

1 **Mycophagy among Japanese macaques in Yakushima: Fungal species diversity**
2 **and behavioral patterns**

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14 A short title: Mycophagy among Japanese macaques

15 **ABSTRACT**

16 Mycophagy (fungus-feeding) by Japanese macaques (*Macaca fuscata yakui*) in
17 Yakushima has been observed by many researchers, but no detailed information is
18 available on this behavior including which fungi species are consumed. To provide a
19 general description of mycophagy and to understand how and whether macaques avoid
20 poisonous fungi, we conducted behavioral observation of wild Japanese macaques in
21 Yakushima and used molecular techniques to identify fungi species. The results indicate
22 that the diet of the macaques contains a large variety of fungi species (67 possible
23 species in 31 genera), although they compose a very small portion of the total diet
24 composition (2.2% of the annual feeding time). Fungi which were eaten by macaques
25 immediately after they picked up were less likely to be poisonous than those which
26 were examined (sniffed, nibbled, carefully handled) by macaques. However, such
27 examining behaviors did not appear to increase the macaques' abilities to detect
28 poisonous fungi. Fungi that were only partially consumed included more poisonous
29 species than those fully consumed with/without examining behavior yet this was not
30 significant. Taste, therefore, might also play an important role in discriminating
31 poisonous from non-poisonous.

32

33 **Key words** Japanese macaque; *Macaca fuscata yakui*; mycophagy; poisonous fungi;
34 fungal diversity

35 INTRODUCTION

36 While mycophagy has been reported in at least 24 primate species (Hanson et
37 al. 2003; Hanya 2004; Hilário and Ferrari 2010), fungi have received less attention
38 compared with other food items. Fungi usually account for only a small part of the diet
39 composition of most primate species, such as mountain gorillas, bonobos, green
40 monkeys, and Japanese macaques (Harrison 1984; Watts 1984; Bermejo et al. 1995;
41 Hill 1997). According to Hanya (2004), Japanese macaques in the high-altitude
42 coniferous forest of Yakushima, where fruits are less abundant, eat fungi more often
43 than their lowland counterparts. However, no data show whether they feed on specific
44 fungal species.

45 A few primates are known to consume large quantities of fungi on a regular
46 basis, for example Goeldi's monkeys and buffy-headed marmosets (Hilário and Ferrari
47 2010; Porter 2001). Goeldi's monkeys who mainly consumed fungi that were
48 predominantly fiber (68 – 83%) have molars with high shearing crests, which might
49 increase their ability to shred fungi into small pieces and thus promote digestion (Porter
50 and Garber 2004; Hanson et al. 2006). Another elucidation for intense mycophagy
51 among Goeldi's monkeys is as an effective tactic to lower the chance of interspecific
52 competition for fruits with large animals (Porter 2001).

53 One problem that studies on mycophagy face is fungal species identification.
54 Morphology-based identification is challenging because fungi are quite variable in
55 appearance and many cryptic species exist, as evidenced by the fact that humans around
56 the world occasionally misidentify fungi and are poisoned as a result (Eren et al. 2010;
57 Ishihara and Yamaura 1992; Unluoglu and Tayfur 2003). In this study, we applied
58 DNA-based species identification, which requires no expertise in mycology. Moreover,

59 this method allows us to identify the species even from debris that an animal left behind.
60 Since we do not have to collect intact fruiting bodies to obtain macroscopic and
61 microscopic traits, it is particularly effective in studies on mycophagy by wild animals.

62 Wild Japanese macaques of Yakushima are an ideal study species for
63 mycophagy because researchers have repeatedly observed them eating fungi (Agetsuma
64 and Noma 1995; Hill 1997; Hanya 2004) and they are well habituated and can be
65 observed from a close distance. Besides, the fungal diversity is high in Yakushima
66 (Tsujino et al. 2009) and many genome sequences of fungi collected there have been
67 added to GenBank, the NIH genetic sequence database
68 (<http://www.ncbi.nlm.nih.gov/genbank/>), over the past several years. Since DNA-based
69 species identification is a matching process, we can expect reliable results with a large
70 database.

71 The purposes of this study were (1) to provide a general description of
72 mycophagy among wild Japanese macaques in Yakushima, and (2) to elucidate whether
73 and how macaques avoid poisonous fungi. For the first study purpose, we estimated the
74 diet composition and determined whether the macaques consume a wide variety of
75 fungi. For the second purpose, we focused on detailed mycophagy behaviors, which
76 may reveal whether macaques avoid specific species, namely 'poisonous mushrooms'.
77 Sherratt et al. (2005) indicated that fungi species that are poisonous to humans are more
78 likely to exhibit an unpleasant odor, as judged by humans. Such poisonous species may
79 have a particular taste as it is possibly associated with the odor (Sherratt et al. 2005).
80 Macaques may decide whether to eat the fungi or not based on on-site assessment of
81 such chemical 'warning' signals. Alternatively, macaques may have previous
82 knowledge about fungi species that are edible to them, as they do about plant food

83 items. In order to confirm these two possibilities, we established a set of predictions
84 suggesting that the fungi that macaques did not feed on would be more likely to be
85 poisonous compared with those eaten. Additionally, we provide information on the
86 fungi that macaques consumed repeatedly to explore the possibility of their knowledge
87 about fungi species through previous feeding experiences.

88

89 **METHODS**

90 All data collection was non-invasive and permitted by the Yakushima Forest
91 Environment Conservation Center and Kagoshima Prefectural Government. The
92 research adhered to the guidelines established by the Field Research Committee of the
93 Primate Research Institute, Kyoto University ('Guidelines for Studying Wild Primates
94 or Using Wild Primates in Research'), and the legal requirements in Japan.

