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Brassicaceae flowers: diversity amid uniformity

Lachezar A. Nikolov*

Department of Molecular, Cell and Developmental Biology, Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA

* Correspondence: nikolov@ucla.edu

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Abstract

The mustard family Brassicaceae, which includes the model plant *Arabidopsis thaliana*, exhibits morphological stasis and significant uniformity of floral plan. Nonetheless, there is untapped diversity in almost every aspect of floral morphology in the family that lends itself to comparative study, including organ number, shape, form, and color. Studies on the genetic basis of morphological diversity, enabled by extensive genetic tools and genomic resources and the close phylogenetic distance among mustards, have revealed a mosaic of conservation and divergence in numerous floral traits. Here I review the morphological diversity of the flowers of Brassicaceae and discuss studies addressing the underlying genetic and developmental mechanisms shaping floral diversity. To put flowers in the context of the floral display, I describe diversity in inflorescence morphology and the variation that exists in the structures preceding the floral organs. Reconstructing the floral morphospace in Brassicaceae coupled with next-generation sequencing data and unbiased approaches to interrogate gene function in species throughout the mustard phylogeny offers promising ways to understand how developmental mechanisms originate and diversify.

Keywords: *Brassica*, *Capsella*, *Cardamine*, *Heliophila*, *Lepidium*, monosymmetry, organ elaboration, organ number, phenotypic plasticity, *Stanleya*.

Introduction

With nearly 4000 species distributed on all continents except Antarctica, the mustard family (Brassicaceae) is one of the largest flowering plant families today (Appel and Al-Shehbaz, 2003; Al-Shehbaz, 2012; BrassiBase, 2019). Despite its species richness, most of the members of the family are readily recognizable by their conserved floral plan with a cross-like appearance, which prompted its classical botanical name, the Cruciferae. The conservation of floral architecture in the mustards is in stark contrast to the floral architecture of their closest relatives, Capparaceae and Cleomaceae, which exhibit significant variation in floral organ number and arrangement (Endress, 1992; Cardinal-McTeague *et al.*, 2016; Bayat *et al.*, 2018). Because the floral plan of Brassicaceae is considered to represent the ancestral condition in the Brassicaceae–Capparaceae–Cleomaceae clade (Endress, 1992; Ronse de Craene, 2010), the strong conservation suggests significant constraint on floral morphology throughout the evolutionary history of the family that allowed only minor architectural deviations.

Brassicaceae flowers are tetramerous, a feature occasionally found in the eudicots (Endress, 2010*a*), with four sepals arranged in medial and lateral positions, alternating with four petals in diagonal positions (Fig. 1) (Ronse de Craene, 2010). The androecium consists of six stamens, two outer, shorter stamens opposite the lateral sepals and four inner stamens with longer filaments opposite the medial sepals and shifted toward the median line. The gynoecium consists of two carpels and

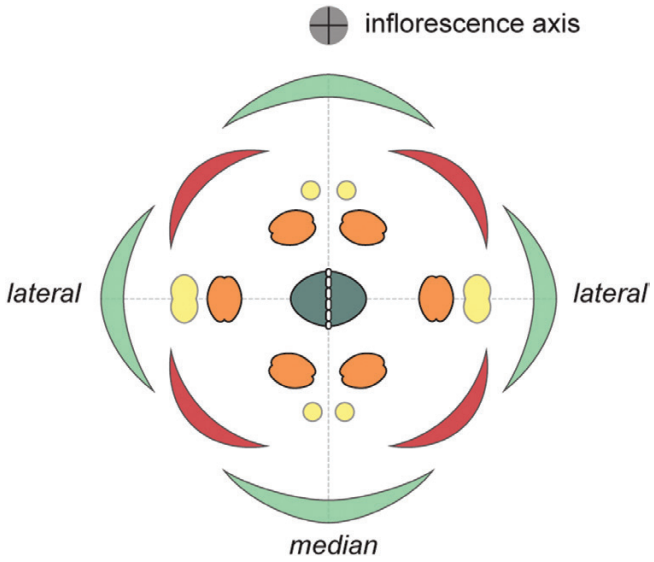

Fig. 1. Generalized floral diagram of Brassicaceae. Four sepals (light green) in median and lateral positions are followed by four alternating petals (red). The androecium (orange) consists of two outer stamens with shorter filaments opposite the lateral sepals and four inner stamens with longer filaments, which are closer together (and sometimes fused at the base) and shifted towards the median line. The bases of the stamens are associated topologically with receptacular nectaries (yellow) of various shapes and arrangements. The gynoecium (dark green) consists of two congenitally fused carpels and is divided into two compartments by a false septum (white dotted line).

has a false septum dividing the ovary into two compartments. This basic organization shows uniformity throughout the family and is associated with a generalist pollination system that includes pollinators from different taxonomical and functional groups (Appel and Al-Shehbaz, 2003). Surprisingly, morphometric analysis of corolla shape variation from 111 mustards and sampling of their insect visitors identified several distinct pollination niches of plants despite their architecturally conserved floral plan (Gómez *et al.*, 2016), hinting at overlooked diversity among Brassicaceae species.

Brassicaceae are uniquely positioned to address fundamental questions in comparative biology about the genetic basis of diversity above and below the species level. The family includes species exhibiting substantial morphological diversity, and encompasses model systems with extensive genetic tools and genomic resources (Hay *et al.*, 2014; Koenig and Weigel, 2015; Provart *et al.*, 2016), which allow exploration of the origin and diversification of floral developmental mechanisms and the contributions of conservation and divergence in the evolution of phenotypic diversity. Addressing these questions requires a comprehensive description of the floral phenotypic space of Brassicaceae at different levels, from variations in the floral display in the context of the inflorescence to the elaboration of individual floral organs and their integration.

