Gene discovery in in the parasitic plant *Cuscuta pentagona*: Clues from ivy leaf morning glory (*Ipomoeae hederacea*) expressed sequence tags.

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Introduction: The plant as a parasite

Just as some microbes and animals make a living parasitizing other organisms, many species of plants make their living by parasitizing other plants. The parasitic habit has arisen several times among flowering plant lineages. One feature that all parasitic plants share is the use of penetrating organs, called haustoria, to connect to the host in order to draw upon its water and nutrient supply. Beyond the occurrence of haustoria, however, parasitic plants can vary widely in their degree of modification and in the degree to which they are dependent upon their hosts. Some parasitic plants can complete their life cycles without engaging in parasitism, while others are wholly dependent upon their hosts.

Species of *Cuscuta*, commonly called the dodders, lack roots, have only vestiges of leaves, and perform very little or no photosynthesis in most tissues. As such, they are wholly dependent upon their hosts for water and mineral nutrients, as well as for a majority of their energy needs. In order to determine the physiological consequences of adopting the parasitic habit, we are investigating the function and fate of genes that are expressed in tissues that have undergone reduction or have been lost in the parasite. During the summer session of 2008, the Biology-STEP molecular research team examined populations of expressed sequence tags (ESTs) from immature leaf tissue of ivy-leaf morning glory, a close relative of dodder, to identify candidate genes that may have been impacted by dodder's adoption of the parasitic habit.

Figure 1. The parasitic angiosperm *Cuscuta pentagona* on its host (left) and its non-parasitic relative *Ipomoea hederacea*. Recent molecular investigations place the genus *Cuscuta* in the family Convolvulaceae with *Ipomoea*. Molecular and physiological investigations may provide clues as to the changes that accompanied the adoption of the parasitic habit, which included the loss of functional leaves and roots, and, likely, the modification of core metabolic processes.

The 2008 Biology-STEP molecular research team

L-R, front: Alicia Ramsey, Jacklyn Miller; middle: Sara Ramsey, Jennifer Hasiak, Kara Tischer, Jessica Walter, Amrit Kandel, Sydney Brommer (laboratory assistant); back: Branden Poe, Daniel Bundrick, Qianli Wang, Joshua DeWitt, and Mark Schoenbeck (instructor).

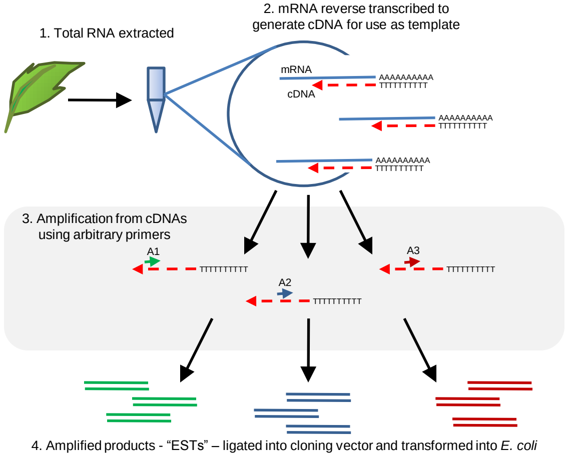

Figure 2. **Generation of leaf tissue ESTs**. Transformed *E. coli* carrying ESTs from *I. hederacea* leaf were maintained as colonies for subsequent analysis.

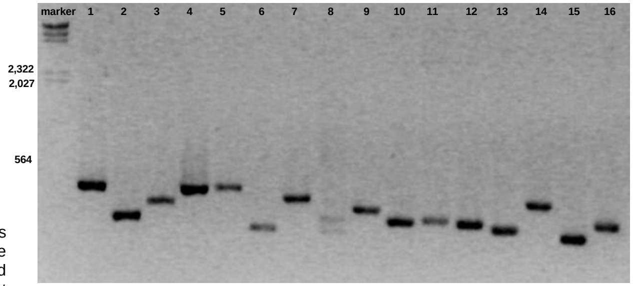

Figure 3. **Size distribution of selected *I. hederacea* ESTs**. Amplification from the cloning vector T7 and Sp6 flanking sites generated products indicative of the inserts' sizes. This image shows an example generated from EST clone pool A2. Students chose multiple ESTs with distinct sizes for subsequent analysis. As a group the students sequenced more than thirty EST clones.

Table I. Putative identities of select *I. hederacea* immature leaf ESTs.

clone	provisional identification [a]
A2-7	40S ribosomal protein
A7-1	cyclophilin –type peptidyl-prolyl cis-trans isomerase
A7-2	ferredoxin family protein
A9-8	DNA dependent RNA polymerase
A10-8	serine-threonine protein kinase
A13-8	heat shock protein
A15-3	thioredoxin peroxidase
A16-4	non-photosynthetic ferredoxin
A21-2	C3C4-type RING protein
A22-1	2-cys peroxyredoxin-like protein

[a] Determined by submitting nucleic acid sequence data to the NCBI BlastX database search function.

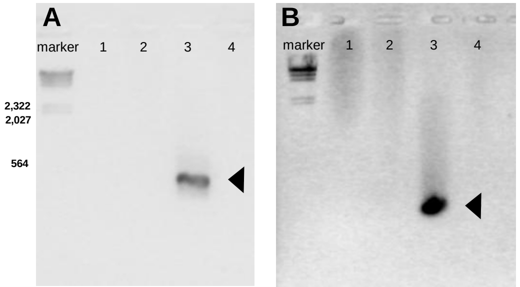

Figure 4. **Attempted recovery of cognate sequences from the *C. pentagona* genome**. Using EST sequence data and available sequences from Genbank, students designed oligonucleotide primers for the amplification of non-photosynthetic ferredoxin (A) and the 40 S ribosomal protein (B). Lanes show the amplified products with 1 – forward primer alone with genomic template, 2- reverse primer alone with genomic template, 3 – forward and reverse primers together with genomic template, and 4 – forward and reverse primers together in the absence of genomic template. Amplification products, indicated by arrowheads, will be the subject of further examination, to determine whether these represent EST-cognate gene sequences in dodder.

Conclusions and future work

Several *I. hederacea* immature leaf ESTs were examined, including putative genes with functions associated with photosynthesis or cellular redox regulation, and others that may be regarded as having non-specialized "housekeeping" functions. Subsequent works will examine whether the loss of leaves and reduction of photosynthetic activity, accompanying the adoption of the parasitic habit, is reflected in the function of these genes in dodder.

Acknowledgements

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