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Temporary translocation of entire mistletoe plants to understand the mechanistic basis of animal foraging decisions --Manuscript Draft--

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1 **TITLE:**

2 Temporary translocation of entire mistletoe plants to understand the mechanistic basis of animal
3 foraging decisions

4

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20 **KEYWORDS:**

21 Spatial learning, seed dispersal, mistletoe, plant-animal interactions, canopy, frugivory, endozoochory

22

23 **SHORT ABSTRACT:**

24 This work outlines a simple experimental procedure to quantify behavioural drivers of foraging
25 decisions in free-living animals, temporarily relocating mistletoe plants to novel locations and
26 measuring visitation rates.

27

28 **LONG ABSTRACT:**

29 Fruiting mistletoes present a model system for understanding decisions made by foraging animals
30 when locating food. Where, when and how animals find food is central to many ecological questions,
31 relating to the basis of individual foraging decisions and the extent to which these decisions are innate
32 or acquired. Ecologists have paid particular attention to frugivores, quantifying preference for fruits
33 with specific shapes, colours or scents which, over evolutionary time, confer selection for suites of
34 traits in their favoured plants whose seeds they disperse. This work outlines a novel experimental
35 approach to manipulating food plant occurrence and measuring the response of wild, free-living
36 animals, ideally suited to studying the evolutionary origin and ecological maintenance of seed
37 dispersal. The “cut and paste” protocol involves removing an entire fruiting mistletoe plant from its
38 host and either returning it to its original location or moving it to a novel location, affixing it to a

39 'pseudo-host' of the same or different tree species. By counting visits to the mistletoe and noting the
40 duration, species and behaviours, a series of comparisons can discern the most important factors
41 affecting foraging decisions and the consequences for both plant and animal. Here, the protocol is
42 illustrated with a case study to determine between-guild differences in mistletoe frugivory. The
43 experimental approach facilitates an ability to tease apart the mechanistic basis of search image
44 formation and refinement, spatial learning, inter-specific differences in foraging strategies and how
45 these changes modify seed dispersal effectiveness. Finally, potential modifications are considered with
46 respect to addressing other questions on foraging ecology, plant-animal interactions and coevolution.

47

48 **INTRODUCTION:**

49 How do animals find food? It's a deceptively simple question, integrating cognition, sensory
50 perception and metabolic demands with habitat structure, inter-specific interactions and variation in
51 resource availability through space and time. Most of the conceptual advances in our understanding of
52 this topic have come from studying captive animals, where resource quality, quantity and accessibility
53 can be manipulated^{1,2}. While useful for establishing sensory capabilities, qualitative preferences and
54 nutritional qualities of food, captive methods do not reveal how animals fulfill these demands in the
55 wild.

56 Early experimental studies on resource use by free-living animals sought to understand the lower
57 bound of food availability an organism will reach before deciding to feed elsewhere (Charnov's
58 marginal value theorem³). Known as "giving up density", this approach quantifies how much risk an
59 animal is willing to tolerate—e.g., how few acorns per square metre a squirrel is prepared to leave
60 behind when feeding in woodlands of differing densities where predators are variously detectable⁴.
61 While this framework has been applied to a wide range of food resources and ecosystems, the
62 necessarily constructed basis of the approach limits its application and can confound interpretation of
63 reported differences⁵. Further, determinants of giving up density relate more to vigilance, habitat
64 preferences and competition than foraging ecology (known collectively as the ecology of fear⁶). This
65 approach is rarely able to capture the attractiveness of a food resource in the wild to a free-living
66 animal. Hence, studies on frugivory are usually based on observation of wild behaviour with
67 implications to both plant and animal being drawn from the resulting behaviours.

