

DO LATEX AND RESIN CANALS SPUR PLANT DIVERSIFICATION?  
RE-EXAMINING A CLASSIC EXAMPLE OF ESCAPE AND RADIATE COEVOLUTION

By

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## ABSTRACT

### DO LATEX AND RESIN CANALS SPUR PLANT DIVERSIFICATION? RE-EXAMINING A CLASSIC EXAMPLE OF ESCAPE AND RADIATE COEVOLUTION

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The association between increased lineage diversification rates and the evolution of latex and resin canals is widely cited as a paradigmatic example of Ehrlich and Raven's 'escape-and-radiate' hypothesis of co-evolution. However, it has been nearly three decades since these plant defenses have been examined as key innovations, and updates to phylogenetic comparative methods, plant molecular systematics, and phenotypic data warrant a reassessment of this classic finding. I gathered data on latex and resin canals across 345 families and 986 genera of vascular plants and conducted a multi-scale test of the association between these traits and lineage diversification rates. At a broad scale (across clades), I used sister-clade comparisons to test whether 28 canal-bearing clades had higher net diversification rates than their canal-lacking sister clades. At a finer scale (within clades), I used ancestral state reconstructions and phylogenetic models of lineage diversification rates to examine the relationship between trait evolution and the timing of diversification rate shifts in two better-characterized clades – Araceae and Papaveraceae. At both scales of analyses, I found poor support for the predicted relationship between diversification and the evolution of latex and resin canals. This re-examination reveals that there is no longer strong evidence for latex or resin canals as general, consistently replicable drivers of species diversity across plants. However, I could not rule out a relationship in all groups, and therefore argue that theoretical and empirical work aimed at understanding ecological factors that condition 'escape-and-radiate' dynamics will allow for more nuanced tests of the hypothesis in the future.

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## **BACKGROUND:**

A fundamental question at the heart of plant biology centers on explaining the rapid accumulation of plant species over recent evolutionary time. The pace of angiosperm evolution is so staggering that it has been called an “abominable mystery” (Darwin, 1879 in Darwin & Seward (1903); Berendse & Scheffer, 2009; Friedman, 2009; Buggs, 2017; Katz, 2018). The ‘mystery’ is well illustrated by the footprint that angiosperms have stamped into the fossil record (Ward, 1885; Buggs, 2017). When plotting species richness through geological time, both of the major angiosperm groups (monocots and dicots, which differ primarily by the number of embryonic leaves) have fossil footprints that resemble a top: the pace of early angiosperm evolution is slow and static, until the late Cretaceous where the abrupt rise of angiosperms explodes, rapidly increasing to a climax in the late Pliocene, and then tapering off in the early Quaternary (Figure 1 from Buggs, 2017). This pattern of early bursts followed by deceleration is consistent with an adaptive radiation (Schluter, 2000), although it is not universal among adaptive radiations (Puttick, 2017). Today, the ‘abominable mystery’ of angiosperm evolution has blossomed into a desire to understand why some groups of plants are very diverse, while others are relatively species poor (Magallón & Castillo, 2009; Donoghue & Sanderson, 2015). To start making sense of these patterns, work over the past 50 years has focused on identifying the pacemakers of macroevolution; or more specifically, traits and processes that are associated with diversification (the net result of speciation and extinction).

According to the “key innovation hypothesis”, traits with great ecological or functional significance may allow a lineage to colonize new adaptive zones such that lineages bearing a key innovation *may* contain more species than closely related lineages lacking the innovation (reviewed in: Rabosky, 2017). A number of traits have been invoked to explain the evolutionary



success and ecological dominance of angiosperms, including: animal pollination and seed dispersal, floral symmetry, floral ‘levers’, nectar spurs, fast growing and rhizomatous growth forms, subsidiary stomatal cells, nitrogen fixation, extrafloral nectaries, and latex and resin canals (Stebbins 1970, 1971; Regal 1977; Mulcahy 1979; Farrell, 1991; Doyle & Donoghue 1993; Hodges & Arnold, 1995; Claßen-Bockhoff et al., 2004; Sargent, 2004; Weber & Agrawal, 2014; Werner et al., 2014; Chen et al., 2017; Foisy et al., 2019). Despite the thrilling stories each of these key innovations tells, it is highly improbable that a single trait was the sole key to the success of angiosperms (Davies et al., 2004), and instead many traits and processes likely played in concert throughout their evolutionary history (Donoghue & Sanderson, 2015; O’Meara et al., 2016). In addition, as these studies are replicated across clades and traits, it is becoming clear that the relationship between key innovations and diversification is not always the same. Latex (Foisy et al., 2019), dioecy (Heilbuth, 2000; Vamosi & Otto, 2002; Sabath et al., 2016; but see Käfer et al., 2014), and polyploidy (Mayrose et al., 2011; but see Schranz et al., 2012; Vanneste et al., 2014) are examples of traits that can be associated with either positive or negative diversification rates.

My thesis focuses on re-visiting a classic key innovation in plants, latex and resin canals. These are plant defenses, stored in pressurized canal systems that have originated many times throughout plant evolution (Agrawal & Konno, 2009). Here, the mechanism by which the trait is hypothesized to be a key innovation is through defending the plant against herbivores. The idea that plant defensive traits could serve as key innovations was first proposed by Ehrlich and Raven in 1964. Their hypothesis was elegant: coevolution between plants and their herbivores could lead interacting clades to diversify in a stepwise fashion. More specifically, the evolution of a novel plant defense could allow plants to escape constraints imposed by herbivores, and

subsequently radiate into new niche space. Then, insects could diversify after evolving a countermeasure to the novel plant defense and the cycle could repeat. Indeed, there is evidence for stepwise radiations in co-evolving plant and herbivore lineages (Wheat et al., 2007; Mckenna et al., 2009), and well-documented patterns that link plant species diversity to the evolution of novel defenses (Farrell et al., 1991). Today, this hypothesis is also well known as the “escape and radiate” hypothesis (Thompson, 1989), and over 50 years later, remains prominent in evolutionary thinking (Janz, 2011; Suchan & Alvarez, 2015). By using updated models of lineage diversification, my master’s thesis research provides an updated example of the nuanced relationship between lineage diversification rates and defensive key innovation traits.

## **INTRODUCTION:**

In their landmark paper, Ehrlich and Raven (1964) changed the field of evolutionary ecology by proposing a novel hypothesis of coevolution. Now termed “escape-and-radiate” coevolution (Thompson, 1989), the hypothesis aims to explain plant diversity by invoking a coevolutionary tango between herbivores and plant defenses. Specifically, they posited that the innovation of novel defenses allows plants to “escape” constraints of herbivory and “radiate” (or persist under reduced extinction rates) in newly opened niche space. Ehrlich and Raven’s ideas remain prominent in current eco-evolutionary thinking, and many studies have tested various associations between plant defensive traits and increased lineage diversification (diversification = speciation – extinction) (reviewed in: Janz, 2011; Suchan & Alvarez, 2015). Despite an extensive literature on the topic, evidence for “escape-and-radiate” dynamics remains mixed, and there remains a paucity of data testing these ideas for the majority of defensive traits (Futuyma & Agrawal, 2009; Suchan & Alvarez, 2015). Here, we revisit one of the most iconic examples of escape-and-radiate dynamics: the association between lineage diversification and the evolution of latex and resin canals, which store and release sticky exudates that defend plants against herbivores (Farrell et al., 1991).

Both latex and resin are potent plant defenses that represent model traits for studying the evolution and ecology of plant-herbivore interactions (Agrawal & Konno, 2009). Although they differ in their anatomy (latex is stored in elongated cells called laticifers, whereas resin is stored within intercellular spaces called canals or ducts) and chemistry (latex is rich in proteins, alkaloids, and sugars, whereas resin is rich in terpenoids or phenolics), both latex and resin are stored in pressurized cellular structures that play active roles in defense (Agrawal & Konno, 2009; Konno, 2011; Prado & Demarco, 2018; Ramos et al., 2019). Notably, pressurization

allows latex and resin to be released when chewing herbivores damage the leaves, acting as an inducible defense via toxicity and by physically gumming-up herbivore mouthparts. Thus, despite anatomical and chemical differences, the functional similarity of laticifers and resin canals in pressurizing and exuding latex and resin, respectively, has led several authors to argue that these traits should be considered as a single defensive syndrome (Dussourd & Denno, 1991; Farrell et al., 1991). There is a rich body of work linking these traits to reduced herbivory across plants (reviewed in: Phillips & Croteau, 1999; Konno, 2011), and documenting the evolution of behavior or physical “counter-defenses” in herbivores (adaptations that allow herbivores to circumvent the latex and resin defenses, such as trenching and notching behaviors that depressurize canals before feeding (Dussourd & Denno, 1991). Together, this body of work suggests a strong role for latex and resin canals as key innovations in the escape-and-radiate process.

Latex and resin canals are also ideal candidates for conducting tests of the “escape-and-radiate” hypothesis because they each show high levels of evolutionary convergence across plant lineages (Langenheim, 2003; Agrawal & Konno, 2009). Latex and resin canals have originated at least 40 independent times (Farrell et al., 1991), and latex occurs in about 10% of flowering plant species (Agrawal & Konno, 2009) while resin occurs in roughly 53% of plant orders (Langenheim, 2003). In 1991, Farrell et al. took advantage of the highly convergent histories of laticifers and resin canals across vascular plants to test for patterns consistent with trait-associated shifts in lineage diversification rates. Farrell et al. (1991) compared 16 pairs of sister-clades that differed in the presence or absence of laticifers/canals, performing a sign test to evaluate whether there was evidence for enhanced species richness in clades with laticifers/ducts relative to sister clades without laticifers/ducts. By controlling for clade age using sisters, their

design allowed for the comparison of net diversification rates across many independent evolutionary events, offering an intuitive and replicated test of the escape-and-radiate hypothesis. Their results were striking: 13 of the 16 comparisons revealed higher species richness when canals were present, supporting the prediction that the origins of plant defensive traits are associated with higher diversification rates in plants. This study was one of the first to show strong support for Ehrlich and Raven's ideas, and remains a prominently cited empirical example (e.g. Schluter, 2000; Magallon & Sanderson, 2001; Coyne and Orr, 2004; Agrawal & Konno, 2009).

While Farrell et al.'s (1991) paper was an instant classic, major progress over the last three decades in the fields of plant molecular systematics, phylogenetic comparative methods, and trait phenotyping warrants a reassessment of the role of latex and resin canals in plant diversification. Farrell et al. (1991) themselves stressed that their results were subject to future research, especially in regard to plant systematics (e.g. "Plant phylogeny is the subject of intense current research, and all of the phylogeny estimates we accept should be regarded as possibly erroneous."). Indeed, since Farrell et al.'s 1991 study, plant systematics has undergone major changes. Several notable milestones include the rise of molecular systematics, major updates to deep phylogenetic hypotheses, the development of "big tree" methods, and the formation of the Angiosperm Phylogeny Group (currently APG IV, 2016). While Farrell et al. (1991) argued that "...taxonomic error should be random with respect to the escalation-diversification hypothesis, making a significant effect harder to detect...", several of the original sister comparisons in the paper have changed dramatically under modern systematic analysis (see: Appendix A; compare Supplementary Tables 3 and 4). Thus, a re-assessment of the relationship between the latex, resin canals, and plant diversification is now due.