Diversity of angiosperm flowers is best described hierarchically (Endress, 1994). At the coarsest level, floral organization (bauplan, groundplan) takes into account the identity, number, and arrangement of floral organs (Endress, 1994). This is the most constrained aspect of floral morphology, which is controlled by deeply hardwired developmental mechanisms, such as the floral organ identity program, and therefore carries a significant burden of phylogenetic contingency. A portion of this constraint is released through variation in the floral construction (gestalt), which includes differences in allometry, stacking and stuffing, and floral symmetry (Endress, 1994). This aspect of floral morphology is more accessible to natural selection and generally exhibits dynamic evolution at various phylogenetic scales. Finally, floral mode (style) directly caters for different pollinators through variations in color, scent, and organ elaboration (Endress, 1994). This is the most labile aspect of floral morphology. The distinction among these hierarchical levels of floral organization is not sharply defined; nonetheless, they provide an instructive conceptual framework, which is adopted here. This review highlights aspects of Brassicaceae diversity in floral organization, construction, and mode. I also discuss the diversity in flower arrangement in the context of the inflorescence and in structures preceding the flower, which do not belong to the flower in a strict sense. Although fruit morphology is very diverse in the family, it is not discussed in detail here. Where possible, I make a connection between induced genetic variation obtained by the perturbation of gene function in Arabidopsis (hereafter for *Arabidopsis thaliana*) and the diversity of shape and form observed in nature (Smyth *et al.*, 1990; Bowman, 1994) to highlight candidate genes with a possible contribution to diversity. I also highlight studies of morphological diversity using unbiased approaches for gene discovery to dissect traits that do not exist in Arabidopsis but can be studied in other Brassicaceae.

Phylogenetic framework for Brassicaceae

The mostly temperate and herbaceous family Brassicaceae belongs to Brassicales, a plant order of 18 families, which are united by the presence of glucosinolates, a distinct chemical signature with a defensive role (Edger *et al.*, 2015, 2018). The closest relatives of Brassicaceae within the order are predominantly tropical shrubs and trees in the families Capparaceae and Cleomaceae (Cardinal-McTeague *et al.*, 2016; Bayat *et al.*, 2018). Brassicaceae appeared relatively recently in the geological record, and their extant centers of diversity include the Irano-Turanian and the Mediterranean floristic regions, as well as the Himalayas, the Cape floristic region, the Andes, Western USA, and Australia and New Zealand (Appel and Al-Shehbaz, 2003; Franzke *et al.*, 2011; Nikolov *et al.*, 2019). The majority of the Brassicaceae diversity falls within 52 natural groupings (tribes) and five main lineages, which are sister to the genus *Aethionema* (tribe Aethionemeae) (Fig. 2) (Al-Shehbaz, 2012; Huang *et al.*, 2016; Nikolov *et al.*, 2019). The improving understanding of the relationships among lineages and tribes provides the phylogenetic framework to study the tempo and mode of morphological evolution in the family (Huang *et al.*, 2016; Nikolov *et al.*, 2019). Brassicaceae exhibit a significant diversity in a number of morphological characters, including leaf shape, trichome morphology, and fruit shape (Appel and Al-Shehbaz, 2003; Beilstein *et al.*, 2006; Bowman, 2006; Nikolov and Tsiantis, 2017). Character mapping on resolved phylogenies has revealed rampant convergent evolution in

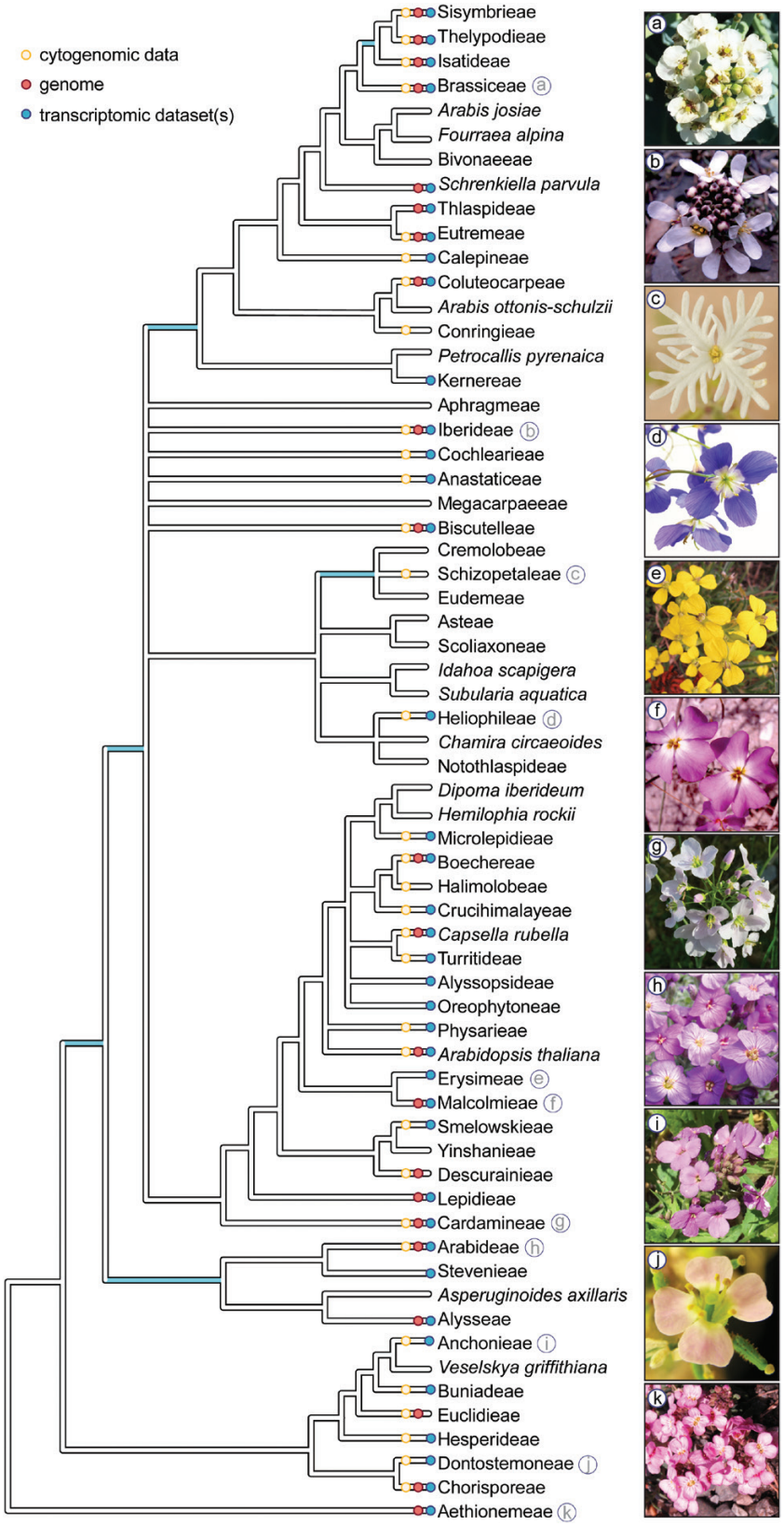

Fig. 2. Phylogeny of Brassicaceae based on 1421 nuclear markers, featuring 50 of the 52 recognized tribes and 12 species unassigned to a tribe, with snapshots of the floral diversity in the family (tree topology from Nikolov *et al.*, 2019; branches in blue have received strong but not uniform support among different analyses). Some of the resources available to study the floral biology of Brassicaceae, including genomic data from comparative chromosome painting (yellow) and genome sequences (red), and floral transcriptome data sets (blue) are highlighted at the terminal branches of the phylogenetic tree [summary after Phytozome v. 13 (https://phytozome.jgi.doe.gov/pz/portal.html); Huang *et al.*, 2016; Koenig and Weigel, 2015; Lopez *et al.*, 2017; Mandáková *et al.*, 2017*a*, *b*, and references therein].

