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[2–6]. The prevailing view in the animal literature is that hybridization constrains lineage diversification because hybrid lineages are often documented to be less fit than parentals[4,5,7]. Under this scenario, hybridization is predicted to increase extinction rates. Further, introgressive hybridization has the potential to "wash away" accumulating divergence among incompletely isolated lineages[8–10], leading to a prediction of decreased speciation rates. In contrast, the prevailing view in the plant literature is that hybridization enhances adaptive potential by introducing novel genetic and phenotypic variation[11–13]. Reinforcement, or the accumulation of post-zygotic reproductive isolation through selection against hybrids[14], has long been considered to expedite the speciation of diverging lineages[9]. Reinforcement has the potential to act even when heterosis occurs, as hybrids may be largely sterile, such as in *Triturus* newts[15,16]. Additionally, hybridization-mediated shuffling of old genetic variants may fuel rapid diversification, as outlined by the combinatorial view of speciation[17]. Accordingly, hybridization is predicted to increase speciation rates and/or decrease extinction rates.

Whereas evidence of hybrid speciation in plants has long been abundant, evidence for widespread hybrid speciation in animals is relatively scarce[6,10,18–21]. In allopolypoid hybrid speciation, a mode of speciation common in plants but rare in animals, nearly complete reproductive isolation may evolve in a single generation due to a change in ploidy[11]. In contrast, homoploid hybrid speciation typically has to occur in the face of continued gene flow, which acts to homogenize the diverging hybrid lineages[10].

Interest in hybrid-mediated speciation has recently burgeoned, but studies have typically

been limited in taxonomic scope. Studies of the effect of hybridization on diversification have most commonly been conducted among closely related pairs or small clades of taxa, and results have been equivocal[1,22–28]. Additionally, studies of hybridization often occur along different stages of the speciation continuum[29], whereby hybridization can appear as a force that either facilitates or impedes speciation. Recent work highlights this uncertainty by demonstrating that the outcomes of hybridization depend on the underlying nature of selection pressures and demography[30]. We suggest that studies at broad phylogenetic and macroevolutionary timescales can help overcome these limitations by providing a phylogenetic context in which to view repeated hybridization events over evolutionary timescales and the consequent impact on lineage diversification rates.

Here, we conduct a taxonomically-broad test of the relationship of hybridization with macroevolutionary diversification rates. We study salamanders (order Caudata, ca. 716 spp as of October 2018[31]), which are particularly suitable for this study because hybridization is pervasive and has been studied extensively (i.e., nearly 1/3 of N. American species hybridize: Supplementary Fig. S1, Supplementary Table S1). Additionally, sufficient sequence data are available to resolve the phylogenetic relationships among most (~63%) taxa within this group[32].

Salamanders present a valuable case study of hybridization, as numerous groups are comprised of speciose, yet morphologically and ecologically conservative species[23,33–38]. As a consequence of this frequent morphological and ecological conservatism, many species have come to be described on the basis of molecular differentiation (e.g. [39–44]). Many salamander groups have diversified through primarily non-ecological means of speciation (i.e. [23,33,35,45–48]). Salamander species have often diverged in allopatry/parapatry following restriction to refugial, isolated populations during periods of climatic fluctuations or via orogeny of mountain ranges

75 (e.g. [49–51]). Following the evolution of incomplete reproductive isolation, young, diverging
76 lineages may then come into secondary contact and hybridize (e.g. [23]). If salamanders are indeed
77 predisposed to the evolution of incomplete reproductive isolation, then hybridization may play
78 an important role in their diversification.

79 If hybridization plays a meaningful role in the diversification process, differences in
80 diversification rates among hybridizing and non-hybridizing taxa are expected. Thus, we test the
81 hypothesis that there is a difference in diversification rates (speciation and extinction rates)
82 between contemporaneously hybridizing and non-hybridizing salamander lineages. Note that we
83 are simply testing whether contemporary hybridization influences diversification rates, not
84 whether ancient hybridization facilitated the present radiation as postulated by the hybrid swarm
85 hypothesis[6,52], because our experimental design cannot address this (See supplement). We
86 replicate this test across four datasets to investigate the robustness of our results: 1) including all
87 available data; 2) exclusion of species that do not exhibit sympatry (defined as <10% geographic
88 range overlap) thereby lacking the opportunity to hybridize; 3) only the family Plethodontidae,
89 which are the most widely hybridizing and diverse of the 10 salamander families; 4) all (nine)
90 salamander families except the Plethodontidae.

91

92 **Materials and Methods**

93 *Data Collection*

94 We used the time-calibrated phylogeny of Amphibia[32] as the source for downstream analyses.
95 This tree[32] was constructed using nine nuclear genes and three mitochondrial genes as data using
96 RAxMLv7.2.8[53], and time-calibrated using treePL[54]. Using the APE package (v.3.4[55]) in R, we
97 extracted the subtree containing salamanders for subsequent analyses. This approach yielded a

98 tree containing 469 of 716 extant species of salamanders (Supplementary Fig. S1[32]) representing
99 approximately two-thirds of the known diversity.

100 Each species was scored as 'non-hybridizable' (NH) or 'hybridizable' (H), based on an
101 extensive literature review of hybridization using the search engines Google Scholar and ISI
102 Web of Knowledge. To do so we paired each species with the terms 'hybrid' and 'introgress' as
103 well as dialectical and structural variants thereof (e.g., 'hybridization', 'introgressed') to search
104 for cases of hybridization. Criteria for hybridizability under the first, "narrow" definition were
105 the documentation of hybridization among natively distributed species, specifically the
106 observation of heterospecific mating or hybrid offspring in the wild as detected by substantial
107 and repeated morphological *and* molecular intermediacy. Criteria for hybridizability under the
108 second, "broad" definition included those criteria described previously, as well as: 1) the
109 observation of hybridization occurring in laboratory settings or among introduced and native
110 species, and/or; 2) inference of historical introgression as determined using molecular lines of
111 evidence (e.g. substantial and replicated genealogical discordance among molecular markers or
112 detection via Approximate Bayesian Computation methods). This latter definition is less
113 conservative than the former. We excluded cases where one or two individuals exhibited cyto-
114 nuclear discordance between markers such as allozymes and mtDNA (as observed for members
115 of *Sirenidae*[56], and several Hynobiids[57–60]), as we did not perceive these cases to meet the criteria
116 of substantial and replicated genealogical discordance. Although inference of hybridization via
117 the detection of genealogical discordance warrants caution, our narrow definition of
118 hybridization does not recognize these species as hybridizable. In total, we retrieved 56 papers
119 (date-range: 1957-2017: Supplementary Table S1). We confirmed hybridization for roughly 11
120 and 13 percent of extant Caudates for the narrow and broad datasets, respectively (78 and 92 of

121 716 species: 17 and 20 percent of sampled taxa; Supplementary Table S1). Documented
122 hybridization was absent from four families (Cryptobranchidae, Sirenidae, Proteidae,
123 Rhyacotritonidae) in the narrow dataset and two families (Rhyacotritonidae, Sirenidae) in the
124 broad dataset.

