# RIBOSOMAL RNA UNIT EVOLUTION

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The fungal genus *Ceratocystis* includes a number of pathogenic species affecting a variety of hosts, making the study of these fungi economically significant. It is vital to correctly identify and classify these species in order to address the management and spread of diseases associated with *Ceratocystis*. Various methods have therefore been developed to identify fungal species, of these DNA sequence based methods are most extensively used in fungal systematics.

For fungal taxonomy, the ribosomal RNA unit is of special importance because various regions of this unit are frequently targeted for DNA-based identification. The RNA unit (cistron) is present in repeated units within the genome of organisms and includes genes that form the ribosome. The genes are separated by means of spacers. The internally transcribed spacer (ITS) region is used as the standard DNA barcoding region for fungal identification.

It has been proposed that the ITS barcoding gene region is under evolutionary forces which allow for the gene sequences to become mixed within a gene cluster. This is known as concerted evolution. The potentially far-reaching results of concerted evolution on fungal taxonomy and diagnostics require a more detailed understanding of this evolutionary process in fungi. Concerted evolution prevents harmful mutations from accumulating in gene clusters. To date, not much of this process is well understood. A chance discovery of a *Ceratocystis* species, *Ceratocystis manginecans*, which contained two distinct ITS sequences within the ribosomal RNA unit, enabled a study of concerted evolution in this fungus. As is the case with many *Ceratocystis* species, *C. manginecans* is a homothallic fungus, which means it can undergo both sexual (meiotic) and asexual (mitotic) life cycles (Fig. 1).

The aim of this research was to investigate if unequal crossing over in the ITS gene regions contributed significantly to gene conversion events within the ribosomal RNA unit. Both life stages (i.e during meiosis or mitosis) in the fungal tree pathogen *Ceratocystis manginecans* were targeted. The DNA isolated from vegetatively cultured isolates of *C. manginecans* for each life cycle was used to amplify the ITS gene region. The ratios of the different ITS sequence types were analysed in order to determine if the changes in the observed ratios were significant (Fig. 2 gives a schematic overview of this process.)

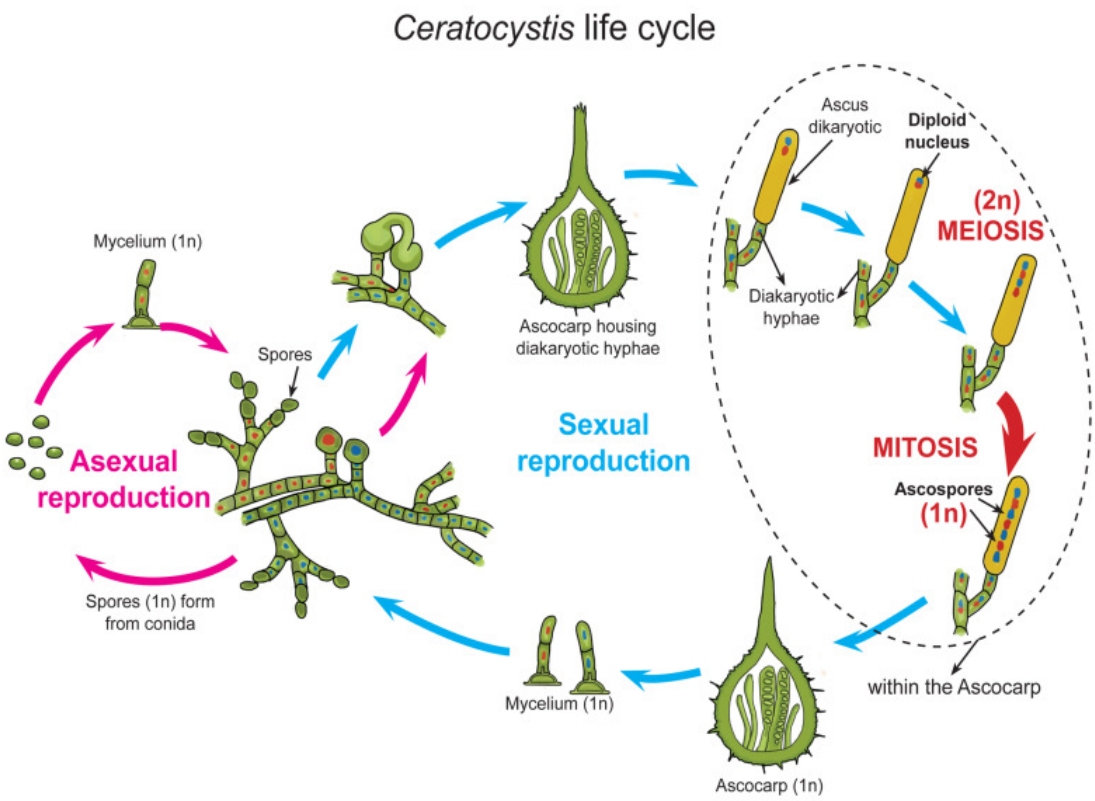


**Figure 1. Life cycle of a *Ceratocystis* species.** Shown here for this typical ascomycete includes both sexual (meiotic) and asexual (mitotic) cycles. In this case both the meiotic and mitotic states occur in a single haploid culture, a condition known as homothallism in fungi.

