

## Predicting network topology of mistletoe–host interactions: do mistletoes really mimic their hosts?

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Network analysis provides a unified framework for investigating different types of species interactions at the community level. Network analysis is typically based on null models that test for specific patterns in network topology. Here we use a novel predictive approach to investigate the topology of a mistletoe–host network. It has been hypothesised that Australian mistletoes mimic the phenotype of their preferred hosts to avoid herbivory. We developed a deterministic model based on phenotypic similarity to predict the topology of a quantitative network between Lauranthaceous mistletoes and their hosts. We quantified mistletoe–host interactions in a semi-arid woodland central Australia, along with the size, shape and colour of leaves produced by both players in the interaction. Traditional null model analyses showed support for negative co-occurrence patterns, web specialisation and strong links between species pairs. However, our deterministic model showed that the observed network topology could not be predicted by phenotypic similarity, suggesting that Australian mistletoes do not mimic their hosts.

A primary goal in community ecology is to identify the processes that govern the arrangement of species assemblages. Recently, species interaction networks have provided a useful way of interpreting community-level patterns. Under this unified framework the arrangement of species interactions (topology hereafter) can describe community structure (Bascompte et al. 2003, Jordano et al. 2003, Lehsten and Harmand 2006, Bascompte and Jordano 2007, Guimarães et al. 2007, Ulrich and Gotelli 2007, Blüthgen et al. 2008, Krishna et al. 2008, Dormann et al. 2009, Vásquez et al. 2009, Blüthgen 2010). The network approach has been applied to a wide range of biological interactions (including herbivory, pollination, seed dispersal, parasite–host relationships and predator–prey relationships) in ecosystems ranging from rainforests to coral reefs (Ings et al. 2009) and has provided further resolution to the on-going debate between assembly rules and stochastic processes in species interactions (Diamond 1975, Connor and Simberloff 1979).

Networks are generated by recording all species interactions (usually between two trophic levels) (Vásquez and Aizen 2003, Vásquez 2005, Vásquez et al. 2009) and evaluated using null model simulations that test a wide variety of network properties (Connor and Simberloff 1979, Gotelli and Graves 1995, Gotelli 2000, Dormann et al. 2009). Observed indices that deviate from those predicted by chance indicate deterministic processes (Gotelli 2000). For example, species-pairs that do not co-occur together and interact only with specialised ‘players’ in the network, or specific geographic locations,

generate checkerboard distributions and are interpreted as negative co-occurrence patterns (Stone and Roberts 1990). However, very different networks can often show similar structural properties (Bascompte et al. 2003). For example plant–pollinator interactions and parasite–host interactions can show similar patterns of nestedness (Fortuna et al. 2010). Inferring which processes lead to the organisation of species interactions, and ultimately the convergence of network topology, presents a new set of challenges.

Here we propose an alternative method for evaluating network topology. In addition to null model evaluations, we derive a deterministic model that predicts species interactions according to phenotype (e.g. morphology or physiology). Under this new analytical approach, biologically informative traits can be used to predict observed network topology. This approach can be used to investigate the connectivity among species interactions limited by trait matching. For example, Stang et al. (2009) recently showed the utility of using morphological information to evaluate network properties (Stang et al. 2006, 2007). However, their approach determined size class distributions that were associated with nestedness, and not specialised interactions. Here we employ a deterministic approach to evaluate a plant–plant network that displays a high degree of phenotypic similarity.

Phenotypic similarity can have substantial effects on species interactions (Thompson 2005) by increasing the removal rate of less favorable fruit, encouraging pollen transfer to flowers without reward (i.e. nectar) or decreasing herbivore browsing (Burns 2005, Dyer and Murphy 2009,

Fadzley et al. 2009, Gaskett and Herberstein 2010). For plant–herbivore interactions, the advantages of deception are beneficial if the plant can maintain traits that lead to aversion by herbivores (Williamson 1982). In the Southern Hemisphere, mistletoes (Loranthaceae) produce leaves that have a striking resemblance to the leaves of their preferred hosts. Along with the widely held view that mistletoe leaves mimic their host leaves, some (but not all) evidence points towards an adaptive strategy for leaf concealment (Barlow and Weins 1977, Ehleringer et al. 1986, Canyon and Hill 1997). These hemi-parasitic plants obtain water and some nutrients from host trees while maintaining their own photosynthetic ability (Aukema 2003). Therefore, mistletoes stand to gain if they conceal high water and nutrient content by displaying similar traits to their host tree (Canyon and Hill 1997, Mathiasen et al. 2008). Visual deception is an important factor that may influence the evolution of leaf symmetry in parasitic plants (Brown and Lawton 1991). However, all hypotheses proposed to explain apparent similarity in mistletoe–host interactions have remained unresolved (Barlow and Weins 1977, Ehleringer et al. 1986, Canyon and Hill 1997). In this study we test the hypothesis that mistletoe leaves mimic their host leaves by quantifying community-level patterns in matching and mismatching leaf traits.

Here, we test whether phenotypic similarity in leaf traits can predict the topology of an interaction network between mistletoes and their hosts. We quantified the distribution of mistletoes among host trees in a semi-arid woodland in central Australia and used traditional null models to establish the topology of the mistletoe–host network. Next, we quantified the size, shape and reflectance properties of the leaves of each mistletoe and host species, which we then used to derive a deterministic model to test whether the observed network topology can be predicted by phenotypic similarity. Overall results are then used to test the hypothesis that Australian mistletoes mimic their hosts at the community level. Specifically we test the following hypotheses:

- 1) Mistletoe–host interactions are structured non-randomly.
- 2) Mistletoe leaves mimic (in shape and colour) host trees in the community.
- 3) Phenotypic similarity can predict the topology of the interaction network.

