

Mistletoes: a hypothesis concerning morphological and chemical avoidance of herbivory

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Summary. Leaves from many mistletoe species in Australia strongly resemble those of their hosts. This cryptic mimicry has been hypothesized to be a means of reducing the likelihood of mistletoe herbivory by vertebrates. Leaf Kjeldahl nitrogen contents (a measure of reduced nitrogen and thus amines, amino acids and protein levels) of mistletoes and their hosts were measured on 48 mimetic and nonmimetic host-parasite pairs to evaluate hypotheses concerning the significance of crypsis versus noncrypsis. The hypothesis that mistletoes mimicking host leaves should have higher leaf nitrogen levels than their hosts is supported; they may be gaining a selective advantage through crypsis (reduced herbivory). The second hypothesis that mistletoes which do not mimic their hosts should have lower leaf nitrogen levels than their hosts is also supported; they may be gaining a selective advantage through noncrypsis (reduced herbivory resulting from visual advertisement of their reduced nutritional status).

Key words: Mimicry – Nitrogen – Herbivory – Mistletoe – Australia

Early botanical explorations of the Australian flora have noted a strong similarity in appearance between the leaves of a number of mistletoe species and their hosts (Drummond 1840; Hemsley 1896; Moore 1899). Not all mistletoes mimic their hosts, but rather there is one subset of mistletoe species that strongly resembles host leaf shape and another subset of mistletoe whose leaves clearly are different in size and shape from those of their hosts. The striking resemblance of host and parasite leaves was best described by Barlow and Wiens (1977), who proposed that it was an example of protective cryptic mimicry. The authors indicated that cryptic mimicry in mistletoes had evolved as a means of avoiding predation by herbivores. Furthermore, they suggested that cryptic mimicry by mistletoes may have been to avoid vertebrate herbivory, since vertebrates more often rely on visual cues as opposed to insects which more frequently use olfactory cues.

Underlying this hypothesis is the notion that host-resembling mistletoes are a more favorable food resource for

some unknown reason. Barlow and Wiens (1977) suggested that perhaps mistletoes were preferable because of higher water contents and that by visually blending in with the background of their hosts, the probability of being found and predated was reduced. However, no data on leaf quality (water content, nutritional, antiherbivore defense, etc.) were available to evaluate their hypothesis.

Mattson (1980) reviewed a number of studies of herbivory, which indicated that leaf tissue protein levels affect feeding patterns in animals (especially vertebrates). There was a preference toward feeding on leaf tissues with higher levels of protein. Animals may prefer different food resources for a variety of reasons (water content, vitamins, carbohydrates, etc.), and separating out the influences of these factors from protein levels may be difficult. Although higher protein contents may be only one component, it is a major component and can form the basis of testable hypotheses concerning the evolutionary bases of cryptic mimicry in mistletoes.

As a refinement of the Barlow-Wiens hypothesis for cryptic mimicry in Australian mistletoes, we suggest that leaf mimicry in mistletoes evolved as a means of reducing predation because host-mimicking mistletoes contain higher protein levels than their hosts. However, we realize that this need not be the only reason for mimicry. It is also possible that under some circumstances, hosts may possess higher concentrations of antiherbivore compounds and that mistletoes could derive some protection in these cases by mimicking their hosts, irrespective of their relative levels of leaf protein.

On the other hand, there may be some advantage to a mistletoe to appear different from its host, if it turned out that the mistletoe was of lower nutritional quality than was its host. If the mistletoe had on average a lower leaf protein content than did its host, it would be advantageous to alert potential predators to this difference by contrasting in leaf size or shape. The contrasting morphologies could be a visual advertisement of the mistletoe's lower nutritional status.

We have two hypotheses concerning cryptic mimicry, nonmimicry and protein levels of Australian mistletoes and their hosts:

1. Mistletoes that mimic their hosts will have higher levels of leaf protein than their hosts (cryptic mimicry). They

may be gaining a selective advantage by blending in with their background (reduced herbivory).

2. Mistletoes that do not mimic their hosts will have lower protein levels than their hosts. They may be gaining a selective advantage through noncrypsis (reduced herbivory resulting from visual advertisement of their reduced nutritional status).