68 Foraging decisions made by frugivores when selecting fruit may hinge on many different traits
69 expressed physically by the plant in terms of abundance, quality and seasonal availability. How easy
70 the fruits are to locate, to consume and to pass through the gut also play a role in the selection by
71 frugivores, making it tricky to separate the potentially learnt behaviour from the inherited. The current
72 work introduces a new approach to manipulating resource availability and location to measure the
73 response of wild, free-living animals as they forage in their natural habitat. This method is ideally
74 suited to addressing questions regarding the cues different animals use to locate food—in the case
75 showcased here, the energy-rich fruit of hemiparasitic mistletoe plants. The approach involves
76 removing entire mistletoe plants from their host trees and relocating them to other trees of the same
77 or different species. Note that the case study presented focuses on fruit, frugivores and the
78 interaction between dietary breadth and the implications for seed dispersal. However, for work on
79 nectarivores or folivores the same approach can be applied to flowering mistletoes or non-
80 reproductive mistletoes, respectively. Mistletoes are an ideal model to use for this approach, being
81 found in woodlands and forests worldwide and visited by a wide range of animals⁷. In terms of fruit,
82 although most research has focused on mistletoe fruit specialists that eat little else⁸, a large range of
83 generalist frugivores and opportunists with a broader diet regularly consume mistletoe fruit⁹. Finally,
84 their size, growth habit, and physiognomy make them especially amenable to experimental
85 manipulation¹⁰.

86 Research in a semi-arid woodland system demonstrated that foliage density affected apparency of
87 mistletoes to fruit eating birds¹¹, but numerous questions remain unanswered. Do birds search for
88 *mistletoes* or *fruiting* mistletoes? For those mistletoe populations dependent on a single host species,
89 do birds preferentially search for the mistletoe or for their principal host? Do groups that forage
90 primarily, occasionally, or opportunistically on mistletoe fruit use divergent cues to find mistletoe
91 fruit?

92 To answer these questions and uncouple the influence of host identity, spatial context and mistletoe
93 location on bird visitation, a novel relocation protocol was devised and that experiment is used as a
94 case study. The protocol is illustrated with step-by-step instructions to determine how birds locate
95 fruit in a structurally complex woodland. In addition to exploring other questions readily addressed
96 using this technique, consideration is given to how this method could be integrated with other
97 ecological field methods to understand the mechanistic basis of foraging ecology in forest and
98 woodland canopies.

99 The initial application of this experimental approach was to determine how birds find food in a
100 heterogeneous woodland canopy by relocating entire mistletoe plants. This protocol spans two days—
101 selecting the mistletoe on day 1 to manipulation, then affixing, observing and detaching the mistletoe
102 on day 2. Conduct replicate trials on successive days; select the mistletoe for the next trial on the
103 second day of the first trial. In the illustrative case study, bird visitation to mistletoes was compared
104 among three different host locations, here referred to as treatments. To do this, a single fruiting Grey
105 Mistletoe (*Amyema quandang*) plant was cut from its host plant and attached to one of three
106 locations: 1) its *original host tree*, 2) a *pseudo-host tree*, or 3) a *novel host tree*. The *original host*
107 treatment kept spatial location and host identity constant while controlling for the effects of cutting.
108 The *psuedo-host* treatment involved temporarily affixing the mistletoe to a different individual of the
109 same species as the host (in the case study, Yarran (*Acacia homalophylla*), but with few to no existing
110 mistletoes to discern the roles of spatial memory *versus* host-association. The *novel host*, an individual
111 of a different tree species that does not host mistletoes (for the case study site, White Cypress Pine
112 (*Callitris glaucophylla*)) clarified whether the search image used by mistletoe fruit consumers relates
113 to the mistletoe itself or to the principal host.

114 ETHICS STATEMENT

115 This experimental protocol was developed and experimentally proved under the provision of and
116 abiding by the Animal Research Authority guidelines of the University of Technology Sydney (UTS ACEC
117 2013-745). The protocol does not require handling of animals. Native plants were experimentally
118 manipulated under the permission of a National Parks and Wildlife scientific licence (SL101337).

119 **PROTOCOL:**

120 **1. Determine suitable site, species and ethical considerations**

121 1.1 Choose the ecosystem type and study location. A suitable site should have abundant
122 mistletoes with one regular mistletoe host and at least one species of tree/shrub that
123 does not normally host mistletoe. The case study was carried out in a semi-arid woodland,
124 New South Wales, Australia. **{Filming could be case study: pan shot of two different host
125 tree species with one mistletoe, maybe still shots of 1.1.1 – 1.1.3 below}**

126 1.2 Identify the ecologically relevant time of year for the study. For example, pre-determine
127 when mistletoes are fruiting. Consider that this might be different times of year,
128 depending on the seasonal profile of a given area. In this case, mistletoe species fruit for
129 some months over spring-summer.

