Hybridizing salamanders experience accelerated diversification

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ABSTRACT

Whether hybridization generates or erodes species diversity has long been debated, but to date most studies have been conducted at small taxonomic scales. Salamanders (order Caudata) represent a taxonomic order in which hybridization plays a prevalent ecological and evolutionary role. We employed a recently developed model of trait-dependent diversification to test the hypothesis that hybridization impacts the diversification dynamics of species that are currently hybridizing. We find strong evidence supporting this hypothesis, showing that hybridizing salamander lineages have significantly greater net-diversification rates than non-hybridizing lineages. This pattern is driven by concurrently increased speciation rates and decreased extinction rates in hybridizing lineages. Our results support the hypothesis that hybridization can act as a generative force in macroevolutionary diversification.

Introduction

A leading unresolved question in evolutionary biology is whether hybridization, defined as the interbreeding between two genetically distinct lineages[1], acts as a creative or destructive evolutionary force[2,3,4,5,6]. The prevailing view in the animal literature is that hybridization constrains lineage diversification because hybrid lineages are often documented to be less fit

29 than parentals[4,5,7]. Under this scenario, hybridization is predicted to increase extinction rates.
30 Further, introgressive hybridization has the potential to "wash away" accumulating divergence
31 among incompletely isolated lineages[8,9,10], leading to a prediction of decreased speciation rates.
32 In contrast, the prevailing view in the plant literature is that hybridization enhances adaptive
33 potential by introducing novel genetic and phenotypic variation[11,12,13]. Reinforcement, or the
34 accumulation of post-zygotic reproductive isolation through selection against hybrids[14], has long
35 been considered to expedite the speciation of diverging lineages[9]. Additionally, hybridization-
36 mediated shuffling of old genetic variants may fuel rapid diversification, as outlined by the
37 combinatorial view on speciation[15]. Accordingly, hybridization is predicted to increase
38 speciation rates and/or decrease extinction rates.

39 Whereas evidence of hybrid speciation in plants has long been abundant, evidence for
40 widespread hybrid speciation in animals is relatively scarce[6,10,16,17,18,19]. In allopolypoid hybrid
41 speciation, a mode of speciation common in plants but rare in animals, nearly complete
42 reproductive isolation may evolve in a single generation due to a change in ploidy[11]. In contrast,
43 homoploid hybrid speciation typically has to occur in the face of continued gene flow, which acts
44 to homogenize the diverging hybrid lineages[10].

45 Interest in hybrid-mediated speciation has recently burgeoned, but studies have typically
46 been limited in taxonomic scope. Studies of the effect of hybridization on diversification have
47 most commonly been conducted among closely related pairs or small clades of taxa, and results
48 have been equivocal[1,20,21,22,23,24,25,26]. Additionally, studies of hybridization often occur along
49 different stages of the speciation continuum[27], whereby hybridization can appear as a force that
50 either facilitates or impedes speciation. Recent work highlights this uncertainty by demonstrating
51 that the outcomes of hybridization depend on the underlying nature of selection pressures and

52 demography[28]. We suggest that studies at broad phylogenetic and macroevolutionary timescales
53 can help overcome these limitations by providing a phylogenetic context in which to view
54 repeated hybridization events over evolutionary timescales and the consequent impact on lineage
55 diversification rates.

56 Here, we conduct a taxonomically-broad test of the relationship of hybridization with
57 macroevolutionary diversification rates. We study salamanders (order Caudata, ca. 716 spp as of
58 October 2018[29]), which are particularly suitable because hybridization is pervasive and has been
59 studied extensively (i.e., nearly 1/3 of N. American species hybridize: Supplementary Fig. S1,
60 Supplementary Table S1). Additionally, sufficient sequence data are available to resolve the
61 phylogenetic relationships among most (~63%) taxa within this group[30]. If hybridization plays a
62 meaningful role in the diversification process, differences in diversification rates among
63 hybridizing and non-hybridizing taxa are expected. We thus test the hypothesis that there is a
64 difference in diversification rates (speciation and extinction rates) between contemporaneously
65 hybridizing and non-hybridizing salamander lineages. Note that we are simply testing whether
66 contemporary hybridization influences diversification rates, not whether ancient hybridization
67 facilitated the present radiation as postulated by the hybrid swarm hypothesis[6], because our
68 experimental design cannot address this (See supplement). We replicate this test across four
69 datasets to investigate the robustness of our results: 1) including all available data; 2) exclusion
70 of species that do not exhibit sympatry (defined as <10% geographic range overlap) thereby
71 lacking the opportunity to hybridize; 3) only the family Plethodontidae, which are the most
72 widely hybridizing and diverse of the 10 salamander families; 4) all (nine) salamander families
73 except the Plethodontidae.

74

Materials and Methods

Data Collection

We used the time-calibrated phylogeny of Amphibia[3] as the source for downstream analyses. This tree[30] was constructed using nine nuclear genes and three mitochondrial genes as data using RAxMLv7.2.8[31], and time-calibrated using treePL[32]. Using the APE package (v.3.4[33]) in R, we extracted the subtree containing salamanders for subsequent analyses. This approach yielded a tree containing 469 of 716 extant species of salamanders (Supplementary Fig. S1[30]) representing approximately two-thirds of the known diversity.

Each species was scored as 'non-hybridizable' (NH) or 'hybridizable' (H), based on an extensive literature review of hybridization using the search engines Google Scholar and ISI Web of Knowledge. To do so we paired each species with the terms 'hybrid' and 'introgress' as well as dialectical and structural variants thereof (e.g., 'hybridization', 'introgressed') to search for cases of hybridization. Criteria for hybridizability under the first, "narrow" definition was the documentation of hybridization among natively distributed species, specifically the observation of heterospecific mating or hybrid offspring in the wild as detected by morphological and molecular intermediacy. Criteria for hybridizability under the second, "broad" definition included those criteria described previously, as well as: 1) the observation of hybridization occurring in laboratory settings or among introduced and native species, and; 2) inference of historical introgression as determined using molecular lines of evidence (e.g. substantial and replicated genealogical discordance among molecular markers or detection via Approximate Bayesian Computation methods). This latter definition is less conservative than the former. Although inference of hybridization through the detection of genealogical discordance warrants caution, our narrow definition of hybridization does not recognize these species as hybridizable.

98 In total, we retrieved 56 papers (date-range: 1957-2017). We confirmed hybridization for
99 roughly 11 and 13 percent of extant Caudates for the narrow and broad datasets, respectively (78
100 and 92 of 716 species: 17 and 20 percent of sampled taxa; Supplementary Table S1).
101 Documented hybridization was absent from four families (Cryptobranchidae, Sirenidae,
102 Proteidae, Rhyacotritonidae) in the narrow dataset and two families (Rhyacotritonidae,
103 Sirenidae) in the broad dataset.

104 In addition to analyzing the entire salamander subclade from the Pyron[30] amphibian
105 phylogeny (469 total species), we compared diversification rates using three additional datasets,
106 produced using both the narrow and broad datasets (i.e., for a total of eight datasets). In the first
107 dataset, we required that "non-hybridizable" taxa were sympatric (>10% range overlap) with
108 another species, thus possessing sufficient opportunity to hybridize. Consequently, species that
109 were not sympatric with any other salamander taxa were excluded from the dataset. Therefore,
110 species classified as non-hybridizing in this analysis may have limited opportunity to hybridize
111 but have not been observed to do so. Although we cannot account for historical species
112 distributions (e.g. species' ranges may have previously overlapped), our primary (narrow)
113 definition of hybridizability necessitates contemporary hybridization. Thus, our designation of
114 sympatry occurs at the same time scale as our designation of hybridizability.