Along with changes in molecular systematics, the last thirty years have also seen the development of novel phylogenetic comparative methods for evaluating links between trait evolution and shifts in diversification rates. New approaches for sister comparisons (Paradis, 2012; Käfer & Mousset, 2014) plus methods for explicitly modelling diversification rates (reviewed in: Morlon, 2014; O’Meara & Beaulieu, 2016) offer benefits over the original sign test approach used by Farrell et al. (1991). For example, modern sister comparison methods, such as diversity contrast tests, now consider the numerical values of species richness across clades (opposed to the qualitative sign of the difference only – as in sign tests) (Wiegmann et al., 1993.; Barraclough et al., 1995, 1996; Sargent, 2004). Modern sister-clade comparison methods also use maximum likelihood to estimate null hypotheses (McConway & Sims, 2004; Paradis, 2012) and to account for stem length biases (Käfer & Mousset, 2014). Given a well-resolved phylogeny, a collection of likelihood-based methods is also available to explicitly model lineage diversification through time (e.g. Alfaro et al., 2009; Pennell et al., 2014; Rabosky, 2014) sometimes simultaneously modelling trait evolution (e.g. Maddison et al., 2007; Beaulieu & O’Meara, 2016). While these clade-based models come with their own set of assumptions, they provide a more robust framework to test for strong associations between the timing of trait evolution and shifts in diversification rates. Ultimately, modern methods can account for several limitations to the original sign tests and create opportunities to apply a set of complementary methods in tandem to evaluate patterns of diversification across multiple scales.

Here, we ask whether updated systematic, phylogenetic, and phenotypic data crystallizes, complicates, or challenges the relationship between lineage diversification and the evolution of latex and resin canals. We test for macroevolutionary patterns consistent with the predictions from Ehrlich and Raven (1964) at multiple phylogenetic scales. First, we use expanded and

updated sister clade comparisons to broadly test whether clades with canals are generally more speciose than clades without canals. Second, zooming in on two groups with more phylogenetic resolution and sufficient phenotypic sampling – the poppies (Papaveraceae) and the aroids (Araceae) – we use ancestral state reconstructions and phylogenetic models of lineage diversification rates to examine whether independent origins of latex and resin canals correspond to positive shifts in diversification rates. This integrative approach allows for a replicated, multi-scale test of the hypothesis that trait evolution is repeatedly associated with increased net diversification rates, while avoiding the drawbacks of studies that rely on any single scale or approach (see: Maddison & FitzJohn, 2014; Rabosky & Goldberg, 2015). Together, this study represents a modern test of the macroevolutionary consequences of latex and resin canals – evolutionarily convergent and ecologically important defensive traits in vascular plants.

## **METHODS:**

To test for associations between latex, resin, and lineage diversification rates, we (1) compiled an updated list of taxa reported with latex or resin canals in the literature, (2) conducted modern sister comparisons (across a broad set of clades), and (3) reconstructed historical patterns of diversification rates and trait evolution in two focal families from the sister comparisons that had higher data resolution. General methods for each approach are detailed below, with specific details available in the supplementary material.

### ***(1) Trait database***

To improve accuracy from the original Farrell et al. 1991 sister comparisons and to expand our dataset to more broadly test the “escape-and-radiate” hypothesis, we compiled current published information about the phenotypic distribution of species with and without latex and/or resin canals. First, we surveyed previously published datasets on the presence and absence of latex and resin canals, including those used by Farrell et al. (1991) (see: Supplementary Table 3), plus 76 additional sources. We focused on comprehensive datasets rather than single-species accounts. Because these traits (especially latex) are taxonomically informative for many plant groups, we scrubbed several taxonomic sources: (1) the Angiosperm Phylogeny Group website (<http://www.mobot.org/MOBOT/research/APweb/>, accessed May through October 2018), (2) a number of floristic and taxonomic treatments (Condit et al., 2010; Dicht & Lüthy, 2006; Evert, 2006; Haston & Condit, 2011; Kubitzki & Kadereit, 2004; Mabberley, 2017; Singh, 2016; Tomlinson, 2016; Willis, J. C. 1973; Lewinsohn, 1991), and (3) a compendium of plant resins (Langenheim, 2003). After data collection, we updated the list of taxa to represent current nomenclature using the R package *taxize* (Chamberlain & Szöcs, 2013), cross-referencing between the Taxonomic Name Resolution Service (TNRS) reference database and the National



Center for Biotechnology Information (NCBI) taxonomy browser. For any taxon that disagreed across these two databases, and for any hits that had a score less than 100%, we manually checked Tropicos (Garden, 2008) for nomenclature. Finally, duplicate taxa were concatenated into a single datum, with more recent data supplanting older data.

***(2) Sister clade comparisons: a broad test of the relationship between latex, resin canals and diversification rates***

We used sister comparisons to perform a broad-scale test of the relationship between plant diversification and latex and resin canals. We combined our phenotypic database with recent literature on plant systematics and richness estimates (Supplementary Table 4) to identify independent and up-to-date sister comparisons between sister-lineages with and without latex and/or resin. Sister comparisons were restricted to cases where: *(i)* monophyly was supported for each clade, *(ii)* there was evidence for an independent origin of the latex and/or resin canals within the focal clade, and *(iii)* there was a well-supported sister clade that entirely (or almost entirely) lacked the focal traits. Under these criteria we aimed to include as many comparisons as possible – including the 16 original comparisons (Supplementary Table 3) performed by Farrell et al. (1991) as feasible. Due to shifts in taxonomy, two of the original comparisons could not be included (see: Celastrales in Appendix A, and compare Supplementary Tables 3 and 4). Rather than having our analyses dependent on a single richness value per clade, we collected a range of minimum and maximum richness estimates, collected from the Angiosperm Phylogeny Group Website (<http://www.mobot.org/mobot/research/apweb/>, accessed September through October 2018), The Plant List database (<http://www.theplantlist.org/>, accessed September through October 2018), and primary literature (Supplementary Table 4).

To test whether clades with the latex and resin canals were more speciose on average than clades without these traits, we applied difference-based contrast tests (e.g. Sargent, 2004; Paradis, 2012). Our data were non-normally distributed, so we used a non-parametric Wilcoxon signed-rank test. We performed a one-sided, paired test to evaluate the hypothesis that richness is higher, on average, in canal-bearing clades relative to canal-lacking sister clades. This analysis was conducted once for our maximum richness estimates for each clade, and then repeated in an independent test using the minimum richness estimates. We performed each difference-based contrast test using the *wilcox.test* function in the R package *stats* (R Core Team, 2013), and cross-validated these results with the *diversity.contrast.test* function in the R package *ape* (Paradis, Claude, & Strimmer, 2004). Note that in order for sister comparisons with similar richness values to be included in these analyses, it was necessary to add a small amount of random noise using the *jitter* function in base R (R Core Team, 2013).

To make this study directly comparable to Farrell et al. (1991), we also applied the above methods to the original richness dataset of Farrell et al. (1991) (Supplementary Table 3). This allowed us to evaluate whether any differences between our results and the results of Farrell et al. (1991) were due to updates made to statistical methods, phenotypic/phylogenetic datasets, or both. Because Farrell et al. (1991) occasionally reported a range of values for some clades, we analyzed both their maximum and their minimum richness estimates in two separate contrast tests, performed as above.

### ***(3) Models of lineage diversification rates: Zooming in on patterns of trait evolution and lineage diversification rate shifts***

Although sister comparisons offer a powerful tool for replicated tests across a broad swath of clades, they do not evaluate whether the timing of diversification rate shifts in a

phylogeny are associated with the evolution of a trait of interest. To evaluate more nuanced relationships between lineage diversification and the timing of trait evolution, we used MEDUSA analyses and ancestral state reconstruction in two plant families: poppies (Papaveraceae) and aroids (Araceae). Both groups showed patterns consistent with escape-and-radiate evolution in the sister comparisons – that is, greater species richness in canal-bearing clades (Supplemental Table 4) – providing good candidate groups to test for linked evaluate the putative relationship between diversification and trait evolution. Defensive traits are also well documented for both of these groups: laticifers across Papaveraceae (Hoot et al., 2015) and laticifers and resin canals across Araceae (French 1987, 1988; Cusimano et al., 2011).

We reconstructed relative time ultrametric maximum clade credibility phylogenies of each group using BEAST (Bouckaert et al., 2014). For details on phylogenetic reconstruction see the supplemental methods. We analyzed lineage diversification rates of each clade using the R package MEDUSA (Alfaro et al., 2009; Brown et al., 2018). To visualize the rate shifts, net diversification rates, and richness data on each family tree (Araceae or Papaveraceae), we plotted the bootstrapped results of MEDUSA diversification analyses onto the respective maximum clade credibility tree using the *plotMultiMedusa* function from the MEDUSA package (Alfaro et al., 2009; Brown et al., 2018). To examine whether shifts in lineage diversification were associated with the evolution of latex/resin, we conducted ancestral state reconstructions and plotted evolutionary gains and losses of latex/resin. Because we are uncertain about the link between these traits and diversification, we reconstructed trait evolution using both state-dependent diversification models and diversification-free models. Specifically, we used the *asr* function in the R package *diversitree* (FitzJohn, 2012) to reconstruct marginal likelihoods of ancestral states for discrete characters under a model of evolution (biSSE) that accounts for

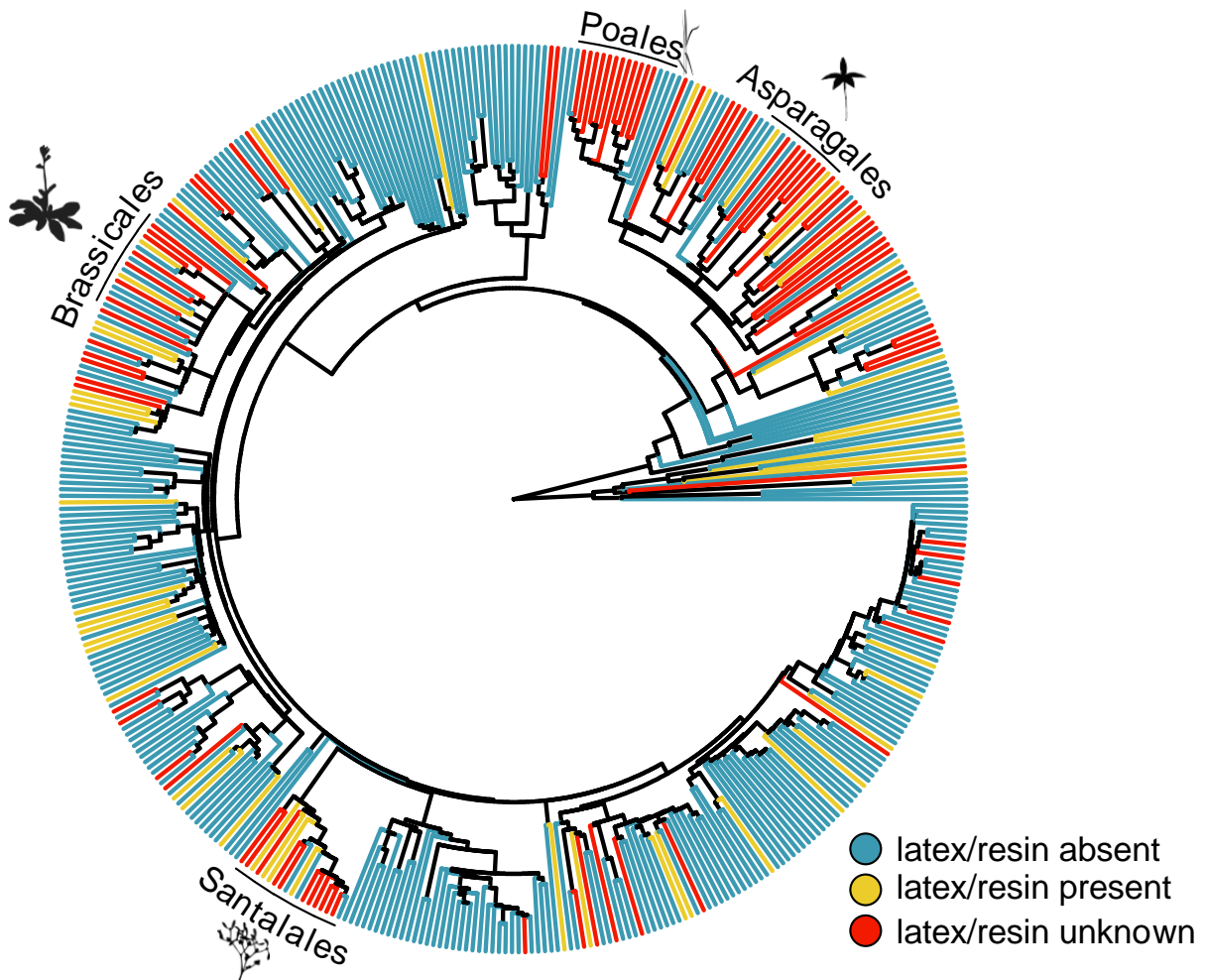
diversification, and the rayDISC and corHMM functions in the R package corHMM (Beaulieu et al., 2017) to reconstruct ancestral states under a diversification-free model (canonical Markov model) (Lewis, 2001). Due to constraints of our dataset, we were not able to reliably make inferences using hiSSE or fiSSE analyses (see: supplementary methods). Finally, to summarize diversification rates over time and with respect to each character state, we adopted the approach of Nakov et al. (2018): we binned the phylogeny into time-intervals of 0.001 units, resampled the parameter estimates at each interval, and then plotted the values by character state. Note that diversification rates are sensitive to the maximum tree depth and are inflated due to the relative time calibration in BEAST (Supplementary methods); however, this should not affect conclusions drawn within the clade.

