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Evolutionary trade-offs in the chemical defense of floral and fruit tissues across genus *Cornus*

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PREMISE: Defense investment in plant reproductive structures is relatively understudied compared to the defense of vegetative organs. Here the evolution of chemical defenses in reproductive structures is examined in light of the optimal defense, apparency, and resource availability hypotheses within the genus *Cornus* using a phylogenetic comparative approach in relation to phenology and native habitat environmental data.

METHODS: Individuals representing 25 *Cornus* species were tracked for reproductive phenology over a full growing season at the Arnold Arboretum of Harvard University. Floral, fruit, and leaf tissue was sampled to quantify defensive chemistry as well as fruit nutritional traits relevant to bird dispersal. Native habitat environmental characteristics were estimated using locality data from digitized herbarium records coupled with global soil and climate data sets.

RESULTS: The evolution of later flowering was correlated with increased floral tannins, and the evolution of later fruiting was correlated with increased total phenolics. Leaves were found to contain the highest tannin activity, while inflorescences contained the highest total flavonoids. Multiple aspects of fruit defensive chemistry were correlated with fruit nutritional traits. Floral and fruit defensive chemistry were evolutionarily correlated with aspects of native habitat temperature, precipitation, and soil characteristics.

CONCLUSIONS: Results provide tentative support for the apparency hypothesis with respect to both flower and fruit phenology, while relative concentrations of secondary metabolites across organs provide mixed support for the optimal defense hypothesis. The evolution of reproductive defense with native habitat provides, at best, mixed support for the resource availability hypothesis.

KEY WORDS chemical defense; Cornaceae; dogwood; evolutionary ecology; flower; macroevolution; optimal defense hypothesis; phylogenetic generalized least squares regression; reproduction; resource availability hypothesis.

Plants are subject to attack by a wide variety of natural enemies. Unlike animals, plants cannot move to flee these pressures. Although they must remain stationary, plants have evolved effective arsenals of physical and chemical defenses of broad diversity across the plant kingdom. One of the most studied defense strategies is the evolution of the use of secondary metabolites, which have been hypothesized to make plants unpalatable or toxic to herbivores or to resist fungal and/or bacterial infection (Harborne and Turner, 1984; Crozier et al., 2006; Bhattacharya et al., 2010; Ayman et al., 2013). There is much speculation as to how plants have evolved the use of these secondary metabolites, and much research points to coevolution with various herbivores, especially considering the variation in how these chemicals affect different herbivorous species (Janzen, 1975; Coley, 1983; Feeny, 1992). There are several prominent evolutionary hypotheses that seek to explain how plants allocate resources to defense in light of diverse ecological strategies, and many overlap in their assumptions and predictions (Rhoades, 1979; Coley et al., 1985; Stamp, 2003). At present, two of the most prominent are the optimal defense hypothesis and the resource availability hypothesis.

The optimal defense hypothesis and the resource availability hypothesis

The optimal defense hypothesis addresses how the defensive needs of a plant influence the evolution of plant defenses, with the energetic costs of producing defenses incurred to maximize plant fitness (Rhoades, 1979; Stamp, 2003). The first part of this hypothesis states that organisms evolve and allocate resources in a way that maximizes individual fitness and therefore is adaptive. This hypothesis has been under some scrutiny because of the inherent difficulty for researchers to reliably demonstrate that a given defense investment is or is not adaptive and therefore falsify the hypothesis (Stamp, 2003). The second part of this hypothesis, which states that plant defenses are costly because they divert resources from other needs such as reproduction, can be broken up into four subhypotheses: (1) that plants evolve defenses proportional to their risk of predation (the apparency hypothesis), (2) that defenses are allocated in proportion to the fitness benefit conferred and inversely to the fitness cost of defense, (3) that defense investment evolves to increase or decrease depending on the relative presence or absence of natural enemies, and (4) that there is a trade-off between investment in plant defense and other metabolic functions like growth (Rhoades, 1979; Stamp, 2003). Under the optimal defense hypothesis, these subhypotheses work together to predict why and how plant defense phenotypes evolve in relation to the biotic and abiotic environment. All of these components involve consideration of the costs of plant defenses and together assume that selection acts on genetic variation for amount and type of defense, that herbivore and disease pressure is a major selective force for production of defense characteristics, and that defenses actually do reduce herbivory or pathogen infection. In general, these assumptions are thought to be reasonably well supported by data (Stamp, 2003; Hahn et al., 2019).

The first prediction of the optimal defense hypothesis is that plants will allocate more defense to tissues that are more important for plant fitness. This prediction has been relatively well supported among vegetative tissues, with most studies examining the defensive chemistry and fitness value of young leaf tissue and old leaf tissue (Barto and Cipollini, 2005; Alves et al., 2007; Traw and Feeny, 2008; McCall and Fordyce, 2010; Heath et al., 2014), but this hypothesis has not been well studied in comparison with reproductive structures (Keith and Mitchell-Olds, 2017). Under the second prediction of the optimal defense hypothesis, the apparency hypothesis has been historically applied to comparisons between persistent (more-apparent) and ephemeral (less-apparent) plant species as a function of life history, predicting that more apparent species should invest more in defenses than less-apparent species (Feeny, 1976; Rhoades and Cates, 1976). Most work done on the apparency hypothesis focuses on vegetative herbivory and growth rate, and the hypothesis has earned mixed support (Endara and Coley, 2011; Massad et al., 2011; Smilanich et al., 2016). However, while the apparency hypothesis has often been applied to the relative apparency of vegetative organs based on their persistence and abundance, very little work has been done on the relationship between apparency and defense in reproductive structures. This gap is surprising given the typically broader diversity in phenology present in reproductive organs relative to vegetative structures among species, with even closely related taxa often exhibiting substantial variation in the seasonal timing and duration of flowering and fruiting.

Distinct from the optimal defense hypothesis, another prominent evolutionary explanation for variation in the allocation of plant defense investment is the resource availability hypothesis. This hypothesis states that the supply of resources in a habitat exerts selection on plant inherent growth rate, such that a species adapting to a low-resource environment will evolve a low inherent growth rate and vice versa, and that plants with low inherent growth rates will tend to invest more in chemical and physical defenses than plants with high inherent growth rates (Coley et al., 1985; Stamp, 2003). This hypothesis suggests that species occupying habitats with high resource availability will tend to evolve a strategy that compensates for herbivory through additional growth (given the relative ease of producing new tissues when resource availability is high), rather than evolve increased levels of defense to protect existing tissues. This prediction has been generally supported empirically for vegetative organs (Endara and Coley, 2011). Thus, the resource availability hypothesis suggests that species which inhabit environments that are resource-rich or otherwise supportive of high inherent growth rate in terms of water availability, nutrient availability, light availability, and conducive temperatures for growth should have lower observed levels of defense than species which inhabit environments that are more resource-poor (e.g., low rainfall, nutrient-poor soils). While this hypothesis has been tested for vegetative organs, to our knowledge it has not been evaluated for reproductive structures, despite the fact that the same logic of the differential cost of compensation based on resource availability should also apply to reproductive structures.

In this context, the study of flowers and fruits may provide insight into the applicability of both the optimal defense hypothesis and the resource availability hypothesis to plant reproductive structures. When examining the evolution of defenses with environment and phenology, phylogenetically explicit studies of closely related groups of species (e.g., within genera) have an advantage over studies that compare distantly related taxa, in that such studies can reconstruct the repeated evolution of phenotypes during diversification among habitats within a group of species otherwise sharing a common evolutionary history, allowing for insights into the drivers of trait evolution in a more controlled manner (Weber and Agrawal, 2012).