115 In the second and third datasets, we tested for a family-specific effect of hybridization on
116 diversification rates. As plethodontids are the most diverse family of salamanders (Fig. 1B) and
117 also the most well studied, the greatest number of instances of hybridization have been
118 documented in this group. Additionally, many plethodontids of the genus *Plethodon* in the
119 Eastern United States have been described as species on the basis of a threshold genetic distance
120 (e.g. [34, 35,36,37]). Coincidentally, nearly half of these species have been observed to hybridize in

121 nature. Thus, we produced datasets including either only members of family Plethodontidae, or
122 the nine families that exclude plethodontid salamanders. In total, eight analyses were conducted,
123 using four trees for each of the narrow and broad datasets, respectively. Information on the total
124 number of species included in each tree, as well as the number of species in each state (i.e., H or
125 NH) may be found in Supplementary Table S1.

126 *Assessment of Trait Dependent Diversification*

127 We applied the HiSSE (Hidden State Speciation & Extinction[38]) trait-dependent diversification
128 model to test for differences in speciation and extinction rates between hybridizing and non-
129 hybridizing lineages. HiSSE infers speciation and extinction rates for a binary character while
130 allowing for heterogeneity in diversification rate to exist within each character state. In the
131 HiSSE model, hidden states are co-distributed with the trait of interest and account for
132 unsampled traits that may simultaneously contribute to the diversification process. The hidden
133 state need not be associated with any single trait but may instead be associated with a set of traits
134 or suite of traits. Inclusion of the hidden state thus ameliorates the confounding effects of
135 unsampled traits on diversification rate estimations by allowing for greater rate heterogeneity in
136 the tree than in previous SSE models[38]. Thus, we are in essence measuring the impact of
137 contemporary hybridization while controlling for other, correlated traits on diversification rate.
138 Finally, by accounting for increased rate heterogeneity in character-dependent and character-
139 independent (null) models through the inclusion of hidden states, model rejection properties are
140 greatly improved[38] relative to previous SSE models.

141 We evaluated a total of 14 competing models using HiSSE, half of which represent a
142 model of character-dependent diversification and the remaining half represented models of
143 character independence (Supplementary Tables S2-S9). Models of trait dependence varied in the

144 number of hidden states included and in the number of free transitions among states. In
145 character-dependent models, our four states where Hybridizing (H) and Non-hybridizing (NH),
146 each being associated with one of the two hidden state (A & B) for four total character
147 states/diversification regimes (H-**A**, H-**B**, NH-**A**, NH-**B**).

148 Additionally, to account for the fact that diversification rates may be biased by
149 incomplete sampling of extant diversity within a phylogeny[39,40,41], we assume that 20% of extant
150 species of salamanders hybridize in nature. This value was chosen because it approximately
151 equals the mean frequency of hybridization across our datasets (19.75). To explore the effect this
152 assumption had on our results, we repeated these analyses assuming 1) we have sampled all
153 extant hybridizing species (Supplementary Fig. S2), 2) our sampling of character states is
154 proportional to their prevalence in nature (Supplementary Fig. S3), and 2) that 30% of extant
155 species hybridize (Supplementary Fig. S4). Thus, while unable to designate all hybridizing taxa
156 as such in our phylogeny due to their not being represented in the literature, we have explicitly
157 addressed this uncertainty in our analysis. We elaborate upon our choice of sampling fractions in
158 the Supplementary Materials.

159 To assess whether the inclusion of extremely young species impacted our diversification
160 rate estimates, we ran a single analysis excluding species younger than 1MY, as per Beaulieu &
161 O'Meara[38] using the narrow dataset and all species. This in turn led to the removal of 14 species.
162 Results were qualitatively identical to those obtained including these young species, so all
163 subsequent analyses were conducted including them. To improve the performance of the
164 Maximum Likelihood optimization procedure implemented in HiSSE, we used simulated
165 annealing to first traverse the likelihood surface to identify optimal starting values for subsequent
166 ML-optimization. Rather than reporting the results of individual model fits, we instead take the

167 approach of investigating model-averaged parameter estimates for each sampled character state[42]
168 (see Supplementary Information for further justification). That is, parameter estimates obtained
169 from each fitted model are averaged together such that their contribution to the average is
170 proportional to their relative support (Akaike weights) among the set of candidate models
171 (Supplementary Table S13). This leads the best supported models to have the greatest impact on
172 the final model averaged parameter estimates. Diversification rates are returned for each sampled
173 state respectively, as not all models include hidden states.

174 To test for significance among diversification rates inferred for each state, we calculated
175 all possible ratios between non-hybridizing and hybridizing species' model-averaged parameter
176 estimates and calculating the proportion of comparisons in which the value for the non-
177 hybridizing lineage is greater than that of hybridizing lineage. Thus, we obtain empirical P-
178 values (reported in Supplementary Table S14) in which a value of 0 means in every comparison,
179 hybridizing lineages were inferred to have rates greater than those of non-hybridizing lineages,
180 and vice versa. This test is extremely conservative and tests the null hypothesis that non-
181 hybridizing species always experience diversification rates greater than hybridizing species.

182 In summary, four phylogenies (all species, sympatric species, plethodontids, & non-
183 plethodontids) were analyzed using two datasets (narrow & broad definitions of hybridization).
184 Each data/tree-set combination was tested assuming the three aforementioned differences in
185 prevalence of hybridization in nature. In all, a total of 32 rounds of model testing were
186 performed, comparing seven models of trait-independent diversification and seven models of
187 trait-dependent diversification for each sampling fraction. Further information on how these
188 data/tree-sets were produced and analyzed may be found in the Supplementary Materials. Lastly,
189 a description of our test of sensitivity to phylogenetic uncertainty may also be found in the

190 Supplement (Supplementary Fig. S6 & S7; Supplementary Table S15)

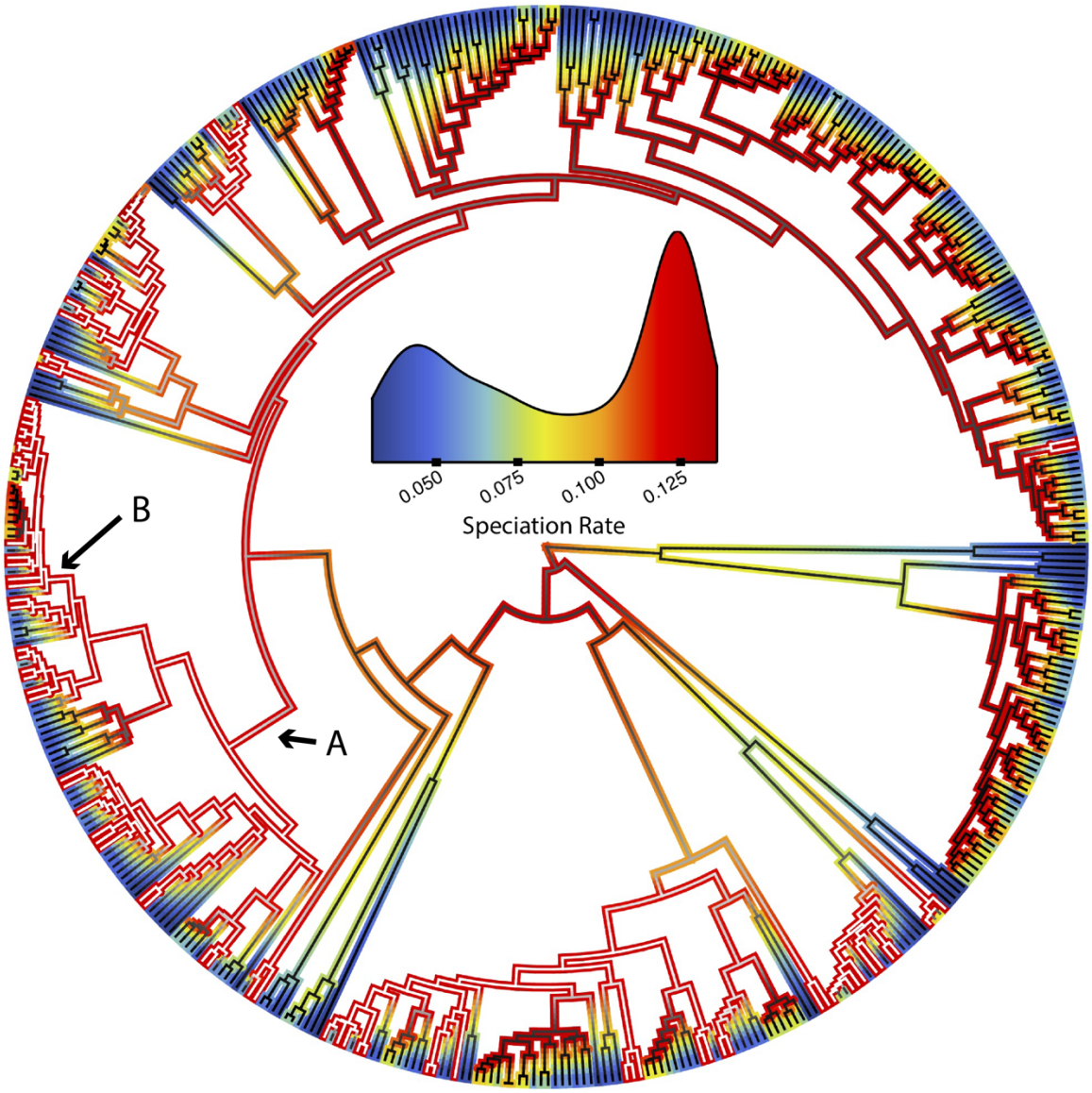
191 To complement our HiSSE analyses in a manner that is largely insensitive to the
192 potentially confounding relationship between branch lengths and propensity to hybridize (see
193 discussion), we conducted sister clade comparisons. Specifically, using all comparisons of three
194 or more taxa (i.e. two sister species hybridize, and the sister lineage does not), we used the
195 method of Barraclough, Harvey and Nee[43] to test the hypothesis that hybridizing clades had
196 greater richness than non-hybridizing clades. To assess confidence, 1000 permutations of
197 contrast signs were conducted. This test was repeated for each of the eight datasets described
198 above (Supplementary Table S16).