many lineages, with similar character states evolving in different parts of the tree (Huang *et al.*, 2016), suggesting lability in the genetic architecture of the underlying traits. Few characters represent true synapomorphies for the larger clades, including tribes, although there is a propensity for certain traits to evolve in particular lineages. Although there is a substantial body of literature on the morphological variation of Brassicaceae flowers in the context of taxonomic studies (Appel and Al-Shehbaz, 2003; Al-Shehbaz, 2012), synthetic treatments of their diversity in a phylogenetic context are currently lacking.

Putting flowers in context: the floral display

The flowers of Brassicaceae are typically arranged in racemes, an indeterminate inflorescence where single flowers are borne on an elongated axis attached by short stalks, the pedicels (Weberling, 1989; Endress, 2010*b*) (Figs 2, 3). Racemes of higher order (e.g. raceme of racemes) are common [e.g. *Armoracia* (Cardamineae), *Isatis* (Isatideae), and *Crambe* (Brassiceae)]. In some cases, the individual racemose units are subtended by cauline leaves (sometimes referred to as inflorescence 'bracts'), especially in the lower part of the inflorescence (e.g. Smelowskieae, Descurainieae, Aphragmeae, Sisymbrieae, and Hesperideae) (Appel and Al-Shehbaz, 2003; Huang *et al.*, 2016). An exceptional floral arrangement is observed in *Asperuginoides axillaris*, a poorly known species and currently unplaced in a tribe, which has solitary flowers in axillary positions (Bani and Adiguzel, 2006). The developmental basis for this condition is not known, but may involve the reduction of racemes to a single flower. In other species, racemes with compacted main axes reduced to single, terminal flowers borne on elongated pedicels appear as solitary flowers that originate directly from the basal rosette (Yoon and Baum, 2004). This trait, known as rosette flowering, has evolved independently in several tribes, including Cardamineae, Cochlearieae, Cremolobeae, Eudemeae, Oreophytoneae, Euclideae, and Chorisporeae (Appel and Al-Shehbaz, 2003).

Establishing floral identity is intrinsically connected to the mechanisms that promote flowering, a process during which a vegetative shoot apical meristem is transformed into an inflorescence meristem capable of producing flowers (reviewed in Wellmer, 2017). The sites of flower initiation correspond to auxin maxima at the flanks of the inflorescence meristem (Stewart *et al.*, 2016). In addition to specifying the position of incipient floral primordia, auxin reinforces floral identity by promoting the expression of the floral meristem identity gene *LEAFY* (*LFY*). *LFY* activates the expression of genes required for floral development and represses shoot identity genes, such as *TERMINAL FLOWER1* (*TFL1*). Overexpression of *TFL1* in Arabidopsis delays flowering and prevents the transition from inflorescence to floral meristems (Hanzawa *et al.*, 2005). Mutations in *TFL1* result in early flowering and the formation of terminal flowers as the inflorescence meristem is completely transformed into floral primordia. Modeling studies have shown that the interplay of factors with activities similar to *LFY* and *TFL1* can produce much of the inflorescence diversity observed in nature (Prusinkiewicz *et al.*, 2007).

The contributions of the candidate genes *LFY* and *TFL1* to rosette flowering have been investigated in a number of Brassicaceae species using a combination of interspecies gene transfers and expression analyses (Shu *et al.*, 2000; Yoon and Baum, 2004; Sliwinski *et al.*, 2006, 2007; Bosch *et al.*, 2008; Liu *et al.*, 2011). In one example (Yoon and Baum, 2004), the entire *LFY* locus from the rosette flowering crucifers *Ionopsidium acaule* (*IacLFY*; Cochlearieae), *Idahoa scapigera* (*IscLFY1* and *IscLFY2*; unplaced to a tribe), and *Leavenworthia crassa* (*LcrLFY*; Cardamineae) was transgenically introduced into the Arabidopsis loss-of-function *lfy* mutant. The *IacLFY* locus was able to rescue the *lfy* phenotype, suggesting functional conservation. In contrast, *IscLFY1* rescued some aspects of the *lfy* phenotype in Arabidopsis, but also produced shortened internodes and occasionally aerial rosettes that resemble the phenotype of the donor, suggesting that *IscLFY1* may contribute to rosette flowering (Yoon and Baum, 2004). Similarly, *LcrLFY* partially rescued the *lfy* phenotype, with some transgenic lines producing terminal flowers as in wild-type *L. crassa* plants. These observations imply different mechanisms for rosette flowering in the studied species and suggest that changes in the activity or expression of *LFY* contribute to rosette flowering.

Getting into floral territory: (cryptic) bracts

Morphologically, an inflorescence is a reproductive shoot built of repeated basic units (metamers) consisting of a node with an attached lateral organ and an internode (Bell, 2008). An axillary meristem at the base of the lateral organ can grow out to produce a secondary reproductive shoot in an iterative manner or can adopt floral identity fate and give rise to a flower. In this case, the lateral organ is referred to as a flower-subtending bract. In some flowering plants, additional leafy structures (prophylls or bracteoles) that precede the flower are initiated on the floral axis (Endress, 2006). Brassicaceae flowers lack noticeable bracts and prophylls. Nonetheless, the existence of a gene expression domain on the abaxial side of the floral primordium defined by *AINTEGUMENTA* expression (typically expressed in very young organ primordia) and the exclusion of *SHOOT MERISTEMLESS* expression (a marker of shoot apical meristem fate) in Arabidopsis strongly suggests that a bract primordium is demarcated during development, but its outgrowth is suppressed (Long and Barton, 2000). Its outgrowth can be induced by ectopic expression of *JAGGED* (*JAG*), which is typically excluded from the cryptic bract (Dinneny *et al.*, 2004; Ohno *et al.*, 2004). Interestingly, although floral bracts are completely reduced in Brassicaceae, paired structures at the base of the pedicel, called squamules, are observed in the early floral development of some species, including *Heliophila* (present in ~60 out of the 95 species of the genus), *Arabis verna*, *Sisymbrium*, *Lunaria*, *Nasturtium officinale*, and *Leavenworthia alabamica*, and have been interpreted as the developed stipules of the cryptic bract (Arber, 1931; Weberling, 1989; Bosch *et al.*, 2008). Bract suppression is not unique to Brassicaceae. In maize, where bracts similarly do not develop in wild-type plants, mutations in five separate loci, *tassel sheath1* (*tsh1*)–*tsh5*, result in bract outgrowth (Whipple *et al.*, 2010). None of the

Tsh loci cloned so far is a homolog of *JAG*, suggesting a different mechanism of bract suppression in the grasses.

