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Lost and Found: The Third Molars of *Callimico goeldii* and the Evolution of the Callitrichine Postcanine Dentition

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1 Lost and found: the third molars of *Callimico goeldii* and
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25 **Abstract**

26 This study tests the hypothesis that the third molars of *Callimico goeldii* represent a
27 reversal in evolutionary tooth loss within the Callitrichinae. Loss of third molars is part of a suite
28 of unusual characters that has been used to unite marmosets and tamarins in a clade to the
29 exclusion of *Callimico*. However, molecular phylogenetic studies provide consistent support for
30 the hypothesis that marmosets are more closely related to *Callimico* than to tamarins, raising the
31 possibility that some or all of the features shared by marmosets and tamarins are homoplastic.
32 Here, I use the binary-state speciation and extinction (BiSSE) model and a sample of 249 extant
33 primate species to demonstrate that, given the shape of the primate phylogenetic tree and the
34 distribution of character states in extant taxa, models in which M3 loss is constrained to be
35 irreversible are much less likely than models in which reversals are allowed to occur. This result
36 provides support for the idea that the last common ancestor of *Callimico* and marmosets was
37 characterized by the two-molared phenotype. The M3s of *Callimico* therefore appear to be
38 secondarily derived rather than plesiomorphic. This conclusion may also apply to the other
39 apparently plesiomorphic traits found in *Callimico*. Hypotheses regarding the re-evolution of M3
40 in the callitrichine clade and the origin and maintenance of the two-molared phenotype are
41 discussed.

42

43

44 **Keywords:** BiSSE; Callitrichinae; Evolutionary tooth loss; Inhibitory-cascade model;

45 Platyrrhini; Reversal

46

47 **Introduction**

48 Marmosets (genus *Callithrix*) and tamarins (genera *Leontopithecus* and *Saguinus*) are
49 characterized by a number of features that are unusual in comparison to other anthropoid
50 primates, such as very small body size, reproductive twinning, clawlike nails on all digits except
51 the hallux, three-cusped maxillary molars, and reduction in the number of molar teeth from the
52 primate-typical three in each jaw quadrant to two through loss of the third molar (e.g., Ford,
53 1980; Martin, 1992).¹ Prior to the application of genetic data to the study of platyrrhine
54 systematics, these features were widely viewed as synapomorphies uniting these species within
55 the subfamily Callitrichinae to the exclusion of all other New World monkeys, including the
56 closely related species *Callimico goeldii*, a callitrichine that shares with marmosets and tamarins
57 clawlike nails and small body size but lacks the derived dental and reproductive features
58 (Rosenberger, 1977, 1981; Ford, 1980, 1986; Martin, 1992; Kay, 1994; Fig. 1 left). Since the late
59 1980s, however, molecular phylogenetic studies have built a strong case for the sister
60 relationship between *Callimico* and the marmosets that was initially proposed by Cronin and
61 Sarich (1975), with *Leontopithecus* and *Saguinus* being successively more distantly related (Fig.
62 1, center and right) (reviewed by Cortés-Ortiz, 2009; Schneider and Sampaio, 2015). Molecular
63 support for this phylogenetic hypothesis has complicated interpretations of the features shared by
64 marmosets and tamarins, suggesting either that these similarities evolved convergently three
65 times within the Callitrichinae, or that *Callimico* re-evolved singleton births, four-cusped molars,
66 and M3s and is therefore secondarily derived in these traits rather than plesiomorphic (Martin,
67 1992; Pastorini et al., 1998; Porter and Garber, 2004). A third possibility is a mixed pattern of
68 reversal and homoplasy within the clade.

69 Pastorini et al. (1998; see also Martin, 1992) argued that the combination of features
70 associated with twinning in marmosets and tamarins (i.e., simplex uterus, shared placental
71 circulation, hematopoietic chimerism), which is unique among mammals, is unlikely to have
72 evolved multiple times in callitrichines. According to these authors, a much more probable
73 scenario is reversal in *Callimico*. Oerke et al. (2002) presented evidence in support of this
74 argument, showing that *Callimico* shares at least one aspect of its reproductive biology—a long
75 delay in embryonic development—with other callitrichines that is not found in other primates
76 and which they argued may reflect descent from an ancestor that twinned. On the other hand,
77 Pastorini et al. (1998) considered homoplasy in other features shared by marmosets and tamarins
78 to be a strong possibility. This suggestion is reasonable and deserves further attention, given that
79 homoplasy appears to have been common in primate evolution (e.g., Lockwood and Fleagle,
80 1999), and perhaps pervasive in platyrrhines (Kay et al., 2008).

81 With respect to M3 evolution in callitrichines, it is notable that tooth loss has occurred
82 repeatedly over the course of primate evolutionary history and is common in other mammalian
83 lineages. The earliest-known euprimate radiations—the adapiforms and omomyiforms—
84 experienced numerous independent reductions of the premolar row from the plesiomorphic
85 pattern of four in each jaw quadrant (Rose and Bown, 1984, 1991; Bown and Rose, 1987;
86 Gingerich et al., 1991; Ni et al., 2004; Smith et al., 2006; Kirk and Williams, 2011; Rose et al.,
87 2011). Among the modern clades, loss of one or more components of the antemolar tooth classes
88 has occurred several times (incisors—*Lepilemur*, *Tarsius*, *Daubentonia*; canines—indriids,
89 *Daubentonia*; premolars—catarrhines, indriids, *Daubentonia*), and additional instances have
90 been identified in extinct members of these groups—e.g., incisor loss in the early anthropoid
91 *Parapithecus* and in the subfossil lemur *Megaladapis* (Kay and Simons, 1983; Jungers et al.,

92 2002). Outside of the Callitrichinae, the only taxon that has lost M3 is the fossil platyrrhine
93 *Xenothrix*, which does not appear to be closely related to callitrichines (Rosenberger, 1977;
94 Rosenberger et al., 1990; MacPhee and Horovitz, 2004). Agenesis of M3 also occurs in many
95 human groups at frequencies that are much higher than those observed in other catarrhines (e.g.,
96 Brothwell et al., 1963; Lavelle and Moore, 1973), suggesting that some populations may be
97 experiencing selection for M3 loss (e.g., Calcagno and Gibson, 1988; Spencer and Demes,
98 1993).