130 1.3 Choose a patch size that satisfies the research question, e.g., if the species being observed
131 is territorial, choose a patch size that reflects that, if the mistletoe species abundance is

- 132 patchy, choose several patches and be prepared to reflect the variance in your statistical
133 analysis.
- 134 1.4 Identify the dominant species in your chosen ecosystem and location. At the case study
135 site, the dominant tree canopy species comprised *Callitris glaucophylla* (White Cypress
136 Pine), *Acacia homalophylla* (Yarran) and *Casuarina cristata* (Belah), with sub-dominant
137 stands of *Allocasuarina luehmannii* (Buloke) and *Eucalyptus populneus* (Poplar box).
138 *Amyema quandang* (Grey Mistletoe; Loranthaceae) is the principal mistletoe in the area,
139 growing almost exclusively on *Acacia homalophylla* (Yarran) at the study site **{Filming
140 could pan around the location, with a voice over describing the habitat (either at Binya
141 State Forest or elsewhere)}**
- 142 1.5 Identify the target mistletoe and become familiar with the animals that forage on it. For
143 example, in the case study, Grey Mistletoe produces pale-yellow fleshy fruits¹² that are
144 eaten by two mistletoe specialist avian frugivores (Mistletoebird; Dicaeidae, *Dicaeum
145 hirundinaceum* and Painted Honeyeater, Meliphagidae, *Grantiella picta*) and four
146 generalist frugivores (Silveryeye, *Zosterops lateralis*, Spiny-cheeked Honeyeater,
147 *Acanthagenys rufogularis* and Singing Honeyeater, *Lichenstomus virescens*, Striped
148 Honeyeater, *Plectorhyncha lanceolata*), with numerous other species opportunistically
149 consuming the fruits and occasionally dispersing seeds^{13,12}.
- 150 1.6 Choose the ideal number of replicates for the study, considering the total number of days
151 required to complete each 2-day replicate trials. In the case study, data for 20 replicates
152 were collected for each of the three relocation treatments (60 individual mistletoes), over
153 the course of 60 days, with one day of observation per replicate, randomised across
154 treatments. To reduce the number of days that the experiment will run, two replicates
155 may be observed by one observer with sufficient distance between the two replicates to
156 minimise interference.
- 157 1.7 Conduct a pre-experiment pilot study to prolong the vigour of the mistletoe once cut from
158 the host plant by comparing visitation to mistletoes with and without the cut ends sealed
159 with glue. If there is no difference in terms of either wilting or bird visitation for the 12-hr
160 duration of each trial, then the mistletoe can be deemed as retaining sufficient vigour until
161 the late afternoon. If bird visitation is significantly lower to cut mistletoes, select a
162 different mistletoe species and/or in a more humid environment where evaporation is
163 slower.
- 164 1.8 Ensure that all relevant permissions are in place, both to collect native plants and to
165 observe wildlife. Since this protocol involves cutting live mistletoe from the canopy, avoid
166 work in populations of mistletoe of conservation concern. Further, given the reliance of
167 many animals on mistletoe as both a food source and a nesting/roosting location, ensure
168 that the experiment will not cause a lasting disruption to the ecological community under
169 investigation. Obtain appropriate permissions from the relevant government agency and
170 consult animal care and ethics committee of the researcher's institution, balancing any
171 short-term impacts with the scientific merit of the proposed study. Note that no wildlife
172 handling is explicitly required for this method.

173

174 2. Identify target individual mistletoe-host pairs

175 2.1 At least one day prior to conducting the experiment, locate suitable mistletoe plants on
176 the appropriate hosts and, if relevant, appropriate *pseudo-hosts* or *novel hosts*.

177 2.2.1 When selecting locations to affix the mistletoe, ensure the branch is sufficiently
178 strong to hold the weight of the mistletoe.

179 2.2.2 When selecting a target individual mistletoe, consider the thickness of the host
180 branch and whether the selected mistletoe is growing at the terminal end of the
181 branch or midway along. Host branches above 70mm diameter or mistletoes growing
182 mid-way along a host branch that still bears host foliage should be excluded from
183 selection or pruned above the haustorium to avoid difficulty in cutting the host branch
184 or transporting the host foliage along with the mistletoe. ***{Filming could give an***
185 ***example of a good vs bad target mistletoe/host branch combination}***

186 2.2.3 Select the new host location that will allow for affixing the mistletoe in the same
187 orientation as it grew e.g., if its branches all droop down in a tear drop shape ensure
188 that they do so at the new location as well (Fig 2). ***{Filming could give a***
189 ***demonstration of appropriate matching of shape}***

190 2.2.4 Inspect each candidate closely to ensure that no active nests are located within
191 them or near them.

