

1 **Title: Decoupled ecomorphological evolution and diversification in Neogene-**
2 **Quaternary horses**

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17 **Abstract:**

18 Evolutionary theory has long posited a connection between trait evolution and diversification
19 rates. Here, we use phylogenetic methods to evaluate the relationship of lineage-specific
20 speciation rates and the mode of evolution of body size and tooth morphology in the Neogene
21 and Quaternary radiation of horses (8 living and 130 extinct species). We show that

22 diversification pulses are a recurrent feature of equid evolution, but that these are not correlated
23 with rapid bursts in phenotypic evolution. Rather, rapid cladogenesis seems repeatedly
24 associated with extrinsic factors that relaxed diversity bounds, such as increasing productivity
25 and geographic dispersals into the Old World. This evidence suggests that diversity dynamics in
26 Equinae were controlled mainly by ecological limits under diversity-dependence, rather than
27 rapid ecomorphological differentiation.

28 **Main Text:**

29 Rapid phenotypic evolution has long been taken to be an important factor in evolutionary
30 radiations ([1](#)). In this model of ‘adaptive radiation’, change of ecologically relevant traits should
31 be faster in early phases of the clade’s expansion ([2](#)), as lineages fill new adaptive zones ([1](#), [3](#)).
32 Since change is predicted to slowdown as these zones are filled ([2](#)), ecomorphological disparity
33 among subclades and zones should be more partitioned than expected under a non-adaptive
34 scenario ([4](#)). However, such adaptive model may not be common in nature ([2](#), [3](#), [5](#), [6](#)), and
35 accelerated diversification could result from extrinsic factors —such as geographical dispersals,
36 increased productivity or habitat heterogeneity ([7-9](#))— that release diversity limits and promote
37 speciation ([10](#)) without involving rapid ecomorphological divergence ([3](#)).

38 The radiation of equids in the Neogene has been cited as a textbook example of ‘adaptive
39 radiation’ for over a century ([11](#)), being crucial in the development of evolutionary theory
40 linking trait evolution and adaptive success ([1](#), [12](#)). The rich equid fossil record provides a
41 suitable dataset for testing these ideas within a phylogenetic framework. Much work has focused
42 on the evolution of body size and dental morphology ([12](#)), as these two traits condense multiple
43 dimensions of a species’ adaptive zone such as population density, range size, diet and

44 environmental pressures. Early studies based on dental proportions suggest that phenotypic
45 change accelerated during an early Miocene radiation ([1](#), [13](#), [14](#)), although recent analyses
46 showed that body size disparity did not increase during diversification pulses ([15](#)). Yet, previous
47 work has been nonphylogenetic and has not directly investigated the connection between
48 diversification dynamics and phenotypic evolution.

49 Here, we assess tree-wide variation in speciation rates in the Neogene and Quaternary radiation
50 of equids (around 18 Ma to the present). We then use phylogenetic maximum-likelihood trait
51 modelling to evaluate the mode of evolution of body size and tooth crown height (hypsodonty)
52 across clades, time, and space, as well as to directly ask whether speciation and rates of
53 phenotypic evolution are coupled in equine lineages. Finally, we used phylogenetically-informed
54 regressions (PGLS) to directly test for correlations among speciation rates, body size and
55 hypsodonty. Our dataset incorporates a significant amount of information from fossils (95% of
56 the 138 species considered are extinct), which considerably improves the ability of tree-based
57 approaches to recover the past ([3](#), [16](#)).

58 We find evidence for repeated speciation bursts across Equinae, but none of these were
59 associated with rapid ecomorphological evolution. Each subclade considered shows an early
60 expansion followed by a slowdown in diversification rates that results in a diversity plateau ([14](#),
61 [17](#)). This pattern, which is mirrored in the early Miocene rise of American tribes (18 to 15 Ma)
62 and when lineages entered the Old World (11 Ma and 4.5 Ma, Fig. 1, A and B), is consistent with
63 a model of logistic growth with a finite upper limit to species richness (Table S1) ([8](#)). If such a
64 burst and slowdown dynamic was the result of early niche differentiation processes (an ‘adaptive
65 radiation’) ([3](#)), we should expect early rapid ecomorphological divergence resulting from rapid
66 trait evolution ([2](#), [6](#)). None of these early-phase expansions, however, was correlated with an

67 early burst in body size or hypsodonty evolution (Table S5 to S14). Furthermore, rates of body
68 size evolution were not significantly different in lineages exhibiting high and low speciation,
69 whereas rates of hypsodonty evolution were significantly lower in lineages with fast speciation
70 rates (Fig. 1, D to E, and Table S15) pointing to a marked decoupling of diversification rates and
71 the evolution of functional traits. In line with these findings, and in contrast to previous notions
72 ([14](#)), we found that rates of phenotypic evolution in American forms were marginally slower
73 during their basal radiation (before ~15 Ma) than afterwards (Table S16).