99 In contrast to these many instances of tooth loss, there is no evidence, other than the
100 phylogenetic position of *Callimico*, for the re-evolution of lost teeth or the evolution of
101 supernumerary teeth in primates.² This observation can be interpreted as support for the idea that
102 tooth loss is irreversible, suggesting that the third molar of *Callimico* is plesiomorphic (Fig. 1,
103 right). However, examples of tooth re-evolution or addition, though uncommon, do exist in other
104 mammals. Among carnivorans, the bat-eared fox (*Otocyon megalotis*) and Eurasian lynx (*Lynx*
105 *lynx*) have each regained or added molar teeth, and each is nested deep within a clade (Canidae
106 and Felidae, respectively) in which all other members possess fewer molars, leaving little doubt
107 regarding the polarity of these teeth (Guilday, 1962; Kurtén, 1963; Werdelin, 1987). Manatees
108 (*Trichechus*), the pygmy rock-wallaby (*Petrogale concinna*), and the silvery mole-rat
109 (*Heliophobius argenteocinereus*) are characterized by continuous dental replacement with
110 supernumerary molars (Gomes Rodrigues et al., 2011). Supernumerary teeth are also found in
111 some armadillos and cetaceans (Vizcaíno, 2009; Uhen, 2010). Outside of mammals, a
112 particularly spectacular case of tooth re-evolution comes from amphibians, in which there is
113 strong evidence that a species of frog reacquired mandibular teeth approximately 220 million
114 years after its clade had lost them (Wiens, 2011). The third molar of *Callimico* is therefore

115 interesting because it potentially represents the first documented instance in primates of a rare
116 phenomenon that is known to have occurred in other groups. Moreover, recognizing the dental
117 formula of *Callimico* as secondarily derived has implications with respect to understanding the
118 evolution of the two-molared phenotype in the Callitrichinae.

119 The current platyrrhine fossil record lacks the resolution needed to test the hypothesis of
120 reversal of M3 loss in *Callimico* (e.g., Rosenberger et al., 1990; Kay, 1994). An alternative
121 approach is to use the distribution of character states in extant primates to infer the number of
122 molars in the last common ancestor of callitrichines. However, the assumptions of the methods
123 typically used to reconstruct ancestral states are sensitive to factors that will bias an analysis of
124 M3 evolution in callitrichines in favor of the reversal hypothesis. In the case of maximum
125 parsimony, the most obvious problem is that this method assumes, a priori, minimal homoplasy.
126 Making such an assumption in the present context is tantamount to accepting that reversal, which
127 requires fewer evolutionary changes, is true (Fig. 1, center and right; but note that reversal
128 requires only one additional step). Maximum likelihood, on the other hand, does not necessarily
129 prefer the most parsimonious scenario and it can be used to quantitatively compare different
130 models of evolutionary change, such as those that permit reversal versus those that prohibit it
131 (Cunningham et al., 1998; Cunningham, 1999). However, this method is expected to mislead in
132 tests of irreversible evolution in cases where character states are associated with different rates of
133 net lineage diversification (i.e., speciation rate minus extinction rate), such that lineages
134 characterized by one state tend to diversify and become more common in a clade over time, thus
135 biasing ancestral-state reconstructions (Goldberg and Igić, 2008; Wiens, 2011; see also
136 Maddison, 2006).

137 Following the recommendations of Goldberg and Igić (2008), several recent studies of
138 evolutionary reversal have applied a likelihood-based model developed by Maddison et al.
139 (2007) called BiSSE (for binary-state speciation and extinction) to a range of taxa and characters
140 (e.g., Kohlsdorf et al., 2010; Lynch and Wagner, 2010; Wiens, 2011; Scott et al., 2012; Bonett et
141 al., 2014; Pyron and Burbrink, 2014). The BiSSE model was initially introduced as a way of
142 examining whether character states influence speciation and extinction rates (Maddison et al.,
143 2007). Because BiSSE estimates speciation and extinction rates for taxa with different character
144 states, it can address the problems associated with state-dependent diversification in analyses that
145 rely on inferred sequences of character evolution (Goldberg and Igić, 2008). In other words, the
146 BiSSE model can be used to reconstruct character-state evolution using maximum likelihood
147 along with information about rates of speciation and extinction, thereby accounting for the
148 effects of state-dependent diversification—when present—on such reconstructions. In this study,
149 I use this approach to examine M3 evolution in the Callitrichinae and test hypothesis that the
150 third molar of *Callimico* is an evolutionary reversal.

151

152 **Methods**

153 I obtained a consensus molecular phylogeny for 249 extant primate species, representing
154 all major taxonomic groups, along with a Bayesian block of 1,000 trees from the *10kTrees*
155 website using the Wilson and Reeder taxonomy and with branch lengths set proportional to time
156 (Arnold et al., 2010). As many primate species as possible were included in the analysis because
157 the BiSSE model requires a large number of taxa to achieve sufficient statistical power to
158 distinguish among models (Davis et al., 2013). This limitation precludes reliable inference using
159 a sample restricted to platyrrhines, which were represented in the sample by only 60 species, a

160 small number by BiSSE standards (Davis et al., 2013). However, a separate platyrrhine-only
161 analysis was conducted for heuristic purposes. Analyses performed on the consensus tree were
162 repeated on each of the trees in the Bayesian block to account for uncertainty in the topology of
163 the consensus tree. These trees are available as text files in the Supplementary Online Material.
164 Note that this study assumes that the molecular phylogeny is accurate with respect to the
165 *Callimico-Callithrix* sister relationship. However, although molecular phylogenetic studies are
166 consistent in their support for this relationship, some researchers view this evidence with
167 skepticism (e.g., Ivan-Perez and Rosenberger, 2014).

168 Species sampling was determined by availability in the *10kTrees* database. In the case of
169 species represented by multiple subspecies, I arbitrarily selected one and excluded the others.
170 This sample represents 66.4% of the species-level taxa listed by Groves (2005). The use of an
171 incomplete phylogeny can be problematic for BiSSE, but a correction for missing taxa is
172 available (FitzJohn et al., 2009) and was used here. This correction requires that sampling of the
173 phylogenetic tree be random (FitzJohn et al. 2009). Following Matthews et al. (2011), I used a
174 *G*-test of proportions to confirm that this assumption was not violated for taxonomic sampling at
175 the generic level ($p = 0.1517$) and with respect to the proportions of two- and three-molared
176 species present in the sample ($p = 0.5054$).