199 To test for possible circularity of causality between diversification rates, species richness
200 and opportunity to hybridize, we quantified the relationships between the three. Specifically, we
201 tested for a relationship between 1) mean diversification rates and the proportion family
202 hybridizing, 2) mean diversification rates and species richness, and 3) proportion family
203 hybridizing and species richness (Supplementary Fig. S8). All analyses were conducted at the
204 family level, and mean diversification rates were obtained using lineage-specific model-averaged
205 diversification (speciation, extinction, net-diversification) rates. Simple linear regressions were
206 conducted in R[44].

207 **Results**

208 Across both datasets (narrow and broad) assuming 20% of species hybridize, three of four
209 analyzed phylogenies consistently found that hybridizing species experience increased speciation
210 rates, decreased extinction rates, and therefore increased net-diversification rates (all significant:
211 Figs. 1, 2 & 3; Tables 1, 2, Supplementary Tables S6 & S7). Net-diversification of hybridizing

212 lineages in these trees were on average 4X greater than that of non-hybridizing lineages. Our
213 results were insensitive to phylogenetic uncertainty (Supplementary Fig. S7; Supplementary
214 Table S15).



215
216 **Figure 1. Inferred speciation rate along the salamander phylogeny for both hybridizing and**
217 **non-hybridizing species.** Branch outlines depict speciation rates corresponding to the inset
218 density plot. Branch interiors depict the probability that a lineage is hybridizable (white) or non-
219 hybridizable (black). The arrow labeled **A** denotes family Plethodontidae, and the arrow labeled **B**
220 denotes the *Plethodon glutinosus* group.
221

Dataset	Tree	Speciation λ		Extinction μ		Net Diversification $r = \lambda - \mu$		Extinction Fraction $\varepsilon = \mu \div \lambda$		Turnover $\tau = \lambda + \mu$	
		H	NH	H	NH	H	NH	H	NH	H	NH
Narrow	All Species	**0.124 ± 5.90e-4**	0.055 ± 1.69e-3	9.39e-3 ± 8.62e-5	**0.038 ± 8.36e-4**	**0.115 ± 5.04e-4**	0.017 ± 2.54e-3	0.076 ± 3.20e-4	**0.794 ± 0.036**	**0.134 ± 6.76e-4**	0.094 ± 8.60e-4
	Sympatric Species	**0.111 ± 8.140e-3**	0.042 ± 1.73e-3	8.57e-4 ± 8.68e-5	**9.45e-3 ± 3.5e-4**	**0.110 ± 8.04e-3**	0.032 ± 2.08e-3	7.54e-3 ± 1.53e-4	**0.283 ± 0.020**	**0.112 ± 8.22e-3**	0.051 ± 1.38e-3
	Plethodontids	**0.098 ± 5.50e-6**	0.041 ± 1.48e-3	4.26e-4 ± 1.20e-8	**0.014 ± 7.66e-4**	**0.098 ± 5.48e-6**	0.028 ± 2.24e-3	4.35e-3 ± 1.63e-7	**0.435 ± 0.051**	**0.098 ± 5.50e-6**	0.055 ± 7.12e-4
	Non-Plethodontids	**0.078 ± 3.38e-3**	0.041 ± 1.73e-3	1.89e-3 ± 4.92e-5	**0.039 ± 7.88e-4**	**0.076 ± 3.42e-3**	2.2e-3 ± 2.5e-3	0.025 ± 1.19e-3	**1.040 ± 0.066**	8.00e-2 ± 3.32e-3	0.080 ± 9.80e-4
Broad	All Species	**0.117 ± 2.72e-3**	0.053 ± 2.04e-3	2.39e-3 ± 1.55e-4	**0.027 ± 7.24e-4**	**0.115 ± 2.88e-3**	0.026 ± 2.74e-3	0.021 ± 1.95e-3	**0.647 ± 0.039**	**0.120 ± 2.58e-3**	0.080 ± 1.37e-3
	Sympatric Species	**0.106 ± 4.60e-3**	0.047 ± 1.85e-3	3.02e-3 ± 1.64e-4	**0.027 ± 1.10e-3**	**0.103 ± 4.74e-3**	0.019 ± 2.96e-3	0.031 ± 2.04e-3	**0.732 ± 0.057**	**0.109 ± 4.46e-3**	0.074 ± 7.58e-4
	Plethodontids	**0.143 ± 1.79e-4**	0.049 ± 1.64e-3	3.48e-6 ± 1.33e-6	**1.40e-3 ± 6.66e-5**	**0.143 ± 1.78e-4**	0.048 ± 1.70e-3	2.42e-5 ± 9.24e-6	**0.033 ± 2.26e-3**	**0.143 ± 1.80e-4**	0.051 ± 1.57e-3
	Non-Plethodontids	0.071 ± 5.42e-3	0.072 ± 2.88e-3	1.44e-4 ± 1.04e-5	**1.66e-4 ± 3.72e-7**	0.071 ± 5.42e-3	0.072 ± 2.88e-3	2.38e-3 ± 4.80e-4	**2.60e-3 ± 2.52e-4**	0.071 ± 5.42e-3	0.072 ± 2.88e-3

Table 1. Model-averaged diversification rate estimates at the tips of the phylogeny ± 2 SE. Results assume 20% of extant species hybridize. Bold indicate parameter estimates that differ significantly among character states, with the boldened values as the larger rate estimate. Significance was determined by calculating all possible ratios between non-hybridizing and hybridizing species' model-averaged parameter estimates and calculating the proportion of comparisons in which the value for the non-hybridizing lineage is greater than that of hybridizing lineage. This comparison thus produced an empirical P-value with which significance could be determined.

