A novel test for host-symbiont codivergence indicates ancient origin of fungal endophytes in grasses

Problem

Suppose we have a set of DNA sequences for host species H and a set of DNA sequences for parasite species P. We would like to study the co-evolution between the host species and the parasite species. More precisely, T_H and T_P are phylogenetic trees reconstructed from the data sets H and P. suppose the hypotheses are:

Null hypothesis: Trees T_H and T_P are independent.

Alternative hypothesis: Trees T_H and T_P are not independent.

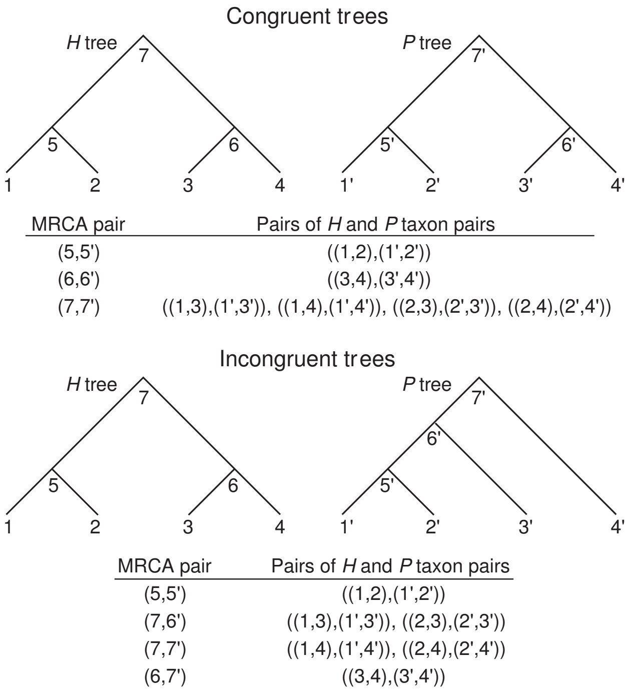
Data

We used 25 grasses and endophytes for full trees. See Table 1 for a list of species. For phylogenetic analysis, sequences from endophyte tub2 and tef1 genes were aligned, then concatenated into a single, contiguous sequence for each endophyte. Likewise, plant chloroplast sequences including two intergenic regions (trnT to trnL, and trnL to trnF) and the trnL intron sequence were aligned individually and concatenated to give a dataset of approximately the same size for each host grass, and then appended to yield a combined sequence alignment of approximately 2200 bp.

Table 1: **Hosts and symbionts**: All listed taxa, as well as trimmed taxon sets T_1–T_4, were assessed for probability of codivergence.

		Included in:			
Grasses	Endophytes	T_1	T_2	T_3	T_4
Brachyelytrum erectum (root)	*Epichloë brachyelytri* (root)	+	+	+	+
Brachypodium sylvaticum	*Epichloë sylvatica* 200751	+	−	+	−
Echinopogon ovatus	*Neotyphodium aotearoae* 829	+	−	+	−
Calamagrositis villosa	*Epichloë baconii* 200745	+	+	+	+
Agrostis tenuis	*Epichloë baconii* 200746	+	+	+	+
Agrostis hiemalis	*Epichloë amarillans* 200744	+	+	+	+
Sphenopholis obtusata	*Epichloë amarillans* 200743	+	+	+	+
Koeleria cristata	*Epichloë festucae* 1157	+	+	−	−
Lolium sp. P4074	*Neotyphodium* sp. FaTG2 4074	+	+	+	+
Lolium sp. P4078	*Neotyphodium* sp. FaTG3 4078	+	+	+	+
Lolium arundinaceum	*Neotyphodium coenophialum* 19	+	+	+	+
Lolium multiflorum	*Neotyphodium occultans* 999	+	+	+	+
Lolium edwardii	*Neotyphodium typhinum* 989	−	−	−	−
Lolium perenne	*Epichloë typhina* 200736	−	−	−	−
Lolium perenne	*Neotyphodium lolii* 135	+	+	−	−
Festuca rubra	*Epichloë festucae* 90661	+	+	+	+
Festuca longifolia	*Epichloë festucae* 28	+	+	+	+
Holcus mollis	*Epichloë* sp. 9924	+	+	+	+
Hordelymus europaeus	*Neotyphodium* sp. 362	+	+	+	+
Bromus ramosus	*Epichloë bromicola* 201558	+	+	+	+
Bromus erectus	*Epichloë bromicola* 200749	+	+	+	+
Bromus purgans	*Epichloë elymi* 1081	+	+	−	−
Hordeum brevisubulatum	*Neotyphodium* sp. 3635	+	+	+	+
Elymus canadensis	*Epichloë elymi* 201551	+	+	+	+
Glyceria striata	*Epichloë glyceriae* 200755	+	+	+	+
Achnatherum inebrians	*Neotyphodium gansuense* 818	+	+	+	+