## Methods

### Location and species

Mistletoe–host interactions were recorded in a semi-arid environment surrounding Fowlers Gap Research Station, approximately 100 km north of Broken Hill in western New South Wales, Australia (31°4'13"S, 141°42'16"E). The area has a mean annual rainfall of just 222.6 mm, and is exposed to extreme temperatures in summer (mean maximum daily temperature = 31.8°C) and relatively low temperatures in winter (mean minimum daily temperature = 4.5°C) (Broken Hill weather station, Australian Government Bureau of Meteorology, <<http://reg.bom.gov.au/>>, last visited on 17 March 2011). Trees and large shrubs

(i.e. potential hosts for mistletoe) are primarily found at the edges of ephemeral creeks. However, some trees can be found along connecting waterways and exposed ridge tops.

The riparian woodland community is dominated by *Eucalyptus camaldulensis* with an understory of *Myoporum* and *Santalum* species. Connecting waterways typically consist of *Eremophilla*, *Casuarina* and *Alectryon* species, while exposed ridge tops are primarily populated by *Acacia aneura*. However, most plant species occurred across multiple regions (Appendix 1), and are therefore considered a single plant community. We recorded 127 mistletoe–host interactions for four mistletoe species occurring within six kilometers of vegetation surrounding Fowlers Gap Research Station. The mistletoes *Amyema preissii*, *A. maidenii*, *A. lino-phylla* and *Lysiana exocarpi* were observed colonising ten host tree species, including *Acacia loderi*, *A. aneura*, *A. salicina*, *A. victoriae*, *Alectryon oleifolium*, *Casuarina pauper*, *Eremophila alternifolia*, *E. longifolia*, *Santalum acuminatum* and *S. lanceolatum*. All *Amyema* species were observed on no more than two host species, while *L. exocarpi* was found on eight host tree species. *L. exocarpi* was observed colonising *Pittosporum angustifolium* and *Senna sturtii*; however they consisted of two rare interactions and removed from analyses for consistency between models. Un-parasitised trees were not included in this study.

### Network topology of mistletoe–host interactions: null model

Mistletoe–host interactions were recorded as one community-level matrix following Burns (2007). Null model simulations were first used to test whether the observed assemblage of mistletoes and their hosts were structured non-randomly. To do this we randomly generated interactions for all species in the matrix by maintaining marginal row totals (mistletoe species) and allowing the marginal totals of each column to vary (host species). All simulations generated mistletoe–host interactions by randomly drawing host trees, without replacement, from a total pool of observed host interactions. The probability of detecting a mistletoe–host interaction was proportional to host abundance. Each randomly simulated matrix consisted of quantitative cell entries containing the incidence between species pairs. All simulations were replicated 1000 times using a fixed random seed and evaluated using three community-level indices including 1) network-level specialisation (i.e.  $H_2'$ ), 2) as negative co-occurrence patterns between species pairs (i.e. c-score) and 3) within each 'cell' of the interaction network (i.e. interaction strength) (Appendix 2). These network indices were selected because they assess different levels of specialization.

First the  $H_2'$  index was used to calculate network-level interactions.  $H_2'$  calculates overall network specialisation by measuring the extent to which interactions within the matrix deviate from what is expected given the abundance of each species (Blüthgen et al. 2006).  $H_2'$  is a standardised measure of specialization that ranges between 0 (generalists) and 1 (specialists) (Blüthgen et al. 2006). Results close to one indicate that the proportion of links between species of each trophic level is lower than expected given the abundance distribution.

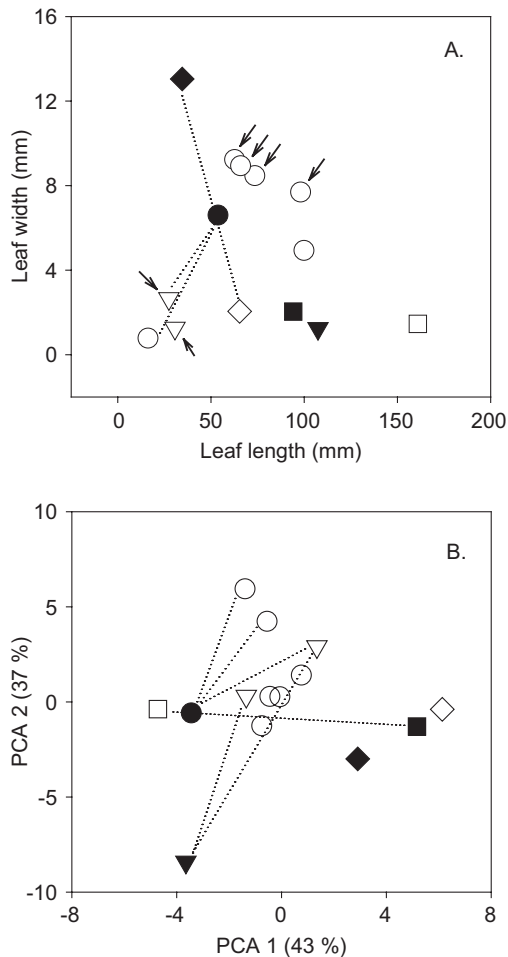


Figure 1. Pair wise comparisons of all mistletoes and their hosts according to (A) leaf size and shape and (B) leaf colour (PCA 1 and 2). Symbols represent species pairs; circles = *L. exocarpi*, triangles = *A. preissii*, diamonds = *A. maidenii*, and squares = *A. linophylla*. Filled symbols represent mistletoe species and open symbols represent host species. Lines indicate species pairs with significantly different traits across all dimensions. All remaining species pairs were similar in only one dimension. Leaf dimensions displayed as length and width only. Arrows indicate mistletoe species that were significantly different in leaf area to their preferred host tree species. All data was  $\log_{10}$  transformed before statistical analysis.

