Annotation of Fungal Transposable Elements

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Abstract

Transposable elements (TE), also called transposons, in fungi have been shown to be regions of high DNA variability and subsequent gene evolution. This flexibility renders them important for fungal adaptation to changing hosts and environments. Several families of TEs have been identified; however, the methods and pipelines used to classify them are relatively new and rely heavily on BLAST output. In this study we augment traditional nucleotide and protein searches with statistics on several notable features of both RNA and DNA transposons. Specifically, we examine long terminal repeats (LTRs), terminal inverse repeats (TIRs) and helitron motifs. These features are used to confirm the results of homology testing. This classification system was tested using previously annotated transposons from three genomes: *Laccaria bicolor* (73 sequences), *Aspergillus nidulans* (38), and *Saccharomyces cerevisiae* (18) and correctly annotated all of them. When run on a wide array of fungi, including several closely related members of the *Ascomycete* and *Basidiomycete* families, the system revealed a relatively low proportion of transposons (<10%, with the exception of *A. alcalcophilum* and *L. bicolor*) but significant diversity between even closely related species.

Introduction

Fungal genomics represent a vast potential for ameliorating energy demands in the US. There are several established genetic characteristics/motifs for annotating fungal and plant genomes. One such characteristic is the transposable element (TE): segments of DNA that often replicate and insert themselves hundreds to thousands of times within a genome. Transposons increase genetic diversity and can affect gene expression via mutations and alteration of regulating mechanisms [1]. Various genomes have also demonstrated the ability to adapt to potentially disadvantageous transposon insertions by down-regulating affected genes or showing a tendency towards greater transposon presence in non-coding DNA segments [2]. TEs have been previously classified into families based on several auxiliary attributes and DNA homology to known repeat sequences. These methods rely heavily on BLAST searching, and can be difficult to transfer to different hardware systems [3].

We implement additional searches based on structural characteristics, including long terminal repeats (LTR), terminal inverse repeats (TIR), and helitrons. These additions greatly increase the number of transposons successfully classfied, both in our validation study as well as in numerous full genomes.

Methods & Validation

The transposon categories and structural characteristics are shown in Figure 1 [3]. The classification system uses RepeatScout [4] (RS) output files (consensus sequences and location coordinates of repeat candidates), and searches for both DNA/protein homology and the occurrence of these features.
BLASTn and BLASTp DNA/protein homology is used to isolate the best hit for each RS repeat sequence. The LTR/TIR search uses Smith-Waterman string matching to find nearly identical strings with minimal length of 70bp and 50bp for LTR and TIR, respectively. The helitron search examines ~10bp regions flanking transposon occurrences for secondary structure motif, and searches the trans.

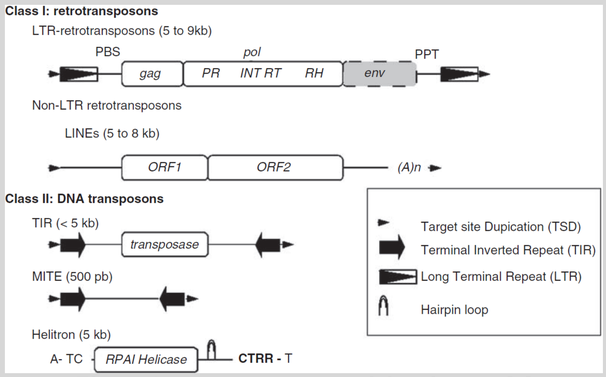


Figure 1. Transposon types and their features [3]

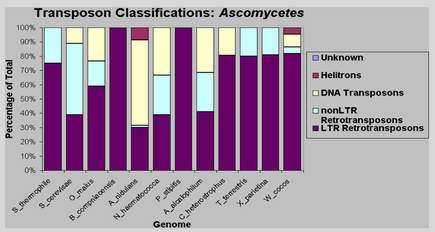
Genome	Curated TEs	Correctly reclassified by homology	Correctly reclassified by LTR/TIR
A. nidulans	38	9	29
S. Cerevisiae	18	6	12
L. bicolor	73	33	40

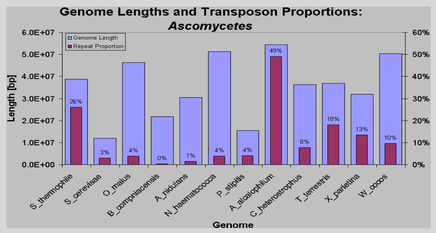
Table 1. System verification results.