125 In addition to analyzing the entire salamander subclade from the Pyron[32] amphibian
126 phylogeny (469 total species), we compared diversification rates using three additional datasets,
127 produced using both the narrow and broad datasets (i.e., for a total of eight datasets). In the first
128 dataset, we required that "non-hybridizable" taxa were sympatric (>10% range overlap) with
129 another species, thus possessing sufficient opportunity to hybridize. Consequently, species that
130 were not sympatric with any other salamander taxa were excluded from the dataset. Therefore,
131 species classified as non-hybridizing in this analysis may have limited opportunity to hybridize
132 but have not been observed to do so. Although we cannot account for historical species
133 distributions (e.g. species' ranges may have previously overlapped), our primary (narrow)
134 definition of hybridizability necessitates contemporary hybridization. Thus, our designation of
135 sympatry occurs at the same time scale as our designation of hybridizability.

136 In the second and third datasets, we tested for a family-specific effect of hybridization on
137 diversification rates. As plethodontids are the most diverse family of salamanders (Fig. 1B) and
138 have been the subject of extensive study[37], the greatest number of instances of hybridization have
139 been documented in this group (Table S1). Additionally, many plethodontids of the genus
140 *Plethodon* in the Eastern United States have been described as species on the basis of a threshold
141 genetic distance (e.g.. [39–42]). Coincidentally, nearly half of these species have been observed to
142 hybridize in nature. To test for an effect of this relative taxonomic oversplitting, we excluded the
143 *Plethodon glutinosus* group (Figure 1B) and repeated analyses under both the narrow and broad

144 definitions of hybridization (see Supplement for additional details). Thus, we produced datasets
145 including either only members of family Plethodontidae, or the nine families that exclude
146 plethodontid salamanders. In total, ten analyses were conducted, using five trees for each of the
147 narrow and broad datasets, respectively. Information on the total number of species included in
148 each tree, as well as the number of species in each state (i.e., H or NH) may be found in
149 Supplementary Table S1.

150 *Assessment of Trait Dependent Diversification*

151 We applied the HiSSE (Hidden State Speciation & Extinction[61]) trait-dependent diversification
152 model to test for differences in speciation and extinction rates between hybridizing and non-
153 hybridizing lineages. HiSSE infers speciation and extinction rates for a binary character while
154 allowing for heterogeneity in diversification rate to exist within each character state. In the
155 HiSSE model, hidden states are co-distributed with the trait of interest and account for
156 unsampled traits that may simultaneously contribute to the diversification process. The hidden
157 state need not be associated with any single trait but may instead be associated with a set of traits
158 or suite of traits. Inclusion of the hidden state thus ameliorates the confounding effects of
159 unsampled traits on diversification rate estimations by allowing for greater rate heterogeneity in
160 the tree than in previous SSE models[61]. Thus, we are in essence measuring the impact of
161 contemporary hybridization while controlling for other, correlated traits on diversification rate.
162 Finally, by accounting for increased rate heterogeneity in character-dependent and character-
163 independent (null) models through the inclusion of hidden states, model rejection properties are
164 greatly improved[61] relative to previous SSE models.

165 We evaluated a total of 14 competing models using HiSSE, half of which represent a
166 model of character-dependent diversification and the remaining half represented models of

167 character independence (Supplementary Tables S2-S9). Models of trait dependence varied in the
168 number of hidden states included and in the number of free transitions among states. In
169 character-dependent models, our four states where Hybridizing (H) and Non-hybridizing (NH),
170 each being associated with one of the two hidden state (A & B) for four total character
171 states/diversification regimes (H-**A**, H-**B**, NH-**A**, NH-**B**).

172 Additionally, to account for the fact that diversification rates may be biased by
173 incomplete sampling of extant diversity within a phylogeny[62–64], we assume that 20% of extant
174 species of salamanders hybridize in nature. This value was chosen because it approximately
175 equals the mean frequency of hybridization across our datasets (19.75). To explore the effect this
176 assumption had on our results, we repeated these analyses assuming 1) we have sampled all
177 extant hybridizing species (Supplementary Fig. S2), 2) our sampling of character states is
178 proportional to their prevalence in nature (Supplementary Fig. S3), and 3) that 30% of extant
179 species hybridize (Supplementary Fig. S4). Thus, while unable to designate all hybridizing taxa
180 as such in our phylogeny due to their not being represented in the literature, we have explicitly
181 addressed this uncertainty in our analysis. We elaborate upon our choice of sampling fractions in
182 the Supplementary Materials.

183 To assess whether the inclusion of extremely young species impacted our diversification
184 rate estimates, we ran a single analysis excluding species younger than 1MY, as per Beaulieu &
185 O'Meara[61] using the narrow dataset and all species. This in turn led to the removal of 14 species.
186 Results were qualitatively identical to those obtained including these young species, so all
187 subsequent analyses were conducted including them. To improve the performance of the
188 Maximum Likelihood optimization procedure implemented in HiSSE, we used simulated
189 annealing to first traverse the likelihood surface to identify optimal starting values for subsequent

190 ML-optimization. Rather than reporting the results of individual model fits, we instead take the
191 approach of investigating model-averaged parameter estimates for each sampled character state[65]
192 (see Supplementary Information for further justification). That is, parameter estimates obtained
193 from each fitted model are averaged together such that their contribution to the average is
194 proportional to their relative support (Akaike weights) among the set of candidate models
195 (Supplementary Table S13). This leads the best supported models to have the greatest impact on
196 the final model averaged parameter estimates. Diversification rates are returned for each sampled
197 state respectively, as not all models include hidden states.

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

206 In summary, four phylogenies (all species, sympatric species, plethodontids, & non-
207 plethodontids) were analyzed using two datasets (narrow & broad definitions of hybridization).
208 Each data/tree-set combination was tested assuming the three aforementioned differences in
209 prevalence of hybridization in nature. In all, a total of 32 rounds of model testing were
210 performed, comparing seven models of trait-independent diversification and seven models of
211 trait-dependent diversification for each sampling fraction. Further information on how these
212 data/tree-sets were produced and analyzed may be found in the Supplementary Materials. Lastly,

213 a description of our test of sensitivity to phylogenetic uncertainty may also be found in the
214 Supplement (Supplementary Fig. S6 & S7; Supplementary Table S15)

215 To complement our HiSSE analyses in a manner that is largely insensitive to the
216 potentially confounding relationship between branch lengths and propensity to hybridize (see
217 discussion), we conducted sister clade comparisons. Specifically, using all comparisons of three
218 or more taxa (i.e. two sister species hybridize, and the sister lineage does not), we used the
219 method of Barraclough, Harvey and Nee[66] to test the hypothesis that hybridizing clades had
220 greater richness than non-hybridizing clades. To assess confidence, 1000 permutations of
221 contrast signs were conducted. This test was repeated for each of the eight datasets described
222 above (Supplementary Table S16).