Second the *c*-score index was used to calculate negative co-occurrence patterns among mistletoe species. *C*-score calculates the average checkerboard score of all species interactions (Stone and Roberts 1990, Gotelli 2000). The *c*-score index was obtained by calculating the number of checkerboard units (cu) for each species pair:  $cu = (O_i - S)(O_j - S)$ , where  $O_i$  is the total number of host species occupied by mistletoe species *i*,  $O_j$  is the total number of host species occupied by mistletoe species *j*, and *S* is the number of host species occupied by both species (Stone and Roberts 1990). All simulated interactions were converted from quantitative counts to binary cell entries before calculating *c*-score. A *c*-score that is greater than predicted by chance indicates that mistletoes use different host species in the community.

Third we tested for non-random patterns in interaction strength. Interaction strength is a quantitative measure of links between species pairs. Interaction strength significantly

greater than predicted by chance indicates that mistletoes infect specific host tree species (i.e. host preferences). We tested interaction strength by calculating the difference between observed and expected values using Wilcoxon signed ranked tests as normality assumptions were not met. All calculations were performed in the R environment (R Development Core Team 2010). *C*-score and  $H_2'$  indices were calculated using functions loaded from the bipartite package in R.

## Phenotypic similarity

Phenotypic similarity in leaf size, shape and colour was quantified for 20 mature leaves collected from four individuals (five leaves from each plant) of each mistletoe and host species. Mistletoe leaves were collected randomly from different host tree species in the community. *Amyema linophylla* was sampled from only two individuals due to low local abundance. Three measurements were obtained. Leaf width was measured at the widest point of the lamina using digital calipers, while leaf length and area were calculated using ImageJ (ver. 1.44p; Rasband 1997–2009). *Casuarina* species have reduced leaves surrounding the internodes of photosynthetic stems. Here we considered the stems to be the primary photosynthetic organ, and thus the appropriate unit for morphological measurements. Tree species free from mistletoe infection and rare host species observed only once in the community were excluded from analysis. All data were  $\log_{10}$  transformed before analysis.

Leaf reflectance properties (i.e. colours) were quantified for all mistletoe and host species using a spectrometer and a pulsed xenon light source. A diffuse white reflectance standard was used to calibrate the spectrometer and spectral curves were measured as a proportion using Spectrasuite software. A fiber optics probe was fitted with an additional matt black tube modified to include a 45° angle and a 1 cm distance between the object and light source. Reflectance spectra were recorded between 400 and 700 nm at 5 nm intervals. Reflectance patterns were analysed in absence of perceptual color space (for instance a bird or insect vision model), because mistletoes in Australia experience herbivory from taxa with a range of different visual systems (including insects, marsupials, ratites and placental mammals), and the degree of selection pressure from each taxon is unknown. All wavelengths were adjusted to retain only the colour (chromatic) signal by converting percent reflectance to a proportion for each wavelength. In other words, the achromatic (brightness) component was standardized among all replicates.

We tested for differences in morphology among all species pairs using two methods. First, we assessed whether mistletoes exhibit overlapping traits with their hosts. Pair wise comparisons of length, width, area, and colour of all mistletoe–host interactions were assessed individually using Tukey HSD with adjusted *p*-values. Leaf colour was analysed using the first three principle components that accounted for over 80% of the variation in principle components analysis following Endler (1990).

Our second approach was two-fold. We tested whether the collective arrangement of leaf traits for each mistletoe–host species pair was more or less similar than random expectations. Next we used these results to predict network

topology. First we sampled twenty leaves (regardless of species identity) randomly without replacement from a pooled dataset of all host leaves sampled ( $n = 200$ ). Twenty leaves were sampled to ensure that predicted distributional trait values were comparable with those observed. Phenotypic similarity was calculated by measuring the Euclidean distance between individual host leaves (e.g. length, width and area) and each mistletoe species (i.e. the distance from the centroid of all three traits). All Euclidean distances were averaged to generate the expected similarity in leaf traits from host tree species with randomly assorted leaves and each mistletoe species. This created a benchmark to examine the degree of phenotypic similarity among observed interactions. Observed phenotypic similarity was compared to 1000 simulations and z-scores were obtained by calculating the number of standard deviations away from the randomised distribution:  $z = (X_o - R_m)/R_{sd}$ , where  $X_o$  is the observed Euclidean distance,  $R_m$  is the mean, and  $R_{sd}$  is the standard deviation of all randomly generated Euclidean distances.

### Predicting network topology: deterministic model

Phenotypic similarity in leaf traits was used to predict network topology and results were used to investigate whether mistletoes mimic their hosts. The analytical approach we used applied weighted probabilities during the randomization procedure to generate each simulated matrix. Therefore, all simulated interactions were selected according to phenotype (i.e. leaf traits) and abundance of individuals in the community. We weighted the likelihood that each interaction could occur in the deterministic model following two approaches that have been proposed in the literature to explain phenotypic similarity in mistletoe–host interactions (Barlow and Weins 1977, Vane-Wright 1980).

First, we tested the hypothesis that being similar to a host is advantageous (mimicry hypothesis hereafter). To test the mimicry hypothesis we adjusted the randomisation procedure by weighting each host species by their similarity in leaf traits with each mistletoe species (i.e. observed Euclidean distance). Each host tree species was weighted relative to the most dissimilar trait combination. To do this we subtracted each Euclidean distance from the largest Euclidean distance in the entire matrix. Therefore, mistletoe–host interactions that were most dissimilar in leaf traits were least likely to occur in each simulated matrix. All Euclidean distances were weighted as a proportion between 0 and 1, where 0 represents the most dissimilar trait combination and 1 represents the most similar trait combination.