In all cases of inferred trait dependent diversification, a HiSSE model was the best supported (Supplementary Tables S2-S9). Of these HiSSE models, two groups emerged: one in which hybridizing species did not harbor a second hidden state (All Species – Narrow, Plethodontids – Narrow & Broad), and one in which both hybridizing and non-hybridizing taxa had two hidden states (Table 2). The former of these (without a second hidden state for hybridizing taxa) is interpretable as meaning that there is less diversification rate heterogeneity experienced by hybridizing taxa than by non-hybridizing taxa. Interestingly, non-hybridizing taxa were sometimes inferred to have slightly negative net-diversification rates (Table 2). In the case of the best-fit model using all taxa assuming 20% of species hybridize, this leads to an expected waiting time of 128 million years before the next net-loss in diversity. These negative rates are not persistent however; examination of ancestral state reconstructions indicates that the

244 hidden state responsible (NH-B) for these rates is distributed primarily along the tips
245 (Supplementary Figs S9 – S12).

246 As the average magnitude of increase in speciation rate across all 32 analyses (95% CI:
247 0.0557 ± 0.0059 species/MY) is significantly greater than the average decrease in extinction rate
248 (95% CI: 0.0145 ± 0.0037 species/MY), we conclude it is primarily differential speciation that is
249 driving the increase in net-diversification in hybridizing lineages. Interpreted as a waiting time,
250 this means that hybridizing species, on average, speciate every 9.2 (95% CI: 8.84 - 9.62) million
251 years and go extinct every 160.7 (118.97 – 247.96) million years.

252 Analysis of the tree containing all species recovered strong signal of trait dependent
253 diversification, with significant differences between hybridization or non-hybridizing lineages
254 (as measured by empirical P-values) found between states for all five parameter estimates (Figs.
255 2 & 3; Tables 1, 2, Supplementary Tables S6 – S7), regardless of whether the broad or narrow
256 criterion was used. Speciation (λ), net-diversification ($r = \lambda - \mu$), and turnover rate ($\tau = \lambda + \mu$)
257 were greater in hybridizing than non-hybridizing lineages, whereas extinction rate (μ) and
258 extinction fraction ($\varepsilon = \mu \div \lambda$) were lower. Increases were on average 125% (95% CI: 117 -
259 133%) for speciation, 576% (486 - 699%) for net diversification and 43% (41 - 45%) for
260 turnover. In contrast, decreases were on average 305% (292 - 317%) for extinction and 945%
261 (893 - 997%) for extinction fraction (Fig. 2). Additional analyses that assume different
262 percentages of extant species hybridize produced qualitatively similar results and are discussed
263 in the supplement (Supplementary Figs. S2-S4, Supplementary Tables S2-14).

264 Analysis of the tree including only sympatric taxa regardless of the hybridization
265 criterion (narrow versus broad) datasets recovered patterns identical to those obtained using the
266 complete phylogeny (Figs. 2 & 3; Tables 1, 2, Supplementary Tables S6-S7). Speciation rate,

net-diversification rate, and turnover increased by 164% (135 - 196%; λ), 244% (199 - 295%; *r*), and 120% (98 - 142%; τ) respectively in hybridizing versus non-hybridizing lineages. In contrast, extinction rate and the extinction fraction decreased in hybridizing lineages by 1003% (864 - 1172%; μ) and 3653% (3319 - 4002%; Ɛ) respectively.

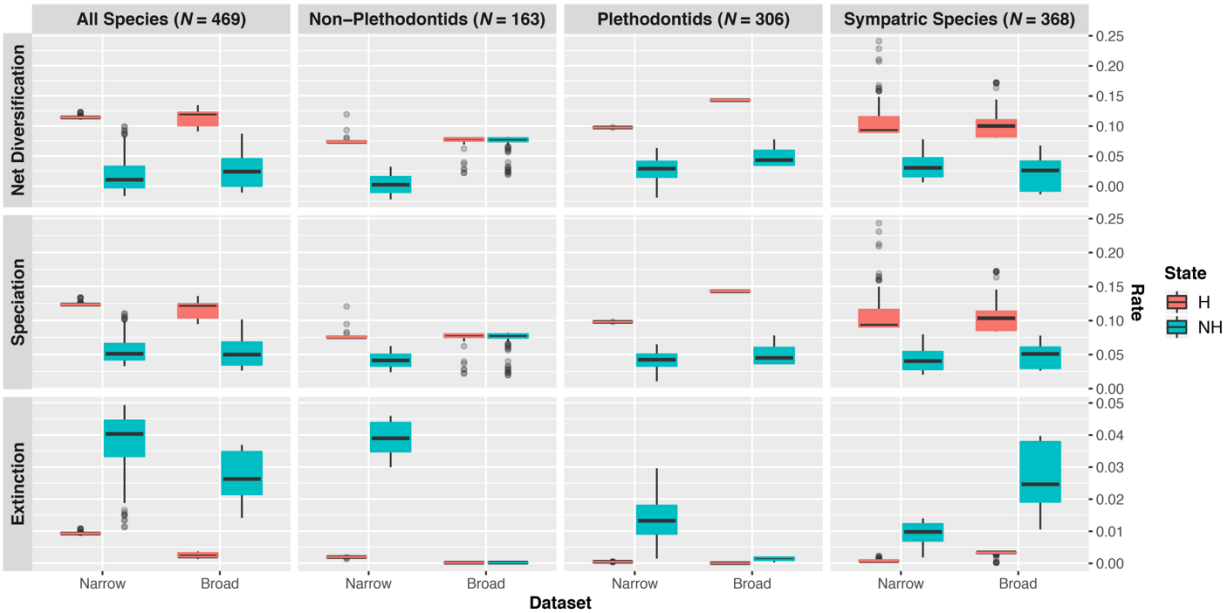


Figure 2. Model-averaged lineage-specific diversification rate estimates at the tips of the phylogeny assuming 20% of species hybridize. Results using different trees are displayed by column, whereas results for different parameters are displayed by row. Hybridizing lineages (H) are displayed in red, whereas non-hybridizing (NH) lineages are displayed in blue. Results for both the narrow and broad datasets are shown; the narrow dataset includes only instances of contemporary hybridization in nature among natively distributed species, whereas the broad datasets includes instances of historical introgression and non-natural hybridization.

Similarly, plethodontids exhibited the same patterns described above (Figs. 2 & 3; Tables 1, 2, Supplementary Tables S6-S7). Hybridizing lineages experienced rates of speciation, net-diversification and turnover that were on average 139% (130 - 148%; λ), 250% (224 - 281%; *r*), and 78% (76 - 81%; τ) greater than those experienced by non-hybridizing lineages. Extinction rate and extinction fraction were reduced by 3186% (3007 - 3366%; μ) and 9000% (8727 - 11073%; Ɛ) in hybridizing lineages relative to non-hybridizing lineages.

