

REVIEW

Complementing Model Species with Model Clades

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ABSTRACT

Model species continue to underpin groundbreaking plant science research. At the same time, the phylogenetic resolution of the land plant Tree of Life continues to improve. The intersection of these two research paths creates a unique opportunity to further extend the usefulness of model species across larger taxonomic groups. Here we promote the utility of the *Arabidopsis thaliana* model species, especially the ability to connect its genetic and functional resources, to species across the entire Brassicales order. We focus on the utility of using genomics and phylogenomics to bridge the evolution and diversification of several traits across the Brassicales to the resources in *Arabidopsis*, thereby extending scope from a model species by establishing a “model clade”. These Brassicales-wide traits are discussed in the context of both the model species *Arabidopsis thaliana* and the family Brassicaceae. We promote the utility of such a “model clade” and make suggestions for building global networks to support future studies in the model order Brassicales.

INTRODUCTION

Model species are essential in plant biology. For example, Barbara McClintock carefully tracked corn kernel variation in *Zea mays* to characterize transposable elements and chromosome segregation (McClintock, 1941, 1950). The genome of *Arabidopsis thaliana* (The Arabidopsis Genome Initiative, 2000) and foundational mutant and germplasm collections like the *Arabidopsis* T-DNA Insertional Mutant Collection (O’Malley et al., 2015) have driven our understanding of gene function in all plants. However, the plant kingdom is vast, comprising nearly 500,000 species and spanning a billion years of evolutionary time (Nic Lughadha et al., 2016). Collaborations among systematists, herbaria, and botanical gardens continue to drive advances in building the plant tree of life (One Thousand Plant Transcriptomes Initiative, 2019; Johnson et al., 2019), in part due to rapid

advances and the falling cost of genome and transcriptome sequencing technologies. These technological advancements enable us to build resources that complement and elevate the usefulness of a model species, encompassing larger taxonomic groups to now produce “model clades” of plants. As species relationships become clearer, the plant biology community is in an ideal position to leverage comparative genomics across these clades to unravel the evolution and function of genes, regulatory networks, repetitive elements, and non-coding DNA. Comparative biologists and phylogeneticists have long been working interdisciplinary on these problems, for example, to examine floral organ evolution across the order Ranunculales (Damerval and Becker, 2017) and the grasses (Schrager-Lavelle et al., 2017). The development of accessible comparative genome and gene family evolution platforms like Phytozome (Goodstein et al., 2012), CoGE (Lyons, 2008), Ensembl Plants (Yates et al., 2022), and GENESPACE (Lovell et al., 2018, 2022) allow the placement of stand-alone genome assemblies in a larger evolutionary and phylogenetic context, creating an opportune time for plant biologists to identify clades with diverse phenotypes that can be anchored to a closely related model species.

The order Brassicales exhibits extensive diversity of species, traits, and environmental adaptations across a broad geographical distribution, and includes several crops like canola, caper, broccoli, kale, papaya, rapeseed, and saltwort. Within the Brassicales, one can find recurrent evolution and diversification of complex traits involving **genomics** (e.g., hybridization, polyploidy, repeat sequence turnover, speciation, and reproductive systems), **physiology** (e.g., extremophytism, photosynthesis modes), **morphology** (e.g., woodiness, tuberization), **metabolomics** (e.g., oil production, secondary metabolite diversification), and **environment** (e.g., wet and hyperarid habitats). To understand the genetic underpinnings of these traits, the predicted functions of orthologous genes in any understudied Brassicales species can be anchored to the massive mutant collections in the Brassicaceae “model species” *A. thaliana*. In this review, we highlight the utility of all Brassicales for benefiting from comparative genomics to understand trait variation and evolution, in the context of producing a “model clade” of plants that builds out from a “model species”. We frame each major section with three key points. First, we describe what is known about a given trait from studying the family Brassicaceae, often derived from research using *A. thaliana*. Second, we describe what is known about the diversity or variation of a given trait across the order Brassicales. Third, we discuss how leveraging comparative genomics across the order will lead to a better understanding of gene, genome, and trait evolution.

SYSTEMATICS AND DIVERSIFICATION OF THE BRASSICALES

Systematics across the Brassicaceae

The Brassicales comprises ca. 4,700 species in 18 families (**Table 1**; Magallon et al., 1999; Swanepoel et al., 2020), with the Brassicaceae, by far the largest family, comprising ca. 4,140 species in 349 genera (Koch et al., 2018; Walden et al., 2020a; German et al., 2023; Hendriks et al., 2023; Table 1). Brassicaceae systematics has long been complicated by high levels of parallel and convergent evolution of morphological characters likely reflecting recent and rapid radiations (Huang et al., 2016; Guo et al., 2017; Walden et al., 2020a; Zuo et al., 2022). Nearly all species are assigned to one of 58 monophyletic tribes (German et al., 2023; Hendriks et al., 2023), which in turn are assigned to two subfamilies: the small monogeneric Aethionemoideae and the large Brassicoideae (98.6% of species; German et al., 2023). Additionally, the five main lineages within Brassicoideae recently received supertribe status (German et al., 2023). While assignment of tribes to supertribes is more or less consistent between reconstruction methods, relationships among supertribes vary. For instance, in plastid phylogenies, the Camelinoideae (formerly lineage I) consistently diverges first (**Figure 1**; Huang et al., 2016; Guo et al., 2017; Mabry et al., 2020; Walden et al., 2020a; Hendriks et al., 2023), whereas nuclear phylogenies show the Hesperoideae (formerly lineage III) diverging first (**Figure 1**; Huang et al., 2016; Nikolov et al., 2019; Mabry et al., 2020; Hendriks et al., 2023). With the availability of whole genome sequences from all main lineages and advances in synteny and coalescent-based methods for phylogenetic inference, additional evolutionary hypotheses have been proposed recently (Zou et al., 2020; Walden et al., 2020b). For example, the similarity of the *Arabidopsis thaliana* genome structure with that of *Aethionema* (Aethionemoideae) may indicate an earlier diverging position of the lineage leading to Arabidoideae (Walden et al., 2020b) and potentially Alysiaceae (both supertribe Araboideae, formerly lineage IV; Hendriks et al., 2023). Such contrasting gene and species tree topologies suggest complex evolutionary histories and lead to natural complications in achieving a stable classification based on the principle of monophyly at many taxonomic levels (Forsythe et al., 2020).

Systematics across the Brassicales

The morphological, biochemical, and diverse genomic composition across the Brassicales permits comparative investigation of evolutionary novelties and macroevolutionary patterns (**Figure 2**). However, these approaches rely on first constructing a well-supported species tree. Most of the families across the Brassicales, including Borthwickiaceae (Zhao et al., 2015) and the recently

described Tiganophytaceae (Swanepoel et al., 2020), are only minimally sampled at the species level. Relationships across the order have been established with phylotranscriptomics (Edger et al., 2015, 2018a; Mabry et al., 2020), target sequence capture (Baker et al., 2022), and more traditional molecular systematic approaches based on fewer genes and larger taxonomic sampling (Rodman et al., 1993, 1996; Cardinal-McTeague et al., 2016). Many relationships amongst the Brassicales are well-supported (Hall et al., 2002, 2004; Craene et al., 2006; Hall, 2008; Edger et al., 2015, 2018a; Cardinal-McTeague et al., 2016; Mabry et al., 2020); however, some key nodes remain unresolved, which may be the consequence of rapid diversification and/or introgression (Edger et al., 2018a). Due to a large number of extant Brassicales species, building a high-resolution species tree requires both a unified research community effort, as well as creative approaches to integrating multiple types of data.

The future of Brassicales systematics research

A complete tree of all Brassicales species, with multiple representative sampling for each species, is desirable and tractable, especially given the growing popularity of reduced representation methods such as targeted sequence capture (Dodsworth et al., 2019; Baker et al., 2021). Targeted sequence capture can be applied very effectively even to degraded DNA obtained from herbarium specimens (Brewer et al., 2019), which vastly increases opportunities for extensive species sampling. These methods have proven useful for understanding species relationships at the order level across angiosperms (Antonelli et al., 2021; Lee et al., 2021; Maurin et al., 2021; Thomas et al., 2021; Zuntini et al., 2021), and efforts are already underway to build comprehensive phylogenomic datasets across the Brassicales by employing a universal sequence capture toolkit (Johnson et al., 2019; Baker et al., 2022). The combination of universal and lineage-specific toolkits (Nikolov et al., 2019; Hendriks et al., 2021; Eserman et al., 2021) offers great promise for a complete species phylogeny of the order. Further, a Department of Energy Joint Genome Institute Community Sequencing Project (<https://jgi.doe.gov/csp-2021-brassicales-genome-initiative/>) is currently funded to assemble and annotate chromosome-scale assemblies for at least 26 species, spanning every family in the order. The future for the phylogenomic community studying the Brassicales likely resides in a dual approach, integrating reduced representation genomic data with high-quality, chromosome-scale genomes and annotations.