Second, although the mimicry hypothesis is the most commonly believed, and the major focus of this manuscript, there are other hypotheses about mistletoe–host resemblance, such as adaptive similarity and the cryptic mimicry hypothesis that quantify mismatched traits (Barlow and Weins 1977, Canyon and Hill 1997). For example, mistletoes with high nutrient content gain more from appearing similar to the foliage of their host trees, while mistletoes with low nutrient content gain more from appearing dissimilar to the foliage of their host trees (Barlow and Weins 1977). Assigning mismatched combinations may have important implications for predicting network topology and are therefore included in this study (i.e. trait mismatching hereafter). To test trait

mismatching we adjusted the randomisation procedure by weighting each host species with the divergence from the mean similarity of all host leaf traits using a standardised distance (i.e. z-score; Appendix 3). To do this we converted each z-score to a positive value. Therefore, mistletoe–host interactions with the most dissimilar leaf traits (positive z-score) were equally likely to occur as those with very similar leaf traits (negative z-score) in each simulated matrix. All Euclidean distances were weighted as a proportion between 0 and 1, where 0 represents trait combinations that are no different to random and 1 represents very similar or dissimilar trait combinations.

Initially we tested a combined model including leaf size, shape and colour. We followed up analyses of leaf size and shape and leaf colour individually as they often show conflicting displays of mimicry. All tests followed the assumptions of the mimicry hypothesis and trait mismatching as outlined above. Observed values that deviate from the expected distribution indicate poor correspondence between leaf traits and species interactions. We did not include a ‘threshold operator’ which recognizes forbidden links following Stang et al. (2009) as we assumed all interactions were possible in the community.

Some mistletoe species colonise several hosts (Downey 1998). Of course we do not expect these generalist mistletoes to resemble all hosts equally. Under the mimicry hypothesis, the prediction is for mistletoes to most closely resemble those hosts with which they have the strongest interactions. We tested how pervasive leaf similarity is in the community by quantifying the relationship between interaction strength and pair wise similarity. In addition we tested the performance of the deterministic approach by quantifying the relationship between predicted interaction strengths and observed interaction strengths of each species pair. We compared the null model approach with the deterministic approach for each combined model defined by the mimicry hypothesis and trait mismatching hypothesis using linear regression. Results indicate the explanatory power of including leaf traits in the evaluation of mistletoe–host network topology. All other analyses were conducted using ‘bipartite’ package in the R environment (R Development Core Team 2010) and PASW ver. 18.

## Results

### Network topology of mistletoe–host interactions: null model

We found non-random structure in network topology of mistletoes and their hosts. Mistletoe–host interactions showed network-level specialisation ( $H_2' = 0.913$ ;  $z = 33.318$ ,  $p < 0.001$ ), negative co-occurrence patterns ( $cu = 0.361$ ;  $z = 2.749$ ,  $p = 0.003$ ), and strong interaction strengths between species pairs ( $z = 2.771$ ,  $p < 0.001$ ). Results did not differ when we included rare interactions ( $H_2' = 0.913$ ;  $z = 33.777$ ,  $p < 0.001$ ;  $cu = 0.307$ ;  $z = 2.725$ ,  $p = 0.003$ ;  $z = -2.981$ ,  $p < 0.001$ ).

### Phenotypic similarity

Initially we predicted that mistletoes would have overlapping leaf traits with preferred host trees; however our analysis showed that single traits were often very different between

species pairs. In leaf size and shape *A. maidenii* was significantly different to its only host, *A. aneura*, while *L. exocarpi* was significantly different to *A. victoriae* and *E. alternifolia* for all traits. All remaining mistletoe–host pairs were similar to their hosts in only one of three leaf dimensions (i.e. length, width, or area) (Fig. 1a). In leaf colour, *A. linophylla* was significantly different to its only host, *C. pauper*, while *A. priessii* was significantly different to its only hosts, *A. victoriae* and *A. loderi*. *L. exocarpi* was significantly different to three hosts including *A. salicina*, *A. oleifolium* and *A. victoriae* (Fig. 1b).

Next we asked whether the collective arrangement of leaf traits were more or less similar than random expectations in the community. For leaf size and shape, eight out of twelve mistletoe–host interactions were more similar than predicted by chance (Fig. 2) indicating that comparisons of single leaf traits can underestimate similarity between mistletoes and their hosts. However, leaf size and shape was also shared with alternative trees (non host trees) in the community (Fig. 2). For leaf colour, only three interactions collectively for *L. exocarpi* and *A. maidenii* were more similar than predicted by chance (Fig. 3).

### Predicting network topology: deterministic model

Phenotypic similarity did not predict observed network topology (Fig. 4). A combined model including leaf size,

shape and colour did not predict network level specialization ( $H_2' = 0.086$ ;  $z = 3.815$ ,  $p < 0.001$ ), negative co-occurrence patterns ( $cu = 0.128$ ;  $z = 2.624$ ,  $p = 0.004$ ) or interaction strengths ( $z = -2.981$ ,  $p < 0.001$ ). Similarly, leaf size and shape, and leaf colour when analysed separately did not predict network-level specialisation ( $H_2' = 0.095$ ;  $z = 27.741$ ,  $p < 0.001$ ;  $H_2' = 0.108$ ,  $z = 29.958$ ,  $p < 0.001$ , respectively), negative co-occurrence patterns ( $cu = 0.128$ ;  $z = 2.499$ ,  $p = 0.006$ ;  $cu = 0.144$ ,  $z = 2.271$ ,  $p = 0.012$ ; respectively) or interaction strengths ( $z = -2.903$ ,  $p < 0.001$ ;  $z = -2.306$ ,  $p < 0.001$ ; respectively) according to the mimicry hypothesis.

