Hyper-specialized primates possess a reduced suite of xenobiotic-metabolizing cytochrome P450 genes.

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Abstract

Subfamilies of cytochrome P450 proteins have been strongly linked to the metabolism of physiologically disruptive compounds such as alkaloids, terpenoids, and other xenobiotics. Consistent with this function, these genes have adaptively evolved in response to environmental pressures exerted on animals, such as herbivores, that consume elevated amounts of toxic xenobiotics or plant secondary metabolites (PSMs). Theory on evolutionary tradeoffs predicts that highly specialized herbivores should exhibit a relatively narrow toolkit of adaptations to accommodate the concomitantly narrow arrays of PSMs in their diets. The bamboo lemurs of Madagascar (genera *Prolemur* and *Hapalemur*) represent an interesting test case for this theory because of their dietary hyper-specialization, as these lemurs consume bamboo and grasses at rates otherwise unseen in the order Primates. To test whether the hyper-specialized folivory of these primates is reflected in a similarly specialized and narrow P450 gene suite, we assembled a dataset of confidently assembled *CYP1-3* genes for two species of bamboo lemur as well as additional lemur species. We tested the predictions that bamboo lemurs would exhibit, first, greater rates of gene loss for xenobiotic-metabolizing P450s and, second, relaxed selection on xenobiotic-metabolizing P450 subfamilies relative to lemurs without such dietary hyper-specialization. We found support for the first prediction, related to gene loss, in the *CYP2B, CYP2C, CYP2D, CYP2J,* and *CYP3A* subfamilies, all of which encode xenobiotic metabolizers. We additionally inferred relaxation of selection for the *CYP2F* and *CYP2J* subfamilies. The evolution of the P450 genes in bamboo lemurs provides support for the evolutionary tradeoff hypothesis, and we further hypothesize that, rather than adapting to

34 a general array of PSMs, bamboo lemurs have instead adapted to the primary toxin in their

35 diet, the highly potent poison cyanide.

36

37 **Keywords:** Lemurs, plant secondary metabolites, *Prolemur*, *Hapalemur*, neutral evolution, gene

38 loss

39

40 **Highlights**

41 - Some of the most specialized diets among primates are those of bamboo lemurs.

42 - Bamboo lemurs have fewer xenobiotic-metabolizing P450 genes than other lemurs.

43 - Natural selection has relaxed on the *CYP2F* and *CYP2J* subfamilies in bamboo lemurs.

44

45 **1. Introduction**

46 Among mammals, a large proportion of xenobiotic compounds are transformed or
47 detoxified by enzymes within the cytochrome P450 superfamily. Because of this role that P450s
48 play in mediating potential environmental toxins and because species vary in their diets and
49 exposure to such compounds, selective pressure has induced non-random additive and
50 adaptive changes in genetic sequence and copy number among species (Chaney et al., 2018,
51 2020; Ingelman-Sundberg, 2022; Kawashima & Satta, 2014; Kirischian et al., 2011; Skopec et al.,
52 2022). The effect of evolving toxin resistance has been especially well-documented for pest
53 insect species because of the economic importance of pesticides and insects' evolutionary
54 responses to those substances (Wondji et al. 2009; Feyereisen 2011; Bass et al. 2014; Wang et
55 al. 2014; Walsh et al. 2018; Joussen and Heckel 2021). Studies of P450 gene adaptation outside
56 of pest insects have often focused on dietary xenobiotics or plant secondary metabolites
57 (PSMs). For example, research on a model system comprising several species of woodrat
58 (*Neotoma* spp.) has shown that PSMs common in their diet (*e.g.*, alkaloids, terpenoids) are
59 metabolized especially by CYP2B enzymes and, to a minor extent, other xenobiotic-
60 metabolizing enzymes (Haley et al., 2007a, 2007b; Orr et al., 2020; Wilderman et al., 2014).

61 Comparisons between specialist and generalist species of these woodrats have shed
62 light on the effects that diet can have on P450 evolution. For instance, the generalist woodrat
63 species (*N. albigula*) was found to have lower copy number but greater sequence diversity of
64 *CYP2B* relative to a specialist (*N. stephensi*). This greater diversity of the generalist is consistent
65 with the broader array of PSMs with which the generalist must contend (Kitanovic et al., 2018).
66 The increased copy number of the specialist, in contrast, is in line with the prediction that genes

67 important for coping with the specialist's narrow diet will be subject to *additive* evolutionary
68 processes (*e.g.*, gene duplication, positive-selection) that will enhance the species' ability to
69 mitigate a narrow range of metabolically harmful compounds (Dearing et al., 2005; Freeland &
70 Janzen, 1974). This is taken to an extreme for specialized koala (*Phascolartcos cinereus*), in
71 which the key xenobiotic metabolizing *CYP2C* subfamily has reached a copy number of 31 via
72 serial duplication (Johnson et al., 2018).

73 Mammalian dietary specialization has also been linked to patterns of gene loss (Blumer
74 et al., 2022; Hecker et al., 2019; Itoigawa et al., 2019; Lauterbur, 2019; Marciniak et al., 2021;
75 Wagner et al., 2022). For example, vampire bats (genera *Desmodus, Diaemus,* and *Diphylla*)
76 have lost sweet and umami taste receptors (Zhao et al., 2010b; Zhao et al., 2012) and have
77 experienced reduction of their bitter-taste sense through pseudogenization of *TAS2R* genes
78 (Hong & Zhao, 2014). Interestingly, losses of gustatory genes have also been found for
79 cetaceans, which have lost nearly all taste-receptor genes apart from those for salty taste (Feng
80 et al., 2014), and for pandas (*Ailuropoda melanoleuca*), which have lost their umami taste-
81 receptors (Zhao et al., 2010a). Theory predicts that genes unimportant to a specialist's dietary
82 adaptation will be subject to a suite of *subtractive* evolutionary processes (*e.g.,* accumulation of
83 deleterious mutations, pseudogenization, or loss of protein coding region or entire gene). Gene
84 loss could either be for adaptive reasons (see Olson, 1999) or because of a release of selective
85 pressure (see Blumer et al., 2022; Huelsmann et al., 2019). Despite these examples and the
86 theory surrounding them, subtractive mechanisms such as gene loss have remained relatively
87 understudied compared to additive mechanisms such as gene duplication and positive or
88 directional selection.

89 In summary, specialized folivory is predicted to have two evolutionary effects on a
90 species' xenobiotic-metabolizing toolkit: First, genes involved in detoxification or avoidance of
91 xenobiotic toxins that are rich in a folivorous specialist's diet should show signals of selection in
92 the form of positive copy-number changes or positive selection. Second, genes *not* involved in
93 such species' defense against toxins in their specialized diet should exhibit signals of negative
94 copy-number changes and relaxation of selection. Although the first prediction is well-
95 supported by prior studies of P450 gene evolution, comparatively few studies have investigated
96 patterns of P450 gene loss or relaxation of selection in specialized folivores.
97 Bamboo lemurs represent a well-suited case for testing predictions about P450 gene
98 loss in specialized folivores due to their narrow diets, which are among the most specialized in
99 the entire primate order. Despite their diminutive body sizes (0.9-2.6 kg: Tan, 2006), they
100 inhabit an ecological niche that is often compared with that of the giant panda (Eronen et al.,
101 2017; McKenney et al., 2017; Tan, 2006) and are the only extant primates known to specialize
102 so intensely on bamboo or grass species (Table S1). The three sympatric species at Ranomafana
103 National Park (*i.e.*, *Hapalemur aureus, H. griseus*, and *Prolemur simus*) are the source of much
104 of the current understanding of bamboo lemur ecology. All three of these populations have
105 diets that are often predominantly composed of a small number of species of bamboo or liana
106 (Grassi, 2006; Overdorff et al., 1997; Tan, 1999), and the sympatry of these species has been
107 understood in terms of niche partitioning, notably by their differential reliance on separate
108 plant parts from the same species of giant bamboo (Glander et al., 1989). Although other
109 species outside of Ranomafana (*e.g.*, *H. meridionalis, H. alaotrensis*) do not actually consume
110 bamboo, they nonetheless eat grasses (Poaceae) or sedges (Cyperaceae) at similarly high