## **RESULTS:**

### ***(1) Trait database***

In total, we gathered data on the presence and absence of latex and resin canals across over 345 families and 986 genera of vascular plants (archived in the Dryad Digital Repository: doi:10.5061/dryad.2mn0j54). Compared to previous databases (Metcalf & Chalk, 1983; Lewinsohn, 1991), this expanded database increases the number of sampled groups by hundreds of additional genera, and nearly 20 additional families. Plotting the data onto a phylogeny for vascular plants (Fig. 1) illustrates wide sampling effort across the plant tree of life. However, there are several notable gaps in available information on the presence/absence of latex/resin canals across plant families, suggesting clades where research is lacking (e.g. several families in the orders Poales, Santalales, Asparagales, and Brassicales; for more, see: Supplementary Table 2 and Appendix A).

**Figure 1:** Family-level phylogeny (Zanne et al. 2015) showing the presence (yellow) or absence (blue) of latex or resin canals across plants. Branches in red indicate clades where the occurrence is unknown. Names denote plant orders for which missing data is particularly common (silhouettes are utilized from [phylopic.org](http://phylopic.org)). Note that this figure does not indicate if traits are synapomorphic, but rather where data are available or lacking. A version of this figure with latex and resin canals depicted as independent traits can be found in the supplementary information.

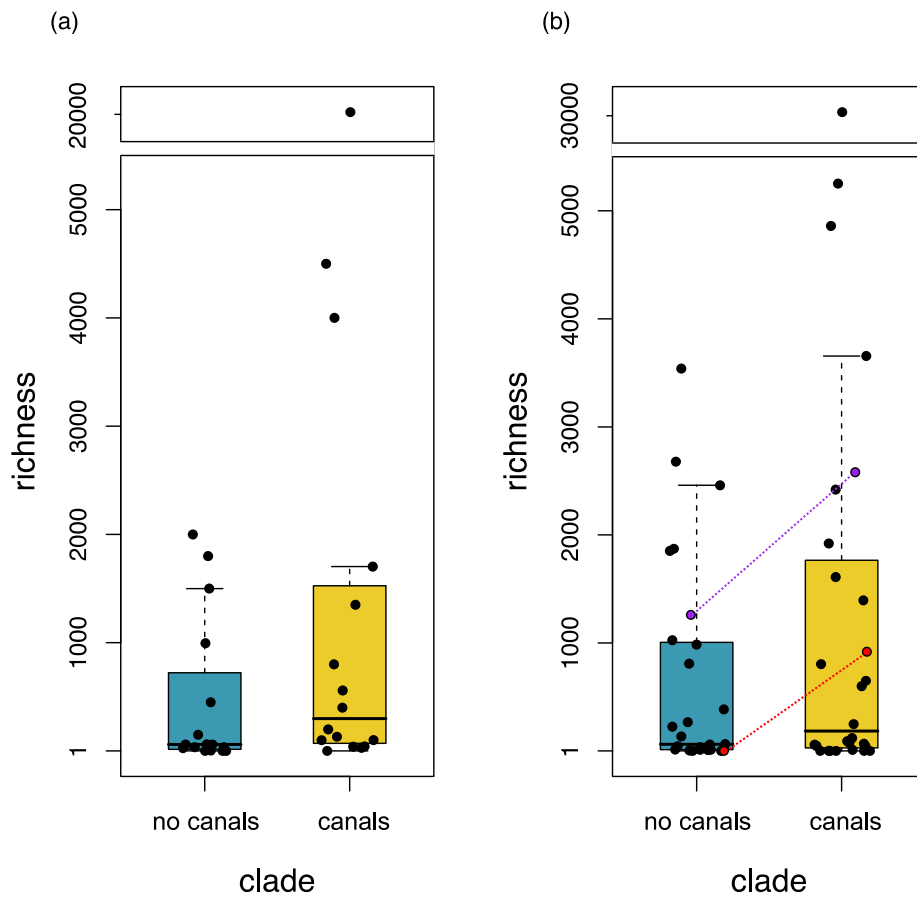


***(2) Sister clade comparisons: a broad test of the relationship between latex, resin canals and diversification rates***

In total, we identified and analyzed 28 independent sister comparisons that fit our criteria (Supplementary Table 4), nearly doubling the original sample size of Farrell et al. 1991 (n=16, Table 1, Supplementary Table 3). Detailed information on each sister comparison, as well as notes on clades that did not fit all of the criteria but offer promise for future study, are detailed in Appendix A. A Wilcoxon signed-rank test on our 28 sister comparisons suggested that latex/resin-bearing clades are not more species-rich than sister groups without these traits; this was true whether analyzing minimum ( $V = 250$ ,  $= 1132 \pm 4655$  species,  $n = 28$ , one-tailed  $p = 0.147$ ; Supplementary Table 4) or maximum ( $V = 245$ ,  $= 1519 \pm 6303$  species,  $n = 28$ , one-tailed  $p = 0.132$ ; Supplementary Table 4) richness estimates.

Repeating our analysis on the original richness data from Farrell et al. (1991) (Table 1, indices 1-16) revealed that the differences between our results and the results of Farrell et al. are a consequence of additional trait data and updated systematics, not a consequence of updated analysis methods. Using the original dataset of Farrell et al. (1991), 13 of the 16 original comparisons under previous taxonomy had higher richness (sign test: 13/16,  $p=0.0106$ ; Supplementary Table 3). When we apply modern contrast tests to the same dataset, we find an even stronger relationship, whether computed with Farrell's minimum richness values ( $V = 120$ ,  $= 1980 \pm 5512$  species,  $n = 16$ , one-tailed  $p = 0.003$ ; Supplementary Table 3) or maximum ( $V = 119$ ,  $= 1799 \pm 5467$  species,  $n = 16$ , one-tailed  $p = 0.003$ ; Supplementary Table 3), suggesting that our results are not due to methodological differences between studies. A box plot summarizing the results for (a) the original data in Farrell et al. (1991) and (b) our extended dataset, can be seen in Fig. 2.

**Figure 2:** A box plot showing the distribution of species richness values across clades with latex/resin canals and their sister clades without canals from (a) Farrell et al. (1991) (Supplementary Table 3) and (b) our updated and extended dataset (Supplementary Table 4). In panel b, purple shows Araceae, while red denotes Papaveraceae, both of which we investigate in additional analyses. Note that the y-axes contain breaks, which have different upper values.





### ***(3) Models of lineage diversification rates: Zooming in on patterns of trait evolution and lineage diversification rate shifts***

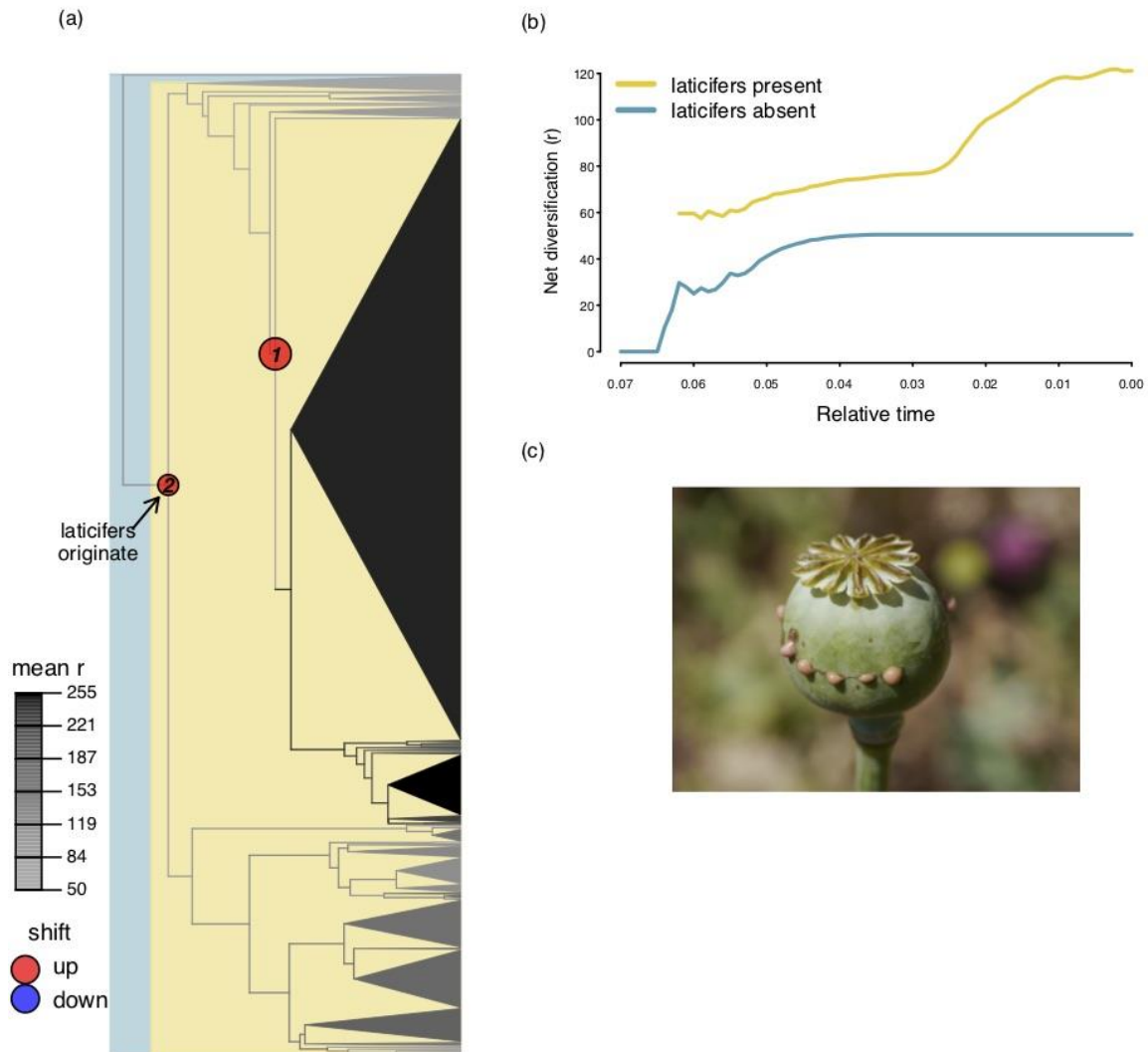
For diversification analyses performed using MEDUSA, we present the results separately for each of the two focal families: first Papaveraceae (3a) and then Araceae (3b). Note that in the sister comparisons, both Araceae and Papaveraceae met the predicted pattern of greater species diversity in canal-bearing clades (Supplemental Table 4). Diversification rate shifts are discussed with respect to particular nodes, numbered based on the output of MEDUSA. This was done with the intent of allowing readers to lookup specific statistics in the supplementary materials. Lastly, ancestral state reconstructions for latex and/or resin inferred from *diversitree* did not differ qualitatively from reconstructions under a diversification-free Markov model – the character histories were the same across models. All reconstructions can be found in Supplementary Figs. 1-4.

