the use of expensive strategies, such as quarantine, herbicide application, and the use of ethylene gas to stimulate suicidal seed germination in parasites. While effective, these methods are too costly to employ in resource-limited parts of the world, where parasite infestations are the most problematic. However, by targeting seed germination, it may be possible to prevent parasites from reaching their host plants in the first place. Crops that stimulate suicidal seed germination in parasites (trap crops), or that inhibit parasite seed germination (allelopathic crops), are promising control methods. Catch crops are also used, which are susceptible hosts that “catch” the parasites in a particular field, and are then harvested or destroyed before the parasites can reproduce. Knowing how parasitic weeds perceive strigolactones can help with the development of more effective trap, catch, and allelopathic crops. It can also contribute to the development of chemical stimulants of suicidal seed germination. If compounds that stimulate parasite seed germination in the absence of a host can be quickly and inexpensively synthesized, they may be useful for reducing the parasite seed bank in farmers’ soil. More effective strategies for the control of parasitic weeds are desperately needed, and understanding how parasites perceive their hosts is an important step in the fight against parasitic weeds.

How do parasite seed sense a host? (43-76)

As stated above, parasitic weeds in the Orobanchaceae germinate in response to strigolactones, which have several important functions in host plants. This adaptation of host-responsive seed germination has become an attractive target for the development of new parasite control strategies. However, until recently, the mechanism of strigolactone perception in parasitic weeds was unknown.

Genetic studies in parasitic Orobanchaceae are difficult, but strigolactone signaling has been studied extensively in several model non-parasitic plants. In Arabidopsis, a protein called D14 is a strigolactone receptor. Strigolactones are not known to stimulate germination in any non-parasitic plants, but a homolog of D14 called KAI2 is involved in seed germination. KAI2 is a receptor for smoke-derived compounds called karrikins, which promote seed germination in many non-parasitic plants. In Arabidopsis, KAI2 also likely responds to an endogenous regulator of germination and development. Other homologous proteins are involved in strigolactone signaling and in seed germination in Arabidopsis, and these two pathways share a common component called MAX2. It is currently hypothesized that strigolactone signaling in vascular plants arose from a duplication of the KAI2 signaling pathway, and, specifically, that D14 is an ancient duplicate of KAI2.

But how do parasitic plants perceive strigolactones? Unlike non-parasitic plants, parasites respond to strigolactones in seed germination. Thus, two hypotheses were developed: 1) D14 is a strigolactone receptor in parasitic plants, but it has evolved a new role in seed germination, or 2) KAI2 is involved in seed germination in parasites as it is in non-parasites, but it has evolved strigolactone responsiveness. Like Arabidopsis, parasitic plants have a single *D14* gene, but they have extra copies of *KAI2*. Duplicate genes are often implicated in adaptive evolution, because as long as one copy retains its original role, others may be freer to diverge in sequence and in function. Indeed, some parasite KAI2 proteins have been evolutionarily conserved (KAI2c), while others have an intermediate (KAI2i) or relatively rapid (divergent, or KAI2d) rate of evolution. Structural models of parasite KAI2 proteins show that conserved KAI2c have ligand-binding pockets that resemble that of Arabidopsis KAI2. KAI2d from parasites have ligand-binding pockets that resemble the non-parasite strigolactone receptor, D14.

A cross-species complementation approach was used to functionally characterize *KAI2* genes from parasitic plants. This means that *KAI2* genes were cloned from parasitic plants and transformed into Arabidopsis *kai2* null mutants. As transgenes, *KAI2c* and *KAI2i* from parasites rescued different subsets of *kai2* mutant phenotypes. This suggests that *KAI2c* and *KAI2i* in parasites each have a subset of Arabidopsis *KAI2* functions. However, *KAI2d* from parasites conferred a germination response to synthetic strigolactone, indicating that KAI2d proteins enable parasitic plants to detect nearby hosts. Other researchers tested KAI2d from parasites using *in vitro* ligand-binding experiments. They too found that KAI2d are strigolactone-responsive.

Thus, strigolactone perception has evolved convergently in non-parasitic vascular plants, in which D14 is strigolactone-responsive, and in parasitic weeds, in which KAI2d are strigolactone-responsive. D14 and KAI2d both likely arose from duplications of KAI2. Therefore, the evolution of strigolactone sensitivity in KAI2 duplicates has occurred multiple times in plant evolution.