These two hypotheses were tested using data collected as a part of an integrated ecophysiological field trip through eastern and central Australia. The purpose of this field tour was to investigate the comparative gas exchange characteristics of mistletoes and their hosts (Ehleringer et al. 1985; Ullmann et al. 1985), not specifically to gather data for evaluating crypsis in mistletoes. However, the data set is sufficiently extensive that it allows us also to address the interesting question of why host-parasite resemblance might have evolved in Australia.

Materials and methods

Levels of leaf Kjeldahl nitrogen are a reliable indicator of reduced nitrogen levels in plants (amides, amines, amino acids, proteins, etc.) and we have tested these two hypotheses through an analysis of nitrogen levels in leaf tissue in 48 host-mistletoe combinations collected from central and southeastern Australia.

Most of the sampling (42 samples) was done as part of an expedition organized by the Research School of Biological Sciences, Australian National University, Canberra, and involved 14 scientists who provided help and assistance in a series of cooperative investigations. Samples were collected in September 1981 at different sites along the route from Mildura (New South Wales), via Broken Hill, Port Augusta (South Australia), Kingoonya, Kulgera (Northern Territory), Ayers Rock, Alice Springs, Tennant Creek, Mount Isa (Queensland) to Townsville. A detailed map of this expedition has been published by Ullmann et al. (1985). Six additional samples were collected by H. Ziegler as part of a separate field tour through New South Wales in August 1981. Each paired sample consisted of outer canopy, mature mistletoe and host leaf tissues; host tissues were collected from branches adjacent to the mistletoes. All samples were initially air dried and then stored for later analyses.

For nitrogen determinations, leaf tissues were oven dried, ground to 40 mesh in a Wiley mill and then Kjeldahl nitrogen contents were determined using an autoanalyzer (Technicon Instruments, Tarrytown, New York).

Determination of whether or not mistletoe-host combinations represented mimics was initially determined using lists from Barlow and Wiens (1977) and further verified by consultation with Dr. B.A. Barlow (CSIRO, Canberra, Australia). The criterion for mimicry was an overall visual similarity of leaf dimensions, leaf presentation and internodal distances between host and mistletoes. This is the same definition as used by Barlow and Wiens (1977). Closer inspection of the host-parasite pairs sometimes revealed constant differences in leaf texture or color. It is not known whether potential herbivores are nocturnal or diurnal in their feeding patterns (which will influence perception of color or texture differences). However, the overall visual similarity in morphology and architecture between mistletoe and host made it difficult to distinguish between them at first glance during the day. Recognition of these mimetic situations by Barlow and Wiens (1977) was subjective, since

it is now known that the visual perception pattern of potential herbivores is identical to that of humans.

In our analyses, the data are presented as Kjeldahl nitrogen contents (mg N g dw^{-1}). For comparisons, mistletoe-host nitrogen data were divided into three categories: those in which mistletoe leaves had more nitrogen than their hosts' leaves, those in which the nitrogen contents were the same, and those in which the mistletoes had less nitrogen than their hosts. As all the samples are included and represented a one time sampling, we allowed a conservative 10% maximum variation for individual sample comparisons. Thus, in order for a mistletoe nitrogen content to differ from that of its host, the difference had to exceed 10%.

The data were analyzed using 1) chi-square analyses, assuming that the frequency of mistletoe nitrogen contents in different categories was equal, 2) correlational analyses of host and mistletoe nitrogen contents, and 3) student's t-test for comparing mean nitrogen contents between mistletoes and hosts.

Results and discussion

Seventeen of the twenty-two mistletoes which exhibited cryptic mimicry had leaf Kjeldahl nitrogen contents which equaled or exceeded those of their hosts (Table 1). The chi-square analysis of these data revealed that the number of cryptic mistletoes with higher leaf nitrogen contents was statistically significant at the $P=0.11$ level ($\chi^2=4.45$). The average leaf Kjeldahl nitrogen content of cryptic mistletoes were significantly greater ($t=9.12$, $p<0.001$) than those of their hosts by a factor of 1.7 (Table 2). The correlation of mistletoe nitrogen content to host nitrogen was positive and significant ($r=0.41$, $P<0.05$). These data support the first hypothesis that cryptic mistletoes may derive a benefit by blending in with their host backgrounds – they are more nutritious to an herbivore, but are perhaps harder to find among the less nutritious background.