111 frequencies (Table S1). A recent report, also based on locations outside of Ranomafana,
112 demonstrated more dietary flexibility for *Prol. simus* than would have previously been expected
113 (Mihaminekena et al., 2024; see Table S1), but bamboo is nonetheless critical forthis species'
114 evolution, likely as a staple fallback food (Marshall & Wrangham, 2007), as evidenced by
115 alimentary traits that are likely related to a specialized folivorous (dentition: Jernvall et al.,
116 2008; Vinyard et al., 2008; gastrointestinal morphology: Yamashita et al., 2008; M.E. Chaney,
117 pers. obs.; see Campbell et al., 2000). Regardless, all three populations subsisted on diets
118 composed of 90% folivorous foods or more, even if one population's diet was only 41.5%
119 bamboo (Mihaminekena et al., 2024). Thus, regardless of habitat or species, bamboo lemurs
120 subsist on highly folivorous diets that are notably low in their diversity.
121 One bamboo PSM is particularly intriguing for its toxicity. Consumption of the specific
122 bamboo parts consumed by *H. aureus* and *Prol. simus* exposes these lemurs to high levels of
123 potent cyanogenic glycosides (Ballhorn et al., 2009; Eppley et al., 2017; Glander et al., 1989).
124 When plant tissue is disrupted by some destructive process like mastication, these glycosides
125 will be hydrolyzed by the plant cell's own β-glycosidase enzymes to release cyanide (Ballhorn,
126 2011; Cressey & Reeve, 2019). At Ranomafana, two bamboo lemur species have been
127 estimated to consume many times the estimated lethal dose of cyanide for mammals of their
128 body sizes (Ballhorn et al., 2009; Glander et al., 1989). Although cyanide, the primary known
129 antifeedant of this bamboo, is detoxified by a metabolic pathway that does not include any
130 cytochrome P450 enzymes (reviewed by Isom et al., 2015), bamboo lemurs likely have to
131 contend with other PSMs known to be present in bamboo species, such as phenolic and

132 polyphenolic compounds (Zhu et al. 2018). Thus, we still expect evolution of bamboo lemur
133 P450 genes to be impacted by their hyper-specialized diet.
134 For this project, we hypothesized that the highly specialized diet observed for bamboo
135 lemurs should be associated with a narrower P450 repertoire, streamlined to those subfamilies
136 that metabolize xenobiotic compounds found in bamboo. Therefore, we predicted that,
137 compared to other generalist Malagasy strepsirrhines, the bamboo lemur species *Prol. simus*
138 and *H. griseus* should exhibit a clear pattern of cytochrome P450 (1) gene loss and (2)
139 signatures of relaxed natural selection, because the specialist diet contains a narrow set of
140 PSMs, of which the primary toxin (*i.e.*, cyanide), is not even metabolized by P450s. Similar to
141 previous work (Chaney et al., 2020), we focus on P450 genes in the *CYP1-3* subfamilies. Briefly,
142 this decision was made because of these subfamilies' monophyletic placement within the
143 cytochrome P450 superfamily of genes (Kirischian et al., 2011) and the presence of genes
144 encoding both xenobiotic and endobiotic metabolizers within that gene-clade (see Guengerich,
145 2015).

146 **2. Materials and Methods**

147 2.1 Generation of *Hapalemur griseus* genome assembly

148 For this study, we constructed a genome assembly for a male *H. griseus* (named Beavis)
149 housed at the Duke Lemur Center in Durham, North Carolina. A fresh whole blood sample was
150 shipped to the Molecular Primatology Laboratory of New York University for processing and
151 sequencing. High molecular weight (HMW) DNA was extracted from 200 µL of whole blood
152 using a MagAttract HMW DNA Kit (QIAGEN Sciences Inc., Germantown, MD) following the
153 manufacturer's protocol. DNA was eluted into a low-bind 1.5 mL tube and stored at 4°C prior

154 to size selection. In order to obtain DNA fragments roughly 40 kilobases or longer, the extracted
155 HMW DNA was run through a Blue Pippin DNA Size Selection System (Sage Science Inc., Beverly,
156 MA) using a 0.75% agarose gel cassette with the R1 size standard. Following this size selection
157 protocol, the HMW DNA concentration was quantified using a Qubit 2.0 fluorometer (Thermo
158 Fisher Scientific Inc., Carlsbad, CA) using the broad range standard. The extraction yielded 3.2
159 ng/µL of HMW DNA.

160 After extraction and quantification, the HMW DNA sample was sent to NYU Langone
161 Medical School's Genome Technology Center for quality control tests, which consisted of DNA
162 quantification and fragment size determination using a Tape Station (Agilent Technologies Inc.,
163 Santa Clara, CA). After passing this checkpoint, the HMW DNA was prepared for sequencing
164 using a Chromium Genome Linked Reads Kit (10X Genomics Inc., Pleasanton, CA) and
165 sequenced on a HiSeq 2500 DNA Sequencing Platform (Illumina Inc., San Diego, CA) with 1200M
166 reads. The linked-read output data were assembled using the Supernova assembler (Weisenfeld
167 et al., 2017). We used the pseudohaplotype output as our genome assembly for all downstream
168 steps (Table 1).

169 <u>2.2 Additional data gathering</u>

170 For both the gene-birth/death and selection-analysis portions of this study, we gathered
171 data from publicly available genome assemblies for 14 species: *Prolemur simus* (Hawkins et al.,
172 2018), *Lemur catta* (Palmada-Flores et al., 2022), *Eulemur flavifrons* and *E. macaco* (Meyer et al.
173 2015), *Propithecus coquereli* (Lowe and Eddy 1997; Guevara et al. 2021), *Indri indri* (accession
174 number: GCA_004363605.1), *Daubentonia madagascariensis* (accession number:
175 GCA_004027145.1), *Mirza coquereli* (accession number: GCA_004024645.1), *Mirza zaza*

176 (Hunnicutt et al., 2020), and *Microcebus murinus* (Averdam et al., 2011; Lecompte et al., 2016),
177 as well as the following additional species of mouse lemur: *Mic. griseorufus, Mic. mittermeieri,*
178 *Mic. ravelobensis,* and *Mic. tavaratra* (Hunnicutt et al., 2020). These assemblies, along with any
179 associated annotation files, were downloaded locally and formatted into BLAST databases
180 within Geneious Prime, version 2022.1.1.

181 We located the loci for all annotated *CYP1-3* homologs in the *L. catta, Prop. Coquereli,*
182 and *Mic. murinus* by using the associated annotation (GFF3) files for each. We defined these
183 loci by the non-P450 genes that bounded them; therefore, those surrounding genes were used
184 initially as queries for local BLAST searches. In this way, each locus was linked to two searches
185 per species. The three reference genomes listed above were used because they are all
186 members of separate strepsirrhine families (Lemuridae, Indriidae, and Cheirogaleidae,
187 respectively), and they were each therefore used as a starting point to extract the desired
188 *CYP1-3* genes or loci for confamilial species. Ideally, a pair of BLAST searches would return
189 results that included the same scaffold. By locating both BLAST hits on each of these scaffolds,
190 we were able to extract genomic regions that were hypothetically orthologous to those P450
191 loci in the *L. catta* assembly. After locating the scaffolds in each assembly corresponding to
192 each P450 locus, we used LASTZ (Harris, 2007) to interrogate the homology of those scaffolds
193 by aligning them to the confirmed P450 locus from the appropriate confamilial reference
194 genome. Positive results from these alignments were checked using the Mauve genome aligner
195 (Darling et al., 2004) on the same sequences. If output from both of these aligners indicated
196 that the reference had homology with the query scaffold(s), then the annotations from the
197 reference genome were used to extract the corresponding sequence in the other species'