#### ***(3a) Papaveraceae: the origin of laticifers is associated with a shift in diversification rates***

Pairing marginal ancestral character reconstructions with MEDUSA models revealed that latex originated once in poppies (Papaveraceae), and that this origin coincided with a positive shift in the net lineage diversification rate (consistent with escape-and-radiate coevolution). Marginal ancestral state reconstructions revealed that laticifers originated once in Papaveraceae and were never lost subsequently (Supplementary Fig. 1). We found no data suggesting resin canals in Papaveraceae. Pairing these analyses with MEDUSA revealed that laticifers originated early on in the group, and that this origin is associated with an increase in diversification rates and followed by a subsequent but unassociated diversification event nested within the laticiferous group (Fig. 3a). The initial shift (node.id=2; mean shift = 20.37,  $\pm$  19.24; Fig. 3a; Supplementary Table 5) represented a three-fold rate increase in diversification, and occurred

concurrently (on the same branch) with the evolutionary origin of laticifers, early on within the lineage, enveloping all extant poppy genera except for the non-laticiferous *Pteridophyllum*. The second and larger increase is nested deeper in Papaveraceae (node id=1; mean shift =  $176.33 \pm 21.10$ ; Fig. 3a; Supplementary Table 5). This more recent shift subtends most of the subfamily Fumarioideae, including the notably species-rich genera *Corydalis* (586 species) and *Fumaria* (57 species); however, the shift is not associated with any evolutionary gains or losses of laticifers (Fig. 3a). Lastly, sampling the parameters through time for each phenotypic state reveals that net diversification rates are always higher in the laticiferous lineages, relative to the non-laticiferous lineage (*Pteridophyllum*) (Fig. 3b).

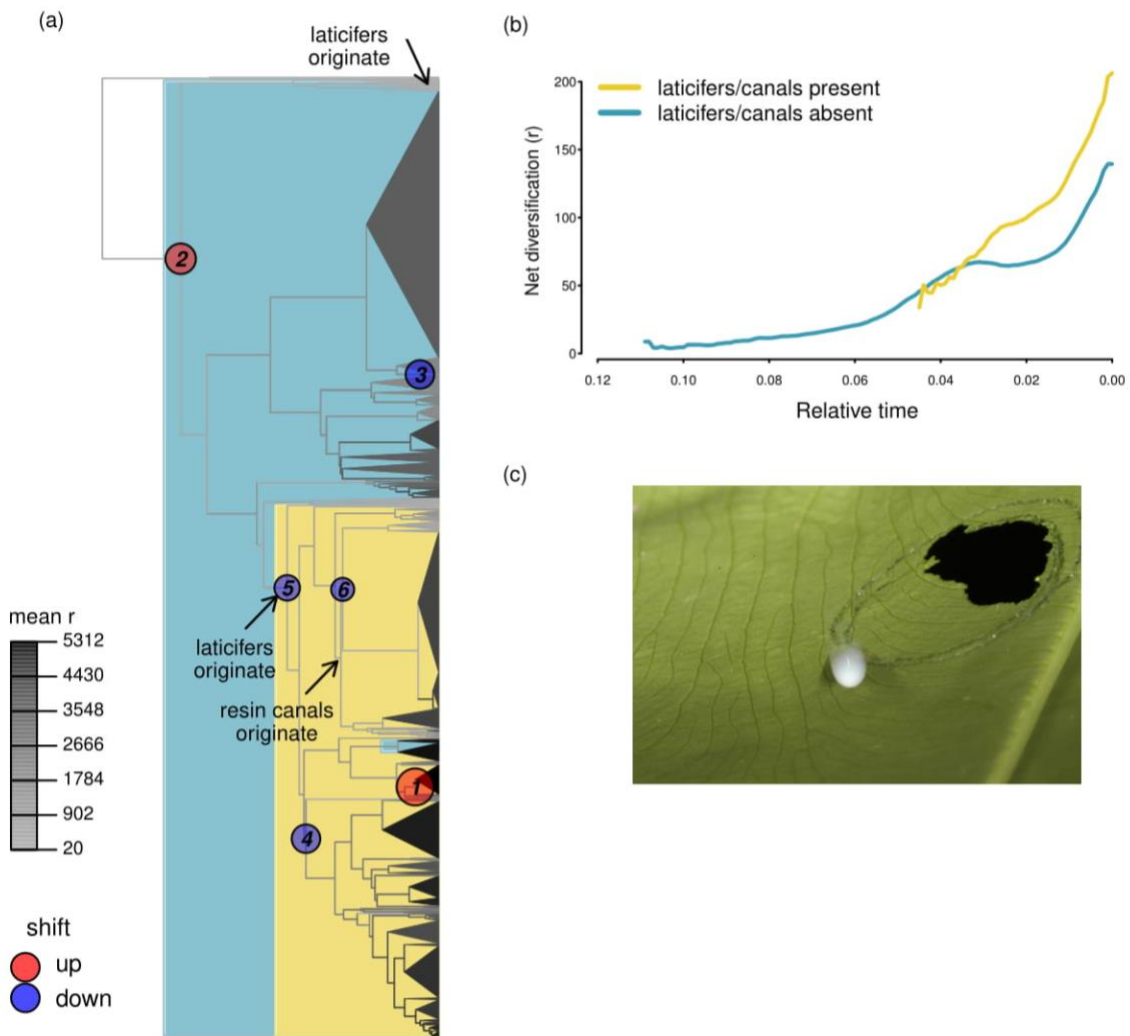
**Figure 3:** The results of MEDUSA diversification analyses in Papaveraceae. The results from 1000 random samples of the posterior distribution are summarized on (a) the maximum clade credibility tree, showing the inferred shifts in mean diversification rates ( $r$ ) and the origins of laticifers. (b) The estimated net diversification rates ( $r$ ) through relative time for lineages with and without laticifers. (c) Rich, latex exuded by *Papaver somniferum* (Papaveraceae), near Chihuahua, Mexico (photo credit: Anurag Agrawal).



*(3b) Araceae: the evolution of latex and resin canals does not correspond with shifts in diversification rates*

While our analyses uncover an eventful history of diversification and trait evolution within Araceae, with laticifers evolving twice and resin canals evolving once within the family, there was no obvious association between any of the three evolutionary origins of latex or resin and positive shifts in net diversification rates (Fig. 4a). In total, MEDUSA estimated six rate shifts, four of which were negative (i.e. diversification slowing down; Fig. 4a). Early on in the lineage, before latex or resin had evolved in the family (Supplementary Figs. 2-4), diversification increased (node id = 2; mean shift =  $105.86 \pm 31.33$ ; Fig. 4a; Supplementary Table 6). Following this initial increase in diversification, there were four subsequent decreases in diversification rates and one large increase in diversification that was 50 times the magnitude of the original increase (node id = 1; mean shift =  $5312.28 \pm 3443.52$ ; Fig. 4a; Supplementary Table 6). Only one of the six total shifts corresponded to an evolutionary change in the focal traits: a gain of laticifers was associated with a 27% *reduction* in diversification rates (node id = 5; mean shift =  $-121.55 \pm 101.70$ ; Fig. 4a; Supplementary Table 6), the opposite of the prediction from escape-and-radiate coevolution. Similarly, following the origin of resin canals, a subsequent reduction in diversification rates was observed (node id = 6; mean shift =  $-119.60 \pm 39.07$ ; Fig. 4a; Supplementary Table 6). Lastly, sampling the rates through time for each phenotypic state illustrates that, despite no association between the timing of trait origination and diversification shifts, overall net diversification rates are still consistently higher when laticifers and/or resin canals are present in Araceae (Fig. 4b).

**Figure 4:** The results of MEDUSA diversification analyses in Araceae. The results from 1000 random samples of the posterior distribution are summarized on (a) the maximum clade credibility tree, showing the inferred shifts in mean diversification rates ( $r$ ) and the origins of resin canals and laticifers. (b) The estimated net diversification rates ( $r$ ) through time for lineages with laticifers and/or resin canals and lineages without. (c) Latex exuding from a trenched leaf of *Colocasia gigantea* (Araceae), in Ba Be National Park, Vietnam (photo credit: Chris Darling).



## **DISCUSSION:**

Testing for evidence of “escape-and-radiate” coevolution has been a major goal in evolutionary ecology ever since Ehrlich and Raven published their 1964 hypothesis (Futuyma & Agrawal, 2009). In this study, we revisit a classic test of this hypothesis, Farrell et al.’s 1991 paper investigating whether the evolution of latex and resin canals spur lineage diversification in plants. We draw on an additional 28 years of systematic, phylogenetic, and phenotypic resources to conduct a multi-scale re-valuation of this question (Farrell et al., 1991). We found poor support for the relationship between the evolution of latex and resin canals and enhanced diversification rates across scales. At a broad scale, sister clade comparisons did not support the prediction of greater species richness in canal-bearing clades. At a finer scale, zooming-in on two clades (that were supported in the sister-clade analyses) using diversification rate analyses revealed that origins of canals were disconnected from the timing of diversification rate increases: while rates were higher overall when canals are present, of the four origins examined only one was associated with an increase in diversification rates. While our study does not rule out a conditional role of latex and resin in driving diversification in some groups, our findings do suggest that the evolution of latex and/or resin canals should not be invoked as a general, consistently replicable explanation for patterns of species diversity across plants. Below we (i) discuss the complementary findings of our sister-clade and clade-specific analyses, (ii) consider the potential for latex and resin canals to be conditional drivers of diversification in plants, and (iii) acknowledge important caveats of our study and highlight ways forward for future research.

***The complementary results of sister-clade and clade-specific diversification analyses in evaluating the latex/resin canal hypothesis***

The power of diversification rate studies (both statistically and theoretically) is increasingly predicated on evolutionary replication (Maddison & FitzJohn, 2014; Donoghue & Sanderson, 2015). In this worldview, our ability to confidently assign a trait a causal role in driving increased diversification requires statistically significant replication. On the other hand, detailed studies within clades are required to link the timing of trait evolution with diversification dynamics, and detailed single (non-replicated) occurrences of a trait associating with high diversification rates have been cited as evidence for a role in driving patterns of diversity in several prominent examples (e.g. the evolution of flowers, mammary glands; Wagner & Lynch, 2010). Thus, while sister comparisons offer a broad view of diversification across plants, in-depth studies of diversification in focal clades are essential to understand the relationship (or lack thereof) between trait evolution and diversification. We contend a multi-scale approach marries the best of both worlds by allowing for more nuanced tests of relationships between traits and diversification, while not losing evolutionary replication as additional groups become well-resolved.

In our study, detailed modelling in two sister clades revealed a complex relationship between traits and the timing of diversification. In Araceae, we found a general disconnect between trait evolution and the tempo of diversification. However, Araceae did show higher diversification rates in laticiferous and canal-bearing clades, suggesting that an underlying relationship should not be ruled out entirely. Indeed, it may be unrealistic to expect “key innovation” traits to associate instantaneously (on the same branch of a phylogeny) with increased diversification rates in all cases due to the many confounding factors possible at the macroevolutionary scale (Rabosky, 2017; Vamosi et al., 2018). In Papaveraceae, we found a clear concordance between the origin of latex and a positive shift in diversification, consistent

with the *a priori* hypothesis. However, a closer look at the biology of this clade uncovers additional traits that arise at that same node as latex (e.g. calcium oxalate seed crystals) that could be associated with the diversification shift early on in the lineage. This suggests that there is as much evidence for latex as a “synnovation” (Donoghue & Sanderson, 2015) as for a “key innovation” in this group. These analyses demonstrate that closer examination of tractable clades from sister comparisons are a fruitful approach for uncovering nuanced effects of latex and resin canals on diversification. Pairing sister clade analyses with additional in-depth clade-based analyses as more data becomes available will be a particularly fruitful approach in future studies.

