Review

Cabbage family affairs: the evolutionary history of Brassicaceae

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Life without the mustard family (Brassicaceae) would be a world without many crop species and the model organism *Arabidopsis* (*Arabidopsis thaliana*) that has revolutionized our knowledge in almost every field of modern plant biology. Despite this importance, research breakthroughs in understanding family-wide evolutionary patterns and processes within this flowering plant family were not achieved until the past few years. In this review, we examine recent outcomes from diverse botanical disciplines (taxonomy, systematics, genomics, paleobotany and other fields) to synthesize for the first time a holistic view on the evolutionary history of the mustard family.

Taxonomy

The mustard family (Brassicaceae or Cruciferae) belongs to the order Brassicales and is readily distinguished from other flowering plant families by a cruciform (cross-shaped) corolla, six stamens (the outer two shorter than the inner four), a capsule often with a septum and a pungent watery sap. However, the taxonomy (see Glossary) of the Brassicaceae (338 genera and 3709 species [1]) has long been controversial because the generic boundaries are often poorly delimited and almost all tribes recognized before 2006 were artificially circumscribed. These difficulties resulted in the lack of agreement on number and boundaries of tribes and genera, and gave rise to several systems of classification which have been proposed during the past two centuries. For a detailed history of these tribes see [2], a comprehensive study that examines all major classifications and assigns 308 genera (92%) of the family to 44 tribes.

Systematics

A substantial abundance of Brassicaceae family-wide molecular phylogenetic data has been published within the past 5 years [2–11]. These findings, as well as others on tribes and genera, solved many existing conflicts between molecular and morphological data and established monophyletic taxa that are well-supported molecularly and

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Glossary

Adh: alcohol dehydrogenase gene (nuclear genome).
Calibration: converting genetic distances to absolute times by means of fossils or nucleotide substitution rates.
Chs: chalcone synthase gene (nuclear genome).
Clade: group of organisms (species, genera, etc.) derived from a common ancestor.
Core Brassicaceae: all recent lineages except tribe Aethionemeae.
Crown group age: age of the clade that includes all recent taxa of a group.
Evo–devo (evolutionary developmental biology): compares underlying developmental processes of characters in different organisms to investigate the links between evolution and development.
Gamosepaly: fusion of sepals.
Geocarpy: burying fruits close to the mother plant.
Homoplasy: non-homologous similarities due to convergence or parallel evolution. In contrast, homologous similarity is inherited through common ancestry.
ITS: internal transcribed spacers of nuclear ribosomal DNA.
Key innovation: a newly evolved trait giving organisms access to new ecological resources and cause rapid and/or adaptive radiation.
K–T extinction: a geological signature caused by one or more catastrophic events such as an asteroid impact dated to the transition between the Cretaceous (K) and the Tertiary period (T), ~65 million years ago, associated with a mass extinction of animal and plant species.
***Mat*K**: maturase K gene (chloroplast genome).
Molecular dating: estimating divergence dates of two or more lineages by comparing their DNA or protein sequence data.
Molecular systematics: use of the structure of molecules to gain information on evolutionary relationships.
Monophyletic group: consists of a last common ancestor and all of its descendants.
***Nad4* intron 1**: first intron of mitochondrial gene for NADH subunit 4.
***Ndh*F**: F subunit of NADH dehydrogenase gene (chloroplast genome).
Paleo-, meso- and neopolyploidy: classification of WGD events according to the time of occurrence within a given clade or taxon and their detectability in the extant genomes.
Paleopolyploidy: refers to ancient WGD events detected in bona fide diploid organisms through DNA or protein sequence homology. More recent **mesopolyploid** duplications are detectable by comparative genomic and phylogenetic analyses identifying the parental genomes in organisms with diploid-like genomes and disomic inheritance. **Neopolyploids** are very recent polyploids with the increased genome size, chromosome number and usually extant parental cytotypes or species.
***Phy*A**: phytochrome A gene (nuclear genome).
Radiation: increase in taxonomic diversity or morphological disparity due to adaptations or the opening of new ecological niches.
***Rbc*L**: large subunit of ribulose–bisphosphate carboxylase gene (chloroplast genome).
Relaxed molecular clock approach: analytical methods for molecular dating that relax the assumption of nucleotide substitution rate constancy among lineages.
Systematics: study of evolutionary relationships between groups of organisms (species, genera, etc.).
Taxon (plural taxa): group(s) of organisms with a taxonomic rank such as order, family, genus, species and variety.