198 genome. In this way, we mined the genome assemblies listed above for as many complete P450
199 genes loci as we could confidently locate.
200 To augment our dataset of codon sequences specifically for our selection analyses (see
201 below), we also devised a *blastn*-based strategy to gather such data from 25 recently published
202 assemblies in the Primate Genome Diversity Project (PGDP; Kuderna et al., 2023). Similar to
203 locating complete P450 loci for the birth-death analysis, we used one reference species for each
204 of three strepsirrhine families comprised by the portion of the PGDP data that we utilized:
205 Cheirogaleidae (reference species: *Mic. murinus*), Indriidae (*Prop. Coquereli*), and Lemuridae *(L.*
206 *catta*). Each of the *CYP1-3* genes for these references was transposed into a multifasta file with
207 each codon separated by sequence headers. Each of these exons were then used to
208 independently query each of the selected PGDP genome assemblies, which resulted in a large
209 dataset of BLAST hits for each of these searches. For each set of results, any orthologous
210 sequence was used only if it met the following criteria: all of the resultant exons were found on
211 the same contig, these exons were in the same relative order as in the reference (after
212 accounting for strandedness), and this locus did not appear elsewhere in the dataset (after all
213 sequence data had eventually been gathered). In order to maximize our statistical power in the
214 downstream selection analysis, we accepted partial sequences, or sequences with incomplete
215 coverage of each query sequence. In order to construct the trees needed for these analyses, we
216 downloaded all sequence between the first and last exons for each set of BLAST results and
217 used this for alignment and phylogenetics (see below).

218 2.3 Inference of gene birth and death

219 For this first portion of the study, we used only species for which each *CYP1-3* locus
220 could be wholly collected from a single scaffold or reasonably reconstructed if not found on a
221 single scaffold using the process described above. In order to model the events of gene birth
222 and death in this subset of lemur species, our alignment strategy followed a similar workflow as
223 outlined in previous work with other datasets (Chaney et al., 2018, 2020), but several
224 modifications were made for this project in order to allow for more standardization and
225 automation across subfamilies. First, all of the P450 genes were extracted from each species'
226 locus according to the annotation file associated with its confamilial reference. Then, all of the
227 genes from a given P450 subfamily were aligned using MAFFT (Katoh & Standley, 2013), and the
228 resulting alignment was stripped of all sites containing any gaps using trimAl (Capella-Gutierrez
229 et al., 2009). After the best-fitting nucleotide substitution model was inferred by jModelTest
230 (Darriba et al., 2012), this stripped alignment was visualized with PhyML 3.0 and the strength of
231 that resulting phylogenetic tree was tested by comparing it to 1000 bootstrap replicates
232 (Guindon et al., 2010).

233 The gene trees constructed with PhyML were then passed to Possvm (Grau-Bové &
234 Sebé-Pedrós, 2021). This program uses the intrinsic information contained in a phylogram to
235 infer speciation and gene-duplication events; it does this using the species-overlap algorithm in
236 the ETE3 toolkit (Huerta-Cepas et al., 2007, 2016). Briefly, this algorithm compares the
237 intersection of species present in both descendants of an internal node of a tree to the union of
238 species present in those descendants; using these values, the algorithm computes a species-
239 overlap score which it then uses to identify each internal node as either a speciation event,

240 having an overlap score, or a duplication event, having a high overlap score (Huerta-Cepas et
241 al., 2007). Once the identities of each node were estimated in this way, we then manually
242 examined each subtree rooted by a node called as a duplication event to infer whether any
243 gene loss had occurred. This was examined on a case-by-case basis using the reasoning that,
244 after a duplication event, each descendant of that node should recapitulate the organismal
245 phylogeny present at the time of duplication. Therefore, any species missing in one of those
246 subtrees must have lost one of the duplicates born in the earlier duplication event as long as
247 the subtree in question was well-resolved in terms of bootstrap support. In cases where
248 multiple species lineages may be absent, we deferred to the parsimonious hypothesis that a
249 loss event would have occurred prior to the divergence of those lineages, rather than a more
250 complicated hypothesis that the same paralog had been independently lost in both species
251 after their split. We visualized the Possvm output using the program Treerecs (Comte et al.,
252 2020) and then, in some cases, manually modified the depicted gene-evolution scenario in
253 order to accommodate the Possvm results.

254 2.4 Selection analysis

255 We used primarily used HyPhy's RELAX program (Wertheim et al., 2015) to test our
256 hypothesis concerning the relaxing of selectional pressure on the P450s covered in this project,
257 but the programs aBSREL (Smith et al., 2018) and BUSTED (Murrell et al., 2015) were also used
258 only in the case of *CYP2C* because of some preliminary tests indicating possible diversifying
259 selection in this gene subfamily (see below: section 3.2). For all runs, we made sure to include
260 estimates of synonymous rate variation because ignoring this parameter and setting it to 1.00,
261 as is often done for many *dN/dS* analyses, has been shown to bias results and, in the case of

262 detecting diversifying selection, inflate the number of false positives (Wisotsky et al.,
263 2021).Each HyPhy run requires, first, a codon alignment stripped of any stop codons and,
264 second, a phylogenetic tree that contains labels for the branches on which *a priori* relaxation of
265 natural selection is hypothesized to have occurred. For the codon alignments, we used
266 preexisting annotations from each of the annotated genome assemblies to extract the likely
267 coding DNA sequence (CDS) from each of the ostensibly complete P450 genes gathered in the
268 first portion of the study. Those annotations were then leveraged to find the likely CDS in
269 unannotated homologous sequences from confamilial relatives.
270 We aligned the CDS from each subfamily by translation using MACSE (Ranwez et al.,
271 2018), and we then checked each alignment by eye using MEGA 11 (Tamura et al., 2021). Once
272 the alignment had been checked by eye and the maximum number of useable sequences were
273 included, it was exported in FASTA format. The trees used for all HyPhy runs were were built
274 from genomic (*i.e.,* combined exonic and intronic) sequence using MAFFT (Katoh & Standley,
275 2013). We compared the rows in each subfamily's final CDS alignment to the leaves of the gene
276 tree constructed earlier with an R script using functions from the *phytools* (Revell, 2012) and
277 *seqinr* (Charif & Lobry, 2007) R packages, and a new tree was written that contained only the
278 intersection of these two sets. This filtered gene tree was then viewed with the web-based
279 *phylotree.js* platform (Shank et al., 2018), which allowed us to easily label branches on each
280 tree. Only foreground branches were labeled for *Prol. simus, H. griseus*, and any internal
281 branches exclusive to the clade formed by these two species. Accordingly, all unlabeled
282 branches were used for the estimation of the background *dN*/*dS* ratios for RELAX. Aside from
283 manually reading the text-file output, the HyPhy Vision platform

284 (http://vision.hyphy.org/RELAX) was used to generate some of the graphs made from the
285 output JSON files. For ancestral-sequence reconstruction of *CYP2F*, we used a HyPhy batch file
286 that makes use of parameters estimated from a selection analysis run beforehand
287 (https://github.com/veg/hyphy-analyses/tree/master/AncestralSequences). Rather than using
288 some other software for ancestral-sequence reconstruction, our use of this HyPhy batch file
289 increased our confidence in the comparability of our RELAX runs and the ancestral sequences
290 used in our *CYP2F* post-hoc analysis. Using these ancestral sequences, we then computed
291 pairwise *dN/dS* values using the Nei-Gojobori (1986)Nei-Gojobori (1986) method to more
292 closely examine signals of neutral evolution or selectional relaxation revealed by the earlier
293 HyPhy RELAX analysis.