177 The BiSSE model computes the likelihood of a phylogenetic tree and the character states
178 of the tip taxa given a particular model of character evolution with up to six parameters: a
179 speciation rate under each state of a binary character (λ_0 and λ_1), an extinction rate under each
180 state (μ_0 and μ_1), and transition rates from each character state to the other (q_{01} and q_{10})
181 (Maddison et al., 2007). The six-parameter model ($k = 6$), hereafter referred to as the
182 *unconstrained model*, estimates all parameters using maximum likelihood. This model can be

183 constrained by fixing one (or more) of the parameters to equal a certain value, thus creating a
184 new model of character evolution with fewer estimated parameters ($k < 6$) that can be compared
185 to the unconstrained model to evaluate which provides a better fit to the data.

186 The initial step in the analysis was to compare the unconstrained model to one in which
187 net diversification rates for three- and two-molared lineages were set equal to each other (i.e., λ_3
188 $= \lambda_2$ and $\mu_3 = \mu_2$, so that $\lambda_3 - \mu_3 = \lambda_2 - \mu_2$) to determine whether diversification is state-
189 dependent. In other words, do lineages with two molars diversify at a different rate than lineages
190 with three molars? This model will be referred to as the *equal-diversification model* and it has k
191 $= 4$ estimated parameters ($\lambda_3, \mu_3, q_{32}, q_{23}$) and two fixed parameters (λ_2, μ_2). Greater support for
192 the equal-diversification model would indicate that diversification rates are not state-dependent,
193 making the BiSSE model unnecessary. In such a situation, a simpler likelihood model, such as
194 the AsymmMk option in Mesquite (Maddison and Maddison, 2011), which only has $k = 2$
195 estimated parameters (the two state-to-state transition rates), would be preferred for evaluating
196 the reversibility of M3 loss, because it may provide more power (Goldberg and Igić, 2008).

197 The second step in the analysis was to evaluate the performance of a model in which
198 transitions from two molars to three were not allowed. In this *irreversible model* (whether BiSSE
199 or AsymmMk), the transition rate from two molars to three molars was set equal to zero ($q_{23} =$
200 0), whereas the transition rate for three to two molars (q_{32}) was free to vary. The irreversible
201 model thus had one fixed parameter and either $k = 5$ estimated parameters (BiSSE) or $k = 1$
202 (AsymmMk), depending on the level of support for state-dependent diversification.

203 All likelihood calculations and parameter estimations were made using the diversitree
204 package (FitzJohn, 2011) for R (R Core Team, 2014). Because the different models of character
205 evolution vary in the number of parameters they estimate, I used Akaike's information criterion

206 with adjustment for sample size (AIC_c) to compare them (Akaike, 1973; Burnham and
207 Anderson, 2002; Johnson and Omland, 2004). A model's AIC value is its log-likelihood value
208 penalized for the number of parameters the model estimates: $AIC = -2 \ln(L) + 2k$, where L is the
209 likelihood and k is the number of parameters (Akaike, 1973). The adjustment for sample size is:
210 $AIC_c = AIC + [2k(k + 1) / (n - k - 1)]$, where n is the sample size, in this case 249 (Burnham and
211 Anderson, 2002). Under this approach, the model with the lowest AIC_c value is considered the
212 best supported. Support for the other models is evaluated by subtracting the AIC_c for the best-
213 supported model from the AIC_c values for each of the competing models, producing a ΔAIC_c
214 value for each model. Typically, ΔAIC_c values between 0 and 2 indicate substantial support for a
215 competing model, values between 4 and 7 indicate considerably less support, and values greater
216 than 10 indicate essentially no support (Burnham and Anderson, 2002, p. 70). Model support can
217 also be characterized using relative likelihoods, computed as $\exp(-0.5 \times \Delta AIC_c)$ for each model,
218 which can then be used to quantify the relative level of support for one model versus another by
219 creating an evidence ratio: the relative likelihood of the better-supported model divided by the
220 relative likelihood of a competing model (Burnham and Anderson, 2002; Johnson and Omland,
221 2004).

222

223 **Results**

224 Table 1 presents the results of the model comparisons for the consensus tree. For the all-
225 primate analysis, the equal-diversification model has a lower AIC_c value than the unconstrained
226 model, but the ΔAIC_c value for the unconstrained model is 1.79, indicating that neither of these
227 models is strongly preferred over the other. State-dependent diversification, therefore, cannot be
228 ruled out, justifying the use of BiSSE with respect to testing for reversal in molar loss in the

229 Callitrichinae. The parameter estimates for the unconstrained model suggest that, if
230 diversification is in fact state-dependent, then lineages with two molars have a higher speciation
231 rate and lower extinction rate—and thus tend to diversify at a greater rate (approximately 2.3
232 times faster)—than lineages with three molars. It is important to keep in mind that variation in
233 molar number is not necessarily the direct cause of differences in diversification rates; some
234 other variable may mediate such a relationship, given that two-molared callitrichines differ from
235 other primates in a number of ways.

236 The irreversible model performed poorly ($\Delta AIC_c > 10$) relative to the two models that
237 allowed transitions from two to three molars in the all-primate analysis. The evidence ratios for
238 the equal-diversification and unconstrained models versus the irreversible model are 457 and
239 187, respectively, indicating that reversibility is well over a hundred times more likely than
240 irreversibility. Because the equal-diversification model is not unambiguously favored over the
241 unconstrained model, I examined a second irreversible BiSSE model in which net diversification
242 rates for two- and three-molared lineages were set equal to each other. This model is identified as
243 the *irreversible, equal-diversification model* ($k = 3$) in Table 1. This model performed slightly
244 better than the $k = 5$ irreversible model but still has a ΔAIC_c value close to 10 versus the
245 (reversible) equal-diversification model, with the latter model being 145 times more likely than
246 the irreversible, equal-diversification model.

247 The platyrrhine-only sample recapitulates the results of the all-primate analysis to some
248 extent. As in the all-primate analysis, the equal-diversification model that allowed reversal had
249 the lowest AIC_c value, but the irreversible, equal-diversification model performed well ($\Delta AIC_c =$
250 2.14; relative likelihood = 0.3437), indicating that it cannot be ruled out. This ambiguity is not
251 surprising; as noted in the methods section, the number of species included in the platyrrhine-

252 only analysis is relatively small ($n = 60$), limiting its power to distinguish among models. These
253 results should therefore be interpreted with caution.