95

96 **Study Site and Animals**

97 Yakushima is a mountainous island located in the southwestern part of Japan
98 (30°N, 130°E) with an area of 505 km². Our study site, Hanyama, is part of the
99 Yakushima National Park and is located in the western coastal area of Yakushima (Fig.
100 1). The study area is covered by subtropical/warm-temperate, evergreen broad-leaved
101 vegetation (Yamagiwa 2008). The mean annual temperature and rainfall (from August
102 2009 to July 2010) were 20.3°C and 3,223.7 mm. The meteorological data were
103 collected at the Yakushima Field Station of Kyoto University in Nagata, which is
104 approximately 8 km from the study site (Shin-ichiro Aiba, unpublished data). The main
105 study subjects were nine adult females of the AT troop, which consists of seven adult
106 males and thirteen juveniles (1–4 years old). They were individually identified by

107 physical features. There is no natural predator of wild Japanese macaques in
108 Yakushima, and the macaques are well habituated but not provisioned.

109

110 **Data Collection**

111 The study period was from August 2009 to September 2010. We collected the
112 data by focal animal sampling, where we recorded the activity and food item of the
113 focal animal with a 1-min interval to estimate the proportion of mycophagy in the total
114 feeding time. We also calculated annual and monthly diet composition based on this
115 data. For detailed data analysis on mycophagy, we continuously recorded the onset and
116 end of all feeding events (see definition and Table 1). We used all-occurrence sampling
117 of mycophagy and recorded the duration of the feeding/foraging bout. We also collected
118 data on mycophagy by non-focal individuals whenever possible. We used this all-
119 occurrence sampling of focal animals and data for non-focal animals to compile a list of
120 the fungi that macaques consumed and to make a detailed analysis of their mycophagy
121 behavior. Macaques ate fungi off the ground, from decaying logs, or on the trunk of
122 living trees. As fungi have brittle flesh, macaques often dropped small fragments even
123 when they completely consumed the whole fruiting bodies. We collected such fungal
124 samples when available except for cases when macaques did not leave any debris. After
125 coming back to the field station, we placed a small section of each fruiting body sample
126 in 99.5% ethanol.

127 We changed focal animals every 180 min with the intention of collecting data
128 from several individuals during a single day. When we lost the focal animal and failed
129 to resume observation within 20 min, we started a new session with a different
130 individual. Here we present the data on the frequency and proportion of mycophagy

131 related to feeding time that we collected over 223 sessions, amounting to 669 hours in
132 total. We categorized all of the food items eaten by the focal animals as follows: fungi,
133 other fungal materials, seeds, fruits, flowers, leaves, shoots, bark/branches, insects, and
134 others. The category “other fungal materials” refers to the white mycelium found
135 frequently under the bark of dead trees. Leaves include both mature and young leaves.
136 Others consist of herbaceous plants, soil, water, and unidentified items. We calculated
137 monthly dietary composition from a 1-min instantaneous recording of feeding events of
138 the focal animal as the proportion of each food item to the total feeding time. We
139 levelled off August and September, months that data were collected during two calendar
140 years, and calculated annual dietary composition as the average for the 12 months.

141

142 **Definitions for Mycophagy Behavior**

143 We defined one *foraging event* as a period starting when a macaque picked up
144 a fungus and ending when the animal finished handling it. While foraging for fungi,
145 macaques sometimes showed additional conspicuous behaviors such as sniffing,
146 nibbling (no spitting out), and careful handling (splitting a fungi into several pieces;
147 rubbing a fungus with the hands). We defined that macaques *examined* fungi when they
148 showed at least one of these behaviors.

149 When a macaque entered within a radius of 1 m from a fungus, we regarded
150 this as the animal encountering the fungus. We divided behavioral sequences of
151 mycophagy into the following four phases: when a macaque (i) encountered, (ii) picked
152 up, (iii) examined, and (iv) consumed a fungus. At each phase, the macaque chose either
153 of the following two behavioral options: (i) picked up or did not pick up, (ii) examined
154 or did not examine, (iii) ate or did not eat, and (iv) consumed a whole piece of fungus or

155 ate it only partially. Using these sequences, we classified behavioral patterns into six
156 categories (Table 1):

157 *Foraging* includes the events during which macaques handle fungi, thus
158 consisting of RE, EX-A, EX-P, NE-A, and NE-P. *Feeding* represents situations when
159 the macaque actually consumes a fungus, thus including EX-A, EX-P, NE-A, and NE-P.

160

161 **Prediction of Proportion of Poisonous Fungi**

162 We predicted that fungi that macaques did not eat were more likely to be
163 poisonous if macaques avoided them. We, therefore, compared the proportion of
164 poisonous fungi between the two choices at each phase.

165 At Phase i, where macaques encountered fungi, the proportion of poisonous
166 species is expected to be higher in fungi that were left untouched (IG) than in those that
167 were picked up (RE + EX-A + EX-P + NE-A + NE-P). At this phase, macaques did not
168 have any physical contact with the fungi. Therefore, they would discriminate poisonous
169 fungi by judging them from knowledge they had acquired through previous feeding
170 experiences.

171 At Phase ii, where macaques picked up fungi, the proportion of poisonous
172 species is expected to be higher in the fungi examined than in those immediately eaten
173 by macaques (RE + EX-A + EX-P > NE-A + NE-P). This suggests macaques would
174 discriminate poisonous fungi based on previous knowledge.

175 At Phase iii, where macaques examined fungi, the proportion of poisonous
176 species is expected to be higher in fungi rejected than in those eaten by macaques (RE >
177 EX-A + EX-P). This suggests that macaques would discriminate poisonous fungi by
178 examining behaviors.