Not all cryptic mistletoes had Kjeldahl nitrogen contents greater than those of their hosts (Table 1). In fact, all of the mistletoes whose leaves mimicked those of *Eucalyptus* species had leaf Kjeldahl nitrogen contents equivalent to or lower than those of their hosts. This is interesting, because *Eucalyptus* leaves are typically characterized as having high oil contents, which are thought to serve as protective antiherbivore mechanisms (Morrow and Fox 1980). Thus, among the cryptic mimics with equivalent or lower protein levels, six of the ten mistletoes are growing on *Eucalyptus* species, and because they blend into their background these mistletoes may experience potentially less herbivore pressure if eucalypts are in general avoided by certain herbivores. A seventh mistletoe (*Amyema mackayense*) was growing on a mangrove, whose leaves secrete salts onto their surfaces, and may therefore suffer limited herbivore damage.

Of the nonmimetic mistletoes, the majority of them (15 out of 26) had leaf Kjeldahl nitrogen contents significantly lower than those of their hosts (Table 1). In fact, on average the nonmimetic mistletoes had leaf nitrogen contents that were almost one-third lower than their hosts ($t=6.31$, $P<0.01$) (Table 2). The chi-square analysis revealed that the number of nonmimetic mistletoes with lower nitrogen contents was significantly lower than expected ($\chi^2=8.38$, $P<0.02$). The correlation of mistletoe nitrogen content to host

Table 1. Leaf kjeldahl nitrogen contents and the parasite/host ratio for nitrogen contents in paired mistletoe-host combinations