74 We find that the subclades of Equinae substantially overlap each other in morphospace
75 occupation across the entire analysis interval (see Fig. 1C, Fig. 2 and Fig. S7). This contrasts
76 with the among-subclades partitioned disparity expected in radiations that are adaptive, as
77 observed in other clades ([4](#), [18](#), [19](#)). The observed disparity through time pattern supports the
78 conclusion of our trait modeling approach. The explosive radiation of equine horses in the early
79 Miocene, and those that followed as the group dispersed into the Old World, were not spurred by
80 early, rapid ecomorphological divergence ([15](#)).

81 The early radiation of American equids tribes (18 to 15 Ma) has been traditionally explained as
82 the direct outcome of morphological adaptation linked to the onset of grass dominated habitats
83 ([17](#), [20](#)). However, recent paleobotanical evidence suggests that grasslands were well developed
84 in North America ~25 Ma ([21](#)), and recent microwear analyses show that the more primitive
85 *Parahippus* forms already had a grass-dominated diet in the earliest Miocene, before 20 Ma ([22](#)).
86 Our findings fit this environmental context, and imply that the early Miocene radiation of equine
87 lineages took place in the absence of rapid ecomorphological shifts (Fig. 1, C and D). One
88 possible explanation is that grazing behaviour evolved ahead of morphological adaptation ([23](#)) in
89 early forms. Morphological change accelerated later on (Fig. 1, C to E; Table S16), probably to

90 accommodate the new feeding style and as a response to enhanced competition within the clade
91 and from other clades. However, the appearance of the grazing behaviour still predates the
92 American radiation by several million years (22). In absence of a clear intrinsic driver, we
93 suggest that the early Miocene radiation of horses might be attributed to external factors,
94 including higher productivity or increased biogeographic provincialism associated to climatic
95 change (8, 9, 24, 25). These could have released diversity limits and promoted speciation by
96 reducing competition (the ‘crowding effect’) among forms with largely overlapping
97 ecomorphotypes and behaviour (Fig. 1C).

98 The long middle Miocene diversity plateau exhibited by New World clades was only surpassed
99 with the first dispersal into the Old World ~11 Ma (Fig. 1B and S5). Large dispersal events like
100 these have been predicted to increase diversity limits by providing new ecological arenas and
101 spurring speciation (8, 26). Dispersals into the Old World always promoted speciation (Fig. 1, A
102 and B) under disparate modes of evolution (Tables S5 to S14, Fig. S7, and external dataset S1).
103 For example, New and Old World hipparions show early-phase speciation (Fig 1B) but under
104 two completely disparate modes of phenotypic evolution probably resulting from different
105 environmental pressures (27) (Fig. S7). Hipparions that dispersed across the Old World likely
106 evolved from a large ancestor (Fig. S3), and their size diffused five times faster around a larger
107 optimal size (~250 kg) than their American relatives (~100 kg) (Fig. 1C, Fig. S7 and Table S5).
108 In contrast, hypsodonty of Old World hipparions changed six times slower than in New World
109 hipparions and their macroevolutionary optimum barely departed from moderate scores over 10
110 myr (Fig. 1C, Fig. S7 and Table S6).