223 To test for possible circularity of causality between diversification rates, species richness
224 and opportunity to hybridize, we quantified the relationships between the three. Specifically, we
225 tested for a relationship between 1) mean diversification rates and the proportion family
226 hybridizing, 2) mean diversification rates and species richness, and 3) proportion family
227 hybridizing and species richness (Supplementary Fig. S8). All analyses were conducted at the
228 family level, and mean diversification rates were obtained using lineage-specific model-averaged
229 diversification (speciation, extinction, net-diversification) rates. Simple linear regressions were
230 conducted in R v3.6.1[67].

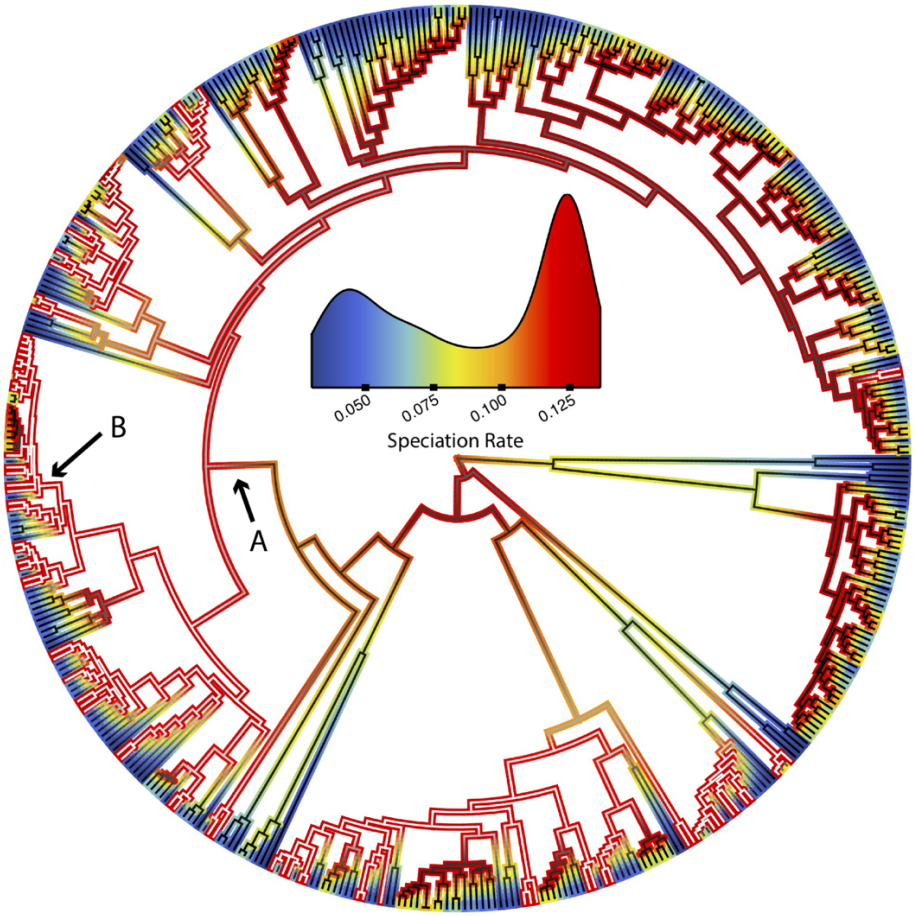

Figure 1. Inferred speciation rate along the salamander phylogeny for both hybridizing and non-hybridizing species. Branch outlines depict speciation rates corresponding to the inset density plot. Branch interiors depict the probability that a lineage is hybridizable (white) or non-hybridizable (black).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

Results

Across both datasets (narrow and broad) assuming 20% of species hybridize, three of four analyzed phylogenies consistently found that hybridizing species experience increased speciation rates, decreased extinction rates, and therefore increased net-diversification rates (all significant: Figs. 1, 2 & 3; Tables 1, 2, Supplementary Tables S6 & S7). Net-diversification of hybridizing lineages in these trees were on average 4X greater than that of non-hybridizing lineages. Our

results were insensitive to phylogenetic uncertainty (Supplementary Fig. S7; Supplementary Table S15).

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

267 expected waiting time of 128 million years before the next net-loss in diversity. These negative
268 rates are not persistent however; examination of ancestral state reconstructions indicates that the
269 hidden state responsible (NH-B) for these rates is distributed primarily along the tips
270 (Supplementary Figs S9 – S12).

271 As the average magnitude of increase in speciation rate across all 32 analyses (95% CI:
272 0.0557 ± 0.0059 species/MY) is significantly greater than the average decrease in extinction rate
273 (95% CI: 0.0145 ± 0.0037 species/MY), we conclude it is primarily differential speciation that is
274 driving the increase in net-diversification in hybridizing lineages. Interpreted as a waiting time,
275 this means that hybridizing species, on average, speciate every 9.2 (95% CI: 8.84 - 9.62) million
276 years and go extinct every 160.7 (118.97 – 247.96) million years.

277 Analysis of the tree containing all species recovered strong signal of trait dependent
278 diversification, with significant differences between hybridization or non-hybridizing lineages
279 (as measured by empirical P-values) found between states for all five parameter estimates (Figs.
280 2 & 3; Tables 1, 2, Supplementary Tables S6 – S7), regardless of whether the broad or narrow
281 criterion was used. Speciation (λ), net-diversification ($r = \lambda - \mu$), and turnover rate ($\tau = \lambda + \mu$)
282 were greater in hybridizing than non-hybridizing lineages, whereas extinction rate (μ) and
283 extinction fraction ($\varepsilon = \mu \div \lambda$) were lower. Increases were on average 125% (95% CI: 117 -
284 133%) for speciation, 576% (486 - 699%) for net diversification and 43% (41 - 45%) for
285 turnover. In contrast, decreases were on average 305% (292 - 317%) for extinction and 945%
286 (893 - 997%) for extinction fraction (Fig. 2). Additional analyses that assume different
287 percentages of extant species hybridize produced qualitatively similar results and are discussed
288 in the supplement (Supplementary Figs. S2-S4, Supplementary Tables S2-14).