***Is there evidence for a conditional role of latex and resin in spurring plant diversification?***

While we did not find a strong replicable relationship between latex and lineage diversification across plants, we cannot rule out a context-dependent relationship between diversification and the evolution of latex and resin canals. Our study thus joins a growing body of work suggesting that more theoretical and empirical work is needed to clarify how consistently and under what conditions hypothesized traits are expected to spur diversification, in coevolutionary (Yoder & Nuismer, 2010) and “key innovation” frameworks (Rabosky, 2017). Because of their high level of evolutionary convergence across plants and their clear role in defense, latex and resin are a promising model trait to examine this topic in future studies.

Several other plant traits are hypothesized to conditionally spur diversification rates in plants, and these traits could be included in future analyses. For example, dioeciousness (Sabath et al, 2016) and mutualisms (Weber & Agrawal, 2014) are all hypothesized to impact lineage diversification in certain conditions. More directly, both the amount and chemical content of latex and resin vary considerably across species that have been investigated (Konno, 2011; Langenheim, 2003), and have been shown to impact the traits defensive efficacy (Agrawal et al.,



2008). As such, one might predict that latex/resin with particularly toxic secondary metabolites, high secondary metabolite diversity, or high secondary metabolite abundance would have larger impacts on a plants' ability to "escape" herbivory, and consequently, impact the relationship between these traits and diversification. Similarly, variation in the evolutionary responses of herbivores, such as trenching behaviour and host switching, could condition diversification under escape-and-radiate dynamics. Interestingly, seven of the sister-clade lineages in this study have canal-cutting herbivores reported (D. Dussourd, personal communication), and in all seven cases the lineage with canals has higher diversity than their sister group.

Extrinsic factors may also drive conditionality in the relationship between latex/resin canals and diversification. For example, variation in rates of diversification or trait evolution between temperate and tropical clades (e.g. Mittelbach et al., 2007; but see: Schluter & Pennell, 2017) could obfuscate patterns in our data if sister clades consistently differ in their regional associations. Previous work by Lewinsohn (1991) found that latex is more common and more abundant in tropical plant species and herbivory pressures have been shown to be higher in the tropics (Coley, 1996; Baskett & Schemske, 2018). A cursory post-hoc examination of the clades in this analysis, in which we evaluated whether sister clade status is confounded with tropicality, did not reveal a detectible signal of strong latitudinal differences across clades (Supplementary Fig. 5). However, a detailed analysis of diversification rates, latitude, and trait evolution is necessary to fully disentangle this issue. In addition to latitude, other external factors hypothesized to impact plant diversification include ploidy, shifts in floral form, and outcrossing (summarized in: Table 1, Vamosi et al., 2018). Ultimately, when evaluating patterns of diversification at such a large scale (across many independent origins of a trait), there is high

potential for shifts in these traits to occur in at least some of the groups examined, potentially overshadowing the role of latex/resin in particular clades.

Finally, in studies that examine a large number of origins, accounting for clade age could prove informative. A high number of sister clade comparisons in our study were relatively young and species poor (Fig. 2), which may confound our ability to detect diversification rate shifts. This is particularly true in light of recent evidence suggesting that diversification rates may be time-dependent across the tree of life (Hena-Diaz et al., 2018). Ultimately, it may be that variation in the relationship between latex, resin and diversification rates across clades is unlikely to be explained by a single factor, or even handful of factors, and instead a myriad of clade-specific factors may shape diversification patterns across plants (Donoghue & Sanderson, 2015).

***Caveats of our approach and ways forward:***

Our study presents an updated test of whether latex and resin canals are broadly associated with increased diversification in plants. However, like that of Farrell et al. (1991), this study includes several major caveats that should be taken into consideration.

First, just as systematic relationships have changed in the 28 years, the relationships in this study may also change with future data and methodological advances. While this is a caveat of any phylogenetic study, it warrants particular mention in light of the differences between the 1991 paper and the current study. That being said, we have confidence in our data, as we only included sister comparisons with currently well-supported phylogenetic relationships. However, to aid future work in this area, we discuss any uncertainty in the systematic relationships of our current sister comparisons, and identify clades we omitted, in Appendix A.

Second, similar to phylogenetic hypotheses, updated trait data on the distribution of laticifers and ducts also have the potential to change future results. We attempted to take a conservative approach to aid in this issue, only making sister comparisons when the available trait data met certain criteria (i.e. laticifers and/or resin canals were known ancestral synapomorphies or derived states). However, for many potential sister-clades this was not possible due to insufficient trait or phylogenetic data (detailed in Appendix A). While these instances were not analyzed in this study, their inclusion in future analyses (with additional data) could alter conclusions. In a similar vein, if canal-bearing clades have higher persistence (over macroevolutionary time) than their canal-lacking sister clades, then it is possible that the extinction of lineages without canals could bias our results. If the “true” sister clade has gone extinct, then the sister comparisons will be conducted on the next closest-related clade. A similar bias may exist if laticiferous or resinous clades go extinct and thus are not available to test. While these biases cannot be accounted for in most sister comparisons, they could be an issue, particularly for deep trait origins (like most origins of latex and resin canals), or for lineages that have low (or negative) diversification rates.

Third, diversification rate analysis methodology is changing rapidly, and more sophisticated analyses of trait-dependent diversification applied to clade-specific studies could illuminate our understanding of this question. We were unable to apply state-dependent diversification models (e.g. hiSSE Beaulieu & O’Meara, 2016) to our analyses due to a lack of phylogenetic resolution and power (see supplementary methods), and while our MEDUSA analyses offer an agnostic characterization of diversification patterns in these clades, diversification analyses with hiSSE (Beaulieu & O’Meara, 2016) and fiSSE (Rabosky & Goldberg, 2017) will more directly test the state-dependent hypothesis that diversification is

dependent on latex and resin canals, as well as disentangle the effects of focal traits from the hidden effects of unmeasured drivers of diversification. These and other sophisticated methods will become increasingly accessible to researchers as more species-level phylogenies and trait data become available for clades representing independent origins of canals.

Fourth, our study (like Farrell et al., 1991) treats laticifers and ducts as a single, discrete character state and assumes that all laticifers and resin ducts play an equivocal role in defense. While we have evidence of defensive functions for laticifers and/or resin canals in many of the groups studied (Konno et al., 2004 (Caricaceae); Konno et al., 2006 (Moraceae); Dussourd & Eisner, 1987 (Apocynaceae); Dussourd 1993, 1995 (Asteraceae, Campanulaceae); Kniep, 1905 (Euphorbiaceae); Harris, 1960; Lewinsohn et al., 1991 and Phillips & Croteau, 1999 (conifers); Nawrot, 2017 (Papaveraceae)), not all occurrences of laticifers or resin canals have been tested for defensive roles. Given the variation in form (e.g. ideoblasts, sacs, canals, trichomes) and function (e.g. defense, pollinator attraction, *etc.*) of secretory structures containing latex and resins, binning laticifers and resin canals into binary states may oversimplify the nature of these traits. Given that anatomical data is limited for the structure of laticifers and resin canals across clades (Farrell et al., 1991), and that the role of defense has not been tested in most occurrences of laticifers (Castelblanque et al., 2017), broader categorization is currently intractable in most clades. Furthermore, additional categories would increase the number of parameters in diversification models, further increasing the demand for more trait data. Because we were retesting a historically impactful study, and for the reasons outlined in Farrell et al. (1991) and Dussourd and Denno (1991), we make the assumption that latex and resin canals are a single defensive syndrome. Currently there is not enough data to test resin canals independently, but a cursory analysis considering latex separately reveals that relationship between latex and

diversification is even weaker than when both traits are considered together ( $V = 230$ ,  $= 1552 \pm 6421$  species,  $n = 27$ , one-tailed  $p = 0.1653$ ). However, future studies that are able to analyze these traits independently and incorporate trait variation within latex and resin canals will illuminate whether these traits show similar or different patterns in relation to diversification dynamics across plants.

Here, we revisited a classic test of Ehrlich and Raven escape-and-radiate dynamics: an association between the evolution of defense exudates (latex and resin canals) and diversification rates across plants. Both at the broad scale (using updated sister-clade approaches across 28 clades) and at the narrower scale (modelling diversification rates and trait evolution in two clades that vary in latex/resin presence and absence), we find that updated tests muddle the previously reported relationship between latex/resin evolution and diversification. Post-hoc analyses revealed that the change in results is due to updates to plant systematics over the last 28 years (not methodological advances in the statistical analyses or the addition of new data). The conclusions of this study, as those of the original study, are provisional and could change with updated phylogenies and trait data. We suggest that, while both theoretical and empirical work grounded in understanding conditionality in “escape-and-radiate” dynamics may allow for more nuanced tests of the hypothesis in the future, there is currently not strong evidence for latex or resin as general, consistently replicable drivers of species diversity across plants.

## CONCLUDING REMARKS:

The key innovation hypothesis has produced some of the most thrilling tales in evolutionary biology. Yet this thesis, along with other recent work on dioecy and polyploidy dioecy, suggests that the relationship between a focal trait and diversification rates can vary substantially across the tree of life (Heilbuth, 2000; Vamosi & Otto, 2002; Mayrose et al., 2011; Schranz et al., 2012; Käfer et al., 2014; Vanneste et al., 2014; Sabath et al., 2016; Foisy et al., 2019). Furthermore, changes in methodological approaches and data availability through time can impact our overall picture of whether traits are likely key innovations. Although testing for traits associated with diversification has led to a firm foundation for understanding the diversification history of plants, by focusing on obvious or interesting traits, we are likely missing out on discovering possible pathways through which diversification history has played out. I propose a few possible approaches, complementary to character-centric approaches, that could help broaden scope when studying a focal clade's diversification history.

First, there are a number of character-agnostic methods for estimating diversification rates along a phylogeny, irrespective of character states (Alfaro et al., 2009; Maliet et al., 2019). These methods are particularly valuable because they require no *a priori* hypotheses about key traits and can be particularly valuable for very large-scale analyses when collecting phenotypic data takes substantial time. In addition, this 'phylogenetic natural history' approach (Uyeda et al., 2018) can be fantastic for hypothesis-generating. Second, if multiple factors are hypothesized to play a role in diversification, then future diversification tests could incorporate pGLMMS or phylogenetic path analyses. These frameworks would have the advantage of being able to accommodate many agents at once, with the ability to identify positive and negative effects on diversification rates, both direct and indirect, painting a more holistic picture of the

diversification process. Phylogenetic path analysis approaches are also valuable for addressing hypotheses that compete biotic and abiotic pacemakers in macroevolution. The diversity of factors that have been investigated with respect to angiosperm diversification have been reviewed elsewhere (Vamosi et al., 2018); and complex scenarios involving synergistic interactions among suites of traits, or in particular environments, are becoming more popular (Vamosi & Vamosi, 2011; Onstein et al., 2014; Spriggs et al., 2014; Bouchenak-Khelladi et al., 2015; O’Meara et al., 2016), suggesting that we can successfully discover more complex stories of angiosperm diversification. Intriguingly, some of the more complex scenarios find that phenotypic evolution can sometimes lag behind diversification events (Folk et al., 2019).

Another excellent opportunity for future research is to explore the mechanisms that link traits and diversification. For instance, although Ehrlich and Raven’s hypothesis of “escape and radiate” coevolution has been around since the 60’s, the underlying mechanisms linking plant defenses to speciation have only recently been outlined (Marquis et al., 2016; Maron et al., 2019). Other hypotheses, like the Grant-Stebbins model of pollinator-mediated diversification (Grant 1949; Grant & Grant 1965; Stebbins 1970), have often acknowledged mechanisms but have neglected testing them in light of magic traits – divergence in reproductive traits or pollinator use is all too easy to invoke reproductive isolation and speciation. Part of these gaps lie in the difficulty of doing work that spans multiple scales, especially from the extremes of microevolution to macroevolution. There are a number of ecological and microevolutionary studies that could be useful following a diversification study (Weber & Agrawal, 2012).