Variation in floral organization: identity, number, and arrangement of floral organs

Organ identity

Much is known about the genetic program controlling floral organ identity in Arabidopsis (reviewed in Causier *et al.*, 2010; Ó'Maoiléidigh *et al.*, 2014; Irish, 2017; Theissen and Rümpler, 2018), which is largely conserved in Brassicaceae. Stable homeotic transformations occur in populations of *Capsella bursa-pastoris* (Camelineae) at several localities in Europe, where all petals are transformed into supernumerary stamens ('decandric' phenotype) (Hintz *et al.*, 2006; Nutt *et al.*, 2006; Hameister *et al.*, 2009). Wild-type and homeotic mutant populations are sympatric and show temporal reproductive isolation, with mutants flowering later in the summer, which has resulted in genetic differentiation between the wild type and the decandric morphs (Hintz *et al.*, 2006; Hameister *et al.*, 2009). It has been argued that the potential of such 'hopeful monsters' for speciation and diversity is substantial (Theissen, 2010). Crosses between the wild type and mutants point towards a single locus, named *Stamenoid petals* (*Spe*), that underlies the decandric phenotype (Hameister *et al.*, 2013). *Spe* is in the same linkage group as a homolog of the floral homeotic gene *AGAMOUS* and it remains to be determined whether both loci are the same (Hameister *et al.*, 2013)

Organ number

In contrast to floral organ identity, the genetic mechanisms controlling floral organ number are not well understood. Organ number variation may result from differences in the initiation and outgrowth of floral organ primordia, or changes in organ boundaries. In Arabidopsis, mutants of genes involved in auxin biosynthesis, transport, and response often exhibit defects in floral organ initiation (Cheng and Zhao, 2007), suggesting that these activities may be at play to generate diversity in nature. An increased number of floral organs generally [but not always, e.g. *PETAL LOSS* in Arabidopsis (Lampugnani *et al.*, 2013)] correlates with larger floral meristem size (Schoof *et al.*, 2000). Mutants of the bZip transcription factor gene *PERIANTHIA* (*PAN*), which develop larger meristems, exhibit striking architectural transformations from tetramerous flowers into flowers with pentamerous symmetry, increasing the number of sepals and petals from four to five (Chuang *et al.*, 1999; Maier *et al.*, 2011). Surprisingly, this change is coupled with a reduction of the number of stamens from six to five, which may be related to the role of *PAN* in the direct transcriptional activation of *AGAMOUS* (Maier *et al*, 2011). *CUP-SHAPED COTYLEDON* (*CUC*) genes, on the other hand, modulate local auxin concentrations in diverse model species, to establish boundaries between organs, and inactivating mutations in these genes result in the lack of organ separation (Blein *et al.*, 2008; Berger *et al.*, 2009; Maugarny *et al.*, 2016).

Petal number

Natural variation in some species and certain environmental conditions may cause an extreme suppression of petal growth and petal absence, as in *Lepidium oxytrichum* and *L. densiflorum* (Lepidieae; Bowman and Smyth, 1998), *Rorippa* and *Cardamine* (Cardamineae; Hay *et al.*, 2014), and *Subularia aquatica*. Species in the genus *Cardamine* exhibit age-, temperature-, and population-dependent variation in the number of petals per flower, from zero to four, suggesting that this variation is under environmental and genetic control (Hay *et al.*, 2014; Monniaux *et al.*, 2016; Hay and Tsiantis, 2016; Theissen and Melzer, 2016). Quantitative trait locus (QTL) mapping based on different *Cardamine hirsuta* accessions provided the first evidence for the polygenic architecture of this trait, with many small to medium effect loci contributing to petal number variation (Pieper *et al.*, 2016). Recently, Monniaux *et al.* (2018) have shown that this variation is governed by changes in the expression of the *AP1* homolog in *C. hirsuta*. The authors argued that Arabidopsis *AP1* is buffering the effect of loci contributing to petal number variation such that this variation remains cryptic and, as a result, the species has a robust petal number. The restriction of *AP1* expression in *C. hirsuta* has released these loci from the epistatic effect of *AP1*, which phenotypically manifests as variability of petal number. It would be interesting to identify these candidate loci and to characterize their mode of action.

Stamen number

When floral organs are arranged in discrete whorls, they are generally initiated in alternate positions to their predecessors and their number does not change from one whorl to the next (Endress, 2006). In this respect, the standard stamen arrangement of Brassicaceae (2 + 4) is unusual as stamens are preceded by four petals. One of the classical interpretations of this condition postulates an ancestrally dimerous flower with four stamens arranged in two whorls (2 + 2), which have undergone organ doubling (dédoublement) of the inner, medial stamens (reviewed in Endress, 1992; Ronse de Craene, 2010). Other authors (most recently Merxmüller and Leins, 1967) interpreted the flower as having five tetramerous whorls, with loss of two medial outer stamens. In Arabidopsis, the primordia of the four inner stamens are initiated independently from one another and develop slightly earlier than the two lateral, outer stamen primordia (Smyth *et al.*, 1990). Molecular genetic data support the uniformity of the third floral whorl, which gives rise to the androecium, and postulates a duplication of the organ position of the medial stamens (Meyerowitz *et al.*, 1991). Irrespective of the different interpretations, six stamens arranged in what appears morphologically as two cycles is the rule in Brassicaceae, and exceptions are rare. *Megacarpaea polyandra* has between 8 and 24 stamens, and their position and development have not been studied (Endress, 1992). The increased number of stamens resembles the *superman* mutant in Arabidopsis, where the increase in stamen number is at the expense of the gynoecium (Prunet *et al.*, 2017). Variation in the number of stamens, between 0 and 10 stamens per flower, is observed in *Hormathophylla spinosa* (Alysseae) (Méndez and Gómez, 2006). This variation has been attributed to flexibility in the reproductive strategy and

offers another example of plastic response manifested as variation in organ number (Méndez and Gómez, 2006). Reduction to four stamens is observed in *Iberis* (Iberideae), *Hornungia* sp. (Descurainieae), and *Cardamine hirsuta* and *Nasturtium officinale* (both Cardamineae (Arber, 1931; Matsuhashi *et al.*, 2012; Hameister *et al.*, 2013). In *C. hirsuta*, variation in stamen number is phenotypically plastic and has been linked to ambient temperature, much like the variation in petal number (Matsuhashi *et al.*, 2012; Monniaux *et al.*, 2016).