MRCALink algorithm



MRCA pair	Pairs of H and P taxon pairs
(5,5')	((1,2),(1',2'))
(6,6')	((3,4),(3',4'))
(7,7')	((1,3),(1',3')), ((1,4),(1',4')), ((2,3),(2',3')), ((2,4),(2',4'))

MRCA pair	Pairs of H and P taxon pairs
(5,5')	((1,2),(1',2'))
(7,6')	((1,3),(1',3')), ((2,3),(2',3'))
(7,7')	((1,4),(1',4')), ((2,4),(2',4'))
(6,7')	((3,4),(3',4'))

Figure 1: Simple examples of congruent and incongruent H and P trees, demonstrating the relationships of MRCA pairs to their corresponding pairs of H and P taxon pairs. In an ultrametric time tree, the distance between any two taxa is twice the age of their MRCA. In each tip clade a MRCA uniquely relates two taxa. However, a MRCA deeper in the tree relates multiple taxon pairs. Therefore, for congruent H and P trees the matrix of all pairwise distances of H taxon pairs against all pairwise distances of P taxon pairs represents each corresponding pair of tip clade MRCAs only once, and each corresponding pair of deeper MRCAs multiple times. This relationship is more complicated in the case of incongruent trees, which nevertheless tend to give greater representation to pairs of deeper MRCAs than to pairs of shallower MRCAs in pairwise distance matrices. The MRCALink algorithm samples corresponding H and P MRCA pairs only once.

The MRCALink algorithm introduced here identifies and stores each corresponding H and P MRCA pair. Crucially, the data for each corresponding MRCA pair is selected only once for subsequent statistical analysis. Trees must be strictly bifurcating for unique identification of valid pairs of H and P MRCAs. Note that the method does not assume an equal number of taxa in H and taxa in P, and also does not assume similar mutation rates in H and P. Given a set of host taxa H and a set of symbiont taxa P ("parasites," in keeping with other literature in the field), there is a map called $L : H \to P$ such that a host $A \in H$ has a parasite or symbiont $L(A) \in P$. Define $MRCA(A, B)$ to be the node representing the Most Recent Common Ancestor (MRCA) of leaves A and B.

Algorithm 1 (The MRCALink Algorithm)**.**

- **Input** *a set of host taxa H, a set of parasite taxa P, a H tree T_H, and a P tree T_P where n_1 is the number of taxa in H and n_2 is the number of taxa in P.*
- **Output** *a set of MRCA pairs of host taxa and parasite taxa.*
- **Algorithm**

```
Assign each node a unique number from $1$ to $2n_1 - 1$ in the host tree and
a unique number from $1$ to $2n_2 - 1$ in the parasite tree such that a node $i$ is
ancestral to a node $j$.
Let $U$ be a set of pairs of $H$ and $P$ node pairs, initially empty.
for ($i$ from $n_1 + 1$ to $2n_1 - 1$) do{
  Set $X_i = l_i \times r_i$ where $l_i$ is the set of all left-descendents of $i$,
    and where $r_i$ is the set of all right-descendents of $i$.
  /* This is just another way of saying $X_i$ is all such pairs of one leaf
    from the left and one from the right. */
  while ($X_i \neq \emptyset$) do{
    Choose $x = MRCA(a, b) \in X_i$ and identify $y_j = MRCA(L(a), L(b))$ for each
      distinct $L(a)$ and $L(b)$.
    Remove $x$ from $X_i$.
    for (each distinct $y_j$) do{
      if ($MRCA(x, y_j) \notin U$) do{
        $U \leftarrow U \cup MRCA(x, y_j)$.
      }
    }
  }
}
Output $U$.
```

Dissimilarity method

We are interested in estimating the probability that the host and symbiont tree have some degree of dependence that may be due to a history of codivergence. To this end, we use the sets of all pairwise differences in H and P or the sets of pairwise differences in H and P from the the MRCA pairs sampled by the MRCALink algorithm. Let the sum of differences in uniquely estimated MRCA ages for trees A and B be $S(A, B)$. The null hypothesis is that our T_H and T_P are independent, so we generate a distribution of S for pairs of unrelated random trees with the same number of leaves and root-to-tip normalized distances (i.e., we normalize the heights of T_H and T_P to 1) as T_H and T_P. Then we compare our $S(T_H, T_P)$ with this distribution. If the p-value is significantly low (< 0.05), we reject the null hypothesis and conclude that there is evidence of codivergence between T_H and T_P. To calculate $S(A, B)$ with all pairwise distances, we take the sum of difference between pairwise distances for A and B over all pairwise distances. To calculate $S(A, B)$ with the set of the MRCA pairs sampled by the MRCALink algorithm we take the sum of differences between pairwise distances for A and B over the set of the MRCA pairs sampled by the MRCALink algorithm.

We generate $10,000$ random trees with the given branch lengths from the BDP via `evolver` from the `PAML` package for each T_H and T_P. For each tree, we used birth rate 0.5, death rate 0.5, and sampling fraction 1, 0.5, 0.001, 0.0005 (sampling fraction is the ratio of sample size to population size). We use the BDP for its biological justifications.

Results are expressed as p, the probability that the pattern of corresponding node ages are independently developed. Thus, we reject the null hypothesis that T_H and T_P

are independent if p is less than 0.05.

Results

Table 2: The p-values obtained by applying the dissimilarity method to all pairwise distances (noted by ALL) and to the MRCALink-derived matrix (noted by MRCA) for full and $T_1 - T_4$ plant and endophyte data sets (see Table 1 for the data sets). SF means a sampling fraction.

Method	Data	SF = 0.0005	SF = 0.001	SF = 0.5	SF = 1.0
ALL	Full	0.7843	0.7831	0.6768	0.3741
MRCA	Full	0.1234	0.1228	0.0813	0.0388
ALL	T_1	0.1165	0.115	0.0345	0.0089
MRCA	T_1	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_2	0.0934	0.0849	0.027	0.0116
MRCA	T_2	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_3	0.0639	0.0607	0.0173	0.0054
MRCA	T_3	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_4	0.0178	0.0199	0.0046	0.0017
MRCA	T_4	< 0.001	< 0.001	< 0.001	< 0.001

Table 3: The p-values obtained using the dissimilarity method with sub-optimal trees with 26 full and $T_1 - T_4$ plant and endophyte data sets (all taxa listed in Table 1) via the Bayesian MCMC method. ALL means the dissimilarity method with all pairwise distances and MRCA means the dissimilarity method with the MRCALink-derived matrix. SF means a sampling fraction. Each sampled tree is assigned number from 1 to 3 to distinguish it from the others.

Method	Data	sample number	SF = 0.0005	SF = 0.001	SF = 0.5	SF = 1.0
ALL	Full	sample 1	0.7002	0.6858	0.4656	0.2942
MRCA	Full	sample 1	0.0107	0.0112	0.0029	0.0018
ALL	Full	sample 2	0.4742	0.4833	0.2452	0.1192
MRCA	Full	sample 2	0.0636	0.0643	0.0253	0.0136
ALL	Full	sample 3	0.6842	0.6833	0.4499	0.2617
MRCA	Full	sample 3	0.193	0.1898	0.1022	0.0608
ALL	T_1	sample 1	0.4505	0.4478	0.2361	0.1152
MRCA	T_1	sample 1	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_1	sample 2	0.0285	0.0327	0.0049	0.0009
MRCA	T_1	sample 2	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_1	sample 3	0.0064	0.007	0.0006	< 0.001
MRCA	T_1	sample 3	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_2	sample 1	0.3459	0.3548	0.190	0.0965
MRCA	T_2	sample 1	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_2	sample 2	0.3547	0.3601	0.1836	0.0991
MRCA	T_2	sample 2	0.0007	0.0001	0.0002	< 0.001
ALL	T_2	sample 3	0.0837	0.0788	0.0218	0.0103
MRCA	T_2	sample 3	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_3	sample 1	0.0695	0.0673	0.0202	0.0072
MRCA	T_3	sample 1	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_3	sample 2	0.0301	0.0293	0.0065	0.0297
MRCA	T_3	sample 2	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_3	sample 3	0.1318	0.1378	0.0498	0.0208
MRCA	T_3	sample 3	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_4	sample 1	0.1062	0.1029	0.0389	0.0147
MRCA	T_4	sample 1	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_4	sample 2	0.02407	0.0261	0.0069	0.0017
MRCA	T_4	sample 2	< 0.001	< 0.001	< 0.001	< 0.001
ALL	T_4	sample 3	0.0174	0.0161	0.0056	0.0015
MRCA	T_4	sample 3	< 0.001	< 0.001	< 0.001	< 0.001