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Taxonomy: description, identification, naming and classification of groups of organisms at various ranks (species, genera, families, etc.).
Tribe: taxonomic category placed between a family or subfamily and a genus.
***Trn*LF**: intergenic spacer between genes encoding for transfer RNA of Leucine and Phenylalanine (chloroplast genome).
Whole-genome duplication (WGD): an event creating an organism with extra copies of the entire genome (also called polyploidy). WGD events are of different age and can be caused by hybridization combining genomes of different species (**allopolyploidy**) or by the multiplication of the same genome (**autopolyploidy**).

well-defined morphologically. Indeed, the systematics of Brassicaceae has been revolutionized based on the crucial reevaluation of morphology with regards to molecular findings. The number of monophyletic tribes increased from the original 25 [12] to 44 [2,13–15]. Similar adjustments were also made on genera and the reevaluation of morphological characters enforcing monophyly lead to the union or segregation of many genera [16–20].

Major lineages

Two studies [21,22] pioneered the molecular work on the Brassicaceae and the family has been split into two major groups, one of which is *Aethionema* (now approx. 35 spp.), the other represents the core of the family (confirmed later in [23,24]). Based on chloroplast *ndh*F sequence data three major lineages have been proposed in the core Brassicaceae [3]. Subsequent studies using ITS DNA [5], *nad4* intron 1 [8] and combined molecular data sets (*ndh*F, *phy*A [4]; *adh*, *chs*, ITS, *mat*K, *trn*LF [6]; *adh*, *chs* ITS, *mat*K, *nad4* intron 1, *ndh*F, *rbc*L, *trn*LF [11]) also supported these monophyletic, well-supported lineages. As shown in Figure 1, lineage I includes 13 tribes, lineage II four tribes and lineage III seven tribes. Although these lineages total only 24 of the currently recognized 44 tribes *sensu* Warwick *et al.* [2], they include the vast majority of taxa of core Brassicaceae. Both lineages I and III include genera with predominantly branched trichomes. Lineage III alone has multicellular and multiseriate glandular papillae in six of its seven tribes; the Euclidieae lacks such glands and Hesperideae has uniseriate glands. Lineage II consists of tribes with primarily simple trichomes, although branched trichomes evolved independently in terminal taxa in tribes Thelypodieae and Sisymbrieae. However, detailed molecular studies are still needed to resolve, for example, generic limits within Anastaticeae, Anchonieae, Brassiceae, Euclidieae and Thelypodieae, and to fully understand the multiple origins of branched trichomes and multicellular glandular papillae.

Morphological homoplasy

Almost every character in Brassicaceae, which has been used for classical taxonomy, exhibits substantial homoplasy especially those of fruits. A most notable example is the formation of angustiseptate fruits, a feature that evolved independently in 25 of the 44 currently recognized tribes. Likewise, fruit indehiscence also appears to have evolved independently in many Brassicaceae lineages. The tribe Euclidieae *sensu* Schulz [25] comprises 14 genera, all with one- or few-seeded indehiscent fruits; these have now been placed in seven tribes [2]. Undoubtedly, evo–devo studies on various morphological characters (e.g. fruit dehiscence–indehiscence in *Lepidium* [26]) help us to understand the genetic basis which lead to the observed morphological homoplasy [27,28]. Although the Brassicaceae are primarily herbaceous, shrubs evolved independently in at least 12 tribes of all major lineages. Furthermore, lianas 1–9 m tall are known in the South American *Polypsecadium* (three spp.; tribe Thelypodieae) and *Cremolobus* (three spp.; Cremolobeae), South African *Heliophila scandens* (Heliophileae) and Australian *Lepidium scandens* (Lepidieae). Divided petals evolved independently in the Chilean *Schizopetalon* (Schizopetaleae), North American *Ornithocarpa* (Cardamineae) and *Dryopetalon* (Thelypodieae), and Eurasian *Berteroa* (Alysseae) and *Draba verna* (Arabideae). Geocarpy evolved in five genera (*Geococcus*, *Cardamine*, *Morisia*, *Lignariella*, *Pegaeophyton*) of four tribes on four continents and gamosepaly is known in ten genera of five tribes. However, there are also some uniquely evolved characters in the Brassicaceae, including dioecism in *Lepidium sisymbrioides* (New Zealand), glochidiate spines in *Asperoginoides* (Southwest Asia), straight embryo in *Leavenworthia* (Southeast USA), wind pollination in *Pringlea* (South Indian Ocean islands) and involute cotyledons that persist to become the major photosynthetic organ in *Chamira circaeoides* (South Africa).

Morphological key innovations and role in adaptation and radiation

The early rapid radiation of Brassicaceae into newly available ecological niches potentially succeeded due to novel morphological and ecological traits associated with shifts in the mating system [29–32], flowering time, flower symmetry and pollination biology [33,34], diaspore dispersal and germination [33,35], and shifts between annual and perennial growth [36]. Owing to the lack of adequate experimental and observational studies, our knowledge of the adaptive significance of these traits is still tentative at best. However, such studies can be used in a predictive framework upon which future experimental studies can be based. Indeed, a few studies using population and landscape genetics approaches have already revealed a significant signal indicating local adaptation, but have as yet provided no direct link to key innovations and radiation [37–40].