Similar results were found when we considered trait mismatches between mistletoes and their hosts. A combined model including leaf size, shape and colour did not predict network level specialization ( $H_2' = 0.127$ ;  $z = 23.740$ ,  $p < 0.001$ ), negative co-occurrence patterns ( $cu = 0.1865$ ;  $z = 1.809$ ,  $p = 0.035$ ) or interaction strength ( $z = -2.668$ ,  $p < 0.001$ ). Similarly, leaf size and shape, and leaf colour when analysed separately did not predict network level specialization ( $H_2' = 0.145$ ;  $z = 21.936$ ,  $p < 0.001$ ;  $H_2' = 0.122$ ,  $z = 26.37$ ,  $p < 0.001$ ; respectively), negative co-occurrence patterns ( $cu = 0.1918$ ;  $z = 1.922$ ,  $p = 0.027$ ;  $cu = 0.194$ ,  $z = 1.594$ ,  $p = 0.055$ ; respectively) or interaction strengths ( $z = -2.667$ ,  $p = 0.002$ ;  $z = -3.059$ ,  $p < 0.001$ ; respectively) according to the trait mismatching hypothesis.

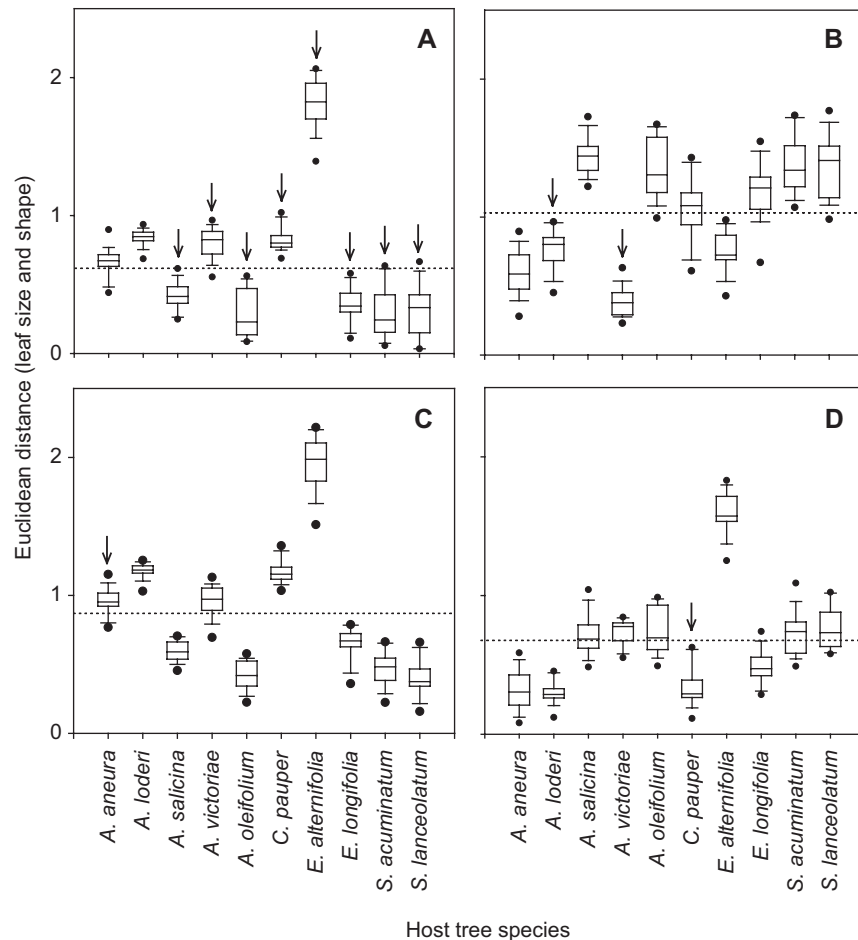


Figure 2. Similarities in leaf dimensions for all species pairs. Arrows indicate observed mistletoe–host interactions for (A) *L. exocarpi*, (B) *A. priessii*, (C) *A. maidenii* and (D) *A. linophylla*. The dashed line indicates expected similarity if leaf shape was distributed randomly among all species interactions. Error bars are 95% CI.