111 Geographic dispersals may affect morphological evolution dissimilarly in different clades.
112 Whereas Old World hipparions show strikingly different trait evolution than their American

113 relatives (see above), American and Old World *Equus* are better modelled as a single clade (Fig.
114 S6). Trait evolution shifts were more likely to happen at the base of the *Equus* clade than when
115 *Equus* entered the Old World (see external dataset S1), suggesting that the Old World radiation
116 of *Equus* did not require a significant shift in phenotypic evolution. Size in Equini evolved under
117 a macroevolutionary diffusion (Table S5), confirming that larger sizes in this lineage did not
118 arise by active selection (Cope's rule) ([15](#)). In *Equus*, body size shows slower evolutionary rates
119 and a marked selection towards smaller sizes, and hypsodonty evolved faster under higher
120 selective pressure than in the rest of Equini (Fig. S6 and Table S5 and S6). Interestingly, both
121 traits followed more selected or constrained evolution in *Equus* than in other Equini lineages.
122 Also, *Equus* show a tendency to increase hypsodonty and decrease body size in more recent
123 lineages that also show slower speciation (see PGLS results in Table S2 to S4; see also Fig. 1C).
124 Taken together, these results are consistent with short-term responses to harsher environmental
125 conditions and frequent shifts in resource availability in Pleistocene times, as previously noted
126 ([28](#), [29](#)).

127 Our multi-layered approach reveals a complex connection between ecological opportunities,
128 diversification dynamics and trait evolution ([5](#), [6](#)). Although early clade expansions are prevalent
129 in Neogene horses, we found no evidence supporting a key role for ecomorphological divergence
130 in these speciation pulses. Rather, ecologically relevant traits show completely disparate
131 evolutionary modes during such diversification events (Fig. 1, C to E). Clade expansion probably
132 occurred as a result of diversity limits being released due to extrinsic factors. Under this
133 scenario, and with the present data, it is difficult to tease apart the nature of the diversity limits
134 rendering the subsequent slowing speciation rate ([3](#)). Given the remarkable overlap in
135 ecomorphological space of Equinae lineages (Fig. 1C, Fig. 2 and Fig. S7), the signal of diversity

136 dependence recovered in our analyses (Table S1) to a great extent could result from intensifying
 137 within-clade competition and an attenuation of the factors that release diversity limits in the first
 138 place (3, 8, 9). Horses now join a growing body of fossil evidence of diversity dependence being
 139 a recurrent pattern in macroevolution. Phenotypic evolution in Equinae is characterized by a
 140 remarkable capacity to shift both at the short and long term (high evolutionary rates in some
 141 clades; shifting macroevolutionary optima in others). Such shifts certainly were shaped by
 142 environmental pressures, but diversity dynamics was the domain of ecological limits.

143

144 **References and Notes:**

145

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216

217 **Fig. 1. Speciation rates and ecomorphological evolution in Neogene-Quaternary horses. (A)**
218 Chronogram showing polytomies and complete stratigraphic ranges (thin grey bars). Branches
219 are coloured according to the mean speciation rate (DR). Vertical shaded bars highlight early
220 Miocene, late Miocene, and Pleistocene. Grey circles indicate dispersals into the Old World. The
221 inset shows the scale and density of speciation rate values across branches. **(B)** Log-scaled
222 median diversity through time across 100 trees. Shaded regions represent the 95% quantile (see
223 also Fig. S1). **(C)** Evolution of Equinae ecomorphospace plotted on the phylogenetic tree of

224 Equinae during different time intervals. For the sake of clarity, *Pseudhipparion simpsoni* was left
225 out of the plots. Note log-transformed values on the y-axes. **(D)** Rates of trait evolution
226 reconstructed for each branch. **(E)** Rates of body size and hypsodonty evolution in lineages of
227 the tree with high and low speciation rates according to our BM_{DR} models

228

229 **Fig. 2. Subclade relative subclade disparity through time (DTT) in the clade containing**
230 **Equinae and *Parahippus*.** The DTT measures the proportion of disparity of the whole clade
231 held by each of the subclades whose ancestral lineages are present at a given time (4). The DTT
232 of body size **(A)** and hypsodonty **(B)** through time is plotted (dark line). The dashed line
233 indicates the median DTT based on simulations of trait evolution under Brownian motion.
234 Shaded area, 95% range of the simulated data. The morphological disparity index (MDI) is the
235 overall difference between the observed among-clades disparity and the null distribution. In
236 adaptive radiations disparity should be highly partitioned among clades, each lineage holding
237 little of the total disparity (little overlap in morphospace), and significant negative MDI values
238 are expected. The significant *p*-value in (B) suggests the observed trend departs from the null
239 expectation. DTT plots for different subclades are shown in Fig. S7.

240 **Supplementary Materials:**

241 Materials and Methods

242 Supplementary Text

243 Figures S1-S7

244 Tables S1-S16

245 External Database S1

246 References (30-107)