Whole-genome duplications in the evolution of Brassicaceae

Analysis of the *Arabidopsis thaliana* genome provided evidence that this species, and probably all the core Brassicaceae taxa, experienced three ancient whole-genome duplication (WGD) events leading to paleopolyploidy, named γ, β and α (Box 1; Figures 1 and 2). The oldest *At*-γ duplication was linked to the diversification of eudicots and perhaps all angiosperms [41,42], whereas the *At*-β event postdates the Caricaceae–Brassicaceae divergence ~70 million years ago (mya) [43,44]. The occurrence of *Adc* [23], the *Shp* (K. Mummenhoff *et al.*, unpublished) and *Dog1* genes (G. Leubner *et al.*, unpublished) duplications in the core Brassicaceae, but not in *Aethionema* (Aethionemeae), argues that the *At*-α is specific for core Brassicaceae. The *At*-α event occurred ~40 mya [45], although younger or older estimates have also been proposed (Figures 1 and 2).

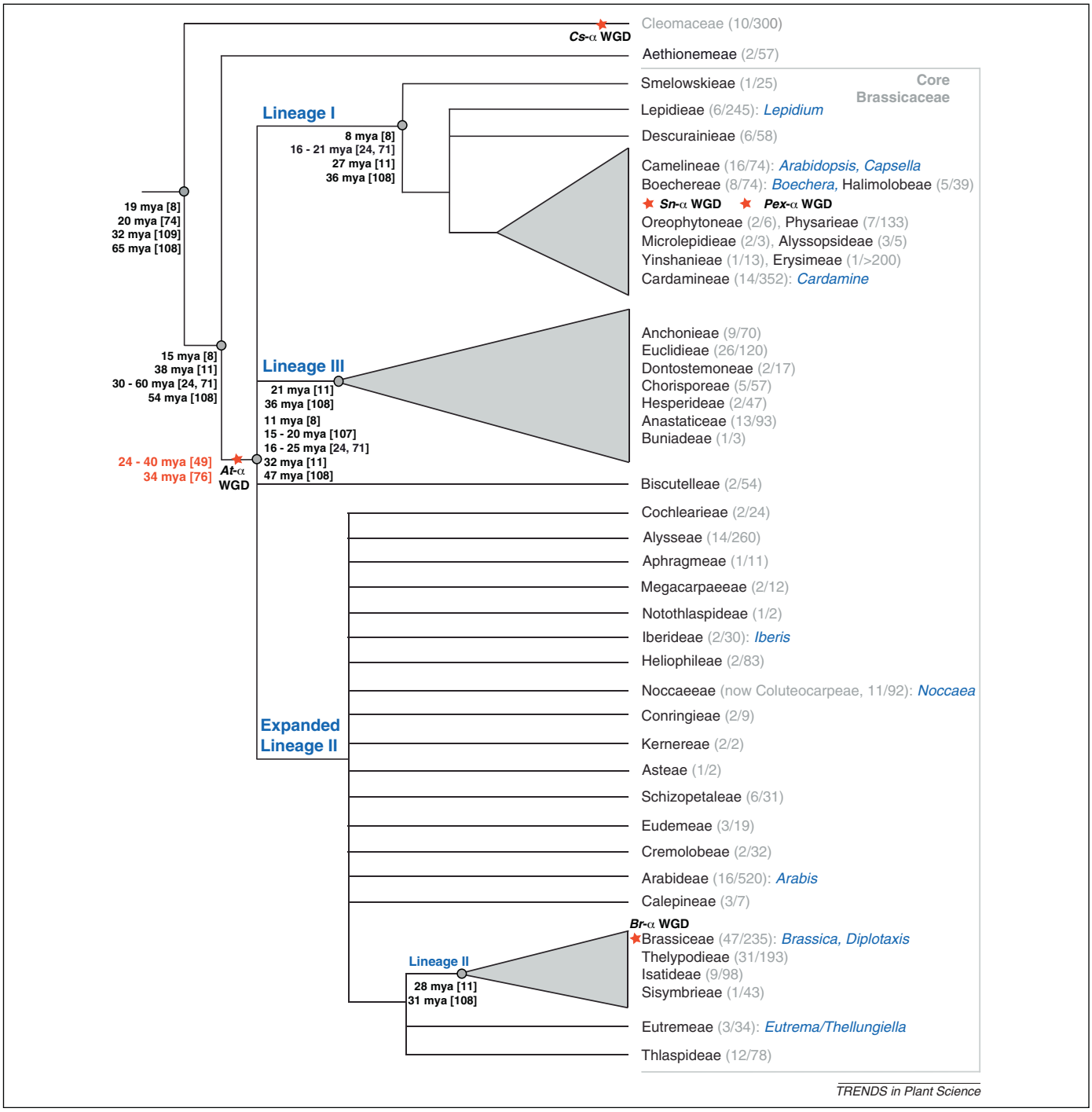

Figure 1. Brassicaceae phylogeny. Synoptic diagram of phylogenetic relationships of Cleomaceae and the various tribes of the Brassicaceae following the most recent phylogenies [2,11]. The number of genera and species of tribes are given in parentheses (genera/species) and important model organisms are specified (see Box 3). In total, these numbers for molecularly surveyed groups add up to 44 tribes, 307 genera and ~3600 species. An additional 38 genera with 71 species have not yet been assigned to tribes. Lineages I–III were originally described by Beilstein *et al.* [3,4], whereas expanded lineage II is introduced herein for the first time. Selected divergence time estimates and discussed whole-genome duplications (WGDs) are indicated (see text and Box 1). The *Ov*-α WGD in *Orychophragmus violaceus* (see text) is not shown as this genus is yet to be assigned appropriately to tribe. WGDs were also detected in *Pachycladon exilis* (*Pex*-α WGD) from New Zealand and Australian *Stenopelatum nutans* (*Sn*-α WGD). Both genera have not yet been assigned to tribes. However, there is evidence for a close relationship to Camelinae–Boecherae–Halimolobeae of lineage I [12,54].

As the age of *At*-α WGD coincides approximately with the radiation of the core Brassicaceae (Figure 1 and below), it is intriguing to link both events [8,41]. The incidence of the *At*-α duplication might explain the drastically increased species number in the core Brassicaceae (approx. 3700) compared to the approximately 35 species of the sister Aethionemeae [8,46]. We argue that the most recent paleopolyploid WGD has played a pivotal role in the cladogenesis and ecological diversification of the core Brassicaceae (Box 1, Box 2 and below). Furthermore, it has been proposed that the eight chromosomes of the Ancestral Crucifer Karyotype [47,48], an ancestral genome shared at least by lineages I and II, originated through the *At*-α duplication of a protokaryotype with four chromosomes only (n=4) [49].

Box 1. The impact of whole-genome duplications (WGDs)