254 Analysis of the all-primate Bayesian tree block confirms that the results obtained using
255 the all-primate consensus tree are robust to phylogenetic uncertainty. Figure 2 shows the
256 distribution of ΔAIC_c values generated from the tree block for the irreversible, equal-
257 diversification model versus the (reversible) equal-diversification model. The values range from
258 6.20 to 17.71, with 95% of the values falling between 9.06 and 12.25. The all-primate BiSSE
259 analysis is therefore unequivocal in supporting the hypothesis that reversion from two molars to
260 three occurred in the lineage leading to *Callimico goeldii*.

261

262 **Discussion**

263 *Re-evolution of M3 in the Callitrichinae*

264 The results of this study support the hypothesis that the last common ancestor of
265 *Callimico goeldii*, marmosets, and tamarins was characterized by the two-molared phenotype,
266 indicating that the third molars of *Callimico* are secondarily derived within the Callitrichinae
267 (Fig. 1, center). The high level of support for reversal of M3 loss provided by the all-primate
268 analysis is somewhat unexpected in light of the fact that the irreversible scenario is only slightly
269 less parsimonious than the reversible one (Fig. 1, center versus right). The platyrrhine-only
270 analysis also preferred reversal, but did not decisively reject irreversibility. However, the latter
271 result should not be interpreted as contradicting the results of the all-primate analysis; the small
272 number of species included in the platyrrhine-only sample ($n = 60$) severely limits the power of
273 the BiSSE analysis to detect asymmetries in model parameters, such as character-state transition
274 rates (Davis et al., 2013).

275 It should not be surprising that some callitrichine lineages retained the capacity to re-
276 evolve third molars after their initial loss, given that teeth are meristic structures and that
277 supernumerary molars occasionally develop in some marmoset and tamarin individuals
278 (Hershkovitz, 1970; Ford, 1980). Experimental studies conducted on mice indicate that the
279 developmental pathways used to generate M3s are also involved in producing the first and
280 second molars (e.g., Tucker and Sharpe, 1999; Stock, 2001; Kavanagh et al., 2007; Tummers and
281 Thesleff, 2009). Because of these pleiotropic links, it is likely that the genetic architecture of M3
282 is protected by selection from loss-of-function mutations even when not expressed
283 phenotypically (Marshall et al., 1994; Collin and Miglietta, 2008).³ A simple genetic change may
284 be all that is required to reactivate the M3 pathway, as has been demonstrated experimentally for
285 pharyngeal teeth in zebrafish (Aigler et al., 2014). The results of this study suggest that
286 *Callimico* realized this potential. Marshall et al. (1994) argued that such reversals may be
287 common early in the evolutionary history of a clade, especially during periods of rapid
288 diversification, with lost traits reappearing in multiple lineages and then disappearing again, a
289 phenomenon they termed *flickering*. An example they provided was the repeated loss and regain
290 of metamorphosis in a clade of ambystomatid salamanders. Although the prevalence of this
291 phenomenon is unclear, the possibility that M3 reacquisition occurred in other callitrichine
292 lineages should be kept in mind when interpreting the platyrrhine fossil record.

293 A potential mechanism for M3 re-evolution in *Callimico* comes from the inhibitory-
294 cascade model of molar development proposed by Kavanagh et al. (2007). According to this
295 model, molar proportions and number are governed by the relative strengths of activator signals
296 secreted by the surrounding mesenchyme and inhibitor signals from earlier-developing molars,
297 such that a lower activator-inhibitor ratio (i.e., relatively greater inhibition) results in small or

298 absent distal molars, whereas a higher ratio (i.e., relatively lower inhibition) results in large distal
299 molars or supernumerary teeth (Kavanagh et al., 2007). This model makes specific predictions
300 about molar proportions. For example, it predicts that M_2 area will constitute approximately one-
301 third of molar occlusal area in species with three mandibular molars, and that M_3 will not be
302 expressed when M_2 is approximately half the size of M_1 or smaller (Kavanagh et al., 2007).

303 The inhibitory-cascade model has been applied to a variety of mammalian clades
304 (Renvoisé et al., 2009; Wilson et al., 2012; LaBonne et al., 2012; Asahara, 2013; Bernal et al.,
305 2013; Halliday and Goswami, 2013). These studies provide support for the hypothesis that
306 variation in the activator-inhibitor ratio has played a major role in the diversification of molar
307 proportions and number in mammals, but they also identified deviations from the model's
308 predictions, suggesting that there are differences among groups in how the inhibitory cascade is
309 expressed and in the relative importance of other factors. Particularly relevant in the present
310 context is the analysis conducted by Bernal et al. (2013) on New World monkeys. These authors
311 found that three-molared platyrrhines conform to the interspecific predictions of the model, but
312 that marmosets and tamarins have M_2 s that are much larger (66–76% of M_1 size) than the
313 threshold for M_3 loss predicted by the model (50% of M_1 size) (Bernal et al., 2013). Despite this
314 deviation from the model's predictions, marmosets and tamarins still have smaller M_2 s relative
315 to M_1 size than any other platyrrhine, which suggests that the inhibitory cascade contributed at
316 least partially to M_3 loss in callitrichines.

317 Moreover, it is notable that, with an M_2 area that is 86% the size of M_1 , the molar
318 proportions of *Callimico* are very similar to those of *Saimiri* and *Cebus* (Bernal et al., 2013).
319 Assuming that *Callimico* evolved from an ancestor that resembled marmosets and tamarins in its
320 molar proportions, one hypothesis for M_3 re-evolution in *Callimico* is that it was a by-product of

321 selection for a larger M₂, which was achieved through a reduction in the strength of inhibitor
322 signals, leading to the reappearance of M₃. A similar argument was made by Werdelin (1987)
323 with regard to what may be the closest living analog for *Callimico*—the Eurasian lynx, which
324 differs from all other extant Felidae in that about 10% of individuals possess two mandibular
325 molars rather than one (Kurtén, 1963). Werdelin (1987) hypothesized that M₂ re-evolution in the
326 Eurasian lynx was a consequence of selection to lengthen M₁: “I suggest, however, that the
327 selection pressure for an enlarged molar region proceeded further in *L. lynx* than an enlargement
328 of M₁ alone could accommodate. Through linkage with M₁ in the molarization field, M₂ may in
329 some individuals have reached the level of phenotypic expression, and these two factors taken
330 together would lead to selection for the presence of M₂” (pp. 262–263). The M₃s of *Callimico*
331 may represent a similar phenomenon.