192 2.2.5 Choose mistletoe plants that can be safely reached and removed from their host
193 before dawn. If ladders are to be used, ensure that the ground beneath each tree is
194 clear of snakes, animal burrows and obstructions.

195 2.2.6 Note phenology (i.e., presence of ripe fruit or open flowers).

196 2.2 Record details of experimental plants. Mark the target plant pair unobtrusively to avoid
197 disturbing animals e.g. an inconspicuous fabric tag, a stick or stake in the ground close by or
198 GPS coordinates.

199 ***{Filming could show the selection of a mistletoe, a person indicating the thickness of the***
200 ***branch, describing the size of the mistletoe, inspecting the fruits, marking of a tree with a***
201 ***fabric tag or a still of a person standing next to a tree with a GPS}***

202

203 3. Cutting the mistletoe

204 3.1 At least one hour before dawn on the day of observations, remove the mistletoe from its
205 host using a clean pruning saw.

206 3.1.1 Depending on the branching pattern of the mistletoe, this can be achieved by
207 cutting either side of the haustorium ***{filming should visually indicate}*** but cutting
208 proximal (i.e., upstream) to the connection with the host ***{filming should indicate}*** and
209 removing the entire mistletoe is simpler and more effective.

210 3.1.2 Take care when cutting, undercutting first to minimize damage to the host tree.
211 It is important to be well-positioned and/or have a second person assisting in this
212 process, as the abscised mistletoe may be heavier than anticipated. For larger
213 mistletoe plants, wind a length of rope around the proximal portion of the host branch
214 (between the trunk and haustorium) before tying it securely to the mistletoe prior to
215 abscission, enabling the plant to be lowered safely to the ground without losing
216 branches which are characteristically brittle.

217 3.1.4 Thoroughly clean the saw with ethanol after each individual mistletoe removal.

218

219 4. Attaching ('pasting') the mistletoe

220 4.1 Once the mistletoe is removed, affix it to the final location using black cable-ties. Make
221 sure that the mistletoe will not swing unnaturally in the wind or fall off if a larger animal lands

222 in it. Make the cable ties as inconspicuous as possible, cut off long ends and leave no rubbish
223 behind for curious animals to find. **{Filming should capture this process perhaps using a**
224 **comparative set of shots of the mistletoe in the original host tree and move on to the**
225 **process of attaching the mistletoe in close detail then zoom out to show the attached**
226 **mistletoe}.**

227 4.2 As per 2.2.3 ensure that the relocated plant is secured in an orientation similar to its
228 original growth habit.

229

230 5. Collect visitation data.

231 5.1 In addition to noting species and duration of visit, behavioural data can be useful for
232 distinguishing different kinds of visits, including actively foraging for insects, visiting and
233 probing flowers, taking fruit, agonistic interactions and loafing. This can be conducted using
234 timed watches with binoculars, or with motion-activated cameras mounted the night before.

235 5.1.1 If using cameras, conduct initial trials using different sensitivity settings and
236 locations to minimise false triggering.

237 5.1.2 For timed watches, multiple mistletoes can be observed simultaneously from the
238 one vantage point. Our case study used this method, with the observation period of
239 approximately 6.5 hours between 7:30 am and 6:30 pm, in two blocks over the
240 morning and afternoon, avoiding the heat of the day where there was little bird
241 activity while still capturing peak foraging activity^{14,15}. During this period, every visit
242 to relocated mistletoes was recorded by direct observation from a distance of 5–10 m,
243 noting the identity of each bird and the duration of each visit (as per¹¹). Visiting
244 species were divided into three diet-based functional groups

245 6. Collect contextual data on the location of mistletoe plants.