In studies of diversification there has been a tendency to put the lion’s share of attention on speciation processes, with extinction processes becoming more of an afterthought. As a result, we know little about the role extinction has played in shaping the distribution of species across

the tree of life, as well as the processes that lead to depauperate lineages (Donoghue & Sanderson, 2015). This may be partially due to the strong critiques of extinction estimates from phylogenies (citations), the difficulty in studying extinction because clades often don't persist into the present, or our fascination with diverse clades and the speciation process. Whatever the reason, considering extinction and other processes that impact the persistence of a lineage through time have recently been recognized as important (Harvey et al., 2019), as they could play a very important role, especially over long timescales. A number of interesting studies could be performed to test hypotheses about a trait's impact on extinction rates. One could simply look at historical data, and ask whether there are key traits that are abundantly present (or absent) in extinct lineages in the fossil record, or in extirpated populations. For example, do extinct plants tend to lack laticifers or resin canals? Alternatively, long-term common gardens could be powerful, in that you could measure differential longevity of plants with different traits. For example, in an enclosed common garden with herbivore pressure, are plants with latex less likely to become extirpated due to oscillating predator-prey dynamics?

Plants are wonderfully diverse, and research on plant diversification in the past 50 years has yielded a rich collection of hypotheses to explain this pattern. The field is reaching a point where many of these ideas are generally well-accepted, and it will soon be time to appraise the relative roles each has played on the macroevolutionary stage. To my knowledge, there is no single system that has evaluated multiple of these hypotheses, however one could potentially do so under a pGLMM framework. The ideas linking traits and species interactions to diversification encompass all types of ecological interactions, and our understanding of how each of these impacts macroevolution is still emerging. Given that coevolution's importance in diversification varies depending on the type of interaction and the scale of the diversification



under consideration (Hembry et al., 2014), it will also be important to compare not just various hypotheses, but also to appraise the relative impacts of different types of species interactions like mutualism, competition, predation, and parasitism, on macroevolutionary processes like diversification and trait evolution (Jablonski, 2008; Weber et al., 2017; Harmon et al., 2019).

In this thesis I revisited the classic finding that latex and resin canals are key innovations in plant evolution, resulting in increased species diversity for clades that harbor these defensive traits. Nearly three decades after the classic work by Farrell et al. (1991), I found that the relationship between latex, resin canals, and lineage diversification is not quite as simple as once thought. However, this doesn't rule out the potential for these traits to be key innovations. First of all, key innovations may be necessary but not sufficient for increases in diversification rates (Levinton, 1988; Rabosky, 2017), meaning that evolving a trait may sometimes only be one step on the road to diversification. Indeed, as focal clades are examined across more clades and scales, we are learning that the relationships linking traits to diversification may experience lags, perhaps depending on particular ecological contexts for diversification to take place (Onstein et al., 2014; Weber & Agrawal, 2014). Future work that incorporates some of the ecological contexts, clade age, and other factors mentioned in Chapter 1 will only add to the ever-growing story of the important role latex and resin canals have played in plant evolution.

## APPENDICES

## APPENDIX A:

### Supplementary notes on latex and resin canals.

To parallel Farrell et al. (1991), who compiled an excellent appendix describing laticifers and resin canals across plants, we include an appendix of the new information gathered throughout the course of this study. Whereas Farrell et al. (1991; Appendix A) collected detailed information on the anatomy within each order (the types of laticifers, etc.), we *(i)* note shifts in systematic hypotheses that have overturned original tests by Farrell et al. (1991) (compare [Supplementary Table 2](#) against [Supplementary Table 3](#)), *(ii)* mention caveats to some of our sister comparisons, *(iii)* consider alternative diversification studies in these clades, and *(iv)* highlight additional plant clades that may be good targets of future studies focused on the evolution of latex and resin. We are hopeful that together this appendix, Farrell's original Appendix A, and our trait database, will be useful resources for future study as more phenotypic data become available, and as phylogenetic relationships crystallize. Below we report groups organized alphabetically by plant order. There are more groups (and likely trait origins) ([insert link to archived data](#)), as well as groups where we lack data on both latex and resin ([Supplementary Table 2](#)), but here we focus on a few groups of particular interest.

#### **Some broader comments**

If interested in directly comparing our sister groups and those of Farrell et al. (1991), compare the indices of [Supplementary Table 2](#) against the matching indices of [Supplementary Table 3](#) in the main text (n.b. any indices that are present only in [Supplementary Table 2](#) were removed from the study for reasons described above, and any indices that present only in [Supplementary Table 3](#) were novel to this study). We decided not to comment in depth about the certainty of phylogenetic relationships in each group, as the amount of details in the appendix was quickly becoming unwieldy; however, we note when uncertainty is obvious, and comment on how the interpretation of latex-associated diversification may be affected in that clade. Lastly, there are many more orders, families, and genera that likely represent independent origins of latex and/or resin. Farrell et al. (1991) estimated at least 40 independent origins of canal systems containing latex or resin – given phylogenetic uncertainties (described below) and the amount of missing phenotypic data across the vascular plant phylogeny ([Figure 1](#)), we still cannot infer many occurrences as phylogenetically independent events (though we estimate at least 33 independent origins of latex, but likely many more). This appendix is by no means an exhaustive summary of all possible origins, and we look forward to the next generation of advances in the field to bring about an even more thorough exploration of the role that latex and resin might play in diversification.

In many of the sister comparisons, latex has been secondarily lost. This can be cursorily explored by filtering through the archived data ([insert link to archived data](#)) (by order or family), and then evaluating the trait values of different taxa therein. In addition, there are several cases (e.g. Celastraceae), where independent origins of latex are complex to infer – however, it seems like these may represent multiple origins, or labile clades, which provide interesting cases to study (in contrast, many cases (e.g. Papaveraceae) appear to evolve latex and then rarely lose it). Studying clades that are more labile may offer some insight into possible context-dependency of “escape-and-radiate” dynamics. We would also like to point out that there are many taxa for which only one trait, either latex or resin, is well documented – filling out these gaps may be

useful for understanding whether latex and resin are interchangeable, or whether they should be treated and analyzed separately.

### Alismatales

There appear to be several independent origins of latex within Alismatales. Two origins within Araceae, one origin in Alismataceae, and another in Aponogetonaceae. There may also be one or two origins of resin within Araceae; however, this exudate has not been chemically verified as resinous (Langenheim, 2003), and therefore we focus primarily on latex in that group.

**Araceae:** are mostly laticiferous, with some resinous species (Farrell et al., 1991; French 1987, 1988; Cusimano et al., 2011), and these traits have been important for classification within the family (French, 1987; L. Lagomarsino personal communication). Farrell et al. (1991) originally compared Aroideae+Philodendroideae+Colocasiodeae against the non-laticiferous Monsteroideae+Pothoideae. Current APG taxonomy places Philodendroideae and Colocasiodeae within Aroideae (2520 species, almost entirely laticiferous, some resinous); however this clade is polytomous with Lasioideae (58 species, non-laticiferous, non-resinous) and Zamioiculcadoideae (21 species, non-laticiferous, non-resinous) (APG IV, 2016). An independent study supports the poor resolution of the relationship between Aroideae, Lasioideae, and Zamioiculcadoideae (Cusimano et al., 2011). Therefore, we took the most conservative test for our sister comparison, and tested the laticiferous clade (Aroideae–Lasioideae–Zamioiculcadoideae) against the non-laticiferous sister clade [Monsteroideae+Pothoideae] – effectively recapitulating the sister comparison of Farrell et al. (1991), but with updated richness estimates. In the future, the polytomy containing (Lasioideae+Zamioiculcadoideae+Aroideae) may offer a more refined sister comparison with respect to the non-laticiferous sister of Aroideae; however, such a test would only strengthen the positive findings of Farrell et al. (1991) and of our study, given that Lasioideae and/or Zamioiculcadoideae is far less diverse than Monsteroideae+Pothoideae. Lastly, we wanted to note the trenching behaviour exhibited by some herbivores of the laticiferous *Colocasia gigantea* (Darling, 2007), which can be seen in Fig. 4c of the main article.

Next, as hypothesized by Grayum (1990), and pointed out by Farrell et al., (1991), we reconstruct a second origin of secretory canals within Araceae: in the monotypic *Orontium* (Orontioideae) (Supplemental Figure 1). Though monotypic, this genus once contained several now-extinct taxa, which, to our knowledge, have no phenotypic data available on latex, resin, or the presence of secretory canals. This lineage may be an interesting case where canals originate, yet extinction remains prevalent.

Lastly, if latex and resin canals are considered independently, then we also find an independent origin of resin that is nested within the laticiferous Aroideae. This resin occurs in the roots of *Culcasia*, *Cercestis*, *Philodendron*, *Homalomena*, *Furtadoa*, and *Dieffenbachia*. Unfortunately, the chemical composition (Langenheim, 2003) and defensive role of this compound (to our knowledge) remain untested. However, our inferences for this group remain the same whether we compare our MEDUSA analyses to the reconstructed evolution for latex (Supplemental Figure 3), “resin” (Supplemental Figure 4), or both traits (Supplemental Figure 2) – none of these traits, alone or together, are strongly associated with diversification shifts in the group. In addition to the putatively resinous taxa within Aroideae, the flowers of *Monstera* (Monsteroideae) have a sticky secretion that has been called “resin”; however, as previously stated, the chemical constituents of this compound have not been identified. Interestingly, some trigonid bees have been observed collecting this putative resin for nest construction (Ramírez & Gómez, 1978; Chouteau et al., 2007). Similarly, several species of *Anthurium* exude a resin-like

substance from the spadix, which attracts two species of euglossine bees, *Eulaema merriana* and *Euglossa flammula*. Ultimately, further study is required to assess whether the resin of Araceae can be chemically classified as a resin, and whether it plays a defensive role against herbivores.

**Alismataceae:** Like Farrell et al. (1991), we also find a single origin of latex within the Alismataceae (115 species). However, since the study by Farrell et al. (1991), Limnocharitaceae is no longer its own family and is now placed within Alismataceae, and the sister to this laticiferous clade is Hydrocharitaceae (135 species) Butomaceae (2 species), rather than strictly Butomaceae (Farrell et al. 1991). Thus, the addition of Hydrocharitaceae the sister clade to Alismataceae has changed the interpretation of this comparison. Interestingly, Butomaceae has rudimentary vascular bundles that are similarly located to the laticifers in the Alismataceae (Stant, 1967). All Hydrocharitaceae have tannin cells to some extent, which play a role in defense - although no members possess laticifers (Ancibor, 1979).

**Aponogetonaceae:** In their original study, Farrell et al. (1991) suggested that the laticiferous Aponogetonaceae is likely the sister group of either the primitively non-laticiferous Alismatales or Najadales or these together. Currently we find the sister clade to the laticiferous Aponogetonaceae to be a taxonomic grab bag of [Scheuchzeriaceae [Juncaginaceae [Maundiaceae [[Posidoniaceae [Ruppiaceae + Cymodoceaceae]] [Zosteraceae + Potamogetonaceae]]]] (APG IV, 2016). Unfortunately, we could not find primary literature describing the presence/absence of latex or resin within Scheuchzeriaceae, Maundiaceae, Posidoniaceae, Ruppiaceae, Cymodoceaceae, or Zosteraceae, and much of the sister group remains misunderstood. Nevertheless, we include this comparison, as the systematics are still consistent with the suggestions of Farrell et al. (1991).

Interestingly, *Lilaea* (4 species, Juncaginaceae) have hypodermal laticifers (APG IV, 2016), whereas other members of Juncaginaceae do not (e.g. *Triglochin*). This may represent an independent origin of laticifers given that they are hypodermal, although more phenotypic data is needed in the surrounding clades. For our sister comparison, we conducted the analyses with these taxa included in the sister clade, to provide the most conservative test.

### Aquifoliales

There appear to be two independent origins of latex (Aquifoliaceae and Cardiopteridaceae), and one origin of resin (Aquifoliaceae) within Aquifoliales.