332 While the inhibitory-cascade model provides a potential explanation for how *Callimico*
333 reacquired its M₃s, it does not explain why. One possibility is selection for an expanded molar
334 row related to this species’ heavy reliance on fungus as a food source (Porter, 2001; Porter et al.,
335 2007). Mycophagy has also been invoked as an explanation for the well-developed molar
336 shearing crests of *Callimico*, which may reflect the need to efficiently reduce fungus into tiny
337 particles to increase the relative surface area exposed to chemical digestion in the gut (Porter and
338 Garber, 2004; Hanson et al., 2006). If this argument is correct, then expanded molar occlusal
339 area, and probably four-cusped maxillary molars, may also be part of this hypothesized
340 adaptation. It is important to emphasize two points: First, data on dental adaptations for
341 mycophagy are lacking and therefore the link between dental form and mycophagy in *Callimico*
342 is speculative and requires testing. Second, the M₃s of *Callimico* are still very small, which
343 raises questions about their adaptive importance. However, the adaptationist hypothesis proposed

344 above does not hinge on the M3s being selectively advantageous; rather, as noted in the
345 discussion of the inhibitory-cascade model, the M3s may simply be a by-product of selection for
346 enlarged M2s.

347

348 *The origin and maintenance of the two-molared phenotype*

349 Support for the idea that M3 re-evolved at least once in the callitrichine radiation raises
350 questions about why it was lost in the first place and why reversal has not occurred in other
351 extant callitrichines. Ford (1980) linked M3 loss—along with the other derived features of
352 marmosets and tamarins—with phyletic dwarfing, hypothesizing that a rapid reduction in overall
353 body size, and particularly jaw size, combined with a less rapid reduction of the postcanine
354 dentition resulted in oversized molar teeth relative to the space available for them in the reduced
355 jaws, leaving no room for M3 to develop. Spatial constraints have been implicated in other
356 instances of tooth loss in primates. For example, Godfrey et al. (2002) suggested that canine and
357 premolar loss in the Indriidae and their subfossil relatives was a consequence of molar
358 megadonty and a rapid dental developmental schedule, which imposed severe limitations on
359 later-developing teeth. The densely sampled fossil record of early Eocene anaptomorphine
360 omomyids from the Bighorn Basin of North America provides perhaps the best-documented link
361 between tooth loss and concomitant changes in other aspects of the masticatory system. In this
362 case, the stratigraphic sequence records the loss of P₂ in a single evolving lineage, which was
363 divided by Rose and Bown (1984) into two chronospecies (*Tetonius matthewi* and
364 *Pseudotetonius ambiguus*) and a series of morphologically intermediate stages (see also Bown
365 and Rose, 1987). Along with loss of P₂, this lineage is characterized by progressive enlargement
366 of the mandibular central incisors, reduction and mesiodistal compression of the remaining

367 antemolar teeth, and anteroposterior shortening of the mandible (Rose and Bown, 1984; Bown
368 and Rose, 1987).

369 Establishing such connections in callitrichines has proven difficult. Marmosets and
370 tamarins, as a group, do not have large postcanine teeth relative to body mass in comparison to
371 other platyrrhines (Martin, 1992; Plavcan and Gomez, 1993a,b), and in fact their postcanine
372 tooth rows are among the shortest relative to the size of the facial skeleton (Scott, 2012; Fig. 3).
373 Moreover, as shown in Figure 3, the maxillary postcanine row of *Callimico* is much longer than
374 those of other callitrichines that are similar to *Callimico* in palate length (species of *Saguinus*) or
375 larger (*Leontopithecus*). This observation, along with the phylogenetic position of *Callimico* and
376 the evidence presented here that the third molars of *Callimico* are secondarily derived, suggests
377 that facial size is not a constraint on the length of the postcanine row in two-molared species. As
378 noted by Martin (1992) and Plavcan and Gomez (1993a,b), interpretations of the relative size of
379 the postcanine dentition of extant callitrichines vis-à-vis the dwarfing hypothesis are complicated
380 by the fact that dwarfing occurred several million years ago, allowing time for selection to adjust
381 the initially oversized postcanine teeth to better fit their functional environment. Nevertheless,
382 the current distribution of relative postcanine size in this clade provides no support for the
383 hypothesis that rapid phyletic dwarfing led to M3 loss.

384 The idea that dwarfing would have resulted in oversized postcanine teeth in callitrichines
385 has also been challenged by recent studies of life history in this group (Marroig and Cheverud,
386 2009; Montgomery and Mundy, 2013). Relatively large postcanine teeth are expected in
387 evolutionarily dwarfed lineages when body-size reduction occurs by early cessation of postnatal
388 growth (i.e., progenesis), such that early-developing structures, such as the dentition, are less
389 affected than later-developing structures, such as adult body size (Shea, 1983; Shea and Gomez,

390 1988). Dwarfism in callitrichines, in contrast, appears to have been achieved primarily through
391 reduction in prenatal growth rates, which should affect tooth size as much as adult body size
392 (Marroig and Cheverud, 2009; Montgomery and Mundy, 2013). Furthermore, in the analysis
393 conducted by Montgomery and Mundy (2013), the smallest callitrichine, the pygmy marmoset
394 (*Callithrix pygmaea*), stood out in two respects: First, it was the only lineage in which
395 progenesis, in combination with reduced prenatal growth rates, was implicated in the process of
396 phyletic dwarfing. Second, it was identified as having experienced the highest rates of dwarfing
397 in the evolutionary history of the Callitrichinae. It is therefore notable that *C. pygmaea* has not
398 lost additional teeth nor does it have relatively large postcanine teeth in comparison to other
399 callitrichines (Kanazawa and Rosenberger, 1988; Plavcan and Gomez, 1993a,b; Fig. 3).

400 An alternative to the dwarfing hypothesis was proposed by Rosenberger (1992) and
401 Spencer (1999), who both suggested that M3 loss was related to an adaptive shift involving
402 greater emphasis on food harvesting or processing using the antemolar dentition. Spencer (1999),
403 in particular, argued that when such a shift involves an increase in the mechanical advantage of
404 the jaw adductors to increase the efficiency of force production at anterior bite points, M3
405 function may in some cases be compromised, resulting in its reduction or loss. The basis for this
406 hypothesis comes from a model of masticatory biomechanics developed by Greaves (1978),
407 which specifies that the resultant force of the jaw adductors must lie within a triangle of support
408 formed by the left and right temporomandibular joints and the bite point. If the resultant falls
409 outside of the triangle, particularly during forceful biting, then the working-side
410 temporomandibular joint will be subjected to potentially injurious tensile forces that it is poorly
411 designed to resist (Greaves, 1978; Spencer, 1995, 1999). Thus, according to this constrained
412 lever model, the resultant force should intersect the occlusal plane posterior to the tooth rows;

413 Greaves (1978) assumed that the anterior limit was located just posterior to the distal margins of
414 the molar row, but Spencer (1995, 1999) presented data indicating that it is located more
415 posteriorly in anthropoid primates, which he interpreted as evidence for the existence of a safety
416 factor, reflecting the importance of protecting the temporomandibular joint from tensile reaction
417 forces.