WGD events (polyploidy) played a crucial role in the genetic diversification and species radiation of several angiosperm lineages [41,42,45,90]. Polyploidy is generally thought to provide raw material for gene neo- and subfunctionalization, extend resilience to deleterious mutations, increase net speciation rate and species richness [42], as well as provide the adaptive advantage for colonizing harsh and unstable environments (see [91] for references). The increased number of species in polyploid lineages can be attributed to the higher adaptive potential of polyploids and to the ratchet-like nature of polyploidy as the repeated hybridization and/or polyploidization events lead by default to the origin of new polyploid taxa and to increased species richness [91,92]. Polyploidization was followed by diploidization including genetic, epigenetic and transcriptional changes towards a diploid-like genome status. As articulated by the Gene Balance Hypothesis [46,93], dosage-sensitive genes (e.g. transcription factors) are over-retained, whereas other gene duplicates are preferentially lost following WGD events. The gene over-retention, neofunctionalization and loss of genes were most probably important stimuli of increased morphological complexity and species diversification following WGDs [46]. For example, reciprocal losses of gene paralogs in two closely related species might also establish reproductive isolation and foster speciation [94]. Some authors [45,89,91] suggested that polyploid genomes might have been particularly successful in periods of ecological upheavals when new ecological niches were occupied by vigorous polyploids and less competitive diploids outcompeted.

The sequencing of the *Arabidopsis thaliana* genome [95] provided the first evidence of multiple paleopolyploid WGD events in Brassicales and Brassicaceae. Several independent analyses confirmed the existence of three major WGD events in the evolution of *Arabidopsis* and the core Brassicaceae [96]. The oldest WGD was called γ (gamma) or 1R, followed by β (beta) or 2R and the most recent one is called α (alpha) or 3R [41,96]. To distinguish between lineage-specific paleopolyploid WGD events, Barker *et al.* [97] refined the existing terminology to specify the species in which a WGD event has been detected. Hence, the three duplications of the core Brassicaceae are referred to as *At*-γ, *At*-β and *At*-α (*At* denotes *A. thaliana*), whereas *Cs*-α refers to a WGD identified in *Cleome spinosa* (Figures 1 and 2).

Interestingly, the age of major WGD events identified in several angiosperm lineages (Fabales, Poaceae, Solanaceae) generally coincides with the mass K–T extinction (~65 mya) caused by catastrophic events, such as an asteroid impact and/or volcanic activity [45]. Polyploids in several angiosperm lineages could have a better adaptive potential to survive and diversify in the changed environment after the K–T extinction. The *At*-β duplication event might have stimulated the radiation of core Brassicales [97], perhaps around the time of the K–T catastrophe, and the *At*-α polyploidy promoted the radiation of the core Brassicaceae.