POLYPLOIDY AND DIPLOIDIZATION DYNAMICS

Polyploidy across the Brassicaceae

Whole genome duplications (WGDs) are prevalent throughout plant evolution and are often associated with the origin of novel traits and shifts in diversification (Schrantz et al., 2012; Van de Peer et al., 2021). Early studies suggested that *Brassica* species (Brassicaceae) were hexaploids relative to *A. thaliana* (Arabidopsidae) and that their genomes experienced chromosomal rearrangements during their return to the diploid state (Lagercrantz, 1998; Babula et al., 2003). These results were confirmed by later studies and the sequencing of *Brassica* genomes (Parkin et al., 2005; Lysak et al., 2005, 2007; Schrantz et al., 2006; *Brassica rapa* Genome Sequencing Project Consortium, 2011; **Figure 3**). This hexaploidy or whole genome triplication (WGT) occurred through two successive hybridizations creating first an allotetraploid and eventually an allohexaploid (Tang et al., 2012; Cheng et al., 2012). However, the timing and placement of the hexaploidy on a resolved phylogeny remains elusive (Lysak et al., 2007; Wang et al., 2011; Arias and Pires, 2012; Huang et al., 2016; Cardinal-McTeague et al., 2016). More than 13 other independent, genus- or tribe-specific, polyploidy events have been identified across the Brassicaceae (Mandáková et al., 2017; Walden et al., 2020b; Huang et al., 2020; Hendriks et al., 2023). A study of nearly half of all Brassicaceae species found that fifty percent of these are recent polyploids (i.e., still recognised as polyploids based on cytological data) with at least one hidden state (characters that are not observed for the purpose of the model but influence the diversification rate; Beaulieu & O'Meara 2016) impacts the net diversification of diploid and polyploid species (Román-Palacios et al., 2020). Another study by Walden et al. (2020a) suggests these unassessed traits from Román-Palacios et al. (2020) may be associated with morphological diversity.

Polyploidy across the Brassicales

Multiple studies have found ancient WGDs spread more broadly across the Brassicales (e.g., At- β event; **Figure 2**; Barker et al., 2009; Edger et al., 2015, 2018a; One Thousand Plant Transcriptomes Initiative, 2019; Mabry et al., 2020). Many families have also experienced family-specific WGDs, including the Brassicaceae (At- α ; Barker et al., 2009; Haudry et al., 2013; Edger et al., 2015, 2018a; One Thousand Plant Transcriptomes Initiative, 2019; Mabry et al., 2020), Cleomaceae (Cs- α or Th- α ; Schrantz and Mitchell-Olds, 2006; Barker et al., 2009; Bayat et al., 2018; Mabry et al., 2020; Hoang et al., 2023) and Resedaceae (Rs- α ; Lysak, 2018; One Thousand Plant Transcriptomes Initiative, 2019; Mabry et al., 2020). Family-specific WGDs have also been identified

in Gyrostemonaceae, Koerberliniaceae, and Tropaeolaceae (One Thousand Plant Transcriptomes Initiative, 2019).

The future of Brassicales polyploidy research

Understanding polyploidy and diploidization in the Brassicales is highly relevant and impactful for several reasons. Firstly, the Brassicales are ideal for studying these processes due to their relatively small genomes ($N = 342$ taxa, Mean = 761.0 Mb/1C, Median = 537.0 Mb/1C, Mode = 392.0 Mb/1C; based on data from the Plant DNA C-values database release 7.1 together with additional data not yet incorporated; Pellicer and Leitch, 2020; Leitch et al., release 7.1, April 2019). We can also produce high quality genomes that allow for fine-scale comparisons (Kreiner et al., 2017), making them well-suited for in-depth genomic analysis. This relevance stems from the potential insights that can be gained into broader biological phenomena.

The impact of this research extends to various areas. Firstly, resolving the current struggles in understanding gene loss and retention after polyploidy in Brassicales can provide critical insights into the evolution of complex genomes. While gene loss after polyploidy is common, it is not random, and the fate of duplicated genes is determined by the interplay of many forces, including dosage-induced constraints (Birchler and Veitia, 2007, 2012; Conant, 2014; Conant et al., 2014; Hao et al., 2022), differentially methylated transposable element (TE) density patterns among subgenomes (Hollister and Gaut, 2009; Freeling et al., 2012), changes in regulatory networks (Blischak et al., 2018; Hu and Wendel, 2019), and epigenetic changes after hybridization (Cheng et al., 2016; Zhao et al., 2017). These processes yield diploidized genomes with genes retained in different copy numbers and patterns of variation (Li et al., 2021). This understanding is not only significant for advancing our knowledge of plant genetics but also has implications for broader evolutionary biology.

Secondly, the knowledge gained from studying polyploidy and diploidization in Brassicales can have practical applications in agriculture. Genes retained after polyploidy harbor more genetic diversity than other genes and were important during the domestication of *Brassica rapa* (Qi et al., 2021). Therefore, a better understanding of the processes involved can lead to more efficient breeding strategies and the development of improved crop varieties.

Thirdly, resolving these struggles can open up new avenues for future research. It can enable the development of advanced tools and methods, such as POInT (the Polyploid Orthology Inference Tool), a likelihood method for modeling subgenome partitioning and biased fractionation (Conant and Wolfe, 2008), which can then be applied not only to Brassicales but also to other polyploid systems. Inferring the biased pattern of gene loss has been challenging for several reasons, including

genomic rearrangement and homoeologous exchanges (Xiong et al., 2011; Mandáková et al., 2017; Edger et al., 2018b; Bird et al., 2020, 2023). Orthology inference using gene synteny can reliably differentiate subgenomes in polyploids (Lyons et al., 2008; Haug-Baltzell et al., 2017). POInT has been used to confirm that biased fractionation cannot be attributed to artifacts in synteny block inference (Conant, 2020). Integrating tools like POInT and GENESPACE (Lovell et al., 2022) can incorporate synteny into phylogenetically robust models of evolution. These approaches are promising for studying the diploidization process, particularly as the wider Brassicales community generates more chromosome-scale genome assemblies from across the order.

EPIGENETICS, SMALL RNAS, AND REPETITIVE ELEMENTS

Epigenetics, small RNAs, and transposable elements across the Brassicaceae

Much of our knowledge of small RNA (sRNA) biology has been derived from mutant screens in *A. thaliana*, including studies of TE mobilization and repression pathways (Miura et al., 2001; Gendrel et al., 2002; Lippman et al., 2004; Martinez et al., 2017). Plant genomes are typically dominated by TEs that can move throughout the genome using a variety of mechanisms (Wicker et al., 2007). While cycles of TE turnover are crucial for shaping plant genomes, most TEs are kept in a transcriptionally repressed state via DECREASED DNA METHYLATION 1 (DDM1) and RNA-directed DNA methylation (RdDM) pathways (Zemach et al., 2013; Matzke and Mosher, 2014; Li et al., 2015; Gallego-Bartolomé et al., 2019). The loss of DDM1 leads to loss of DNA methylation, accompanied by TE mobilization and developmental defects (Kakutani et al., 1999; Miura et al., 2001). The *A. thaliana* genome, which was the first plant genome to be sequenced (The Arabidopsis Genome Initiative, 2000), has been paramount to the dissection of these pathways; however, there is immense variation in DNA methylation levels within the Brassicaceae (Seymour et al., 2014; Bewick et al., 2016), such as the loss of gene body methylation in *Eutrema salsugineum* (Eutremeae) due to a loss of the CHROMOMETHYLASE 3 (CMT3) gene (Bewick et al., 2016). A recent study by Lu et al., (2019) investigated epigenetic variation across thirteen angiosperm species, two of which are in the Brassicaceae (*A. thaliana* and *E. salsugineum*). They found that species-specific accessible chromatin regions (ACRs) were strongly enriched for TE sequences. Therefore, species-specific TEs may regulate gene expression across the order, emphasizing the importance of sequencing across the clade.

Epigenetics, sRNAs, and TEs across the Brassicales

In the Brassicales, sRNAs have been poorly sampled in non-Brassicaceae species, though there are several emerging studies related to classes of sRNAs. One example is research on phased, secondary, small interfering RNAs (phasiRNAs), which display an intriguing pattern of loss in the Brassicales. In *A. thaliana*, phasiRNAs are expressed in vegetative tissues (Fei et al., 2013); additionally, 21nt and 24nt reproductive phasiRNAs, triggered by miR2118 and miR2275, respectively, have been found broadly across monocots (Patel et al., 2018; Kakrana et al., 2018) and eudicots (Xia et al., 2019), and shown to play a role in anther fertility in corn and rice (Teng et al., 2020). Intriguingly, miR2275 is not present in any of the sampled Brassicales (Polydore et al., 2018; Xia et al., 2019). The loss of miR2275 has been reported in several other lineages, including legumes and Solanaceous species, allowing us to place the Brassicales in a larger context of sRNA loss, thus enabling comparisons across the tree of life to understand the anther fertility phenotype compared to other monocots and eudicots. Other floral-related microRNAs in the Brassicales are implicated in sex determination and sexual dimorphism in papaya (Aryal et al., 2014). Further examples of lineage-specific sRNA evolution in the Brassicales are tandem microRNA loci, miR826 and miR5090, involved in nitrogen starvation adaptation (He et al., 2014).

With multiple documented WGDs, the Brassicales are a model clade for testing the interplay between polyploidy, genome size, and repetitive element proliferation. A recent study characterized TE abundances from low-coverage genome shotgun data in 71 phylogenetically diverse taxa across the order, placing transposon content in the context of WGD and phylogeny (Beric and Mabry et al., 2021); surprisingly, little connection was found between phylogeny and TE expansion or contraction, nor any correlation between patterns of WGD and TE abundance. While WGDs might be expected to lead to global TE re-activation, TE purging mechanisms seem to work efficiently in the Brassicales (Hawkins et al., 2009). Interestingly, the elimination of TEs is less effective in some Brassicales clades, resulting in large diploid genomes (1500 - 4200 Mb) that exceed the modal C-value of the Brassicales by 4- to 10-fold (Hloušková et al., 2019; Zuo et al., 2022).

The future of Brassicales epigenetics, sRNA, and TE research

The future of epigenetics, sRNA, and repetitive element studies within the Brassicales hinges on our ability to generate diverse sRNA and methylome sequencing datasets, and anchor analyses onto chromosome-scale assemblies in a comparative genomic context. Genomes from across the order will provide opportunities for investigation of how sRNA biogenesis and methylation pathways

of *A. thaliana* function in phylogenetically distant relatives, as well as provide insight into polyploidy, transposon biology, and lineage-specific sRNA emergence and loss.

REPRODUCTIVE BIOLOGY

Reproductive biology across the Brassicaceae

Thirty years ago, homeotic genes discovered in *A. thaliana* formed the foundation of the “ABC Model” (Bowman et al., 1991a, 1991b; Drews et al., 1991). *Arabidopsis thaliana* is also a model for understanding the evolutionary genetic underpinnings of the loss of self-incompatibility (SI) which promotes outcrossing. SI is a widespread trait that is found in 40% of flowering plant species and is based on recognition of self-pollen (e.g., Brassicaceae, Papaveraceae), or non-self pollen (e.g., Solanaceae; Igic et al., 2008; Fujii et al., 2016). The mechanisms underlying sporophytic SI in the Brassicaceae were discovered in *Brassica* and involve a pollen-specific ligand (*S-LOCUS PROTEIN 11*, SP11; or *S-LOCUS CYS-RICH PROTEIN*, SCR) and pistil-specific receptor (*S LOCUS RECEPTOR KINASE*, SRK) tightly linked at the S-locus (Stein et al., 1991; Schopfer et al., 1999; Takasaki et al., 2000; Takayama et al., 2000). The loss of SI arose in *A. thaliana* at least three times (Shimizu et al., 2008; Tsuchimatsu et al., 2017) through loss-of-function mutations in SCR, followed by rearrangements of the S-locus (Shimizu et al., 2008; Tsuchimatsu et al., 2010, 2017). The loss of SI in the Brassicaceae has occurred independently in at least five other species (Shimizu and Tsuchimatsu, 2015; Nasrallah, 2017). Further, there is an epigenetic component to SI plasticity. In *Brassica*, both DNA methylation (Shiba et al., 2006) and 24nt sRNAs (Tarutani et al., 2010) target recessive alleles in allelic pollen S-determinants. Both genomic and ecological factors can influence the repeated evolution and breakdown of SI, so understanding the diversity of pathways leading to SI-loss can be gained by studying systems across the Brassicales, although to date most involve the primary loss of the male-specific determinant of SI (Shimizu and Tsuchimatsu, 2015).

Reproductive biology across the Brassicales

Most species in the Brassicales are hermaphroditic or monoecious (Renner, 2014). However, roughly 1% are dioecious, where unisexual flowers develop on separate plants (Renner, 2014). Dioecy has evolved in seven different families, representing at least five origins (Figure 2; Renner, 2014; Soza et al., 2014). While structurally hermaphroditic, Cleomaceae commonly vary the ratio of functionally male to functionally female flowers according to environmental conditions (Zohoungbogbo et al., 2018).

In some Brassicales, sex chromosomes have been identified, with the most detailed studies in papaya (Caricaceae). The papaya Y is defined by the presence of a ~10 Mb non-recombining sex-determining region (SDR) that is only a fraction (~10%) of the entire sex chromosome (Liu et al., 2004; Na et al., 2012; Wang et al., 2012; Yue et al., 2022); this is similar to other plant sex chromosomes like asparagus and kiwifruit (Akagi et al., 2018; Harkess et al., 2020). The closely related genus *Vasconcellea* also has evidence of XY chromosomes; however, evidence suggests this is an independent origin (Wang et al., 2012; Carvalho and Renner, 2015). While papaya is almost exclusively dioecious in the wild, it is often described as trioecious because most cultivars are essentially gynodioecious (Brown et al., 2012; Fuentes and Santamaría, 2014).

The Brassicales are also an excellent model for the evolution of diverse fruit structures. The Brassicaceae fruits exhibit the greatest diversity in size and shape (Crane et al., 2006); however, fruit characters are highly homoplastic (Franzke et al., 2011) with clear evidence of parallel evolution of indehiscent fruits (Mühlhausen et al., 2013) and independent origins of dehiscence in Capparaceae and Cleomaceae (Hall et al., 2002). Moreover, the diverse fruit characters in the Brassicaceae play major ecological roles in fruit/seed dispersal (Sperber et al., 2017; Arshad et al., 2019; Bhattacharya et al., 2019a; Nichols et al., 2020). The recent evidence of plasticity in *Aethionema* in response to stress, with corresponding glucosinolate allocation to fruit tissues (Bhattacharya et al., 2019b), demands a holistic evo-devo analysis among other members of the Brassicales to understand the genetic and epigenetic basis of diversity in fruit characters.

The future of Brassicales reproductive biology research

The repeated evolution of dioecy, SI, and diverse fruit types across the Brassicales makes it a powerful model order for comparative developmental and genomic analyses. For instance, are the same sterility genes or pathways involved in dioecious flower development across the Brassicales? Do the same genes control fruit shape and dehiscence? These comparisons will be valuable as they may be potential targets for controlling sex and fruit characteristics in breeding programs for the many economically important traits found across the order. Moreover, the many origins of sex chromosomes will provide further insight into their early evolution from ancestral autosomes (Carey et al., 2021).

SPECIALIZED METABOLITE DIVERSITY

Specialized metabolite diversity across the Brassicaceae

Glucosinolates, or mustard oils, are a clade-defining characteristic of plants in the Brassicales. The presence of major ecological model systems within this group, including *Arabidopsis*, *Boechera* (Boechereae), *Brassica*, and *Streptanthus* (Thelypodieae), has allowed mechanistic ecology studies to identify the forces shaping specialized diversity in the Brassicaceae. Competition experiments between *A. thaliana* accessions from central and northern Europe in the presence of multiple herbivores showed structural variation in the glucosinolate *METHYLTHIOALKYLMALATE* (MAM) locus linked to the presence/absence of two different aphids across the continent (Kroymann et al., 2003; Züst et al., 2012). Recreating natural variation in isogenic lines with field trials in both *Arabidopsis* and *Boechera* showed specialized metabolism is under strong selective pressure that fluctuates locally, creating local bet-hedging (Schranz et al., 2009; Manzaneda et al., 2010; Kerwin et al., 2015, 2017). Manipulating glucosinolates within *Brassica* showed how intraspecific glucosinolate variation shaped inter and intraspecific competition and enabled stochastic variation in species occupancy across a landscape (Lankau and Strauss, 2007). Combining ecological parameters, biochemistry, and phylogenetics within *Streptanthus* has been used to understand how specialized metabolism is shaped within the family in relation to serpentine soils (Cacho et al., 2015, 2021). Interestingly, the above ecological model systems and other less studied Brassicales can co-occur within the same or similar environments. This raises the potential to develop studies investigating how specialized metabolism within one species may influence the specialized metabolism within another.

Specialized metabolite diversity across the Brassicales

Extending the ecology work via the combination of genomics and chemistry across the entire order Brassicales has begun to illustrate how specialized metabolite pathways evolve. Glucosinolates are estimated to have first occurred at the base of the Brassicales ~108 Ma and span the phylogeny from the leafy greens of the Brassicaceae to the seeds of the Caricaceae, where they co-occur with the hypothesized evolutionary progenitor cyanogenic glycosides (Olafsdottir et al., 2002). Glucosinolates then diversified further ~43 Ma with the evolution of the aliphatic glucosinolates (Beekwilder et al., 2008; Zhang et al., 2015; Czerniawski et al., 2021). Some glucosinolates, like 2-hydroxy-2methylbutyl glucosinolate (glucocleomin), are family-specific innovations (e.g., Cleomaceae), and others like 3-benzoyloxybutyl glucosinolate (glucomalcomiin) appear to be relatively species-specific (e.g., *A. thaliana*). In contrast, glucosinolates like methyl glucosinolate

(glucocapparin) are found in the Cleomaceae and the Capparaceae but not in the Brassicaceae, suggesting a loss or replacement in the Brassicaceae that has yet to be characterized genetically.

Although some of these innovations in glucosinolate amino acid substrate types have been mapped on the phylogeny (Edger et al., 2018a; Kumar et al., 2019; Petersen et al., 2019), the underlying genomic mechanisms influencing these transitions have yet to be phylogenetically characterized. What is clear is that the evolution of the glucosinolate pathway has involved whole-genome, local duplication, and gene transposition events, each at different levels. In the glucosinolate pathway, whole-genome duplications gave rise to variation in the CYP79 family that determines the specific amino acid used for the glucosinolate backbone (Edger et al., 2015, 2018a) and the origin of the MAM locus derived from primary metabolism (Abrahams et al., 2020). In contrast, local structural diversity, duplications, and inversions create enzymatic variation leading to novel enzymes that create the structural modifications to the glucosinolate backbone, determining the ultimate biological activity (Kroymann et al., 2003; Hansen et al., 2008; Chan et al., 2010).

The future of Brassicales specialized metabolite diversity research

Moving beyond glucosinolates to other specialized pathways, the identified diversity and innovations at different time scales, both within and between metabolic pathways, allow for unique comparisons using the Brassicales. For example, is there a difference in how older and younger genes evolve within a pathway and is this difference consistent across pathways? In addition to investigating how pathways are gained, the Brassicales provide the ability to study how pathways can be lost and independently recreated (Haribal et al., 2001; Rajniak et al., 2015). Additional questions related to specialized metabolism can also be addressed when sampling across the Brassicales. For example, does the evolution of cardiac glycosides within the *Erysimum* lineage (Erysimeae) influence the selective pressure on specialized metabolism in other species when they coexist or within the same species when the two pathways coexist (Züst et al., 2020)? Developing extensive functional, biochemical, genetic, and genomic resources within the Brassicales will allow testing these questions about how specialized metabolism is driven to novelty.

THE EVOLUTION OF WOODINESS

Woodiness across the Brassicaceae

The initiation and activation of a vascular cambium can lead to massive secondary growth in woody plants, in which the wood cylinder is the main component in stems and roots of shrubs, trees, and lianas. The herbaceous *A. thaliana* is an excellent model for elucidating the genetic controls of

wood formation. Woody mutants show that the genetic mechanism turning on the wood pathway in stems can be simple, e.g. by knocking out two flowering control genes (Melzer et al., 2008; Lens et al., 2012; Davin et al., 2016), or by overexpressing a single gene that is downstream of these two flowering control genes (Rahimi et al., 2022). Interestingly, about 10% of Brassicaceae species have developed into woody shrubs, treelets, and even lianas under natural conditions, and these ca. 400 woody species are the result of nearly 100 independent transitions from herbaceousness towards phylogenetically derived woodiness (e.g., Mohammadin et al., 2017; Zizka et al., 2022; Lens et al., unpublished dataset). This makes the Brassicaceae a perfect model to better understand why flowering plants became woody during evolutionary history.

Woodiness across the Brassicales

Woodiness represents the ancestral state in the Brassicales (**Figures 2 & 4**). Across the order, evolutionary transitions from ancestral woodiness towards herbaceousness, and reversals back to phylogenetically derived woodiness, have occurred repeatedly. Transitions towards herbaceousness evolved at least seven times, leading to among others the predominantly herbaceous sister families Brassicaceae (Al-Shehbaz, 1984; Franzke et al., 2011) and Cleomaceae (Byng, 2014; Patchell et al., 2014) that include by far the majority of perennial or annual herbaceous species within the order. Other families including herbs are Emblingiaceae (Hall et al., 2004; Byng, 2014), Gyrostemonaceae (Byng, 2014), Limnanthaceae (Meyers et al., 2010; Edger et al., 2018a), Resedaceae (Martín-Bravo et al., 2007), Tovariaceae (Hall et al., 2004), and Tropaeolaceae (Andersson and Andersson, 2000). Within the predominantly herbaceous Brassicales clades, we also identified ca. 100 evolutionary reversals from herbaceousness towards derived woodiness (e.g., Lens et al., unpublished dataset), accounting for about 15% of the total number of reversals within flowering plants. Nearly all of these independent reversals are in the Brassicaceae, but they also occurred in Cleomaceae (at least four transitions; Patchell et al., 2014), and Resedaceae (at least three; Martín-Bravo et al., 2007).

Although the tallest Capparaceae trees reach 40 m in height, other trees in the order are typically much smaller (e.g., Akaniaceae, other Capparaceae; Bayer and Appel, 2003; Mercado Gómez and Escalante, 2018). Across the entire order, multiple invasions into seasonally dry (Brassicaceae, Gyrostemonaceae, Resedaceae), (hyper)arid (Brassicaceae, Cleomaceae, Gyrostemonaceae, Koeberliniaceae, Moringaceae, Salvadoraceae, Setchellanthaceae, Tiganophytaceae), and temperate habitats (Brassicaceae, Cleomaceae, Limnanthaceae) gave rise to a general reduction in plant size, leading to small shrubs and herbs in many lineages (Al-Shehbaz, 1984; Martín-Bravo et al., 2007; Byng, 2014; Salariato et al., 2016; Swanepoel et al., 2020). It is noteworthy

that woody Gyrostemonaceae, which may reach 10 m, remain relatively short-lived (usually <10 years) in arid areas (Baker et al. 2005). The successful attempts to establish and diversify into (hyper)arid habitats is unusual across the largely tropical rosids, making the Brassicales in general and the species-rich Brassicaceae, in particular, stand out as ecologically distinct lineages (Cornwell et al., 2014).

The future of Brassicales woodiness research

Ongoing efforts to start building a species-dense Brassicales phylogeny (Nikolov et al., 2019; Hendriks et al., 2023), required to identify all the species pairs with contrasting life forms, makes the Brassicales a new, representative model clade to investigate the environmental and genetic drivers of the multiple woodiness shifts. The emerging phylogenetic framework will inform the first step towards reconstructing the evolution of niche dimensions and allow for solid statistical testing of hypotheses explaining why plants became woody (Lens et al., 2013; Dória et al., 2018; Zizka et al., 2022). Moreover, identification of the ca. 100 contrasting life form pairs in the Brassicales will serve as a great tool to help discover key regulatory genes controlling wood formation, and subsequently validate these results in *A. thaliana* (Melzer et al., 2008; Lens et al., 2012; Davin et al., 2016; Rahimi et al., 2022). Using newly developed approaches such as phylogenetic inter-species genome-wide association studies (Kiefer et al., 2019), we will be able to assess whether or not the same genes are involved in the independent transitions, thereby contributing to our general understanding of parallel and convergent evolution as a principal evolutionary phenomenon (Elmer and Meyer, 2011).

PHOTOSYNTHESIS

Photosynthesis across the Brassicaceae

Modifications to C_3 photosynthesis have occurred repeatedly in angiosperms and typically are characterized by spatial (C_4) or temporal (Crassulacean Acid Metabolism) sequestration of CO_2 fixation (Edwards, 2019). Within the Brassicaceae, so far only *Moricandia* (Brassicaceae) has been reported to include five C_3 - C_4 intermediate species as well as three C_3 species, while no species has been shown to have C_4 photosynthesis (Apel et al., 1998; Schlüter et al., 2017). Schlüter et al. (2017) suggest various constraints on the path to C_4 , including anatomical limitations to efficient metabolite exchange, limitation in nutrients other than carbon, and limited pressure to reduce photorespiration as these species inhabit cooler climates than those occupied by C_4 species. Other Brassicaceae lineages deviate from typical C_3 physiology (e.g., carbon isotope ratios not typical of C_3) including *Diplotaxis tenuifolia* (Brassicaceae; Apel et al., 1998; Ueno et al., 2003, 2006), *Brassica gravinae*

(Brassicaceae; Ueno, 2011), and *Hirschfeldia incana* (Brassicaceae; Garassino et al., 2022). Such species and close relatives need detailed analysis to understand how C₃ photosynthesis has been modified.

Photosynthesis across the Brassicales

In the Brassicales, at least four instances of partial- or full-C₄ photosynthesis have evolved. In Cleomaceae, C₄ NAD-ME photosynthesis has been characterized extensively in *Gynandropsis gynandra* (Marshall et al., 2007; Voznesenskaya et al., 2007; Koteyeva et al., 2011; Bräutigam et al., 2011; Brown et al., 2011; Kajala et al., 2012; Koteyeva et al., 2014; Williams et al., 2016; Huang et al., 2021). This research indicates that C₄ photosynthesis has evolved from components found in C₃ species. Identification of a bipartite transcription factor module driving gene expression in the *A. thaliana* bundle sheath provides insight into *trans*-factors important for C₄ photosynthesis (Dickinson et al., 2020). Significant natural variation of C₄ traits exist in *Gynandropsis* (Reeves et al., 2018); and the *G. gynandra* genome has gene families retained in duplicate following polyploidy that facilitated C₄ evolution (Hoang et al., 2023). The *Coalisina* clade (Cleomaceae; Roalson and Hall, 2017), includes C₃, intermediate, and C₄ species (Voznesenskaya et al., 2007). *Coalisina angustifolia* has NAD-ME C₄, whereas *C. paradoxa* has been characterized as intermediate / C₂ (Koteyeva et al., 2011). Only one taxon within the *C. angustifolia* complex has been characterized fully (*C. angustifolia* subsp. *angustifolia*; Voznesenskaya et al., 2007). Denser sampling of this clade would inform us about the C₃ to C₄ transition as it appears to include C₃, C₃-C₄ intermediate, and fully C₄ species, and would provide an interesting comparative lineage to *Moricandia* where there are C₃ and C₃-C₄ intermediates but no known C₄ species. *Areocleome* is the least studied of Cleomaceae C₄ lineages and includes at least one species restricted to arid Australia (Barrett et al., 2017). *Areocleome oxalidea* has NAD-ME-type C₄ physiology (Voznesenskaya et al., 2007; Koteyeva et al., 2011) but the underlying C₄ genetics remain unstudied. The sister genus *Arivela* may have C₃-C₄ intermediate characteristics as it is sympatric with *Areocleome* and more widespread in the monsoonal tropics. Other Cleomaceae lineages including *Cleomella sparsifolia*, *Tarenaya siliculifera*, *Sieruela allamanii*, and *S. gallaensis* appear to deviate from typical C₃ physiology (Voznesenskaya et al., 2007; Parma et al., 2021). These species differ significantly in their distributions from dry southwestern North America to tropical Brazil, and tropical to subtropical Africa, providing opportunities to consider C₃ pathway modifications across environments.

The future of Brassicales photosynthesis research

Understanding the modifications in photosynthetic pathways and carbon-concentrating mechanisms holds significant relevance and impact in several key aspects. Gaining insights into these fundamental physiological processes is crucial for advancing our comprehension of plant biology and evolution. This knowledge helps us unravel the intricacies of how plants have adapted to various environmental conditions, contributing to a deeper understanding of the natural world.

Photosynthetic pathway modifications occur in numerous lineages, and in some cases co-occur and shift between the two major modification types (Holtum et al., 2017; Edwards, 2019; Winter et al., 2019). Further, C_4 photosynthesis has multiple biochemical forms (Sage, 2004). As our understanding of these basic physiological processes has expanded, four foundational hypotheses have solidified: (1) there have been multiple independent origins of C_4 /CAM (Sage et al., 2011), (2) independent origins of carbon concentrating mechanisms can occur through the co-option of similar molecular components (Christin et al., 2007; Brown et al., 2011) and these components exist in the ancestral C_3 state (Brown et al., 2011; Williams et al., 2016; Reyna-Llorens et al., 2018), (3) precursor anatomical modifications that appear unrelated to photosynthesis act as facilitators of biochemical adaptations (Christin et al., 2013; Williams et al., 2013), and (4) a number of carbon concentrating mechanisms do not fully fit our concepts of C_4 /CAM (e.g., C_2 ; Lundgren, 2020). Our understanding of carbon-concentrating adaptations has become better attuned to these nuances as knowledge on independent origins has accumulated.

Resolving the current struggles in this field would open up exciting future directions for research. Access to genomes and a comprehensive understanding of photosynthetic pathway modifications would enable researchers to explore the genetic and molecular underpinnings of these processes in even greater detail. This, in turn, could lead to the discovery of novel molecular components and mechanisms that drive photosynthetic adaptations. The relevance of this research extends to agriculture and environmental conservation. By understanding the multiple independent origins of C_4 /CAM photosynthesis and the co-option of molecular components, we can potentially engineer crops to be more efficient in photosynthesis and better adapted to changing environmental conditions. This has implications for food security and sustainable agriculture, particularly in the face of climate change. Additionally, the recognition of carbon concentrating mechanisms that do not fit traditional C_4 /CAM concepts, such as C_2 , presents an intriguing avenue for further investigation. Unraveling the molecular basis of these mechanisms could challenge and expand our current understanding of photosynthesis and carbon fixation in plants.

EXTREMOPHYTISM AND STRESS TOLERANCE

Extremophytism across the Brassicaceae

Many Brassicaceae (and Brassicales) species thrive in extreme environments including desert, arctic, and saline ecosystems. To survive in such habitats, extremophytes possess genetic adaptations for tolerating severe abiotic stresses. Such adaptations are exemplified in two halophytic Brassicaceae, *Schrenkiella parvula* (Schrenkielleae) and *Eutrema salsugineum* (Eutremeae), quintessential models (Zhu, 2015) for studying tolerance to ionic and nutrient stresses (Kazachkova et al., 2018; Tran et al., 2021; Pantha et al., 2021; Wang et al., 2021). Curated genomes, multiple tissues, and environmental response “omics” datasets have been generated for both species as well as transformation protocols that facilitate independent genetic analyses (Oh et al., 2010; Dassanayake et al., 2011; Wu et al., 2012; Oh et al., 2014; Wang et al., 2019; Pantha et al., 2021; Tran et al., 2022; Wijesinghe et al., 2022a, 2022b; Sun et al., 2022).

Because multiple evolutionary paths can provide unique solutions to different environmental challenges, the Brassicaceae from various extreme environments are being studied including: (1) the desert species *Anastatica hierochuntica* (Anastaticaceae), which is tolerant to heat, low nutrient, and salt stress (Eshel et al., 2017, 2022); (2) the metal hyperaccumulator *Arabidopsis halleri* (Arabidopsidae; Briskine et al., 2017; Honjo and Kudoh, 2019); and (3) three Arctic species, *Cardamine bellidifolia* (Cardamineae), *Cochlearia groenlandica* (Cochlearieae), and *Draba nivalis* (Arabideae; Birkeland et al., 2020). Together, these models should provide a window into evolutionary adaptations that confer stress tolerance and facilitate an extremophyte lifestyle.

Extremophytism across the Brassicales

Beyond the Brassicaceae, only a few studies have examined extremophyte Brassicales. For instance, *Batis maritima* (Bataceae), a perennial, succulent halophytic shrub that inhabits mangrove swamps, salt marshes, and salt flats (Marcone, 2003; Debez et al., 2010) tolerates up to 1 M NaCl and has evolved different salt tolerance mechanisms compared to the two annual Brassicaceae halophyte models, *E. salsugineum* and *S. parvula*. For example, *B. maritima* accumulates high levels of shoot Na⁺ while *E. salsuginium* minimizes entry of Na⁺ (Kant et al., 2006; Debez et al., 2010).

Drought responses were investigated in the stress-tolerant, evergreen, African tree species, *Dobera glabra* (Salvadoraceae). When compared to co-occurring non-Brassicaceae species, *D. glabra* displayed the lowest water potential, illustrating its ability to thrive in drought-prone areas (Gebrekirstos et al., 2014).

Molecular studies of non-Brassicaceae Brassicales are extremely sparse. Mercati et al. (2019) reported a *de novo* transcriptome assembly from the drought-tolerant, xerophytic crop, *Capparis spinosa* (Capparaceae), and they generated the first set of SSR markers distinguishing subspecies of *C. spinosa*. A chromosome-level *C. spinosa* var. *herbacea* genome assembly showed expansion of gene families involved in photosynthesis and response to abscisic acid (Wang et al., 2021). Phylogenetic analysis of a Brassicales-specific gene encoding *PROTEIN PHOSPHATASE7-LIKE* (*PP7L*) that is involved in chloroplast development and abiotic stress tolerance suggested that *PP7L* evolved about 43-92 MYA, after the divergence of Caricaceae from the other Brassicales families, but before the separation of Cleomaceae from Brassicaceae (Xu et al., 2019).

The future of Brassicales extremophyte research

Core stress response pathways exist in all land plants. Yet, selective pressures imposed by extreme environments have driven novel evolutionary innovations to modify these core pathways or add alternatives. These evolutionary innovations are being revealed by comparative analyses of the premier model species *A. thaliana* (“stress-sensitive”) with the Brassicaceae extremophyte models. Yet these Brassicaceae species are all annuals, and alternative stress tolerance strategies could be found in perennial extremophytes that survive harsh stresses over multiple years. Thus, the gap in the adaptive trait space left by the Brassicaceae models can be narrowed by studies of perennial Brassicales extremophytes, which possess the added advantage of shared ancestry with *A. thaliana*, many of whose core stress response pathways have been identified.

The lack of genomic resources for the Brassicales extremophytes has limited research into their adaptations to extreme environments. Emerging technologies in large-scale and single molecule/cell sequencing, offer unprecedented molecular tools that require little prior genetic information to produce primary genomic resources. Single-molecule/hyperspectral imaging, and phenomics can then facilitate linkage of novel genomic variation to unique extremophyte traits. Thus, the broader phylogenetic footprint capturing the life history strategies of the Brassicales extremophytes could facilitate new discoveries for sustainable crop production, land reclamation, biodiversity conservation in this era of climate change, and global threats to the environment and food insecurity.

SUPPORTING MODEL ORDERS WITH GLOBAL COMMUNITIES

Issues identified not only in the determination of relationships among or within the Brassicales, but also in analysis of all of the phenotypes discussed here, create many opportunities to

leverage the trait diversity across the order. To fully take advantage of these opportunities for both those currently investigating this diverse plant order and those who wish to, there must be a cohesive development of a global network for plant material, data generated, and researchers. Turner-Hissong et al. (2020) highlight ways to curate community efforts in organizing not only germplasm and data, but also outreach and broader impacts. Although these suggestions are primarily directed at crop systems, many ideas are useful here too. For example, there is a need to develop reliable stock sources or live genetic material which researchers can both order and submit samples, to increase reproducibility across studies. Some examples of this exist, such as the *Arabidopsis* Biological Resource Center (ABRC; <https://abrc.osu.edu/>), the Universidad Politécnica de Madrid Plant Germplasm Bank (UPM-PGB; which includes the César Gómez Campo Collection), the USDA Germplasm Resources Information Network (GRIN), and the Royal Botanic Gardens (Kew) Millennium Seed Bank and its DNA and tissue Bank collections. However, one must first know about these resources to utilize them. However, even with these resources available, cultivating them demands substantial time, effort, funding, and space. Numerous species lack established protocols, particularly those accessible to the public, on optimizing plant growth for generating sufficient seeds for trait-focused studies. It is essential to meticulously document the origins of genetic resources and acknowledge the knowledge's source by returning it to the country of origin

Following germplasm curation, data curation of all types is crucial for continual progress. As we generate these large, multi-omic datasets, there is an increasing need for scalable, systematic community efforts to organize these resources, all of which will require standards for data collection and curation and ideally utilize established open-access repositories. Fortunately, there are already databases in existence, such as BrassiBase (Koch et al. 2018), which consolidate information encompassing taxonomy, systematics, evolution, available germplasm resources, and specimen collections and could be expanded to include all of the Brassicales in the future. Furthermore, networks like the Multinational *Brassica* Genome Project (MBGP; brassica.info) could play a pivotal role if expanded to Brassicales. Currently, MBGP organizes annual meetings at international conferences and is dedicated to establishing a *Brassica* Information System, which includes the formulation of standards for describing data entities and experimental resources. Additionally, platforms like Phytozome, which serves as a central hub for accessing, visualizing, and analyzing JGI-sequenced plant genomes, along with the recently published and updated JGI Gene Atlas (Sreedasyam et al. 2023), can greatly contribute to the development of a cohesive community focused on the Brassicales model clade. These types of databases provide a place where not only data can be

accessed, but researchers can also keep others updated on current projects, results, and future plans. Centralizing access to these plant materials and data generated would significantly decrease the barriers for scientists, especially early career scientists and those with less access to modern resources, and ultimately encourage equity and recruit new and diverse researchers to study the model order Brassicales. Nonetheless, persistent challenges exist, including a decline in taxonomic expertise, restricted access to materials from several small endemic families, and a noticeable bias toward collaboration with researchers primarily from the global North.

Greater accessibility to plant genomics at lower costs has expanded global collaboration opportunities. However, it is important to acknowledge that the discovery of genes and pathways in plant species can have significant economic implications, so these initiatives must also address the growing complexity surrounding genetic resources and intellectual property rights. There are growing calls for decolonization of science in the Global North that extracts materials and knowledge from the Global South and Indigenous groups without reciprocity (Radcliffe, 2017; Baker et al., 2019; Armstrong and McAlvay, 2019; Carroll et al., 2020; The First Nations Information Governance Centre, 2022). Within the order Brassicales, *Pentadiplandra brazzeana* was at the center of a biopiracy controversy when scientists from the University of Wisconsin patented a protein, brazzein, extracted from the berries of Gabonese plants as a sweetening agent with no benefit-sharing agreement with Gabon or Gabonese people (Dwivedy et al., 2019; Blakeney, 2019). A growing number of countries have implemented the Nagoya Protocol on Access and Benefit-sharing (Buck and Hamilton, 2011) and other measures to govern the exchange and use of biological materials. Moving forward, we urge all researchers working on Brassicales collection and sequencing efforts to consider genuine collaboration with Indigenous groups and scientists in the Global South to work to align project goals with local interests (Baker et al., 2019). We understand the need to build a foundation of respect, reciprocity, and accountability within our growing collaborative efforts in keeping with the principles of Indigenous data sovereignty and equitable benefit sharing. The authors here commit to these core principles throughout our contacts and research.

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Table 1. The distribution of families within the Brassicales, along with the noted genera and species numbers. Genera and species numbers from the Angiosperm Phylogeny Website (Stevens 2001 onwards). The recently proposed family Borthwickiaceae (found in China and Myanmar) is included within Resedaceae.

Family	Genera/Species	Geographical Distribution
Akaniaceae	2/2	<i>Akania</i> – Australia <i>Bretschneidera</i> – Asia
Bataceae	1/2	Tropical America, Austral-Asia
Brassicaceae	349/4,140	Cosmopolitan (mostly temperate)
Capparaceae	16/480	Cosmopolitan (mostly tropical)
Caricaceae	6/34	Tropical Africa and America (mostly New World)
Cleomaceae	26+/270	Cosmopolitan
Emblingiaceae	1/1	Southwest Australia
Gyrostemonaceae	5/18+	Australia
Koeberliniaceae	1/2	North, Central, and South America
Limnanthaceae	2/8	North America
Moringaceae	1/12	Africa, Madagascar, and Asia
Pentadiplandraceae	1/1	Western Africa
Resedaceae	8/96	North Africa, Eurasia, North America
Salvadoraceae	3/11	Africa to Southeast Asia
Setchellanthaceae	1/1	Mexico, North America
Tiganophytaceae	1/1	Namibia, Africa
Tovariaceae	1/2	Tropical America
Tropaeolaceae	1/105	North and South America

FIGURE LEGENDS

Figure 1. Phylogeny indicating the lineage relationships of the Brassicaceae using nuclear and plastid data. Placed tribes are noted with corresponding color. Summary, classification, and relationships based on Hendricks et al. 2023.

Figure 2. Phylogeny of 17 families in the Brassicales and character trait matrix. The presence of a given trait is marked by a filled dark green square. Traits that are unknown or absent in light green. Possible locations of At- β are marked in blue stars with family-specific whole-genome duplications in orange. Traits displayed are **A)** C4, **B)** Extremophythism, **C)** Glucosinolates, **D)** Ancestral woodiness, and **E)** Dioecy. Additional proposed families, which are not included here, still need to be analyzed using multiple single-copy nuclear genes (e.g. Tiganophytaceae and Borthwickiaceae). Areas of the phylogeny with no or low support are indicated by thin branch lines. Topology and support based on Edger et al. (2018a).

Figure 3. Whole genome synteny of chromosome-scale Brassicaceae genomes. The synteny plot was generated using GENESPACE v0.9.1 (Lovell et al., 2022) with “diamondMode” and “orthofinderMode” set to fast, using *Arabidopsis thaliana* (Arabidopsidae; Lamesch et al., 2012), *A. arenosa* (Arabidopsidae; Barragan et al., 2021), *Brassica rapa* (Brassicaceae; v1.3; downloaded from Phytozome), *Camelina sativa* (Camelineae; Kagale et al., 2014), *Cardamine hirsuta* (Cardamineae; Gan et al., 2016), and *Arabis alpina* (Arabideae; Willing et al., 2015) genome annotations. The four diploid genomes (*A. arenosa*, *A. thaliana*, *A. alpina*, and *C. hirsuta*) show 1:3 syntenic relationships with the younger hexaploid *C. sativa* genome ($n = 20$) and evolutionarily older hexaploid genome of *B. rapa* ($n = 10$).

Figure 4. Light microscope cross sections through Brassicales stems showing the difference between herbaceous (A) and woody stems (B-D). Double-pointed arrows indicate the wood cylinder. All images are at the same magnification (scale bar = 500 μ m). **A)** Basal inflorescence stem part of *Arabidopsis thaliana* (Brassicaceae). **B)** *Reseda* sp. (Resedaceae). **C)** *Capparis spinosa* (Capparaceae). **D)** *Sinapidendron angustifolium* (Brassicaceae).

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Phylogeny and Tribal Assignments of the Brassicaceae

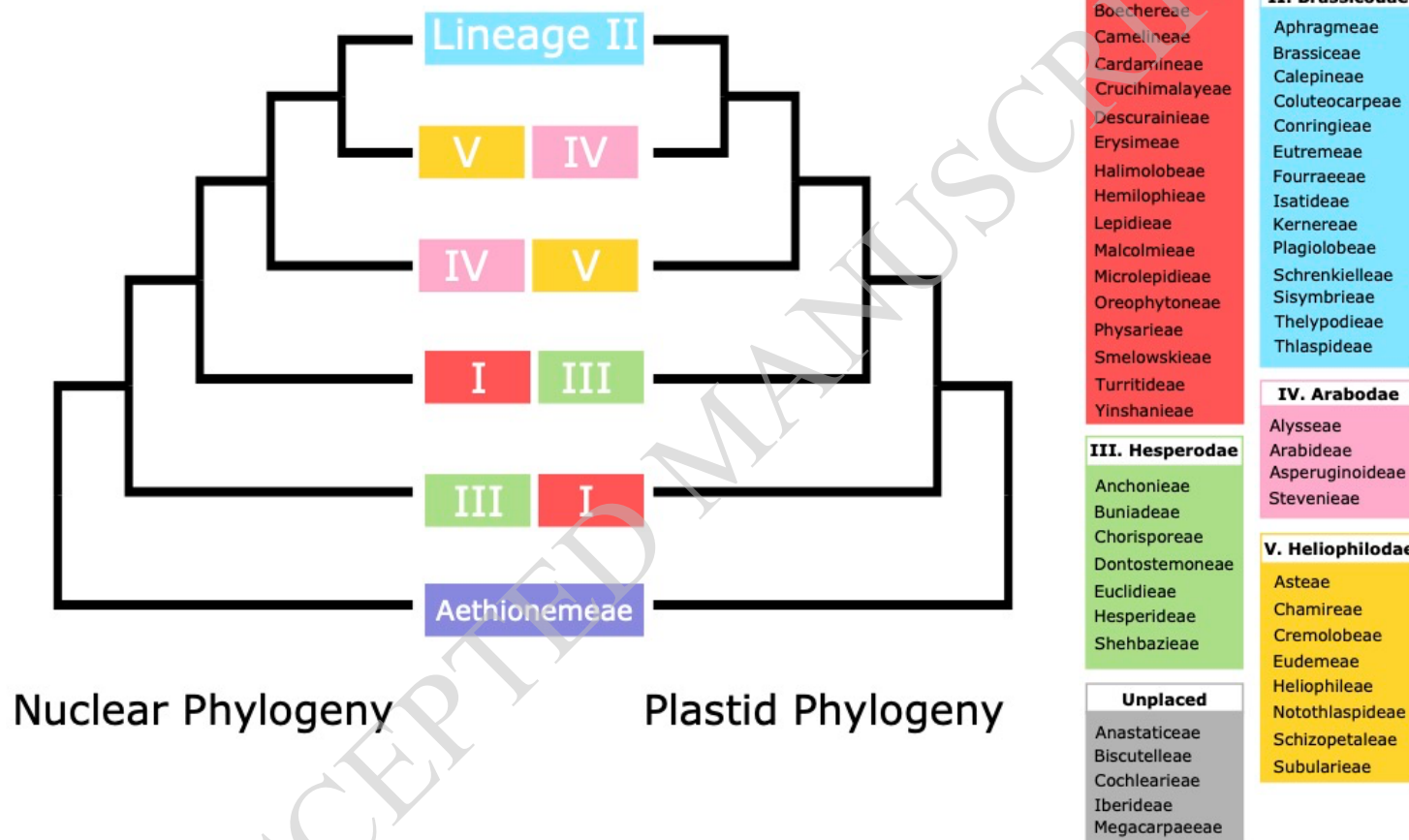


Figure 1. Phylogeny indicating the lineage relationships of the Brassicaceae using nuclear and plastid data. Placed tribes are noted with corresponding color. Summary, classification, and relationships based on Hendricks et al. 2023.

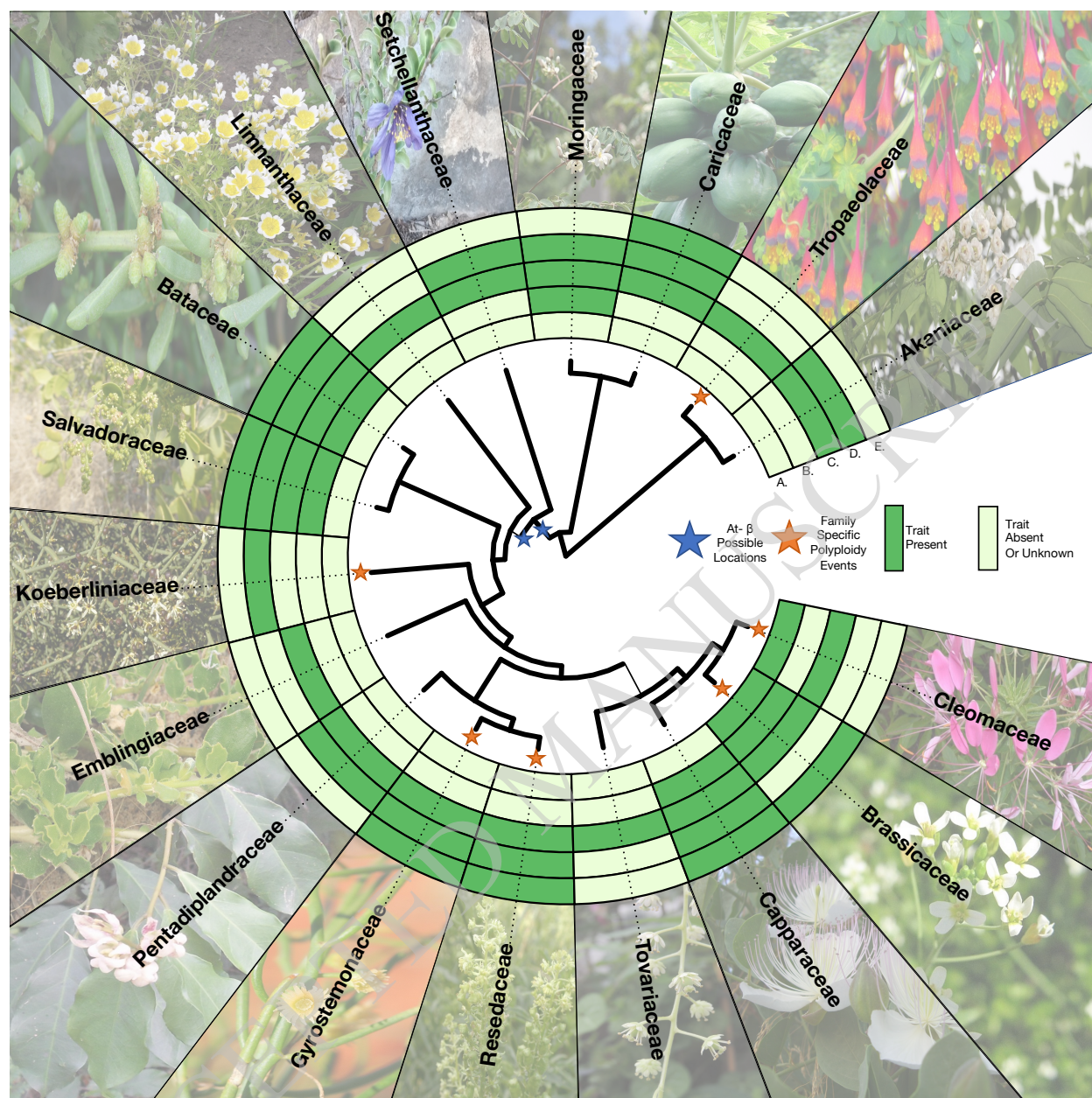


Figure 2. Phylogeny of 17 families in the Brassicales and character trait matrix. The presence of a given trait is marked by a filled dark green square. Traits that are unknown or absent in light green. Possible locations of At- β are marked in blue stars with family-specific whole-genome duplications in orange. Traits displayed are **A)** C₄, **B)** Extremophythism, **C)** Glucosinolates, **D)** Ancestral woodiness, and **E)** Dioecy. Additional proposed families, which are not included here, still need to be analyzed using multiple single-copy nuclear genes (e.g. Tiganophytaceae and Borthwickiaceae). Areas of the phylogeny with no or low support are indicated by thin branch lines. Topology and support based on Edger et al. (2018a).

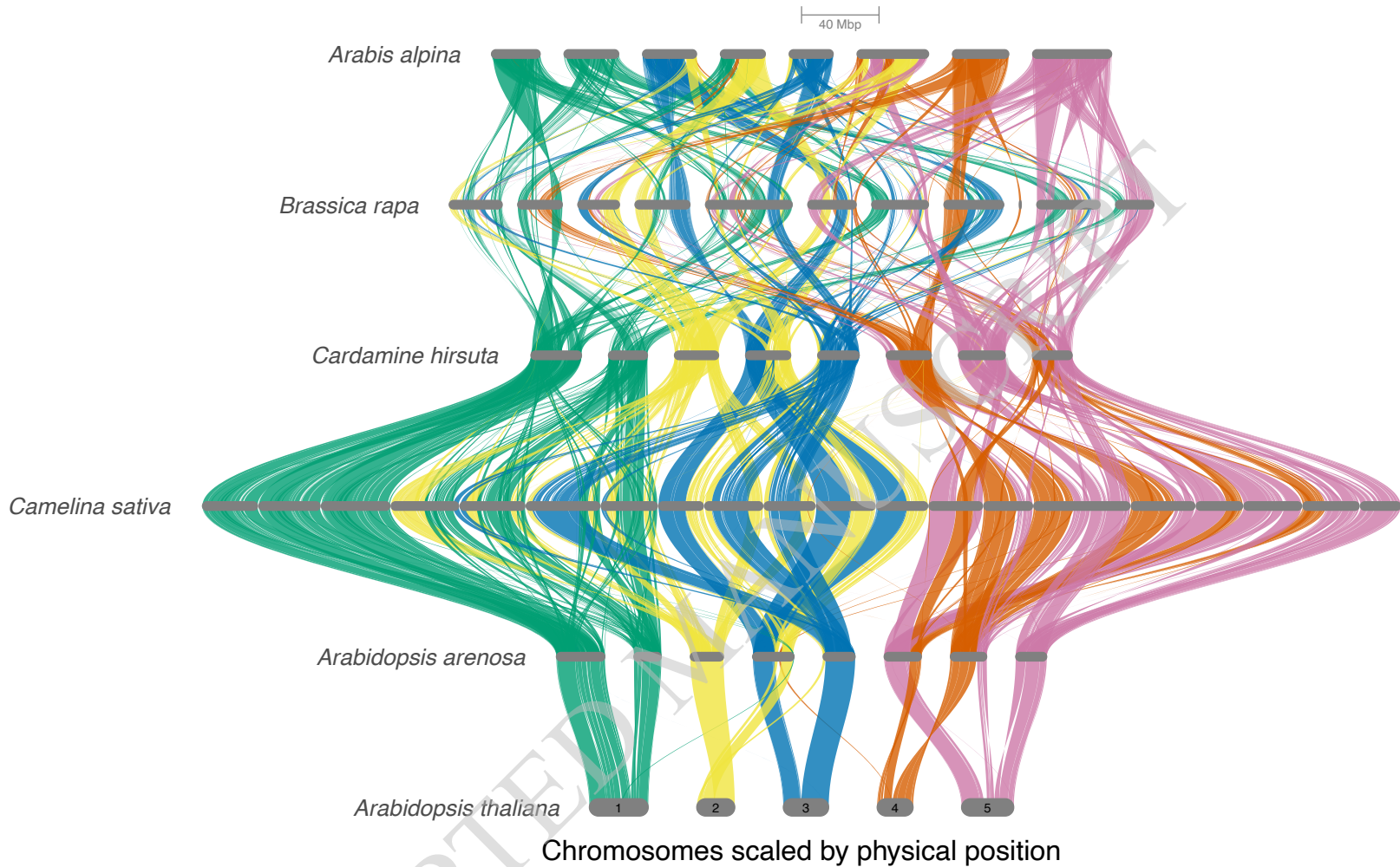


Figure 3 Whole genome synteny of chromosome-scale Brassicaceae genomes. The synteny plot was generated using GENESPACE v0.9.1 (Lovell et al., 2022) with “diamondMode” and “orthofinderMode” set to fast, using *Arabidopsis thaliana* (Arabidopsidae; Lamesch et al., 2012), *A. arenosa* (Arabidopsidae; Barragan et al., 2021), *Brassica rapa* (Brassicaceae; v1.3; downloaded from Phytozome), *Camelina sativa* (Camelineae; Kagale et al., 2014), *Cardamine hirsuta* (Cardamineae; Gan et al., 2016), and *Arabis alpina* (Arabideae; Willing et al., 2015) genome annotations. The four diploid genomes (*A. arenosa*, *A. thaliana*, *A. alpina*, and *C. hirsuta*) show 1:3 syntenic relationships with the younger hexaploid *C. sativa* genome ($n = 20$) and evolutionarily older hexaploid genome of *B. rapa* ($n = 10$).

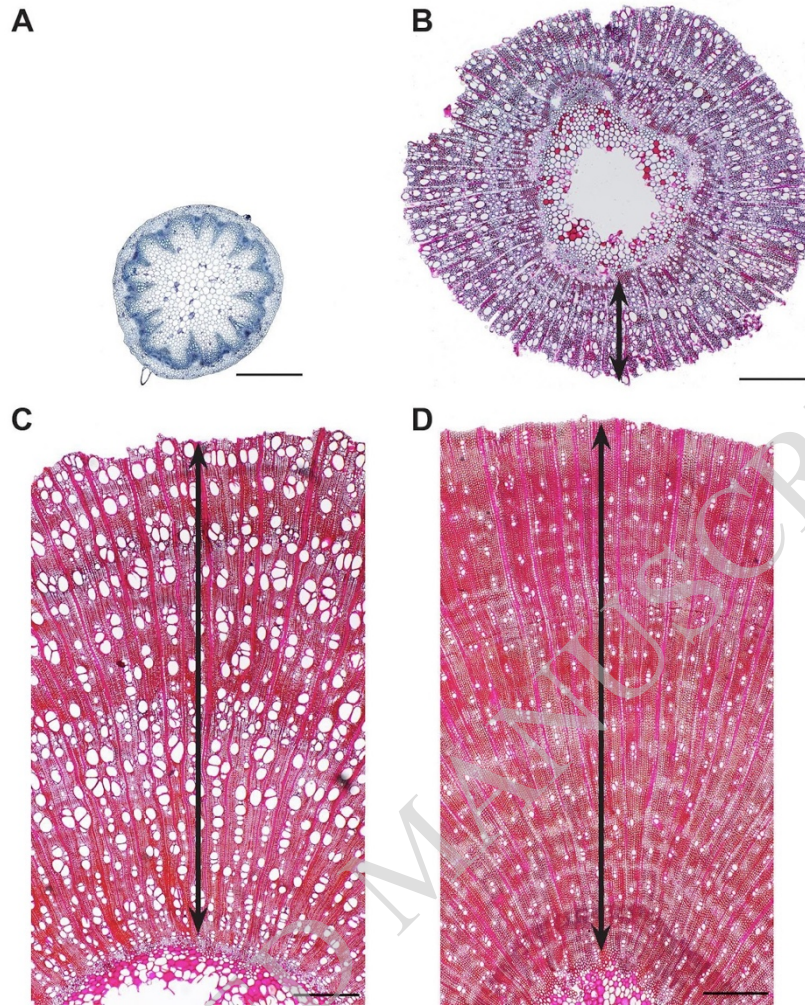


Figure 4. Light microscope cross sections through Brassicales stems showing the difference between herbaceous (A) and woody stems (B-D). Double-pointed arrows indicate the wood cylinder. All images are at the same magnification (scale bar = 500 μ m). **A)** Basal inflorescence stem part of *Arabidopsis thaliana* (Brassicaceae). **B)** *Reseda* sp. (Resedaceae). **C)** *Capparis spinosa* (Capparaceae). **D)** *Sinapidendron angustifolium* (Brassicaceae).