418 Primate lineages that are known to regularly process hard or tough objects using their
419 incisors, canines, or premolars are characterized by reduction of M3 (Spencer, 1995, 2003;
420 Wright, 2005), anteroposterior shortening of the molar row (Singleton, 2004), or a relatively high
421 frequency of M3 agenesis (Spencer and Demes, 1993). Within the framework of the constrained
422 lever model, these morphologies are thought to be responses to the jaw-muscle resultant force
423 encroaching on the distal tooth rows, either by anterior migration of the jaw adductors or
424 posterior migration of the dentition (Spencer and Demes, 1993; Spencer, 1995, 1999, 2003;
425 Singleton, 2004; Wright, 2005). Such configurational changes increase the mechanical advantage
426 of the jaw adductors, but they also increase the likelihood that the resultant force will fall outside
427 of the triangle of support or close to its edge when biting on the distal molars. Spencer (1995,
428 1999, 2003; Spencer and Demes, 1993) argued that, in this situation, teeth that are too close to
429 the muscle resultant force will exhibit evidence of functional impairment in the form of a
430 reduction in size or loss, reflecting the need to avoid loading the temporomandibular joint in
431 tension. Loss of molar occlusal area is therefore an expected compromise of evolutionary
432 specialization for intensive use of the antemolar dentition in food harvesting or processing
433 (Spencer, 1995, 1999).

434 The extent to which the constrained lever model explains M3 loss in callitrichines is
435 unclear. Spencer (1995, 1999) found that the relative anteroposterior positions at which the force

436 vectors of the jaw adductors intersect the occlusal plane between the distal molars and the
437 temporomandibular joints are similar in marmosets, tamarins, and three-molared anthropoids.
438 This similarity means that the jaw-muscle resultant force is relatively closer to M2, M1, P4, etc.,
439 in two-molared species than in three-molared species, which suggests that the jaw adductors of
440 two-molared species have greater mechanical advantage than those of three-molared species at
441 homologous teeth (e.g., at M1). Note that this conclusion is tentative, given that the lengths of
442 the lever arms of the jaw adductors depend on other factors, such as muscle orientation (Spencer,
443 1995).

444 It is important to bear in mind, however, that the possibility that M3 loss occurred in
445 callitrichines for some other reason, and was then followed by an increase in jaw-muscle
446 mechanical advantage, cannot be excluded in the absence of a well-supported adaptive
447 explanation linking selection for greater jaw-adductor mechanical advantage at anterior bite
448 points with feeding behavior in marmosets and tamarins. Marmosets are well-known for gouging
449 trees with their incisors and canines to obtain exudates, but this behavior and its associated suite
450 of anatomical traits, absent in other callitrichines, are viewed as apomorphic within the clade
451 (e.g., Coimbra-Filho and Mittermeier, 1976; Sussman and Kinzey, 1984; Garber, 1992;
452 Rosenberger, 1992; Vinyard et al., 2009). The conclusion that *Callimico* is descended from a
453 two-molared ancestor supports the idea that marmosets and tamarins did not lose their third
454 molars independently, indicating that the two-molared phenotype did not evolve together with
455 gouging and its attendant dental adaptations in the last common ancestor of marmosets.

456 Rosenberger (1992) argued that callitrichine premolar morphology reflects an adaptive
457 emphasis on puncturing and crushing insects using these teeth. If Rosenberger's interpretation is
458 correct, then this feeding behavior may have been associated with selection for greater jaw-

459 muscle mechanical advantage to either increase bite-force magnitudes or the efficiency of bite-
460 force production at the premolars. In this scenario, *Callimico* represents an adaptive shift back to
461 emphasis on molar processing, perhaps related to mycophagy. An obvious problem with linking
462 molar loss with insectivory in callitrichines is that other insectivorous primates do not tend to
463 have relatively small molar rows in comparison to species in other dietary categories and some
464 have relatively very large molar rows (Vinyard and Hanna, 2005; Scott, 2012). Thus, this idea
465 remains speculative. Comprehensive tests of this hypothesis for the origin of the two-molared
466 phenotype will come from detailed studies of how callitrichines differ from other insectivorous
467 primates in feeding behavior, and from an expanded callitrichine fossil record documenting the
468 degree to which reduction and loss of M3 was correlated with changes in the positions of the jaw
469 adductors.

470 On the other hand, the hypothesis that selection for increased jaw-muscle mechanical
471 advantage at anterior bite points is currently counterbalancing selection for increased molar
472 occlusal area in marmosets and tamarins, thus constraining M3 re-evolution in these lineages
473 (i.e., maintaining the two-molared phenotype), is potentially falsifiable using craniodental data
474 from extant taxa. Spencer (1995, 1999) did not include *Callimico* in his study, but the
475 constrained lever model predicts that this species will resemble three-molared anthropoids and
476 differ from marmosets and tamarins in jaw-muscle mechanical advantage at homologous teeth.
477 Failure to match this expectation would result in rejection of this hypothesis. It is worth noting
478 that there is variation in relative postcanine size among marmosets and tamarins that may be
479 relevant to testing this hypothesis. For example, some species of *Leontopithecus* have relatively
480 large postcanine teeth (Kanazawa and Rosenberger, 1988; Plavcan and Gomez, 1993a,b; Scott,
481 2012; Fig. 3), which may be associated with reduced jaw-muscle mechanical advantage relative

482 to other two-molared taxa. Also potentially relevant is the observation that some exudativorous
483 strepsirrhines have reduced M3s (Burrows and Nash, 2010) or relatively very small molar rows
484 (Scott, 2012).