179 At Phase iv, where macaques consumed fungi, the proportion of poisonous
180 species is expected to be higher in fungi that were partially eaten than in those
181 completely eaten by macaques (EX-P > EX-A; NE-P > NE-A). Since macaques ingest a
182 few bites of the fungi before making a decision, this suggests that they would screen out
183 poisonous fungi by taste.

184 We used Fisher's exact tests to compare the proportion of poisonous fungi at
185 each phase using the free statistical package R (version 2.13.2).

186

187 **DNA Extraction, PCR Amplification, and Sequencing**

188 We extracted total DNA from each fruiting body sample following a modified
189 method described in Sato and Murakami (2008). We added 300 μ L CTAB buffer to the
190 crushed fungal tissue. After incubating at 55°C for 30 min, we added 300 μ L CIA
191 (chloroform-isoamyl alcohol; 24:1 [vol/vol]) to the mixture and centrifuged it for 15
192 min at 4,200 rpm. We transferred 100 μ L clear supernatant to a new microtube, and then
193 with 100 μ L isopropanol added we centrifuged the mixture for 30 min at 4,200 rpm.
194 After washing with 70% ethanol, we dissolved the precipitated DNA in a 50 μ L TE
195 buffer. We amplified Internal transcribed spacer 2 (ITS2) regions from nuclear
196 ribosomal DNA using the ITS3 and ITS4 primers described by White et al. (1990). We
197 carried out PCR amplification using 1 μ L total DNA in a 20- μ L reaction mixture
198 containing Ampdirect Plus (Shimadzu, Kyoto, Japan), 10 pmol of both forward and
199 reverse primers, and 0.5 units of BIOTAQ HS DNA Polymerase (Shimadzu, Kyoto,
200 Japan). Cycling parameters for PCR were as follows: we conducted denaturation at
201 95°C for 10 min, followed by 40 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30
202 s, and followed by a final extension of 2 min at 72°C. We added 5 μ L of 0.05 \times ExoSAP-

203 IT (USB Corp., Cleveland, Ohio) to the PCR products for purification. We incubated
204 the PCR products at 37 °C for 15 min and then deactivated the enzymes at 80°C for 15
205 min. We sequenced the purified PCR products using the same primers used for
206 amplification. We performed nucleotide sequencing using an ABI 3130 Genetic
207 Analyzer (Applied Biosystems, Foster City, California USA) with BigDye-Terminator
208 v3.1 (Applied Biosystems), following the manufacturer's protocols.

209

210 **Fungal Species Identification**

211 We compared the nucleotide sequences obtained with the sequences compiled
212 in the NCBI GenBank database. To establish the list of fungi species that the macaques
213 interacted with or ignored, we included the genetic sequences of fungi that were
214 consumed by both focal and non-focal animals as we collected data by all-occurrence
215 sampling. For each sample, we chose the species with the highest bit score when max
216 identity was 97% or over. Among 478 fungal samples we collected, we successfully
217 obtained the genetic sequences from 398 samples and identified 235 samples to the
218 species level. For credibility of species identification, we did not provide the species
219 name for those with max identity lower than 97% (82% for the lowest) but treated them
220 as *sp.* and used them only for analysis at the genus level. The five most possible
221 candidates suggested by a BLAST search were of the same genus at the rate of 86% or
222 belonged to one of the genera that were already in our list. Therefore, we believe that
223 we have provided a fairly accurate estimate of the number of fungus genera.

224

225 **Toxicity of Fungi**

226 We divided fungi into two groups, poisonous and non-poisonous, based on
227 information from a reference book (Imazeki et al. 1988). We admit that the information
228 on toxicity in the reference books is specific to humans. Due to the similarities in
229 digestive systems between a Japanese macaque and a human, such as the lack of a
230 foregut fermentation system, we assumed that fungi poisonous to humans are also
231 poisonous to macaques.

232

233 **RESULTS**

234 **General Features of Mycophagy**

235 During the observation, all the focal animals consumed fungi. Mycophagy
236 accounted for only 2.2% of the total feeding time of Japanese macaques over the study
237 period, whereas fruits, leaves, and seeds constituted substantial portions of it (42.6%,
238 18.1%, and 11.4%, respectively). There were seasonal changes in their monthly dietary
239 composition (Fig. 2). Frequency of mycophagy reached its highest during May–August,
240 when the macaques mainly fed on fleshy fruits (*Myrica rubra*) (Table 2). Macaques
241 rarely ate fungi between September and January.

242 Mycophagy was associated with the feeding time of other fungal materials,
243 fruits and others (other fungal materials: $r = 0.58$, $P < 0.05$, fruits: $r = 0.83$, $P < 0.001$,
244 others: $r = 0.7$, $P < 0.05$). There was no such correlation between mycophagy and the
245 consumption of other food items (seeds: $r = -0.51$, $P = 0.09$, flowers: $r = 0.52$, $P = 0.08$,
246 leaves: $r = 0.55$, $P = 0.06$, shoots: $r = 0.45$, $P = 0.14$, bark/branches: $r = -0.01$, $P = 0.97$,
247 insects: $r = -0.2$, $P = 0.54$).

248 The total numbers of fungi species and genera that the macaques actually
249 consumed (at least partially) were 67 and 31, respectively (Table 3, Appendix).

250 Eighteen species (18 genera) were ignored by macaques and 25 species (11 genera)
251 were rejected. Among these 110 species (51 genera), 40 species (23 genera) are
252 described in the reference book (Imazeki et al. 1988). The number of fungi ignored by
253 macaques is underestimated because, by definition, fungi were supposed to be large
254 enough to be found with the naked eye. Furthermore, considering that all of the fungi
255 we recorded were located less than 1-m away from macaques, it was unlikely that
256 neither macaques nor the observers missed many fungi. Therefore, we assume that those
257 fungi were left untouched because the macaques were not interested in them, rather than
258 simply overlooking them.