294 3. Results

295 The evidence presented here largely displays a trend in which the common lineage of
296 bamboo lemurs has a diminished or expected number of gene copies relative to generalist
297 species, with several xenobiotic-metabolizing P450 subfamilies exhibiting a resolved gene-tree
298 topology indicating genic loss in one or both of the bamboo lemur species included here.
299 Because of the other species included in this data set, there are also some insights to be gained
300 from examining other portions of the gene trees outside of the portions focused on our species
301 of interest.

302 3.1 Heightened gene loss within the bamboo-lemur lineage

303 The topologies of the *CYP1A* and *CYP1B* subfamilies show strong bootstrap support and
304 near-perfect correspondence to the species phylogeny for their member orthologs (Figs. S1 &
305 S2). The only exception to this derives from the *Propithecus coquereli* lineage, which saw the

306 duplication of the *CYP1A1* ortholog after the divergence of the *Prop. coquereli* and *Indri indri*
307 lineages.
308 The shape of the *CYP2A* gene tree implies substantially more genic turnover than either
309 of the *CYP1* subfamilies (Fig. 1, top-left; Fig. S3). While the *Microcebus murinus* lineage shows a
310 very straightforward trajectory of no duplications, all other species have either duplicated or
311 lost orthologs according to the pattern of relatedness displayed in this tree. Due to the mid-
312 point rooting of this tree by Possvm, it is unclear whether two or three orthogroups are truly
313 present in this case (see Fig. S3), but we opt for a parsimonious interpretation of two
314 orthogroups here (see Fig. 1). Nonetheless, at least one duplication was certainly specific to the
315 *Prop. coquereli* lineage, depicted here by the common node of *CYP2A13L1* and *CYP2A13L2*. The
316 lemurid portion of the tree indicates a gene duplication in the common ancestor of all three
317 species and another duplication that was specific to *L. catta*. Conversely, the topology displayed
318 here implies genic loss for *E. macaco* and for *Prol. simus.*
319 The only members of *CYP2B* examined in this study are *CYP2B6* homologs because
320 another group of orthologs in the *L. catta* genome assembly (*CYP2B11*) did not show a CDS
321 length that was remotely close to the normal length of a functional primate P450 gene (*i.e.*, *ca.*
322 1500 nt). In the *CYP2B6* tree (Fig. 1, bottom-left; Fig. S4), we find two major orthogroups among
323 the five species analyzed. One of these shows a topology that, in itself, appears to recapitulate
324 the organismal phylogeny with no duplications. However, the other orthogroup exhibits a
325 signature of interesting dynamism. There are two serial duplication events in the *Prop.*
326 *coquereli* lineage (*CYP2B6L1, 3,* and *4P*). In the lemurid portion of this subtree, there are two
327 more serial duplications prior to the speciation of *L. catta* and *Prol. simus.* The pattern in this

328 part of the tree most parsimoniously indicates, first, that the bamboo-lemur common ancestor
329 lost genes orthologous to the *CYP2B6L3* and *CYP2B6L4* genes in the *L. catta* assembly and,
330 second, that the *H. griseus* lineage further lost its final remaining gene in this orthogroup.
331 The *CYP2C* genes yielded a phylogeny whose complexity is underscored by the large
332 number of duplications within it (Fig. 1, right; Fig. S5). Possvm separated this phylogeny into
333 five coherent orthogroups. The first of these (*i.e.,* OG-0) contained a duplication prior to the
334 divergence of *L. catta* and the bamboo lemurs. Following the *Prolemur-Hapalemur* divergence,
335 *Prol. simus* lost one of these genes but the other paralog was lost by *H. griseus*; thus, both
336 species retained separate paralogs. OG-1 and OG-2 lack *Prol. simus* genes but do contain genes
337 belonging to *H. griseus*; this pattern indicates two lineage specific events of gene loss for *Prol.*
338 *simus.* While this latter species, the greater bamboo lemur, showed a pattern of complete loss
339 for these orthogroups, *Mic. murinus* experienced a duplication in OG-2. All lemurid genes were
340 ostensibly lost from OG-3, while the mouse-lemur lineage saw a single-gene expansion of this
341 orthogroup. The final clade of genes, OG-4, contained three duplication events and,
342 parsimoniously, three instances of gene loss. While *L. catta* retained every one of the
343 duplicated genes from this sequence of events, bamboo lemurs likely lost the ortholog to *L.*
344 *catta CYP2C18L1*, and both *Prol. simus* and *H. griseus* further lost one more gene each.
345 However, these two final losses were lineage-specific, and each involved a separate genic
346 lineage. Thus, with the exception of genes in one orthogroup (*i.e.*, OG-3), bamboo lemurs
347 exhibited all of the losses detected in this tree.
348 Similar to *CYP2C*, the *CYP2D* subfamily also showed an increased amount of evolutionary
349 dynamism with regard to gene birth and death (Fig. 2, top-left; Fig. S6). Along a basal branch of

350 the phylogeny, there are two serial duplications, resulting in three *Mic. murinus* homologs
351 equally related to all lemurid *CYP2D* homologs. Each of the black lemur (*E. macaco*) species has
352 two very similar *CYP2D* genes which arose from the duplication specific to the *Eulemur* lineage.
353 Prior to the divergence of the *L. catta* lineage from that of the bamboo lemurs, there is some
354 uncertainty in these results whether only a single duplication occurred or whether two
355 occurred. This uncertainty stems from two nodes displaying low bootstrap support (73.0% and
356 57.5%; see Fig. S6) in the original PhyML tree. The topology displayed in Figure S6 describes two
357 duplications and parsimoniously implies just as many deletions. In light of the low bootstrap
358 support for this pattern, however, it is conceivable that the true topology could place the *L.*
359 *catta CYP2D17L2* gene into the clade with the two bamboo-lemur genes. Such a revision would
360 reduce the number of deletions and duplications. The other duplicated gene in the lemurid
361 lineage, while secondarily duplicated in the *L. catta* lineage, was lost in the *H. griseus* lineage.
362 Lastly, no functional homologs for the *CYP2D* subfamily could be retrieved for *H. griseus*
363 because no open reading frames devoid of stop codons could be found in the extracted
364 sequence for this species' single *CYP2D* gene. However, this isolated instance may be due to an
365 assembly artifact, as the *CYP2D* locus was located on the ends of two separate scaffolds in the
366 original Supernova assembly. In all, the general pattern of this subfamily is similar to that of
367 *CYP2C*: evidence of heightened genic turnover with an emphasis on loss in the specific
368 lineage(s) of the bamboo lemurs.
369 The *CYP2J* subfamily divided into three orthogroups (Fig. 2, top-right; Fig. S7). The first
370 of these (OG-0) contains only a single duplication node before the speciation node for the split
371 of *L. catta* from the bamboo lemurs. The complete lack of any bamboo-lemur genes in this

372 portion of the tree parsimoniously indicates a loss of that duplicate prior to the *Prolemur-*
373 *Hapalemur* divergence. The remaining two *CYP2J* orthogroups (OG-1 and OG-2) arose as the
374 product of a duplication event prior to the first speciation event of all species involved in this
375 analysis. While no more duplications were called in the OG1-OG2 clade, several deletions are
376 implied by the tree's topology. First, one gene appears to have been lost in the *Prop. coquereli*
377 lineage. Second, another deletion was ostensibly lost in the ancestral lemurid lineage. Last, *H.*
378 *griseus* lost one more homolog after that lineage's split from that of *Prol. simus.* Thus, the
379 *CYP2J* subfamily displays more of the above-mentioned pattern wherein the bamboo lemurs
380 stand out for their likelihood of losing P450 genes.

381 The topologies of the remaining *CYP2* subfamilies (*i.e.*, *E, F, G, R, S, U,* and *W*) show no
382 evidence of duplication (Figs. S8-S13). Degeneration of one *CYP2F* gene may have occurred in
383 the *Mic. murinus* lineage (see Fig. S9), but anthropoids and at least some lorisiforms appear to
384 possess only a single *CYP2F* gene as well (Chaney et al., 2020; Chaney, 2023). Therefore, the
385 ancestral primate number of *CYP2F* orthologs is uncertain without the inclusion of a
386 phylogenetically broader set of species. With this single possible exception, bamboo lemurs
387 have clearly maintained the same number of gene copies in each of these subfamilies as the
388 other lemur species analyzed here.

