

Intraindividual sequence variation in pre-rRNA cistrons of the ectoparasitic plant *Cuscuta*



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Abstract
Cuscuta, commonly called dodder (family Convolvulaceae), is an ectoparasite of numerous plants, sucking nutrients from its hosts using haustoria, which penetrate into the vascular tissue of the host. We have demonstrated that several species of *Cuscuta*, especially *C. pentagona*, show considerable variation in the sequence of its pre-rRNA cistrons. The phylogenetic relationship between these sequences is presented and the possible significance of this diversity is discussed.

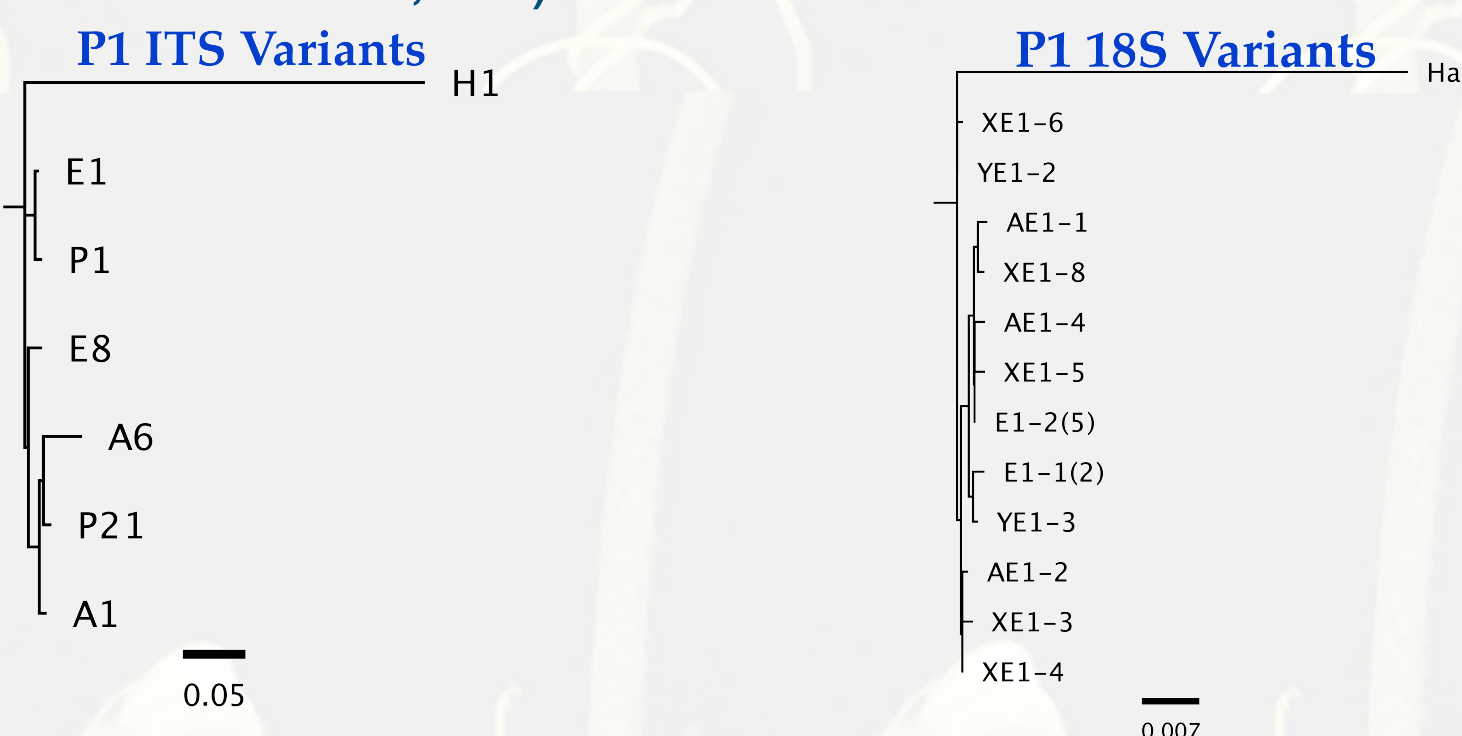
Cuscuta pentagona, the fiveangled dodder, shows a high degree of intraindividual sequence variation in its rRNA sequences