259

260 **Behavioral Patterns**

261 Japanese macaques generally consumed fungi without examining behaviors
262 (Table 2). The proportion of poisonous fungi species in the total number of fungi they
263 picked up was not significantly different from that in the total number of fungi they
264 ignored. (Phase i: $P = 1$, Fig. 3). The proportion of poisonous species was significantly
265 higher among the fungi examined by the macaques compared with the fungi they ate
266 immediately (Phase ii: $P < 0.0001$). However, when they exhibited examining
267 behaviors, the proportion of poisonous species did not differ between the rejected fungi
268 and the eaten fungi (Phase iii: $P = 1$). A marginally significant trend was found in fungi
269 that were completely consumed compared with those only partially eaten by macaques
270 with examining behaviors (Phase iv: $P = 0.086$). The same pattern was found when they
271 did not exhibit examining behaviors while eating, yet this difference was also
272 marginally significant (Phase iv: $P = 0.074$).

273 To clarify behavioral consistency for a particular fungi species, we analyzed
274 repeated consumption of the same species. We pooled behavioral patterns for each
275 species, and thus no individual difference is mentioned here. When the macaques
276 encountered the same species multiple times, they tended to show similar behavioral
277 patterns. For example, when they encountered the same species twice (18 species), the
278 proportion showing the same behavioral pattern was 61%, which was much higher than
279 the chance level (1/6) ($P < 0.0001$, binomial test). The same was true for fungi eaten
280 three times (25% of eight species, $P = 0.019$) and four times (25% of five species, $P =$
281 0.023). The same pattern was not found for species eaten more than four times (five
282 times: 0% of three species, $P = 1$; six times: 0% of six species, $P = 1$; nine times: 0% of
283 one species, $P = 1$; 27 times: one species, $P = 1$). When combining the six categories
284 into two, based on whether the macaques consumed the fungus (NE-A, NE-P, EX-A,
285 EX-P) or not (IG, RE), there was a stronger tendency for macaques to show the same
286 response to the same species. The proportion of showing only either of the two
287 behaviors became significantly higher than the chance level for the species the
288 macaques encountered twice (15 out of 18 species, $P < 0.01$, binomial test), three times
289 (five out of eight species, $P < 0.05$), five times (three out of three species, $P < 0.001$),
290 six times (two out of six species, $P < 0.05$), nine times (one out of one species, $P <$
291 0.005), and 27 times (one out of one species, $P < 0.0001$). The only exception was the
292 species encountered exactly four times, where there was no such tendency (two out of
293 three species, $P = 0.12$).

294

295 **DISCUSSION**

296 **General Features of Mycophagy among Japanese Macaques**

297 Mycophagy among Japanese macaques in Yakushima comprises only a small
298 portion of feeding time, which might be explained by their relatively large body size
299 compared with other intensively mycophagous primates. Mycophagy by the macaques
300 constituted only 2.2% of the annual feeding time and 4.5% during the peak month
301 (June). These findings correspond to general patterns in mycophagy among primates,
302 where fungi are generally not important food resources (Hanson et al. 2003). For
303 example, fungi account for 1.2% of the annual feeding time in green monkeys, 0.2% in
304 mountain gorillas, and 1.7% in bonobos (Bermejo et al. 1995; Harrison 1984; Watts
305 1984). On the other hand, Goeldi's monkeys and buffy-headed marmosets are
306 exceptionally strong mycophagous primates. The average feeding time on fungi by
307 Goeldi's monkeys is 31%, which reaches 65% during the peak month (Porter 2001), and
308 that by buffy-headed marmosets is 64.8% and 70.2%, respectively (Hilário and Ferrari
309 2010). These animals are much smaller (Goeldi's monkeys: 400-535 g, buffy-headed
310 marmosets: 406 g) compared with other primates known to eat fungi, such as gorillas
311 (97.9–159.2 kg), bonobos (31.0–39.0 kg), and vervet monkeys (3.3–4.6 kg) (Rowe and
312 Mittermeier 1996). Patchy distribution and the smaller food-patch size of fungi make it
313 difficult for them to be the main or fallback food resources for larger animals.
314 Interestingly, there is also a relationship between mycophagy and body size among
315 marsupials, where large species tend to become less mycophagous (Johnson 1996).
316 Considering the fact that Japanese macaques (8.3–18.0 kg) are larger than most of those
317 small mycophagous primates and marsupials, their low mycophagy frequency appears
318 to be consistent with this pattern. The fact that fungi constituted 14% of the diet of
319 Japanese macaques in a harsh environment with lower fruit availability (Hanya 2004)
320 indicates that environmental factors also contribute to mycophagy frequency. Fungi are

321 nonetheless still not as important to those macaques as they are to small mycophagous
322 animals.

323 There was a positive correlation between the consumption of fungal fruiting
324 bodies and fungal materials. This consistency may reflect fungal emergence patterns as
325 both are categorized as fungi. Previous studies showed that fungal fruiting emergence in
326 evergreen broad-leaved forests in Japan is a seasonal event that is affected by
327 temperature and rainfall (Tsujino et al. 2009; Murakami 1989). Mycophagy was also
328 associated with the feeding time of fruits. Macaques consumed fungi most often during
329 May–August while their main food items were fleshy fruits of *M. rubra*. *M. rubra* fruits
330 are highly preferred by Japanese macaques (Agetsuma and Noma 1995), and they
331 reached up to 60% of their diet during the peak month in this study. Fungi therefore are
332 not a fallback food, which is defined as a food eaten during times when preferred foods
333 are scarce. During the research period, we observed macaques fighting over a fungus at
334 least twenty times. These findings suggest the notion that fungi are valuable foods for
335 macaques. The high protein values of some fungi (Agrahar-Murugkar and
336 Subbulakshmi 2005) might be related to this tendency, while simple-stomach macaques
337 without strong fermentation system may not readily derive such proteins from fungi
338 (Hanson et al. 2006). Since the nutritional quality varies among fungi species, further
339 analysis of the nutritional values of fungi might help us to understand why macaques
340 desire fungi.