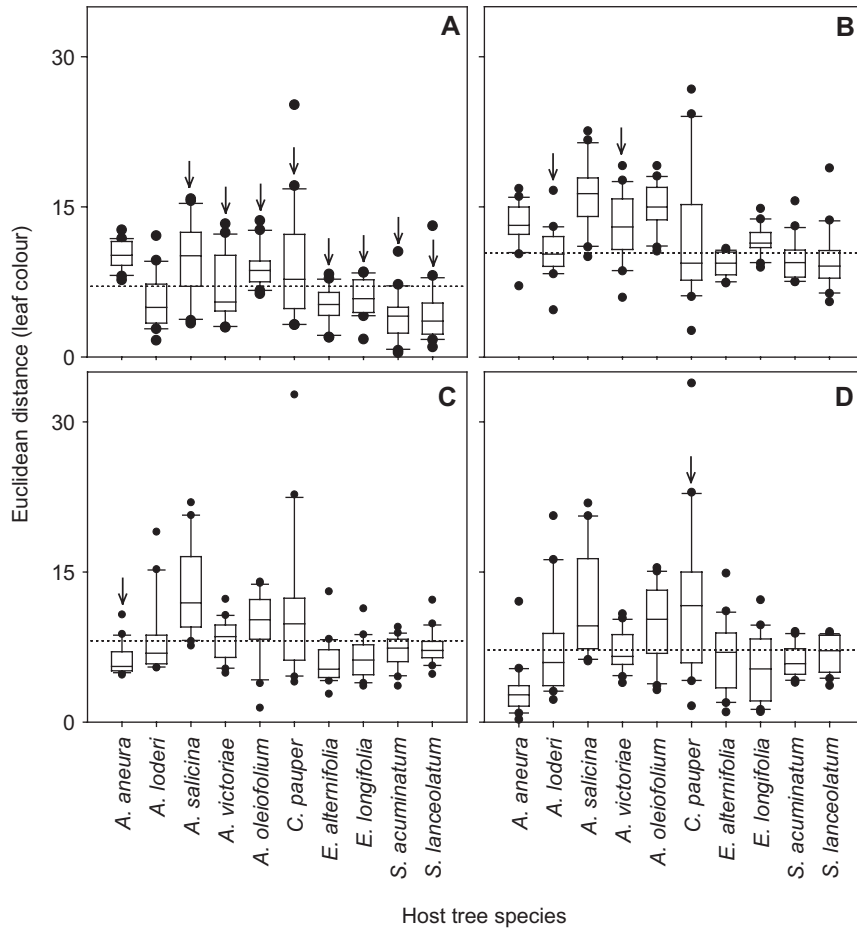


Figure 3. Similarities in leaf colour for all species pairs. Arrows indicate observed mistletoe-host interactions for (A) *L. exocarpi*, (B) *A. preissii*, (C) *A. maidenii* and (D) *A. linophylla*. The dashed line indicates expected similarity if leaf colour was distributed randomly among all species interactions. Error bars are 95% CI.

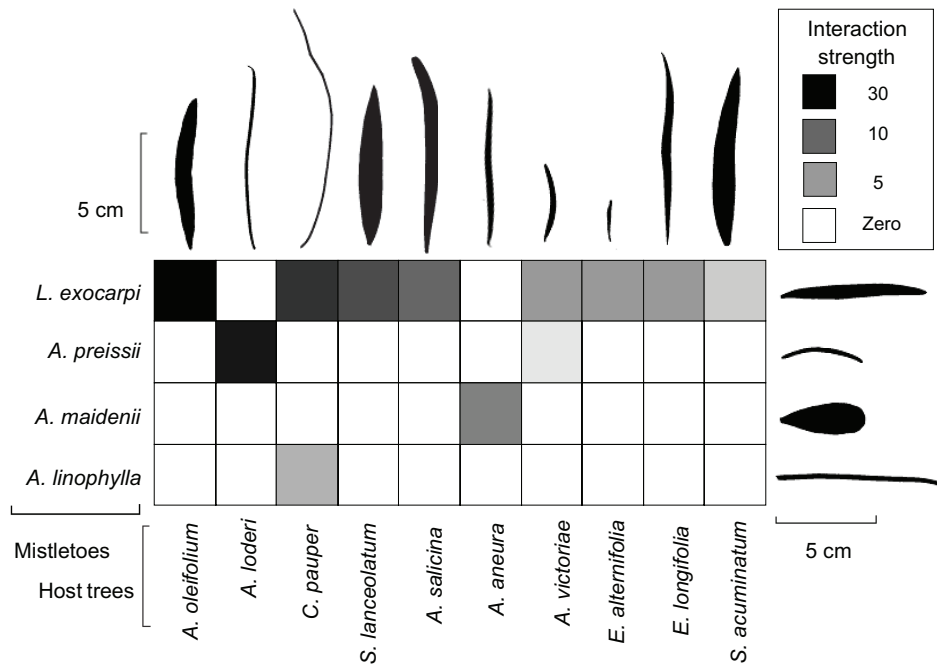


Figure 4. Network topology and leaf dimensions of all mistletoes and host trees (rows and columns, respectively) found at Fowlers Gap Research Station. Cell shade indicates interaction strength. Leaf size and shape depict the average dimensions of each plant species. The network image was developed using the 'visweb' function in the bipartite library loaded in the R environment.

Neither the similarity in leaf size and shape, nor similarity in leaf colour, between mistletoe–host species pairs (measured as Euclidean distances) was correlated with interaction strength ( $r^2 = 0.053$ ,  $F_{1,38} = 2.125$ ,  $p = 0.153$ ;  $r^2 = 0.013$ ,  $F_{1,38} = 0.483$ ,  $p = 0.491$ ; respectively) indicating that apparent similarities in leaf traits are not a pervasive feature in the community. Predicted interaction strength from the null model (i.e. based on abundances alone) explained 47% of the variation in observed interaction strength ( $r^2 = 0.472$ ,  $F_{1,38} = 33.962$ ,  $p < 0.001$ ). Predicted interaction strength from the deterministic model for the mimicry hypothesis (which included phenotypic similarity in addition to abundance), explained 43% of the variation in observed interaction strength ( $r^2 = 0.431$ ,  $F_{1,38} = 28.731$ ,  $p < 0.001$ ). However, predicted interaction strength from the deterministic model for the trait mismatching hypothesis (which included phenotypic similarity in addition to abundance), explained just 23 percent of the variation in observed interaction strengths ( $r^2 = 0.23$ ,  $F_{1,38} = 11.338$ ,  $p = 0.002$ ).

## Discussion

The topology of the mistletoe–host network could not be predicted by similarity in leaf traits. Mistletoe leaves are often suggested to match their hosts (Barlow and Weins 1977, Williamson 1982, Ehleringer et al. 1986, Canyon and Hill 1997). However, size, shape and colour of leaves are also similar to non host trees. As such, the deterministic model did not predict co-occurrence patterns, interaction strengths or network specialisation. However, mistletoe–host interactions did show negative co-occurrence patterns, interaction strength and network specialisation indicating strong, exclusive host preferences.