389 The final subfamily of this part of the analysis further underscores the pattern described
390 for other subfamilies above (Fig. 2, bottom-left; Fig. S14). While OG-0 within the *CYP3A* tree
391 possesses only speciation nodes, one loss is evident in the absence of *Prop. coquereli* from this
392 clade. The other part of the tree exhibits a very complex topology because of the number of
393 duplication nodes contained within it. Many of these outside of the lemurid portion of the tree

394 are taxon-specific: *Prop. coquereli* and *Mic. murinus* each experienced two and four exclusive
395 duplication events, respectively. The lemurid portion of OG-1 shows at least three duplication
396 events prior to the divergence of *L. catta* and from the ancestral bamboo-lemur lineage. One
397 more duplication event, leading to the *L. catta* genes *CYP3A4L1* and *CYP3A4L2*, would be most
398 parsimoniously interpreted as having occurred specifically in ring-tail lemur lineage, but it is not
399 ruled out that this duplication may have occurred in an ancestral species and been lost by both
400 bamboo lemur species. Regardless, at least two separate events of genic loss seem to have
401 occurred in the lineage of the bamboo lemurs. Thus, while *L. catta* appears to exhibit six
402 separate *CYP3A* homologs, bamboo lemurs possess half that number; furthermore, we
403 conclude that this pattern is attributable nearly wholly to gene loss by bamboo lemurs rather
404 than lineage-specific expansion of *CYP3A* for *L. catta.*

405 <u>3.2 Selection relaxed on two P450 xenobiotic metabolizers</u>

406 Out of all P450 subfamilies studied here, only *CYP2F* and *CYP2J* showed significant
407 signals of selectional relaxation within the bamboo-lemur clade (Table S2). The *CYP2F*
408 subfamily's estimated *dN/dS* distributions were interesting in that all rate classes in the test-
409 branch distribution collapsed to a ratio of 1.00, indicating neutral evolution of these genes since
410 their divergence from their *L. catta* orthologs. After examining all bamboo-lemur sequences
411 and comparing them to their ancestral reconstructions, we found that all *dN – dS* calculations
412 for the bamboo-lemur *CYP2F1* and *CYP2F2* ancestral sequences and all of their descendent
413 sequences were virtually zero (*CYP2F1* range: -9.2e-3, -9.4e-4; *CYP2F2* range: 0.018, -6.6e-3).
414 This result is consistent with the above RELAX results, which indicate that both *CYP2F* orthologs
415 appear to be evolving neutrally in the bamboo-lemur clade.

416 The *CYP2C* subfamily exhibited a significant signal of selectional *intensification* (p = 6.8e-
417 8, k = 1.42; see Table S3). Examination of the omega distribution for the test branches showed
418 that this appears to be driven by a heightened estimate for the highest *dN/dS* rate class, and
419 follow-up analysis with aBSREL and BUSTED showed this result was driven by a site in the *H.*
420 *griseus* ortholog for *CYP2C21* (Fig. S15). Indeed, aBSREL detected diversifying selection only
421 along the this terminal branch (likelihood ratio = 41.92, $p < 0.00001$), with a very high estimate
422 for the highest *dN/dS* rate class (> 1000). Some, but not all, of this variation was shared by the
423 closely related *H. meridionalis*, but this species' terminal branch did not show any positive
424 result from our aBSREL or BUSTED analyses.

425 **4. Discussion**

426 In this study, we have demonstrated that the genomes of two bamboo lemur species (*H.*
427 *griseus* and *Prol. simus*) harbor, at a conservative maximum, the modal number of cytochrome
428 P450 genes across the other lemur species included here (Table S1). Indeed, in most cases
429 where losses of P450 genes were inferred, they are most parsimoniously attributed to a
430 bamboo lemur-specific branch of the phylogeny in question (see Figs. 1-2). Thus, bamboo
431 lemurs appear to show a narrower repertoire of xenobiotic-metabolizing P450s insofar as they
432 have generally fewer genes than other species of lemur, especially in comparison to their
433 closest relative, *Lemur catta.* Bamboo lemurs also show signals of relaxation of natural selection
434 for the *CYP2F* and *CYP2J* subfamilies (see Fig. 3), which are known to be involved in xenobiotic
435 metabolism (Danielson, 2002; Guengerich, 2015).

436 These results are perhaps best understood in terms of an evolutionary tradeoff on
437 detoxification strategies (Dearing et al., 2005; Freeland & Janzen, 1974; Ueno, 2001). In a

438 pivotal paper outlining several nutritional-ecological hypotheses, Freeland and Janzen (1974)
439 formalized the notion that generalist and specialist herbivores should differ inversely with
440 regard to the breadth of their arsenals for the metabolism of toxic plant secondary metabolites.
441 In their view, herbivores should possess a comparatively wide array of detoxification
442 mechanisms to handle low doses of a commensurately broad variety of toxins, and specialist
443 herbivores should, conversely, possess a smaller array of such mechanisms able to handle large
444 doses of a narrow variety of toxins (Dearing et al., 2005; Freeland & Janzen, 1974; Marsh et al.,
445 2006). The general pattern of copy-number differences and the more limited result of
446 selectional relaxation shown in this study seems to support this hypothesis in the case of
447 bamboo lemurs, alongside this taxon's high degree of specialization (see Table S1) and the
448 stupendous amounts of cyanide consumed by them (Ballhorn et al., 2009; Glander et al., 1989).
449 In this set of results, the *CYP2F* subfamily stood out from other P450 subfamilies by
450 having a particular signal of selectional relaxation that suggested neutral evolution, as all of the
451 rate classes for *CYP2F* bamboo-lemur orthologs collapsed toward *dN/dS* = 1.00. Follow-up
452 comparisons of *dN* and *dS* were consistent with this result (Table S4). Although this P450
453 subfamily is most highly expressed in the respiratory tract, hepatic expression has been
454 demonstrated, albeit at lower levels (Duff et al., 2015; Fagerberg et al., 2014; Yue et al., 2014).
455 Human CYP2F1 and its model-rodent homologs can activate coumarins through epoxidation
456 reactions, leading to cytotoxicity and necrosis at varying species-dependent levels (Born et al,
457 2002; Vassalo et al., 2004). Similarly, CYP2F enzymes have been linked with bioactivation of the
458 toxin naphthalene, also through an oxidation reaction (Lanza et al., 1999; Li et al., 2011; Zhang
459 & Ding, 2008). These biochemical associations are notable here because chromatographic

460 assays and microbiome studies of various species of bamboo have implied or directly indicated
461 the presence of both naphthalene (Li et al., 2002; Takahashi et al., 2010) and coumarins (Coffie
462 et al., 2014) in those bamboo species (reviewed by Gagliano et al., 2022). Generalizing from
463 these findings, we would hypothesize, first, that naphthalene and coumarins are present in
464 Malagasy bamboo consumed by bamboo lemurs and, second, that bamboo lemurs would be
465 susceptible to toxicity from their metabolite oxides (napthalene: see Yost et al., 2021;
466 coumarins: see Foroozesh et al., 2019). Therefore, neutral evolution of bamboo lemurs' *CYP2F*
467 orthologs may have allowed for marginally lowered efficiency of the bioactivation of these
468 PSMs into more toxic metabolites. Experimental testing of this hypothesis would help to clarify
469 this interesting result.