341 Japanese macaques in Yakushima consumed an enormous variety of fungi
342 (possibly 67 species of 31 genera). This could rise further if fungal samples were
343 available from all mycophagy events. Yet, the numbers are still astonishing compared
344 with other mycophagous primates such as Goeldi's monkeys, which consume only four

345 species (Hanson et al. 2003). Even northern flying squirrels, primarily mycophagous
346 mammals, feed on fungi of only 23 genera (Lehmkuhl et al. 2004). This fact indicates
347 that Japanese macaques interact with more species of fungi via mycophagy than do
348 frequent mycophagous mammals. Why do Japanese macaques consume such a diversity
349 of fungi? Since the macaques move/feed both on the ground and in the trees, they have
350 more chances to find fungi compared to highly arboreal species. Diversified mycophagy
351 among macaques might also reflect the high fungal diversity in Yakushima (Tsujino et
352 al. 2009). Our reasoning is based on the assumption that macaques are opportunistic
353 when eating fungi rather than being selective mycophagists. In fact, when macaques
354 encounter a fungus, they usually ingest it without exhibiting examining behaviors,
355 although they also exhibit avoidance of poisonous fungi to some degree (see below).
356 Since few sites have accumulated a body of data on fungus availability in regard to
357 species diversity comparable to that in Yakushima (Tsujino et al. 2009), further studies
358 are needed before drawing concrete conclusions.

359

360 **Behavioral Patterns: Exclusion of Poisonous Fungi**

361 Our findings indicate the possibility that Japanese macaques can discriminate
362 poisonous fungi effectively at two different phases: first when they hold the fungi in
363 their hands (Phase ii), and then when they actually take a few bites (Phase iv).
364 Therefore, macaques seemed to use mixed tactics to avoid poisonous fungi based on
365 both previous knowledge and on-site assessment of taste, and neither alone was
366 sufficient to discriminate poisonous fungi perfectly.

367 When the macaques picked up fungi, they generally did not exhibit examining
368 behaviors but immediately started eating them. In most cases, such fungi were found to

369 be non-poisonous. However, the fungi examined by the macaques were more likely to
370 be poisonous than those eaten immediately. These findings suggest the possibility that
371 macaques visually discern poisonous fungi based on knowledge they have acquired
372 through previous feeding experiences.

373 There were nine cases of the macaques eating poisonous fungi after showing
374 examining behaviors, indicating that poisonous fungi were not always excluded at the
375 first phase. At the second phase, the rest of the poisonous fungi were screened out
376 except for one case. The macaques are likely to screen out poisonous fungi by actually
377 taking a few bites on the spot, possibly judging from the taste and smell. We failed to
378 find statistical significance for this finding because most of the poisonous fungi had
379 already been excluded at the first phase.

380 Macaques did not selectively pick up non-poisonous fungi off the ground or
381 from the trees and decaying logs (Phase i). It appears that examining behaviors were not
382 useful for eliminating poisonous fungi (Phase iii). Examination of bibliographic
383 descriptions of fungi in North America and Europe reveal that poisonous species are
384 more likely to emit distinctive odors, which might have evolved as warning odors to
385 enhance avoidance learning by mycophagists (Sherratt et al. 2005). Our research shows
386 that macaques cannot perceive such warning odors. Therefore, warning odors emanating
387 from poisonous fungi do not function as a deterrent to mycophagy for Japanese
388 macaques.

389

390 **Behavioral Consistency and Possibility of Group Learning**

391 Macaques tended to display the same foraging behavior for the same fungus
392 species. This consistent behavior can be explained on two different levels, at the

393 individual or among-group level. For example, there were some cases where the same
394 individual consumed a particular fungus repeatedly. Among the nine individuals who
395 ate *Crepidotus mollis*, the most frequently eaten species, all the nine animals showed the
396 same tendency as to whether they ate or did not eat. In a detailed evaluation with six
397 categories, seven individuals consumed it more than twice and four of them always
398 showed the same behavior (NE-A). Although there were some consistencies as we
399 suggested, their behavioral patterns were not universal and hence we should admit other
400 interpretations. Macaques may make on-site assessments of fungi on a case-by-case
401 basis rather than depending on unequivocal cues.

402 In this study, we have shown that Japanese macaques may have a certain
403 degree of knowledge about poisonous fungi species. This finding would lead us to a
404 legitimate question about the possible process of knowledge acquisition and how and
405 whether macaques would share such information. There were cases where mothers
406 joining their offspring in eating fungi, and vice versa. We also observed a 1-year-old
407 male, who was watching his mother eating a fungus, got a piece left by his mother. We
408 could not obtain enough evidence to evaluate their memory or learning performance.
409 However, these instances imply possibilities for group learning, which is crucial,
410 especially for immatures. A study on red squirrels suggests that naive animals, who
411 have not yet learned what not to eat, may fail to avoid poisonous fungi (Camazine et al.
412 1983). Further research to explore how young animals acquire knowledge about fungi
413 may reveal the mechanism of group learning.