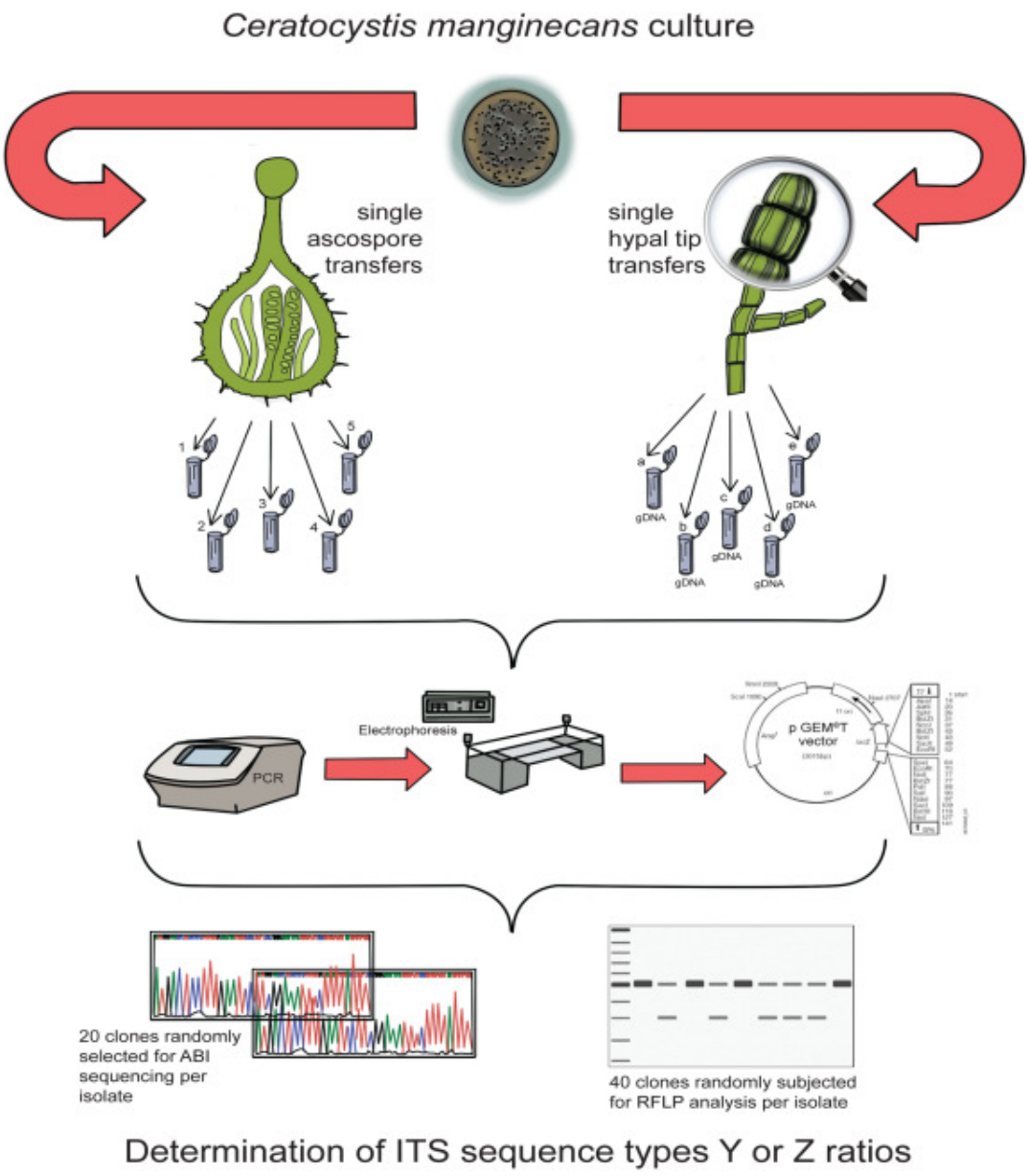


**Figure 2. Diagrammatic representation of the methodology employed in the experimental design.** The fungus *Ceratocystis manginecans* isolate (CMW 17568) was derived from single meiospores (ascospores) to generate the meiotic progeny, and the four sequential single hyphal tip isolations generated the mitotic generations of the fungus.

The relative ratios of the two ITS sequence types changed when the fungal isolates were cultured vegetatively as well as during sexual reproduction. These active changes were

shown to occur more frequently during meiosis than mitosis. Using this non-model organism the study showed experimental evidence for unequal crossing over and gene conversion as the ultimate forces acting on this gene regionn dictating a concerted evolutionary effect. It can thus be suggested that this process is true for all eukaryotes.

The full scientific paper is available on the PLoS ONE webpage:
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