ORIGINAL ARTICLE

Limited evidence for a positive relationship between hybridization and diversification across seed plant families

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Hybridization has experimental and observational ties to evolutionary processes and outcomes such as adaptation, speciation, and radiation. Although it has been hypothesized that hybridization and diversification are positively correlated, this idea remains largely untested empirically, and hybridization can also potentially reduce diversity. Here, we use a hybridization database on 170 seed plant families, life history information, and a time-calibrated phylogeny to test for phylogenetically-corrected associations between hybridization and diversification rates, while also taking into account life-history traits that may be correlated with both processes. We use three methods to estimate diversification rates and two metrics of hybridization. Although hybridization explains only a small amount of overall variation in diversification rates, we show that diversification and hybridization are sometimes positively correlated, although the effect sizes are very small. Moreover, the relationship remains detectable when incorporating the correlations between diversification and two other life history characteristics, perenniality and woodiness. We discuss potential mechanisms for this association under four different scenarios: hybridization may drive diversification, diversification may drive hybridization, both hybridization and diversification may jointly be driven by other factors, or, as an alternative, that there is in fact no relationship between the two. We suggest future studies to disentangle the causal structure.

KEY WORDS: Adaptive radiation, extinction, perenniality, speciation, traits, woodiness.

Hybridization is a biological phenomenon wherein heterospecific mating typically forms offspring that are genetically and often phenotypically distinctive. The prevalence of natural hybridization is increasingly recognized in animals (involving up to 25% of species in some groups (Mallet 2005)), fungi (reviewed in Albertin and Marullo 2012), and in plants, with hybridization occurring in 40% of plant families (Whitney et al. 2010) and involving up to 25% of species in some groups (Mallet 2005).

Hybridization increases the availability of genetic variation on which natural selection can act (Schluter et al. 2004; Barrett and Schluter 2008) and can result in novel phenotypic traits or in novel combinations of traits, for example, via transgressive segregation (Kim and Rieseberg 1999; Bell and Travis 2005; Dittrich-Reed and Fitzpatrick 2013). Moreover, introgressive hybridization, or repeated backcrossing from one lineage into another,

can result in the transfer of adaptive traits between lineages that confer advantages in novel environments (explored in Anderson 1953; Dowling and Secor 1997; Suarez-Gonzalez et al. 2018).

Both theory and empirical observations support the idea that hybridization can promote increased rates of evolution and/or speciation (Anderson and Stebbins 1954; Stelkens et al. 2014; Marques et al. 2019; Taylor and Larson 2019). Hybridization has been linked to multiple evolutionary and ecological processes, such as adaptation (e.g., Lewontin and Birch 1966; Campbell and Snow 2007; Hovick et al. 2012; Stankowski and Streisfeld 2015; Mitchell et al. 2019b), speciation (e.g., Rieseberg 2003; Mallet 2007; Rieseberg et al. 2007; Soltis and Soltis 2009; Abbott et al. 2013), and evolutionary radiation (e.g., Anderson and Stebbins 1954; Stebbins 1959; Barton 2001; Seehausen 2004; Yakimowski and Rieseberg 2014; Berner and Salzburger 2015;

Marques et al. 2019). For example, experimental hybridization between Texas sunflowers increased the speed of adaptive evolution over eight generations when compared to non-hybrid controls (Mitchell et al. 2019b). Hybridization has also led to speciation within this same genus. Historical interbreeding between two sunflowers (*Helianthus annuus* and *H. petiolaris*) generated three species of hybrid origin that are adapted to novel and extreme environments in a repeatable fashion (reviewed in Rieseberg et al. 2007). On a macroevolutionary scale, hybridization has been linked to rapid speciation and evolutionary radiations. For example, in the Hawaiian silverswords, an ancient hybrid founder may have provided the evolutionary novelty necessary to promote adaptive radiation (Barrier et al. 1999).

Based on the association between hybridization and evolutionary change at different scales, it has been hypothesized that hybridization may be linked to overall net diversification (Dowling and Secor 1997; Seehausen 2004), yet this idea remains untested (but see Tank et al. 2015; Landis et al. 2018 for related work linking polyploidy to diversification). Importantly, the direction of causality between hybridization and diversification could go either way; high rates of hybridization could result in high rates of diversification, or high rates of diversification could result in the increased prevalence of hybridization within a lineage. For instance, in the latter scenario, high rates of diversification may mean that species have low amounts of genetic divergence between them, allowing for higher rates of hybridization. Additionally, a positive detectable relationship between hybridization and diversification could be an artifact, since gene flow can lead to underestimated divergence times and inflated estimates of diversification rates (Leaché et al. 2014).

Conversely, hybridization can also reduce diversity through either demographic or genetic swamping (Wolf et al. 2001, reviewed in Rhymer and Simberloff 1996; Todesco et al. 2016). There are documented cases where human-induced changes have increased the potential for gene flow that breaks down reproductive barriers, for instance in Lake Victoria where increased turbidity reduced sexual selection and led to decreased diversity in cichlid fishes (Seehausen et al. 1997) or in Canada where land use changes appeared to break down reproductive barriers between benthic and limnetic species of three-spine sticklebacks (Kraak et al. 2001). Whether hybridization increases or decreases diversity may be dependent upon phylogenetic scale, time scale, and how diversity is measured or assessed.

Net diversification (r), the collective result of speciation (s) minus extinction (e), can be estimated from time-calibrated phylogenies (Ricklefs 2007). The accuracy of estimating net diversification rates is limited and speciation may be more robustly estimated, especially at the tips of trees (Louca and Pennell 2019; Title and Rabosky 2019), however we focus here on net diversification as it has the capacity to account for both the diversity-generating and diversity-reducing effects of hybridization. Diversification is an emergent property of a given lineage and is influenced by individual, population, or species-specific factors that can increase or decrease the likelihood of either speciation or extinction (Barraclough 1998; Langerhans and DeWitt 2004; Bouchenak-Khelladi et al. 2015). It is therefore necessary to investigate the relative effects of different factors on diversification in a multivariate context, while acknowledging that additional yet unmeasured factors (and their interactive effects) are likely at play, and that the explanatory power of any one factor is likely small.

Here, we present a test of the hypothesized relationship between hybridization and diversification in seed plants. Both diversity (Davies et al. 2004) and rates of hybridization (Ellstrand et al. 1996; Whitney et al. 2010; Beddows and Rose 2018; Mitchell et al. 2019a) are unevenly distributed across plant lineages. Moreover, both diversification and hybridization have been linked to numerous aspects of plant biology, including life form and life history. For instance, plant groups dominated by herbaceous life forms or short generation times tend to have faster diversification rates (Eriksson and Bremer 1992; Dodd et al. 1999), while both perenniality and woodiness are positively associated with hybridization (Mitchell et al. 2019a; Ellstrand et al. 1996; Beddows and Rose 2018).

Using a database containing two measures of hybridization and life history information on 170 seed plant families (spermatophytes), as well as a time-calibrated family-level phylogeny, we estimate family-level diversification rates using three different methods and ask 1) Are diversification rates and hybridization rates related across seed plants?, and 2) Does hybridization remain predictive of diversification when accounting for other correlated traits?

Materials and Methods
HYBRIDIZATION RATES, PERENNIALITY, AND WOODINESS

For hybridization rates in seed plant families, as well as two life history characteristics (perenniality and woodiness) known to be positively correlated with hybridization rates, we used the hybrid and trait database previously examined in Mitchell et al. (2019a). This database includes data from eight regional floras: the Great Plains of the U.S. (McGregor and Barkley 1986); the British Isles (Stace 1997); Hawai'i (Wagner et al. 1999); the Intermountain Region of the western U.S. (Cronquist et al. 1972); the Northeastern U.S. (Magee and Ahles 1999); California (Hickman 1993); Europe (Tutin et al. 1964); and Victoria, Australia (Walsh and Entwisle 1994). These floras do not fully represent global diversity in terms of geography or biome, but we were limited to floras for which the authors include reference to hybrids. In summary,

for each plant family in each flora the number of non-hybrids and interspecific hybrids was assessed as in Whitney et al. (2010). For counting purposes, a "hybrid" was defined as a hybrid type derived from a unique combination of two parental species (as in Ellstrand et al. 1996). Thus, in each flora, each pair of hybridizing species was counted as generating a single hybrid, even if there was evidence that the pair had hybridized multiple times. Recognition of an interspecific hybrid does not imply that it was formally or taxonomically recognized in the flora (though some were). Therefore, "hybrids" described in theses floras likely represent a mix of incidental hybrids observed in nature and cases of evolutionary significant hybridization.

From these data, two metrics of hybridization (hybridization propensity and hybrid ratio) were estimated. Hybridization propensity (HybProp) is calculated as

$$Hybridization\ propensity\ =\ 100\ \times\ \left(\frac{number\ of\ hybrids}{\frac{n(n-1)}{2}}\right) \quad (1)$$

where $n =$ the number of nonhybrid species in the family. It thus represents the percentage of possible hybrid combinations that have been actually realized in nature. Hybrid ratio (HybRatio) is calculated as

$$Hybrid\ ratio\ =\ \frac{number\ of\ hybrids}{n} \quad (2)$$

and has been used in previous studies examining patterns of hybridization (e.g. Beddows and Rose 2018; Mitchell et al. 2019a). Note the scale difference: by convention, hybridization propensity is a percentage bounded between 0 and 100, while hybrid ratio is unbounded (in the Mitchell et al. 2019a dataset, it ranges from 0 to 0.15 with outliers up to 1.2). Hybridization propensity may also vary depending on the phylogenetic scale of clades analyzed, though we chose to analyze only families here. Both hybridization metrics represent the outcome of numerous ecological and evolutionary factors, including geographic extent and overlap, density, demography, speed, and strength of the evolution of reproductive isolating barriers, and thus are a measure of present-day "overall" evidence of hybridization within a taxon, rather than indicating hybrid mechanisms that may be more lineage- or case-specific. Some of these factors are discussed in Mitchell et al. (2019a).

The initial dataset contained hybridization data on 282 plant families. We eliminated observations of genera where a single non-hybrid species was observed in a single flora to avoid including groups with no chance for hybridization and families for non-seed plants, resulting in 170 seed plant families for use in subsequent analyses (Fig. 1). The elimination of genera with a single non-hybrid species is a conservative choice, as these monotypic groups have hybridization scores of zero and likely have overall low diversity. See Mitchell et al. (2019a) for details on collection of these data.

We used the values of perenniality and woodiness estimated by Mitchell et al. (2019a). Briefly, the number of annual, biennial, and perennial species, and the number of herbaceous vs. woody species were counted in the floras. Each species was assigned a score (for perenniality: 0 if annual, 0.5 if biennial, 1 if perennial; for woodiness: 0 if herbaceous, 1 if characterized by above-ground woody biomass; with intermediate species characterized by varying life histories, for example, split between categories). Perenniality and woodiness were then estimated as percentages of species within a family possessing the characteristic.

PLANT PHYLOGENY

We used the plant phylogeny of Zanne et al. (2014a) to assess diversification rates for seed plants (while incorporating family-level species richness, see below) and account for phylogenetic non-independence of lineages. Briefly, we downloaded the dated phylogeny from the Dryad Digital Repository (Zanne et al. 2014b) and updated the taxonomy using the TPL function in the R package *Taxonstand* (Cayuela et al. 2012), resulting in a phylogeny with 30,913 species representing 411 plant families. To build family-level phylogenies for downstream analyses, we generated 100 trees where a random species from each family was chosen as a representative. The phylogenies and additional metrics were visualized using the R package *ggtree* (Yu et al. 2017).

SPECIES RICHNESS

We created taxonomic richness matrices based on the number of species in each family listed in The Plant List (<www.theplantlist.org>, as of March 2019).

DIVERSIFICATION RATES

We estimated diversification rates using three different methods: BAMM, Medusa, and method-of-moments estimation.

BAMM. We used BAMM (Bayesian Analysis of Macroevolutionary Mixture Models) (Rabosky 2014) to model speciation and extinction rates using reversible jump Markov chain Monte Carlo to explore model space. Due to computational limitation associated with running BAMM on a phylogeny of 30,913 species, we divided the full phylogeny into 6 subclades and a "backbone" phylogeny consisting of the remaining species and one representative from each subclade, similar to the process used by Igea et al. (2017). Prior values were obtained using the *BAMMtools* R package (Rabosky et al. 2014). We set the expected number of shifts to 50 (as suggested for phylogenies with >5000 tips) and accounted for richness by providing a sampling fraction for each family calculated as 1 over the number of species in the family. To account for the fact that the "sampling fraction" was sometimes

Figure 1. Phylogeny containing 170 seed plant families analyzed in this study. Rings depict the standardized values of the log-transformed rates for (moving from inside to outside) the hybridization measures (HybProp and HybRatio; orange) and the net diversification rates (BAMM, Medusa, and MS; blue) which have been placed into five bins each based on quantiles. Phylogenetically corrected analyses typically find a positive association between hybridization and diversification (Figs, 2, 3, Tables 1, 2), and non-phylogenetically corrected analyses also typically show positive relationships (Fig. S2, Table S5). Lighter = relatively lower values, darker = higher values.

low, we set extinctionProbMax = 0.9999999. We ran BAMM on each of the seven phylogenies (six sub-trees plus the backbone) on the high-performance computing facilities maintained by the University of Wisconsin – Eau Claire Blugold Supercomputing Cluster for 200 million generations sampled every ten thousand generations. We combined event data files using scripts from Igea et al. (2017), with a burnin of 150 million generations. Convergence was checked visually using the R package *coda* (Plummer et al. 2006); we ensured that the effective sample sizes for the number of shifts and log-likelihoods were over 200 (although for

one subtree the values were just below this threshold). We extracted the average net diversification rate for each family for use in phylogenetic generalized least squares models.

Medusa. We used Medusa (Alfaro et al. 2009) to model diversification using stepwise AIC using piecewise birth-death models. We used the medusa() function in the R package *geiger* (Harmon et al. 2008) using the 100 family-level phylogenies and family species richness. The program compares stepwise models until the improvement, measured using the sample-size corrected Akaike information criterion (AICc), does not exceed the internally computed threshold (for a phylogeny with 411 tips, threshold of approximately 7.87). Because results are highly dependent on threshold value (May and Moore 2016), we ran Medusa using three different threshold values (7.87, 4, and 2) to compare outputs; note that a previous version of Medusa used a default threshold value of 4. We extracted the net diversification rates associated with each tip (family) from each of these threshold runs.

Method-of-moments (MS). We used the stem-group method-of-moments estimation (MS) of diversification using the stem ages of each family (tip edge lengths from the family-level phylogenies) and family species richness to calculate a measure of net diversification rates (Rohatgi 1976; Magallon and Sanderson 2001) for each of the 100 family-level phylogenies. We calculated MS using three different relative extinction rates (epsilon, $\epsilon = 0$, 0.5, or 0.9) and the equation $r = \ln(n (1 - \epsilon) + \epsilon)/t$, where $r = $ the net diversification rate, $n = $ the extant species richness and $t = $ the stem group age (in millions of years).

ASSOCIATIONS BETWEEN HYBRIDIZATION RATES AND DIVERSIFICATION

To evaluate the robustness of different methods of both hybridization estimation and diversification estimation, we tested associations using permutation tests, phylogenetic generalized least squares (PGLS), and correlations without phylogenetic correction for comparison. Here, our approach is to conduct the same comparison using the same two traits (hybridization versus diversification rate) multiple ways, rather than performing multiple comparisons using different traits. We therefore do not correct for "multiple comparisons", although we discuss some drawbacks associated with this approach in the Discussion.

Permutation tests. We used STRAPP (STructured RAte Permutations on Phylogenies) through the function traitDependent-BAMM in the R package *BAMMtools* (Rabosky et al. 2014) to estimate correlations between hybridization rates and diversification rates estimated in BAMM (Rabosky and Huang 2015). This procedure takes as input the estimated diversification rates from BAMM, then computes a test statistic (Spearman correlation coefficient) comparing character states and diversification rates, and finally compares this test statistic to a null distribution formed by permuting diversification rates at the tips of the tree across blocks

of taxa (accounting for independent shifts in diversification) (Rabosky and Huang 2015). We assigned family-level hybridization metrics to each species-level tip in the phylogeny within that family to estimate the two-tailed Pearson correlations using all the rate data possible. This may obscure variation and reduce statistical power, as diversification rate shifts within a family are shared across the same trait (hybridization) value (D. Rabosky, pers. comm.). We performed this analysis using log-transformed hybrid measures and log-transformed net diversification rates. We also ran this analysis by drawing a single random species from each family and repeating the process 100 times to obtain estimates without multiple observations per family. Finally, we repeated this process using percent perennial and percent woody species to compare the effect sizes of hybridization on diversification with the effect sizes of other factors known to be related to both diversification and hybridization.

Phylogenetic Generalized Least Squares (PGLS). We used phylogenetic generalized least squares regression (PGLS) (Grafen 1989; Martins and Garland Jr 1991) to detect associations between diversification rates and rates of hybridization in plant families while accounting for evolutionary history. The use of PGLS in understanding relationships with diversification rates is largely unexplored, and similar models may be inadequate (such as state-dependent diversification), producing biased p-values, so these need to be interpreted with caution (Maddison 2006). We trimmed the family-level seed plant phylogeny to include only the families for which we had hybridization data, resulting in a phylogeny of 170 families. We standardized all variables to make results comparable between analyses and to enable us to compare the relative effects of different variables in these models. We ran univariate PGLS using the pgls() function in the R package *caper* (Orme et al. 2013), modeling diversification rates as outcomes and using each hybridization metric as our predictor with estimated lambda values and kappa and delta set to 1. We performed these regressions for each of the 100 phylogenies produced using one species per family when analyzing models and report results from the approximate median "50th" result when sorted by estimate strength as well as the full range of values. We did this using Medusa and MS diversification rates, but because the BAMM diversification rates were calculated from the species-level tree, we report only the median result.

For each combination of diversification and hybridization metrics, we also ran multivariate regressions using standardized data to detect associations between diversification rates and hybridization metrics when including two other predictors: perenniality and woodiness.

Analyses without phylogenetic correction. We also estimated relationships between hybridization and diversification rates without phylogenetic correction in order to compare results. We ran two-sided Pearson's correlation tests to determine the

strength and direction of associations between our family level log-transformed hybridization metrics and diversification rates. We also ran these correlations between hybridization metrics and species richness to understand patterns in overall present-day diversity.

SISTER-CLADE COMPARISON

We further examined the relationship between hybridization and diversification using the more traditional approach of sister-clade comparisons (Slowinski and Guyer 1993; Barraclough 1998) to ask whether families with higher rates of hybridization also had higher diversification rates. We identified sister families present in the 411-taxon phylogeny and consistent with relationships on the Angiosperm Phylogeny Website <http://www.mobot.org/MOBOT/research/APweb/, accessed 23 April 2019>. We restricted comparisons to sister pairs where families were not monotypic, for which we had data, and where both hybridization propensity and hybridization ratio differed consistently (i.e., the same family had higher values for both metrics), as there were some cases where the metrics were inconsistent in identifying the more hybridization-prone family. This resulted in 10 sister-clade comparisons. We then asked whether differences between sister families in diversification rate were associated with the differences between sister families in hybridization rates using two-sided Pearson correlation tests on log-transformed values. We also assessed whether differences in family level species richness (rather than diversification rates) were associated with hybridization by running similar correlation tests. For these, we used the ratio of the species richness in the more hybridization-prone family divided by the richness of the less hybridization-prone family as a measure of difference.

Results

HYBRIDIZATION RATES

Hybridization rates across families in this dataset have been presented and discussed previously in Mitchell et al. (2019a). Briefly, out of 170 seed plant families, 95 contained hybrids. The average family-level hybridization propensity was 1.42 and the average hybridization ratio was 0.07 (Fig. 1). See Mitchell et al. (2019a), Fig. 2, and Table S2 for details.

DIVERSIFICATION RATES

We used three different methods to estimate diversification rates across the family-level seed plant phylogeny: method-of-moments BAMM, Medusa, and MS (Fig. 1).

Due to computational limitations, we estimated diversification rates using BAMM on seven sub-trees (six sub-trees plus a backbone) for the species-level phylogeny of 30,913 plant species. After combining information from these individual anal-
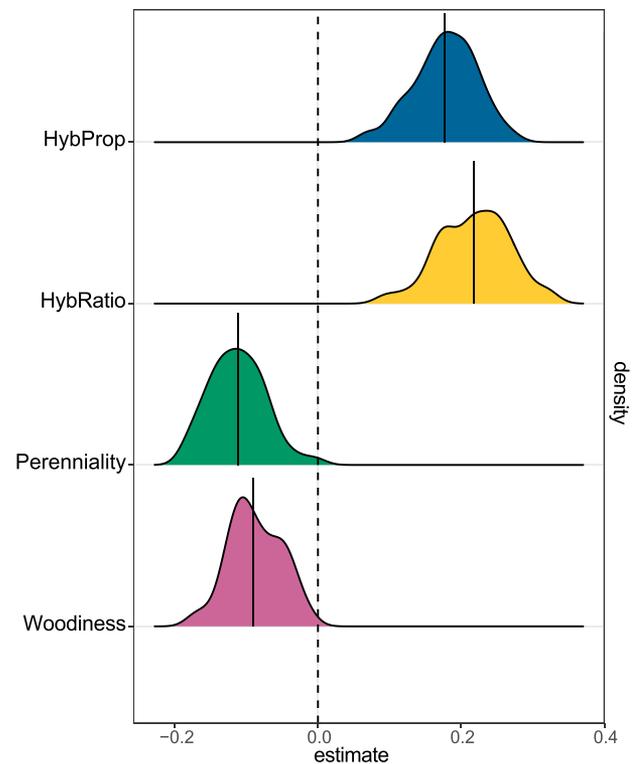
Figure 2. Distributions of estimated associations between hybridization and diversification using permutation tests across 100 phylogenies. Density plots display the full distribution of estimates, vertical solid lines indicate the median value, and vertical dashed line indicates zero (no association). HybProp (blue), HybRatio (gold), Perenniality (green), and woodiness (pink).

yses, we estimated a mean of 918 shifts (range 857 – 984 shifts). We obtained family-level diversification rates by averaging across species within each family. The mean net diversification rate across families was 0.222 species per Ma (range = −0.030 – 3.747) using all 411 families in the phylogeny, and the mean rate was 0.413 species per Ma across the 170 families for which we had hybridization data (range = 0.013 – 3.757).

Using the computed AICc threshold value of 7.87 for our 411-family phylogeny, Medusa detected an average of 46 rate shifts in diversification across the 100 family-level trees (range = 42 – 50 shifts), while the previously standard threshold value of four detected an average of approximately 78 rate shifts (range = 76 – 79 shifts) and a threshold of two detected an average of approximately 94 rate shifts (range = 90 – 96 shifts). Estimated diversification rates for Medusa runs using different thresholds were highly correlated (Table S1). We chose to examine diversification rates computed using the threshold of four for presentation in the main results, as this threshold resulted in an intermediate number of estimated shifts and results were similar across thresholds (Table S3). The mean net diversification rate was 0.077 species per Ma (range = 0.000 – 0.212) across all 411

Table 1. Results for permutation tests of associations between net diversification (from BAMM) and hybridization and life history traits. "Full Phylogeny" refers to the analysis of a species-level tree where each species is assigned the hybridization values of its family. "100 Phylogenies" refers to analysis using 100 trees each with one randomly chosen representative per family. Bold values are significant at $p < 0.05$. * significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at p < 0.001.

	Full Phylogeny		100 Phylogenies	
Metric	Estimate	p-value	50th estimate	50th p-value
HybProp	0.077	0.089	**0.177** (range $= 0.069 - 0.271$)	**0.027*** (range $= 0.000 - 0.437$)
HybRatio	**0.124**	**0.016***	**0.219** (range $= 0.093 - 0.325$)	**0.008**** (range $= 0.000 - 0.237$)
Perenniality	**−0.101**	**0.034***	−0.113 (range $= -0.183 -0.004$)	0.129 (range $= 0.024 - 0.790$)
Woodiness	**−0.207**	**<0.001****	−0.091 (range $= -0.171 -0.012$)	0.306 (range $= 0.053 - 0.804$)

families in the phylogeny and the mean rate was 0.091 species per Ma (range $= 0.001 = 0.212$) across the 170 families for which we had hybridization data.

We estimated the MS net diversification rates using relative extinction fractions of $\epsilon = 0.0$, 0.5, and 0.9. Across the 100 family-level phylogenies, we estimated average diversification rates of 0.053, 0.044, and 0.026 for values of $\epsilon = 0$, 0.5, or 0.9, respectively across all 411 families (ranges $= 0.000 – 0.442$, $0.000 – 0.387$, and $0.000 – 0.261$). Across the 170 families for which we had hybridization data, we estimated average diversification rates of 0.087, 0.075, and 0.054 (ranges $= 0.000 – 0.442$, $0.000 – 0.387$, and $0.000 – 0.261$). We chose the tree with the highest average correlation with all other trees for further analysis. Diversification rates were highly correlated across values of ϵ since these are deterministic calculations (Table S2). We chose to examine rates computed with the moderate value of $\epsilon = 0.5$ for presentation in the main analyses.

Net diversification rates across the three different methods were highly correlated across the full 411-family dataset (BAMM – Medusa: $r = 0.488$, $p < 0.001$; BAMM – MS: $r = 0.642$, $p < 0.001$; Medusa – MS: $r = 0.744$, $p < 0.001$) as well as the 170 family dataset (BAMM – Medusa: $r = 0.474$, $p < 0.001$; BAMM – MS: $r = 0.532$, $p < 0.001$; Medusa – MS: $r = 0.763$ $p < 0.001$) (Figs. 1, S1).

SOME EVIDENCE FOR PHYLOGENETIC CORRELATION BETWEEN HYBRIDIZATION AND DIVERSIFICATION

Using permutation tests implemented using the STRAPP procedure (see Methods) on the full phylogeny with species-level tip data and net diversification rates estimated from BAMM, we found positive associations between diversification rates and hybridization rates across seed plants, but the associations were only statistically significant when examining HybRatio. For HybProp, the estimate was 0.077 ($p = 0.089$) and for HybRatio was 0.124 ($p = 0.016$) (Table 1). For comparison, we ran these permutation

tests on two measures of life history, perenniality, and woodiness. We detected significant negative associations between both traits and diversification rates (perenniality: -0.101, $p = 0.034$, woodiness: -0.207, $p < 0.001$, Table 1).

We repeated these permutation tests on 100 family-level phylogenies which were generated using a single randomly chosen species to represent each family. This resulted in a distribution of the associations (and p-values) between net diversification rates and hybridization and life history traits. The median (fiftieth) estimate for HybProp was significant (0.177, range 0.069 – 0.271; $r = 0.027$ range 0.000 – 0.437), as was the median estimate for HybRatio (0.219, range 0.092 – 0.325; $p = 0.008$, range $= 0.000 – 0.237$) (Table 1, Fig. 2). For perenniality and woodiness, however, these median estimates were both negative but not significant (-0.113, $p = 0.129$; -0.091, $p = 0.306$, respectively), although some of the estimates within the distribution were statistically significant (Table 1, Fig. 2).

Univariate phylogenetically generalized least squares (PGLS) regressions of hybridization measures on diversification rates were positive across all combinations of diversification estimates, hybrid metrics, and phylogenies. These positive relationships indicate that families with higher hybridization rates also had higher diversification rates when accounting for phylogenetic nonindependence, but these were not always statistically significant (Table 2, Fig. 3) and the effect sizes were very small (see adjusted-R^2 values). Using HybProp, none of the median estimates from the distribution using the 100 phylogenies, or the BAMM estimates, were significant ($p < 0.05$), although some estimates using MS were. Using HybRatio, the median estimate with MS diversification rates was significant ($p = 0.038$) and some of the estimates using Medusa were as well, but the BAMM estimates were not (Table 2). Although hybridization explained very little variation in diversification rates, the overall patterns were largely robust to the use of alternate parameters, thresholds, or priors for estimating diversification rates (Table S3).

Table 2. Results for univariate PGLS tests. Values reported are for the median (50th) estimate and ranges on 100 Phylogenies. Note that for BAMM we only report the median estimate, as diversification rates were not estimated from family-level phylogenies. Bold values are significant at $p < 0.05$.

Method	Hybrid Metric	Estimate	t-value	p-value	Adj R^2	Estimate range	t range	p range	R^2 range
MS	HybProp	0.113	1.768	0.079	0.012	(0.071 - 0.139)	(1.046 - 2.217)	(0.028 - 0.297)	(0.001 - 0.023)
	HybRatio	**0.128**	2.088	**0.038**	0.020	(0.088 - 0.155)	(1.351 - 2.592)	(0.010 - 0.182)	(0.005 - 0.033)
Medusa	HybProp	0.045	0.970	0.333	0.000	(0.019 - 0.103)	(0.410 - 1.933)	(0.055 - 0.682)	(−0.005 - 0.016)
	HybRatio	0.045	1.070	0.286	0.001	(0.025 - 0.101)	(0.586 - 2.036)	(0.043 - 0.558)	(−0.004 - 0.018)
BAMM	HybProp	0.081	1.058	0.292	0.001				
	HybRatio	0.129	1.680	0.095	0.011				
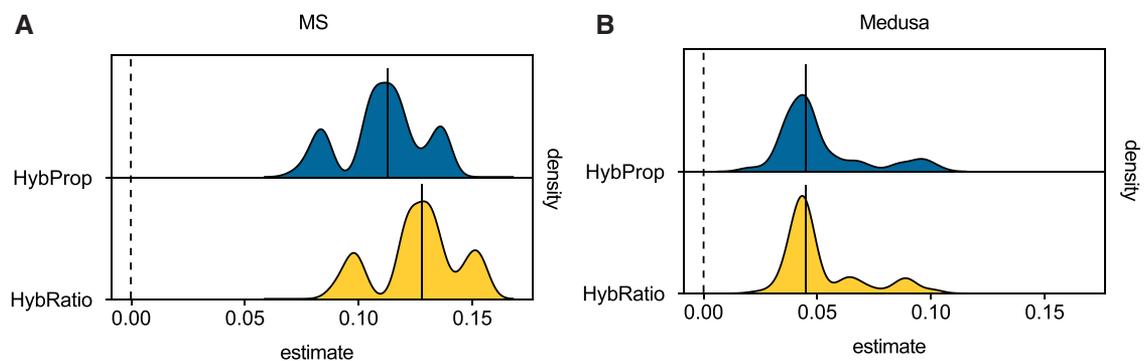
Figure 3. Distributions of estimated associations between hybridization and diversification estimated using the method-of-moments, MS (A) or Medusa (B) using univariate PGLS regressions across 100 phylogenies. Density plots display the full distribution of estimates, vertical solid lines indicate the median value, and vertical dashed line indicates zero (no association) for diversification rates. HybProp (blue), HybRatio (gold).

RELATIONSHIPS BETWEEN HYBRIDIZATION AND DIVERSIFICATION ARE NOT DRIVEN BY PERENNIALITY OR WOODINESS

Multivariate PGLS regressions incorporating family-level perenniality and woodiness measures echoed the trends observed in the permutation tests and univariate analyses. Relationships between diversification rates and hybridization measures were consistently positive, though none of the median estimates were significant and the models overall had very low R^2 values (Fig. 4, Table 3). Only some estimates from the distributions contained significant associations, and these were between MS diversification and HybRatio, or Medusa diversification and both hybridization metrics (Table 3).

Diversification rates trended towards negative associations with woodiness, but relationships were not significant (Figs. 2, 4, Table 3). Perenniality was negatively associated with diversification when diversification was estimated using Medusa, but not with MS. The standardized partial regression coefficients were similar in magnitude for hybridization metrics and woodiness, along with perenniality (but only when diversification rates were

estimated using Medusa, in other analyses the estimated relationship with perenniality was lower) (Fig. 4, Table 3).

For completeness, we also examined correlations between hybridization and diversification without accounting for phylogenetic nonindependence, using mean family diversification rates across the 100 phylogenies. All correlations were positive, and all but two were significant at $p < 0.05$ (exceptions being correlations between hybridization propensity and both the MS and Medusa diversification rates) (Fig. S2a, Table S4). We also found significant positive correlations between both metrics of hybridization and family level species richness (Fig. S2b, Table S4).

ONE SISTER-CLADE COMPARISON COMBINATION SUPPORTS A POSITIVE HYBRIDIZATION - DIVERSIFICATION RELATIONSHIP

We identified 10 pairs of sister families in our dataset with consistent differences in hybridization rates. In the majority of comparisons, the family with the higher hybridization rates also had higher diversification rates (nine, eight, and nine out of ten for

Table 3. Results for multivariate PGLS tests. Values reported are for the median (50th) estimate and ranges on 100 phylogenies. Note that for BAMM we only report the median estimate, as diversification rates were not estimated from family-level phylogenies. Bold values are significant at $p < 0.05$.

Method	Hybrid Metric	Covariate	Estimate	t-value	p-value	Estimate range	t range	p range	Adjust-R²	R² range
MS	HybProp	Hybridization	0.081	1.181	0.239	(0.056 – 0.110)	(0.769 – 1.607)	(0.110 – 0.443)	0.005	(−0.007 – 0.019)
		Perenniality	0.021	0.289	0.773	(−0.109 – 0.081)	(−1.427 – 1.157)	(0.155 – 0.995)		
		Woodiness	−0.127	−1.602	0.111	(−0.142 – −0.068)	(−1.819 – −0.833)	(0.070 – 0.406)		
	HybRatio	Hybridization	0.114	1.890	0.060	(0.076 – 0.134)	(1.179 – 2.251)	(0.026 – 0.240)	0.014	(−0.00 – 0.026)
		Perenniality	0.020	0.281	0.779	(−0.114 – 0.077)	(−1.495 – 1.107)	(0.136 – 0.999)		
		Woodiness	−0.127	−1.609	0.109	(−0.147 – −0.072)	(−1.896 – −0.889)	(0.059 – 0.375)		
Medusa	HybProp	Hybridization	0.063	1.334	0.184	(0.034 – 0.111)	(0.717 – 2.129)	(0.035 – 0.475)	0.013	(−0.007 – 0.027)
		Perenniality	−0.059	−1.043	0.299	(−0.105 – 0.012)	(−1.697 – 0.210)	(0.092 – 0.834)		
		Woodiness	−0.065	−1.020	0.309	(−0.088 – −0.019)	(−1.351 – −0.288)	(0.179 – 0.773)		
	HybRatio	Hybridization	0.067	1.574	0.117	(0.038 – 0.111)	(0.906 – 2.178)	(0.031 – 0366)	0.017	(−0.005 – 0.031)
		Perenniality	−0.053	−0.952	0.343	(−0.105 – 0.012)	(−1.702 – 0.206)	(0.091 – 0.837)		
		Woodiness	−0.073	−1.154	0.250	(−0.093 – 0.025)	(−1.441 – −0.364)	(0.151 – 0.717)		
BAMM	HybProp	Hybridization	0.101	1.287	0.200				0.004	
		Perenniality	0.055	0.632	0.528					
		Woodiness	−0.140	−1.583	0.115					
	HybRatio	Hybridization	0.146	1.882	0.062				0.015	
		Perenniality	0.053	0.607	0.545					
		Woodiness	−0.143	−1.638	0.103					
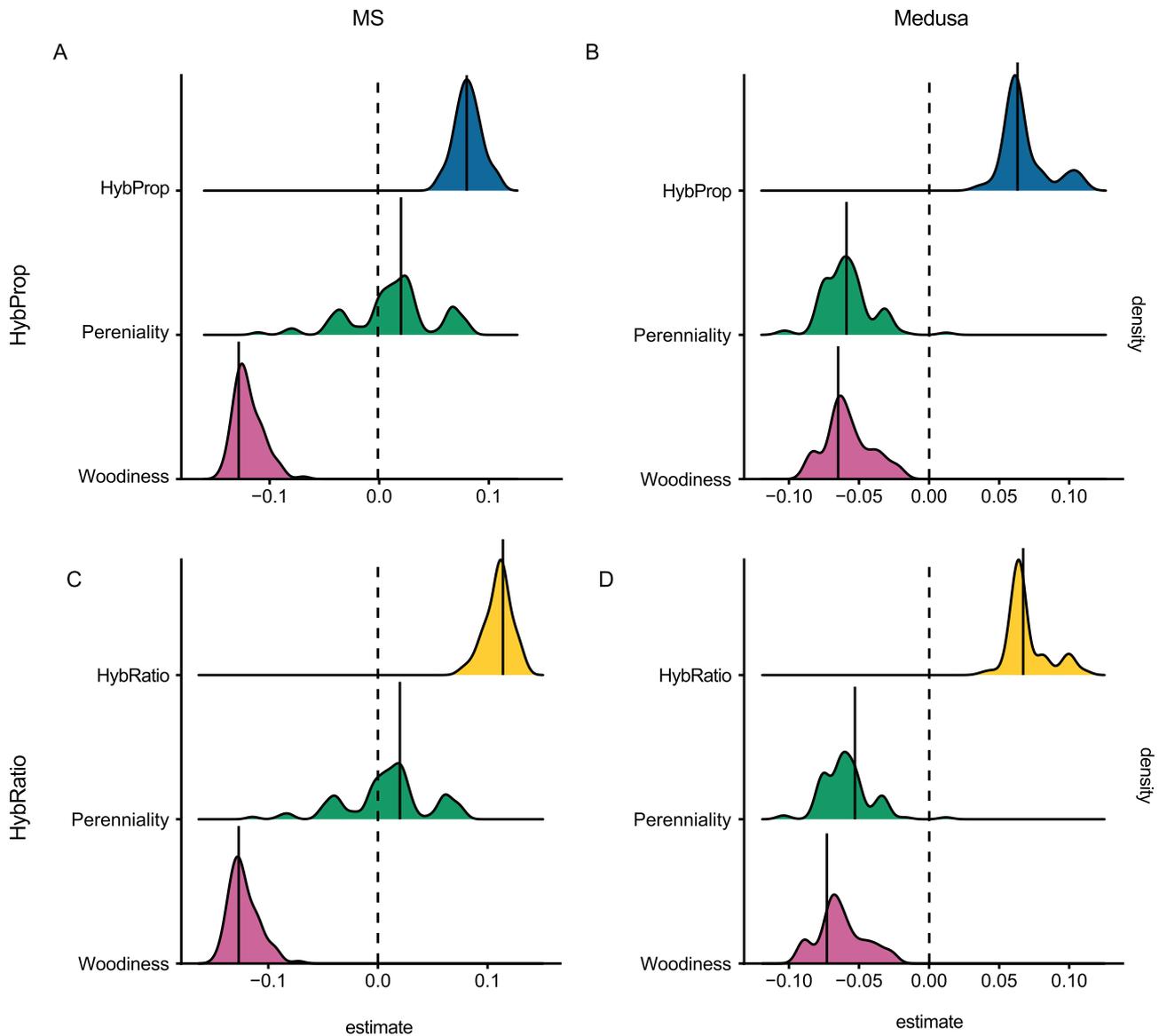
Figure 4. Distributions of estimated associations between hybridization, perenniality, woodiness, and diversification using multivariate PGLS regressions across 100 phylogenies for MS and HybProp (A), Medusa and HybProp (B), MS and HybRatio (C), and Medusa and HybRatio (D). Density plots display the full distribution of estimates, vertical solid lines indicate the median value, and vertical dashed line indicates zero (no association). HybProp (blue), HybRatio (gold), Perenniality (green), and Woodiness (pink).

diversification rates estimated using MS, Medusa, and BAMM, respectively) (Table S5). The difference between the sister families in terms of hybridization was positively associated with the difference between the families in diversification rate in one instance: using HybProp and MS ($r = 0.715$, $t = 2.891$, $p = 0.020$, Table 4). In all other instances, there was no significant correlation in either direction ($p > 0.05$, Table 4). We also found that differences in family species richness (family size) between sister clades were related to differences in hybridization propensity ($r = 0.714$, $t = 2.88$, $p = 0.020$), though this pattern was not observed when using hybrid ratio ($r = 0.034$, $t = 0.096$, $p = 0.926$) (Fig. S3, Table 4).

Discussion
HYBRIDIZATION AND DIVERSIFICATION ARE CORRELATED, BUT WEAKLY SO
The idea that hybridization can promote processes such as adaptation, speciation, and radiation has long been hypothesized, but direct links between hybridization and diversification have been largely untested. Here, we found generally positive associations between hybridization rates and diversification rates among seed plant families using three measures of diversification and two measures of hybridization. These relationships were sometimes statistically significant when using permutation tests (Table 1),

Table 4. Pearson correlations from sister-clade comparison between family differences in hybridization rates and family differences in diversification rates. df = 8 for all cases. Bold values are significant at $p < 0.05$.

Hybridization Metric	Diversification metric	Correlation	p-value	t-value
HybProp	MS	**0.715**	**0.020**	2.891
	Medusa	−0.038	0.918	−0.107
	BAMM	0.139	0.703	0.396
HybRatio	MS	0.097	0.790	0.275
	Medusa	0.004	0.992	0.010
	BAMM	−0.317	0.372	−0.946

but generally not so when using PGLS (Table 2, Table 3). According to PGLS models, hybridization accounted for negligible amounts of the observed variation in diversification rates (Figs. 2, 3, 4, Table 2, Table 3). Although the family with higher hybridization rates also had higher estimated diversification rates in most cases (Table S5), only one instance of our sister-clade comparisons supported the idea that plant families exhibiting more hybridization tend to diversify faster, as families with higher hybridization rates also had more rapid diversification (estimated by MS and with HybProp) (Table 4).

The question about potential relationships between hybridization and diversification is ultimately asking about species richness, and whether either contemporary patterns of richness across lineages or within exemplary lineages such as adaptive radiations may be explained in part by hybridization (Seehausen 2004, 2013). We found that hybridization and species richness were positively related when examining relationships without accounting for phylogeny and in some sister clade comparisons (Figs. S2, S3, Table S4). Further work is necessary to disentangle the biological and mathematical relationships between diversification, hybridization, and species richness.

POTENTIAL EXPLANATIONS FOR RELATIONSHIP BETWEEN HYBRIDIZATION AND DIVERSIFICATION

The weak positive associations presented here do not necessarily imply that hybridization causes increased diversification. The relationship could be due to causation in either direction or due to correlation with an additional unmeasured or untested variable; finally, the inferred relationship itself could be spurious. Here, we discuss potential mechanisms underlying the putative relationship, emphasizing that these mechanisms are speculative and cannot be tested with our observational data.

Scenario 1: Increased hybridization leads to faster diversification. Interbreeding between species results in increased genetic and phenotypic diversity which can promote adaptation and speciation. Hybridization can lead to increased genetic diversity (Stebbins 1959; Hedrick 2013; Suarez-Gonzalez et al. 2018; Marques et al. 2019) and trait diversity, for example, transgressive

segregation (Kim and Rieseberg 1999; Seehausen 2004; Kerbs et al. 2017). Hybridization has been shown experimentally to speed up adaptive evolution in annual sunflowers (Mitchell et al. 2019b) and can result in hybrid speciation (for instance in sunflowers, reviewed in Rieseberg 2006) and the Louisiana irises (Arnold et al. 2012), reviewed more broadly in (Mallet 2007; Soltis and Soltis 2009; Abbott et al. 2013). Marques et al. (2019) recently synthesized the idea that the reassembly of old genetic variation into new combinations can provide a substrate for speciation. In contrast to novel mutations arising within a lineage, alleles incorporated from other lineages have previously been tested in other genomic and ecological backgrounds (Mitchell et al. 2019b), and thus may be more likely to promote adaptation. Such adaptation in turn could reduce the chance for extinction and/or promote ecological speciation.

It has also been postulated that hybridization events can lead to adaptive radiation, broadly defined as "the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage", via similar mechanisms (Seehausen 2004, 2013). For instance, Barrier et al. (1999) inferred that the founder of the Hawaiian silversword alliance (an iconic plant radiation) was a hybrid and suggest that enhanced genetic diversity in the founder may have promoted adaptive radiation. In this scenario, hybridization is hypothesized to facilitate ecological speciation by providing increased genetic variation and multiple genotypes that can fill available niches, allowing for colonization of novel environments or habitats (Schluter 2000; Seehausen 2004).

In contrast, there is evidence that hybridization could act to decrease diversification. In certain scenarios, hybridization can result in "extinction-by-hybridization" (Rhymer and Simberloff 1996; Wolf et al. 2001; Todesco et al. 2016; Campbell et al. 2019) in which the genetic material of one species is replaced by that of another. At present little data exists to assess how common this process is relative to the above-described processes by which hybridization could enhance diversification.

Scenario 2: Faster diversification leads to increased hybridization. Rapid speciation within a lineage results in many closely related species and may also result in incomplete

reproductive isolation (e.g., Baldwin and Sanderson 1998; Barrier et al. 1999). Most hybridization takes place within genera (Whitney et al. 2010), so if fast diversification rates result in many congeneric species, then there is enhanced opportunity for hybridization due to the large number of potential partners and their likely geographic proximity. Taxa that are more closely related have weaker reproductive barriers than distantly related taxa (Coyne and Orr 1997, 2004; Moyle and Nakazato 2010), and these reduced barriers should result in increased interspecific mating (Ellstrand et al. 1996).

Scenario 3: Diversification and hybridization are jointly driven by other factors not tested or described here. A correlation between two variables can arise in the absence of a direct causal relationship between them, if they are both driven by a third factor or set of factors. This may especially be the case since both estimates of hybridization may be the result of numerous ecological and evolutionary processes (as described in the Methods). We discuss two of these potential factors (woodiness and perenniality) below.

Scenario 4: Diversification and hybridization are not actually related. Although we detected some positive relationships between hybridization and diversification, the p-values are modest, not all meet the traditional cut-off of alpha = 0.05, and the associated R^2 values are low, perhaps indicating that these two traits are not actually related. Moreover, given the number of comparisons performed to account for differences in estimation and methodology, we might expect some associations with $p < 0.05$ by chance. Although there are critiques of null hypothesis testing in the fields of ecology and evolution in favor of other approaches such as information theoretic models or Bayesian statistics (e.g., Anderson et al. 2000; Hobbs and Hilborn 2006; Stephens et al. 2007), null hypothesis testing is still commonly used and valuable especially in exploratory analyses. It may be that the modest p-values here indicate that no true relationship between hybridization and diversification exists. Under this scenario, the diversity-generating and diversity-reducing effects of hybridization may in effect cancel each other out, or hybridization may in fact have no measurable effect on diversification.

HYBRIDIZATION, DIVERSIFICATION, AND LIFE HISTORY/LIFE FORM

Previous work has linked both hybridization and diversification to other aspects of plant biology, such as life form and life history. Plant groups with woody growth forms and perennial life histories tend to contain more hybrids (Mitchell et al. 2019a; Grant 1958, 1981; Stebbins 1959; Ellstrand et al. 1996; Beddows and Rose 2018), while plant groups with herbaceous life forms and faster generation times (e.g., annual life histories) have faster diversification (Eriksson and Bremer 1992; Baker et al. 2014) or speciation rates (Dodd et al. 1999). We incorporated peren-

niality and woodiness into multivariate analyses to ask whether hybridization still had positive associations with diversification rates when these traits were included, and to compare the effect sizes of these potential correlates. We found that hybridization remains positively associated with diversification in these analyses; in contrast, woodiness was generally negatively associated with diversification, while perenniality was sometimes negatively associated with diversification and other times had little to no association (Figs. 2, 4, Table 1, Table 3). The effect of hybridization was always positive (Fig. 3, Table 3) and the association between hybridization and diversification (measured as the standardized partial regression coefficients) was equivalent to the magnitude of the woodiness – diversification association and equivalent to or greater than the perenniality – diversification association (Fig. 4, Table 3). The relationships between woodiness and diversification were in the expected direction (Eriksson and Bremer 1992; Dodd et al. 1999) (woody groups tended to have lower diversification rates) (Figs. 2, 4, Table 1, Table 3). Interestingly, the negative association between perenniality and diversification was not always present nor significant (Table 1, Table 3), contrary to expectations (Eriksson and Bremer 1992; Baker et al. 2014) .

OTHER CORRELATES OF DIVERSIFICATION

Although we find detectable associations between hybridization and diversification, our models explain only a small amount of the variation in diversification rates among plants (low adjusted-R^2 values, Table 2, Table 3). Although statistical issues associated with PGLS may be responsible here, it could also be that the case that hybridization biologically explains little of the variation in diversification rates observed across seed plants. Diversification is an integrative property of a lineage, which is influenced by multiple dimensions of life history, environmental factors, and physiology of the organisms within a lineage. Diversification rates or species richness in plants have been tied to numerous attributes (in addition to life history). These include aspects of the genome such as polyploidy (Vamosi and Dickinson 2006; Wood et al. 2009; Tank et al. 2015; Landis et al. 2018) or genome size (Puttick et al. 2015); dispersal mode or geography such as biotic vs. abiotic dispersal (Larson-Johnson 2016), latitudinal gradients (Davies et al. 2004; Jansson and Davies 2008), biome or habitat differences (Moore and Donoghue 2007; Valente et al. 2010; Goldberg et al. 2011; Onstein et al. 2016), geographic area (Vamosi and Vamosi 2010, 2011), and geographic isolation (Baldwin and Sanderson 1998); species interactions such as defense mutualisms (Weber and Agrawal 2014) and pollination mode (Eriksson and Bremer 1992; Hodges and Arnold 1995; Hodges 1997; Dodd et al. 1999; Vamosi and Vamosi 2010, 2011); and other reproductive aspects such as heterostyly (De Vos et al. 2014), self-incompatibility (Goldberg et al. 2010), and dioecy (Käfer et al. 2014). Future multivariate analyses or phylogenetic path

analyses (van der Bijl 2018) with more complete datasets are needed to disentangle and weight the relative contributions of these attributes, hopefully resulting in models with greater explanatory power.

CHOICE OF HYBRIDIZATION AND DIVERSIFICATION METRICS

Our two measures of hybridization (HybProp and HybRatio) and three methods used to estimate diversification rates (MS, Medusa, and BAMM) yield similar results. Previous work on the correlates of hybridization also found similar results for the two hybridization metrics (Mitchell et al. 2019a; Beddows and Rose 2018). Our results with hybridization propensity and hybrid ratio are similar, though analyses using hybrid ratio tend to show slightly stronger relationships with diversification (Table 1, Table 2).

We acknowledge that the use of floras to estimate both hybridization metrics is biased in two major ways (see also Mitchell et al. 2019a). First, although the eight floras analyzed here span multiple continents, they represent only a small geographic area and limited biomes; for instance, tropical regions were not analyzed. This is unfortunately due to the fact that many regional floras do not include mention of hybrids. Future work could investigate patterns of hybridization and diversification within, rather than across, these regional floras. Secondly, the recognition of a "hybrid" is at the discretion of the author(s) of each flora and is largely based on morphological observations, without any formal molecular confirmation, and likely represent both transient or incidental cases of hybridization and more long lasting and evolutionarily impactful cases of hybridization and introgression. Either way, increased levels of hybridization within a taxonomic group are likely indicative of more instances of evolutionarily significant hybridization in the group's past. Additionally, although there is recognition of cryptic hybridization from molecular evidence (without strong morphological evidence) (e.g. Owens et al. 2020, reviewed in Taylor and Larson 2019), a comprehensive analysis including these cases is not currently feasible.

There is much debate surrounding the methods of estimation of diversification rates (May and Moore 2016; Moore et al. 2016; Rabosky et al. 2017; Meyer and Wiens 2018; Meyer et al. 2018; Rabosky 2018), and moreover, inferring trait-dependent diversification is difficult and may be associated with high Type I error rates due to model inadequacy, resulting in statistically significant associations between neutral traits and speciation rates (Rabosky and Goldberg 2015). For our purposes, the tight correlations among the methods (Fig. S1) and consistency in results across methods (Figs. 3, 4) suggest a more robust relationship between hybridization and diversification, although PGLS results suggest that this relationship explains relatively little of the variation in diversification rates (Table 2, Table 3). The permutation

tests that take full advantage of the posterior distributions of estimates from the BAMM diversification analyses were sometimes significant (Table 1), but unfortunately, we cannot estimate effect sizes in the same way that we can using PGLS. The differences in significance using different methods for estimating diversification may be due to the fact that both BAMM and Medusa estimate rate shifts across the phylogeny (Alfaro et al. 2009; Rabosky 2014), meaning that multiple closely-related families can share a diversification rate, while the method-of-moments (MS) estimation is based on branch lengths and richness (Magallon and Sanderson 2001), resulting in a uniquely-estimated diversification rate for each family. Our results suggest that the choice of method to estimate diversification is not crucial to our understanding of the overall direction of relationships between hybridization and diversification, but is important in determining whether the relationship is statistically significant.

Finally, all estimates of diversification rates are dependent on input phylogenies. Historical hybridization or introgression can also lead to phylogenetic conflict and complicate phylogenetic inference (Degnan and Rosenberg 2009), so incorrect inference of evolutionary relationships (associated with hybridization as well as other statistical factors) could lead to miscalculation of diversification rates, potentially in ways that influence our analyses since hybridization is our focus.

Conclusions

Here, we present limited evidence that hybridization and diversification rates are positively related across seed plant families. Evidence for this relationship is slightly stronger when estimated using permutation tests associated with diversification rates from BAMM than it is when estimated using PGLS, but statistical support still varies depending on hybrid metrics analyzed. Although the explanatory power of hybridization is weak in the PGLS analyses, the relationship is sometimes detectable when other factors (perenniality, woodiness) collinear with hybridization rates and previously linked to diversification rates are accounted for. The magnitude of the correlation between diversification and hybridization is on par with that of woodiness and either similar to or greater than that of perenniality. Although we cannot determine the directionality of causation via data at this taxonomic scale, our results are consistent with experimental evidence indicating that hybridization has the potential to speed up adaptive evolution (Mitchell et al. 2019b), so hybridization may enable more rapid diversification. More likely, hybridization and diversification may have positive effects on each other through a variety of mechanisms. The use of hypothesis-driven phylogenetic path analysis (van der Bijl 2018) may be one way to disentangle causality and incorporate both direct and indirect mechanisms

related to diversification rates overall. Detailed evidence suggests that hybridization events may trigger evolutionary radiations, such as in the Hawaiian silverswords (Barrier et al. 1999), Galapagos finches (Grant et al. 2005), Hawaiian crickets (Shaw 2002), and African cichlids (Genner and Turner 2012; Keller et al. 2013; Meier et al. 2017). Exploration of hybridization at the base of additional radiations and incorporation of ancient or cryptic hybridization events are needed to provide further confirmation of this hypothesis. Overall, a deeper theoretical, empirical, model-based, and experimental knowledge of hybridization, diversification, other factors, and the interplay between them is necessary to more completely understand these evolutionary phenomena.

AUTHOR CONTRIBUTIONS

K.D.W. and N.M. conceived of the study. N.M. performed the analyses. N.M. and K.D.W. wrote the manuscript. All authors contributed to revisions.

ACKNOWLEDGMENTS

DATA ARCHIVING

All relevant data and code generated for this work are available via the Dryad Digital Repository https://doi.org/10.5061/dryad.0gb5mkm1h (Mitchell and Whitney 2021).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Associate Editor: Y. Brandvain
Handling Editor: T. Chapman

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Net diversification rates calculated using three different methods are correlated.

Figure S2. Scatterplots of relationships between log-transformed hybridization measures and net diversification rates without correcting for phylogeny

Figure S3. Sister-clade comparison associations between differences in hybridization and differences in species richness across families

Table S1. Medusa diversification estimates calculated using different AICc thresholds are corelated.

Table S2. Method-of-moments (MS) diversification estimates are correlated calculated different values of ϵ are correlated.

Table S3. Supplemental results for univariate PGLS tests.

Table S4. Non phylogenetically-informed correlations between diversification rates and hybridization.

Table S5. Sister family pairs used for hybridization and diversification comparisons.

Table S6. Pearson correlations from sister-clade comparison between family differences in hybridization rates and family differences in diversification rates