Definition of Hybridization	Dataset	Model	Akaike Weight	Speciation				Extinction				Net Diversification			
				NH-A	*NH-B*	*H-A*	*H-B*	*NH-A*	*NH-B*	*H-A*	*H-B*	*NH-A*	*NH-B*	*H-A*	*H-B*
Narrow	**All Species**	**HiSSE: No H-B, all transitions**	**0.715**	**0.114**	**0.033**	**0.120**	**NA**	**4.24E-7**	**0.041**	**0.010**	**NA**	**0.114**	**-0.008**	**0.110**	**NA**
		HiSSE: No double transitions	0.263	0.025	0.148	0.131	0.181	0.076	0.019	0.007	0.015	-0.051	0.129	0.124	0.166
	Sympatric Species	**HiSSE: All parameters free**	**0.980**	**0.086**	**0.019**	**0.255**	**0.078**	**1.77E-10**	**0.014**	**0.002**	**0.001**	**0.086**	**0.005**	**0.253**	**0.077**
	Plethodontids	**HiSSE: No H-B, all transitions**	**0.523**	**0.063**	**0.009**	**0.099**	**NA**	**1.31E-10**	**0.027**	**2.04E-10**	**NA**	**0.063**	**-0.018**	**0.099**	**NA**
		HiSSE: No H-B, no double transitions	0.475	0.073	0.011	0.097	NA	1.50E-10	0.001	0.033	NA	0.073	0.010	0.064	NA
	Non-plethodontids	**HiSSE: No double transitions**	**0.648**	**0.022**	**0.070**	**0.073**	**0.204**	**0.067**	**0.003**	**0.026**	**0.001**	**-0.044**	**0.068**	**0.047**	**0.203**
		HiSSE: No H-B, no double transitions	0.206	0.089	0.014	0.067	NA	1.83E-10	0.010	1.37E-10	NA	0.089	0.004	0.067	NA
		HiSSE: No H-B, all transitions	0.085	0.017	0.092	0.071	NA	0.031	0.058	0.002	NA	-0.015	0.035	0.069	NA
Broad	**All Species**	**HiSSE: No H-B, all transitions**	**0.631**	**0.106**	**0.026**	**0.065**	**0.126**	**2.19E-10**	**0.025**	**0.003**	**2.59E-10**	**0.106**	**1.00E-4**	**0.062**	**0.126**
		HiSSE: No H-B, no double transitions	0.229	0.089	0.014	0.067	NA	1.83E-10	0.010	1.37E-10	NA	0.089	0.004	0.067	NA
		HiSSE: No H-B, all transitions	0.130	0.028	0.152	0.111	NA	0.024	0.069	0.006	NA	0.004	0.083	0.106	NA
	Sympatric Species	**HiSSE: No double transitions**	**0.637**	**0.027**	**0.075**	**0.099**	**0.194**	**0.044**	**0.013**	**0.005**	**4.00E-10**	**-0.018**	**0.063**	**0.094**	**0.194**
		HiSSE: All parameters free	0.361	0.096	0.024	0.055	0.146	1.98E-10	0.032	1.13E-10	3.01E-10	0.096	-0.008	0.055	0.146
	Plethodontids	**HiSSE: No H-B, no double transitions**	**0.970**	**0.036**	**0.085**	**0.143**	**NA**	**0.002**	**1.74E-10**	**2.95E-10**	**NA**	**0.035**	**0.085**	**0.143**	**NA**
	Non-plethodontids	**CID-2: Three transitions, no double**	**0.949**	**0.014**	**0.082**	**0.014**	**0.082**	**2.86E-11**	**1.69E-10**	**2.86E-11**	**1.69E-10**	**0.014**	**0.082**	**0.014**	**0.082**

Table 2. Best-fit models assuming 20% of species hybridize. Included are models that received >5% Akaike weights for their respective analyses. For each dataset, the best fit model is bold. Maximum-likelihood parameter estimates for speciation, extinction, and net diversification are reported. Non-hybridizing is abbreviated as NH, Hybridizing as H; A and B indicate the two hidden states.

In contrast, analysis of non-plethodontids revealed greater ambiguity as to the impact of hybridization on diversification rates. Whereas the narrow dataset inferred trait-dependent diversification in which hybridization drove increased diversification, the broad dataset did not (Figs. 2 & 3; Tables 1, 2, Supplementary Tables S6-S7). Using the narrow dataset, all rates except turnover were found to differ significantly (Supplementary Tables S6-S7, S13-S14). Speciation rate and net diversification were 90% (75 - 107%; λ) and 3355% (1444 - 26573%; r) greater in hybridizing lineages, whereas extinction rate and extinction fraction were reduced by 1964% (1871 - 2062%; μ) and 4060% (3619 - 4545%; $\mathcal{E}$) in hybridizing lineages relative to non-hybridizing lineages. Analysis of the broad dataset revealed no significant differences and parameter estimates between hybridizing and non-hybridizing species.

Sister clade contrasts broadly supported results of the HiSSE analysis. That is,

hybridizing clades were found to have significantly greater species richness than non-hybridizing clades for all datasets except for plethodontid salamanders. Details on significance of these tests may be found in Supplemental Table S16.

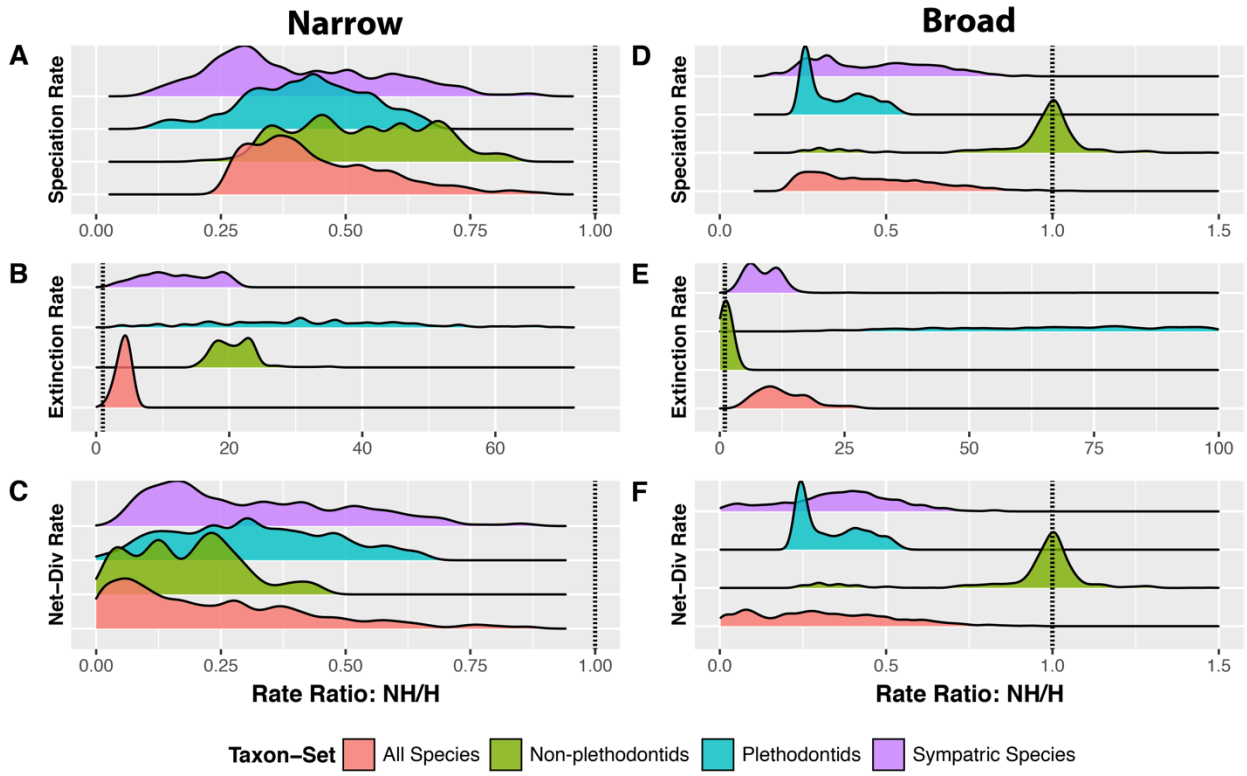


Figure 3. Comparison of model averaged parameter estimates among character states. Results reported here are those assuming 20% of species hybridize. A, B and C illustrate the distributions of non-hybridizing to hybridizing lineages diversification rates as estimated at the tips of the phylogeny using the narrow dataset, whereas D, E and F are those using the broad dataset. A value > 1 corresponds to a comparison in which non-hybridizing lineages experience rates greater than those of hybridizing lineages and vice-versa. Dotted vertical lines are placed at 1, at which rates are equal among states.

Rapidly diversifying species may have greater opportunity to hybridize due to an associated increase in species richness. We did not recover evidence supporting this interpretation. Both mean diversification rate (Adjusted R^2: $\lambda = -0.095$; $\mu = -0.084$; $r = -0.092$) and proportion of family hybridizing (Adjusted $R^2 = -0.112$) are decoupled from family species richness, despite a significant correlation (P: $\lambda = 0.0002$; $\mu = 0.0004$; $r = 0.0002$) between mean

323 diversification rate and proportion family hybridizing (Adjusted R^2: $\lambda = 0.822$; $\mu = 0.786$; $r =$

324 0.811; Supplementary Fig. S8).