485

486 *Broader implications of reversal of M3 loss*

487 Although this study has focused on a specific primate clade, the issues raised in the
488 preceding sections have implications for primates more generally. As discussed in the
489 introduction, the primate premolar row has experienced more losses than any of the other tooth
490 classes, both in terms of the number of teeth lost in some lineages and in the number of
491 independent evolutionary events. This portion of the dentition is therefore the most likely to
492 provide additional instances of tooth loss and regain in primates. There is, however, currently no
493 evidence of premolar re-evolution in any primate group. On one hand, this observation is not
494 surprising, given that reversal in tooth loss is a rare phenomenon. It may simply be the case that
495 premolar reacquisition has not occurred because it has not been advantageous from an adaptive
496 standpoint. On the other hand, given that there are two documented cases of M3 loss in primates
497 (in callitrichines and the fossil platyrrhine *Xenothrix*) and fairly strong support for one
498 reacquisition, it is worth asking whether some types of teeth are more likely to be reacquired
499 than others.

500 An obvious difference between M3 and other teeth, including the premolars, is that the
501 latter are situated within the dental arcade, surrounded by other teeth, whereas third molars are at
502 the distal end of the tooth row, bordered only mesially by the second molars. With the possible
503 exception of the indriids (Godrey et al., 2002), premolar loss in primates has proceeded from
504 mesial to distal. The mesial mandibular premolars of most extant primates have taken on

505 different and more specialized roles than the other premolars. In many strepsirrhines, the
506 mandibular mesial premolar is caniniform, having assumed the functional role previously played
507 by the mandibular canine, which has been incorporated into the tooth comb. In anthropoid
508 primates, the mesial mandibular premolar of most species is a key component of the canine-
509 honing complex. In these cases, the re-evolution of lost premolars may be too functionally
510 disruptive, resulting in strong selection against it. In contrast, adding another tooth to the end of
511 the postcanine row, as appears to have occurred in *Callimico*, may be evolutionarily less
512 difficult.

513 A recent study conducted by Seiffert et al. (2010) indicates that assumptions about the
514 reversibility of premolar loss may in some cases have an important effect on phylogenetic
515 hypotheses involving Eocene primates. In their examination of the 37-million-year-old primate
516 *Nosmips aenigmaticus*, Seiffert et al. (2010) found that cladistic analyses that permitted reversal
517 in premolar loss consistently identified this species as a stem anthropoid, whereas those that
518 specified irreversibility placed it within the Strepsirrhini, either as a sister of the crown clade or
519 nested within the adapiforms. Seiffert et al. (2010) expressed skepticism regarding the
520 connection to stem anthropoids, partly because they preferred the irreversibility assumption.
521 Given the current lack of evidence for reversals in premolar loss in primates and the rarity of
522 reversals in tooth loss in mammals in general, it is reasonable to consider such evolutionary
523 transformations unlikely. However, if functional specialization is a constraint on premolar re-
524 evolution, as hypothesized above, then this early stage of primate evolutionary history—prior to
525 functional differentiation and specialization within the premolar row—may be where primate
526 paleontologists are most likely to find reversals in premolar loss.

527

528 **Conclusions**

529 This study supports the hypothesis that the third molars of *Callimico goeldii* are an
530 evolutionary reversal in tooth loss, the first such instance identified in primates. Together with
531 evidence that *Callimico* shares certain aspects of its reproductive biology with marmosets and
532 tamarins (Oerke et al., 2002) and arguments against the independent evolution of chimeric
533 twinning in *Callithrix*, *Leontopithecus*, and *Saguinus* (Martin, 1992; Pastorini et al., 1998), the
534 results presented here suggest that the unusual features shared by marmosets and tamarins were
535 present in the last common ancestor of callitrichines, and that the apparently plesiomorphic traits
536 of *Callimico* are therefore secondarily derived. The inhibitory-cascade model proposed by
537 Kavanagh et al. (2007), which links molar gains and losses to shifts in molar proportions,
538 provides a plausible developmental mechanism for M3 re-evolution. In *Callimico*, such changes
539 may be related to the need to expand molar occlusal area in conjunction with mycophagy, though
540 this hypothesis is speculative. The possibility that other instances of M3 reacquisition occurred
541 during the course of callitrichine evolutionary history, particularly during the initial
542 diversification, should be kept in mind when interpreting the platyrrhine fossil record. This
543 caveat also applies to earlier parts of the primate fossil record, specifically in cases where
544 premolar number is used to evaluate phylogenetic hypotheses.

545 The origin of the two-molared phenotype in the ancestral callitrichine remains poorly
546 understood. The hypothesis linking M3 loss in callitrichines to spatial constraints imposed by
547 rapid evolutionary dwarfing receives no support from extant species, especially given that the
548 postcanine tooth row of *Callimico* is much longer relative to the size of the facial skeleton than
549 are the postcanine rows of tamarins with faces that are similar in size to that of *Callimico* or
550 larger. An alternative explanation is that the two-molared phenotype arose, and is maintained in

551 marmosets and tamarins, as a by-product of selection for increased jaw-muscle mechanical
552 advantage related to intensive use of the antemolar teeth in harvesting or processing food. This
553 hypothesis raises a number of questions about callitrichine feeding behavior and craniodental
554 morphology that can potentially be addressed through comparisons between *Callimico* and the
555 two-molared species.

556

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560 helpful comments that improved the manuscript.

561

562 **Footnotes**

563 ¹ Recent taxonomic treatments of the marmosets recognize up to four genera: *Callithrix sensu*
564 *stricto*, *Cebuella*, *Mico*, and *Callibella* (e.g., Rylands et al., 2009). For simplicity, and because
565 these genera constitute a monophyletic group with respect to tamarins and *Callimico*, I follow
566 Groves (2005) and group them all in *Callithrix*.

567

568 ² Supernumerary teeth occur at very low frequencies in many primate species, including
569 callitrichines, and are usually considered developmental anomalies (e.g., Hershkovitz, 1970;
570 Lavelle and Moore, 1973; Ford, 1980; Jungers and Gingerich, 1980; Swindler, 2002; Ackermann
571 et al., 2006).

572

573 ³ In this context, it is worth noting that as long as the underlying developmental program
574 remained intact following loss of phenotypic expression, instances of reversal of M3 loss in
575 callitrichine lineages would not represent a violation of Dollo's law, which states that complex
576 structures, once lost, cannot be regained in their original form (e.g., Gould, 1970).