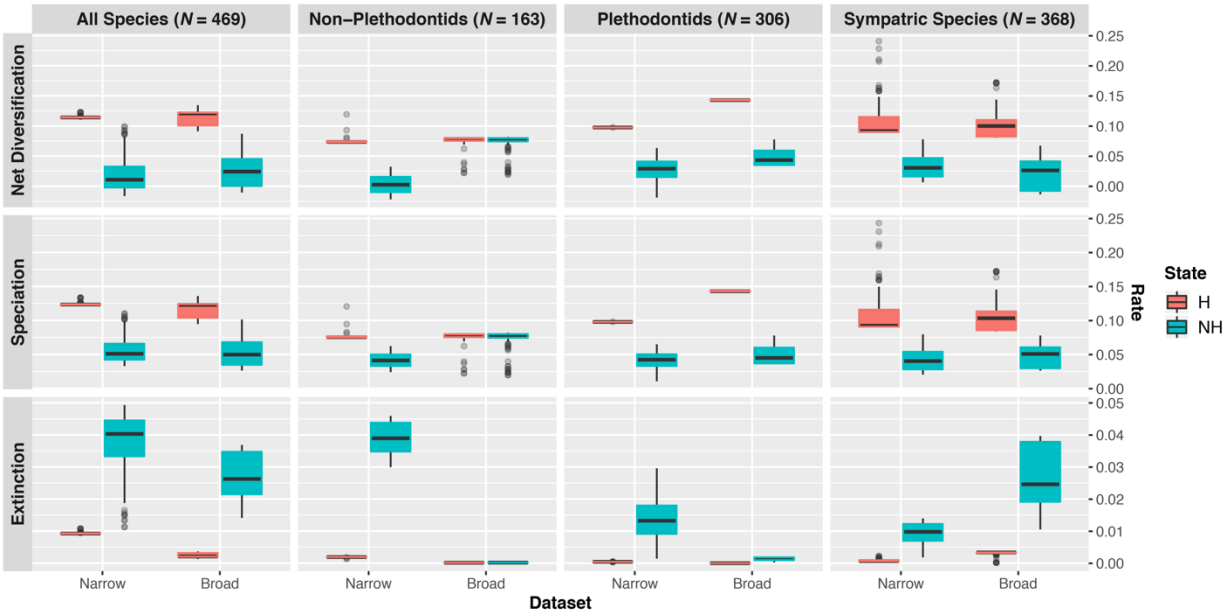

Figure 2. Model-averaged lineage-specific diversification rate estimates at the tips of the phylogeny assuming 20% of species hybridize. Results using different trees are displayed by column, whereas results for different parameters are displayed by row. Hybridizing lineages (H) are displayed in red, 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.

Analysis of the tree including only sympatric taxa regardless of the hybridization criterion (narrow versus broad) datasets recovered patterns identical to those obtained using the 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.

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%; Ɛ) 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.

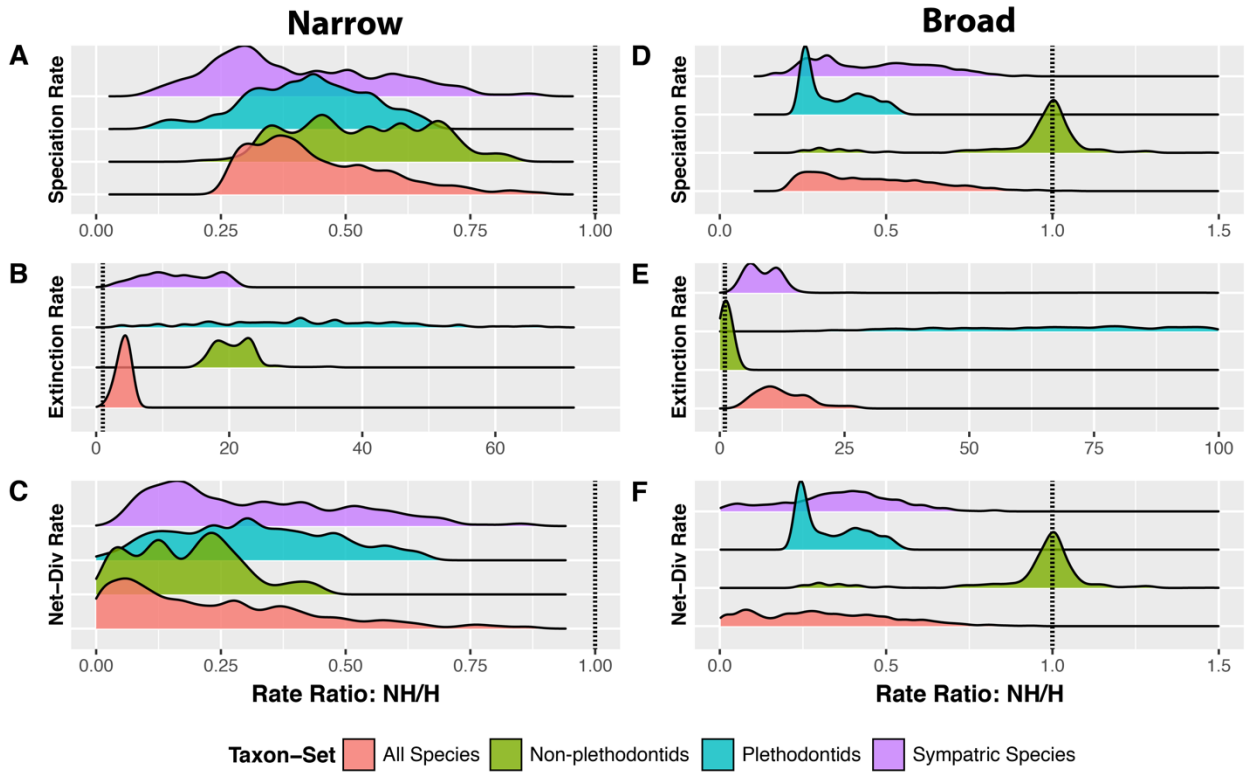

Figure 3. Comparison of model averaged parameter estimates among character states. Results reported here are those assuming 20% of species hybridize. A, B and C illustrate the distributions of non-hybridizing to hybridizing lineages diversification rates as estimated at the tips of the phylogeny using 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 diversification rate and proportion family hybridizing (Adjusted R^2: $\lambda = 0.822$; $\mu = 0.786$; $r = 0.811$; Supplementary Fig. S8).

Discussion

Here, we show strong support that contemporary hybridization is correlated with elevated diversification rates in the order Caudata. Net-diversification of hybridizing species tends to be significantly greater than that of non-hybridizing species, driven primarily by a coincident increase in speciation and decrease in extinction rates (Figs. 2, 3; Tables 1, 2). The accelerated diversification of hybridizing salamanders appears ephemeral, however; rate differences rapidly become less pronounced deeper in the tree due to turnover of the hidden states (Fig. 1, Supplementary Figs. S9-S12). Possible mechanisms leading to this result include frequent range expansions and contractions (i.e. [68]) that have been documented in salamanders (e.g. [69–71]) and the process of reinforcement which has long been recognized to contribute to the diversification process[14,72–74]. We outline the potential contribution of each below.