Deviation from the canonical 2 + 4 stamen number is commonly observed in the large genus *Lepidium* (Lepidieae), where half of the species have two stamens in the median position and several species have four stamens (Bowman and Smyth, 1998; Bowman *et al.*, 1999; Lee *et al.*, 2002). Stamen number reduction partially correlates with petal loss and has occurred several times throughout the phylogeny of the genus along with a number of reversals to an ancestral state (Bowman *et al.*, 1999). In development, reduction to four stamens may occur via two mechanisms, either through failure to initiate the two lateral stamens, or through the congenital fusion of the two medial stamen primordia into one (a hypothetical reversal of dédoublement; Bowman and Smyth, 1998). A combination of these two mechanisms results in flowers with two stamens in several species. Phylogenetic analysis has revealed that allopolyploidization among *Lepidium* species is common, and allopolyploids often lack lateral stamens (Lee *et al.*, 2002). The hybrids of a species with lateral stamens (*L. oleraceum*) and a species without lateral stamens (*L. hyssopifolium*) did not develop lateral stamens in 80% of the examined F_1 flowers, demonstrating that this is a semi-dominant trait. The semi-dominant mode of inheritance may have facilitated the spread of the phenotype throughout the genus via interspecies hybridization and introgression. Segregation in subsequent generations of the synthetic hybrids revealed the quantitative nature of the trait and suggested that several as yet unidentified loci contribute to lateral stamen loss (Lee *et al.*, 2002).

Interestingly, the genus *Lepidium* also includes the sole dioecious members of Brassicaceae, *L. sisymbrioides* and *L. solandri*, which develop only unisexual flowers (Heenan *et al.*, 2007; Soza *et al.*, 2014). Unisexual flowers also form in several monoecious species in the family [*Cardamine pratensis* (Cardamineae), *Hirschfeldia incana* (Brassiceae), and *Pachycladon wallii* (as *Cheesemania wallii*; Microlepideae)] (Endress, 1992). In *L. sisymbrioides*, both carpel and stamens are initiated normally but, in staminate flowers, the gynoecium undergoes developmental arrest at an intermediate stage. In pistillate flowers, the male sporogenous tissue aborts at a similar stage, suggesting different mechanisms for organ degeneration (Soza *et al.*, 2014). The next step toward the characterization of the alternative mating strategy of *L. sisymbrioides* is identifying the factors causing organ arrest and degeneration, and determining how they contribute to the sex determination process.

Variation in floral construction: floral organ size, floral symmetry, and organ fusion

Petal size variation among species

Although retarded in development, showy petals that are distinctly longer than the sepals are common in Brassicaceae (Huang et al., 2016). In some lineages, including *Capsella* (Camelineae), *Rorippa* (Cardamineae), *Cardamine* (Cardamineae), *Lepidium* (Lepidieae), and *Cakile* (Brassiceae), petals are reduced and smaller than the sepals. In the genus *Capsella*, petal size reduction is associated with the transition from outcrossing to selfing as part of the so-called 'selfing syndrome', which also includes overall reduction in floral size, reduced pollen-to-ovule ratio, and reduction in nectar and scent production (Sicard and Lenhard, 2011). QTL analysis based on a cross between the outbreeding species *Capsella grandiflora* and the selfing species *Capsella rubella* identified seven loci that explain ~60% of petal size variation (Sicard *et al.*, 2011). One of these QTLs maps to polymorphisms in an intron of a homolog of *STERILE APETALA* (*SAP*) in Arabidopsis, encoding an F-box protein that modulates the stability of a repressor complex controlling organ size (Sicard *et al.*, 2016; Li *et al.*, 2018). The intron includes a tissue-specific enhancer, and its variant in *C. rubella* leads to a decrease in the time for cell proliferation and a reduced number of petal cells (Sicard *et al.*, 2016). The allelic variant of *SAP* conferring reduced petal size in *C. rubella* is present in the outcrossing progenitor species *C. grandiflora*, providing an explanation for the rapid evolution of reduced floral size during the transition to selfing, which was also associated with a pronounced population bottleneck that facilitated variant fixation (Guo *et al.*, 2009). Another QTL conferring reduced petal size in *C. rubella* maps to polymorphisms in *CYP724A1*, which encodes an enzyme from the brassinosteroid biosynthetic pathway (Fujikura *et al.*, 2018). Increased brassinosteroid levels contribute to petal size reduction by limiting cell proliferation. The increased activity of *CYP724A1* in the selfing species was found to result from the more efficient splicing of the variant in *C. rubella* compared with *C. grandiflora*, which was attributed to two single nucleotide polymorphisms in two exons of the gene. The *C. rubella* allele originated by *de novo* mutations in this lineage after its divergence from *C. grandiflora* (Fujikura *et al.*, 2018). These two examples highlight the role of both *de novo* mutations and standing variation in the evolution of morphological diversity.

Floral symmetry

Brassicaceae flowers have a single plane of symmetry early in development because the abaxial sector (i.e. the sector away from the inflorescence axis) is larger than the adaxial sector (i.e. the sector facing the inflorescence axis), possibly due to the lack of an associated bract (Endress, 1992). Later in development, the flowers of most Brassicaceae have two planes of symmetry (i.e. they are disymmetric). In some species, morphologically distinct abaxial and adaxial domains persist into maturity, giving rise to monosymmetric flowers. Floral monosymmetry in Brassicaceae has evolved apparently independently from disymmetric ancestors several times, in *Iberis* spp. and *Teesdalia nudicaulis* (Iberideae), *Calepina irregularis* (Calepineae), *Notoceras bicorne* (Anastaticeae), *Pennellia* (Halimolobeae), *Ionopsidium* (Cochlearieae), and *Streptanthus* (Thelypodieae) (Appel and Al-Shehbaz, 2003; Busch *et al.*, 2012). Consistent with its disparate origins, the pattern of floral monosymmetry, which manifests in the petal and stamen whorls in Brassicaceae, varies among species (Appel and Al-Shehbaz, 2003). In *Iberis amara*,

the four petals are initiated simultaneously but diverge in size during development, resulting in abaxial petals that are significantly larger than the adaxial petals (Busch and Zachgo, 2007). In *Streptanthus cutleri*, the exaggerated adaxial petal blades and the complete lack of abaxial petal blades create the impression of 'rabbit ears' (Rollins, 1993).