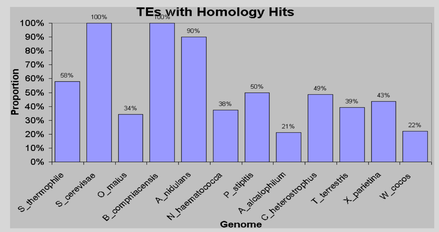
System Verification:

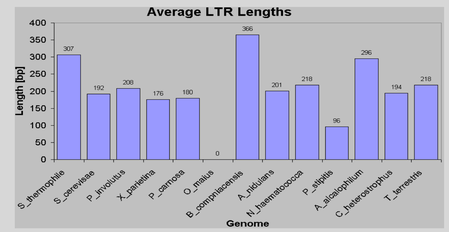
- Curated transposons were reclassified to five categories: LTR, nonLTR, DNA (TIR and MITE), Helitron, and unknown. All sequences with homology results were correctly classified. Those without homology results were classified based on presence/absence of LTRs and TIRs, as shown in Table 1. Helitrons were not found in these data sets.

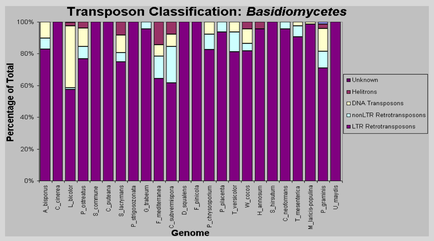
Results

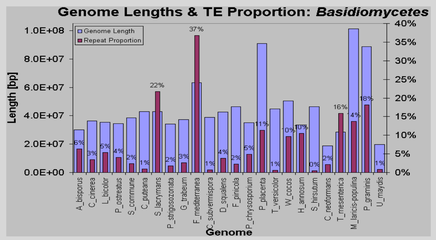


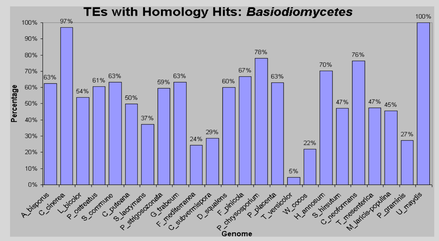


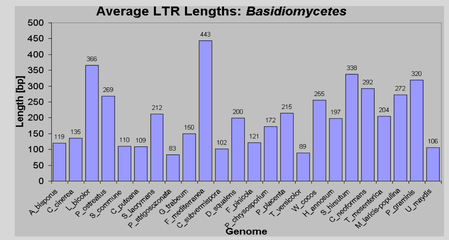












Results:

- For both *Ascomycetes* and *Basidiomycetes*, TEs are dominated by LTRs, followed by non-LTR retrotransposons. However, LTR lengths varied greatly for all species.
- For most species, repeat fraction was consistently low (<10%), regardless of genome size.
- Homology accounted roughly half of all classifications in both groups (53.7% and 54.5%); a significant number of results were derived from the added feature classifications.

***Septoria* Case Study:**

- Two closely related *Septoria* fungi with significantly different repeat fractions were studied. Table 2 shows *S. populicola's* larger genome cannot account for the repeat fraction increase. We hypothesize there are undetected repeats in *S. musiva*. Combining the libraries yields marginal repeat fraction increases in both genomes.
- Decreasing the RS threshold from the standard 150x yields comparable increases for both. We conclude that the repeat content of *S. populicola* and *musiva* are significantly different.

Genome	Length	Repeat Proportion [genome specific lib]	Repeat Proportion [combined lib]
S. musiva	29.3Mbp	3.5%	4.2%
S. populicola	33.2Mbp	20.7%	20.8%

Tables 2-3. *Septoria* repeat fractions for separate and combined repeat libraries (Table 2) and decreasing RepeatScout threshold (Table 3).

Repeat Frequency Threshold	*S. musiva*	*S. populicola*
150x	4.2%	20.8%
100x	6.1%	20.8%
50x	7.7%	23.1%
10x	9.9%	25.6%
1x	10.4%	26%

Conclusions

- We developed a system for transposon classification, and validated it using curated sequences.
- For tested genomes, Class I retrotransposons dominate the classification, but even closely related genomes may show significantly different repeat content, as illustrated by *Septoria*.

References

[1] F. Kempken , "Fungal transposons: from mobile elements toward molecular tools," *Appl Microbiol Biotechnol*, vol. 52, pp. 756-760, 1999.

[2] C. Feschotte et al. "Exploring Repetitive DNA Landscapes USING REPCLASS, a Tool That Automates the Classification of Transposable Elements in Eukaryotic Genomes," *Genome Biol Evol*, vol. 2009, pp. 205-220, 2009.

[3] E. Lerat, "Identifying repeats and transposable elements in sequenced genomes: how to find you way through the dense forest of programs," *Heredity*, vol. 104, pp. 520-533, 2010.

[4] A. Price, N. Jones, and P. Pevzner. "*De novo* identification of repeat families in large genomes," *Bioinformatics*, vol. 16, pp. 351-358, 2005.

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