The deterministic model did not predict host specificity when the deterministic model was defined by mimicry or trait mismatching in leaf size, shape and colour. In fact a model that incorporated biological traits performed worse than null model evaluations. This indicates that leaves of mistletoes in this study are not unique among their preferred host trees and appear to be shared traits in the community. Poor correspondence between *c*-score and interaction strength indicated that the deterministic model generated alternative patterns of mistletoe–host interactions to that observed at Fowlers Gap. Shared leaf traits throughout the community may also indicate that mistletoes and trees use similar strategies for dealing with harsh environmental conditions. Traits such as leaf toughness, trichome density or internode length may provide further resolution to evolution of leaf size and shape in mistletoes. Predicting network topology of mistletoe–host interactions according to leaf traits is the first attempt that we are aware of at identifying mimicry in a plant community.

Surprisingly, few network evaluations have previously incorporated morphological and physiological information, despite many interaction types being highly suited to this sort of approach. For example, plant–pollinator interactions are the most widely investigated networks (Bascompte 2003, Bascompte et al. 2003, Jordano et al. 2003, Vázquez 2005, Bascompte and Jordano 2007) which are generally composed of complementary traits (e.g. proboscis length

and corolla depth) (Stang et al. 2006, Rezende et al. 2007). However only one study to-date has applied phenotype under a unified framework that interprets community-level patterns (Stang et al. 2009). This approach may have important implications to many ecological food webs including predator–prey, plant–herbivore and symbiotic relationships and the evaluation of single or multiple processes that are underlying network topology.

Traditional null model analysis showed support for network-level specialisation, negative co-occurrence patterns and interaction strength. These results indicate that mistletoes infect specific host trees exclusively in this plant community. The mechanisms underlying host specificity in parasitic plants are poorly known. One explanation for host specific interactions is that mistletoes have undergone local adaptation to host availability (Barlow and Weins 1977, Norton and Carpenter 1998). Although seed transplant experiments also show that some trees can resist mistletoe infection (Rodl and Ward 2002) indicating that host provenance is a combination of host availability and host quality (Watson 2009). At present it remains unclear to what extent host resistance influences mistletoe communities. Disentangling the spatial aggregation of individuals and network topology may reveal exciting insights into mistletoe communities. Work is currently underway to address the role of host exploitation underlying network topology.

Plant–plant interaction networks have received much recent attention (Blick and Burns 2009, 2011, Burns and Zotz 2010, Sfair et al. 2010, Silva et al. 2010). The fast expanding literature on plant–plant interaction networks indicate that different arboreal plant groups show different network topologies (Blick and Burns 2009). Mistletoes are primarily composed of host specific interactions and share similar network topology to lianas (Blick and Burns 2009, 2011, but see Sfair et al. 2010). In contrast, epiphytes interact with hosts and other epiphytic species to form positive co-occurrence patterns, or more specifically, ‘nested’ patterns (Burns 2007, Burns and Zotz 2010, Silva et al. 2010). Differences in network topology likely reflect differences in deterministic processes. For example lianas found in a temperate New Zealand rainforest indicate that habitat partitioning is an important determinant of their associations with host trees (Blick and Burns 2011). Interestingly, the network structure observed for mistletoes and their hosts in semi-arid Australia was similar to that previously observed for mistletoe–host interactions growing under very different conditions in New Zealand (Blick and Burns 2009). Further network evaluations of plant–plant interactions will reveal how consistent these trends are across different environments.

It remains an open question whether host specificity influences the evolution of leaf size and shape. In this study, leaf size and shape differed between mistletoes that produce broad leaves (*Amyema maidenii* and *Lysiana exocarpi*) and mistletoes that produce terete leaves (*A. linophylla* and *A. preissii*). Host specificity is known to vary drastically among these species. *L. exocarpi* has been recorded on 109 host species, *A. preissii* has been recorded on 73 host species, *A. maidenii* has been recorded on 37 host species, and *A. linophylla* has been recorded on eight host species (Downey 1998). Despite having a wide host range, it is suggested that mistletoes show phenotypic similarity to preferred host trees.

Barlow and Weins (1977) qualitatively assessed these mistletoes as preferentially mimicking *Heterodendrum oleifolium* (now *Alectryon oleifolium*), *Acacia* spp., *A. aneura* and *C. cristata*, respectively. However, our results only partially support this assessment. Currently, it remains unclear how spatial and temporal variations in host use influence leaf size and shape of different mistletoe species.

All current hypotheses that explain phenotypic similarity between mistletoes and their hosts suggest that herbivore damage will generate convergence in plant traits between species. However, plant–herbivore interactions form complex food webs consisting of both specialist and generalist herbivores (Novotny et al. 2010) that influence reproductive traits and leaf characteristics differently in spatially separated populations (Parra-Tabla and Herrera 2010, Muola et al. 2010). Recent studies have revealed the extent of damage caused by herbivory on mistletoe populations (Bach and Kelly 2004, Sweetapple 2008) suggesting that population level selection of plant traits is plausible. However, the only studies to examine this showed that mistletoe selection by herbivores was unrelated to leaf size and shape (Canyon and Hill 1997). Furthermore, as our results showed considerable variation in phenotypic similarity in leaf size, shape and colour, we suggest that a universal hypothesis for mimicry in mistletoes is unlikely.