Floral symmetry in diverse species is under the control of transcription factors in the TCP [*Teosinte Branched 1*, *CYCLOIDEA* (*CYC*), and *Proliferating Cell Factors*] gene family (reviewed in Hileman, 2014; Spencer and Kim, 2018). In the model species *Antirrhinum majus* (Plantaginaceae), differential expression of two closely related TCP transcription factor genes, *CYCLOIDEA* and *DICHOTOMA*, demarcates the adaxial region of the flower, and inactivating mutations in these genes result in loss of monosymmetry and radialization of the flower (Luo *et al.*, 1996). In *Iberis amara* (Brassicaceae), the unequal petal growth of adaxial and abaxial petals correlates with the expression levels of a *CYC* homolog, *IaTCP1*, which are higher in the smaller, adaxial petals before anthesis (Busch and Zachgo, 2007). In peloric *I. amara* floral variants where all petals have abaxial identity, *IaTCP1* levels are reduced compared with their levels during normal development. A similar correlation between the difference of *CYC* expression in adaxial and abaxial petals and the extent of corolla monosymmetry at anthesis was observed in several other species in the family, and this relationship appears to be dosage dependent (Busch *et al.*, 2012). In the studied monosymmetric species, *CYC* expression is largely homogeneous throughout the floral meristem during early development, and adaxial *CYC* expression is established later during petal differentiation (Busch and Zachgo, 2007; Busch *et al.*, 2012). This expression pattern is probably derived from an ancestral Brassicaceae pattern that features early adaxial *CYC* expression that disappears later in development (Busch *et al.*, 2012). These results demonstrate the role of temporal expression changes in the repeated evolution of *CYC*-controlled monosymmetry. Recent transcriptome analysis comparing the transcriptional profiles of adaxial and abaxial petals in *I. amara* identified differentially expressed genes in the two domains, such as the enrichment of cell wall modification and cell–cell signaling genes in the adaxial petals, which probably include downstream targets of *IaTCP1* that await functional validation (Busch *et al.*, 2014). The monosymmetric flowers of *Iberis* are arranged in dense corymboid inflorescences that function as pseudanthia where the larger abaxial petals radiate to increase the visibility of the floral display. The fixed monosymmetry may explain the higher speciation rates in *Iberis* (27 species) compared with its sister genus *Teesdalia* (three species), which includes species with both monosymmetric (*T. nudicaulis*) and disymmetric (*T. coronopifolia*) flowers (Busch *et al.*, 2012).

Floral organ fusion

Although floral organ synorganization is not pronounced in Brassicaceae like in other species-rich families, such as Orchidaceae and Apocynaceae (Endress, 2016), there are a number of cases where organs are congenitally united. Sepals united at their bases are present in Eudemeae (*Brayopsis*), Alysseae (*Alyssum*), Megacarpaeeae, Sisymbrieae, Euclidieae, and Erysimeae (Endress, 1992; Al-Shehbaz, 2001; Appel and Al-Shehbaz, 2003). In addition to the putative congenital fusion of medial stamens in *Lepidium* discussed above (Bowman and Smyth, 1998), the bases of the medial stamens are united in Aethionemeae, Microlepideae, Cremolobeae, Thelypodieae, Hesperideae, Anchonieae, and Dontostemoneae (Endress, 1992; Appel and Al-Shehbaz, 2003). The developmental and genetic basis of this variation is not known.

Variation in floral mode: organ elaboration and floral color

Most of the diversity in Brassicaceae flowers concerns the elaboration of individual organs that probably affects the visibility and the attractiveness of the floral display (Yuan *et al.*, 2013), and controls the access of pollinators and nectar robbers to floral rewards. In this role, individual organs are functionally integrated and show correlated variation with each other (Smith, 2015). For example, cup-shaped (saccate) lateral sepals, petals with a narrower (clawed) base, and well-developed lateral nectary glands often co-occur in Brassicaceae flowers as a functional unit operating as a nectar repository. In *Heliophila* (Heliophileae; Mummenhoff *et al.*, 2005), asymmetrically positioned appendages on the petal base facing the lateral plane of the flower and appendages on the filaments of the lateral stamens limit the access to nectar at the base of the flower and may contribute to the grasp of pollinating insect visitors (Fig. 3B). The selfing syndrome provides another example of floral integration (Sicard and Lenhard, 2011). The striking association of multiple floral traits raises questions about the genetic basis of floral integration and the potential role of pleiotropy in co-ordinated morphological changes (Smith, 2015).

Elaboration of sepals, petals, and stamens

In planar organs, such as sepals and petals, uniform modification of growth can lead to scaling differences (allometry), and anisotropic growth along a particular axis can result in altered organ dimensions among species (Coen *et al.*, 2017). A combination of uniform and anisotropic growth shapes the petals, which are usually differentiated into a narrower portion, the claw, and an expanded blade-like portion, the limb. There is much variation in petal shape in the family, from typically obovate, elliptic, and spatulate to obcordate (heart-shaped) petals (Appel and Al-Shehbaz, 2003). QTL analysis of petal shape variation based on recombinant inbred lines derived from different Arabidopsis ecotypes (Col-0×Est-1 and L*er*-0×Col-4) identified 23 loci that affect petal morphology (Abraham *et al.*, 2013). The study also demonstrated that variation in petal length, width, area, and shape can be decoupled, and highlighted the role of *ERECTA*, a leucine-rich repeat receptor-like serine-threonine kinase gene, as a major effect locus for petal shape, probably acting through differences in petal cell proliferation (Abraham *et al.*, 2013).