414

415 In conclusion, Japanese macaques in Yakushima consume a large variety of
416 fungi, and mycophagy occurs with fungal fruiting. They eat a fungus when available,

417 yet it is an act of minor importance from a quantitative viewpoint. When macaques
418 ingest a fungal fruiting body immediately without showing examining behaviors, it is
419 highly unlikely to be poisonous. Macaques therefore might have knowledge of the fungi
420 that are edible to them. They eliminate poisonous fungi by taste, while examining
421 behaviors do not function effectively to exclude poisonous species. Behavioral
422 consistency observed in repeated eating of certain species also indicates that the
423 macaques might share common knowledge of some fungi species as food resources.
424

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- 513

Table 1: Definition of mycophagy by Japanese macaques

Behavioral categories	Definitions
Ignore (IG)	Not touching a mushroom that was found within a 1-m radius of the animal *
Reject (RE)	Examining a mushroom but not eating it. Might take a bite but spit it out (no ingestion)
Examine, eat all (EX-A)	Showing examining behaviors while/before consuming the whole mushroom
Examine, eat partially (EX-P)	Same as Ex-A except for mushroom consumption is only partially
Not examine, eat all (NE-A)	Consuming the whole mushroom without examining behaviors
Not examine, eat partially (NE-P)	No examining behavior observed while ingesting a mushroom. Only a part of the mushroom or some of the multiple mushrooms available are ingested

* Note that the number mushrooms may be underestimated by the observer's oversight

Table 2: Observation time and the number of mycophagy events of each behavioral category

Month		Behavioral observation (hour)	Number of mycophagy events						Total
			IG	RE	EX-A	EX-P	NE-A	NE-P	
2009	Aug	12	0	1	1	0	15	3	20
	Sep	21	0	0	0	0	0	0	0
	Oct	51	0	0	0	0	1	0	1
	Nov	57	0	1	0	0	1	2	4
	Dec	42	0	0	2	1	3	0	6
2010	Jan	45	0	0	0	0	0	0	0
	Feb	27	0	1	0	0	5	1	7
	Mar	39	0	0	2	0	15	0	17
	Apr	111	0	0	1	0	34	3	38
	May	99	2	6	4	1	78	17	108
	Jun	45	3	5	4	5	44	3	64
	Jul	36	2	2	0	1	23	3	31
	Aug	57	0	0	3	0	22	3	28
	Sep	27	0	0	0	0	2	2	4
	Total	669	7	16	17	8	243	37	328

All the data presented here was collected by focal observation on adult female macaques. Data include the cases where fungal samples were failed to be collected

Table 3: Number of fungal samples, genera and species identified under each behavioral category

Behavior		All samples		Identified		
Category	Description	Sample	Genus	Sample	Genus	Species
IG	Ignore (no touch)	36	27	26	24	24
RE	Examine, not eat	53	22	37	12	34
NE-A	Not examine, eat all	210	33	116	24	51
NE-P	Not examine, eat partially	57	23	37	19	26
EX-A	Examine, eat all	19	9	9	6	7
EX-P	Examine, eat partially	17	5	10	4	7
Total number of samples		392 (25)	-	235 (18)	-	-
Genera or species foraged or ingored *		-	66 (5)	-	51 (3)	110 (7)
Genera or species consumed **		-	41 (4)	-	31 (3)	67 (6)

Data were collected by all-occurrence sampling on both focal and non-focal animals

Samples with the maximum identify $\geq 97\%$ were defined as identified

* Includes all the 6 behavioral categories (IG, RE, NE-A, NE-P, EX-A, EX-P)

** Either whole (NE-A, EX-A) or partial (NE-P, EX-P) consumption was observed. Numbers in parentheses are those only family, order, or class were available

Figure legends

Figure 1: Home range of AT troop in the study area, on the western coastal area of Yakushima, Kagoshima Prefecture, Japan, estimated by the minimum convex polygon (MCP).

Figure 2: Seasonal variations in dietary composition (August 2009 – September 2010)

Figure 3: Proportion of poisonous mushrooms among each mycophagy behaviors. The number of samples collected is shown in each bar. IG, ignore; NE-A, not examine and eat all; NE-P, not examine and eat partially; RE, reject; EX-A; examine and eat all; EX-P, examine and eat partially (see Table 1 for detailed behavioral description)