It is increasingly clear that the paleopolyploid *At*-α duplication was followed by later lineage-specific mesopolyploid WGD events. In Brassicaceae, at least four independent lineage-specific WGD events have been revealed by comparative genetic and/or cytogenetic analysis, and from molecular phylogenies (Figures 1 and 2). The whole-genome triplication (*Br*-α) in *Brassica* was suggested by Lagercrantz and Lydiate [50], later corroborated by several independent studies [51], and shown to have occurred before the radiation of the whole tribe Brassiceae (~230 species in approximately 46 genera [52,53]). Interestingly, an independent mesotetraploid WGD has also been revealed in *Orychophragmus* (*Ov*-α; [53]), traditionally but not unequivocally associated with Brassiceae. Whereas the Brassiceae-specific duplication could have been partly implicated from the variation of elevated chromosome numbers (n=7–18), finding a mesopolyploid WGD episode in the evolution of Australian crucifer genera with low (n=5

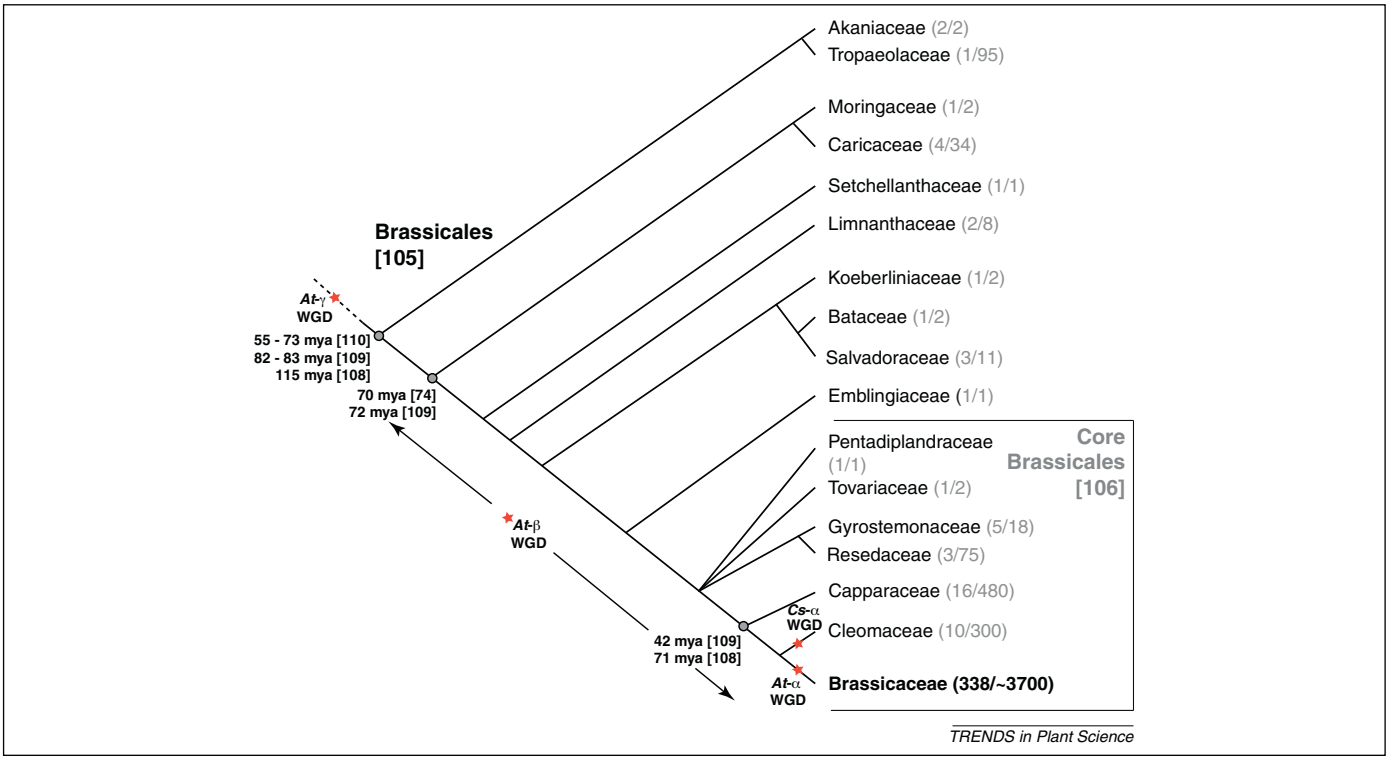

Figure 2. Brassicales phylogeny. Brassicales phylogeny [105] with numbers of genera and species given in parentheses. Selected divergence time estimates are indicated (see text). The *At*-γ WGD was linked to the diversification of eudicots and perhaps all angiosperms [41,42], whereas the *At*-β WGD event postdates the Caricaceae–Brassicaceae divergence [43,44] dated to ~70 mya [74,109]. The *At*-α WGD is specific for the Brassicaceae and the *Cs*-α WGD occurred independently in the Cleomaceae [76].