Epilogue: in defense of parasites (77)

Parasitic plants are not all bad! In fact, they play very important roles in natural ecosystems. Parasitic plant litter is often very nutrient-rich and can increase the diversity of soil microbes. Parasitic plants can also increase plant diversity if they parasitize dominant species, thus allowing other plants to compete. They provide a food source for herbivores and nesting sites for birds. Some parasitic plants are even used by humans for food and for medicine. Although parasitic weeds are extremely destructive, the evolutionary history, diversity, and ecological importance of parasitic plants should not be ignored.

Slide concepts

Slide	Concepts
1	It's not easy being not green: the making of a parasitic plant
2	Outline
3	Part 1: Parasitism as a life strategy
4	Different ways to live together
5	Parasites are found in multiple kingdoms
6	A few remarkable parasitic plants
7	Parasitic plants are broadly classified by how they gain nutrients from a host
8	Haustorial parasitism has arisen 12 times in angiosperms
9	What drives the repeated evolution of parasitism?
10	What are common adaptations in parasitic plants?
11	Convergent evolution in parasitic plants
12	The spectrum of plant parasitism
13	The cost of becoming a parasite: when cheating leads to dependence
14	Obligate parasites must adapt to their host
15	Part 1: Recap
16	Discussion (Part 1)
17	Part 2: Host-parasite coevolution
18	How do the hosts of parasites fare?
19	Host-parasite interactions
20	The Red Queen Hypothesis: Antagonistic coevolution
21	Orobanchaceae: a family of root parasites
22	Obligate parasitic weeds in the Orobanchaceae have evolved host-triggered germination
23	Recognition of host root exudates is an important component of host range
24	Why do potential hosts produce and exude parasite germination cues from roots?
25	~20 strigolactones have been discovered in plants
26	Other chemicals in root exudates can stimulate parasite germination
27	Targeting parasite seed germination: making rice "invisible" to parasites
28	Getting attached: host invasion by parasite haustoria
29	Host resistance to parasite invasion
30	Post-attachment interactions between host and parasite
31	Post-attachment resistance in maize
32	Gene-for-gene resistance to parasites
33	Part 2: Recap
34	Discussion (Part 2)
35	Part 3: The fight against parasitic weeds
36	Parasitic weeds in the Orobanchaceae threaten food security

37	Some control methods are too expensive to be practical
38	Integrated pest management strategies target multiple stages of the parasite life cycle
39	Using plants to target parasite seed germination
40	Targeting parasite seed germination with chemistry
41	Part 3: Recap
42	Discussion (Part 3)
43	Part 4: Host perception in the parasitic Orobanchaceae
44	How do the seeds of root parasites sense strigolactones and germinate?
45	Strigolactones are plant hormones with diverse roles
46	A strigolactone receptor was found through genetic screens for mutants with excess shoot branches
47	Strigolactone signaling in *Arabidopsis thaliana*
48	Arabidopsis germination is controlled by homologs of the strigolactone signaling pathway
49	Karrikins are germination stimulants found in smoke that are butenolide molecules, like strigolactones
50	Karrikins and strigolactones are rhizosphere signals that convey opposite messages to seeds
51	Fire followers and parasites have evolved distinct germination responses to karrikins and strigolactones
52	How did parallel MAX2-dependent signaling mechanisms arise?
53	Strigolactone signaling in higher plants may have evolved after an ancient duplication of *KAI2* and *SMAX1* genes
54	*D14* likely arose from *KAI2* duplication
55	D14 and KAI2 perceive different signals and have different functions in growth
56	Promoter swapping shows that *KAI2* and *D14* functional differences are not simply due to expression
57	How might strigolactone-specific germination have evolved in parasites?
58	Study species for a molecular evolutionary analysis of *KAI2* and *D14*
59	*KAI2* copy number is increased in several parasite genomes
60	Common evolutionary fates following gene duplication
61	Duplicate gene evolution → adaptation
62	Assessing positive selection on genes
63	Parasite *KAI2* paralogs fall into three groups
64	*KAI2* paralogs in a divergent clade evolved more rapidly
65	KAI2 and D14 have different ligand-binding pocket shapes
66	Homology models suggest divergent KAI2 mimic D14
67	Homology models suggest divergent KAI2 mimic D14
68	Cross-species complementation strategy to test the function of parasite *KAI2*
69	Divergent *KAI2* from two parasite species confer strigolactone responses to

	Arabidopsis seed
70	In-depth cross-species complementation analysis of *KAI2/HTL* from *Striga hermonthica*
71	KAI2/HTL in *Striga hermonthica* show different affinities for strigolactones *in vitro*
72	Implications for the evolution of parasitic weeds
73	Proposed model for germination control in parasites
74	Convergent evolution of strigolactone perception
75	Part 4: Recap
76	Discussion (Part 4)
77	Epilogue: In defense of parasites
78	Conclusions