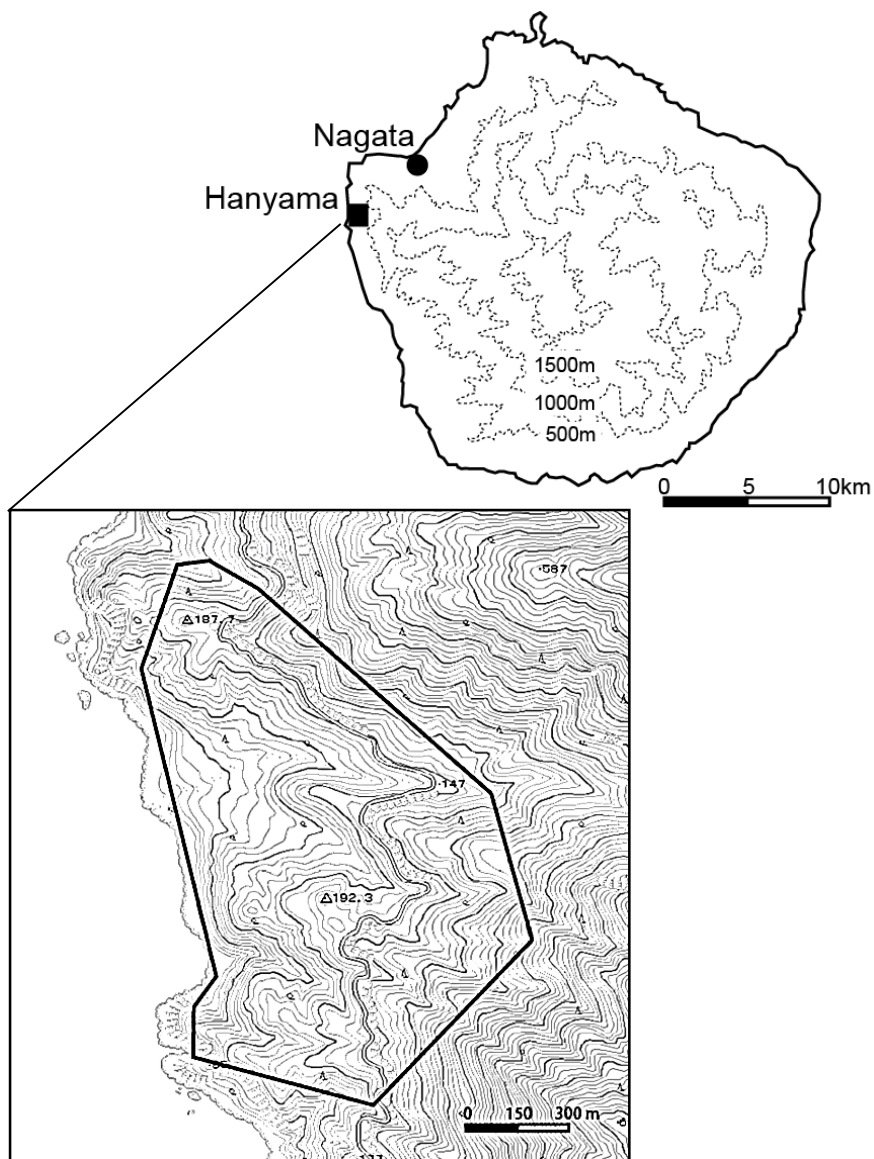


Figure 1

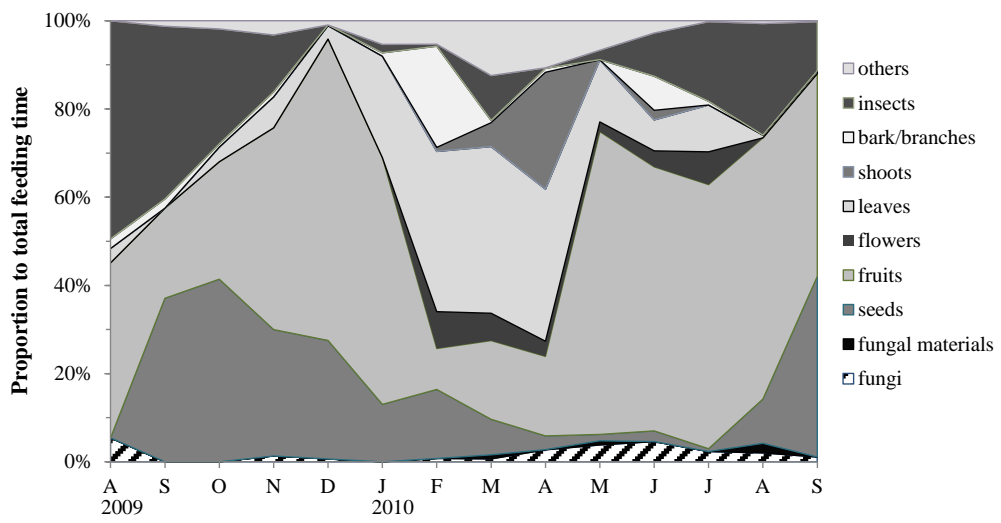


Figure 2

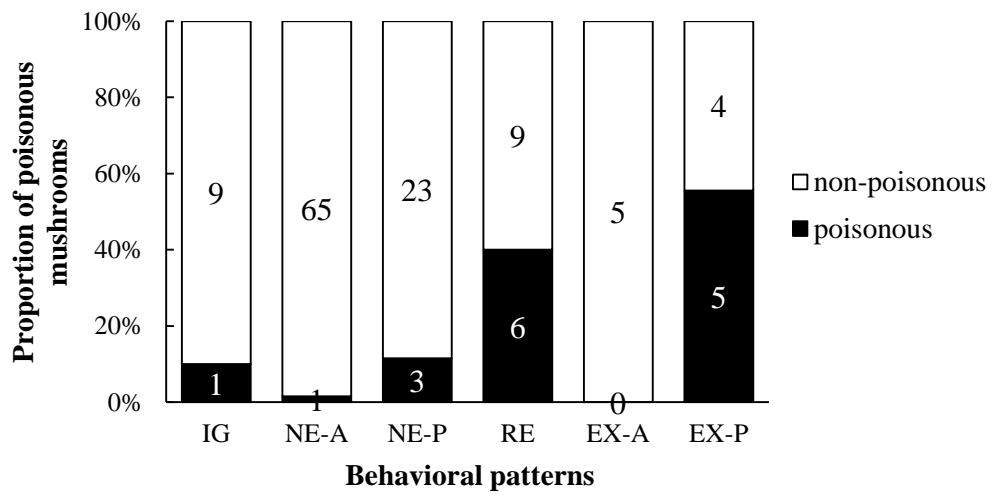


Figure 3

Appendix. List of mushroom species foraged or ignored by Japanese macaques in Yakushima