577

578

579

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- 825

826 **Figure captions**

827 **Fig. 1.** Models of M3 evolution in the Callitrichinae. **Left:** Evolutionary relationships inferred
 828 from morphology, with *Callimico* as the sister taxon of marmosets and tamarins; the two-
 829 molared phenotype evolved in the last common ancestor of marmosets and tamarins. **Center and**
 830 **right:** Evolutionary relationships inferred from molecular data, with *Callimico* as the sister taxon
 831 of *Callithrix*; the two-molared phenotype either evolved once (center) or multiple times (right),
 832 and *Callimico* is either derived in having third molars (center) or plesiomorphic (right).

833

834 **Fig. 2.** Distribution of ΔAIC_c values for the irreversible, equal-diversification model versus the
 835 (reversible) equal-diversification model, generated from the Bayesian block of 1,000 trees. The
 836 ΔAIC_c for the consensus tree is indicated by the white arrow (9.95). Most of the values (95%)
 837 fall between 9.06 and 12.25, indicating that there is essentially no support for the irreversible
 838 model when accounting for uncertainty in phylogenetic relationships.

839

840 **Fig. 3.** Plot of \log_e mean maxillary postcanine length (mesial premolar to distal molar, in
 841 millimeters) versus \log_e mean palate length (alveolare to staphylion, in millimeters) for females
 842 of 27 platyrrhine species. Black circles are callitrichines; open circles are representatives from all
 843 other genera recognized by Groves (2005), except *Brachyteles* and *Oreonax*. The dashed line is
 844 the regression slope ($b = 0.92$) for three-molared platyrrhines computed using phylogenetic
 845 generalized least squares in the caper package for R (Orme et al., 2013). The data and *10kTrees*
 846 consensus phylogeny that were used to make this figure are available in the Supplementary
 847 Online Material.

848

Table 1. Model comparisons.

Model	k	BiSSE parameters						AIC_c	ΔAIC_c	Relative likelihood
		λ_3	λ_2	μ_3	μ_2	q_{32}	q_{23}			
All primates ($n = 249$)										
Unconstrained	6	0.288	0.322	0.223	0.172	0.0004	0.0092	1527.75	1.79	0.4093
Equal div.	4	0.294	0.294	0.226	0.226	0.0006	0.0078	1525.96	0.00	1.0000
Irreversible	5	0.286	0.318	0.220	0.155	0.0011	0.0000	1538.21	12.25	0.0022
Irreversible, equal div.	3	0.294	0.294	0.226	0.226	0.0016	0.0000	1535.91	9.95	0.0069
Platyrrhines only ($n = 60$)										
Unconstrained	6	0.381	0.473	0.286	0.281	0.0037	0.0086	362.97	3.86	0.1447
Equal div.	4	0.407	0.407	0.293	0.293	0.0044	0.0078	359.11	0.00	1.0000
Irreversible	5	0.380	0.407	0.283	0.223	0.0085	0.0000	364.77	5.66	0.0591
Irreversible, equal div.	3	0.407	0.407	0.292	0.292	0.0113	0.0000	361.24	2.14	0.3437

Symbols are as follows: k = number of estimated parameters in the model; λ_3 and λ_2 = speciation rates for three- and two-molared lineages, respectively; μ_3 and μ_2 = extinction rates for three- and two-molared lineages, respectively; q_{32} and q_{23} = transition rates from three to two molars and from two to three molars, respectively. See methods section for further details.

Figure 1

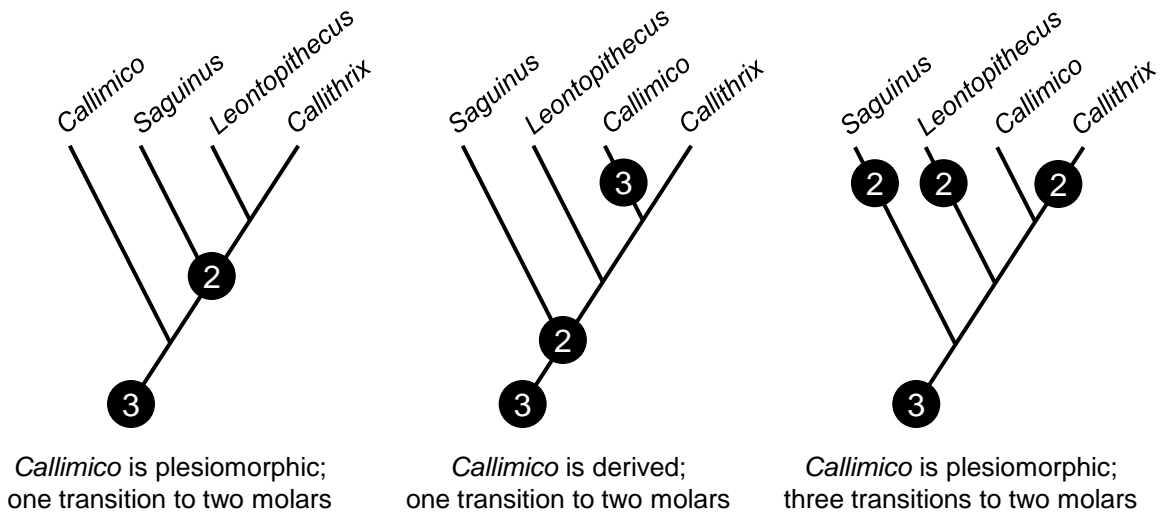


Figure 2

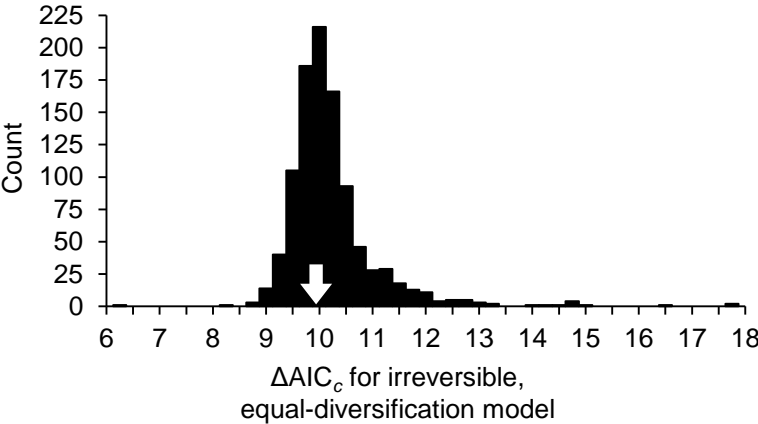


Figure 3