246 6.1. In addition to noting whether each plant is a control (i.e., cut and returned to its original
247 location) or a relocated plant, record attributes of the host (species, height, diameter),
248 mistletoe (size, foliage density, phenology, height, aspect, number of fruits) and context
249 (distance to nearest mistletoe, distance to other fruiting / flowering plants). **{Filming could**
250 **include a still of a data sheet with either more still shots or moving film of one or two people**
251 **measuring and recording the data}**

252 6.2 Photo-point monitoring of both mistletoe and pseudo-host can be an effective
253 complement to conventional quantitative data collection, with image analysis software readily
254 able to generate estimates of canopy closure and other physiognomic attributes. **{Filming**
255 **could include a still shot of the data collection sheet and either still or moving shots with a**
256 **couple of people measuring the trees distances, heights, diameter etc}**

257

258 7. End of observation tasks

259 7.1. For seed dispersal studies, estimate the number of fruits removed by counting the total
260 number of ripe fruits before and after the experimental period. Check the ground for fruit caps
261 or fallen fruits before removing the mistletoe.

262 7.2 At the end of every data collection day, once visitation data have been collected, remove
263 the relocated mistletoe and collect all cable ties and any flagging tape or tags. **{Filming should**
264 **capture counting fruit caps on the ground below the mistletoe then the cutting down of the**
265 **mistletoe and the removal of any rubbish}**

266

267 REPRESENTATIVE RESULTS

268 A total of 392 hours of observation was collected across the 60 replicates, with 26 of the replicate
269 mistletoes receiving visits from 15 species of bird. To determine if the visiting birds preferred one
270 treatment over another, visitation data were analysed using generalised linear models (GzLMs)¹⁷ with
271 negative binomial distributions (after ^{18, 19}). Four variables were included as covariates: host height,
272 host canopy cover, number of mistletoes on the host and number of fruits on the treatment mistletoe.
273 When only treatment was included in the model, there was a significant difference in the number of
274 visits by birds to each of the mistletoe relocation treatments. The number of visits to the *original host*
275 mistletoes was significantly higher than to either mistletoes relocated to *pseudo-hosts* or *novel hosts*
276 (Table 1A). When the covariates, host canopy cover and the number of fruits were included, the
277 difference remained significant, but the habitat characteristics did not influence visitation (Table 1B).
278 Thus, across all birds, spatial cues were more important than resource density, accessibility or
279 apparency, consistent with the inference that prior experience is more influential than proximate
280 sensory cues in finding ripe fruit.

281

282

| |
|----------------------------|
| Insert Tables 1 and 2 here |
|----------------------------|

283

284 To determine whether the dietary breadth of birds influenced their search strategy to locate fruiting
285 mistletoe, dietary functional group (mistletoe specialist, generalist frugivore and opportunist) were
286 included as a second predictor alongside treatment. A median test was conducted to determine if the
287 three dietary guilds differed in the number of visits made to the treatment mistletoes. Further
288 analyses were then conducted separately for each functional group using a Poisson GzLM with a
289 loglinear link. Models for the three groups were created initially including the four selected covariates
290 to find the best model using the same information theoretic approach as described above. The
291 resultant guild models were then compared for overall fit to determine the set of covariates that were
292 most influential for each different dietary group.

293 The specialist dietary guild included one species: Mistletoebird. The generalist frugivore guild included
294 four species: Spiny-cheeked Honeyeater, Silveryeye, Singing Honeyeater and Striped Honeyeater. The
295 opportunist guild included nine species: Splendid Fairy Wren, Inland Thornbill, Yellow Thornbill, Rufous
296 Whistler, Australian (Mallee) Ringneck, Double-barred Finch, Grey Shrike-thrush, Noisy Miner and Red-
297 capped Robin. The mistletoe specialist visited treatment mistletoes on 19 occasions, there were 19
298 visits by generalist frugivores and 34 by opportunists. Visitation by the three guilds differed
299 significantly after accounting for visits to treatment mistletoes, and there was a significant interaction
300 between guild and treatment (Table 2A). The generalists visited the *original host* mistletoes
301 significantly more than the *pseudo-host* or *novel host* mistletoes.

302 Specialist visitation did not significantly differ among treatments; however, the number of fruits was
303 positively related to visitation ($P = 0.001$; Table 2B). The number of fruits on treatment mistletoes was
304 not significantly different across treatments (one-way ANOVA: $F_{(2, 56)} = 0.266$, $P = 0.768$).

305 The individual model for generalist frugivores excluded the *novel host* treatment mistletoes as no visits
306 by generalists were recorded at those mistletoes by this dietary group (Table 2B). The best model
307 included host canopy cover and the number of mistletoes on the host tree as covariates. Generalist
308 frugivores visited the *original host* mistletoes significantly more than they visited the *pseudo-host*
309 mistletoes (Table 2C). The percentage of host canopy cover and the number of mistletoes on the host
310 tree significantly influenced the visiting generalists as main effects (Table 2C).