Species	Edibility/ toxicity	Behavioral category						Total	Sampling																	
		IG	RE	NE-A	NE-P	EX-A	EX-P		2009						2010											
								7	8	9	10	11	12	1	2	3	4	5	6	7	8	9				
<i>Agaricus moelleri</i>			1					1			○															
<i>Agaricus nivescens</i>	NA	1						1	○																	
<i>Agaricus subrutilescens</i>	Edible		2					2	○									○								
<i>Agrocybe aegerita</i>			1					1											○							
<i>Agrocybe chaxingu</i>			1	1				2	○										○							
<i>Agrocybe cylindracea</i>	Edible			1	4			5		○		○	○					○	○							
<i>Amanita alboflavescens</i>				1				1													○					
<i>Amanita esculenta</i>	NA			1				1							○	○										
<i>Amanita fritillaria</i>						1		1											○							
<i>Amanita griseoverrucosa</i>			1					1		○																
<i>Amanita imazekii</i>			1					1		○																
<i>Amanita orientigemmata</i>			1					1	○																	
<i>Amanita pseudoporphyria</i>	Poisonous		2				1	3	○	○																
<i>Amanita sepiacea</i>	NA		1				1	2										○								
<i>Amanita solitaria</i>			1					1	○																	
<i>Amanita spissacea</i>	Poisonous		1		1		4	6	○										○							
<i>Amanita virgineoides</i>	NA		1					1														○				
<i>Amanita sp. A</i>		1	1		1			3										○	○							
<i>Amanita sp. B</i>			1					1	○																	
<i>Amauroderma rugosum</i>					1			1											○							
<i>Armillaria mellea</i>					1			1					○													
<i>Auricularia aff. auricula-judae</i>	Edible			3				3											○		○	○				
<i>Auricularia auricula-judae</i>	Edible			4				4							○			○	○							
<i>Auricularia polytricha</i>	Edible			8	1			9		○					○		○	○								
<i>Boletus fraternus</i>				1				1											○							

Species	Edibility/ toxicity	Behavioral category						Total	Sampling													
		IG	RE	NE-A	NE-P	EX-A	EX-P		2009						2010							
								7	8	9	10	11	12	1	2	3	4	5	6	7	8	9
<i>Campanella alba</i>			1				1											○				
<i>Chaetocalathus galeatus</i>		1					1									○						
<i>Collybia confluens</i>		1					1												○			
<i>Collybia sp.</i>			1				1												○			
<i>Conocybe lactea</i>	NA		1				1														○	
<i>Coriolopsis strumosa</i>				2			2												○			
<i>Crepidotus mollis</i>	NA			22	4		1	27	○			○				○	○	○	○		○	
<i>Crepidotus sp.</i>				1			1											○				
<i>Cyptotrama asprata</i>		1					1											○				
<i>Dictyopanus gloeocystidiatus</i>		1					1												○			
<i>Entoloma sp.</i>				2			1	3	○									○				
<i>Filoboletus manipularis</i>	NA	2					2											○				
<i>Ganoderma mastoporum</i>		1					1	1	○													
<i>Gymnopilus crociphyllus</i>		1					1											○				
<i>Gymnopilus liquiritiae</i>	NA			2	1		3											○				
<i>Hericium coralloides</i>			1				1											○				
<i>Hohenbuehelia sp.</i>				1			1												○			
<i>Hygrocybe reidii</i>		1					1										○					
<i>Hypholoma fasciculare</i>	Poisonous			1	1		2	2	○													○
<i>Inocybe sphaerospora</i>	NA	1					1													○		
<i>Lactarius furcatus</i>			1				1	1	○													
<i>Lactarius gerardii</i>	NA		1				1													○		
<i>Leccinum sp.</i>				2			2													○	○	
<i>Lentinula edodes</i>	Edible				1		1				○											
<i>Lenzites elegans</i>		1					1										○					
Marasmiaceae sp. A *				4	1		5										○		○		○	
Marasmiaceae sp. B *		1					1												○			

Species	Edibility/ toxicity	Behavioral category							Sampling														
		IG	RE	NE-A	NE-P	EX-A	EX-P	Total	2009					2010									
									7	8	9	10	11	12	1	2	3	4	5	6	7	8	9
<i>Russula pectinatoides</i>	NA		1					1											○				
<i>Russula vesca</i>	NA			1			1	2	○	○													
<i>Russula sp. A</i>			1					1	○														
<i>Russula sp. B</i>				2				2								○	○						
<i>Russula sp. C</i>			1					1	○														
<i>Russula sp. D</i>					1			1														○	
<i>Russula sp. E</i>			1	3				4												○			○
<i>Russula sp. F</i>			1					1	○														
<i>Russula sp. G</i>			1	1	1	1		4	○										○				
<i>Russula sp. H</i>			1					1													○		
<i>Russula sp. I</i>				1				1											○				
<i>Russula sp. J</i>				1	2			3	○													○	
<i>Russula sp. K</i>				1				1													○		
<i>Russula sp. L</i>				1				1											○				
<i>Russula sp. M</i>				1				1												○			
<i>Russula sp. N</i>			1					1	○														
Russulaceae sp. A *				2				2											○				
Russulaceae sp. B *			1	5				6	○										○				
Russulaceae sp. C *				1				1		○													
Russulaceae sp. D *				2				2										○	○				
<i>Schizophyllum commune</i>				3				3								○		○	○				
<i>Simocybe serrulate</i>			1					1											○				
<i>Stereum ostrea</i>	NA			3	2			5										○	○	○			
<i>Strobilomyces seminudes</i>	NA		1		1			2												○	○		
<i>Trichaptum biforme</i>	NA		2					2										○	○				
<i>Tylopilus ballouii</i>				1				1														○	
<i>Tylopilus sp.</i>				1				1												○			

Species	Edibility/ toxicity	Behavioral category							Total	Sampling														
		IG	RE	NE-A	NE-P	EX-A	EX-P	2009		2010														
									7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	
<i>Xerocomus chrysenteron</i>	Edible			1	1			2											○					
<i>Xerocomus subtomentosus</i>				1				1														○		
<i>Xerocomus sp.</i>		1						1														○		
<i>Zelleromyces sp.</i>		1						1										○						

Numbers in behavioral categories indicate the number of mycophagy events where mushroom samples were available. * = mushrooms identified only to the family level. NA = no information on edibility/toxicity provided by the reference book. Blank = species not addressed under the reference book.