470 *CYP2J,* the other subfamily to show significant relaxation of selection pressure for
471 bamboo lemurs, is largely responsible for the metabolism of arachidonic acid, an endogenous
472 molecule, and other polyunsaturated fatty acids (Xu et al., 2013; Q.-Y. Zhang & Ding, 2008).
473 However, CYP2J enzymes have been shown to be important in the metabolism of some
474 xenobiotic compounds such as linoleic acid and a variety of pharmaceutical compounds
475 (Capdevila & Wang, 2013; Falck et al., 2009; Hildreth et al., 2020; Michaelis et al., 2003;
476 Pidkovka et al., 2013; Tang et al., 2009; M.-Z. Zhang et al., 2013; cited in Guengerich, 2015). We
477 suggest therefore that bamboo-lemur diets are likely sparse in such xenobiotics that would be
478 targeted by CYP2J enzymes.

479 The difference between bamboo lemurs and the sifaka *Propithecus coquereli* stands out
480 as a second confirmatory signal of Freeland and Janzen's (1974) tradeoff hypothesis because,
481 while both of these taxa are generally classified as folivorous, they differ in their amount of

482 dietary specialization. As mentioned previously, all species of bamboo lemur that have been
483 studied are heavily reliant on either grasses (usually bamboo) or sedges, and very few plants,
484 often only a single species, are required to describe a large majority of their diets (see Table
485 S1). Conversely, sifakas (*Propithecus* spp.) consume much more varied foods (Hemingway,
486 1998; Norscia et al., 2006; Powzyk & Mowry, 2003; Rowe et al., 2016), seasonally trending
487 toward gramnivory or frugivory depending on the species in question (Irwin 2006). Despite this
488 dietary diversity, sifakas do possess clear gastrointestinal adaptations related to hindgut
489 fermentation, such as a greatly elongated intestine and an expanded, haustrated cecum
490 (Campbell et al., 2000). For this reason, sifakas are often described as *anatomical folivores*, with
491 the implication that they are more behaviorally flexible with their diet choice than one might be
492 led to believe upon viewing their gastrointestinal anatomy. Meanwhile, bamboo lemurs of all
493 three major species possess a relatively simpler gastrointestinal anatomy, with an overall
494 relatively shorter intestine and a shorter, though completely haustrated, colon (Campbell et al.
495 2000; Yamashita et al. 2008). All of this information serves to position sifakas and bamboo
496 lemurs on opposite sides of a specialist-generalist continuum among herbivores.
497 Such dietary differences between these two taxa helps to clarify an ultimate explanation
498 for the opposite pattern of gene birth and death seen in the *CYP2B6* gene cluster for bamboo
499 lemurs and *Prop. coquereli* (see Fig. 1). This is also true, but to a less pronounced degree, for
500 the *CYP2A* subfamily (see Fig. 1). In both of these cases, bamboo lemurs exhibit clade-specific
501 gene losses and *Prop. coquereli* shows an opposite signal of lineage-specific gene duplications.
502 Indeed, (Guevara et al. 2021), utilizing this same genome assembly for *Prop. coquereli,*
503 independently found that these two P450 subfamilies had been enriched in so-called “rapidly

504 evolving genes," or genes with increased *dN/dS* ratios according to PAML branch-site models
505 (Yang, 2007). Because both *CYP2A* and *CYP2B* subfamilies are involved in the metabolism of
506 xenobiotic compounds (Guengerich 2015; Orr et al. 2020), at least these two P450 subfamilies
507 stand out as candidate gene groups supporting Freeland and Janzen's (1974) prediction, that
508 specialist and generalist herbivores should differ oppositely in the diversity of their means for
509 detoxifying plant secondary metabolites. This interesting natural experiment, of comparing
510 bamboo lemurs and sifakas (or indriids in general), deserves future investigation.
511 A second line of inquiry is the contrast between *Lemur catta* and the bamboo lemurs.
512 The number of xenobiotic-metabolizing P450 genes in the *L. catta* genome was always greater
513 than or equal to those in either bamboo lemur genome. The highly varied and very seasonal
514 diet documented for *L. catta* (Jolly, 1966; LaFleur, 2012; Sauther, 1992; Sauther et al., 1999;
515 Simmen, Sauther, et al., 2006; Soma, 2006) stands out as a potential explanation for this large
516 number of detoxification mechanisms. Indeed, Sauther et al. (1999) argue that the best dietary
517 classification for *L. catta* should be "opportunistic omnivores" (p. 122) because of the breadth
518 of this species' dietary repertoire and the drastic spikes of certain foods based on their seasonal
519 availability. This species also appears to be relatively inured to plant secondary metabolites
520 based on the high tannin and alkaloid content of their foods (Gould et al., 2009; Simmen,
521 Sauther, et al., 2006) and their relatively high rejection threshold for the bitter alkaloid quinine
522 (Simmen, Peronny, et al., 2006). This constellation of information portrays a pattern of an
523 animal that is exposed to a broad array of plant secondary metabolites and, perhaps because of
524 the annual variety of their foods, exposed to them at relatively low levels.

525 While *L. catta* possesses a sizeable number of xenobiotic-metabolizing P450 genes in
526 comparison to both species of bamboo lemur (see Table S1), these genes are not the products
527 of species-specific duplications, as is plain from the phylogenetic pattern of relatedness among
528 all the genes falling into the clade of ringtail and bamboo lemurs. Instead, many of these
529 duplicated genes seem to have arisen during the internode of a lemurid common ancestor, or
530 that of a common ancestor of the *Hapalemur* and *Lemur* genera, and then subsequently lost by
531 one or both of the bamboo lemurs species included here. This result accords well with
532 experimental work on bitter taste receptors (TAS2R16), which shows that the species of
533 bamboo lemur included here (as well as *H. aureus*) show a decreased sensitivity to several
534 bitter β-glycoside compounds (Itoigawa et al., 2021). Revealingly, however, the missense
535 mutations that most altered the response of the TAS2R16 protein were separate for *Prol. simus*
536 and *Hapalemur* spp., and ancestral reconstructions of the last common ancestors for both the
537 genus *Hapalemur* and for all bamboo lemurs showed a more sensitive response to these bitter
538 compounds than any of the present-day species-specific TAS2R16 isoforms (Itoigawa et al.,
539 2021). Given this earlier work and the pattern of P450 gene birth and death presented here, a
540 generalist dietary pattern is clearly the best estimate for an ancestral ecology of the
541 *Hapalemur-Lemur* last common ancestor. Furthermore, the complex pattern of loss and
542 retention of separate P450 paralogs by *Prol. simus* and *H. griseus* seems to agree with the
543 conclusion of Itoigawa et al. (2021) that these species' varying, but in all cases intense,
544 specialization on cyanogenic bamboo may have evolved in parallel rather than what would be
545 predicted based on parsimony, namely that this specialization would have evolved in the
546 common ancestor of all extant bamboo-lemur species.

547 On a methodological note, the pattern of gene gain and loss reported here is certainly
548 not the conclusion that would be reached by using an approach where the gene-count table is
549 parsimoniously compared to an organismal phylogeny to estimate ancestral states of gene
550 counts, as with the commonly used program for studying gene-family evolution, CAFE (De Bie
551 et al., 2006). This software program takes phylogeny into account only insofar as the species
552 themselves are concerned but does not include any information about the genes' own
553 phylogenetic relatedness, and for this reason, we see it as an undesirable alternative to
554 examining a phylogeny built from the genes themselves. While CAFE is very useful in its ability
555 to be easily automated, manual examinations of gene trees, or perhaps the use of programs
556 such as Possvm (Grau-Bové & Sebé-Pedrós, 2021) that make direct use of gene trees via the
557 species-overlap method (Huerta-Cepas, et al., 2007, 2016), would yield more accurate ancestral
558 reconstructions of gene copy-number differences among species.
559 A lingering question remains as to what compounds, or families of compounds, are
560 leading to the evolutionary expansions and contractions on display in this study. However,
561 there is currently a clear disconnect in the literature that inhibits any discussion between those
562 who have precise knowledge of P450 substrate compounds (*e.g.,* Guengerich 2015) and those
563 who perform phytochemical assays on foods of wild primates (*e.g.,* Eppley et al. 2017; Thurau
564 et al. 2021; Windley et al. 2022). The disconnect arises from differing levels of scale on which
565 each of these two groups operates: pharmacologists often use precise molecules (*e.g.*,
566 benzo[a]pyrene, aflatoxin B1) in their experiments and parlance, while phytochemists and
567 primatologists speak in the more general terms of compound families (*e.g.*, alkaloids,
568 condensed tannins). This nomenclatural gulf between these two camps is a foggy one, and it

569 serves to cloud any inferences that may be gained about the functions of free-ranging, non-
570 model primate species.