311 The best individual model for opportunists included host height and host canopy cover as covariates.
312 Opportunists visited the *original host* mistletoes significantly more than they visited the *pseudo-host*
313 or *novel host* mistletoes and were significantly influenced by the height and canopy cover of the host
314 tree (Table 2D).

315

316 **DISCUSSION**

317 Our novel method represents a cost-effective means of understanding the mechanistic basis of
318 foraging differences among species and feeding guilds, revealing the critical role of prior learning and
319 spatial awareness in determining how birds find ripe fruit in structurally complex environments. By
320 uncoupling spatial location from other proximate cues, it was possible to demonstrate that generalist
321 frugivores visit plants in known locations, rather than relying on associations with particular habitats,
322 whereas specialists used more proximate cues of resource availability regardless of spatial context.

323 These findings lead to the next question of how and when do foraging birds develop this memory and
324 how much of a role does it play in the pattern of seed dispersal that drives the spatial pattern of
325 mistletoe occurrence in the landscape? Although our case study used direct observation to collect
326 visitation data, the protocol described herein could be readily applied using motion-triggered
327 cameras^{20,21}, allowing simultaneous monitoring of multiple sets of mistletoes and yielding new insights
328 into between-species, -guild, -habitat and -biome differences.

329 Several refinements are worth considering to maximise data quality, comparability and ease of
330 application. First, recognize that wild animals can respond both positively and negatively to novelty, so
331 be careful to minimise any extraneous changes to their environment both in establishing the
332 experiment and during data collection. By conducting mistletoe relocation pre-dawn, any disturbance
333 to diurnal animals will be minimized. Although logistically more challenging, it appeared that birds
334 were more likely to visit plants if they had been affixed in the dark, presumably due to disturbance
335 from movement. Second, minor details can be surprisingly important. Trim off the ends of the zip ties,
336 wear dull clothing and keep all movements to a minimum, especially during data collection. For
337 studies on fruit removal and seed dispersal, ripe fruits are readily knocked off the peduncle, so count
338 fruits after mistletoes are relocated. Also, check the ground after each period of collecting visitation
339 data for fruit caps on the ground, indicative of fruit removal.

340 Although these representative results and overarching question related to fruit removal, this protocol
341 could be readily applied to address questions regarding nectarivores and pollination or folivores and
342 arboreal herbivory. In addition to manipulating mistletoe locations (e.g., high versus low to quantify
343 herbivory from ground-based versus arboreal herbivores; *in situ* versus translocated mistletoes to
344 quantify the influence of resident and transient nectarivores in effecting short- and long-distance
345 pollen transport), resource density can also be manipulated. Thus, by making high and low resource
346 density patches by manipulating mistletoe densities and/or flower/fruit/leaf numbers, different
347 resource use strategies can be discriminated. By integrating these experiments with before and after
348 measurements of the relevant resource, giving up densities can also be estimated, enabling resultant
349 inferences regarding foraging ecology to be contextualized within the broader framework of habitat
350 preferences and predator vigilance.

351

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354 thanks to the numerous volunteers who dedicated their time to observing the birds.

355

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402

403 **DISCLOSURE STATEMENT**

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Figure 1. Grey Mistletoe (*Amyema quandang*) in different stages of removal. (A) A mistletoe being removed from its host by cutting below the haustorium with a cleaned pruning saw; and (B) detail of the same mistletoe removed from the host complete with the distal end of the host branch. (C) The three main branches of another, larger mistletoe clump removed from the haustorium; (D) Detail of the three branches showing the cable ties affixing them to one another.