Cornus as a study system for reproductive defense evolution

The genus *Cornus* (Cornaceae) contains 40–60 species, known variously as dogwoods, cornels, and osiers, and hereafter collectively referred to as dogwoods (Eyde, 1988; Xiang et al., 2006). Dogwoods are widespread within the temperate zones of North America, Europe, and Asia (Xiang and Boufford, 2005; Xiang et al., 2006; Murrell and Poindexter, 2017). These trees and shrubs bear an extremely wide range of morphological variation, especially among their reproductive structures (Feng et al., 2011). *Cornus* can be split into four distinct clades: the big-bracted group, the blue-or-white-fruited group, the cornelian cherries, and the dwarf dogwoods (Xiang et al., 2006; Xiang and Thomas, 2008). The big-bracted group have large, showy-bracted inflorescences and typically produce red fruits (Fan and Xiang, 2001; Xiang, 2006). The blue-or-white fruited group have minute bracts and inflorescences in colorful cymes and produce blue, white, or black fruits (Fan and Xiang, 2001; Xiang et al., 2006). The cornelian cherries have inflorescences in umbellate cymes with red, oblong fruits (Fan and Xiang, 2001; Xiang et al., 2006). The dwarf dogwoods have inflorescences on minute cymes with red fruits (Fan and Xiang, 2001; Xiang et al., 2006). The phylogenetic relationships among *Cornus* have been studied, and current relationships are reasonably well supported (Xiang et al., 2006).

There has been some previous work characterizing the chemical composition of reproductive organs within the Cornus genus.

Species including *C. alternifolia*, *C. amomum*, *C. controversa*, *C. kousa*, *C. florida*, and C. mas have been observed to contain a large abundance of anthocyanins, which are generally associated with attractive and colorful fruits (Seeram et al., 2002; Vareed et al., 2006; Ma et al., 2010). In addition to an abundance of anthocyanins, the fruits of *C. mas*, widely known as the cornelian cherry, have a considerable amount of other phenolics including flavonoids and tannins (Marinova et al., 2005; Pantelidis et al., 2007; Pawlowska et al., 2010; Bijelic et al., 2011). Such phenolics are also found in C. officinalis (Hatano et al., 1990), and *C. kousa* has yet a different suite of flavonoids (Vareed et al., 2007). Phenolics are a class of secondary metabolites that have been shown to have significant defensive properties (Coley, 1983; Feeny, 1992; Hammerschmidt, 2005; Arif et al., 2009; Ayman et al., 2013). Tannins and flavonoids are subclasses of phenolics with defensive properties and have been a focus of clade-wide studies of defensive secondary metabolites in leaves (Pearse and Hipp, 2012; Johnson et al., 2014).

Birds have been identified as the primary disperser across the *Cornus* genus (Borowicz and Stephenson, 1985; Eyde, 1988; Willson and Whelan, 1993; Guitian et al., 1996; Rossell et al., 2001; Xiang, 2005), suggesting that the more palatable and juicier a fruit is, the more attractive it is to a bird that will disperse seeds (Eyde, 1988). However, these traits may also make the fruit more attractive to insect frugivores who do not contribute to seed dispersal and more prone to fungal and bacterial infection (Borowicz and Stephenson, 1985; Willson and Whelan, 1993; Guitian et al., 1996). This sets up the potential for a need for higher plant investment in chemical defense in highly attractive and nutritive fruits.

This study explores the evolution of *Cornus* reproductive chemical defenses in relation to fruit and flower phenology, fruit nutritional content, and native habitat environmental characteristics using a phylogenetic comparative framework to specifically test whether the evolution of chemical defenses in *Cornus* supports the predictions of the optimal defense hypothesis and the resource availability hypothesis. The specific research questions addressed in this study are: (1) How do concentrations of floral and fruit chemical defenses evolve in relation to flower and fruit phenology, and do such relationships support the optimal defense hypothesis? (2) How do the concentrations of fruit chemical defenses evolve in relation to fruit nutritional content, and do such relationships support the optimal defense hypothesis? (3) How do the concentrations of chemical defenses compare across *Cornus* fruits, inflorescences, and leaves, and do such relationships support the optimal defense hypothesis? (4) How has defense chemistry evolved within *Cornus* in relation to native habitat climate and soil conditions, and do such relationships support the resource availability hypothesis?

MATERIALS AND METHODS

Sample collection

During the 2016 growing season, 66 individual plants of 25 different taxa within *Cornus* were tracked for flower and fruit phenology, as well as sampled for floral and fruit tissue at the Arnold Arboretum of Harvard University in Boston, Massachusetts, in the United States. These 25 taxa represent at least half of the species diversity of the genus *Cornus* and include representatives of three of the four major clades of the genus and species from across diverse biomes of North America, Europe, and Asia. The number of focal individuals of each species ranged from one to four, depending on availability in the living collections of the Arnold Arboretum. Whole-leaf samples from the same plants had been collected in May of the 2015 growing season as part of a separate study (Mason et al., 2020). The 281 acres of the Arnold Arboretum have very similar climate and soil conditions and can serve as an experimental common garden, minimizing the effect of environmental variation on species' trait differences relative to field sampling across continents. A common garden experiment leaves much of the trait variation among species observed in a study to be interpreted as due to underlying genetic differences among species.

Phenology was assessed on all plants roughly once per week over 6 months from mid-April until mid-September, capturing nearly the entire growing season. On each date, each plant was categorized as dormant (no leaves or reproductive structures present), vegetative (leaves present but no open flowers or fruits of any kind present), flowering (defined as post-anthesis, with completely open petals and anthers present on any inflorescence), intermediate (defined as post-flowering with immature developing fruits), or fruiting (defined as the presence of mature, ripe fruits as indicated by color change).

Flowering start date was defined as the date of first anthesis of flowers on any inflorescence, while the flowering period (weeks observed flowering) was calculated as the number of weeks that plants continued to bear open, nonsenesced flowers. Fruiting start date was defined as the date of the first presence of any ripe fruit (indicated by abrupt and characteristic color changes), while the fruiting period (weeks observed fruiting) was calculated as the number of weeks that plants continued to produce mature, ripened fruit.

Samples of multiple whole inflorescences and multiple ripe fruits were collected from each plant when first observed (flowering start date and fruiting start date) under the definitions above. For inflorescences, entire inflorescences were collected, including peduncles and bracts. Of the 25 taxa observed (Appendix 1; Appendix S2), inflorescence samples were obtained for 50 individual plants representing 21 taxa. No individuals of *C. walteri*, *C. glabrata*, or *C. alba* flowered during this study and were therefore not included in the analysis. Eight individuals of six other species also did not flower during the study, but for these taxa other individuals were present and able to be sampled (Appendix S2), while two species, *C. officinalis* and *C. controversa*, flowered but did not produce sufficient quantities of inflorescences to obtain samples for phenotyping. Mature, ripened fruits were able to be collected from 19 individuals representing 12 taxa (Appendix S2). Sufficient quantities of ripe fruits were not present for sampling in some species either because fruits were rapidly removed by birds in the few days between first ripening and the timing of weekly sampling or because plants did not produce mature fruit in the Arnold Arboretum in 2016, perhaps due to lack of adequate pollination. Both low fruit set and rapid fruit removal have been observed in *Cornus* in field settings as well (Borowicz and Stephenson, 1985; Guitian et al., 1996). In some species, only a portion of the individual plants representing a given species produced or were able to be sampled for fruits (Appendix S2). For all purposes in this study, each individual plant was considered as a biological replicate and the level of organization at which traits were quantified, whether for flowers or fruits.

Trait quantification

Upon collection, inflorescence and fruit samples were kept on ice until transported to the laboratory on the same day. Samples were weighed for fresh mass, dried at 60°C to constant mass in a forced air-drying oven, weighed for dry mass, and the difference between floral and fruit fresh and dry masses were used to calculate water content. Dried samples were then ground into a powder using a mortar and pestle and/or a coffee grinder, as needed. This dried homogenized tissue was later used for the quantification of both defense chemistry and fruit nutritional content.

Total phenolics, total flavonoids, and tannin activity were estimated on both floral and fruit samples using methanolic extracts in conjunction with laboratory methods that express complex chemical profiles in units of reactive equivalents of a common compound standard that is representative of the chemical class. Total phenolics were estimated using the colorimetric Folin-Ciocalteau assay (Singleton et al., 1999) and expressed as caffeic acid equivalents (Chemical Abstracts Service [CAS] no. 331-39-5). Phenolics have been shown to contribute to resistance against microorganisms and insect and vertebrate herbivores (Coley, 1983; Feeny, 1992; Hammerschmidt, 2005; Arif et al., 2009; Ayman et al., 2013). Total flavonoids were estimated using the colorimetric aluminum complexation assay (Pękal and Pyrzynska, 2014) and expressed as quercetin equivalents (CAS no. 117-39-5). Flavonoids are a subclass of phenolic secondary metabolites that are important for many biotic interactions; their functions include UV protection, pollinator and disperser attraction, herbivore resistance, and fungal defense-related signaling (Snyder and Nicholson, 1990; Harborne, 1993; Gould, 2006; Halbwirth et al., 2009; Moore et al., 2014). Tannin activity was quantified using the radial diffusion assay (Hagerman, 1987; Graça and Bärlocher, 2005), which measures the protein precipitation activity of tannins in a leaf extract expressed as tannic acid equivalents (CAS no. 1401-55-4). Tannins are another subclass of phenolic secondary metabolites that contribute to plant defense in several ways, including forming complexes with plant proteins to inhibit assimilation by herbivores, damaging digestive track linings through protein precipitation, and the creation of oxidative stress in herbivores (Bernays et al., 1989; Butler, 1989; Cipollini and Levey, 1997; Nomura and Itioka, 2002; Shimada, 2006; Roslin and Salminen, 2008; Spalinger et al., 2010; Moles et al., 2011; Salminen and Karonen, 2011). These same three traits were quantified in a parallel manner for leaves as reported by Mason et al. (2020).

Key aspects of fruit nutritional content were quantified from the dried homogenized fruit samples. Fruit nutritional content was considered in this study to encompass traits that contribute to consistency (e.g., juiciness, oiliness), flavor (e.g., sweetness, tartness), and nutritive value (e.g., animal-relevant macronutrients and vitamins) and thus likely promote consumption by birds (Johnson et al., 1985; Levey, 1987). The five traits assessed were titratable acidity, lipid content, protein content, water content, and sugar content, all of which have been shown to contribute to fruit nutritional content (Johnson et al., 1985; Levey, 1987; Bairlein, 1996; Jordano, 2000; Levey and Martínez del Rio, 2001). Fruit titratable acidity was quantified using NaOH in an acid-base titration (Sadler and Murphy, 2010). Total lipid content was quantified using the gravimetric method of Moles et al. (2011), using petroleum ether to extract lipids and estimate the mass of lipids in the sample. Total fruit protein content was quantified using the colorimetric Bradford protein assay (Jones et al., 1989). Total fruit sugar content in sucrose equivalents was measured using the Brix method with a handheld refractometer (Kitinoja and Hussein, 2005). The distinction between nutritional content and defensive chemistry is not completely mutually exclusive as some secondary metabolites may contribute to nutrition or nutrient acquisition (Cipollini et al., 2014). For example, total lipids in fruits may represent both nutritive oils and non-nutritive surface waxes, and titratable acidity in fruits may represent both vitamin content (e.g., ascorbic acid) and other forms of acidity that may provide defense against pathogens such as the use of non-protein amino acids (Fürstenberg-Hägg et al., 2013; Killiny and Hijaz, 2016) and non-vitamin organic acids like malic acid, which has been shown to occur in some *Cornus* fruits (Kim et al., 2003).

Species native habitat environmental data were obtained from Mason et al. (2020), as deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.wh70rxwhr). In brief, that study used the following approach: occurrence data from the native distributions of each species was obtained as latitude–longitude coordinates from digitized herbarium records via the Global Biodiversity Information Facility (GBIF, 2019) and Integrated Digitized Biocollections (iDigBio, 2019). Occurrence points were manually curated to remove non-native records (such as those from botanical gardens), duplicates, and any questionable records. Taxonomic synonyms within the genus were harmonized using The Plant List (2013). During this curation, four taxa were excluded from consideration for environmental data because they are not accepted at the species rank by this shared source (*C. australis*, *C. coreana*, *C. pumila*, and *C. kousa* var. *chinensis*), such that the GBIF and iDigBio databases would not be expected to reliably list all occurrences for these taxa. Once the final set of occurrence points was obtained, climate data were extracted for each occurrence point from the WorldClim interpolated global climate model (Hijmans et al., 2005) at a resolution of 30 arc seconds, yielding mean annual temperature, diurnal range, isothermality, maximum temperature of the warmest and coldest months, minimum temperature of the coldest month, mean annual precipitation, precipitation of the wettest and driest months, precipitation of the warmest and coldest quarters, and the frost free period (in months). Soil data were extracted for each occurrence point from the SoilGrids250m interpolated global soil model (Hengl et al., 2017), including average soil bulk density, average soil cation exchange capacity, volumetric percentage of coarse fragments, average soil organic matter content, average soil pH in water, soil silt content, soil sand content, and soil clay content. Soil data were averaged across the seven available soil depths (0, 5, 15, 30, 60, 100, and 200 cm) to cover the rooting zone of these trees and shrubs. A summary of eight focal environmental variables can be found in Appendix S1, demonstrating the interspecific and intraspecific variation in habitat environmental characteristics across *Cornus*.

Phylogenetic comparative analyses

Species means for each trait were taken across all individuals sampled within a given taxa (Appendix S3), and environmental variables were averaged across occurrence points (Appendix S4). For phylogenetic comparative analyses, the most taxonomically thorough phylogeny available was used (Xiang et al., 2006), as reconstructed by Mason et al. (2020) and deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.wh70rxwhr). In brief, the originally published *matK* and ITS sequences published by Xiang et al. (2006) for the taxa of interest were concatenated, and a phylogeny reconstructed using maximum likelihood under a GTRCAT model in RAxML v. 8.2.10 (Stamatakis, 2014). The blue-and-white-fruited clade was used to root the tree to generate polarity consistent with substantial evidence from previous studies (Xiang et al., 2006, 2008; Xiang and Thomas, 2008). Two taxa were manually added to the tree, *C. australis* as sister to *C. sanguinea* and *C. coreana* as sister to *C. walteri* following taxonomic information, because these taxa are considered subspecies or synonyms by some sources (The Plant List, 2013). Such manual grafting is a relatively standard method used in phylogenetic comparative analyses where genetic data are missing for taxa whose affinities are well understood (Beaulieu et al., 2012). Using the resulting phylogeny, trait–trait and trait–environment macroevolutionary correlations were assessed with phylogenetic generalized least squares (PGLS) regression using the R package Rphylopars to estimate trait covariance while accounting for missing trait data (Goolsby, 2017), within the R computing environment (R v. 3.6.0, R Core Team, 2019). In addition, ancestral state reconstruction was performed using the Rphylopars package (Goolsby, 2017) to visualize trait-trait relationships across the genus for floral and fruit phenology in relation to defensive chemistry and for fruit defensive chemistry in relation to fruit nutritional content. The use of Rphylopars in reconstructing ancestral states permits a robust estimate of missing data, particularly since missing data is present only at the tips of the tree and not entire clades (Goolsby, 2017). For phylogenetic comparative analyses, the phylogeny used, R code, input data, and output results are all available in the supplement (Appendix S5). Finally, total flavonoids, total phenolics, and tannin activity were compared across flowers, fruits, and leaves using paired *t*-tests, in order to test for differences in average defense investment among organs.

RESULTS

Phenology and reproductive defense

The timing of flowering and fruiting varied strongly across the phylogeny (Fig. 1). The cornelian cherry group was observed to flower at the very start of the growing season before leaf-out (mid-April), though only *C. mas* was observed to subsequently produce fruit. Members of the big-bracted clade bloomed at different times, with *C. kousa* and *C. kousa* subsp. *chinensis* blooming about 2 weeks later in the season than *C. florida*. All three were observed to produce fruit later in the season; however, *C. kousa* began fruiting about 2 weeks earlier than the others. Members of the blue-and-white-fruited clade flowered and fruited at a wide variety of times across the growing season.

Several phenological traits were significantly evolutionarily correlated with floral and fruit traits. There was a negative correlation between flowering start date and floral total flavonoids (R^2 = 0.43, Fig. 2; Appendices S6, S7), and a positive correlation between flowering start date and floral tannin activity (R^2 = 0.35, Fig. 2, Appendices S6, S7). In fruits, there was a positive correlation between fruiting start date and both fruit total phenolics (R^2 = 0.45, Fig. 2, Appendices S6, S7) and fruit protein content (R^2 = 0.28, Appendix S7), and a negative correlation between fruiting start date and titratable acidity (R^2 = 0.33, Fig. 2, Appendices S6, S7). There was also a positive correlation between the number of weeks observed fruiting and fruit sugar content (R^2 = 0.26, Appendix S7), but no significant correlations between the number of weeks observed flowering or fruiting and any chemical defenses.

Fruit defense and fruit nutritional content

Fruit chemical defenses and nutritional traits differed substantially across the 12 species sampled. Total phenolics ranged from 2.9 to 8.3 mg/g (caffeic acid equivalents), total flavonoids ranged

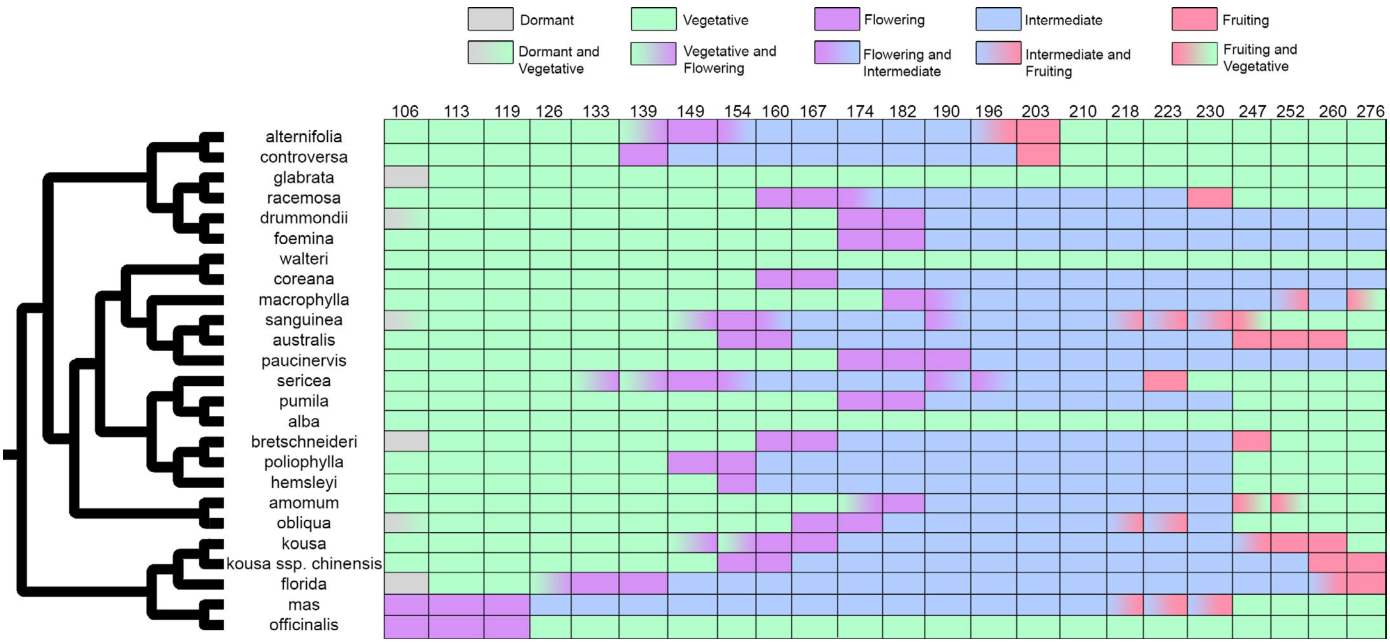

FIGURE 1. The phenology of each species under study by Julian date (days since start of year, top row) as observed in the Arnold Arboretum of Harvard University in 2016, placed in relation to the *Cornus* phylogeny of Xiang et al. (2006). At each date, species were scored as dormant (no leaves or reproductive structures present), vegetative (leaves present but no open flowers, nor fruits of any kind present), flowering (defined as post-anthesis, with completely open petals and anthers present on any inflorescence), intermediate (defined as post-flowering with immature developing fruits), or fruiting (defined as the presence of mature, ripe fruits as indicated by color change). Split categories indicate that different individuals of the same species were in two different stages on a given date. See Appendix S7 for full phenology data for all individuals.

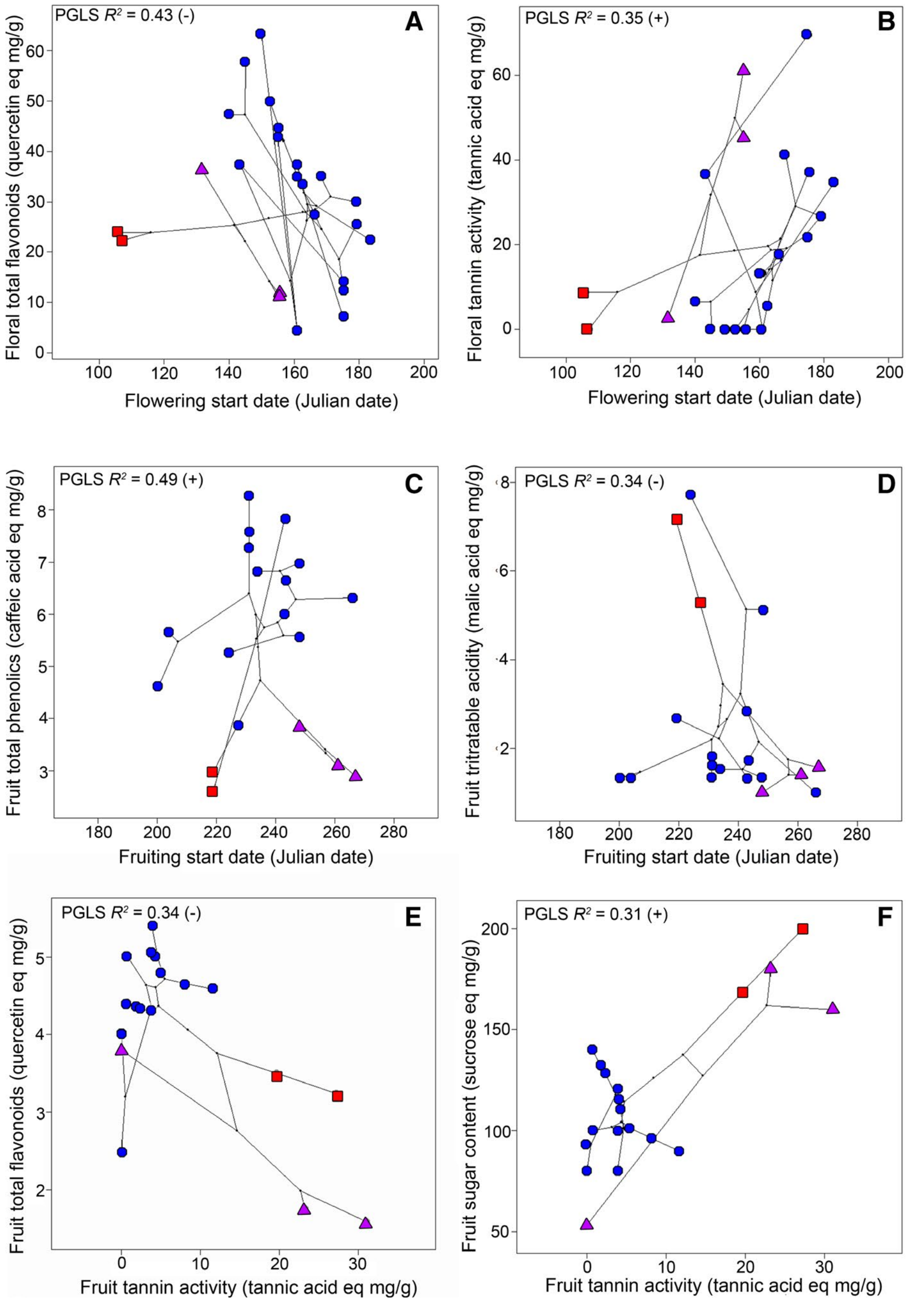

FIGURE 2. Evolution of focal traits in bivariate trait space plotted using the phylomorphospace function in the R package phytools (Revell, 2012). (A) A negative evolutionary correlation between floral total flavonoids and flowering start date (phylogenetic generalized least squares (PGLS) regression, R^2 = 0.43), (B) a positive evolutionary correlation between floral tannin activity and flowering start date (PGLS, R^2 = 0.35), (C) a positive evolutionary correlation between fruit total phenolics and fruiting start date (PGLS, R^2 = 0.49), (D) a negative evolutionary correlation between titratable acid and fruiting start date (PGLS R^2 = 0.34), (E) a negative evolutionary correlation between fruit total flavonoids and fruit tannin activity (PGLS R^2 = 0.35), and (F) a positive evolutionary correlation between fruit sugar content and fruit tannin activity (PGLS R^2 = 0.31). Species are color-coded by their clade: red squares = cornelian cherries, purple triangles = big-bracted clade, blue circles = blue-and-white-fruited clade.

from 1.6 to 5.4 mg/g (quercetin equivalents), and tannin activity ranging from undetectable to 31 mg/g (tannic acid equivalents). Fruit sugar content varied among species from 53 to 200 mg/g (sucrose equivalents), and fruit lipid content ranged across from 5.4 to 24 mg/g (Appendix S3). Fruit water content varied from 48 to 84% of fresh mass, and species spanned a 7-fold variation in titratable acidity (Appendix S3). An evolutionary trade-off was observed between fruit total flavonoids and fruit tannin activity (R^2 = 0.34, Fig. 2, Table 1, Appendix S8). The species with the highest measured tannin activity were in the big-bracted clade and the cornelian cherry group, whereas the highest total flavonoids were in the blue-and-white-fruited clade. Among species, fruit sugar content was related to this trade-off, with a strong positive correlation between fruit sugar content and fruit tannin activity (R^2 = 0.31, Fig. 2, Table

TABLE 1. Macroevolutionary trait–trait correlations between fruit nutritional content and defense chemistry as assessed by phylogenetic generalized least squares regression. R^2 and directionality of significant correlations ($p < 0.05$) are presented. See Appendix S8 for trait data and Appendix S10 for full analysis.

Trait	Fruit total flavonoids	Fruit total phenolics	Fruit tannin activity	Fruit protein content	Fruit lipid content	Fruit sugar content	Fruit titratable acidity
Fruit water content	–	(+) 0.15	–	–	–	–	–
Fruit titratable acidity	–	(–) 0.18	(+) 0.17	–	–	–	
Fruit sugar content	(–) 0.33	–	(+) 0.31	–	–		
Fruit lipid content	(+) 0.17	(–) 0.21	–	(+) 0.19			
Fruit protein content	–	(+) 0.15	(–) 0.17				
Fruit tannin activity	(–) 0.34	–					
Fruit total phenolics	(–) 0.19						

1, Appendix S8), and a negative correlation between fruit sugar content and fruit total flavonoids ($R^2 = 0.33$, Table 1). Alongside these relationships, several other weaker ones were observed between fruit defense chemistry and fruit protein content, lipid content, water content, and titratable acidity (Table 1).

Chemical defense variation across organs

Total flavonoids, total phenolics, and tannin activity varied considerably across flowers, leaves, and fruits (Fig. 3). Among species, flowers have the highest total flavonoids, significantly higher than both fruit total flavonoids and leaf total flavonoids (Fig. 3, Appendix S9). Tannin activity exhibited the largest variation among organs, with leaves having the highest tannin activity, significantly higher than both flowers and fruits (Appendix S9). In several species, flowers and fruits had undetectable levels of tannin activity, though floral tannin activity was significantly higher than fruit tannin activity (Appendix S9). Total phenolics were much less variable among organs, though with leaves significantly higher than fruits (Appendix S9).

Reproductive defenses and native habitat environment

Cornus species exhibit substantial variation in many native habitat environmental conditions (Appendix S1). The focal taxa varied dramatically in native habitat temperature regimes, with species means for variables like mean annual temperature, minimum temperature of the coldest month, and maximum temperature of the warmest month each varying by over 10°C among species (Appendices S1, S4). Species occupying the warmest climates were *C. foemina* and *C. drummondii*, with mean maximum temperature of the warmest month above 32°C, and *C. foemina*, *C. glabrata*, and *C. paucinervis* with mean minimum temperature of the coldest month above freezing (Appendix S4). Species occupying the coldest climates were *C. alba*, *C. alternifolia*, and *C. sericea*, with mean annual temperature below 8.5°C, and *C. alba* and *C. bretschneideri* with mean minimum temperature of the coldest month below −12°C (Appendix S4). With respect to reproductive defenses, maximum temperature in the warmest month was strongly negatively correlated with floral total flavonoids ($R^2 = 0.84$, Table 2) and floral total phenolics ($R^2 = 0.30$, Table 2) as well as negatively correlated with fruit tannin activity ($R^2 = 0.54$, Table 2). However, other metrics of temperature yielded conflicting patterns. Mean annual temperature was positively correlated with floral total phenolics ($R^2 = 0.50$, Table 2), and the minimum temperature of the coldest month was strongly positively correlated with floral total flavonoids ($R^2 = 0.66$, Table 2) and floral total phenolics ($R^2 = 0.71$, Table 2).

Cornus species also varied substantially in metrics of native habitat precipitation, varying 3-fold in mean annual precipitation, 30-fold in average precipitation of the warmest quarter, and 25-fold in average precipitation of the driest month (Appendices S1, S4). Species occupying the wettest habitats were *C. controversa*, *C. macrophylla*, and *C. kousa,* with mean annual precipitation above 150 cm and mean precipitation in the warmest quarter over 60 cm (Appendix S4). Species occupying the driest habitats were *C. mas* and *C. bretschneideri* with mean annual precipitation below 75 cm and *C. bretschneideri*, *C. glabrata*, and *C. hemsleyi* with mean precipitation of the driest month below 1 cm (Appendix S9). Across *Cornus*, evidence suggests the evolution of higher reproductive defenses with increasing precipitation, whether annual, in the driest part of the year, or during the growing season. Specifically, mean annual precipitation was positively correlated with both floral total flavonoids ($R^2 = 0.44$, Table 2) and floral total phenolics ($R^2 = 0.77$, Table 2). Precipitation of the driest month was positively correlated with floral total flavonoids ($R^2 = 0.18$, Table 2), floral total phenolics ($R^2 = 0.36$, Table 2), and fruit total phenolics ($R^2 = 0.55$, Table 2). Precipitation of the warmest quarter was also positively correlated with floral total flavonoids ($R^2 = 0.19$) and floral total phenolics ($R^2 = 0.44$, Table 2).

Cornus species also exhibited variation in native habitat soil conditions, including variables like mean soil organic matter content (10 to 41 ppt), silt content (31 to 46%), and sand content (29 to 49%). Soil organic matter content was positively correlated with floral total flavonoids ($R^2 = 0.61$, Table 2), floral total phenolics ($R^2 = 0.33$, Table 2), fruit total phenolics ($R^2 = 0.32$, Table 2), and fruit tannin activity ($R^2 = 0.42$, Table 2), though negatively with fruit total flavonoids ($R^2 = 0.24$, Table 2). Soil sand content was positively correlated with floral total flavonoids ($R^2 = 0.35$, Table 2), floral total phenolics ($R^2 = 0.40$, Table 2), and fruit total phenolics ($R^2 = 0.63$, Table 2). Inverse correlations were observed with soil silt content, which was negatively correlated with floral total flavonoids, floral total phenolics, and fruit total phenolics (Table 2).

DISCUSSION

The evolution of reproductive defenses with phenology

Secondary metabolites often make up a significant portion of plant defenses (Harborne and Turner, 1984; Crozier et al., 2006; Pais et al., 2018), and these defenses are found in differing concentrations among plant tissues. The metrics of tannin activity, total flavonoids, and total phenolics assessed here estimate secondary metabolite classes that are part of the phenylpropanoid pathway (Crozier et al.,

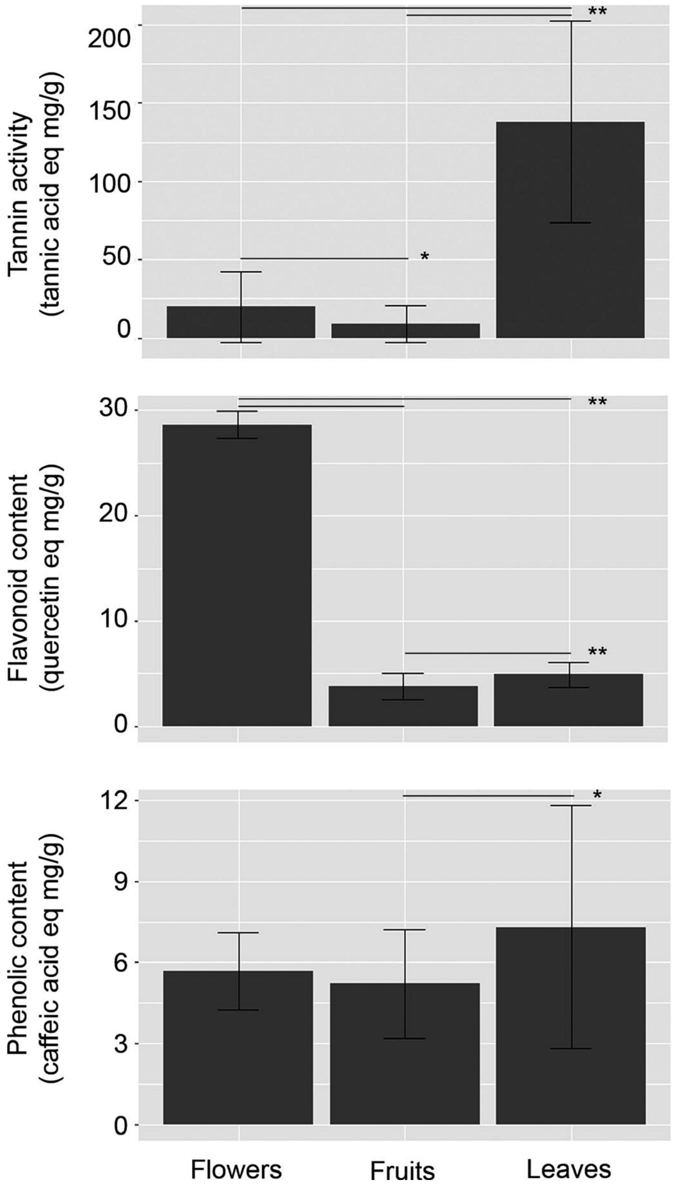

FIGURE 3. Bar plot comparing mean tannin activity, total flavonoids, and total phenolics in flowers, fruits, and leaves of the *Cornus* species studied. Error bars represent the standard deviation among samples of each organ. For tannin activity, leaves were significantly higher than fruits and flowers by paired *t*-test (Appendix S5). For total flavonoids, flowers were significantly higher than leaves and fruits by paired *t*-test (Appendix S5). For total phenolics, there were no significant differences (Appendix S5). Significant differences in means between flowers, fruits, and leaves are signified by single asterisk ($p < 0.05$) or double asterisk ($p < 0.001$).

2006; Fraser and Chapple, 2011). In this pathway, condensed tannins are manufactured from a precursor compound flavan-3,4-diol, which is a flavonoid (Gutierrez-Gonzalez et al., 2010). We observed that the evolution of a later starting date for flowering was associated with the evolution of higher floral tannin activity and lower floral total flavonoids across the phylogeny, indicating that species differentiation by flowering phenology was associated with the relative balance of flavonoids versus tannins in inflorescences. In addition, across *Cornus*, the evolution of later fruiting start date was associated with the evolution of higher fruit total phenolics, and together with the results above, this indicates that later-blooming and later-fruiting species have evolved an increased level of specific phenylpropanoid chemical defenses. One explanation may be the increase in insect abundance and pathogen prevalence as the growing season progresses (Bale et al., 2002; Evans et al., 2008; Wang et al., 2009; Huot et al., 2017, Hahn et al., 2019). Mason et al. (2020) directly observed higher rates of leaf herbivory later in the growing season on this same collection of *Cornus* species in 2015. This strong variation in quantitative defenses in relation to reproductive phenology is highly suggestive in relation to the apparency hypothesis (Feeny, 1976), but relies on assumptions about the relative seasonal abundance of natural enemies to interpret. If earlier phenology corresponds to lower herbivore or pathogen abundance, then this may suggest that the evolution of increased tannin activity in the inflorescences of later blooming species (at the expense of flavonoid production) and the evolution of increased total phenolics in later fruiting species may have both been in response to an increase in the likelihood of herbivory or disease later in the growing season.

Conversely, among *Cornus* species, we did not observe any significant macroevolutionary correlations between our focal chemical defenses and the duration of flowering or fruiting. Our results do not support the apparency hypothesis, which would predict that species which have evolved a protracted flowering or fruiting period would be more apparent to natural enemies and thus be expected to evolve higher defense investment in these structures so closely linked to plant fitness. However, we did observe a much lower degree of variation in the duration of flowering or fruiting among *Cornus* species (range of 1–3 and 1–4 weeks, standard deviation of 0.6 and 1.0 weeks, respectively; Appendix S8) relative to the variation observed for flowering or fruiting start dates (ranges of 67 and 77 days, and standard deviations of 20.8 and 21.4 days, respectively; Appendix S8). This low degree of variation may suggest that *Cornus* is not a conducive system to test the apparency hypothesis in relation to the duration of flowering or fruiting. A caveat in using a common garden approach to assess evolutionary relationships between reproductive defenses and phenological traits is that reproductive structures formed at different times throughout the growing season will form under different environmental conditions, such as temperature. However, in a common garden experiment, species should segregate by phenology relative to their behavior in native habitat in response to the temperature and photoperiod thresholds that control phenology (Premoli et al., 2007; Panchen et al., 2014; Zohner and Renner, 2014; Mason et al., 2017). Here in *Cornus*, flowering start date in the Arnold Arboretum was strongly positively correlated with the average native habitat maximum temperature of the warmest month ($R^2 = 0.52$, Appendix S10), suggesting a temperature-based control of budbreak and flowering phenology among dogwood species that has been previously demonstrated within C. florida (Reader, 1975) and *C. sericea* (Kobayashi and Fuchigami, 1983).

The evolution of fruit defenses alongside fruit nutritional content

An evolutionary trade-off was observed among *Cornus* fruits between flavonoids and tannins. Our observations may indicate that in some species a large proportion of flavonoid precursors are converted into tannins through additional biosynthesis within the phenylpropanoid pathway, while in others they are not (Crozier

TABLE 2. Macroevolutionary correlations between fruit and floral phenology and defense traits and native habitat environmental characteristics as assessed by phylogenetic generalized least squares regression. R^2 and directionality of significant correlations ($p < 0.05$) are presented. See Appendix S8 for trait data, Appendix S9 for environmental data, and Appendix S10 for full analysis.

Environmental characteristic	Floral total flavonoids	Floral total phenolics	Fruit total flavonoids	Fruit total phenolics	Fruit tannin activity
Soil organic matter content	(+) 0.61	(+) 0.33	(–) 0.24	(+) 0.32	(+) 0.42
Soil sand content	(+) 0.35	(+) 0.40	–	(+) 0.63	–
Soil silt content	(–) 0.38	(–) 0.47	–	(–) 0.57	–
Mean annual temperature (Bio1)	–	(+) 0.50	–	–	–
Maximum temperature of the warmest month (Bio5)	(–) 0.84	(–) 0.30	–	–	(–) 0.54
Minimum temperature of the coldest month (Bio6)	(+) 0.66	(+) 0.71	(–) 0.18	–	–
Mean annual precipitation (Bio12)	(+) 0.44	(+) 0.77	–	–	–
Precipitation of the driest month (Bio14)	(+) 0.18	(+) 0.36	–	(+) 0.55	–
Precipitation of the warmest quarter (Bio18)	(+) 0.19	(+) 0.44	–	–	–

et al., 2006; Gutierrez-Gonzalez et al., 2010). One hypothesis is that the amount of sugar in a fruit may have some influence over this process, as indicated by the positive correlation between sugar content and fruit tannin activity. This relationship may support our prediction that higher nutritional content predicts higher defenses within the fruits of genus *Cornus*, for instance, because increased sugar content may make fruits more prone to microbial attack, and that an increase in tannins contributes to fruit defense against microorganisms (Field and Lettinga, 1992). An alternate explanation for this positive evolutionary correlation is the phenomenon of tannin-carbohydrate complexes, which form to reduce the effects of tannins in ripening and ripe fruits so long as they remain intact (Ozawa et al., 1987; Luthar and Tisler, 1992; Cipollini and Levey, 1997). If this is the case, high sugar content in fruits with high tannin activity could be at least partially a byproduct of monosaccharide complexation during tannin biosynthesis rather than a coordinated strategy to defend vulnerable high-sugar fruits, and these explanations cannot be differentiated by this study. Future manipulative work assessing the relative susceptibility of fruits to microbial attack, as well as specific investigation of tannin manufacture and storage, would help differentiate these two explanations.

Among *Cornus* species, we found several additional weak evolutionary correlations between fruit chemical defenses and fruit nutritional traits other than sugar content. We hypothesized that increased attractiveness to bird dispersers through traits like higher water content, lipid content, and protein content would also require increased defenses to protect against insects and pathogens. Among *Cornus* fruits, we found mixed support for these predictions, with no relationships especially strong (Table 1). High concentrations of lipids have been found in the pulp of some *Cornus* species, including *C. florida*, *C. racemosa*, and *C. drummondii* (Borowicz, 1988; Willson and Whelan, 1993; Rossell et al. 2001), and high lipid content has been associated with a higher probability of being eaten by birds (Borowicz, 1988; Willson and Whelan, 1993). This prior research suggests that lipid content may be more dictated by fats and oils in the fruit rather than surface waxes, where waxes would perhaps contribute more to fruit defense than palatability, and that lipid content may be a major determinant of preference in *Cornus* bird dispersers. Fruit lipid content has also been shown in a number of plant species to negatively correlate with fruit carbohydrate content within the pulp (Debussche et al., 1987; Herrera, 1987; Jordano, 2000; Levey and Martínez del Rio, 2001) and has been suggested to be the cause of selective pressures of different lipid and carbohydrate-processing digestion in frugivorous birds (Levey and Martínez del Rio, 2001). Here within the collection of *Cornus* species we were able to sample for fruits, we did not observe a trade-off between lipid content and sugar content, but we did observe high-sugar fruits to be well-defended by high tannin activity and high-lipid fruits to have higher total flavonoids (but lower total phenolics).

Optimal defense and variation among organs

The optimal defense hypothesis predicts that plants should allocate more defense investment to tissues that are most relevant to fitness, which given the importance to reproduction, might lead to the prediction that flowers and fruits should be more chemically defended than leaves (Godschalx et al., 2016). However, there is evidence that in at least some species, floral tissue may not necessarily be more valuable to fitness than leaves, and that floral tissue may have lower concentrations of chemical defenses (Keith and Mitchell-Olds, 2017; McCall and Fordyce, 2010; Alves et al., 2007; Godschalx et al., 2016). This pattern is rationalized by the observation that floral tissue loss may actually reduce fitness less than the loss of leaves, especially young leaves with a long productive lifespan remaining (Godschalx et al., 2016). There has also been evidence of plants tolerating fruit and flower loss through strategies such as vegetative over-compensation, which occurs when plants exhibit higher growth rates to compensate for herbivory (Reichman and Smith, 1991; Wise et al., 2008; McCall and Irwin, 2006; Keith and Mitchell-Olds, 2017).

We found in *Cornus* that leaves had far higher tannin activity than both inflorescences and fruits, which does not support the prediction of the optimal defense hypothesis, but also that inflorescences had substantially higher total flavonoids than leaves, which would appear to support the hypothesis. To differentiate these conflicting results, we need further evidence as to which organs contribute the most to fitness among *Cornus*, as well as data on the relative fitness cost of the biosynthesis of tannins relative to flavonoids. One possible explanation for higher tannin activity in leaves is that tannins are known to be effective against herbivory (Levin, 1976; Salminen and Karonen, 2011), and early season leaf loss due to herbivory may have a bigger plant fitness cost through the reduction of photosynthetic productivity and subsequent reproductive organ production than the loss of a portion of those reproductive organs once produced. This possible explanation needs to be further tested within *Cornus* to provide evidence that young leaves actually do contribute more to fitness than do flowers, especially given the wide variation in phenology among species. While later-flowering species may use same-season photosynthate generated by new leaves for subsequent reproduction, those species that flower at the very beginning of the growing season before or during leaf-out almost certainly use stored carbon resources for reproduction. The relative fitness value of leaf and reproductive defense investment against herbivory therefore almost certainly varies among species based on phenology. This variation could be potentially untangled in future work through a combination of organ removal studies (e.g., Godschalx et al., 2016) coupled with isotope analysis to determine the source of carbon for tissue construction (e.g., Kimak and Leuenberger, 2015).

Flowers may produce more flavonoids than other organs because of the dual role of flavonoids in contributing to floral color (including ultraviolet reflectance) as well as defense against fungal pathogens. Given their complexity and the additional biosynthetic steps in their manufacture, tannins are expected to be more energetically expensive to manufacture than flavonoids (Cipollini et al., 2014). However, the most common classes of tannins (e.g., condensed tannins) are typically considered more stable than small phenolics such as flavonoids and last considerably longer both in a living leaf and in leaf litter (Coley et al., 1985; Hemingway and Karchesy, 1989; Kleiner et al., 1999). Flavonoids would be expected to degrade more easily (Strack et al., 1982; Reichardt, et al., 1991) and thus have a higher turnover in plant tissues, and so to maintain a high concentration for a long period a plant would need to continuously remanufacture these compounds. Since leaf lifespan tends to be substantially longer than flower lifespan, as observed during our study, defending a leaf with more energetically expensive to produce but longer-lasting chemical defenses such as tannins may be a less costly strategy relative to the constant remanufacturing of less expensive compounds like flavonoids. Conversely, flavonoids would seem to be a more energetically prudent defensive strategy for more ephemeral reproductive organs, consistent with the high total flavonoids observed here in inflorescences. If these assumptions are valid, this would suggest that the relative costs and benefits of manufacturing chemical defenses across organs have been optimized by natural selection in *Cornus*, as expected under the optimal defense hypothesis. Stable isotope tracer studies would be one method to examine the specific turnover dynamics of flavonoids versus tannins in different organs (Freund and Hegeman, 2017).

The evolution of reproductive defenses with resource availability

The majority of *Cornus* species inhabit North America, Asia, and Europe (Xiang et al., 1996, 2005), with distributions scattered across the northern hemisphere. Concomitant with this variation in geographic distributions, *Cornus* species occupy ranges that differ across major environmental gradients. We find strong evolutionary correlations between native habitat temperature, precipitation, and soil characteristics and species' floral and fruit chemical defenses, indicating that as species have diversified among habitats, there have been consistent phenotypic responses to these environmental gradients.

As lineages move into sandier habitats, we observe the evolution of higher floral and fruit total phenolics as well as floral total flavonoids, while we observe lower values of all of these traits as lineages move into soils with higher silt content. Other studies that have tested the resource availability hypothesis have made similar findings, where species adapted to sandier soils (where nutrient supply is often insufficient to support high inherent growth rates to compensate for herbivory) have evolved higher flavonoid and phenolic concentrations relative to species occupying environments with less sand (Fine et al., 2004; Hahn et al., 2019). We also find that as lineages have moved onto soils with a higher organic matter content, species have evolved higher floral total flavonoids and total phenolics, higher fruit total phenolics and tannin activity, which appears to run counter to the resource availability hypothesis if organic matter content is inferred to predict soil fertility. However, the availability of organic matter-derived nutrients is highly dependent on complex interactions of other factors, like temperature, water availability, and soil microbial activity, and high organic matter soils can also be highly limiting in mineral nutrients, such as peat bogs where at least some *Cornus* species are occasionally found (Ruch et al., 2013; Kiviat et al., 2019).

Species occupying habitats with elevated precipitation exhibit higher floral total flavonoids and phenolics regardless of the metric used, and species with higher precipitation in the driest month exhibit higher fruit total phenolics. These patterns run counter to the predictions of the resource availability hypothesis, which predicts that improved access to water resources through increased rainfall (especially during the growing season and driest periods of the year) should enable species to evolve a higher inherent growth rate to compensate for herbivory. While many studies have found that increased water availability can lead to an increase in photosynthetic activity (e.g., Li et al., 2007; Potts et al., 2017), this increase in photosynthesis does not always translate into increased growth rate unless water is the primary limiting resource (Koricheva et al., 1998), and in some systems increased water availability has been found to result in improved plant chemical defense (Pezzola et al., 2017). It is possible that the evolution of higher chemical defenses in reproductive structures is favored in wetter environments in response to increased pressure from fungal and bacterial pathogens (Fitt et al., 1989; Bradley et al., 2003), and higher phenolic contents in leaves have been found to evolve repeatedly in response to diversification into wetter environments in other plant genera (Pearse and Hipp, 2012; Mason et al., 2016).

Warmer temperatures are also an important environmental characteristic that allows plants to grow more rapidly (e.g., a higher number of growing degree days) and typically provide a longer growing season. There is evidence that increased temperature also tends to correlate with increased populations of insects and pathogens, which may shape the evolution of plant defenses (Whang et al., 2010; Huot et al., 2017; Hahn et al., 2019). As *Cornus* species have diversified into habitats with warmer temperatures that may support an increase in growth rate, we observe a mix of changes in chemical defenses. In relation to maximum summer temperatures, we observe the evolution of lower reproductive defense as lineages have moved into warmer environments, which supports the predictions of the resource availability hypothesis. However, if considering minimum winter temperatures, we primarily observe the evolution of higher reproductive defenses in warmer environments. One possible explanation for this pattern is adaption to the absence of harsh frosts or prolonged cold periods, which typically kill off a portion of insect and pathogen populations (Ayres and Lombardero, 2000; Bale et al., 2002; Dukes et al., 2009). A more nuanced understanding of how temperature limits growth rates in *Cornus* is needed to untangle whether the resource availability hypothesis is relevant in this group of species, but it is surprising to obtain such strong and contradictory results based on minimum versus maximum temperature metrics.

With respect to testing the resource availability hypothesis, one major difficulty lies in validating the link between tissue defense investment and plant inherent growth rate. This link is key to the theoretical underpinnings of the hypothesis but difficult to assess in many systems without multi-year observations of growth or destructive harvesting to obtain dendrochronological data. Mixed results in assessing the resource availability hypothesis using environmental resource data and tissue chemistry alone (as in this study) are likely related to differences in the relative energetic costs of investment in different kinds of defense (Poorter, 1994). Incorporation of plant inherent growth rate, while difficult in many systems, would provide even stronger insights into how resource supply drives the evolution of plant defense.

CONCLUSIONS

Fruits and flowers play an integral part in determining plant fitness and shaping plant evolution. Despite this, disproportionately few studies have explicitly considered the evolution of chemical defenses in reproductive organs relative to the large body of work on vegetative organs, primarily leaves. Further research into fruit and floral defenses is needed to advance plant defense theory, especially the optimal defense hypothesis, which explicitly includes predictions of the relative allocation of defenses among vegetative and reproductive organs. Our results provide mixed support for this aspect of the optimal defense hypothesis depending on whether tannins or flavonoids are considered. In relation to the apparency hypothesis, we find no support for the evolution of chemical defenses in response to the duration of flowering or fruiting, but tentative support in relation to flowering and fruiting start dates. Our results also provide recurring evidence for an evolutionary trade-off between tannins and flavonoids, including inverse relationships with phenology in flowers, and a direct negative evolutionary correlation in fruits, and the large differences among organs, suggesting an underlying trade-off between investing resources into one subclass of phenolic compounds over another, perhaps driven by the role of flavonoids as tannin precursors in the phenylpropanoid pathway. We also find mixed support for the resource availability hypothesis in the evolution of reproductive defenses depending on which metrics of precipitation, temperature, or soil characteristics are considered, suggesting that the resource availability hypothesis has limited applicability to reproductive defenses in *Cornus*.

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AUTHOR CONTRIBUTIONS

C.M.M. designed the study and organized sampling. C.S.W. led phenology tracking and tissue sampling, and completed floral phytochemical analysis. D.D.L.P. completed fruit phytochemical analysis. E.W.G. and C.M.M. constructed the phylogeny and extracted environmental data, and E.W.G. led the programming of scripts for phylogenetic comparative methods. D.D.L.P. led data analysis with assistance from E.W.G., J.A.D., and C.M.M. D.D.L.P. led interpretation of results and created figures. D.D.L.P. and C.M.M. led the writing of the manuscript with input from E.W.G., J.A.D., and C.S.W.

DATA AVAILABILITY

The supplemental files and data sets that accompany this article are available from the Dryad Digital Repository (https://doi.org/10.5061/dryad.jm63xsj7h) (De La Pascua et al., 2020). The previously published phylogeny and environmental data (Mason et al., 2020) can be found in the Dryad Digital Repository at https://doi.org/10.5061/dryad.wh70rxwhr.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Interspecific and intraspecific variation in selected native habitat environmental characteristics among focal *Cornus* species.

APPENDIX S2. Phenology assessed on 66 *Cornus* plants in the Arnold Arboretum in 2016.

APPENDIX S3. Species mean trait data collected in this study on phenology, floral tissue, and fruit tissue, along with leaf trait data from Mason et al. (2020).

APPENDIX S4. Species means for environmental variables used in this study, as generated from data published in Mason et al. (2020).

APPENDIX S5. R code and phylogeny used for the phylogenetic generalized least squares (PGLS) regression and ancestral state reconstruction analyses, as well as data output files.

APPENDIX S6. Ancestral state reconstructions of phenology and fruit and floral chemical defense across the genus *Cornus*.

APPENDIX S7. Macroevolutionary correlations between floral phenology and other floral phenotypic traits, as well as between fruit phenology and other fruit phenotypic traits, as assessed by phylogenetic generalized least squares regression.

APPENDIX S8. Ancestral state reconstructions of fruit chemical defense and fruit nutrition across the genus *Cornus*.

APPENDIX S9. Paired *t*-test for differing sample sizes testing differences in flavonoid content, phenolic content, and tannin activity means for fruit, flower, and leaves.

APPENDIX S10. Macroevolutionary correlations between native habitat environmental characteristics and phenology and fruit nutritional traits as assessed by phylogenetic generalized least squares regression.

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APPENDIX 1.. Accession information for living collections sampled in this study within the Arnold Arboretum of Harvard University.

Cornus alba L. — 321-97*B, 321-97*A. ***Cornus alternifolia*** L.f. — 612-2007*A, 612-2007*B,612-2007*D, 612-2007*E. ***Cornus amomum*** Mill. — 113-85*MASS, 1418-83*A. ***Cornus australis*** C.A.Mey. — 41-2002. ***Cornus bretschneideri*** L.Henry — 141-2005*A, 149-2007*A. ***Cornus controversa*** Hemsl. — 17-98*A, 17-98*B, 17-98*C, 245-2001*A. ***Cornus coreana*** Wangerin — 706-80*A, 706-80*B. ***Cornus drummondii*** C.A.Mey. — 1031-80*MASS, 1717-81*A. ***Cornus florida*** L. — 115-2007*B, 18-2000*A, 18-2000*D, 29-68*A. ***Cornus foemina*** Mill. — 15-55*A, 15-55*B. ***Cornus glabrata*** Benth. — 1260-66*A, 1260-66*B, 1260-66*C. ***Cornus hemsleyi*** C.K.Schneid. & Wangerin — 92-81*D, 92-81*E. ***Cornus kousa*** F.Buerger ex Hance — 1079-89*A, 1207-82*A, 1916-80*C, 382-86*A. ***Cornus kousa*** **var.** ***chinensis*** (Osborn) Q.Y.Xiang — 274-2000*A. ***Cornus macrophylla*** Wall. — 1277-83*B, 1277-83*D, 278-94*B, 422-94*A. ***Cornus mas*** L. — 1173-67*A, 1314-85*A, 645-79*A, 748-75*A. ***Cornus obliqua*** Raf. — 110-79*A, 110-79*B, 1467-83*A, 198-89*A. ***Cornus officinalis*** Siebold & Zucc. — 10933*C, 918-85*A. ***Cornus paucinervis*** Hance — 1235-70*A. ***Cornus poliophylla*** C.K.Schneid & Wangerin — 739-78*A, 739-78*B, 739-78*C. ***Cornus pumila*** Koehne — 222- 76*B, 228-2000*A, 228-2000*B. ***Cornus racemosa*** Lam. — 212-81*MASS, 599*A. ***Cornus sanguinea*** L. — 205-78*MASS, 343-78*A. ***Cornus sericea*** L. — 113-79*MASS, 1224- 84*MASS, 291-92*A, 769-93*B. ***Cornus walteri*** Wangerin — 435-80*B, 435-80*D, 435-80*E.