325

326 **Discussion**

327 Here, we show strong support that contemporary hybridization is correlated with elevated

328 diversification rates in the order Caudata. Net-diversification of hybridizing species tends to be

329 significantly greater than that of non-hybridizing species, driven primarily by a coincident

330 increase in speciation and decrease in extinction rates (Figs. 2, 3; Tables 1, 2). The accelerated

331 diversification of hybridizing salamanders appears ephemeral, however; rate differences rapidly

332 become less pronounced deeper in the tree due to turnover of the hidden states (Fig. 1,

333 Supplementary Figs. S9-S12). Possible mechanisms leading to this result include frequent range

334 expansions and contractions (i.e. ref [45]) that have been documented in salamanders (e.g. refs

335 [46,47,48]) and the process of reinforcement which has long been recognized to contribute to the

336 diversification process[14,49,50,51]. We outline the potential contribution of each below.

337 Salamanders often exhibit substantial genetic differentiation at small geographic

338 distances (e.g., 200m[52]) owing to limited dispersal abilities and low rates of gene flow[53], thus

339 leading to an abundance of opportunities to evolve in allopatry. Additionally, terrestrial species

340 such as the *Plethodontid* salamanders of the southeastern United States experience elevational

341 range expansions and contractions associated with climatic change[48]. Perhaps this combination

342 of the primarily sessile nature of many salamander species and frequent repeated secondary

343 contact leads to hybridization occurring regularly across evolutionary timescales. Under these

344 scenarios, hybridization may then play a creative evolutionary role in the diversification process

345 similar to that observed in haplochromine cichlids[22,23]. Allopatric speciation of haplochromine

346 cichlids has occurred in lakes that frequently have dried, split, and reformed, whereas sympatric
347 speciation has occurred within lakes in which lineages exhibited extreme habitat specificity and
348 have been reproductive isolated at fine spatial scales[54]. Under these circumstances, hybridization
349 may have afforded genetic rescue from the consequences of small population size by providing
350 increased standing genetic variation and thus expedited adaptation to novel stressors, as in Lake
351 Victorian cichlids post-colonization[23].

352 There is now clear evidence for latitudinal and elevational range shifts mediated by
353 climate change[55,56,57,58,59] and a consequent increase in frequency of hybridization among
354 previously isolated taxa[60,61,62,63]. An informed understanding of the influence of hybridization on
355 macroevolutionary diversification may thus provide invaluable context for contemporary
356 processes. This possibility of climate-change mediated hybridization has already been
357 demonstrated in plethodontid salamanders (*P. shermani* & *P. teyahalee*[48]), as well as in
358 ecologically divergent subspecies of salamandrid salamanders (*S. salamandra*[64]). Thus, it seems
359 likely that salamander species worldwide, particularly those found at high elevations due to their
360 more limited potential geographic distributions, may experience a heightened frequency of
361 hybridization as climate change advances. While generalizations regarding the outcome of
362 hybridization should be made with caution[28], our study indicates that perhaps speciation
363 reversal[65,66,67] need not be the expectation. Rather, our study implies that hybridization may
364 facilitate adaptation to novel conditions under climate change, leading to diversification of new
365 salamander lineages.

366 Here, we show the novel result of a strong correlation of contemporary hybridization with
367 elevated speciation and net diversification at a large taxonomic scale. However, reinforcement,
368 defined as the strengthening of prezygotic reproductive isolation in sympatry[14], is intrinsically

369 intertwined with hybridization. Reinforcement has been documented both experimentally[49] and
370 observationally[51] to accelerate the initiation and/or completion of the speciation process[50,68,69].
371 For instance, reinforcement is likely to play an important role in the speciation process due to
372 strong interspecific sexual selection and mate choice in plethodontids[70]. Indeed, patterns of
373 sexual isolation among populations of *Plethodon jordani* and *P. teyahalee* match expectations of
374 reinforcement[14], with sexual selection being stronger in sympatry than in allopatry[71]. Although
375 we cannot currently quantify the contribution of reinforcement to diversification rate differences
376 using our data, we urge further research measuring the degree of association between
377 contemporaneous hybridization and reinforcement among taxa. Nonetheless, were reinforcement
378 to play a role in the production of the patterns observed in this study, the very occurrence/process
379 of hybridization would be the ultimate driver (i.e., cannot have reinforcement without
380 hybridization). Under such a scenario, our study design is well-suited to identify such a signal.

381 Although a generative role of hybridization is robustly supported across three of our four
382 datasets, evidence for such a role outside of the Plethodontidae is more limited (Table 1,
383 Supplementary Tables S2-S14). We find two possible explanations for this finding. Firstly, the
384 positive association between diversification rates and hybridization may be unique to
385 Plethodontid salamanders. However, family Plethodontidae is the largest extant family of
386 salamanders, comprising approximately 2/3rds of the present diversity (471 of 716 species:
387 amphibiaweb.org). Thus, our observation of hybridization facilitating the diversification process
388 applies to the majority of salamanders and implies that, at a minimum, contemporaneous
389 hybridization does not impede the diversification process of extant salamanders.

390 Secondly, it is highly probable that our analysis of non-plethodontid salamanders is
391 lacking in power. SSE models have long been known to lose much of their power when dealing

392 with small number of OTUs (trees < 300 taxa[72]). For example, for trees of 300 species, BiSSE
393 attains a power of at most 50%, with power dropping below 15% of trees of 100 taxa[72]. Our
394 phylogeny of non-plethodontids includes only 167 species; that we detected a positive
395 relationship between hybridization and speciation rates using our narrow (most conservative)
396 dataset despite such reduced power is a testament to the strength of the signal in our data.
397 Whereas our larger datasets [complete (469 spp), sympatric (368 spp) and plethodontids (306
398 spp)] have greater power, our lack of detection of a relationship between hybridization and
399 diversification rates in non-plethodontids using our broad definition of hybridization is perhaps
400 unsurprising, given the low power of the analysis (also see Supplementary Materials for an
401 elaboration of power). Although the power of HiSSE under such scenarios has not been
402 specifically established, accuracy of parameter estimation does decay with decreasing tree size[38].
403 Consequently, we cautiously interpret the results of the analysis of non-plethodontids.
404 Interestingly, sister clade comparisons consistently supported a positive relationship between
405 hybridization and species richness in non-plethodontids, despite not supporting such a
406 relationship in plethodontids (Supplementary Table S16). These results are insensitive to branch-
407 lengths, thereby ameliorating potential concerns related to the relationship between
408 hybridization, branch-lengths, and diversification rates[21].

409 Importantly, parameter estimates are largely reasonable. For instance, the greatest
410 speciation rate inferred by any analysis (Plethodontids assuming 30% of taxa hybridize using the
411 full tree: Supplementary Table S13), of 0.159 species/million years (MY) can be interpreted as a
412 waiting time, such that on average, hybridizing species speciate every 6.29 MY. Extinction rates
413 appear less reliably estimated however; some estimates functionally equal zero, leading to the
414 large percent decrease in extinction rates observed for hybridizing relative to non-hybridizing

415 species. In some cases, extinction rates in non-hybridizing taxa leads to negative net-
416 diversification rates. That being said, averaged extinction rates across all analyses for hybridizing
417 and non-hybridizing taxa led to more reasonable waiting times of 127.9 and 54.2 MY
418 respectively. Further, recent studies have documented even more negative net-diversification
419 rates than inferred herein[73]. Taken together, it appears that extinction plays an important role in
420 the diversification of salamanders, leading to a reduction in net-diversification rates towards the
421 present relative to hybridizing species.

422 An important question regarding the interpretation of our results is the relationship
423 between lineage diversification rates, species richness, and opportunity to hybridize. Because the
424 relationship between lineage diversification rate and opportunity to hybridize are not necessarily
425 independent, rapidly diversifying lineages may simply have greater opportunity to hybridize due
426 to increased diversification rates. Although a legitimate concern, we did not find evidence that
427 the increased diversification rates we observe are due to increased family-level species richness
428 leading to increased opportunity to hybridize (Supplementary Fig. S8). Further, it is unlikely that
429 non-random taxonomic sampling has biased our results, as there is no relationship between clade
430 specific sampling fraction and frequency of hybridization (Supplementary Fig. S5).

431 Although the ability of methods to accurately infer extinction rates has been debated
432 recently[40,74,75], we emphasize that our results are robust to this concern. Our central result, that
433 hybridizing lineages experience increased net diversification, is driven by both increased
434 speciation rates and decreased extinction rates. Further, in nearly all cases, the magnitude of
435 increase of speciation rate is greater than that of the decrease in extinction. Thus, our results are
436 likely robust even to inaccuracies in the estimation of extinction rate.

437 An important distinction between our study and most previous studies investigating the

438 influence that hybridization exerts on the diversification process is that of the time-scale at which
439 hybridization is being assessed. Following Seehausen's[6] landmark paper "Hybridization and
440 Adaptive Radiation," tests and discussion of his hypothesis, that ancient, widespread
441 hybridization facilitates adaptive radiation became abundant in the literature (e.g. refs
442 [16,20,21,76,77]). Whereas much of the subsequent studies focused on *ancient* hybridization, our study
443 instead focuses on the effects of *contemporary* hybridization.

444 In a pertinent study, Wiens et al.[21], tested the hybrid swarm hypothesis in the *Plethodon*
445 *glutinosus* group (indicated in Fig. 1B) using two nuclear and two mitochondrial genes. They did
446 not recover strongly supported evidence of genealogical discordance at the base of this group;
447 these results were interpreted as not being supportive of Seehausen's hypothesis. Further, they
448 identified a positive relationship between age of species and reproductive isolation. They argue
449 that the observed relationship between diversification rate and hybridization in this group was a
450 consequence of this relationship. Although a legitimate concern, we argue that this hybridization
451 is likely to still have biologically relevant consequences on diversification rates. Specifically,
452 hybridization may either 1) facilitate the divergence of these young species i.e. through
453 reinforcement/strengthening of prezygotic isolation, or 2) erode their divergence leading to
454 species collapse. Whereas the former hypothesis predicts increased speciation rates, the latter
455 predicts increased extinction rates. We find strong, consistent evidence in favor of the former.

456 We explicitly tested the hypothesis that contemporary hybridization plays a creative role
457 in the diversification process in the broadest taxonomic and temporal scale study to date, and our
458 observations strongly supported the predictions of this hypothesis. Specifically, hybridization
459 was found to be correlated with both increased speciation rates and decreased extinction rates,
460 resulting in increased net diversification rates relative to non-hybridizing lineages. Although

461 other factors certainly contribute to the observed diversification dynamics, we have shown that
462 hybridization plays a significant role, while accounting for hidden, correlated states in our
463 analysis. Nearly all studies of hybridization have focused on individual case studies in which
464 hybridization results in species collapse[66] or promotes diversification in a single species
465 group[12,13,20,25]. Such studies are necessarily limited in the extent to which their results may be
466 generalized[28], particularly because results were equivocal across studies. Consequently, we
467 advocate that our approach can be applied at broad taxonomic and evolutionary timescales to
468 facilitate robust tests of the role of hybridization in the lineage diversification process. We
469 anticipate our results are broadly generalizable to animal groups in which homoploid
470 hybridization occurs because only 17 species of salamanders are known to be polyploid[78], and
471 our dataset includes only seven (*Ambystoma mexicanum, A. barbouri*, *A. jeffersonium*, *A.*
472 *laterale*, *A. texanum*, *A. tigrinum*, and *Lissotriton vulgaris*) hybridizing polyploid taxa (none of
473 which are plethodontids). Our study adds to the growing evidence that hybridization may fuel
474 rapid diversification and is a compliment to speciation genomics studies characterizing the
475 genomic basis of this process (e.g. ref [15]). Herein we have shown that hybridization may act as a
476 generative force across a phylogenetic order, and additional studies at such macroevolutionary
477 scales are needed to determine if this pattern holds more generally across the tree of life.

478

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Author Contributions

A.H.P. and J.E. conceived of and designed the study, A.H.P. conducted all analyses, and wrote the manuscript. A.H.P, M.J.M, B.E., J.E., L.J.H, and A.S. contributed to revisions of the manuscript. A.H.P., J.E., & M.J.M. conducted the literature review and B.E. contributed to early analyses.

References

1. Barton, N. H. & Hewitt, G. M. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* **16,** 113–148 (1985).
2. Darwin, C. *On the Origin of Species by Means of Natural Selection. (Murray*. (1859).
3. Fisher, R. *The Genetical Theory of Natural Selection. (Clarendon Press*. (1930).
4. Mayr, E. *Animal species and evolution. Harvard University Press*. (1963).
5. Futuyma, D. J. On the role of species in anagenesis. *Am. Nat.* **130,** 465–473 (1987).
6. Seehausen, O. Hybridization and adaptive radiation. *Trends Ecol. Evol.* **19,** 198–207 (2004).
7. Grant, P. R. & Grant, B. R. Hybridization of bird species. *Science* **256,** 193–197 (1992).
8. Seehausen, O., Van Alphen, J. J. & Witte, F. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277,** 1808–1811 (1997).
9. Turelli, M., Barton, N. H. & Coyne, J. A. Theory and speciation. *Trends Ecol. Evol.* **16,** 330–343 (2001).
10. Buerkle, C. A., Morris, R. J., Asmussen, M. A. & Rieseberg, L. H. The likelihood of homoploid hybrid speciation. *Heredity.* **84,** 441–451 (2000).
11. Dowling, T. E. & Secor, C. L. The role of hybridization and introgression in the

diversification of animals. *Annu. Rev. Ecol. Syst.* **28,** 593–619 (1997).

12. Rieseberg, L. H., Archer, M. A. & Wayne, R. K. Transgressive segregation, adaptation and speciation. *Heredity.* **83,** 363 (1999).

13. Soltis, P. S. & Soltis, D. E. The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.* **60,** 561–588 (2009).

14. Coyne, J. A. & Orr, H. A. *Speciation. (Sinauer Associates Sunderland*. (MA, 2004).

15. Marques, D. A., Meier, J. I. & Seehausen, O. A combinatorial view on speciation and adaptive radiation. *Trends in Ecol. & Evol.* 2019.

16. Mallet, J. Hybrid speciation. *Nature* **446,** 279–83 (2007).

17. Mavárez, J. & Linares, M. Homoploid hybrid speciation in animals. *Mol. Ecol.* **17,** 4181–4185 (2008).

18. Gross, B. & Rieseberg, L. H. The ecological genetics of homoploid hybrid speciation. *J. Hered.* **96,** 241–252 (2005).

19. Schumer, M., Rosenthal, G. G. & Andolfatto, P. How common is homoploid hybrid speciation? *Evolution.* **68,** 1553–1560 (2014).

20. Mavárez, J. *et al*. Speciation by hybridization in Heliconius butterflies. *Nature* **441,** 868–871 (2006).

21. Wiens, J. J., Engstrom, T. N. & Chippindale, P. T. Rapid diversification, incomplete isolation, and the "speciation clock" in North American salamanders (genus *Plethodon*): testing the hybrid swarm hypothesis of rapid radiation. *Evolution.* **60,** 2585–2603 (2006).

22. Keller, I. *et al.* Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. *Mol. Ecol.* **22,** 2848–2863 (2013).

23. Meier, J. I. *et al.* Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nat. Commun.* **8,** 14363 (2017).

24. Grant, P. R. & Grant, B. R. *Darwin's finches on Daphne Major island*. (Princeton University Press, 2014).

25. MacLeod, A. *et al*. Hybridization masks speciation in the evolutionary history of the Galápagos marine iguana. *Proc. R. Soc. B* **282,** (2015).

26. Yakimowski, S. B. & Rieseberg, L. H. The role of homoploid hybridization in evolution: a century of studies synthesizing genetics and ecology. *Am. J. Bot.* **101,** 1247–1258 (2014).

27. Seehausen, O. *et al*. Genomics and the origin of species. *Nat. Rev. Genet.* **15,** 176–192 (2014).

28. Gompert, Z. & Buerkle, C. A. What, if anything, are hybrids: enduring truths and challenges associated with population structure and gene flow. *Evol. Appl.* **9,** 909–923 (2016).

29. Amphibiaweb.org. *Species by the numbers.* (2018). at <http://www.amphibiaweb.org/amphibian/speciesnums.html>

30. Pyron, R. A. Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Syst. Biol.* **63,** 779–797 (2014).

31. Stamatakis, A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22,** 2688–2690 (2006).

32. Smith, S. A. & O'meara, B. C. treePL: divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* **28,** 2689–2690 (2012).

33. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20,** 289–290 (2004).

34. Highton, R. Speciation in eastern North American salamanders of the genus *Plethodon*. *Annu. Rev. Ecol. Syst.* **26,** 579–600 (1995).

35. Highton, R. Geographic protein variation and speciation in the *Plethodon* dorsalis complex. *Herpetologica* **1997,** 345–356 (1997).

36. Highton, R. Geographic protein variation and speciation in the salamanders of the *Plethodon cinereus* group with the description of two new species. *Herpetologica* **1999,** 43–90 (1999).

37. Highton, R. & Peabody, R. in *The Biology of Plethodontid Salamanders.* (eds. Bruce, R. C., Jaeger, R. G. & Houck, L. D.) 31–93 (Kluwer Academic/Plenum Publishers, 2000).

38. Beaulieu, J. M. & O'Meara, B. C. Detecting hidden diversification shifts in models of trait dependent speciation and extinction. *Syst. Biol.* **65,** 583–601 (2016).

39. Stadler, T. How can we improve accuracy of macroevolutionary rate estimates? *Syst. Biol.* **62,** 321–329 (2013).

40. Beaulieu, J. M. & O'Meara, B. C. Extinction can be estimated from moderately sized molecular phylogenies. **69,** 1036–1043 (2015).

41. Rabosky, D. L. & Goldberg, E. E. Model inadequacy and mistaken inferences of trait-dependent speciation. *Syst. Biol.* **64,** 340–55 (2015).

42. Caetano, D. S., O'Meara, B. C. & Beaulieu, J. M. Hidden state models improve state-dependent diversification approaches, including biogeographical models. *Evolution.* (2018).

43. Barraclough, T. G., Harvey, P. H. & Nee, S. Rate of rbcL gene sequence evolution and species diversification in flowering plants (angiosperms). *Proc. R. Soc. B* **263,** 589–591 (1996).

44. R Core Team *R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing*. (Vienna, 2017).

45. Hewitt, G. M. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58,** 247–276 (1996).

46. Kozak, K. H., Blaine, R. A. & Larson, A. Gene lineages and eastern North American palaeodrainage basins: phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Mol. Ecol.* **15,** 191–207 (2006).

47. Shepard, D. B. & Burbrink, F. T. Phylogeographic and demographic effects of Pleistocene climatic fluctuations in a montane salamander, *Plethodon fourchensis*. *Mol. Ecol.* **18,** 2243–2262 (2009).

48. Walls, S. C. The role of climate in the dynamics of a hybrid zone in Appalachian salamanders. *Glob. Chang. Biol.* **15,** 1903–1910 (2009).

49. Rice, W. R. & Hostert, E. E. Laboratory experiments on speciation: what have we learned in 40 years? **47,** 1637–1653 (1993).

50. Servedio, M. R. & Noor, M. A. The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.* **34,** 339–364 (2003).

51. Matute, D. R. & Ortiz-Barrientos, D. Speciation: The strength of natural selection driving reinforcement. *Curr. Biol.* **24,** 955–957 (2014).

52. Zamudio, K. R. & Wieczorek, A. M. Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Mol. Ecol.* **16,** 257–274 (2007).

53. Emel, S. L. & Storfer, A. A decade of amphibian population genetic studies: synthesis and recommendations. *Conserv. Genet.* **13,** 1685–1689 (2012).

54. Rico, C. & Turner, G. F. Extreme microallopatric divergence in a cichlid species from Lake Malawi. *Mol. Ecol.* **11,** 1585–1590 (2002).

55. Parmesan, C. & Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421,** 37–42 (2003).

56. Root, T. L., *et al.* Fingerprints of global warming on wild animals and plants. *Nature* **421,** 57–60 (2003).

57. Kelly, A. E. & Goulden, M. L. Rapid shifts in plant distribution with recent climate change. *Proc. Natl. Acad. Sci.* **105,** 11823–11826 (2008).

58. Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B. & Thomas, C. D. Rapid range shifts of species associated with high levels of climate warming. *Science.* **333,**

1024–1026 (2011).

59. Elsen, P. R. & Tingley, M. W. Global mountain topography and the fate of montane species under climate change. *Nat. Clim. Chang.* **5,** 772–776 (2015).

60. Garroway C.J., *et al.* Climate change induced hybridization in flying squirrels. *Glob. Chang. Biol.* **16,** 113–121 (2010).

61. Hoffmann, A. A. & Sgrò, C. M. Climate change and evolutionary adaptation. *Nature* **470,** 479–85 (2011).

62. Chunco, A. J. Hybridization in a warmer world. *Ecol. Evol.* **4,** 2019–2031 (2014).

63. McQuillan, M. A. & Rice, A. M. Differential effects of climate and species interactions on range limits at a hybrid zone: potential direct and indirect impacts of climate change. *Ecol. Evol.* **5,** 5120–5137 (2015).

64. Pereira, R. J., Martínez-Solano, I. & Buckley, D. Hybridization during altitudinal range shifts: nuclear introgression leads to extensive cyto-nuclear discordance in the fire salamander. *Mol. Ecol.* **25,** 1551–1565 (2016).

65. Seehausen, O. Conservation: losing biodiversity by reverse speciation. *Curr. Biol.* **16,** 334-337 (2006).

66. Seehausen, O., Takimoto, G., Roy, D. & Jokela, J. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.* **17,** 30–44 (2008).

67. Vonlanthen, P. *et al*. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* **482,** 357–362 (2012).

68. Hoskin, C. J., Higgie, M., McDonald, K. R. & Moritz, C. Reinforcement drives rapid allopatric speciation. *Nature* **437,** 1353–1356 (2005).

69. Pfennig, K. S. Reinforcement as an initiator of population divergence and speciation. *Curr. Zool.* **62,** 145–154 (2016).

70. Arnold, S. J., Reagan, N. L. & Verrell., P. A. Reproductive isolation and speciation in plethodontid salamanders. *Herpetologica* **49,** 216–228 (1993).

71. Reagan, N. L. *Evolution of sexual isolation in salamanders of the genus* Plethodon *(Doctoral dissertation*. (University of Chicago, 1992).

72. Davis, W. C. & Twitty, V. C. Courtship behavior and reproductive isolation in the species of *Taricha* (Amphibia, Caudata). *Copeia* **1964,** 601–610 (1964).

73. Freyman, W. A. & Höhna, S. Stochastic character mapping of state-dependent Diversification reveals the tempo of evolutionary decline in self-compatible *Onagraceae* lineages. *Syst. Biol.* **68,** 505–519 (2019).

74. Rabosky, D. L. Extinction rates should not be estimated from molecular phylogenies. *Evolution.* **64,** 1816–1824 (2010).

75. Rabosky, D. L. Challenges in the estimation of extinction from molecular phylogenies: a response to Beaulieu and O'Meara. *Evolution.* **70,** 218–228 (2016).

76. Kearney, M. Hybridization, glaciation and geographical parthenogenesis. *Trends Ecol. Evol.* **20,** 495–502 (2005).

77. Willis, B. L., van Oppen, M. J., Miller, D. J., Vollmer, S. V & Ayre, D. J. The role of hybridization in the evolution of reef corals. *Annu. Rev. Ecol. Evol. Syst* **37,** 489–517 (2006).

78. Schmid, M., Evans, B.J. & Bogart, J. P. Polyploidy in amphibia. *Cytog. and Gen. Res.*, *145*, 315–330 (2015).

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