Box 2. Ancient polyploidy, glucosinolate diversification and Pierinae herbivore radiation

Glucosinolates are amino acid-derived, secondary metabolites representing an antiherbivore defense system of members of the Brassicales and are most highly diversified in the mustard family [102]. Tissue damage through insect feeding brings the compartmentalized myrosinase enzyme into contact with nontoxic glucosinolates. Glucosinolate hydrolysis results in breakdown products such as isothiocyanates, which are highly toxic to many insect herbivores [98]. However, insect herbivores specialized in feeding on plants containing glucosinolates must have a mechanism to overcome the toxicity of the host. Despite the fragmentary data, there is some evidence of temporal association between the diversification of the host–plant and insect–herbivore clades [99]. The radiation of Brassicales and the origin of an evolutionary novel defense system were most probably followed by radiation of insect feeding on these plants (plant–insect coevolution). Indeed, the radiation of the butterfly subfamily Pierinae has been dated within ~10 million years [100] after the diversification of Brassicales ~90–85 mya [74]. The Pierinae butterflies possess a glucosinolate detoxification adaptation based on a nitrile-specifier protein, which redirects the formation of toxic isothiocyanates (upon myrosinase-catalyzed glucosinolate hydrolysis) towards nitrile breakdown products. This adaptation enabled the Pierinae butterflies to feed on Brassicales species and might have acted as a key innovation for the diversification of the Pierinae into significantly more species as compared to its sister group [100]. Furthermore, the origin and diversification of methionine-derived glucosinolates (120 different variants), unique to the mustard family [101], might be linked to the *At*-α WGD [102] as several genes in the biosynthesis and regulation of methionine-derived glucosinolates are known to be duplicated or diversified through neofunctionalization (e.g. *MAM* genes encoding methylthioalkylmalate synthases [103,104 and references therein].

and 6) and the lowest (n=4) known chromosome numbers in the family came as a complete surprise. Comparative cytogenetic analysis and single-copy gene phylogenies revealed that the genera *Arabidella*, *Ballantinia*, *Blennodia* and *Stenopetalum* (21 spp., i.e. a quarter of the crucifers endemic to Australia), loosely assigned to the polyphyletic Camelineae, have most probably undergone a common *Sn*-α WGD [54]. Similarly, a younger *Pex*-α WGD event preceded the species radiation of *Pachycladon* in New Zealand (nine spp. in New Zealand, one in Tasmania) [111]. Both allopolyploidization events could have accelerated the diversification and facilitated the adaptive radiation in both Camelineae lineages. The mesopolyploid WGD in the Australian Camelineae (~6–9 mya) coincided with the development of extensive aridity in central Australia during the late Miocene [55]. The WGD event(s) could have provided the whole lineage with an adaptive advantage to thrive in arid conditions. The 0.8–1.6 million years old WGD in the ancestry of *Pachycladon* is consistent with the Pleistocene; 0.01–2.6 mya colonization of alpine habitats in the Southern Alps of New Zealand [56,111]. In a similar way, the Pleistocene allopolyploid origin and radiation of Australian *Lepidium* species coincided with dramatic climatic fluctuations in Australia when a cooling climate and a drying trend created novel habitats and thus highly invasible terrain providing the necessary ecological space into which allopolyploid *Lepidium* could have radiated [57]. At present, the adaptive advantage of polyploidy is well demonstrated by *Draba* (Arabideae), the largest crucifer genus with more than 370 species, in which the frequent hybridization and allopolyploidization, apparently combined with the duplication of the *COR15* gene which plays an important role in protecting plants from cold stress, resulted in increased species richness and successful colonization of alpine habitats of high mountains and the Arctic [58,59]. Episodes of genome duplication occurring in different ages significantly fostered radiation, ecogeographic diversification and species richness in most speciose Brassicaceae genera and tribes (e.g. Alysseae, Arabideae, Brassiceae, Cardamineae, Erysimeae, Heliophileae or Lepidieae).