When growth along the medio-lateral axis is suppressed, an organ does not expand significantly laterally. Linear petal blades

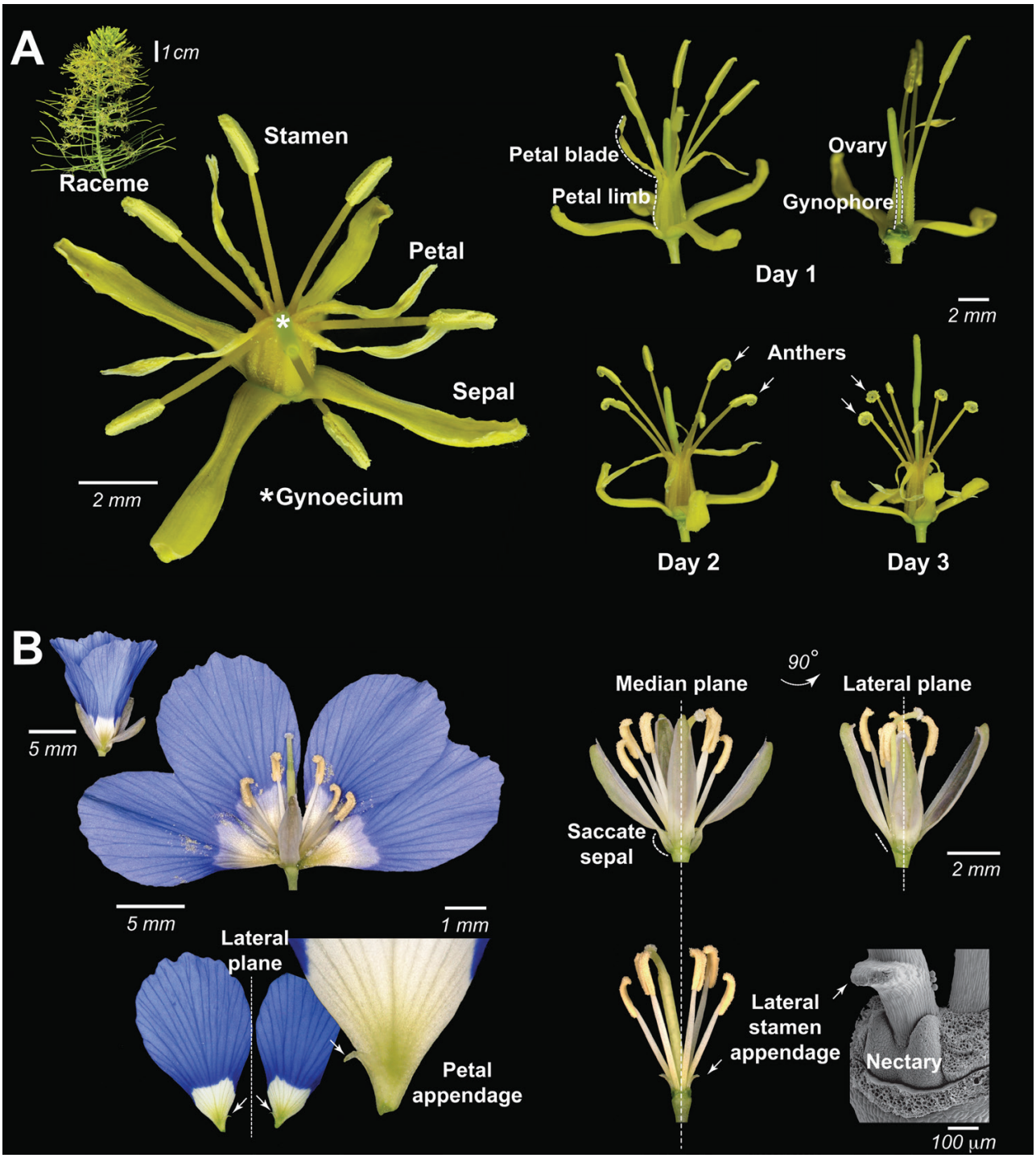

Fig. 3. Examples of floral diversity in the Brassicaceae. (A) Floral morphology of *Stanleya elata* (Thelypodieae). A series of flowers 1, 2, and 3 d after flower opening are shown on the right. Flowers are borne singly on a pyramidal racemose inflorescence (upper left corner), where the internodes elongate acropetally post-anthesis. Sepals are yellow, showy, and spreading, and contribute significantly to the floral display. The petals are differentiated into a limb and a blade. The limbs are wide, erect, and together with the base of the anther filaments create a barrier, which limits the access to copious amounts of nectar in the center of the flower. The petal blade is narrow, elongated, and ribbon-like. The six stamens are of approximately equal height. The bases of the filaments are broad and papillate, and appear post-genitally coherent. After dehiscence, the anthers (arrows) curl from the tip to the base to release pollen towards the center of the flower (introrse anther dehiscence). The gynoecium has a short style and a pronounced gynophore, which elongates post-anthesis irrespective of pollination and contributes to the pyramidal shape of the inflorescence. (B) Floral morphology of *Heliophila coronopifolia* (Heliophileae). Flowers are disymmetric, with median and lateral planes of symmetry, and the floral organs exhibit elaboration along these planes. Lateral sepals are saccate, forming a pronounced pouch which serves as a nectar repository. The petal bases are asymmetric, each developing a petal appendage facing the lateral plane. The lateral petal appendages of two neighboring petals and an appendage differentiating on the filaments of the lateral stamens form a protective barrier that limits access to the nectaries, which differentiate in lateral positions. Anthers are extrorse, with pollen released away from the center of the flower. The gynoecium has a pronounced style and a very short gynophore.

develop in the tribes Physarieae, Biscutelleae, Calepineae, Cremolobeae, Chorisporeae, and Thelypodieae (*Stanleya elata*, Fig. 3A) (Appel and Al-Shehbaz, 2003). On the other hand, increased growth along the medio-lateral axis results in winged stamen filaments in some species of Alysseae, Aethionemeae, Crucihimalayeae, Iberideae, Eutremeae, and Dontostemoneae (Appel and Al-Shehbaz, 2003). Concentration of growth in the interior domain of planar organs may result in bulging to achieve their three-dimensional form. The final form of Arabidopsis sepals is a result of a mechanical feedback that is mediated by the arrangement of microtubules and their response to stress (Hervieux *et al.*, 2016). One of the features that globally affects the distribution of tensile stresses in the sepals is the presence of specialized giant cells in the epidermis, which differentiate after endoreduplication triggered by fluctuations in the levels of the transcription factor gene *Arabidopsis thaliana MERISTEM LAYER1* (Meyer *et al.*, 2017). The contribution of giant cells to sepal form in Brassicaceae offers an interesting example of how growth and morphological diversity at the cellular and the organ scales relate to each other. The contribution of cellular diversity, which also includes floral mucilage cells (Matthews and Endress, 2006), myrosin cells (Li and Sack, 2014), and glands and trichomes on the floral organs that create variation in the floral indumentum (Ó'Maoiléidigh et al., 2018), to floral diversity deserves further studies.