Mistletoe	Host	Mistletoe nitrogen (mg g ⁻¹)	Host nitrogen (mg g ⁻¹)	Mistletoe to host N ratio
Cryptic mimics				
<i>Lysiana murrayi</i> (Tate) Tiegh.	<i>Acacia brachystachya</i> Benth.	46.8	16.6	2.82
<i>Amyema preissii</i> (Miq.) Tiegh.	<i>Acacia brachystachya</i> Benth.	33.8	15.0	2.25
<i>Amyema gibberulum</i> (Tate) Dans.	<i>Hakea eyreana</i> (S. Moore) D. McGillivray	11.2	5.7	1.96
<i>Amyema maidenii</i> (Blakely) Barlow	<i>Acacia kempeana</i> F. Muell.	29.2	17.6	1.66
<i>Amyema maidenii</i> (Blakely) Barlow	<i>Acacia kempeana</i> F. Muell.	22.8	13.8	1.65
<i>Amyema linophyllum</i> (Fenzl) Tiegh.	<i>Casuarina cristata</i> Miq.	13.1	8.7	1.51
<i>Lysiana subfalcata maritima</i> (Hook.) Barlow	<i>Ceriops tagal</i> (Perr.) C.B. Robinson var. <i>Australis</i> C.T. White	13.9	9.4	1.48
<i>Lysiana murrayi</i> (Tate) Tiegh.	<i>Acacia aneura</i> F. Muell.	31.4	22.1	1.42
<i>Lysiana subfalcata subfalcata</i> (Hook.) Barlow	<i>Atalaya hemiglaucula</i> (F. Muell.) F. Muell. ex Benth.	23.6	17.2	1.37
<i>Amyema gibberulum</i> (Tate) Dans.	<i>Hakea eyreana</i> (S. Moore) D. McGillivray	7.6	5.6	1.36
<i>Amyema quandang</i> (Lindl.) Tiegh.	<i>Acacia papyrocarpa</i> Benth.	20.0	15.7	1.27
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Heterodendrum oleaefolium</i> Desf.	22.3	17.5	1.27
<i>Amyema maidenii</i> (Blakely) Barlow	<i>Acacia kempeana</i> F. Muell.	15.2	14.1	1.08
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Pittosporum phylliraeoides</i> DC. var. <i>microcarpa</i> S. Moore	12.5	12.3	1.02
<i>Amyema sanguineum</i> (F. Muell.) Dans.	<i>Eucalyptus</i> sp.	8.6	8.7	0.99
<i>Amyema miquelii</i> (Lehm. ex Miq.) Tiegh.	<i>Eucalyptus largiflorens</i> F. Muell.	9.8	10.2	0.96
<i>Amyema miquelii</i> (Lehm. ex Miq.) Tiegh.	<i>Eucalyptus crebra</i> F. Muell.	10.1	11.2	0.90
<i>Amyema cambagui</i> (Blakely) Dans.	<i>Casuarina cunninghamiana</i> Miq.	17.9	20.5	0.87
<i>Amyema miquelii</i> (Lehm. ex Miq.) Tiegh.	<i>Eucalyptus sideroxylon</i> A. Cunn ex Wodls.	10.8	13.0	0.83
<i>Diplatia grandibractea</i> (F. Muell.) Tiegh.	<i>Eucalyptus leucophloia</i> Brooker	8.5	11.9	0.71
<i>Diplatia grandibractea</i> (F. Muell.) Tiegh.	<i>Eucalyptus leucophloia</i> Brooker	4.5	6.5	0.69
<i>Amyema mackayense</i> (Blakely) Dans.	<i>Avicennia marina</i> Vierh.	8.3	17.7	0.47
Nonmimics				
<i>Lysiana murrayi</i> (Tate) Tiegh.	<i>Acacia kempeana</i> F. Muell.	30.6	13.8	2.22
<i>Amyema preissii</i> (Miq.) Tiegh.	<i>Acacia kempeana</i> F. Muell.	20.8	13.8	1.51
<i>Amyema gibberulum</i> (Tate) Dans.	<i>Grevillea wickhamii</i> Meisn.	11.8	8.5	1.39
<i>Dendrophthoe vitellina</i> Tiegh.	<i>Casuarina glauca</i> Sieb. ex Spreng.	16.5	12.5	1.32
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Acacia tetragonophylla</i> F. Muell.	16.0	12.2	1.31
<i>Lysiana spathulata</i> (Blakely) Barlow	<i>Acacia monticola</i> J.M. Black	19.8	16.0	1.24
<i>Lysiana casurinae</i> (Miq.) Tiegh.	<i>Acacia acradenia</i> F. Muell.	13.7	11.5	1.19
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Acacia victoriae</i> Benth.	31.3	26.6	1.18
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Templetonia egena</i> (F. Muell.)	16.1	14.9	1.08
<i>Lysiana spathulata</i> (Blakely) Barlow	<i>Callitris columellaris</i> (F. Muell.)	7.1	6.9	1.03
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Acacia aneura</i> F. Muell. ex Benth.	15.6	15.8	0.99
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Acacia tetragonophylla</i> F. Muell.	12.3	14.5	0.85
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Acacia victoriae</i> Benth.	23.9	28.5	0.84
<i>Lysiana spathulata</i> (Blakely) Barlow	<i>Acacia aneura</i> F. Muell. ex Benth.	14.6	17.3	0.84
<i>Lysiana casurinae</i> (Miq.) Tiegh.	<i>Gossypium robinsonii</i> F. Muell.	14.3	18.6	0.77
<i>Amyema quandang</i> (Lindl.) Tiegh.	<i>Acacia brachystachya</i> Benth.	13.1	17.8	0.74
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Myoporum platycarpum</i> R. Br.	10.1	13.8	0.73
<i>Lysiana subfalcata</i> (Hook.) Barlow	<i>Cassia oligophylla</i> F. Muell.	9.4	13.3	0.71
<i>Amyema miquelii</i> (Lehm. ex Miq.) Tiegh.	<i>Angophora costata</i> Druce	10.3	15.3	0.67
<i>Lysiana spathulata</i> (Blakely) Barlow	<i>Acacia farnesiana</i> (L.) Willd.	20.1	30.2	0.67
<i>Amyema maidenii</i> (Blakely) Barlow	<i>Acacia cowleana</i> Tate	11.9	18.5	0.64
<i>Amyema maidenii</i> (Blakely) Barlow	<i>Acacia coriacea</i> DC.	9.8	16.3	0.60
<i>Lysiana spathulata</i> (Blakely) Barlow	<i>Acacia monticola</i> J.M. Black	10.6	17.8	0.60
<i>Lysiana exocarpi</i> (Behr) Tiegh.	<i>Acacia brachystachya</i> Benth.	10.9	20.9	0.52
<i>Amyema quandang</i> (Lindl.) Tiegh.	<i>Acacia aneura</i> F. Muell. ex Benth.	9.7	20.9	0.46
<i>Lysiana spathulata</i> (Blakely) Barlow	<i>Acacia cowleana</i> Tate	8.1	18.5	0.44