We argue that several mesopolyploid lineage-specific WGD events remain to be unveiled. For instance, the elevated base chromosome number (n=14) conserved across the tribe Thelypodieae most probably resulted from a tribe-specific WGD event. Hence, the percentage of polyploids in Brassicaceae given as 37% [60] and based exclusively on chromosome counts is grossly underestimated. If we account for the as yet undetected mesopolyploid WGD events, the percentage of extant and 'recent' polyploids will greatly exceed 50%. By contrast, for several crucifer lineages and tribes the available data indicate the absence of mesopolyploid WGD events. Several lineages within the lineage I possess only the *At*-α duplication: Boechereae, Eurasian Camelineae, Cardamineae, Descurainieae [47,54,61,111], as well as the tribes associated with lineage II (Calepineae, Conringieae, Eutremeae, Isatideae, Noccaeeae: now Coluteocarpeae, Sisymbrieae and probably also Thlaspideae) [52,62].

Box 3. The Brassicaceae as model plant family

In addition to the well-known model plants *Arabidopsis thaliana* and *Brassica* species, several other Brassicaceae taxa are currently used as study objects in modern plant biology. Here, we give a selection of these taxa and some key words to characterize some of the addressed research interests:

Arabidopsis halleri, *Noccaea caerulescens* (heavy metal tolerance and hyperaccumulation), *Arabidopsis lyrata*, *A. suecica* (self-incompatibility and genome evolution), *Arabis alpina* (perennial habit and plant–insect and plant–pathogen interactions), *Cardamine hirsuta* (plant architecture, adaptation along water-usage gradient), *Capsella rubella* and *C. grandiflora* (self-incompatibility), *C. bursa–pastoris* (flowering time, floral architecture), *Boechera* spp. (apomixis and plant–insect and plant–pathogen interactions), *Diplotaxis* spp. (mating system changes), *Iberis* spp. (flower and fruit architecture), *Lepidium* spp. (seed physiology, fruit structure), *Eutrema–Thellungiella* ssp. (salt stress). For the phylogenetic position of model plants see Figure 1.

Origin, age and early diversification of the Brassicaceae

The pioneers of crucifer systematic, Hayek [63], Schulz [25] and Janchen [64], believed in a New World origin of the Brassicaceae from Cleomaceae through a 'basal' tribe Thelypodieae (Stanleyeae). This view was later adopted by other authors [65–68]. Dvorák [69], however, proposed an Old World origin of the family, again evolving from the Cleomaceae, but 'via' the tribe Hesperideae. Indeed, molecular studies ([70] and others) revealed a sister group relationship of the mustard family to the Cleomaceae. A basal split between the tribe Aethionemeae and the rest of the family was confirmed in various studies using different markers or marker sets of all three genomes [2,3,5,8,11,22–24,70,71]. Based on this early phylogenetic branching

(tribe Aethionemeae vs. core Brassicaceae), Franzke *et al.* [8] agreed with Hedge [72] who suggested the Irano–Turanian region, where the highest Brassicaeae species diversity is found, as a possible site of origin for the Brassicaceae family. This region is extremely diverse ecologically, altitudinally and geologically. Franzke *et al.* [8] further suggested that the cradle of the family was Turkey, a country where nearly all recent Aethionemeae species grow and has one of the richest crucifer flora worldwide, harboring 560 recent Brassicaceae species [73].

The age of the family is still not clear. The split between Brassicaceae and Cleomaceae has been estimated to approximately 20 mya [74], a result that is in accordance with an age of 19 million years (myr) calculated with a relaxed molecular clock approach ([8]; secondary fossil calibration), based on mtDNA. The crown age of Brassicaceae (node at the split between *Aethionema* and the core Brassicaceae) was estimated to be 15 mya [8]. Most other age estimates for the crown node are significantly older: 30–60 myr ([24,71]; based on synonymous mutation rates) and 37.6 myr ([11]; primary fossil calibration). In these studies a multi-dataset from three genomes has been used. In addition, age estimations of the Brassicaceae based upon the timing of genome duplication in *Arabidopsis* yielded ages ranging from 24 to 40 mya [49,75,76].

Two different evolutionary scenarios have been currently formulated based on different age estimates. Franzke *et al.* [8] suggested that the typically open- and dry-adapted Brassicaceae radiated approximately 19 mya from humid-adapted Capparaceae–Cleomaceae ancestors in or near the eastern Mediterranean region. Palynological data from the latest Burdigalian (approx. 16 mya) of Turkey support this concept because here a mosaic pattern of subtropical and drier open areas is indicated by the palynoflora that also includes typical open vegetation elements, such as Chenopodiaceae and Poaceae [77]. The radiation of the core Brassicaceae was dated to 11 mya and the authors suggested that newly opened habitats and/or niches created by the Miocene climatic changes acted as an 'extrinsic' factor for the radiation of the family.

According to Couvreur *et al.* [11] the Brassicaceae originated as a tropical–subtropical family approximately 37 mya (Eocene) when a warm and humid climate predominated worldwide, including the Irano–Turanian region, the presumed ancestral area of the family (see above). Subsequently, the Brassicaceae evolved to a dry-adapted family. The radiation of the core Brassicaceae was dated to approximately 32 mya by the authors corresponding to the terminal Eocene cooling event that generally induced an increase in dry-adapted flora in Europe [78].