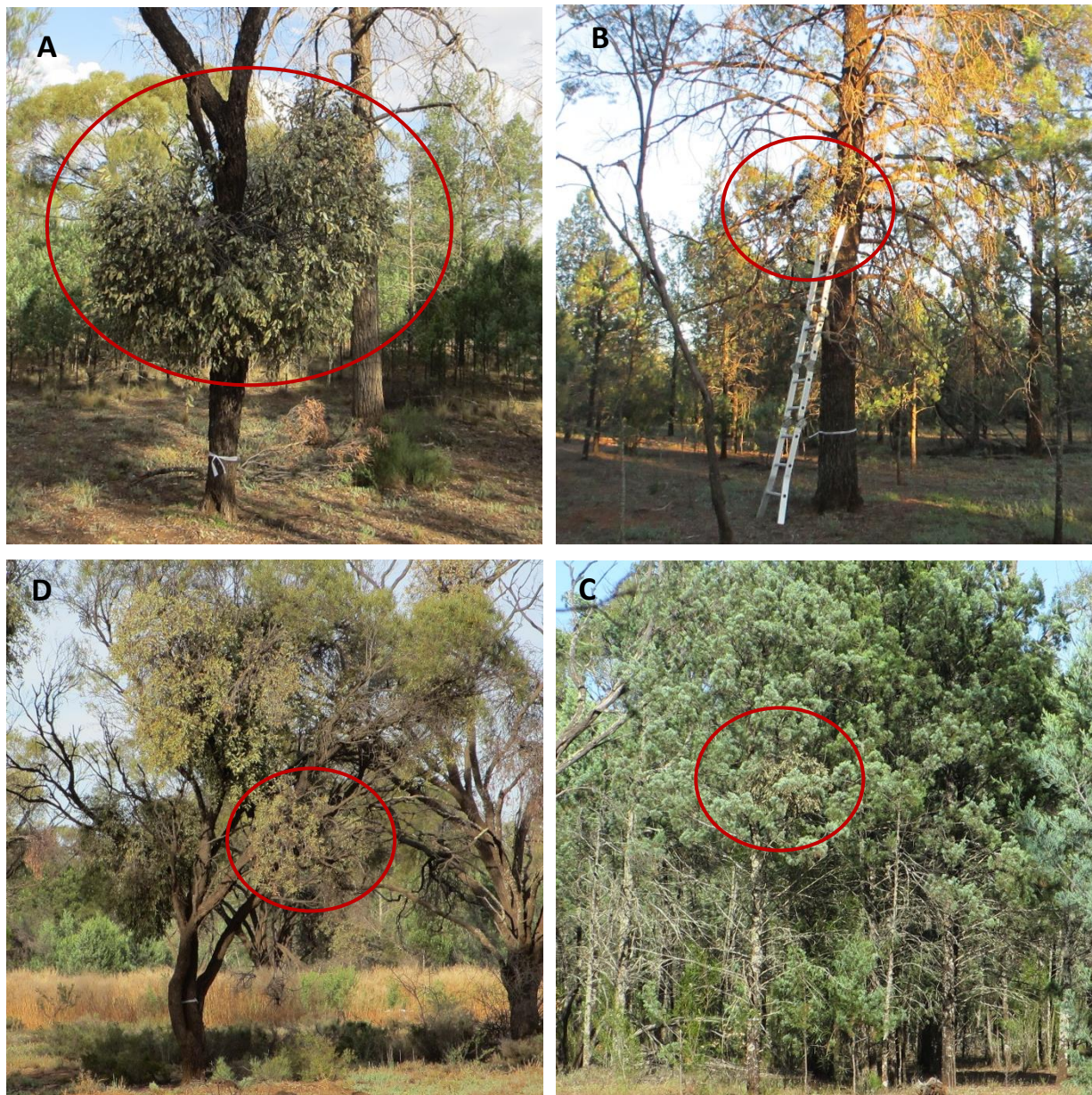


Figure 2. Grey Mistletoe (*Amyema quandang*) in the three different treatments. (A) *In situ* mistletoe before being removal from its Yarran (*Acacia homalophylla*) host and then replaced in its original location; (B and C) *Different Species* treatment mistletoe after being removed from its original host and relocated in this White Cypress tree (*Callitris glaucophylla*). (D) *Same Species* mistletoe after being removed from its original host and relocated to a new Yarran tree already hosting other mistletoes. Treatment mistletoes circled.

| Source of variation | Wald chi-square | Degrees of freedom | Main effects | Direction of relationship |
|---|-----------------|--------------------|--------------|---------------------------|
| A. Number of visits to three treatment mistletoes | | | | |
| Treatment | 11.246 | 2 | 0.004 | IS > SS; IS > DS |
| B. Number of visits to three treatment mistletoes including habitat covariates | | | | |
| Treatment | 9.086 | 2 | 0.011 | IS > SS; IS > DS |
| Host canopy cover | 1.759 | 1 | 0.185 | - |
| Number of fruits | 0.189 | 1 | 0.664 | - |