571 **5. Conclusion**

572 Here, we have found that bamboo lemurs have, on average, fewer *CYP1-3* gene copies
573 than other lemurs, and this is especially true when they are viewed alongside their closest
574 relative, the ringtail lemur. Viewed another way, bamboo lemurs have, at most, the same
575 number of P450 genes per subfamily as *L. catta*, and they often have fewer such genes. The
576 retention of these specific P450 homologs was not parsimonious when compared with the
577 organismal phylogeny, and the complexity of this pattern may imply some information about
578 bamboo-lemur evolution in general, namely that their specialization on cyanogenic bamboo
579 may have evolved in parallel rather than as a sort of ecological synapomorphy early after the
580 divergence of these two lineages. Only two P450 subfamilies (*CYP2F* and *CYP2J*), both of which
581 are linked with xenobiotic metabolism in humans (Guengerich, 2015), showed a statistical
582 decrease in the intensity of selection on the CDS for the genes within those subfamilies. As in
583 previous work on the evolution of the cytochrome P450 superfamily of genes, this topic
584 provides an interesting and complex window into biological evolution. Future work that
585 includes other species of bamboo lemur (*e.g.*, *H. alaotrensis, H. aureus*) will doubtless only
586 clarify that view.

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7. Author contributions

MEC had the following roles for this study: conceptualization, data curation, formal analysis, investigation, methodology, visualization, and writing (original draft). AJT had the roles of funding acquisition, resources, software, supervision, and writing (reviewing and editing). CMB was responsible for methodology, project administration, resources, supervision, and writing (reviewing and editing).

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1007 Data Accessibility and Benefit-sharing

1008 FASTQ reads from the genomic sequencing of *Hapalemur griseus* have been deposited

1009 in the NCBI SRA (accession number XXXXX), and the alignments and trees necessary to

1010 reproduce this work are available on Dryad.

1011

1012 Table 1: Select statistics from *de novo* assembly of *Hapalemur griseus* (Beavis) using the

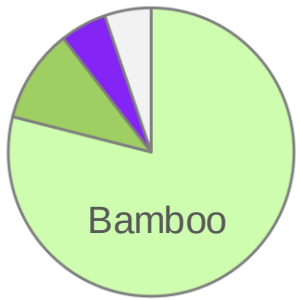
1013 Supernova assembler.

Raw coverage	Contig N_{50}	Scaffold N_{50}	Est. genome size	Primate BUSCO genes recovered (Simão et al. 2015)
69.49x	277.73 kb	20.47 Mb	2.61 Gb[1]	3927/4104 (95.7%)

1014

Specialized folivory and its effects on xenobiotic-metabolizing cytochrome P450 genes

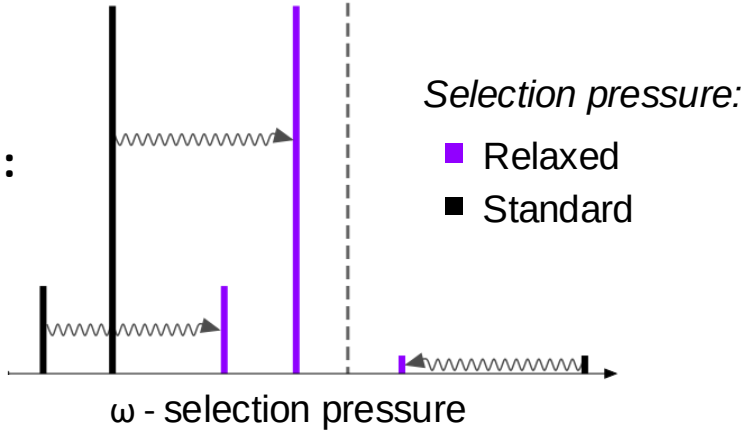
Monotonous diet largely consisting of bamboo



Relaxation of natural selection:

CYP2F

CYP2J



Evolutionary loss of *CYP* orthologs:

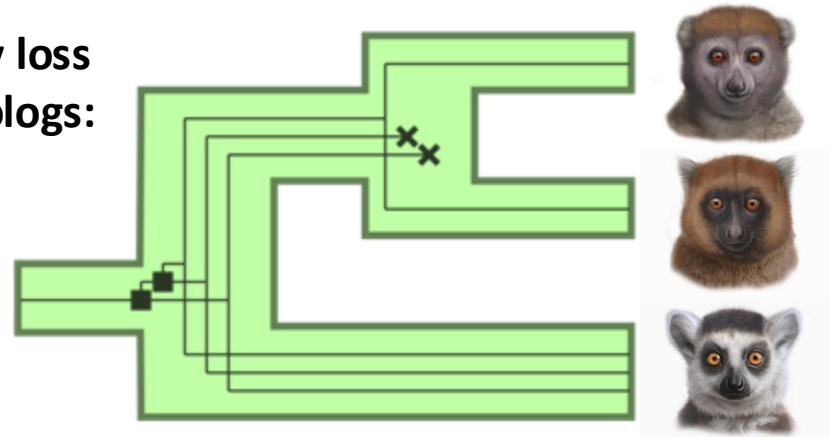
CYP2B

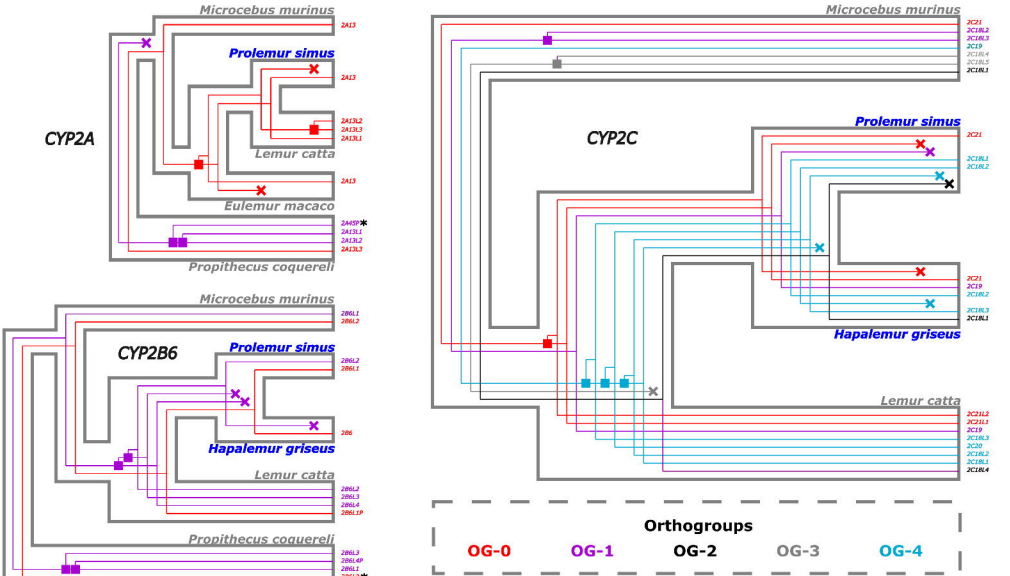
CYP2C

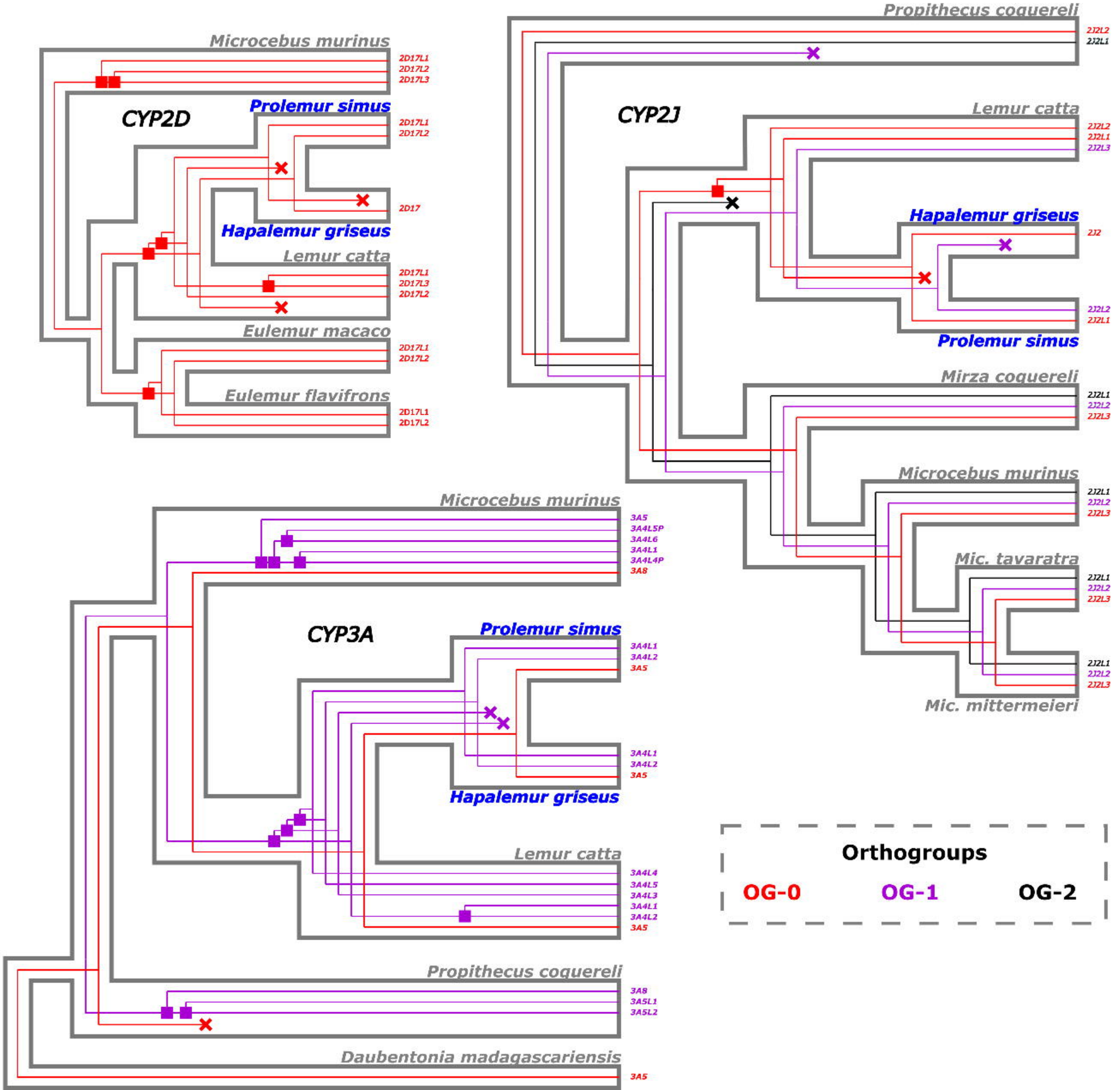
CYP2D

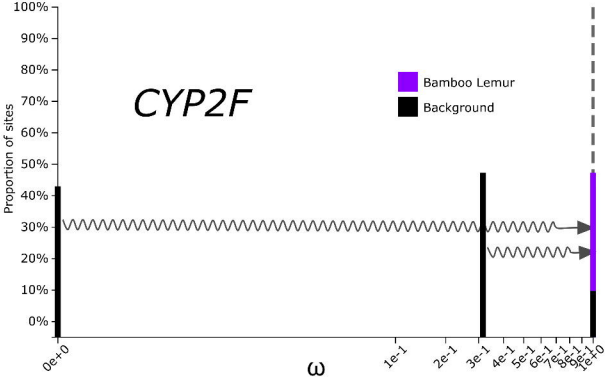
CYP2J

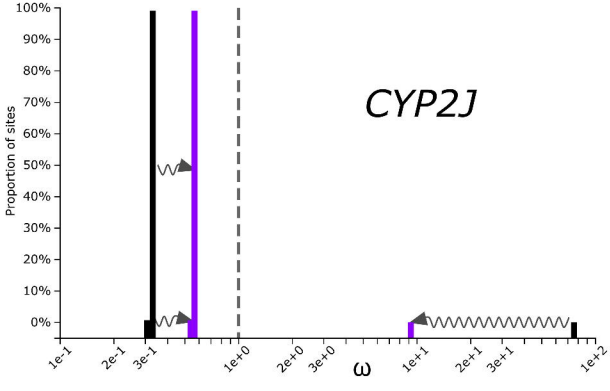
CYP3A




CYP2A
Microcebus murinus
2A13
Prolemur simus
2A13
2A13L2
2A13L3
2A13L1
Lemur catta
2A13
Eulemur macaco
2A459
2A13L1
2A13L2
2A13L3
Propithecus coquereli
CYP2B6
Microcebus murinus
2B6L1
2B6L2
Prolemur simus
2B6L2
2B6L1
2B6
Hapalemur griseus
Lemur catta
2B6L2
2B6L3
2B6L4
2B6L1P
Propithecus coquereli
2B6L3
2B6L4P
2B6L1
2B6L2
CYP2C
Microcebus murinus
2C21
2C18L2
2C18L3
2C19
2C18L4
2C18L5
2C18L1
Prolemur simus
2C21
2C18L1
2C18L2
2C21
2C19
2C18L2
2C18L3
2C18L1
Hapalemur griseus
Lemur catta
2C21L2
2C21L1
2C19
2C18L3
2C20
2C18L2
2C18L1
2C18L4
Orthogroups
OG-0
OG-1
OG-2
OG-3
OG-4


CYP2D
Microcebus murinus
2D17L1
2D17L2
2D17L3
Prolemur simus
2D17L1
2D17L2
2D17
Hapalemur griseus
Lemur catta
2D17L1
2D17L3
2D17L2
Eulemur macaco
2D17L1
2D17L2
Eulemur flavifrons
2D17L1
2D17L2
CYP2J
Propithecus coquereli
2J2L2
2J2L1
Lemur catta
2J2L2
2J2L1
2J2L3
Hapalemur griseus
2J2
2J2L2
2J2L1
Prolemur simus
Mirza coquereli
2J2L1
2J2L2
2J2L3
Microcebus murinus
2J2L1
2J2L2
2J2L3
Mic. tavaratra
2J2L1
2J2L2
2J2L3
2J2L1
2J2L2
2J2L3
Mic. mittermeieri
CYP3A
Microcebus murinus
3A5
3A4L5P
3A4L6
3A4L1
3A4L4P
3A8
Prolemur simus
3A4L1
3A4L2
3A5
3A4L1
3A4L2
3A5
Hapalemur griseus
Lemur catta
3A4L4
3A4L5
3A4L3
3A4L1
3A4L2
3A5
Propithecus coquereli
3A8
3A5L1
3A5L2
Daubentonia madagascariensis
3A5
Orthogroups
OG-0
OG-1
OG-2


CYP2F
Bamboo Lemur
Background
Proportion of sites
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0e+0
1e-1
2e-1
3e-1
4e-1
5e-1
6e-1
7e-1
8e-1
9e-1
1e+0
ω


CYP2J
Proportion of sites
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1e-1
2e-1
3e-1
1e+0
2e+0
3e+0
1e+1
2e+1
3e+1
1e+2
ω