With the exception of the basal split between tribe Aethionemeae and the core Brassicaceae, the lack of resolution in the family-wide phylogenetic analysis using molecular markers was interpreted as the outcome of an early rapid radiation in the Brassicaceae [5,8]. Couvreur and colleagues [11] tested this hypothesis by showing a lineage through time plot that indeed suggests an early rapid radiation in the family, giving rise to the core Brassicaceae. Here, the detected diversification rates are comparable with those found in highly diversified clades such as Lamiales and Asterales [79]. The colonization of the newly formed arid and semi-arid areas worldwide was most probably also promoted by a widespread autogamous breeding system [80] and easily dispersible diaspores that allowed intercontinental dispersal of mucilaginous seeds by adhering to birds [57]. Franzke *et al.* [8] and Couvreur *et al.* [11] hypothesized that the *At*-α WGD was a major factor driving the early rapid evolution of core Brassicaceae.

Fossil records of the Brassicaceae are generally poor and current available reliable fossils of Brassicaceae argue for a minimal age of the family of approximately 16 million years. The oldest known reliable Brassicaceae pollen are reported from the early Middle Miocene (approx. 16 mya) of Turkey [81] and from the Upper Miocene (approx. 5–11 mya) of France [82,83]. Fossil records of pollen from the Latest Cretaceous (approx. 65–70 mya) of New Zealand [84] are regarded as doubtful [82]. The oldest known reliable macrofossils (fruits, seeds) that have been assigned to the genera *Draba*, *Sinapis*, *Thlaspi*, *Cochlearia* and *Clypeola* were reported from the *upper Pliocene* (approx. 1.8–3.6 mya) and the Upper Miocene of Germany, respectively [25].

A significantly older fruit fossil from the Oligocene of Montana (USA) was attributed to the Brassicaceae genus *Thlaspi* [85] and was associated with the fossil Ruby flora, which was first estimated to occur in the late Oligocene (approx. 23–28 mya). Other studies [86] argue that the age of Ruby flora is late Eocene to Oligocene (approx. 34–37 mya). However, the precise age of Ruby flora is not known [87]. Recently, the assignment of this fossil to extant *Thlaspi* (six spp.) was confirmed [88] and dated to early Oligocene (approx. 28–34 mya). The reliability of generic assignment of this single fossilized fruit is debatable. Although fruit and seed morphology of this fossil resembles that of *Thlaspi* s.str., it cannot be ruled out that the specimen is not Brassicaceae due to convergence of winged fruits from unrelated angiosperm families. An assignment to *Thlaspi* is also somehow problematic because the genus and tribe Thlaspideae are strictly restricted to Europe and Western Asia, whereas the fossil is western North American. An assignment of fossil fruits to a distinct recent taxon is generally problematic due to the well-known high occurrence of homoplasy in fruit characters throughout the family [12]. Very recently this fossil was used for age estimates for the mustard and related families (Brassicales) [108] that yielded up to two- to threefold older estimates than the results of previous analyses [8,11,24,71] (Figures 1 and 2). As these high ages within the Brassicales [108] are also not congruent with recent well-founded molecular dating estimates within the angiosperms [109,110], the analysis of Beilstein *et al.* [108] might have been biased towards higher dating results, potentially due to the incorporation of this old putative *Thlaspi* fossil.

Quintessence

A synopsis of our recent knowledge on patterns and mechanism which drove the radiation of the Brassicaceae now allows a holistic view on a family-wide evolutionary history. Radiation of the family most probably started in the Irano–Turanian region. Although the majority of molecular datings indicate a pre-Miocene origin of the family,

evidence from paleobotany and paleoecology favors a Miocene radiation. Nevertheless, in both cases the radiation of Brassicaceae was largely 'extrinsically' driven by Miocene climate changes that created open and drier habitats and these new ecological niches became characteristically occupied by members of the family. Poorly resolved early cladogenesis argues for a rapid colonization of the newly formed arid and semi-arid areas worldwide. Mucilaginous seeds which adhere to birds thus enabling intercontinental dispersals, as well as an autogamous breeding system, represent putative preadaptations of early Brassicaceae taxa promoting this triumphal procession. The most important 'intrinsic' motor for the increase of this family of over 3700 extant species was obviously the *At*-α WGD during its very early history which provided the genetic raw material for biological radiation and diversification rates that are among the highest reported for flowering plants. The Brassicaceae could therefore serve as a prime example supporting the hypothesis advanced by Van de Peer *et al.* [89] that the availability of ecological niches could be the 'single most important determinant for the survival and long-term evolutionary success of a WGD', a success story of a life with 'Sauerkraut' and a small weedy 'super model'.

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