Salamanders often exhibit substantial genetic differentiation at small geographic distances (e.g., 200m[75]) owing to limited dispersal abilities and low rates of gene flow[76], thus leading to an abundance of opportunities to evolve in allopatry. Additionally, terrestrial species such as the *Plethodontid* salamanders of the southeastern United States experience elevational range expansions and contractions associated with climatic change[71]. Perhaps this combination of the primarily sessile nature of many salamander species and frequent repeated secondary contact leads to hybridization occurring regularly across evolutionary timescales. Under these

368 scenarios, hybridization may then play a creative evolutionary role in the diversification process
369 similar to that observed in haplochromine cichlids[24,25,77]. Allopatric speciation of haplochromine
370 cichlids has occurred in lakes that frequently have dried, split, and reformed, whereas sympatric
371 speciation has occurred within lakes in which lineages exhibited extreme habitat specificity and
372 have been reproductive isolated at fine spatial scales[78]. Under these circumstances, hybridization
373 may have afforded genetic rescue from the consequences of small population size by providing
374 increased standing genetic variation and thus expedited adaptation to novel stressors, as in Lake
375 Victorian cichlids post-colonization[25].

376 Such hypotheses of repeated contractions, expansions, and secondary contact of
377 salamander populations have been well supported, across both North[79–81] and South America[50,82],
378 as well as in Europe[83–85]. Often associated with glaciation/deglaciation or orogeny of mountain
379 ranges, geological events may act as species pumps for salamanders (i.e. [86]). However, while
380 periodic geographic range expansion and contraction may initiate speciation, the reproductive
381 isolation that evolves may be incomplete, predisposing the young species/diverging lineages to
382 hybridization (i.e. [23]). As a result, hybridization may commonly occur in salamanders during
383 periods of climatic fluctuations.

384 There is now clear evidence for latitudinal and elevational range shifts mediated by
385 climate change[87–91] and a consequent increase in frequency of hybridization among previously
386 isolated taxa[92–95]. An informed understanding of the influence of hybridization on
387 macroevolutionary diversification may thus provide invaluable context for contemporary
388 processes. This possibility of climate-change mediated hybridization has already been
389 demonstrated in plethodontid salamanders (*P. shermani* & *P. teyahalee*[71]), as well as in
390 ecologically divergent subspecies of salamandrid salamanders (*S. salamandra*[96]). Thus, it seems

391 likely that salamander species worldwide, particularly those found at high elevations due to their
392 more limited potential geographic distributions, may experience a heightened frequency of
393 hybridization as climate change advances. While generalizations regarding the outcome of
394 hybridization should be made with caution[30], our study indicates that perhaps speciation
395 reversal[97–99] need not be the expectation. Rather, our study implies that hybridization may
396 facilitate adaptation to novel conditions under climate change, leading to diversification of new
397 salamander lineages.

398 Here, we show the novel result of a strong correlation of contemporary hybridization with
399 elevated speciation and net diversification at a large taxonomic scale. However, reinforcement,
400 defined as the strengthening of prezygotic reproductive isolation in sympatry[14], is intrinsically
401 intertwined with hybridization. Reinforcement has been documented both experimentally[72] and
402 observationally[74] to accelerate the initiation and/or completion of the speciation process[73,100,101].
403 For instance, reinforcement is likely to play an important role in the speciation process due to
404 strong interspecific sexual selection and mate choice in plethodontids[102]. Indeed, patterns of
405 sexual isolation among populations of *Plethodon jordani* and *P. teyahalee* match expectations of
406 reinforcement[14], with sexual selection being stronger in sympatry than in allopatry[103]. Although
407 we cannot currently quantify the contribution of reinforcement to diversification rate differences
408 using our data, we urge further research measuring the degree of association between
409 contemporaneous hybridization and reinforcement among taxa. Nonetheless, were reinforcement
410 to play a role in the production of the patterns observed in this study, the very occurrence/process
411 of hybridization would be the ultimate driver (i.e., cannot have reinforcement without
412 hybridization). Under such a scenario, our study design is well-suited to identify such a signal.

413 Although a generative role of hybridization is robustly supported across three of our four

414 datasets, evidence for such a role outside of the Plethodontidae is more limited (Table 1,
415 Supplementary Tables S2-S14). We find two possible explanations for this finding. Firstly, the
416 positive association between diversification rates and hybridization may be unique to
417 Plethodontid salamanders. However, family Plethodontidae is the largest extant family of
418 salamanders, comprising approximately 2/3rds of the present diversity (471 of 716 species:
419 amphibiaweb.org). Thus, our observation of hybridization facilitating the diversification process
420 applies to the majority of salamanders and implies that, at a minimum, contemporaneous
421 hybridization does not impede the diversification process of extant salamanders.

422 Secondly, it is highly probable that our analysis of non-plethodontid salamanders is
423 lacking in power. SSE models have long been known to lose much of their power when dealing
424 with small number of OTUs (trees < 300 taxa[104]). For example, for trees of 300 species, BiSSE
425 attains a power of at most 50%, with power dropping below 15% of trees of 100 taxa[104]. Our
426 phylogeny of non-plethodontids includes only 167 species; that we detected a positive
427 relationship between hybridization and speciation rates using our narrow (most conservative)
428 dataset despite such reduced power is a testament to the strength of the signal in our data.
429 Whereas our larger datasets [complete (469 spp), sympatric (368 spp) and plethodontids (306
430 spp)] have greater power, our lack of detection of a relationship between hybridization and
431 diversification rates in non-plethodontids using our broad definition of hybridization is perhaps
432 unsurprising, given the low power of the analysis (also see Supplementary Materials for an
433 elaboration of power). Although the power of HiSSE under such scenarios has not been
434 specifically established, accuracy of parameter estimation does decay with decreasing tree size[61].
435 Consequently, we cautiously interpret the results of the analysis of non-plethodontids (163 spp).
436 Interestingly, sister clade comparisons consistently supported a positive relationship between

437 hybridization and species richness in non-plethodontids, despite not supporting such a
438 relationship in plethodontids (Supplementary Table S16). These results are insensitive to branch-
439 lengths, thereby ameliorating potential concerns related to the relationship between
440 hybridization, branch-lengths, and diversification rates[23].

441 Importantly, parameter estimates are largely reasonable. For instance, the greatest
442 speciation rate inferred by any analysis (Plethodontids assuming 30% of taxa hybridize using the
443 full tree: Supplementary Table S13), of 0.159 species/million years (MY) can be interpreted as a
444 waiting time, such that on average, hybridizing species speciate every 6.29 MY. Extinction rates
445 appear less reliably estimated however; some estimates functionally equal zero, leading to the
446 large percent decrease in extinction rates observed for hybridizing relative to non-hybridizing
447 species. In some cases, extinction rates in non-hybridizing taxa leads to negative net-
448 diversification rates. That being said, averaged extinction rates across all analyses for hybridizing
449 and non-hybridizing taxa led to more reasonable waiting times of 127.9 and 54.2 MY
450 respectively. Further, recent studies have documented even more negative net-diversification
451 rates than inferred herein[105]. Taken together, it appears that extinction plays an important role in
452 the diversification of salamanders, leading to a reduction in net-diversification rates towards the
453 present relative to hybridizing species.

454 An important question regarding the interpretation of our results is the relationship
455 between lineage diversification rates, species richness, and opportunity to hybridize. Because the
456 relationship between lineage diversification rate and opportunity to hybridize are not necessarily
457 independent, rapidly diversifying lineages may simply have greater opportunity to hybridize due
458 to increased diversification rates. Although a legitimate concern, we did not find evidence that
459 the increased diversification rates we observe are due to increased family-level species richness

460 leading to increased opportunity to hybridize (Supplementary Fig. S8). Further, it is unlikely that
461 non-random taxonomic sampling has biased our results, as there is no relationship between clade
462 specific sampling fraction and frequency of hybridization (Supplementary Fig. S5).

463 Although the ability of methods to accurately infer extinction rates has been debated
464 recently[63,106,107], we emphasize that our results are robust to this concern. Our central result, that
465 hybridizing lineages experience increased net diversification, is driven by both increased
466 speciation rates and decreased extinction rates. Further, in nearly all cases, the magnitude of
467 increase of speciation rate is greater than that of the decrease in extinction. Thus, our results are
468 likely robust even to inaccuracies in the estimation of extinction rate.

469 An important distinction between our study and most previous studies investigating the
470 influence that hybridization exerts on the diversification process is that of the time-scale at which
471 hybridization is being assessed. Following Seehausen's[6] landmark paper "Hybridization and
472 Adaptive Radiation," tests and discussion of his hypothesis, that ancient, widespread
473 hybridization facilitates adaptive radiation became abundant in the literature (e.g. [18,22,23,108,109]).
474 Whereas much of the subsequent studies focused on *ancient* hybridization, our study instead
475 focuses on the effects of *contemporary* hybridization.

476 In a pertinent study, Wiens et al.[23], tested the hybrid swarm hypothesis in the *Plethodon*
477 *glutinosus* group (indicated in Fig. 1B) using two nuclear and two mitochondrial genes. They did
478 not recover strongly supported evidence of genealogical discordance at the base of this group;
479 these results were interpreted as not being supportive of Seehausen's hypothesis. Further, they
480 identified a positive relationship between age of species and reproductive isolation. They argue
481 that the observed relationship between diversification rate and hybridization in this group was a
482 consequence of this relationship. Although a legitimate concern, we argue that this hybridization

483 is likely to still have biologically relevant consequences on diversification rates. Specifically,
484 hybridization may either 1) facilitate the divergence of these young species i.e. through
485 reinforcement/strengthening of prezygotic isolation, or 2) erode their divergence leading to
486 species collapse. Whereas the former hypothesis predicts increased speciation rates, the latter
487 predicts increased extinction rates. We find strong, consistent evidence in favor of the former.

488 We explicitly tested the hypothesis that contemporary hybridization plays a creative role
489 in the diversification process in the broadest taxonomic and temporal scale study to date, and our
490 observations strongly supported the predictions of this hypothesis. Specifically, hybridization
491 was found to be correlated with both increased speciation rates and decreased extinction rates,
492 resulting in increased net diversification rates relative to non-hybridizing lineages. Although
493 other factors certainly contribute to the observed diversification dynamics, we have shown that
494 hybridization plays a significant role, while accounting for hidden, correlated states in our
495 analysis. Nearly all studies of hybridization have focused on individual case studies in which
496 hybridization results in species collapse[98] or promotes diversification in a single species
497 group[12,13,22,27]. Such studies are necessarily limited in the extent to which their results may be
498 generalized[30], particularly because results were equivocal across studies. Consequently, we
499 advocate that our approach can be applied at broad taxonomic and evolutionary timescales to
500 facilitate robust tests of the role of hybridization in the lineage diversification process. We
501 anticipate our results are broadly generalizable to animal groups in which homoploid
502 hybridization occurs because only 17 species of salamanders are known to be polyploid[110], and
503 our dataset includes only seven (*Ambystoma mexicanum, A. barbouri*, *A. jeffersonium*, *A.*
504 *laterale*, *A. texanum*, *A. tigrinum*, and *Lissotriton vulgaris*) hybridizing polyploid taxa (none of
505 which are plethodontids). Our study adds to the growing evidence that hybridization may fuel

506 rapid diversification (e.g. [52]) and is a compliment to speciation genomics studies characterizing
507 the genomic basis of this process (e.g. [17,111]). Herein we have shown that hybridization may act
508 as a generative force across a phylogenetic order, and additional studies at such
509 macroevolutionary scales are needed to determine if this pattern holds more generally across the
510 tree of life.

511

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517

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521 **Author Contributions**

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

526

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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 (80-.).* **256,** 193–197 (1992).
8. Seehausen, O., Van Alphen, J. J. & Witte, F. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science (80-.).* **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 (Edinb).* **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 (Edinb).* **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. Francillon-Vieillot, H., Arntzen, J. W. & Geraudie, J. Age, Growth and Longevity of Sympatric Triturus cristatus, T. marmoratus and Their Hybrids (Amphibia, Urodela): A Skeletochronological Comparison. *J. Herpetol.* **24,** 13 (1990).
16. Arntzen, J. W., Jehle, R., Bardakci, F., Burke, T. & Wallis, G. P. Asymmetric Viability of Reciprocal-Cross Hybrids Between Crested and Marbled News (Triturus cristatus and T. marmoratus). *Evolution (N. Y).* **63,** 1191–1202 (2009).
17. Marques, D. A., Meier, J. I. & Seehausen, O. *A combinatorial view on speciation and adaptive radiation*. (Trends in ecology & evolution, 2019).
18. Mallet, J. Hybrid speciation. *Nature* **446,** 279–83 (2007).
19. Mavárez, J. & Linares, M. Homoploid hybrid speciation in animals. *Mol. Ecol.* **17,** 4181–4185 (2008).
20. Gross, B. & Rieseberg, L. H. The ecological genetics of homoploid hybrid speciation. *J. Hered.* **96,** 241–252 (2005).
21. Schumer, M., Rosenthal, G. G. & Andolfatto, P. How common is homoploid hybrid speciation? *Evolution (N. Y).* **68,** 1553–1560 (2014).
22. Mavárez, J. *et al.* Speciation by hybridization in Heliconius butterflies. *Nature* **441,** 868–871 (2006).
23. Wiens, J. J., Engstrom, T. N. & Chippindale, P. T. Rapid diversification, incomplete isolation, and the ``speciation clock'' in North {A}merican salamanders (genus Plethodon): testing the hybrid swarm hypothesis of rapid radiation. *Evolution (N. Y).* **60,** 2585–2603 (2006).
24. 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).

25. Meier, J. I. *et al.* Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nat. Commun.* **8,** 14363 (2017).
26. Grant, P. R. & Grant, B. R. *No Title*. (Darwin's finches on Daphne Major island. (Princeton University Press, 2014).
27. MacLeod, A. *et al.* Hybridization masks speciation in the evolutionary history of the Galápagos marine iguana. *Proc. R. Soc. B* **282,** 20150 (2015).
28. 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).
29. Seehausen, O. & others. Genomics and the origin of species. *Nat. Rev. Genet.* **15,** 176–192 (2014).
30. 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).
31. Berkeley, U. of C. *Species by the numbers.* (2018). at <http://www.amphibiaweb.org/amphibian/speciesnums.html>
32. Pyron, R. A. Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Syst. Biol.* **63,** 779–797 (2014).
33. Wiens, J. J. Speciation and Ecology Revisited: Phylogenetic Niche Conservatism and the Origin of Species. *Evolution (N. Y).* **58,** 193–197 (2004).
34. Kozak, K. H., Weisrock, D. W. & Larson, A. Rapid lineage accumulation in a non-adaptive radiation: Phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: Plethodon). *Proc. R. Soc. B Biol. Sci.* **273,** 539–546 (2006).
35. Wake, D. B. Problems with Species: Patterns and Processes of Species Formation in Salamanders. *Ann. Missouri Bot. Gard.* **93,** 8–23 (2006).
36. Vences, M. & Wake, D. B. in *Amphibian Biology, Vol. 6, Systematics* (eds. Heatwole, H. H. & Tyler, M.) 2613–2669 (Surrey Beatty & Sons, Chipping Norton, Australia, 2007).
37. Wake, D. B. What Salamanders have Taught Us about Evolution. (2009). doi:10.1146/annurev.ecolsys.39.110707.173552
38. Blankers, T., Adams, D. C. & Wiens, J. J. Ecological radiation with limited morphological diversification in salamanders. *J. Evol. Biol.* **25,** 634–646 (2012).
39. Highton, R. Speciation in eastern North {A}merican salamanders of the genus Plethodon. *Annu. Rev. Ecol. Syst.* **26,** 579–600 (1995).
40. Highton, R. Geographic protein variation and speciation in the Plethodon dorsalis complex. *Herpetologica* **1997,** 345–356 (1997).
41. 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).
42. 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).
43. Wake, D. B. & Jockusch, E. L. in *The Biology of Plethodontid Salamanders* 95–119 (Springer US, 2000). doi:10.1007/978-1-4615-4255-1_4
44. Patton, A. *et al.* A New Green Salamander in the Southern Appalachians: Evolutionary History of Aneides aeneus and Implications for Management and Conservation with the

Description of a Cryptic Microendemic Species. *Copeia* **107,** (2019).
45. Rundell, R. J. & Price, T. D. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* **24,** 394–399 (2009).
46. Czekanski-Moir, J. E. & Rundell, R. J. The ecology of nonecological speciation and nonadaptive radiations. *Trends Ecol. Evol.* (2019).
47. Garcia-Paris, M., Good, D. A., Parra-Olea, G. & Wake, D. B. Biodiversity of Costa Rican salamanders: Implications of high levels of genetic differentiation and phylogeographic structure for species formation. *Proc. Natl. Acad. Sci.* **97,** 1640–1647 (2000).
48. Parra-Olea, G. & Wake, D. B. Extreme morphological and ecological homoplasy in tropical salamanders. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 7888–7891 (2001).
49. Vieites, D. R., Min, M. S. & Wake, D. B. Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 19903–19907 (2007).
50. Parra-Olea, G., Windfield, J. C., Velo-Antón, G. & Zamudio, K. R. Isolation in habitat refugia promotes rapid diversification in a montane tropical salamander. *J. Biogeogr.* **39,** 353–370 (2012).
51. Bryson, R. W. *et al.* Phylogenomic insights into the diversification of salamanders in the Isthmura bellii group across the Mexican highlands México View project Phylogeography and contact zones in Ctenosaura pectinata View project Phylogenomic insights into the diversification of salamanders in the Isthmura bellii group across the Mexican highlands. *Artic. Mol. Phylogenetics Evol.* (2018). doi:10.1016/j.ympev.2018.03.024
52. Gillespie, R. G. *et al.* Comparing Adaptive Radiations Across Space, Time, and Taxa. *J. Hered.* **89154,** 14853 (2020).
53. Stamatakis, A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22,** 2688–2690 (2006).
54. Smith, S. A. & O'meara, B. C. treePL: divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* **28,** 2689–2690 (2012).
55. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in {R} language. *Bioinformatics* **20,** 289–290 (2004).
56. Liu, F. G. R., Moler, P. E. & Miyamoto, M. M. Phylogeography of the salamander genus Pseudobranchus in the southeastern United States. *Mol. Phylogenet. Evol.* **39,** 149–159 (2006).
57. Fu, J. & Zeng, X. How many species are in the genus Batrachuperus? A phylogeographical analysis of the stream salamanders (family Hynobiidae) from southwestern China. *Mol. Ecol.* **17,** 1469–1488 (2008).
58. Yoshikawa, N., Matsui, M., Nishikawa, K., Misawa, Y. & Tanabe, S. Allozymic Variation in the Japanese Clawed Salamander, Onychodactylus japonicus (Amphibia: Caudata: Hynobiidae), with Special Reference to the Presence of Two Sympatric Genetic Types. *Zoolog. Sci.* **27,** 33–40 (2010).
59. Yoshikawa, N., Matsui, M. & Nishikawa, K. Genetic Structure and Cryptic Diversity of Onychodactylus japonicus (Amphibia, Caudata, Hynobiidae) in Northeastern Honshu, Japan, as Revealed by Allozymic Analysis. *Zoolog. Sci.* **29,** 229–237 (2012).
60. Yoshikawa, N. & Matsui, M. Two new Salamanders of the genus Onychodactylus from Eastern Honshu, Japan (Amphibia, Caudata, Hynobiidae). (2014). doi:10.11646/zootaxa.3866.1.3
61. 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).
62. Stadler, T. How can we improve accuracy of macroevolutionary rate estimates? *Syst. Biol.* **62,** 321–329 (2013).
63. Beaulieu, J. M. & O'Meara, B. C. Extinction can be estimated from moderately sized molecular phylogenies. **69,** 1036–1043 (2015).
64. Rabosky, D. L. & Goldberg, E. E. Model inadequacy and mistaken inferences of trait-dependent speciation. *Syst. Biol.* **64,** 340–55 (2015).
65. Caetano, D. S., O'Meara, B. C. & Beaulieu, J. M. Hidden state models improve state-dependent diversification approaches, including biogeographical models. *Evolution (N. Y).* (2018).
66. Barraclough, T. G., Harvey, P. H. & Nee, S. Rate of rbcL gene sequence evolution and species diversification in flowering plants (angiosperms). Proceedings of the Royal Society of London. *Ser. B. Biol. Sci.* **263,** 589–591 (1996).
67. R Core Team. R: A Language and Environment for Statistical Computing. (2018). at <https://www.r-project.org/>
68. Hewitt, G. M. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58,** 247–276 (1996).
69. Kozak, K. H., Blaine, R. A. & Larson, A. Gene lineages and eastern North {A}merican palaeodrainage basins: phylogeography and speciation in salamanders of the Eurycea bislineata species complex. *Mol. Ecol.* **15,** 191–207 (2006).
70. 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).
71. Walls, S. C. The role of climate in the dynamics of a hybrid zone in Appalachian salamanders. *Glob. Chang. Biol.* **15,** 1903–1910 (2009).
72. Rice, W. R. & Hostert, E. E. Laboratory experiments on speciation: what have we learned in 40 years? **47,** 1637–1653 (1993).
73. Servedio, M. R. & Noor, M. A. The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.* **34,** 339–364 (2003).
74. Matute, D. R. & Ortiz-Barrientos, D. Speciation: The strength of natural selection driving reinforcement. *Curr. Biol.* **24,** 955–957 (2014).
75. 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).
76. Emel, S. L. & Storfer, A. A decade of amphibian population genetic studies: synthesis and recommendations. *Conserv. Genet.* **13,** 1685–1689 (2012).
77. Meier, J. I. *et al.* The coincidence of ecological opportunity with hybridization explains rapid adaptive radiation in Lake Mweru cichlid fishes. *Nat. Commun.* **10,** (2019).
78. Rico, C. & Turner, G. F. Extreme microallopatric divergence in a cichlid species from Lake Malawi. *Mol. Ecol.* **11,** 1585–1590 (2002).
79. Church, S. A., Kraus, J. M., Mitchell, J. C., Church, D. R. & Taylor, D. R. Evidence for Multiple Pleistocene Refugia in the Postglacial Expansion of the Eastern Tiger salamander, Ambystoma Tigrinum Tigrinum. *Evolution (N. Y).* **57,** 372–383 (2003).
80. Crespi, E. J., Rissler, L. J. & Browne, R. A. Testing Pleistocene refugia theory: phylogeographical analysis of Desmognathus wrighti, a high-elevation salamander in the southern Appalachians. *Mol. Ecol.* **12,** 969–984 (2003).

81. Steele, C. A. & Storfer, A. Phylogeographic incongruence of codistributed amphibian species based on small differences in geographic distribution. *Mol. Phylogenet. Evol.* **43,** 468–479 (2007).
82. Zamudio, K. R. & Savage, W. K. Historical Isolation, Range Expansion, and Secondary Contact of Two Highly Divergent Mitochondrial Lineages in Spotted Salamanders (Ambystoma maculatum). *Evolution (N. Y).* **57,** 1631–1652 (2003).
83. Alexandrino, J., Froufe, E., Arntzen, J. W. & Ferrand, N. Genetic subdivision, glacial refugia and postglacial recolonization in the golden-striped salamander, Chioglossa lusitanica (Amphibia: Urodela). *Mol. Ecol.* **9,** 771–781 (2000).
84. Steinfartz, S., Veith, M. & Tautz, D. Mitochondrial sequence analysis of Salamandra taxa suggests old splits of major lineages and postglacial recolonizations of Central Europe from distinct source populations of Salamandra salamandra. *Mol. Ecol.* **9,** 397–410 (2000).
85. Mattoccia, M., Marta, S., Romano, A. & Sbordoni, V. Phylogeography of an Italian endemic salamander (genus Salamandrina): glacial refugia, postglacial expansions, and secondary contact. *Biol. J. Linn. Soc.* **104,** 903–992 (2011).
86. Kozak, K. H. & Wiens, J. J. Niche conservatism drives elevational diversity patterns in Appalachian salamanders. *Am. Nat.* **176,** 40–54 (2010).
87. Parmesan, C. & Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421,** 37–42 (2003).
88. Root, T. L., Hall, P., K. R., S. & S. H. Rosenzweig C. & Pounds, J. A. Fingerprints of global warming on wild animals and plants. *Nature* **421,** 57–60 (2003).
89. Kelly, A. E. & Goulden, M. L. Rapid shifts in plant distribution with recent climate change. *Proc. Natl. Acad. Sci.* **105,** 11823–11826 (2008).
90. 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 (80-.).* **333,** 1024–1026 (2011).
91. 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).
92. Garroway, C. J. *et al.* Climate change induced hybridization in flying squirrels. *Glob. Chang. Biol.* **16,** 113–121 (2010).
93. Hoffmann, A. A. & Sgrò, C. M. Climate change and evolutionary adaptation. *Nature* **470,** 479–85 (2011).
94. Chunco, A. J. Hybridization in a warmer world. *Ecol. Evol.* **4,** 2019–2031 (2014).
95. 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).
96. 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).
97. Seehausen, O. Conservation: losing biodiversity by reverse speciation. *Curr. Biol.* **16,** 334-R337 (2006).
98. Seehausen, O., Takimoto, G., Roy, D. & Jokela, J. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.* **17,** 30–44 (2008).
99. Vonlanthen, P. & others. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* **482,** 357–362 (2012).

100. Hoskin, C. J., Higgie, M., McDonald, K. R. & Moritz, C. Reinforcement drives rapid allopatric speciation. *Nature* **437,** 1353–1356 (2005).
101. Pfennig, K. S. Reinforcement as an initiator of population divergence and speciation. *Curr. Zool.* **62,** 145–154 (2016).
102. Arnold, S. J., Reagan, N. L. & Verrell., P. A. Reproductive isolation and speciation in plethodontid salamanders. *Herpetologica* **49,** 216–228 (1993).
103. Reagan, N. L. *Evolution of sexual isolation in salamanders of the genus Plethodon (Doctoral dissertation)*. (University of Chicago, 1992).
104. Davis, M. P., Midford, P. E. & Maddison, W. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. *BMC Evol. Biol.* **13,** 38 (2013).
105. 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).
106. Rabosky, D. L. Extinction rates should not be estimated from molecular phylogenies. *Evolution (N. Y)*. **64,** 1816–1824 (2010).
107. Rabosky, D. L. Challenges in the estimation of extinction from molecular phylogenies: a response to Beaulieu and O'Meara. *Evolution (N. Y)*. **70,** 218–228 (2016).
108. Kearney, M. Hybridization, glaciation and geographical parthenogenesis. *Trends Ecol. Evol.* **20,** 495–502 (2005).
109. 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).
110. Schmid, M., Evans, B. J. & Bogart, J. P. Polyploidy in Amphibia. *Cytogenetic and Genome Research* **145,** 315–330 (2015).
111. Runemark, A., Vallejo-Marin, M. & Meier, J. I. Eukaryote hybrid genomes. *PLOS Genet.* **15,** e1008404 (2019).