Overall, plant mimicry has received much less attention than has animal mimicry (Schaefer and Ruxton 2009). As a result, understanding the evolution of deception in plants has lagged behind our understanding of deception in animals (Williamson 1982, Schaefer and Ruxton 2009). There have been few studies on cryptic leaf displays and even fewer on leaf mimicry (Brown and Lawton 1991). Leaf size and shape are known to be influenced by a wide range of factors including environmental and biotic interactions (Brown and Lawton 1991) that current mimicry hypotheses do not account for. Because mistletoes differ in host specificity (Downey 1998) and geographic range (Grenfell and Burns 2009), trait matching between mistletoe and host leaves may be better explained under the geographic mosaic of coevolution (Thompson 2005). Under this framework we suggest that 1) local adaptation in mistletoe leaves will not sweep through the entire population because herbivores differ among communities, 2) there will be common mismatches between leaf traits when multiple hosts are exploited in a community and 3) trait mixing between plants from neighboring communities will decrease selection for cryptic displays.

Overall we show that mistletoes do not mimic preferred host trees and we suggest that a unified framework consisting of geographic isolation (local environmental constraints), host specificity and herbivore interactions is required to explain the patterns of trait matches and mismatches at the community level.

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## Appendix 1

Mistletoe–host interactions in different habitats.

	Host	Mistletoes
Exposed ridge	<i>Acacia aneura</i>	<i>Amyema maidenii</i>
Connecting waterways	<i>Acacia salicina</i> <i>Senna sturtii</i> <i>Pitosporum angustifolium</i> <i>Eremophila alternifolia</i> <i>Acacia victoriae</i>	<i>Lysiana exocarpi</i>
Creepline	<i>Acacia loderi</i> <i>Acacia salicina</i> <i>Acacia victoriae</i> <i>Eremophila longifolia</i> <i>Casuarina pauper</i> <i>Alecryon oleiofolium</i>	<i>Amyema preissii</i> <i>Amyema linophyllum</i> <i>Lysiana exocarpi</i>

## Appendix 2

R script used to simulate network properties. Network indices calculated using functions loaded in the bipartite package. C.score is given here as an example.

### Null model

```
Data <-read.table("F:\\file.txt",header = T)
attach(Data)
names(Data)
"host"
library(bipartite)
set.seed(34263)
for(i in 1:1000){result <-matrix(c(
(as.vector(table(sample(host,9)))),(as.vector(table(sample(host,26))))),
(as.vector(table(sample(host,87)))),(as.vector(table(sample(host,3))))),
nrow=10,ncol = 4)
res <-ifelse(result > = 1,1,0)
R <-matrix(res,byrow = T,nrow = 4)
CS <-C.score®
write((CS),file = "file.txt",append=TRUE)}
```

### Deterministic model

```
set.seed(34263)
for(i in 1:1000){
LE <-c(rep(0.3,5),rep(0.15,2),rep(0.01,4),
AM <-c(rep(0.2,5),rep(0.345,2),rep(0.780,4),
AP <-c(rep(0.1,5),rep(0.5,2),rep(0.874,4),
AL <-c(rep(0.4,5),rep(0.91,2),rep(0.411,4),
#example probabilities for 3 host species of 4 mistletoes. These are
manually calculated and imported into this script.

result <-matrix(c(
(as.vector(table(sample(host,9,prob=AM)))),(as.vector(table(sample(host,26,prob=AP)))),(as.vector(table(sample(host,87,
prob=LE))))),(as.vector(table(sample(host,3,prob = AL))))),nrow=3,ncol=4)
# Random sample from list AND
Probabilities. Row total must match the number of host species – eg
3 in this example

res <-ifelse(result > =1,1,0)
R<-matrix(res,byrow=T,nrow=4)
CS <-C.score(R)
#command to execute c-score – function
loaded in bipartite package

write((CS),file="file.txt",append=TRUE)}
```

## Appendix 3

Z-scores show the similarity (Euclidean distances) between mistletoe leaves and host leaves and measured as the deviation away from a random distribution. Negative values represent interactions that are more similar than predicted by chance.

<i>Amyema maidenii</i>	<i>Amyema preissii</i>	<i>Lysiana exocarpi</i>	<i>Amyema linophylla</i>	Leaf size and shape
0.81	-2.66	-0.01	-2.49	<i>Acacia aneura</i>
5.70	-1.87	3.10	-5.01	<i>Acacia loderi</i>
-3.70	3.20	-2.36	0.27	<i>Acacia salicina</i>
0.78	-6.17	1.32	0.81	<i>Acacia victoriae</i>
-4.36	1.51	-2.16	0.43	<i>Alectryon oleiofolium</i>
3.56	0.13	1.95	-2.53	<i>Casuarina pauper</i>
5.85	-1.93	6.56	6.06	<i>Eremophila alternifolia</i>
-2.06	0.81	-2.42	-1.59	<i>Eremophila longifolia</i>
-3.33	1.54	-1.82	0.41	<i>Sanatlum acuminatum</i>
-3.90	1.76	-2.09	0.63	<i>Sanatlum lanceolatum</i>

<i>Amyema maidenii</i>	<i>Amyema preissii</i>	<i>Lysiana exocarpi</i>	<i>Amyema linophylla</i>	Leaf colour
-1.46	0.59	2.48	-1.76	<i>Acacia aneura</i>
-0.03	-0.64	-0.55	-0.05	<i>Acacia loderi</i>
1.07	1.24	0.76	0.74	<i>Acacia salicina</i>
-0.02	0.30	-0.01	-0.24	<i>Acacia victoriae</i>
0.48	1.36	0.99	0.58	<i>Alectryon oleiofolium</i>
0.40	-0.03	0.36	0.58	<i>Casuarina pauper</i>
-1.13	-2.28	-1.05	-0.33	<i>Eremophila alternifolia</i>
-1.04	-0.22	-0.59	-0.65	<i>Eremophila longifolia</i>
-0.86	-1.15	-1.28	-0.90	<i>Sanatlum acuminatum</i>
-0.62	-0.76	-1.01	-0.43	<i>Sanatlum lanceolatum</i>