nitrogen was not significant ($r = 0.19$, $P > 0.5$). These trends support our second hypothesis that mistletoes that have lower protein levels and are thus less nutritious may have some increased selective advantage through having a leaf morphology which contrasts with that of their host. The contrasting leaf morphologies may be a signal to potential herbivores of their lower nutritional status.

As a precautionary note, it is useful to ask if the segregation of mistletoes to different hosts is associated with some additional pattern such as nutritional status. The results from Table 2 indicate that nutritional differences were not significant between mimics and nonmimics. Both for cases where mistletoe leaf Kjeldahl nitrogen contents exceeded those of their hosts and for cases where it was less than

Table 2. Average leaf Kjeldahl nitrogen contents of mistletoes (M) and hosts (H) in cryptic mimetic and nonmimetic combinations for conditions in which mistletoe leaf Kjeldahl nitrogen contents are greater, equal (within 10%) or less than those of their host. Data are means \pm 1 SE are based on values presented in Table 1. Units are mg g^{-1}

		Cryptic mimics	Nonmimics
M > H	M	23.0 \pm 3.2 (12)	20.1 \pm 2.6 (8)
	H	13.7 \pm 1.5 (12)	14.4 \pm 1.9 (8)
	ratio	1.7	1.4
M = H	M	11.2 \pm 1.2 (5)	12.9 \pm 2.9 (3)
	H	11.3 \pm 0.9 (5)	12.5 \pm 2.8 (3)
	ratio	1.0	1.0
M < H	M	10.0 \pm 1.2 (5)	12.6 \pm 1.1 (15)
	H	13.9 \pm 2.4 (5)	18.8 \pm 1.3 (15)
	ratio	0.7	0.7

that of their hosts, the average leaf nitrogen content of a mistletoe mimic did not differ from that of a nonmimic (Table 2). A further concern might be if host nitrogen fixation capacities were associated with mimicry versus nonmimicry. Ehleringer et al. (1985) have shown that relative leaf water-use efficiencies in Australian mistletoes are dependent on whether or not the host exhibited nitrogen fixation capacities, but an analysis of the present data shows that there is no trend between mistletoe mimicry and host nitrogen fixation capacity.

Mistletoes that were cryptic mimics were not restricted to those hosts. It is important to note that six of the mistletoe species in this study occurred on both hosts for which they were cryptic mimics as well as hosts for which they were nonmimics (Table 1). If there are now strong herbivore pressures on mistletoes, we might not expect such a high frequency of mimics surviving on the hosts they did not mimic. That they do occur might be interpreted as indicating that the cryptic mimicry evolved at some time in the past and that perhaps those vertebrate herbivores are no longer extant.

Barlow and Wiens (1977) proposed that cryptic mimicry in Australian mistletoes evolved as a protective concealment from vertebrate herbivores. They note evidence that mistletoes are palatable to a number of vertebrate herbivores and suggest that among the extant herbivores the most likely candidates are possums. The purpose of our study is not to identify potential vertebrate herbivores be they extant or extinct. Instead, we have tested the hypothesis that if host-leaf resemblance in mistletoes evolved as a means of concealment, then certain patterns with respect to leaf nutritional status should be expected between mistletoe and host leaves. That these predictions are indeed supported by nutritional analyses should be interpreted as supporting the Barlow-Wiens hypothesis that host-leaf resemblance by Australian mistletoes is an adaptive feature that reduces the likelihood of mistletoe predation.

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