**Aquifoliaceae:** are resinous and laticiferous. Older phylogenies placed Stemonuraceae (95 species) as the sister, however recent taxonomy suggests the sister to Aquifoliaceae (500 species) is [Helwingiaceae (3 species) + Phyllonomaceae (4 species)] (APG IV, 2016). We know that neither Helwingiaceae nor Phyllonomaceae has latex, and that Helwingiaceae lacks resin (Bremer et al., 2001). While speciose and consistent with the predictions under Ehrlich and Raven's (1964) hypothesis, we caution that this diversity may not be attributable strictly to escape-and-radiate dynamics. This is because diversification in this group has likely been strongly influenced by a complex biogeographic history, peppered with dispersal and extinction (Cuénoud et al., 2000; Manen et al., 2010). In fact, the tremendous diversity of Aquifoliaceae is entirely attributable by a single extant genus, *Ilex* (all 500 species of extant Aquifoliaceae).

**Cardiopteridaceae:** represent a second and independent origin of latex within Aquifoliales. This family is not particularly speciose (43 species), relative to its sister group Stemonuraceae (93 species); however, it is worth noting that latex may not be ancestral to the entire family. Of the genera sampled, only one genus (*Cardopteris*) has latex; the others (*Citronella*, *Gonocaryum*) lack latex.

## Asparagales

There appear to be at least two independent origins of latex (Asparagaceae, Amaryllidaceae), and at least three origins of resin (*Dracaena* (Asparagaceae), Orchidaceae, Asphodelaceae) within the Asparagales.

**Asparagaceae:** the mucilage cells in Scilloideae likely derived from laticifer-like ideoblasts (Lynch et al., 2006). There are some *Dracaena* species with resin, but more data are needed to resolve the polyphyly of *Dracaena* and *Sansevieria* (Lu et al., 2014), and to understand whether resin is a synapomorphy of the genus.

**Amaryllidaceae:** contains two laticiferous groups [Agapanthoideae+Allioideae], and one group which has secondarily lost latex (Amaryllidoideae).

**Orchidaceae:** we did not conduct analyses in Orchidaceae because we are missing a tremendous amount of genus level data, as many orchids are well known for producing resins, and because many of these resins play a role in attracting pollinators, rather than deterring herbivores.

## Asterales

It appears that there are at least two independent origins of latex (Asteraceae, Campanulaceae) and at least three independent origins of resin (Asteraceae, Goodeniaceae, Rouseaceae) in Asterales.

**Asteraceae and Campanulaceae:** In contrast to Farrell et al. (1991) Campanulaceae is now recognized as a distinct family from Asteraceae, and not part of Asteraceae (APG IV, 2016). As a result, there are now two sister comparisons where Farrell et al. (1991) had one comparison: Asteraceae vs. Calyceraceae, and Campanulaceae vs. Rouseaceae [Rousseoideae + Carpodetoideae].

The exceptional diversity of the Asteraceae “might well depend more on their chemical arsenal than on specialized floral structure” (Cronquist, 1981). However, resins are only but one of many chemicals in this family, and the resin-producing plants tend to be shrubby and distributed in warm arid areas. Several resin-producing genera have many species (e.g. *Baccharis* (400), *Haplopappus* (150), *Brickellia* (110), and *Olearia* (100); Langenheim, 2003).

**Goodeniaceae:** are another resin-producing family in the Asterales. The family includes the resinous desert shrub *Coopernookia* and the lactiferous *Goodenia*.

**Rouseaceae:** one species (*Roussea simplex*) has radially elongated schizogynous resin canals. We note that this presents a challenge in comparing the laticiferous Campanulaceae against their sister (Rouseaceae), if latex and resin are treated as interchangeable. However, because resin is present only in one species (*Roussea simplex*) of Rouseaceae (13 species), we felt this comparison was conservative and more realistic than comparing [Rouseaceae+Campanulaceae] against all remaining Asterales and introducing pseudoreplication.

## Brassicales

The occurrence of latex and resin is poorly understood within Brassicales. There are records of terpenoid resins in *Didymotheca* (Gyrostemonaceae) (Langenheim, 2003) and of articulated laticifers are found in *Carica* (Caricaceae). There are also records of schizogenous “gum” canals in Moringaceae (Olson, 2001); however, while this exudate is sometimes referred to as “resin”, it may not be resinous. We found some data on the absence of latex and resin in several families (*Bataceae*, Brassicaceae, Capparaceae, Limnanthaceae, Resedaceae,

Tropaeolaceae); however, many more families lack data all together (Akaniaceae, Cleomaceae, Koeberliniaceae, Pentadiplandraceae, Salvadoraceae, Setchellanthaceae, Tovariaceae).

### Caryophyllales

There appears to be one or two origins of latex (both in Cactaceae) and perhaps three origins of resin (Cactaceae, Plumbaginaceae, Rhabdodendraceae) within Caryophyllales.

**Cactaceae:** The basic phylogenetic relationships within Cactaceae are still rather uncertain (see: Butterworth, 2006 and Nyffeler & Eggli, 2010 for summaries). The “Mammilloid” clade is one of the most spectacular radiations of Cactaceae in North America (APG IV, 2016), and includes the richest genera within Cactaceae, namely *Mammillaria* (ca. 145 species), *Coryphantha* (55 species) and *Escobaria* (23 species; Hunt, 1979). The derived position of the “Mammilloid” clade within Cactaceae may be consistent with a recent diversification (Hernandez-Hernandez et al., 2011); interestingly, within this clade, *Mammillaria* and *Coryphantha* are laticiferous (Metcalf and Chalk, 1983), while the sister *Escobaria* lacks latex. Increasing taxonomic and phenotypic sampling within Mammilloids will offer another interesting group for more detailed diversification analyses.

Outside of the “Mammilloid” clade, latex may have originated a second time in *Leuchtenbergia*. However, the sister to this genus has not been resolved and this origin remains a possibility for future study.

**Plumbaginaceae:** have been reported to be resinous (*Plumbago*, Langenheim, 2003). The calyx of *Plumbago capensis* has large trichomes. In developed trichomes, the resin is secreted into the head, and then bursts through blisters in the cuticle of the head (Rachmilevitz and Joel 1976). Because fruits are shed from the plant together with a persisting calyx, the sticky resin may facilitate fruit dispersal by animals (Fahn and Werker 1972).

**Rhabdodendraceae:** have secretory canals with resin (APG IV, 2016).

### Celastrales

**Celastraceae:** the presence and absence of latex is fairly well-documented for many genera within Celastraceae (archived data link), and it is likely that there were multiple origins of latex within the family. However, current phylogenetic relationships within Celastraceae are far from resolved (Matthews and Endress, 2005), to the extent that the Angiosperm Phylogeny Group states “Celastraceae have turned out to be a somewhat problematic group, and relationships within the family are still unclear... for a reworking of the classification of Celastraceae, we await the conclusion of Mark Simmons's march through the family”. Not only are the relationships among genera highly uncertain, but many genera are not clearly monophyletic, and therefore it is not possible to infer independent origins of latex. As a consequence, this group was not included in our analyses. This presents a challenge in comparing our analyses to those of Farrell et al. (1991), who did include Celastraceae in their original study (Supplementary Table 2., indices 6 and 7). In the original study there were two independent tests within this family, both of which were more species-rich in the laticiferous clades, relative to the non-laticiferous sister clades. Thus, our study lacks two origins of latex, which possibly support the hypothesis of Ehrlich and Raven (1964). We hope that in future studies on this topic, more phenotypic data and clearer phylogenetic hypotheses will help allow for tests of latex-mediated diversification within Celastraceae.

### Cornales

It appears there is one origin of latex (Cornaceae) and one origin of resin (Nyssaceae) within Cornales. Nyssaceae is sometimes considered part of Cornaceae, but this does not change the fact that resin arose independently. The phylogenetic relationships within Cornales are not well resolved, and so these origins of latex and resin were omitted from our study. Future phylogenetic resolution will make this a useful group for future tests of “escape-and-radiate” dynamics.

### Ericales

There appear to be two independent origins of latex (Sapotaceae and Fouquieriaceae), and at possibly several independent origins of resin (Roridulaceae and Styracaceae) within Ericales.

**Sapotaceae:** have articulated laticifers and their sister group is [Ebenaceae + Primulaceae s.l.]. The addition of Primulaceae following the breakup of the old Primulaceae (APG III, 2009) has shifted the richness to be higher in the sister group of this comparison. So, relative to Farrell, we find that the Sapotaceae does not support the prediction of Ehrlich and Raven (1964), at least based on a sister comparison. More detailed diversification analyses would be valuable in this clade. Over shorter time spans, homostylous clades may show accelerated diversification in Primuloideae (de Vos et al. 2014), and this may explain, at least in part, why the sister group is so diverse. According to Farrell (1991), some Primulaceae may have latex; however, we find no data to support this (Cronquist, 1981).

**Fouquieriaceae:** *Fouquieria* is the sole genus currently recognized in Fouquieriaceae, and likely represents a second and independent origin of latex within the Ericales. Most members of this desert plant genus (11 species) have latex, except for one (*F. columnaris*; previously *Irdia columnaris*) which lacks latex. Recent molecular phylogenetic analyses suggest that *F. columnaris* is actually nested within the *Fouquieria* clade (De-Nova et al., 2018), and thus represents a secondary loss of latex. Therefore, the sister group to Fouquieriaceae is Polemoniaceae (385 species), which lack milky latex (Lawrence, 1951). We note that diversification has been well-studied in Polemoniaceae, especially with respect to *Phlox* (*anthocyanins* and *plant mating systems*; Landis, 2018).

There are a few resinous groups within Ericales (Roridulaceae and Styracaceae), which likely represent independent origins; however, to conduct reliable sister comparisons, more data are needed in their sister clades (Actinidiaceae and Diapensiaceae, respectively). There are also some resinous species of Myrsinaceae (now Primulaceae); but again, more data are needed to infer an independent origin.

### Fabales

It is not clear how many independent origins of latex and resin there are in Fabales. It seems likely that there may be multiple origins within Fabaceae. There have been numerous studies of diversification within Fabales (e.g. Koenen et al. (2013)), yet despite a good phylogeny, more phenotypic data is needed to adequately test the putative roles of latex and resin.

### Garryales

There is likely one origin of latex within Eucommiaceae, and possibly a second derived within Garryaceae; however, the presence/absence of latex is poorly characterized in the latter.



**Eucommiaceae:** has a single extant genus, *Eucommia*, which has a distinctive strand of latex that is recognizable from broken leaves and is diagnostic for the genus in taxonomic keys. These strands have been observed in the single extant species, *E. ulmoides*, as well as in both fossil leaves and fruits (Friis et al., 2011). Garryaceae are sister to Eucommiaceae; however, the presence and absence of latex is not well characterized within this group. Some sources suggest a latex rubber in *G. wrightii* (Roth et al., 1985), while most other sources state nothing about the presence or absence of latex (Friis et al., 2011; Byng, 2014; APG IV, 2016).

### Gentianales

There appear to be two independent origins of latex (Apocynaceae, Rubiaceae) and two origins of resin (Apocynaceae, Rubiaceae) within Gentianales.

**Apocynaceae:** have non-articulated laticifers (but see *Mandevilla*: Lopes et al., 2009), and many species have been well-studied in the context of latex as plant defenses (e.g. *Asclepias*). In the original study by Farrell et al. (1991), Asclepiadaceae was considered separately from Apocynaceae; however, the former family is now placed within the latter; however, this has no impact on the sister comparison test as their original comparison was [Asclepiadaceae+Apocynaceae], which we're now just comparing as Apocynaceae (which includes Asclepiadaceae). It is worth noting that Farrell et al. (1991) were uncertain about the sister to Apocynaceae; this has since been resolved as just Gentianaceae (i.e. Loganiaceae is more distantly related now). This has no impact on comparing this clade to Farrell et al. (1991). There is at least one account of resin in Apocynaceae (*Cryptostegia*, *Plumeria*; Langenheim, 2003); however, we characterize this comparison as just laticiferous as the resin has not been well studied or characterized across the clade. Lastly, we wanted to mention that some Gentianaceae have mucilage cells.

**Rubiaceae:** several genera have been documented with resin and latex (link to archived data); however, more data are needed to determine whether these traits are synapomorphies of the family. In any event, this comparison represents a pseudoreplicate because Rubiaceae are sister to the remaining Gentianales, including the laticiferous Apocynaceae (discussed previously). Thus, to avoid pseudoreplication we omitted the unclear case of Rubiaceae from our analyses. Nevertheless, Gentianales may be an interesting clade for more sophisticated diversification analyses, as trait data and phylogenetic resolution accumulate.

### Gnetales

Consistent with Farrell et al. (1991) we find that there is a single origin of latex (*Gnetum*) within Gnetales, and that the sister group is still the non-laticiferous *Welwitschia*. However, in our study, we find a greater range of richness values for *Gnetum*. Importantly, we also find that this comparison is a pseudoreplicate with Pinales+Cupressales, as the sister to Pinales has been updated to Gnetales (though, this position is precarious, and future studies may see a shift in this relationship). As a consequence, we maintain the *Gnetum* sister comparison, and remove the Pinales+Cupressales pseudoreplicate (see: Pinophyta below).

### Malpighiales

We find the occurrence of latex in at least 15 families, and resin in at least 7 families (link to archived data); however, inferring independent origins is rather tricky due to complete lack of data in many families. We perform three fairly conservative tests and note that this group

has tremendous future potential as more trait data become available and as phylogenetic relationships are resolved. An additional group (Phyllanthaceae) is briefly described.

**Euphorbiaceae:** have non-articulated (Euphorbioideae; but see *Sapium*: Demarco et al., 2013) and articulated (Crotonoideae) laticifers; it is unclear whether these are homologous or an evolutionary transition in laticifers anatomy. Nevertheless, because Farrell et al. (1991) considered them as an independent origin of latex, we also consider them to be homologous. In contrast to Farrell et al. (1991) we find data to suggest that Crotonoideae (*Bertya*, *Beyeria*) have resin.

**Clusiaceae:** some groups have latex, and floral resins have been well studied as exaptations to attract pollinators (Armbruster et al., 2009). Sister to Clusiaceae is the non-resinous Bonnetiaceae, which have a mucilaginous epidermis (APG IV, 2016). However, we note that there are several closely related groups with latex (Calophyllaceae, Hypericaceae, Podostemaceae), and it is unclear whether latex is ancestral to this clade [[Bonnetiaceae + Clusiaceae] [Calophyllaceae [Hypericaceae + Podostemaceae]]]. More data, particularly in Ochnaceae, will help resolve this. Because of the role of resin in this group's pollination ecology, and because it is unclear whether latex arose in Clusiaceae, or ancestrally, we omitted this test. Interestingly, some authors have suggested that latex may play a role in the pollination ecology of *Symphonia* by local interactions with the floral oils that attract visitors (Bittrich and Amaral, 1996). We also find that the sister to Clusiaceae, Bonnetiaceae, have a mucilaginous epidermis. Clusiaceae and related families seem a promising and interesting group for future study into the role that latex and resin play in diversification, whether through herbivory or not.

**Goupiaceae:** are laticiferous and sister to the non-laticiferous Violaceae. The family is monotypic, with the sole genus of the family being *Goupia*. The genus was previously included in Celastraceae until being placed in its own distinct family.

**Phyllanthaceae:** latex is present in *Phyllanthus reticulata*, this trait is reportedly unusual for the group (Balaji et al., 1996); however, more trait data on the presence and absence of latex in this group is needed to be certain whether this represents an independent origin of latex.

### Malvales

There appear to be at most two origins of latex (Bixaceae, Malvaceae) and at most 5 origins of resin; however, better phylogenetic resolution and trait data are necessary to delimit independent tests.

**Dipterocarpaceae:** all resinous Dipterocarpaceae (Dipterocarpoidea) are sister to the non-resinous Monotoideae, which have been reported to have mucilage canals. This was the only clearly independent origin of resin or latex that we could infer within Malvales, given the data available, and the uncertainty of phylogenetic relationships among families within the order. The sister comparison we conducted between Dipterocarpoidea and Monotoideae is identical to the test of Farrell et al. (1991), with the only exception of higher contemporary richness in the Dipterocarpoidea.

It seems likely there may be independent origins of resin within Cistaceae (*Cistus*) and Thymelaeaceae; however, more data within these families will help resolve these. It will also be worth collecting more trait data within the respective families of *Thespesia* (Malvaceae) and *Bixia* (Bixaceae), to understand whether these truly represent independent origins of latex. These groups were not analyzed because they did not meet the criteria for our sister comparisons.

### Marsileales

Consistent with Farrell et al., (1991), we find a single origin of latex within Marsileales (*Regnellidium* remains the only latex-bearing fern!). Since Farrell et al. (1991), however, the sister to *Regnellidium* has been resolved as *Pilularia*. Farrell et al. (1991) considered *Pilularia* or *Marsilea* as possible sister groups to *Regnellidium*, and therefore this sister comparison remains comparable to the original analysis, just with more systematic certainty now.

### **Nymphaeales**

We found a single origin of latex [Cabombaceae + Nymphaeaceae], and no data on resin within Nymphaeales. The phylogenetic relationships were not included on the Angiosperm Phylogeny Group website (APG IV, 2016); however, recent analyses suggest that the two laticiferous groups (Nymphaeaceae and Cabombaceae) are closely related, and sister to the non-laticiferous Hydatellaceae (Borsch et al., 2008) Mucilage is present in all three families in Nymphaeales.

### **Pandanales**

There appear to be two origins of latex (Cyclanthaceae, Velloziaceae) and one origin of resin (Velloziaceae) within Pandanales. Because they were pseudoreplicates (Cyclanthaceae was nested within the sister to Velloziaceae) we randomly selected one origin (Cyclanthaceae) to analyze. Carludovicoideae often contains what is likely mucilage.

### **Pinophyta**

In concordance with Farrell et al. (1991), we find a single origin of resin canals shared by Pinales and Cupressales; though, it has been secondarily lost in some Taxaceae species (present in *Cephalotaxus*) – these species (24) were removed from the tally of resinous species for the Pinales+Cupressales origin of resin canals.

Notably, establishing the sister to conifers has been particularly difficult (APG IV, 2016), although Pinales is more diverse than all sister groups that have been suggested (Cupressales, Cycadales, Ginkgoales, Gnetales). Since Farrell et al. (1991), Gnetales has replaced Ginkgoales as the sister to Pinales (APG IV, 2016). This presented a challenge given that Gnetales (see: above) has another independent origin of resin nested within Pinales+Cupressales; therefore, to avoid pseudoreplication with respect to Gnetales, we randomly selected to remove the Pinales+Cupressales origin from the analysis.

### **Ranunculales**

There are likely two independent origins of latex (Lardizabalaceae, Papaveraceae) and one origin of resin (*Podophyllum* (Berberidaceae)) within Ranunculales. More trait data is needed within Berberidaceae to understand whether *Podophyllum* is the derived origin of resin, or whether other taxa in the family are also resinous.

**Papaveraceae:** all Papaveraceae have laticifers, except for *Pteridophyllum* (1 species). Previously *Pteridophyllum* was considered a distinct family (Pteridophyllaceae); however recent systematics now places it within Papaveraceae (APG IV, 2016).

The secondary burst in diversification revealed by our MEDUSA analyses within Papaveraceae occurs within Fumarioideae (Figure 3a) and seems to correspond to a well-characterized transition in floral symmetry (dissymmetric → zygomorphic) (Hidalgo & Gleissberg, 2010; Hoot et al., 2015; Sauquet et al., 2015). The role of floral symmetry in

affecting diversification rates has been well studied (Sargent, 2004). This shift in diversification also corresponds with a reduction in the number of floral spurs (from two to one).

Papaverales used to be broken into two families: the Papaveraceae and the Fumariaceae, based on the presence and absence of latex respectively (Cronquist, 1981). Now known as Papaveroideae and Fumarioideae respectively, the latter subfamily has been historically reported to have “watery juice” present in non-articulated ideoblasts (possibly reduced laticifers) (Cronquist, 1981). However, because recent databases have characterized Fumarioideae as having laticifers (Hoot et al., 2015), we use the most recent characterization performed by experts on Ranunculales taxonomy. We recognize that the presence of laticifers do not necessarily imply a defensive function if latex is absent, and so if the “watery juice” of Fumarioideae is not serving a defensive role, then this has implications for diversification. In any case, we find no shifts corresponding with the transition to “watery juice” in Fumarioideae.

**Lardizabalaceae:** likely represents an independent origin of latex relative to Papaveraceae. While the sister is well supported as Circaeasteraceae, we could not find data on the presence or absence of latex in this small family (1-2 species). As a result, the likely origin of latex within Lardizabalaceae was not tested as it did not meet the criteria for a sister comparison.

### Rosales

There appear to be two origins of latex (Dirachmaceae, [Cannabaceae [Moraceae + Urticaceae]] within Rosales; however better phylogenetic resolution is needed to delimit whether Rhamnaceae and/or Elaeagnaceae is sister to Dirachmaceae. There and also multiple groups with resin (Rhamnaceae, Rosaceae, Moraceae, Cannabaceae), however more phenotypic data as well as improved systematic hypotheses are necessary to test these as independent events. Therefore, we only conduct a test which remains mostly unchanged since Farrell et al. (1991), by comparing the non-resinous and non-laticiferous Urticaceae to their sister laticiferous and mostly resinous sister clade [Cannabaceae [Moraceae + Urticaceae]]. Since Farrell, this comparison has been moved to Rosales (Urticales is obsolete now). It is worth noting that not all Cannabaceae are laticiferous (laticifers are only present in *Cannabis* (1 species) and *Humulus* (~5 species), and this may represent an independent origin of latex. The sister of [*Cannabis* + *Humulus*] is likely *Celtis* (~73 species) but needs to be better resolved.

### Santalales

It is highly likely that there are multiple origins of latex within Santalales; however, better phylogenetic resolution and phenotypic sampling is necessary to infer independent evolutionary events. We found no data on resin in the group but added several new families of latex since Farrell et al. (1991). Santalaceae and Opiliaceae likely represent a secondary loss of latex; however, with the data available it is not clear whether latex was the ancestral state. More phenotypic data on Misodendraceae, Octonemaceae, Ximeniaceae, Strombosiaceae, and Erythralaceae will help clarify the likely ancestral state of latex in this group. Furthermore, increasing the amount of genus-level data, and searching taxonomic keys will help clarify whether latex is synapomorphic in any of these groups. Lastly, greater phylogenetic resolution for the relationships between Ximeniaceae, Aptandraceae, Olacaceae and the remaining Santalales will be essential for inferring independent origins of latex.

### Solanales

It appears that latex has evolved once (Convolvulaceae) and resin has evolved twice (Solanaceae, Convolvulaceae) in Solanales. Because *Anthocercis* (9 species) is the only known resin producer in Solanaceae, we treated Solanaceae as a non-laticiferous sister comparison. However, more phenotypic data within Solanaceae may reveal that there are other resinous taxa in the family, though that seems unlikely to represent much more than a very small fraction of this very large family (Langenheim, 2003).

### **Zingiberales**

It appears that latex (Musaceae) and resin (Cannaceae) have each originated once within Zingiberales. Because Cannaceae is nested within the sister to Musaceae, to avoid pseudoreplication, we selected one sister comparison (Cannaceae) to remove from analysis; we decided to retain Musaceae, as it was one of the original comparisons performed by Farrell et al. (1991).

## APPENDIX B:

Latex and resin database, containing presence/absence data for latex and resin canals, with references. Because these data do not format into a readable format in Word, these data are archived on the Dryad Digital Repository: <https://doi.org/10.5061/dryad.2mn0j54>.

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