Background

- 🌻 *Cuscuta*, commonly called dodder, is a member of the family Convolvulaceae and is an ectoparasite of various plants, sucking nutrients from the hosts with haustoria.
- 🌻 The genetic material for the primary rRNAs of eukaryotes consists of hundreds or thousands of tandemly repeated copies of this pre-rRNA cistron.
- 🌻 These genes appear to be under concerted evolution due to a homogenizing mechanism that keeps all of these copies identical.
- 🌻 Intraindividual variation in rRNA sequences is the result of a failure of this homogenizing mechanism and has been demonstrated in freeliving (*Acanthamoeba*) and (parasitic (*Plasmodium*) protists, a unicellular fungus (*Zygosaccharomyces*), freeliving (*Dugesia*) and parasitic (*Trypanosoma cruzi*) flatworms, a parasitic nematode (*Rotylenchulus reniformis*), an insect vector of malaria (*Anopheles punctulatus*), an ostrocod arthropod (*Darwinula stevensoni*), the stone flounder (*Kareius bicoloratus*), all nine North American sturgeons (family Acipenseridae), and two angiosperms (*Panax ginseng* and *Halophila stipulacea*, a marine angiosperm).

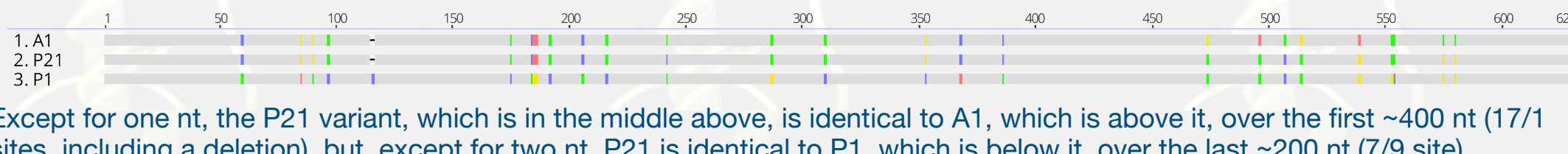
Results

- 🌻 While searching for an appropriate genes for RFLP species determination, we noticed that clones from single museum specimens of a segment of the pre-rRNA from several species of *Cuscuta* showed apparent intraindividual variation, most notably seen in *C. pentagona*, the fiveangled dodder.
- 🌻 A more detailed analysis of pre-rRNA of *C. pentagona* freshly collected from one site in Marengo County AL confirmed this high degree of intraindividual variation.
- 🌻 We used Q5® Hot Start High-Fidelity polymerase PCR P1 (NEB) in order to reduce PCR error.
- 🌻 During DNA extraction, samples were washed with water then ethanol to decrease the chance of contaminating DNA.
- 🌻 Two primer sets were used:
 - 🌻 ITS5 and ITS4 amplify both internal transcribed spacers (ITS) and the entire 5.8S rRNA plus small segments of the 18S and 25S rRNAs.
 - 🌻 EukA and EukB amplify the entire 18S rRNA gene.
- 🌻 Amplicons were TOPO-TA cloned (ThermoFisher) and sent for colony sequencing to GeneWiz (South Plainfield NJ).
- 🌻 Six and 12 unique sequences were isolated from plant 1 (P1) using the ITS primers and the Euk (18S) primers, respectively.
- 🌻 Neighbor-joining trees (outgroups are the host, H1, and the common sunflower, Ha):



- 🌻 Sequencing of three other nearby plants revealed 4 additional 18S variants (data not shown).
- 🌻 Except for one single nucleotide deletion (present in two clones), all 18S variants were single base substitutions.
- 🌻 Alignments of sequences suggest the possibility that recombination events are responsible for some variants:

ITS Variants: Possible Recombination



Discussion

- 🌻 The high degree of intraindividual variation seen in *C. pentagona* may prove helpful in the investigation of the process of rRNA gene homogenization.
- 🌻 Recombination between pre-rRNA genes has been proposed to be a factor in maintaining the homogeneity of these cistrons. Our results show evidence of such recombination.
- 🌻 While recombination seems to be the most likely explanation of variants like A1, P21, and P1 above, further analysis is needed to rule out the possibility that they may have arisen by PCR jumping.
- 🌻 Several organisms with this intraindividual variation are parasites, as is ours. Is there a relationship?
- 🌻 Krieger and Fuerst (2004, J Appl Ichthyol 20:1) demonstrated that although a lake sturgeon has 17 different 18S variants, only a single one is expressed. We are currently using RT-PCR and colony sequencing to determine if the numerous 18S variants discovered in *C. pentagona* are all expressed.

Acknowledgements

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