Local modulation of growth also contributes to changes in organ shape. Mild local repression of growth at the tip of the petal to form a notch (retuse petal apex) is present in *Malcolmia* (Malcolmieae), *Dontostemon* (Dontostemoneae), *Aurinia* and *Lepidotrichum* (both Alysseae), and others (Appel and Al-Shehbaz, 2003; Huang *et al.*, 2016). In extreme cases, the tip of the petal is deeply divided (bifid petals) as in *Berteroa* and *Galitzkya* (members of the same clade of Alysseae) and in the unrelated *Draba verna* (previously *Erophila verna*; Arabideae) (Appel and Al-Shehbaz, 2003). The margin of the petal blade in several unrelated species is elaborated to feature marginal protrusions that resemble small teeth (dentate petal margin in *Megacarpaea polyandra*; Megacarpaeeae), are long and narrow (fimbriate or filiform margin in *Ornithocarpa fimbriata* and *O. torulosa*; Cardamineae), or form pronounced lobes (pinnatifid/pinnately lobed petals in *Schizopetalon* (Schizopetaleae), *Dryopetalon* (Thelypodieae), and *Megacarpaea delavayi* (Megacarpaeeae)) (Endress, 1992; Appel and Al-Shehbaz, 2003). The balance of local growth and repression in sculpting these diverse shapes is not known. Advances in the genetic basis of shape variation in other lateral organs, such as leaves (Nikolov and Tsiantis, 2017), may suggest candidate genes that contribute to petal shape variation in Brassicaceae.

Elaboration of the gynoecium contributes to fruit diversity

Fruit shape is one of the most diverse traits in Brassicaceae, and the genetic basis of fruit diversity has been addressed in a number of model systems (Mummenhoff *et al.*, 2009; Mühlhausen *et al.*, 2013; Avino *et al.*, 2014; Eldridge *et al.*, 2016; Łangowski *et al.*, 2016; Lenser *et al.*, 2016; Galstyan and Hay, 2018). Brassicaceae fruits primarily differ in the ratio of length to width at maturity (silique versus silicle) and in the mode of fruit dehiscence (dehiscent versus indehiscent), which arise from post-anthetic anisotropic growth (Eldridge *et al.*, 2016) and modification of the dehiscence zone, respectively (Zúñiga-Mayo *et al.*, 2019). Although fruit diversity is outside the scope of this review, some aspects of this diversity are pre-determined by the structure of the gynoecium, and will be discussed here.

The gynoecium is functionally divided into stigma, style, and ovary along its apical–basal axis, and is attached to the receptacle via a stalk, the gynophore. The stigma in Brassicaceae can be entire or divided into separate lobes, which extend sideways and downward [*Sinapis* sp. (Brassiceae), *Sisymbrium* sp. (Sisymbrieae), and *Berteroa incana* (Alysseae)] or come in close contact (covinnent stigma) creating slit-like receptive surfaces [most members of Lineage III and *Lunaria* spp. (Biscutelleae)] (Huang *et al.*, 2016). The length of the style in relation to the ovary also varies among species and can be relatively short (Arabidopsis) or long (Brassica), often forming a beak in fruit. In some Erysimeae, Stevenieae, Aphragmeae, *Asta*, *Lunaria annua* and *L. rediviva*, Cremolobeae, and almost all Thelypodieae, the gynophore is well developed, which may represent a shared plesiomorphy with Capparaceae and Cleomaceae (Endress, 1992; Appel and Al-Shehbaz, 2003; Huang *et al.*, 2016).

Patterning along the apical–basal axis of the gynoecium is under hormonal control. An auxin maximum at the stigma and the fact that auxin synthesis, transport, and signaling mutants in Arabidopsis, such as *PIN-FORMED1*, *PINOID*, *ETTIN* (*ETT*), and *MONOPTEROS*, often exhibit patterning defects along the apical–basal axis suggest the existence of an auxin gradient that controls the differentiation of the zones of the gynoecium (Larsson *et al.*, 2013). *ETT* appears to be the central component of this network, and its role is conserved between Arabidopsis and *Brassica* (Simonini *et al.*, 2018). A model where the interaction between ETT and its partners INDEHISCENT, BREVIPEDICELLUS, REPLUMLESS, and SEUSS is tuned in different species can explain the evolution of diverse morphologies (Simonini *et al.*, 2018). An opposing cytokinin gradient also has been proposed, and it appears that the crosstalk between these hormonal activities is required for the precise patterning of the gynoecium (Marsch-Martínez *et al.*, 2012; Müller *et al.*, 2017; Zúñiga-Mayo *et al.*, 2019). Modifying the thresholds along the gradients that zone the gynoecium has the potential to create substantial morphological diversity.

Floral color

The color of Brassicaceae flowers is primarily determined by the color of petals and, rarely, the sepals (Figs 2, 3). Floral color evolution has received much attention in the literature in part due to the ecological significance of the trait in modulating plant–pollinator interactions (Wessinger and Rausher, 2012). Invisible to the human eye but particularly striking to certain pollinating insects, such as bees, are the patterns of UV reflectance, which act as nectar guides (Horovitz and Cohen, 1972). Petals appear white because of the refraction of light from the air-filled mesophyll, and this is the biochemical ground state of color variation. Hues of pink, purple, and blue result from the

accumulation of anthocyanins in Halimolobeae, Malcolmieae, Aphragmeae, Anchonieae, Calepineae, Coluteocarpeae, Heliophileae, Iberideae, Hesperideae, and Donstostemoneae (Appel and Al-Shehbaz, 2003). In well-established models for floral color evolution, such as *Penstemon* (Plantaginaceae), *Clarkia* (Onagraceae), *Phlox* (Polemoniaceae), and *Iochroma* (Solanaceae), change in color is largely due to the expression and activity of enzymes that transform colorless compounds into colored anthocyanins, the most common of which are red pelargonidin, and blue–violet cyanidin and delphinidin (reviewed in Wessinger and Rausher, 2012). Although there is significant intraspecific and interspecific variation of anthocyanin-based color in Brassicaceae, studies on the genetic basis of this trait are rare. Polymorphic populations with differently colored sympatric morphs [*Hesperis matronalis* (Hesperideae); Majetic *et al.*, 2007] and morphs that exhibit a latitudinal gradient [*Parrya nudicaulis* (Chorisporeae) in Alaska; Dick *et al.*, 2011] are attractive systems to study how color variation affects interactions with mutualists and the environment. The color of anthocyanins, which are water-soluble vacuolar pigments, is determined by the acidity of the vacuolar content and the activity of proton pumps in the vacuolar membrane (Koes *et al.*, 2005). The transition in floral color in different species and during development as a result of changes in the acidity of the vacuolar sap is not well understood.

Yellow and orange corollas characterize a number of Brassicaceae clades, including Brassiceae, