

*promoting access to White Rose research papers*



**Universities of Leeds, Sheffield and York**  
**<http://eprints.whiterose.ac.uk/>**

---

This is the Author's Accepted version of an article published in **Statistical Applications in Genetics and Molecular Biology**

White Rose Research Online URL for this paper:

<http://eprints.whiterose.ac.uk/id/eprint/78448>

---

**Published article:**

Mramba, LK, Barber, S, Hommola, K, Dyer, L, Wilson, J, Forrister, ML and Gilks, WR (2012) *Permutation tests for analysing cospeciation in multiple phylogenies: applications in tri-trophic ecology*. *Statistical Applications in Genetics and Molecular Biology*, 12 (6). 679 - 701. ISSN 1544-6115

<http://dx.doi.org/10.1515/sagmb-2012-0073>

---

Short running title :  
Analysing Cospeciation in Multiple Phylogenies

# Permutation Tests for Analysing Cospeciation in Multiple Phylogenies: Applications in Tri-trophic Ecology

Lazarus K. Mramba<sup>\*‡</sup>, Stuart Barber<sup>†</sup>, Kerstin Hommola<sup>†</sup>, Lee A. Dyer<sup>±</sup>, Joseph  
S. Wilson<sup>±</sup>, Matthew L. Forister<sup>±</sup>, and Walter R. Gilks<sup>†‡</sup>

<sup>‡</sup> University of Florida, School of Forest Resources and Conservation, P.O. Box  
110410, Gainesville FL 32611-0410, United States of America. Email: lmramba@ufl.edu

<sup>\*</sup> Kenya Medical Research Institute - Wellcome Trust Research Programme, P.O.  
Box 230, 80108, Kilifi, Kenya

<sup>†</sup> Department of Statistics, School of Mathematics, University of Leeds, Leeds LS2  
9JT, United Kingdom

<sup>±</sup> Biology Department, MS 314, University of Nevada, Reno, Nevada 89557, United  
States of America

<sup>‡</sup> Biomathematics and Bioinformatics Department, Rothamsted Research, Harpenden,  
Hertfordshire, AL5 2JQ, United Kingdom.

## Abstract

There is a need for a reliable statistical test which is appropriate for assessing cospeciation of more than two phylogenies. We have developed an algorithm using a permutation method that can be used to test for and infer tri-trophic evolutionary relationships of organisms given both their phylogenies and pairwise interactions.

An overall statistic has been developed based on the dominant eigenvalue of a covariance matrix, and compared to values of the statistic computed when tree labels are permuted. The resulting overall  $p$ -value is used to test for the presence or absence of cospeciation in a tri-trophic system. If cospeciation is detected, we propose new test statistics based on partial correlations to uncover more details about the relationships between multiple phylogenies.

One of the strengths of our method is that it allows more parasites than hosts or more hosts than parasites, with multiple associations and more than one parasite attached to a host (or one parasite attached to multiple hosts). The new method does not require any parametric assumptions of the distribution of the data, and unlike the old methods, which utilise several pairwise steps, the overall statistic used is obtained in one step.

We have applied our method to two published datasets where we obtained detailed information about the strength of associations among species with calculated partial  $p$ -values and one overall  $p$ -value from the dominant eigenvalue test statistic.

Our permutation method produces reliable results with a clear procedure and statistics applied in an intuitive manner. Our algorithm is useful in testing evidence for three-way cospeciation in multiple phylogenies with tri-trophic associations and determining which phylogenies are involved in cospeciation.

**Keywords:** permutation test, phylogenies, cospeciation, tri-trophic ecology

**Author notes:** The authors would like to thank the editor and referees whose comments have substantially improved this paper. LKM gratefully acknowledges Kenya Medical Research Institute (KEMRI) Kilifi, Kenya, for funding his masters research studies. The Dyer and Forister labs acknowledge support from the United States National Science Foundation, awards DEB 1020509 and DEB 1145609.

# 1 Introduction

New developments in the study of host-parasite phylogenies have given insights into the complexity and necessity of reliable statistical methods that can be used to infer the history of an association between hosts and parasites (Page, 2003). However, there are no reliable statistical tests appropriate for assessing cospeciation of more than two phylogenies (Choi and Gomez, 2009).

Ewens and Grant (2001) defined phylogenetic trees to be diagrammatic representations of the evolutionary relationships that occur between taxonomic groups. Similar phylogenies are consistent with cospeciation whereas dissimilar phylogenies imply a lack of cospeciation. Parasites have long been used to make inferences about the phylogenetic histories of host lineages (Klassen, 1992). If parasites cospeciate with their hosts, then the parasite phylogeny reflects the host phylogeny, assuming that the rate at which the parasite diversifies is not more rapid than that of the host. There is a general expectation derived from Fahrenholz's rule (Fahrenholz, 1913), that parasites diversify together with their hosts. The rule states that parasites' phylogenies should reflect hosts' phylogenies. However, dissimilarities between host and parasite phylogenies might occur due to processes such as parasites switching host lineages, duplication (where the parasites independently speciate from their hosts) or extinction (parasites that fail to speciate with their hosts, or fail to colonise the descendants of a whole diversifying host-lineage) (Paterson, Palma, and Gray, 1999; Paterson and Gray, 1997; Page, 1990, 1996).

Page (2003) points out that a basic test of cospeciation is one that evaluates a significant similarity between the topologies of host and parasite phylogenies that is not due to chance alone. Questions about the timing of speciation are also relevant, particularly in situations with high topological similarity between host and parasite phylogenies (Percey, Page, and Cronk, 2004). However, this issue is not addressed in the current paper.

Randomisation techniques, also referred to as permutation methods, have been used to investigate associations between hosts and their parasites by other researchers including Lapointe and Legendre (1990, 1992a,b); Page (1994a,b, 1995); Siddall (1996, 2001); Charleston and Page (2002); Hommola, Smith, Qiu, and Gilks (2009) and Merkle and Wieseke (2010). The methods developed by these researchers involved bi-trophic associations between host and parasite lineages and form the foundation for the current paper. Lapointe and Legendre (1992a, 1990) assessed differences between phylogenies by randomising the tree labels and branch lengths or randomising the leaf positions and fusion level positions. Planet (2006) also focused on measuring and testing similarity between phylogenies. This paper seeks to extend the permutation methods developed by Hommola et al. (2009) and Siddall (1996, 2001) to more than two phylogenetic associations in order to analyse

cospeciation among three phylogenies by computing their patristic distances and using three-way association matrices.

In brief, Hommola’s method considers the correlation between patristic distances on the host and parasite trees for pairs of host-parasite interactions. If this correlation is high, cospeciation is inferred while if it is low cospeciation is unlikely to have occurred. To determine how large a correlation is likely to occur by chance, the tip labels of the trees are shuffled many times and the correlation re-computed each time. If the original correlation is larger than those obtained after shuffling, it is considered to be significant.

Permutation methods, such as those used by Hommola et al. (2009); Siddall (2001); Lapointe and Legendre (1992b) and ourselves, are useful in that they do not require parametric or distributional assumptions. One approach suggested by Lapointe and Legendre (1992b) is to analyse the statistical significance of the matrix correlation coefficient by comparing independent phylogenetic trees and creating tables of critical values of Pearson’s cross product matrix correlation coefficient. Our method does not require tables of critical values since the critical values are directly computed from the permuted statistics and compared with the observed statistics. Whereas Legendre and Lapointe (2004) required matrices of equal sizes, one of the strengths of our method, as in Hommola et al. (2009), is that this is not a requirement. We can have more parasites than hosts and there can be multiple associations, with more than one parasite taxon attached to a host and vice-versa.

Although we have presented our method in terms of phylogenetic trees, no trees are in fact needed and our method can be applied directly to a set of distance matrices. However, we find the trees extremely helpful in visualising the process and hence have presented the method in this context.

We describe the hypotheses that we investigate in Section 2, along with our proposed test statistics. Exactly how to permute the data is critical, and is discussed in Section 2.2. Interpretation of results can be subtle, and we illustrate it in Section 3 through a series of scenarios ranging from no cospeciation to perfect three-way cospeciation. Simulations to assess the power of our methods are presented in Section 4, and two real data sets are analysed in Section 5. Concluding comments are made in Section 6.

## 2 Methodology

### 2.1 Notation and hypotheses

We consider three phylogenies  $X$ ,  $Y$  and  $Z$ , and association matrices  $A^{XY}$ ,  $A^{XZ}$  and  $A^{YZ}$  representing the interactions among the phylogenetic trees. We consider

simple binary interactions; element  $A_{ij}^{XY}$  is one if species  $i$  of phylogeny  $X$  has been observed to interact with species  $j$  of phylogeny  $Y$  and zero otherwise. Here, “interaction” might refer to a host-parasite relationship, a symbiotic relationship, or some other relationship according to the context of the data.

We wish to test the hypotheses

$H_0$ :  $X$ ,  $Y$  and  $Z$  have evolved independently;

$H_{YZ.X}$ : Cospeciation between  $Y$  and  $Z$  is not due entirely to their common cospeciation with  $X$ ;

$H_{XZ.Y}$ : Cospeciation between  $X$  and  $Z$  is not due entirely to their common cospeciation with  $Y$ ;

$H_{XY.Z}$ : Cospeciation between  $X$  and  $Y$  is not due entirely to their common cospeciation with  $Z$ ;

$H_1$ : Cospeciation is present somewhere in the  $X, Y, Z$  system.

We note that the hypothesis  $H_1$  is not simply a combination of  $H_{YZ.X}, H_{XZ.Y}$  and  $H_{XY.Z}$ . In practice, one would first assess whether one can reject  $H_0$  in favour of  $H_1$  and only if  $H_0$  is rejected then consider the other, more detailed, alternatives.

Our method uses the patristic distances on each phylogenetic tree. Patristic (path-length) distances have been defined as additive phylogenetic distances obtained by summing up the branch lengths on a path between two leaves of a tree. Patristic distances describe the genetic changes in a tree if the phylogeny is based on molecular sequences (Fourment and Gibbs, 2006). Let  $D^X$  denote the matrix of patristic distances on tree  $X$ , so  $D^X = (d_{i,j}^X)$  where  $d_{i,j}^X$  is the patristic distance between tips  $i$  and  $j$  of tree  $X$ , with equivalent matrices  $D^Y$  and  $D^Z$  for trees  $Y$  and  $Z$  respectively. All phylogenetic trees generated in this paper are non-ultrametric; that is, we do not require that all tips on a tree are equidistant from the root of the tree. In fact, our methods do not require the presence of trees, but only that the distances are available.

If  $X$ ,  $Y$  and  $Z$  demonstrate perfect cospeciation, one would expect their interactions to reflect this. Let  $x$ ,  $y$ , and  $z$  represent tips from trees  $X$ ,  $Y$  and  $Z$  respectively. We write  $(x_i, y_i, z_i)$  for a triple such that edges  $x_i-y_i$ ,  $x_i-z_i$  and  $y_i-z_i$  all exist in the interaction matrices  $A^{XY}$ ,  $A^{XZ}$  and  $A^{YZ}$  respectively. In such cases,  $(x_i, y_i, z_i)$  picks out a “triple” or triangle of interactions; Figure 1 gives an illustration. Let  $T$  be an  $n \times 3$  matrix whose  $i$ th row is  $t_i = (x_i, y_i, z_i), i = 1, \dots, n$ , the  $i$ th observed triple.

Suppose  $t_i$  and  $t_j$  are distinct rows of  $T$ . We define an  $n(n-1)/2 \times 3$  matrix  $D$  where each row represents the distances on each tree between a distinct pair of

triples  $t_i$  and  $t_j$  for  $i < j$ . The row of  $D$  corresponding to the pair of triples  $t_i, t_j$  is

$$\left( d_{x_i, x_j}^X, d_{y_i, y_j}^Y, d_{z_i, z_j}^Z \right).$$

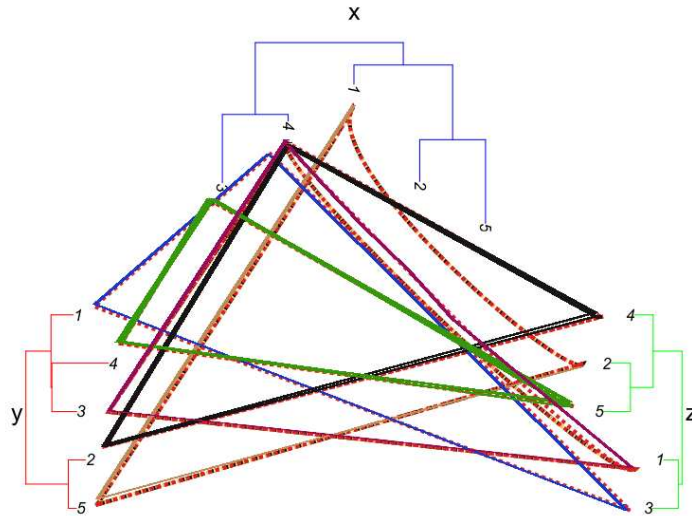


Figure 1: Trees  $X$ ,  $Y$  and  $Z$  are simulated phylogenies generated under the null hypothesis  $H_0$  of no cospeciation. The lines represent  $n = 5$  randomly generated three-way relationships among the trees.

For illustration, we give an example of constructing the matrix  $D$  for the system given in Figure 1, which were randomly generated under  $H_0$  and thus show no evidence of cospeciation. The interaction matrix  $T$ , with  $n = 5$  triangular interactions, for this tri-trophic system is

$$T = \begin{matrix} & X & Y & Z \\ \begin{matrix} t_1 \\ t_2 \\ t_3 \\ t_4 \\ t_5 \end{matrix} & \begin{pmatrix} 4 & 3 & 1 \\ 1 & 5 & 2 \\ 3 & 4 & 5 \\ 4 & 2 & 4 \\ 4 & 1 & 3 \end{pmatrix} \end{matrix},$$

and the three patristic distance matrices  $D^X$ ,  $D^Y$ ,  $D^Z$  for each tree are

$$D^X = \begin{matrix} & \begin{matrix} 1 & 2 & 3 & 4 & 5 \end{matrix} \\ \begin{matrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{matrix} & \begin{pmatrix} 0.00 & 1.25 & 1.67 & 1.11 & 1.63 \\ 1.25 & 0.00 & 2.57 & 2.00 & 1.15 \\ 1.67 & 2.57 & 0.00 & 0.69 & 2.95 \\ 1.11 & 2.00 & 0.69 & 0.00 & 2.39 \\ 1.63 & 1.15 & 2.95 & 2.39 & 0.00 \end{pmatrix} \end{matrix},$$

$$D^Y = \begin{matrix} & \begin{matrix} 1 & 2 & 3 & 4 & 5 \end{matrix} \\ \begin{matrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{matrix} & \begin{pmatrix} 0.00 & 1.64 & 0.74 & 1.22 & 1.50 \\ 1.64 & 0.00 & 1.70 & 2.19 & 0.39 \\ 0.74 & 1.70 & 0.00 & 1.25 & 1.56 \\ 1.22 & 2.19 & 1.25 & 0.00 & 2.05 \\ 1.50 & 0.39 & 1.56 & 2.05 & 0.00 \end{pmatrix} \end{matrix},$$

$$\text{and } D^Z = \begin{matrix} & \begin{matrix} 1 & 2 & 3 & 4 & 5 \end{matrix} \\ \begin{matrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{matrix} & \begin{pmatrix} 0.00 & 2.54 & 1.14 & 1.87 & 2.77 \\ 2.54 & 0.00 & 2.85 & 1.73 & 1.34 \\ 1.14 & 2.85 & 0.00 & 2.18 & 3.08 \\ 1.87 & 1.73 & 2.18 & 0.00 & 1.96 \\ 2.77 & 1.34 & 3.08 & 1.96 & 0.00 \end{pmatrix} \end{matrix}.$$

The corresponding matrix  $D$  has  $n(n-1)/2 = 5(4)/2 = 10$  rows and three columns. Each row will contain the triples:  $d_{x_i, x_j}^X$ ,  $d_{y_i, y_j}^Y$ ,  $d_{z_i, z_j}^Z$ , so we obtain

$$D = \begin{matrix} & \begin{matrix} d^X & d^Y & d^Z \end{matrix} \\ \begin{matrix} t_1, t_2 \\ t_1, t_3 \\ t_1, t_4 \\ t_1, t_5 \\ t_2, t_3 \\ t_2, t_4 \\ t_2, t_5 \\ t_3, t_4 \\ t_3, t_5 \\ t_4, t_5 \end{matrix} & \begin{pmatrix} 1.11 & 1.56 & 2.54 \\ 0.69 & 1.25 & 2.77 \\ 0.00 & 1.70 & 1.87 \\ 0.00 & 0.74 & 1.14 \\ 1.67 & 2.05 & 1.34 \\ 1.11 & 0.39 & 1.73 \\ 1.11 & 1.50 & 2.85 \\ 0.69 & 2.19 & 1.96 \\ 0.69 & 1.22 & 3.08 \\ 0.00 & 1.64 & 2.18 \end{pmatrix} \end{matrix}.$$

## 2.2 Permutations

The test statistics and  $p$ -values defined in Sections 2.3 and 2.4 below do not specifically test for the different hypotheses of interest, although different statistics will



be more or less powerful against different alternatives. Instead, the hypothesis we are testing is determined by the specific permutation algorithm used.

Each of the algorithms is based on permuting or shuffling the tip labels on one or more of our phylogenetic trees. When we do this, the labels retain their existing interaction edges as they are permuted but the tree structure and the structure of the interactions are unchanged. This corresponds to shuffling the rows and columns of the patristic distance matrices corresponding to the trees being permuted.

Permutation of the tip labels of all three trees tests the null hypothesis  $H_0$  of no cospeciation against the alternative  $H_1$ , simply that cospeciation exists somewhere in the tri-trophic system. In this case, the permutation effectively simulates data under the null that none of the trees reflect cospeciation; a small  $p$ -value would indicate that the data are not compatible with this assumption and we would declare the result “significant”.

If a significant result on this full randomisation is obtained, then we try to deduce where the cospeciation has occurred. To do this, consider permuting only the tip labels on tree  $X$ . The permuted  $p$ -values which are produced reflect a situation where the phylogenies  $Y$  and  $Z$  are unchanged, as are their interactions. Each also has the same degree of connectedness with  $X$  as the original data. However, this permutation destroys any cospeciation with  $X$ . Therefore, significant  $p$ -values following permutation of  $X$  indicate a system where tree  $X$  is involved in cospeciation above and beyond any cospeciation between  $Y$  and  $Z$ . Similarly, we can permute the tip labels on any two of the three trees to investigate their interaction with each other and with the third tree.

### 2.3 A dominant eigenvalue test statistic

Consider two triples  $t_i$  and  $t_j$ . If the phylogenies in our tri-trophic system have evolved together, the pairs  $(x_i, x_j)$ ,  $(y_i, y_j)$  and  $(z_i, z_j)$  should have similar patristic distances. Therefore, under the hypothesis  $H_1$  of cospeciation somewhere within our system, we would expect the columns of  $D$  to be correlated. In this case, the dominant (i.e. largest) eigenvalue of the covariance matrix of  $D$  would be large relative to the other eigenvalues, in much the same way as using eigenvalues to indicate the relative importance of components in a principal components analysis. (Indeed, in the limiting case of three identical trees with perfect cospeciation, only the dominant eigenvalue would be non-zero.) We therefore use the dominant eigenvalue of  $\text{var}(D)$ , say  $\lambda^{obs}$ , as a statistic to test  $H_0$  against  $H_1$ . Here and throughout we designate quantities calculated from the real (unpermuted) data with the superscript “obs”.

Rather than attempt to specify the sampling distribution of  $\lambda$ , we use a permutation test; precisely how the permutations are done is discussed in Section 2.2. For each permutation  $k = 1, \dots, N$ , the dominant eigenvalue  $\lambda_k$  is obtained. The  $p$ -value for testing  $H_0$  against  $H_1$  is then calculated as the proportion of permutations where the dominant eigenvalue exceeds that seen in the observed data;

$$P_\lambda = \frac{1}{N} \sum_{k=1}^N I(\lambda_k \geq \lambda^{obs}), \quad (1)$$

where  $I(A)$  is the indicator function which takes value 1 if condition  $A$  is true and zero otherwise. If  $p \leq \alpha$ ,  $H_0$  is rejected at the  $100\alpha\%$  level and cospeciation somewhere in the tri-trophic system is inferred. Otherwise, there is not sufficient evidence to reject  $H_0$ .

## 2.4 Partial correlation test statistics

The dominant eigenvalue test statistic is a somewhat “blunt instrument” as it does not attempt to determine where in the tri-trophic system coevolution has occurred. We therefore consider test statistics based on partial correlations which are more finely tuned to distinguishing between the hypotheses  $H_{YZ.X}$ ,  $H_{XZ.Y}$  and  $H_{XY.Z}$ . Like Siddall (1996, 2001) and Hommola et al. (2009), we consider correlation between the columns of  $D$ . However, those authors considered only bi-trophic systems, comparing only distances on two trees  $X$  and  $Y$ . We have three trees to consider and hence use *partial* correlations as our test statistics.

Let the sample partial correlation coefficients from the observed data be denoted by  $r_{yz.x}^{obs}$ ,  $r_{xz.y}^{obs}$  and  $r_{xy.z}^{obs}$ . For example,  $r_{yz.x}^{obs}$  is the partial correlation between distances  $d^Y$  and  $d^Z$  when we control for their correlations with distance  $d^X$ . After each permutation  $k = 1, \dots, N$ , we obtain corresponding partial correlations  $r_{yz.x,k}$ ,  $r_{xz.y,k}$  and  $r_{xy.z,k}$ . To test the significance of the observed partial correlations,  $p$ -values are computed in a similar manner to (1):

$$P_{yz.x} = \frac{1}{N} \sum_{k=1}^N I(r_{yz.x,k} \geq r_{yz.x}^{obs}),$$

$$P_{xz.y} = \frac{1}{N} \sum_{k=1}^N I(r_{xz.y,k} \geq r_{xz.y}^{obs}),$$

$$P_{xy.z} = \frac{1}{N} \sum_{k=1}^N I(r_{xy.z,k} \geq r_{xy.z}^{obs}).$$

Labels permuted	$P_\lambda$	$P_{yz.x}$	$P_{xz.y}$	$P_{xy.z}$
$X$	$X$ involved in cospeciation	—	$X$ and $Z$ cospeciate	$X$ and $Y$ cospeciate
$Y$	$Y$ involved in cospeciation	$Y$ and $Z$ cospeciate	—	$X$ and $Y$ cospeciate
$Z$	$Z$ involved in cospeciation	$Y$ and $Z$ cospeciate	$X$ and $Z$ cospeciate	—
$XY$ $XZ$ $YZ$ $XYZ$	Cospeciation occurs somewhere in the system			

Table 1: Simplified schematic diagram of the interaction between the various permutations and test statistics. Text indicates how one should interpret individual significant  $p$ -values.

The hypothesis in question,  $H_{YZ.X}$ ,  $H_{XZ.Y}$  and  $H_{XY.Z}$ , is determined by the choice of permutation method (see Section 2.2), not by the choice of  $p$ -value. However, as we shall see in Section 4 the partial correlation test statistics are *more powerful* for their corresponding alternative hypotheses. For example, data for which hypothesis  $H_{YZ.X}$  is true will be most likely to be detected by  $P_{yz.x}$ , but may also result in significant values of  $P_{xz.y}$  and  $P_{xy.z}$ . Moreover, the “overall”  $p$ -value  $P_\lambda$  will be more powerful for the situation where there is genuine cospeciation in all pairs  $XY, XZ, YZ$ , implying truly tri-trophic cospeciation (Forister and Feldman, 2010). The interactions between the test statistics and possible permutations are indicated in table 1, although in practice the interpretation may be more subtle.

### 3 Simulated examples

We now illustrate our method and what can be learned from different test statistics under different randomisations by generating and analysing data sets under a series of different assumptions. In Section 3.1, we consider data where there is no cospeciation; Section 3.2 analyses data where two of the trees are closely related while the third is unrelated; and finally Section 3.3 examines data where all three trees are closely related. The latter two scenarios will form the basis of our power simulations in Section 4.

### 3.1 Simulations under the null

To investigate type I error, data were generated under  $H_0$ . For each data set, three random trees  $X$ ,  $Y$ , and  $Z$  were generated each with 10 tips and 9 internal nodes using the `rtree` command in the `ape` package (Paradis, Claude, and Strimmer, 2004), and a random triangular interaction matrix was simulated. For each triangle the tips were uniformly sampled on each tree and any duplicates replaced until 10 distinct triangles were created. The  $p$ -values  $P_\lambda$ ,  $P_{yz,x}$ ,  $P_{xz,y}$  and  $P_{xy,z}$  were calculated for each data set, using 100 permutations; each permutation involved shuffling the tip labels of all three trees. For the statistic to be reliable,  $p$  values generated under  $H_0$  should be uniformly distributed. Figure 2 shows the resulting empirical cumulative distribution function of our proposed  $p$ -values, demonstrating a uniform distribution in each case. Similar results were obtained when permutations of just one tree or pairs of trees were used.

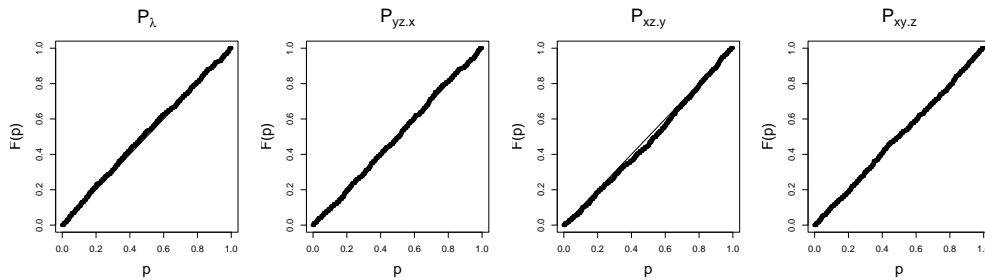


Figure 2: Empirical cumulative distribution function plots of the  $p$ -values  $P_\lambda$ ,  $P_{yz,x}$ ,  $P_{xz,y}$  and  $P_{xy,z}$  using 1000 data sets simulated under the null hypothesis.

It is helpful to consider one specific realisation in more detail, as an example data set. Three random phylogenies were generated with 15 tips on each tree. The three-way interaction matrix, again generated randomly, is given in Appendix A, along with plots of the phylogenetic trees. As one would expect for data showing no cospeciation, the columns of the observed matrix  $D$  were essentially uncorrelated; plotting these distances in pairwise scatter plots shows a cloud of data points with no observable structure (Figure 3).

Figure 4 shows the null distributions of the test statistic generated by 10,000 permutations of the tip labels of all three trees  $X$ ,  $Y$  and  $Z$  (similar plots, not shown, occur when we permute the tip labels of only a subset of the trees). The dashed vertical red lines on the histograms are the observed values of the respective test statistics, whereas the dotted blue lines represent the 95<sup>th</sup> percentile of the randomised null distribution. The results here indicate that there is no evidence to reject the null and that these species have evolved independently over time as the red lines

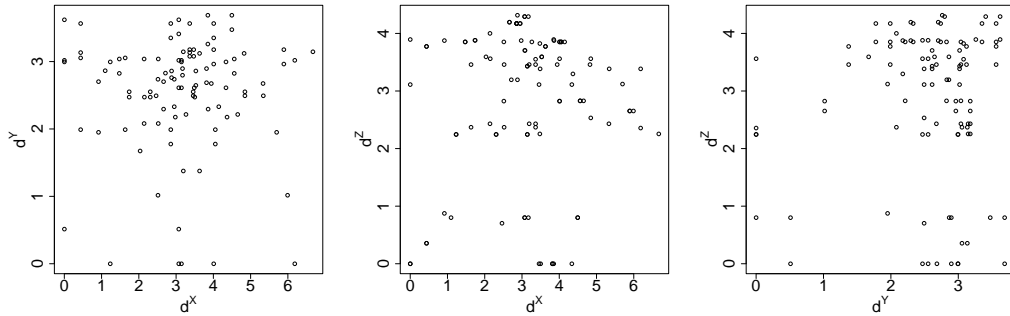


Figure 3: Pairwise scatterplots of permuted distances on three phylogenetic trees simulated assuming no cospeciation has occurred. Each permutation shuffled the tip labels of all three trees; 100 permutations were conducted.

Labels permuted	$P_\lambda$	$P_{yz,x}$	$P_{xz,y}$	$P_{xy,z}$
X	0.933	0.894	0.486	0.092
Y	0.973	0.884	0.075	0.175
Z	0.282	0.943	0.233	0.088
XY	0.930	0.909	0.458	0.059
XZ	0.895	0.966	0.334	0.079
YZ	0.286	0.966	0.202	0.156
XYZ	0.900	0.972	0.330	0.047

Table 2: The  $p$ -values obtained for one realisation of data simulated with no cospeciation.

are to the left of the blue lines. The  $p$ -values from these histograms, as well as those from permuting other trees, are summarised in Table 2. From these results, it is evident that the phylogenies are not related in a way that can be distinguished from a random relationship. One  $p$ -value is just below the conventional cut-off of 0.05, but considering that all the other  $p$ -values are above 0.05 we ascribe that to the fact that, under  $H_0$ , the  $p$ -values are uniformly distributed. (We note that using a multiple testing correction would adjust all these  $p$ -values to be greater than 0.05.) Note that the values of  $P_{xy,z}$  are lower than the other  $p$ -values; this is due to the particular realisation having, by chance, stronger correlation between X and Y than either of those trees have with Z. However, since the overall statistic  $P_\lambda$  is clearly non-significant for all combinations of which trees are permuted, the values of  $P_{xy,z}$  do not indicate a significant bitrophic relationship. Overall, we would conclude, correctly, that these species have evolved independently.

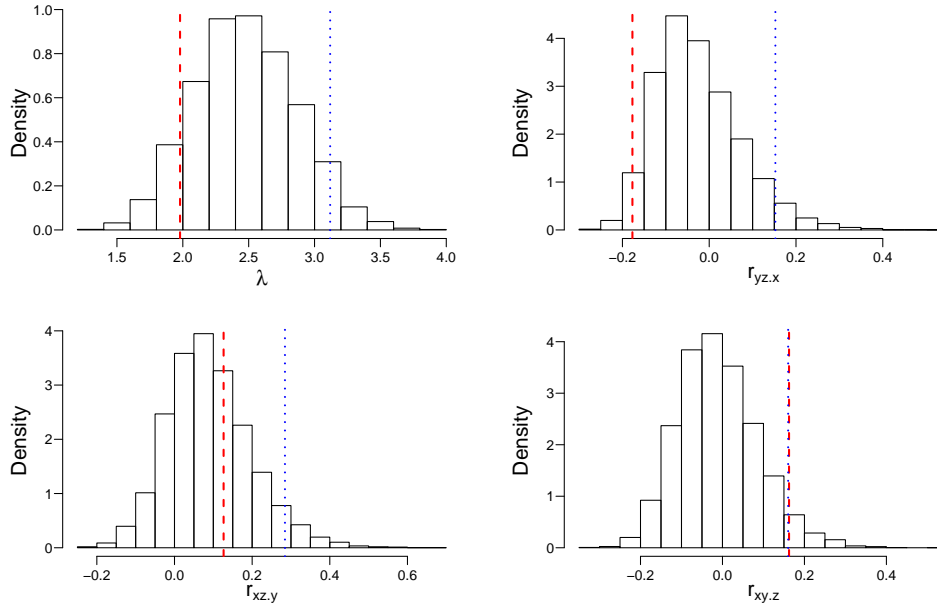


Figure 4: Histograms of test statistics based on 10000 permutations of data simulated under the null hypothesis of no cospeciation. The dashed red lines indicate the observed test statistic whereas the dotted blue lines represent the corresponding critical values, the 95<sup>th</sup> percentile of the the permuted values. The results here indicate that there is no evidence to reject the null and that we would conclude these species have evolved independently over time, as the observed test statistics are below the critical values.

### 3.2 Perfect bi-trophic cospeciation with an unrelated third phylogeny

We now consider an example exhibiting strong cospeciation between two trees which have essentially the same topologies and direct associations between each corresponding tip, while the third phylogeny is totally independent. The phylogeny for  $X$  is the same one simulated in Section 3.1 with 15 tips, and  $Y$  is a copy of  $X$  with independent  $N(0, 0.01^2)$  noise added to the branch lengths (subject to a minimum branch length of 0.1 to avoid invalid trees). The tree  $Z$  was independently simulated with 11 tips. The trees are shown in Appendix A, along with the interaction matrix  $T$  which has  $i$ th row  $t_i = (i, i, j)$  for  $i = 1, \dots, 15$ , with the tip  $j$  on tree  $Z$  generated uniformly from the set  $\{1, 2, \dots, 11\}$ . Figure 5 displays pairwise scatterplots of  $d^X$ ,

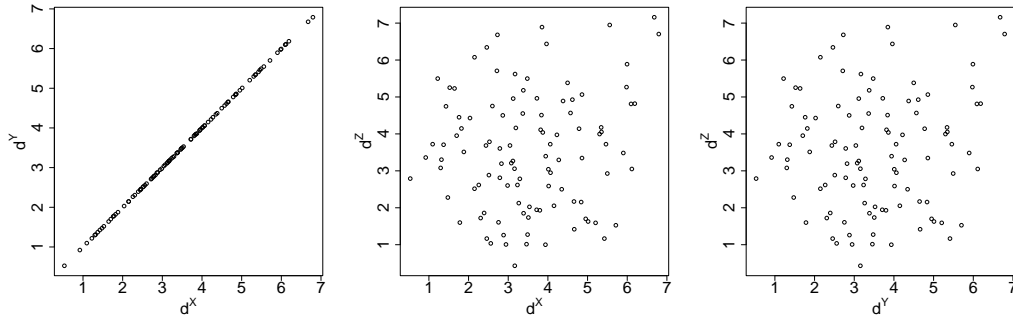


Figure 5: Pairwise scatterplots of permuted distances on three phylogenetic trees simulated assuming strong cospeciation between  $X$  and  $Y$  but no cospeciation with  $Z$ . Each permutation shuffled the tip labels of all three trees; 100 permutations were conducted.

$d^Y$  and  $d^Z$ . The near-perfect correlation between  $d^X$  and  $d^Y$  is clear, as is the lack of correlation with  $d^Z$ .

We applied our tests to these data, using 10,000 permutations. Table 3 displays a summary of the  $p$  values obtained. It is clear that it matters which tree labels are permuted because each choice of permutation tests a different hypothesis as explained in Section 2.2 above. Looking first at the column of global  $p$ -values  $P_\lambda$ , we see that the null hypothesis of no cospeciation is rejected in each case except when only tree  $Z$  is permuted. Since tree  $Z$  is not involved in the cospeciation, permuting it reveals no effects of cospeciation. The same conclusion can be found from the  $p$ -value  $P_{xy,z}$  since again permuting tree  $Z$  has no effect on the partial correlation between  $X$  and  $Y$ . The other  $p$ -values,  $P_{yz,x}$  and  $P_{xz,y}$ , generally fail to reject  $H_0$  since examining the partial correlations between  $Y, Z$  and  $X, Z$  have little power to detect cospeciation when  $Z$  is not involved in the cospeciation. Therefore, these  $p$ -values under the different permutation allow us correctly to conclude that there is cospeciation between  $X$  and  $Y$  but that  $Z$  is not involved.

### 3.3 Simulating three identical phylogenies with near-perfect cospeciation

Finally, we examine a simulated example where there is strong cospeciation between all three phylogenies. The same base tree was used as in Sections 3.1 and 3.2, and  $X, Y$  and  $Z$  were defined to be copies of this base tree with independent normal noise added as in Section 3.2; the trees are shown in Figure A.1. The interaction

Labels permuted	$P_\lambda$	$P_{yz.x}$	$P_{xz.y}$	$P_{xy.z}$
X	0.000	0.005	0.861	0.000
Y	0.000	0.127	1.000	0.000
Z	0.531	0.193	0.801	0.333
XY	0.000	0.133	0.862	0.000
XZ	0.000	0.129	0.859	0.000
YZ	0.000	0.131	0.865	0.000
XYZ	0.000	0.128	0.861	0.000

Table 3: The  $p$ -values obtained for data simulated with strong cospeciation between X and Y but neither X nor Y having coevolved with Z.

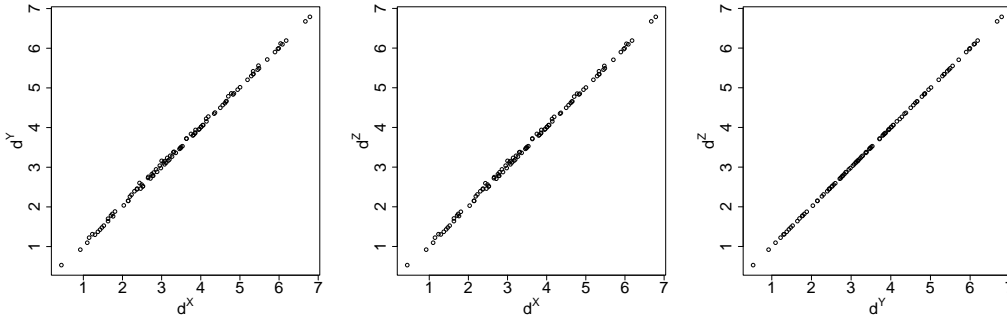


Figure 6: Pairwise scatterplots of permuted distances on three phylogenetic trees simulated assuming strong cospeciation between each of the three phylogenies. Each permutation shuffled the tip labels of all three trees; 100 permutations were conducted.

matrix  $T$  was defined to contain triangular links  $t_i = (i, i, i)$  for  $i = 1, \dots, 15$ , and is also shown in Appendix (A).

Figure 6 displays a strong relationship among the three species by showing almost perfectly correlated distances along the  $X = Y = Z$  line. (We note that perfect cospeciation would result in undefined partial correlations and the dominant eigenvalue always taking value 1.)

Permuting the tree labels 10,000 times gave the  $p$ -values in Table 4. The global  $p$ -value  $P_\lambda$  performs as expected; permuting any combination of trees yields strong evidence to reject  $H_0$  and we conclude that all trees are involved in the cospeciation. However, the partial correlation  $p$ -values require more careful interpretation. The  $P_{xz.y}$  values suggest never rejecting  $H_0$ ; this is due to the sample correlation  $\text{cor}(d^X, d^Z) = 0.99753$  being slightly lower than both  $\text{cor}(d^X, d^Y) = 0.99757$  and  $\text{cor}(d^Y, d^Z) = 1.0000$  (all to 5 dp). Hence, once the common correlations with  $Y$



Labels permuted	$P_\lambda$	$P_{yz.x}$	$P_{xz.y}$	$P_{xy.z}$
X	0.000	1.000	0.957	0.027
Y	0.000	0.000	1.000	0.012
Z	0.000	0.000	0.964	1.000
XY	0.000	0.000	0.996	0.018
XZ	0.000	0.000	0.995	0.019
YZ	0.000	0.000	0.996	0.019
XYZ	0.000	0.000	0.996	0.019

Table 4: The  $p$ -values obtained for data simulated with strong cospeciation between  $X$ ,  $Y$  and  $Z$ .

are accounted for, there is no evidence of further cospeciation of either  $X$  or  $Z$  with  $Y$ . A weaker version of this phenomenon is seen in the values of  $P_{xy.z}$ . Thus, we see that in the presence of very similar levels of cospeciation between the phylogenies, these statistics can be very sensitive to detect the strongest of the cospeciations. Repeating the simulation 100 times gave the off-diagonal mean empirical  $p$ -values of around 0.04, 0.03, 0.08 for  $P_{yz.x}$ ,  $P_{xz.y}$ , and  $P_{xy.z}$  respectively.

The  $p$ -values of 1.000 on the diagonal of Table 4 are worth remarking upon. These are for  $P_{yz.x}$  when the  $X$  labels are shuffled; for  $P_{xz.y}$  when the  $Y$  labels are shuffled; and for  $P_{xy.z}$  when the  $Z$  labels are shuffled. These large  $p$ -values can be explained as follows. For each of these cases, the statistic is designed to detect cospeciation that is due to direct cospeciation between two of the trophic levels, but the randomisation is designed to detect the relevance of only the remaining trophic level. Thus each of these  $p$ -values reports the statistical significance of no information. We shall see a similar effect in a real data set in Section 5.1.

## 4 Power Simulations

### 4.1 Simulation study design

To assess the power of our procedure, we simulate data sets exhibiting varying degrees of cospeciation. Starting with three identical trees with 10 tips exhibiting perfect triangular interactions  $t_i = (i, i, i)$  for  $i = 1, \dots, 10$ , we followed two approaches adopted from Hommola et al. (2009) and Legendre, Desdevies, and Bazin (2002). In the first approach, the interaction matrix  $T$  was supplemented with further random interactions, while in the second randomly chosen interactions in  $T$  were replaced by random triangles. The random triangles had their tips uniformly selected on each tree, with duplicates being removed and replaced until the desired

number of distinct triangles was achieved. This process was repeated for a base system with perfect bitrophic cospeciation and an unrelated third tree.

In each case, the number of triangles added or replaced was steadily increased to move from a situation representing the hypothesis  $H_1$  of cospeciation to the hypothesis  $H_0$  of no cospeciation. The number of triangles added or replaced ranged from 1 to 10. Thus when adding triangles, the simulated data ranged from near-perfect cospeciation to a situation where the interactions were 50% cospeciated and 50% randomly generated. On the other hand, replacing all 10 triangles is more extreme and results in a data set generated under  $H_0$ .

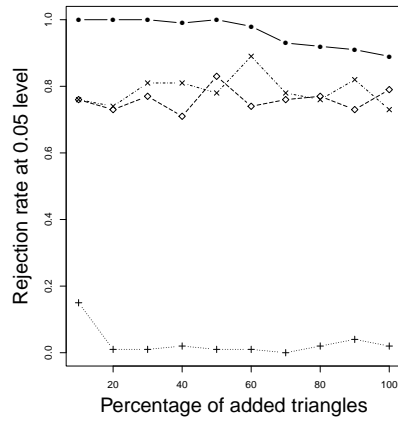
Broadly similar patterns were seen in simulations using trees with 20 and 30 tips when replacing the perfect interactions by random ones. When adding random triangles, power levels remained high for longer due to the stronger evidence for cospeciation seen in larger data sets.

## 4.2 Power curves based on tri-trophic cospeciation

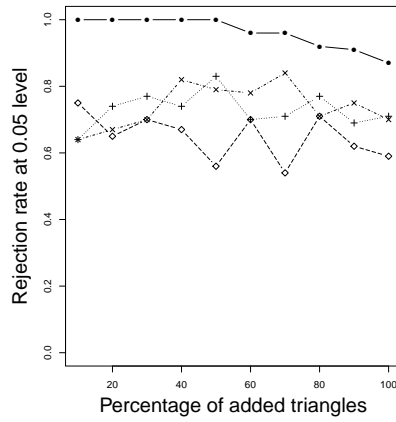
For three identical trees with 10 tips and interactions  $t_i = (i, i, i)$  for  $i = 1, \dots, 10$ , similar to that in Section 3.3, 1,  $\dots$ , 10 uniform random triangles were added. In each case, 100 data sets were simulated, each time permuting the labels 10000 times. We assess the power of our test statistics to reject the null hypothesis of no cospeciation at the 5% significance level.

Figure 7(a)–(c) displays the power curves obtained under randomisation of  $X$  only,  $X$  and  $Y$ , and all three trees respectively. It is evident from these plots that the power of the dominant eigenvalue test statistic to reject  $H_0$  remains high until about 5 tips have been added, and decays only slowly thereafter. The other statistics are somewhat less powerful, although arguably less affected by the number of triangles added.

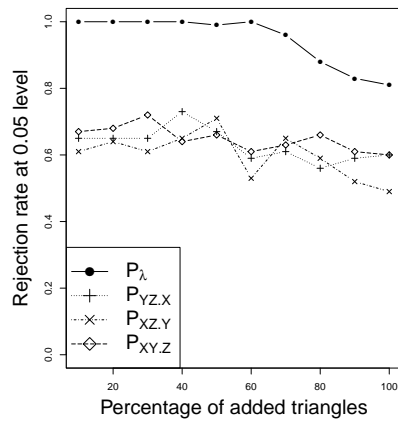
It is noteworthy that in Figure 7(a) the power of  $P_{yz,x}$  is uniformly poor. This is due to the interaction between randomising  $X$  only and the nature of  $P_{yz,x}$ . The statistic  $r_{YZ,X}$  is designed to be most powerful for detecting the hypothesis  $H_{YZ,X}$ : Cospeciation between  $Y$  and  $Z$  is not due entirely to their common cospeciation with  $X$ . However, without randomising at least one of  $Y$  and  $Z$ , it is impossible to determine whether the observed relationships between  $Y$  and  $Z$  suggest  $H_0$  or  $H_{YZ,X}$ , and so there is little power to reject  $H_0$ . In panels (b) and (c), once  $Y$  and  $Z$  are randomised, the power curves of the three partial correlation test statistics are much more similar. Due to the symmetry of the simulated data, with complete cospeciation between all three trees, the same patterns were observed for other choices of tree to randomise.



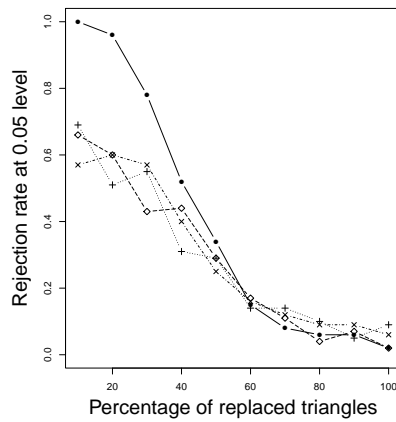
(a) Only  $X$  randomised.



(b)  $X$  and  $Y$  randomised.



(c)  $X$ ,  $Y$  and  $Z$  randomised.



(d)  $X$ ,  $Y$  and  $Z$  randomised.

Figure 7: Simulated power curves generated by adding or replacing interactions in a system starting with three identical 10-tip trees exhibiting perfect cospeciation.

Figure 7(d) displays the simulated power curves when, rather than adding random triangles, we replace the existing triangles with random ones. The overall pattern is similar to panel (c) when few triangles are replaced, descending to low power as 10 triangles are replaced. This is to be expected as replacing only one triangle retains almost all of the perfect cospeciation, while replacing all 10 of the triangles results in data generated under the null hypothesis of cospeciation.

### 4.3 Power curves based on bi-trophic cospeciation

We now consider power curves generated from data where  $X$  and  $Y$  are perfectly cospeciated and  $Z$  is unrelated, as in Section 3.2. Otherwise, the simulations were conducted in the same manner as in Section 4.2, where the underlying data were more symmetric. The results are shown in Figure 8. The weaker cospeciation in this example compared to that in Section 4.2 results in the dominant eigenvalue statistic retaining less power as more triangles are added.

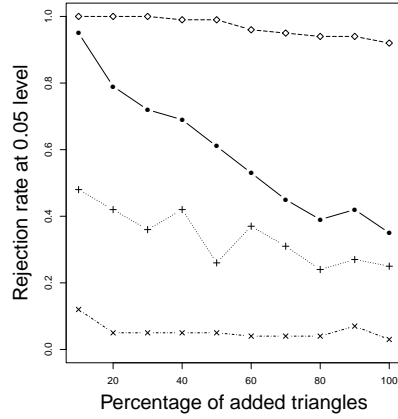
In this case,  $X$  and  $Y$  have cospeciated while  $Z$  is unrelated. This is shown clearly in panels (a) and (b). When  $X$  is the only tree randomised,  $r_{XY.Z}$  is the most powerful statistic, being closely attuned to both the true hypothesis  $H_{XY.Z}$  and the randomisation of  $X$ . The statistic  $r_{YZ.X}$  has much less power, and  $r_{XY.Z}$  essentially no power as they are more attuned to other forms of cospeciation. In contrast, when only  $Z$  is randomised, no statistic has power to detect the cospeciation between  $X$  and  $Y$ . This pattern continues as we randomise  $Y$  and  $Z$  in addition to  $X$ ; similar results are obtained if we start by randomising  $Y$ .

Replacing triangles, rather than adding them, changes the results in a similar way as in Section 4.2 (not shown). The relative power of different statistics and effects of specific randomisations are unchanged, but power decays to very low levels as the data become more like that which would be generated under  $H_0$ .

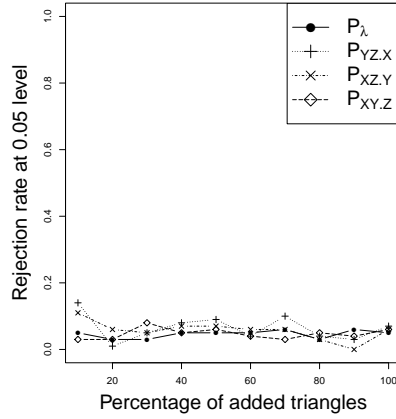
## 5 Application to real datasets

### 5.1 Termite-protist-bacteria data

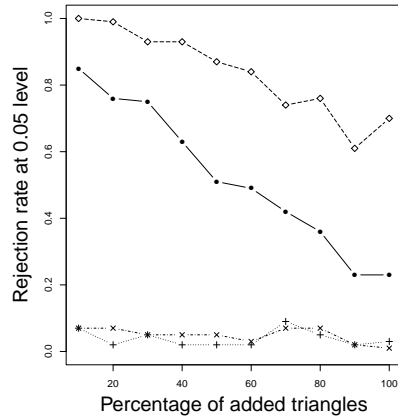
A termite-protist-bacteria phylogenetic dataset was published by Noda, Kitade, Inoue, Kawai, Hiroshima, Hongoh, Constantino, Uys, Zhong, Kudo, and Ohkuma (2007). The symbiotic system consists of termites, gut symbionts (“cellulolytic protists found in the guts of the termites”) and intracellular bacterial symbionts of these protists. Our results agree with Noda et al. (2007), as we found strong evidence for cospeciation among these organisms. However, Noda et al. (2007) made inferences based on two-way comparisons, as they lacked a three-way analysis.



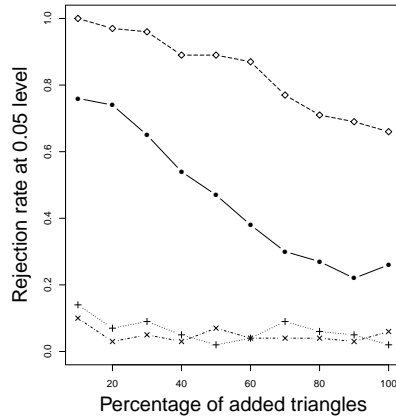
(a) Only  $X$  randomised.



(b) Only  $Z$  randomised.



(c) Both  $X$  and  $Y$  randomised.



(d) All of  $X$ ,  $Y$  and  $Z$  randomised.

Figure 8: Simulated power curves generated by adding or replacing interactions in a system starting with two identical 10-tip trees  $X$  and  $Y$  exhibiting perfect cospicuation, along with an unrelated tree  $Z$ .

The sequence dataset was obtained for each trophic level by downloading individual sequences from GenBank. Phylogenetic trees for each group were reconstructed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) following the methods outlined by Noda et al. (2007). Our phylogenetic reconstructions for each of the three trophic levels matched the trees that were published by Noda et al. (2007).

Figure 9 displays the three phylogenies and the observed triangular interactions. The labels of bacteria, protist and termite are given in Appendix B, in Tables B.1, B.2 and B.3 for the termites, protists and bacteria respectively, along with their interaction matrix  $T$ . We refer to the termites as tree  $X$ , protists as  $Y$ , and bacteria as  $Z$ .

Applying the pairwise permutation analysis method of Hommola et al. (2009) on this dataset suggested a statistical significant association between each of the three possible pairs. In particular, protist and bacteria had  $p = 0.0268$ , termites and bacteria had  $p = 0.000$  and protist and termites  $p = 0.0162$ . We applied our algorithm, performing 1000 permutations. The results are shown in Table 5.

Labels permuted	$P_\lambda$	$P_{yz.x}$	$P_{xz.y}$	$P_{xy.z}$
$X$	0.000	1.000	0.000	0.027
$Y$	0.005	0.392	0.996	0.007
$Z$	0.000	0.438	0.000	0.999
$XY$	0.000	0.452	0.001	0.013
$XZ$	0.000	0.456	0.000	0.014
$YZ$	0.000	0.446	0.000	0.020
$XYZ$	0.000	0.436	0.000	0.011

Table 5: Summary table of the  $p$ -values obtained by analysing the data from Noda et al. (2007).

Table 5 shows clear evidence of cospeciation. The final row (“ $XYZ$ ”) contains the results of shuffling tip labels in all three trees. With the exception of  $P_{yz.x}$ , the  $p$ -value for each of the statistics is less than 0.05, which is indicative of cospeciation somewhere within the tritrophic system. To gain insight into the nature of this cospeciation, we examine the first three rows of the table. The first row (“ $X$ ”) shows the results when the tip labels of only tree  $X$  are shuffled. Again with the exception of  $P_{yz.x}$ , each of the statistics is significant. Thus, *in addition to any cospeciation directly between  $Y$  and  $Z$* , the system shows evidence of direct involvement of tree  $X$ . Small  $p$ -values in rows “ $Y$ ” and “ $Z$ ” similarly provide evidence that  $Y$  and  $Z$  are likewise directly involved. Thus we conclude that all three trophic levels have mutually cospeciated.

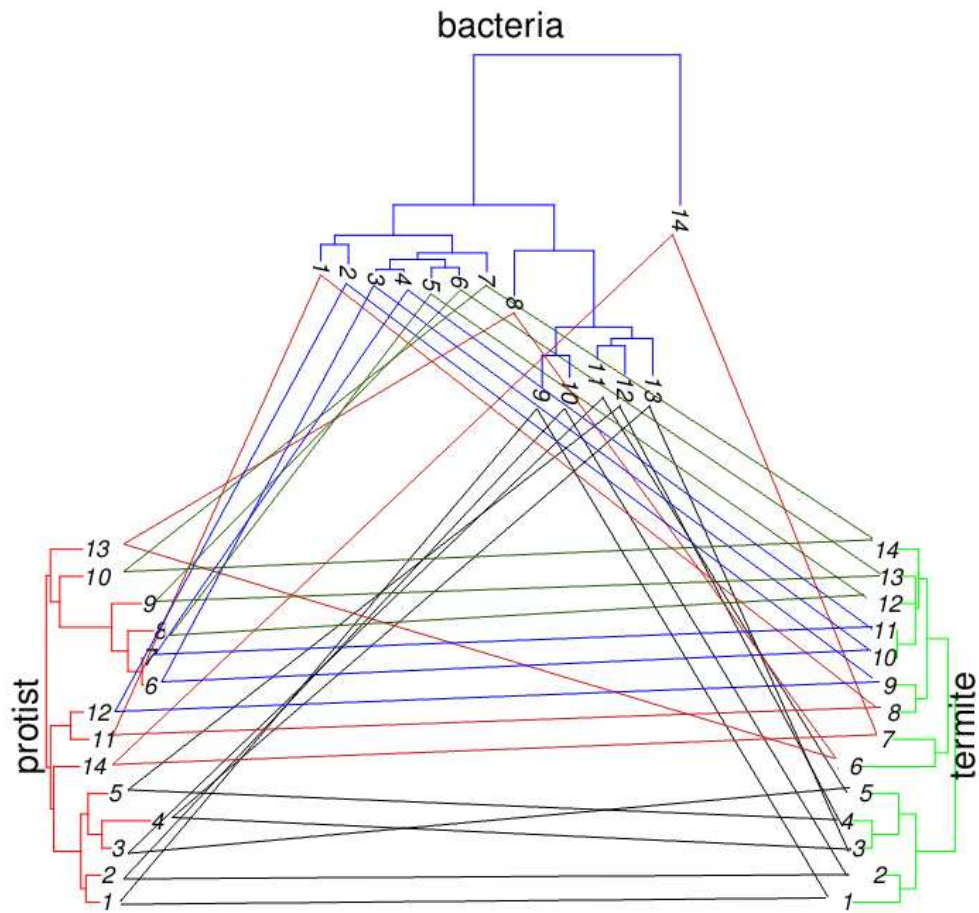


Figure 9: Termite, protist and bacteria phylogenies, with observed interactions between the three trees.

The initial column of Table 5, containing  $p$ -values for the largest eigenvalue, would have been sufficient to draw the above conclusions in these data. It is useful, nonetheless, to consider the results in the following columns. Recall that  $P_{yz,x}$  was designed to be most powerful in detecting any cospeciation between  $Y$  and  $Z$  that is not due to their common cospeciation with  $X$ . We note that none of the  $p$ -values for  $P_{yz,x}$  are significant, unlike those for  $P_{xz,y}$  and  $P_{xy,z}$ . This suggests that  $X$  (termites) has predominated in the cospeciation; the speciation of the termites are more strongly associated with that of protists and bacteria than they are with each other. However, as discussed above, there is still evidence of direct cospeciation between  $Y$  (protist) and  $Z$  (bacteria).

## 5.2 Tree-moth-wasp data

In this section, we show how our method works using the Lopez-Vaamonde, Godfray, West, Hansson, and Cook (2005) dataset that comprises leaf-mining moths, parasitoid wasps and host trees. According to Lopez-Vaamonde et al. (2005), this triplex system provided no evidence for three-way cospeciation but that parasitoids showed phylogenetic conservation of their association with host plant genera.

Lopez-Vaamonde et al. (2005) investigated coevolution using only tree topologies and our method requires trees with branch lengths. Therefore, we reconstructed phylogenetic trees for each group using sequences downloaded from GenBank. Phylogenetic reconstructions were implemented in BEAST version 1.4.8 (Drummond and Rambaut, 2007) and topologies were constrained based on the trees presented by Lopez-Vaamonde et al. (2005). The three phylogenies with their triangular interactions are displayed in Figure 10. The labels are given in Appendix C, in Tables C.1, C.2 and C.3 for host tree, moth and parasitoid respectively, along with the interaction matrix  $T$  for these data. We refer to the host tree as  $X$ , the moth as  $Y$  and the parasitoid as  $Z$ .

We applied the pairwise permutation analysis method of Hommola et al. (2009) on this dataset. The results showed that there was a statistically significant association between the host tree and the parasitoid with a  $p$ -value of 0.003. However, there were no significant associations between the moth and the host tree ( $p = 0.6163$ ) or between the moth and the parasitoid ( $p = 0.5711$ ). The results of our tri-trophic analysis are in Table 6.

Labels permuted	$P_\lambda$	$P_{yz.x}$	$P_{xz.y}$	$P_{xy.z}$
$X$	0.134	0.908	0.156	0.152
$Y$	0.963	0.054	0.998	0.249
$Z$	0.031	0.082	0.035	0.982
$XY$	0.957	0.028	0.213	0.132
$XZ$	0.127	0.248	0.010	0.238
$YZ$	0.957	0.062	0.067	0.265
$XYZ$	0.954	0.048	0.012	0.139

Table 6: Summary table of the  $p$ -values obtained by analysing the data from Lopez-Vaamonde et al. (2005).

The evidence for cospeciation in Table 6 is mixed. The final row, containing the results of shuffling tip labels in all three trees, reports two  $p$ -values less than 0.05, and two others which are much larger. These apparently conflicting results may be explained by the fact that these different statistics are designed to detect different aspects of coevolution. The  $p$ -value for the largest eigenvalue,  $P_\lambda$ , in



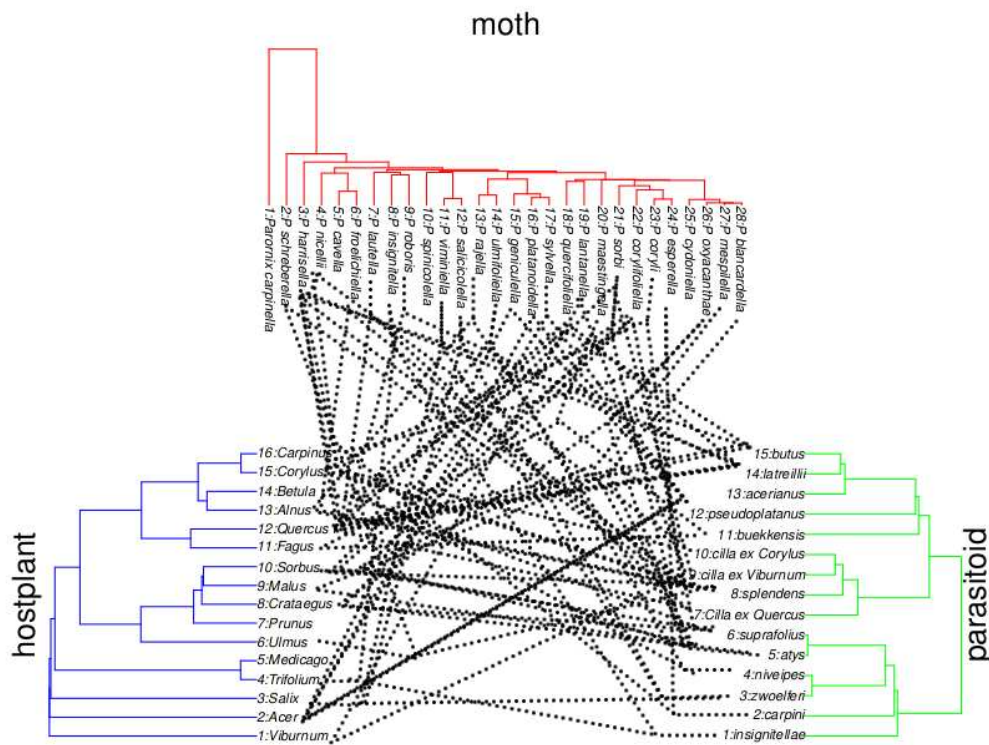
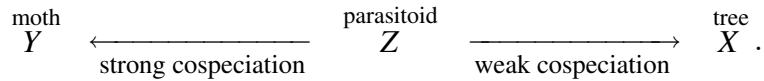


Figure 10: Tree, moth and wasp phylogenies.

the final row, which looks for evidence of cospeciation *non-specifically* within the tri-trophic system, finds none. The remaining  $p$ -values in the final row suggest *direct* cospeciation between  $X$  and  $Z$  and between  $Y$  and  $Z$ , but not between  $X$  and  $Y$ . Nevertheless, these are only *suggestions*, since the randomisation of the final row allows us only to distinguish between the null hypothesis  $H_0$  and the general alternative  $H_1$ . More critical appraisal of these suggestions may be gleaned from the first three rows of the table, which respectively indicate that  $Z$  (the parasitoid) is directly involved in cospeciation; that the evidence for direct involvement of  $Y$  (moth) is marginal; and that there is no clear evidence for direct involvement of  $X$  (host tree). These results, taken together, suggest that the parasitoid has been central in the cospeciation of the tri-trophic system, its speciation being related more strongly to that of the moth than the tree, and accounts effectively for the relationship between the moth and the tree. We can summarise these relationships

with the following diagram:



## 6 Discussion

Evolutionary histories of hosts and parasites have most often been analysed at a bi-trophic level (Hommola et al., 2009; Legendre et al., 2002; Page, 1996; Paterson and Gray, 1997; Huelsenbeck, Rannala, and Larget, 2000). Evolutionary histories can be shared by more than two trophic levels, (Ahmad, Aslam, and Razaq, 2004; Forister and Feldman, 2010; Micha, Kistenmacher, Mölck, and Wyss, 2000), which is referred to as a phylogenetic cascade by Forister and Feldman (2010).

We have demonstrated in this paper that the relationship among three phylogenies can be closely examined by using permutation of the individual tree tip labels and test statistics based on dominant eigenvalues and partial correlations. As explained in Section 2.2, randomisation can be done on one, two or all three of the trees. When we permute all the trees, obtaining a small  $p$ -value shows that there is a relationship between the trees and the interaction matrices, but does not clarify *where* in the system the relationship exists. For example, it could be due to pairwise interactions between  $X$  and  $Y$  and  $X$  and  $Z$ . Observed interactions between  $Y$  and  $Z$  might only be due to their separate interaction with  $X$ .

A significant result obtained on this full randomisation would require one to test the null hypothesis that Tree  $X$  exists independently, whilst allowing for the possibility that  $Y$  and  $Z$  truly interact. We test this by randomising only Tree  $X$ , leaving everything else untouched. So if we now get a significant result, it can only be due to Tree  $X$  interacting with another tree. This randomisation can be repeated, but now randomising Tree  $Y$  only, and then Tree  $Z$  only. If all results come up significant, then each of the trees can be inferred to be involved in cospeciation. Also, simultaneously randomising trees  $X$  and  $Y$  can be done, keeping Tree  $Z$  unperturbed: this would test the null hypothesis that there is no interaction between  $X$  and  $Y$ .

Our tri-trophic host-parasite approach is a successful extension of the host-parasite permutation method presented by Hommola et al. (2009) to test for cospeciation of one host-one parasite lineages. Unlike a simple pairwise application of Hommola et al's work, our method is sensitive to a range of different departures from the null. This is achieved both by consideration of which trees to permute to evaluate different hypothesis, and the use of  $p$ -values based on partial correlations to see the direction and strength of the associations. The dominant eigenvalue statistic is useful in testing the overall cospeciation of the three phylogenies under

the different hypotheses and can be extended to test for more than the three trophic levels.

Calculating patristic distances from the phylogenies is an idea similar to that presented by Campbell, Legendre, and Lapointe (2011) who used distance matrices to test cospeciation in phylogenetic analysis. More importantly, this paper makes it possible to analyse associations from matrices of different sizes.

Our results from analysing real datasets allow for quantitative evidence to support or reject hypotheses of cospeciation described by both Noda et al. (2007) and Lopez-Vaamonde et al. (2005). Our conclusions agree with and extend those of Noda et al. (2007) and Lopez-Vaamonde et al. (2005), addressing the presence or absence of cospeciation in (parts) of the entire tritrophic system rather than only looking at bitrophic cospeciation. In addition, our method helps quantify the overall  $p$ -values obtained by examining the different labels of trees permuted and the partial correlation coefficients. Thus we are not only able to say that there is (or there is not) a statistically significant association among the species but quantify how the strength or weaknesses of these associations arise.

We have confined our attention to the use of linear correlations. While the dominant eigenvalue test statistic  $\lambda$  specifically refers to linear patterns in the distances, the partial correlations can be computed for nonlinear correlations such as Kendall's  $\tau$  or Spearman's  $\rho$ . Other correlation measures could be used if nonlinear relationships between the correlations on different trees were of interest. However, we would be cautious of interpreting these partial correlations in the absence of an overall test statistic.

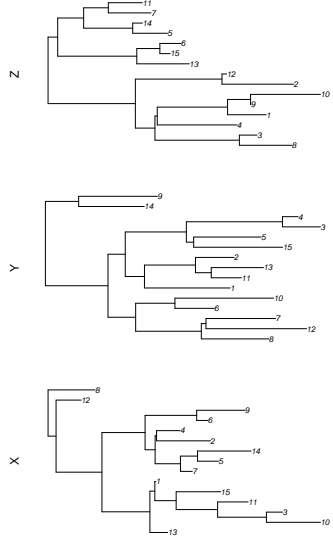
Our method is simple and non-parametric and can be applied to lineages interacting in a tri-trophic relationship. In conclusion, we propose the combined use of  $P_\lambda$ ,  $P_{YZ,X}$ ,  $P_{XZ,Y}$  and  $P_{XY,Z}$  to detect and quantify three-way cospeciation among species.

All computation for this paper has been done using R R Development Core Team (2012) and the ape library developed by Paradis (2006). Software to implement our methods is available from [www1.maths.leeds.ac.uk/~stuart](http://www1.maths.leeds.ac.uk/~stuart).

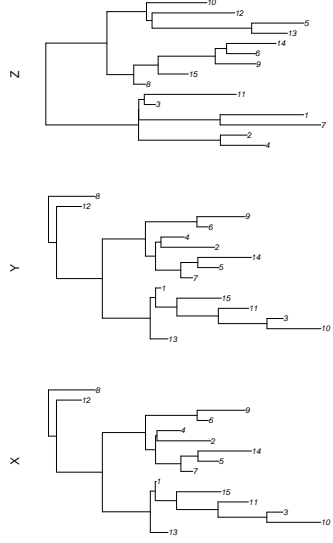
## A Example datasets

The phylogenetic trees simulated for Sections 3.1–3.3 are shown in Figure A.1 (a)–(c) respectively and their corresponding triangular interaction matrices  $T_{(a)}-T_{(c)}$  are given in (2). Here, (a) refers to the example with no cospeciation; (b) refers to strong cospeciation between  $X$  and  $Y$  while  $Z$  is independent of both; and (c) refers to the example where all three trees are strongly cospeciated.

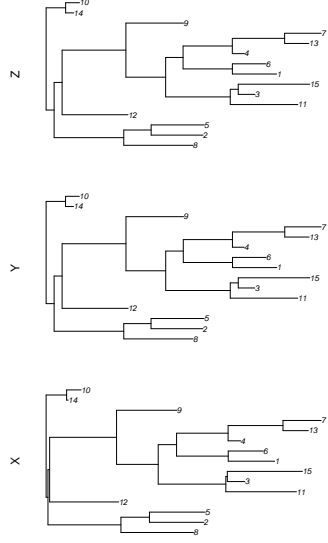
$$T_{(a)} = \begin{pmatrix} 1 & 5 & 15 \\ 1 & 14 & 15 \\ 5 & 13 & 12 \\ 5 & 14 & 11 \\ 6 & 2 & 8 \\ 7 & 1 & 2 \\ 8 & 3 & 8 \\ 8 & 4 & 8 \\ 9 & 9 & 3 \\ 10 & 13 & 9 \\ 11 & 6 & 1 \\ 12 & 6 & 11 \\ 13 & 3 & 3 \\ 13 & 7 & 6 \\ 15 & 5 & 11 \end{pmatrix} \quad T_{(b)} = \begin{pmatrix} 1 & 1 & 1 \\ 2 & 2 & 10 \\ 3 & 3 & 14 \\ 4 & 4 & 4 \\ 5 & 5 & 6 \\ 6 & 6 & 7 \\ 7 & 7 & 8 \\ 8 & 8 & 3 \\ 9 & 9 & 9 \\ 10 & 10 & 2 \\ 11 & 11 & 11 \\ 12 & 12 & 15 \\ 13 & 13 & 12 \\ 14 & 14 & 13 \\ 15 & 15 & 5 \end{pmatrix} \quad T_{(c)} = \begin{pmatrix} 1 & 1 & 1 \\ 2 & 2 & 2 \\ 3 & 3 & 3 \\ 4 & 4 & 4 \\ 5 & 5 & 5 \\ 6 & 6 & 6 \\ 7 & 7 & 7 \\ 8 & 8 & 8 \\ 9 & 9 & 9 \\ 10 & 10 & 10 \\ 11 & 11 & 11 \\ 12 & 12 & 12 \\ 13 & 13 & 13 \\ 14 & 14 & 14 \\ 15 & 15 & 15 \end{pmatrix} . \quad (2)$$



(a) No cospeciation.



(b) Strong cospeciation between X and Y independently of Z.



(c) Strong cospeciation between all three trees.

Figure A.1: Trees generated with varying degrees of cospeciation.

## B Labels for the termite-bacteria-protist dataset

Table B.1: Termite labels, *X*.

1	<i>Rhinotermes_marginalis</i>
2	<i>Rhinotermes_hispidus</i>
3	<i>Schedorhinotermes_sp_Australia</i>
4	<i>Parrhinotermes_sp</i>
5	<i>Schedorhinotermes_sp_Laos</i>
6	<i>Termitogeton_planus</i>
7	<i>Psammotermes_allocerus</i>
8	<i>Heterotermes_longiceps</i>
9	<i>Heterotermes_tenuis</i>
10	<i>Coptotermes_formosanus_japan</i>
11	<i>Coptotermes_formosanus_china</i>
12	<i>Coptotermes_sp_Malaysia</i>
13	<i>Coptotermes_sp_Laos</i>
14	<i>Coptotermes_testaceus</i>

Table B.2: Protist labels, *Y*.

1	<i>AB262494_Psudotrichonympha_sp</i>
2	<i>AB262495_Psudotrichonympha_sp</i>
3	<i>AB262496_Psudotrichonympha_sp</i>
4	<i>AB262497_Psudotrichonympha_sp</i>
5	<i>AB262498_Psudotrichonympha_sp</i>
6	<i>AB032211_Psudotrichonympha_sp</i>
7	<i>AB262486_Psudotrichonympha_sp</i>
8	<i>AB262487_Psudotrichonympha_sp</i>
9	<i>AB262488_Psudotrichonympha_sp</i>
10	<i>AB262489_Psudotrichonympha_sp</i>
11	<i>AB262490_Psudotrichonympha_sp</i>
12	<i>AB262491_Psudotrichonympha_sp</i>
13	<i>AB262492_Psudotrichonympha_sp</i>
14	<i>AB262493_Psudotrichonympha_sp</i>

Table B.3: Bacteria labels, *Z*.

1	<i>AB262559_Br02HtL_S4</i>
2	<i>AB262560_Br78HtT_S1</i>
3	<i>AB218918_CfPt1_2</i>
4	<i>AB262555_CNCpF_S1</i>
5	<i>AB262556_Ma79Cp_S1</i>
6	<i>AB262557_La10Cp_S3</i>
7	<i>AB262558_Br75CpT_S1</i>
8	<i>AB218919_TpPtN_4</i>
9	<i>AB262562_Br84RhM_S5</i>
10	<i>AB262563_Br76RhH_S1</i>
11	<i>AB262564_La19Sc_S1</i>
12	<i>AB262566_My26Pa_S1</i>
13	<i>AB262565_Au05Sc_S1</i>
14	<i>AB262561_SA16PsA_S4</i>

$$T = \begin{matrix} & \begin{matrix} \text{termite} & \text{protist} & \text{bacteria} \end{matrix} \\ \begin{matrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \\ 10 \\ 11 \\ 12 \\ 13 \\ 14 \end{matrix} & \begin{pmatrix} 1 & 1 & 9 \\ 2 & 2 & 10 \\ 3 & 4 & 13 \\ 4 & 5 & 12 \\ 5 & 3 & 11 \\ 6 & 13 & 8 \\ 7 & 14 & 14 \\ 8 & 11 & 1 \\ 9 & 12 & 2 \\ 10 & 6 & 3 \\ 11 & 7 & 4 \\ 12 & 8 & 5 \\ 13 & 9 & 6 \\ 14 & 10 & 7 \end{pmatrix} \end{matrix} .$$

## C Labels and interaction matrix for the tree-moth-wasp dataset

Table C.1: Tree labels,

<u>X.</u>	
1	Viburnum
2	Acer
3	Salix
4	Trifolium
5	Medicago
6	Ulmus
7	Prunus
8	Crataegus
9	Malus
10	Sorbus
11	Fagus
12	<i>Quercus robur</i>
13	Alnus
14	Betula
15	Corylus
16	Carpinus

Table C.2: Moth labels,

<u>Y.</u>	
1	<i>Parornix carpinella</i>
2	<i>P_schreberella</i>
3	<i>P_harrisella</i>
4	<i>P_nicellii</i>
5	<i>P_cavella</i>
6	<i>P_froelichiella</i>
7	<i>P_lautella</i>
8	<i>P_insignitella</i>
9	<i>P_roboris</i>
10	<i>P_spinicolella</i>
11	<i>P_viminiella</i>
12	<i>P_salicicolella</i>
13	<i>P_rajella</i>
14	<i>P_ulmifoliella</i>
15	<i>P_geniculella</i>
16	<i>P_platanoidella</i>
17	<i>P_sylvella</i>
18	<i>P_quercifoliella</i>
19	<i>P_lantanella</i>
20	<i>P_maestingella</i>
21	<i>P_sorbi</i>
22	<i>P_corylifoliella</i>
23	<i>P_coryli</i>
24	<i>P_esperella</i>
25	<i>P_cydoniella</i>
26	<i>P_oxycanthae</i>
27	<i>P_mespilella</i>
28	<i>P_blancardella</i>

Table C.3: Wasp labels

<u>Z.</u>	
1	insignitellae
2	carpini
3	zwoelferi
4	niveipes
5	atys
6	suprafolius
7	<i>Cilla_ex_Quercus</i>
8	splendens
9	<i>cilla_ex_Viburnum</i>
10	<i>cilla_ex_Corylus</i>
11	buekkensis
12	pseudoplatanus
13	acerianus
14	latreillii
15	butus



$$T = \begin{pmatrix} & \text{tree} & \text{moth} & \text{wasp} \\ 1 & 19 & 9 & \\ 2 & 15 & 12 & \\ 2 & 16 & 12 & \\ 2 & 17 & 12 & \\ 3 & 11 & 3 & \\ 3 & 12 & 3 & \\ 4 & 8 & 1 & \\ 6 & 2 & 8 & \\ 8 & 26 & 5 & \\ 8 & 22 & 6 & \\ 9 & 28 & 5 & \\ 10 & 21 & 5 & \\ 10 & 27 & 5 & \\ 10 & 22 & 6 & \\ 10 & 21 & 8 & \\ 11 & 20 & 11 & \\ 12 & 7 & 7 & \\ 12 & 18 & 14 & \\ 12 & 3 & 14 & \\ 12 & 7 & 14 & \\ 12 & 9 & 14 & \\ 12 & 18 & 15 & \\ 12 & 3 & 15 & \\ 13 & 13 & 8 & \\ 13 & 6 & 8 & \\ 14 & 14 & 4 & \\ 14 & 5 & 4 & \\ 14 & 22 & 6 & \\ 15 & 23 & 8 & \\ 15 & 4 & 8 & \\ 15 & 4 & 10 & \\ 16 & 24 & 2 & \end{pmatrix}.$$

## References

- Ahmad, F., M. Aslam, and M. Razaq (2004): "Chemical ecology of insects and tritrophic interactions," *Research (Science)*, 15, 181–190.
- Campbell, V., P. Legendre, and F. J. Lapointe (2011): "The performance of the Congruency Among Distance Matrices(CADM) test in phylogenetic analysis," *BMC Evolutionary Biology*, 11:64.
- Charleston, M. and R. D. M. Page (2002): "Tree Map 2.0b A Macintosh program for the analysis of how dependent phylogenies are related, by co-phylogeny mapping," *Division of Environmental Evolutionary Biology, Institute of Biomedical Life Sciences, University of Glasgow, Glasgow, UK*, URL <http://www.it.usyd.edu.au/~mcharles/software/treemap/treemap.html>.
- Choi, K. and S. M. Gomez (2009): "Comparison of phylogenetic trees through alignment of embedded evolutionary distances," *BMC Bioinformatics*, 10, 423.
- Drummond, A. J. and A. Rambaut (2007): "BEAST: Bayesian evolutionary analysis by sampling trees," *BMC Evolutionary Biology*, 7, 214.
- Ewens, W. J. and G. R. Grant (2001): *Statistical Methods in Bioinformatics, Statistics for Biology and Health*, Springer.
- Fahrenholz, H. (1913): "Ectoparasiten und Abstammungslehre," *Zoology*, 41, 371–374.
- Forister, M. L. and C. R. Feldman (2010): "Phylogenetic Cascades and the Origins of Tropical Diversity," *Tropical Biology and Conservation*, 1–9.
- Fourment, M. and M. J. Gibbs (2006): "Patristic: A program of calculating patristic distances and graphically comparing the components of genetic changes," *BMC Evolution Biology*, 6, 1.
- Hommola, K., J. E. Smith, Y. Qiu, and W. R. Gilks (2009): "A Permutation Test of Host-Parasite Cospeciation," *Molecular Biology Evolution*, 26, 1457–1468.
- Huelsenbeck, J. P., B. Rannala, and B. Larget (2000): "A Bayesian framework for the analysis of cospeciation," *Evolution*, 54, 352–364.
- Klassen, G. J. (1992): "A history of the macroevolutionary approach to studying host-parasite associations," *Parasitology*, 78, 573–87.
- Lapointe, F.-J. and P. Legendre (1990): "A statistical framework to test the consensus of two nested classifications," *Systematic Zoology*, 39, 1–13.
- Lapointe, F.-J. and P. Legendre (1992a): "A statistical framework to test the consensus among additive trees (cladograms)," *Systematic Biology*, 41, 158–171.
- Lapointe, F.-J. and P. Legendre (1992b): "Statistical significance of the matrix correlation coefficient for comparing independent phylogenetic trees," *Systematic Biology*, 41, 378–384.
- Legendre, P., Y. Desdèviès, and E. Bazin (2002): "A statistical test for host-parasite coevolution," *Systematic Biology*, 51, 217–234.

- Legendre, P. and F.-J. Lapointe (2004): "Assessing congruence among distance matrices: single-male scotch whiskies revisited," *Aust N Z J Stat*, 46, 615–629.
- Lopez-Vaamonde, C., H. C. J. Godfray, S. A. West, C. Hansson, and J. M. Cook (2005): "The evolution of host use and unusual reproductive strategies in *Achrysocharoides* parasitoid wasps," *Evolutionary Biology*, 18, 1029–1041.
- Merkle, D. M. and N. Wieseke (2010): "A parameter-adaptive dynamic programming approach for inferring co-phylogenies," *BMC Bioinformatics*, 11, S60.
- Micha, S. G., S. Kistenmacher, G. Mölck, and U. Wyss (2000): "Tritrophic interactions between cereals, aphids and parasitoids: Discrimination of different plant-host complexes by *Aphidius Rhopalosiphi*," *European Journal of Entomology*, 97, 539–543.
- Noda, S., O. Kitade, T. Inoue, M. Kawai, M. K. K. Hiroshima, Y. Hongoh, R. Constantino, V. Uys, J. Zhong, T. Kudo, and M. Ohkuma (2007): "Cospeciation in the triplex symbiosis of termite gut protists (*Pseudotriconympha* spp.), their hosts, and their bacterial endosymbionts," *Molecular Ecology*, 16, 1257–1266.
- Page, R. D. M. (1990): "Temporal congruence and cladistic analysis of biogeography and cospeciation," *Systematic Zoology*, 39, 205–26.
- Page, R. D. M. (1994a): "Parallel phylogenies: Reconstructing the history of host-parasite assemblages," *Cladistics International Journal*, 10, 155–173.
- Page, R. D. M. (1994b): "Maps between trees and cladistic analysis of historical associations among genes, organisms and areas," *Systematic Biology*, 43, 58–77.
- Page, R. D. M. (1995): "Tree Map," *Division of Environmental Evolutionary Biology, Institute of Biomedical Life Sciences, University of Glasgow, Glasgow, UK*.
- Page, R. D. M. (1996): "Temporal congruence revisited: Comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice," *Systematic Biology*, 45, 151–67.
- Page, R. D. M. (2003): *Tangled trees: Phylogeny, cospeciation, and coevolution*, The University of Chicago Press, Chicago and London.
- Paradis, E. (2006): *Analysis of phylogenetics and evolution with R*, Use R, New York: Springer.
- Paradis, E., J. Claude, and K. Strimmer (2004): "APE: analyses of phylogenetics and evolution in R language," *Bioinformatics*, 20, 289–290.
- Paterson, A. M. and R. D. Gray (1997): "Host-parasite cospeciation, host switching and missing the boat," in D. H. Clayton and J. Moore, eds., *Host-Parasite Evolution: General Principles and Avian Models*, Oxford: Oxford University Press, 236–250.
- Paterson, A. M., R. L. Palma, and R. D. Gray (1999): "How frequently do avian lice miss the boat?" *Systematic Biology*, 48, 214–23.
- Percey, D. M., R. D. Page, and Q. C. B. Cronk (2004): "Plant-Insect Interactions: Double-Dating Associated Insect and Plant Lineages Reveals Asynchronous Ra-

- diations,” *Systematic Biology*, 53, 120–127.
- Planet, P. J. (2006): “Tree disagreement: Measuring and testing incongruence in phylogenies,” *Biomedical Informatics*, 39, 86–102.
- R Development Core Team (2012): *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, URL <http://www.R-project.org/>, ISBN 3-900051-07-0.
- Ronquist, F. and J. P. Huelsenbeck (2003): “MrBayes 3: Bayesian phylogenetic inference under mixed models,” *Bioinformatics*, 19, 1572–1574.
- Siddall, M. E. (1996): “Phylogenetic covariance probability: confidence and historical associations,” *Systematic Biology*, 45, 48–66.
- Siddall, M. E. (2001): “Computer-intensive randomization in systematics,” *Cladistics*, 17, 35–52.