Table 1. Generalized linear models exploring the influence of treatment on the number of visits to ti

reatment mistletoes (A). Model (B) includes host canopy cover and the number of fruits on the treatment

t mistletoes as covariates. Treatment mistletoes were one of three treatments: In-situ (IS), cut from the o

original host tree and reattached exactly where it was, Same Species (SS), moved to another tree of the sa

ime species as the original host and Different Species (DS), moved to a species that never hosts Grey Mist

tletoe. Significance is shown in bold.

| Source of variation | Wald chi-square | Degrees of freedom | Main effects | Interaction |
|---------------------|-----------------|--------------------|--------------|-------------|
|---------------------|-----------------|--------------------|--------------|-------------|

A. Number of visits to treatment mistletoes across guilds (specialist, generalist and

| | | | | |
|---------------------------|--------|---|--------------|--------------|
| Dietary guild | 6.469 | 2 | 0.039 | - |
| Treatment | 11.685 | 1 | 0.001 | - |
| Treatment x dietary guild | 8.301 | 1 | - | 0.016 |

B. Specialist visits to treatment mistletoes influenced by the number of f

| | | | | |
|------------------|--------|---|--------------|---|
| Treatment | 2.743 | 2 | 0.254 | - |
| Number of fruits | 11.086 | 1 | 0.001 | - |

C. Generalist visits to treatment mistletoes influenced by mistletoe density and c

| | | | | |
|---------------------------------------|--------|---|--------------|---|
| Treatment | 13.764 | 1 | 0 | - |
| Host canopy cover | 5.883 | 1 | 0.015 | - |
| Number of mistletoes on the host tree | 9.679 | 1 | 0.002 | - |

D. Opportunist visits to treatment mistletoes influenced by host height and car

| | | | | |
|-------------------|-------|---|--------------|---|
| Treatment | 9.719 | 2 | 0.008 | - |
| Host height | 4.203 | 1 | 0.04 | - |
| Host canopy cover | 5.212 | 1 | 0.022 | - |

* negative binomial with log link

Table 2. Generalized linear models for models comparing the number of visits to treatment i

Direction of relationship

opportunistic)*

-

IS > SS; IS > DS

Gen DS < Gen IS, SS

fruits

-

-

canopy cover

IS > SS

-

-

canopy cover

IS > SS; IS > DS

-

-

mistletoes across dietary guilds including various covariates. (A) overall dietary guild; (B) Specialist; (C) G

Generalists; (D) Opportunists. Treatment mistletoes were cut from their original host tree and either re-p

placed in-situ (*In-situ* , IS), placed in a host of the same species as the original host (*Same Species* , SS) or,

. placed in a species that does not host mistletoe (*Different Species* , DS). Models used a Poisson error di:

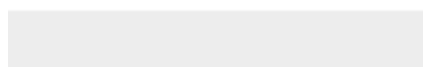
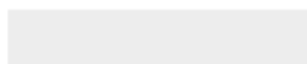
tribution with a log-linear link, unless otherwise indicated. Significance is shown in bold.



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Table of Materials

JOVE_MC_DMW_Materials.xlsx



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14 March 2022

Re: Major revision of JOVE manuscript

On behalf of my coauthors and with their express consent, I am submitting a thorough revision of our manuscript entitled “Temporary translocation of entire mistletoe plants to understand the mechanistic basis of animal foraging decisions”, to be considered for publication in the special issue of JOVE “Methods and applications in parasitic plant research”.

In further revising submission, we adhered closely to the instructions to authors and restructured the entire text to conform to the journal guidelines. In addition to clarifying the context and broader relevance of our approach, we explain the method in clear step-by-step instructions, including suggestions regarding production of the associated video. All substantive comments from all three reviewers were addressed, explanations of our approach expanded to emphasise both the novelty and simplicity of our approach, shortening the representative results section and distilling down our key findings to two short paragraphs. Further, all text was reworded to conform to the editorial style, minimizing notes and using imperative tense throughout.

The revised manuscript and eventual video will be of great interest to ecologists, environmental scientists, behavioural ecologists and evolutionary empiricists, our method complimenting existing experimental approaches in the evolutionary biology / foraging ecology space.

I look forward to hearing from the editorial office in due course

Cordially,



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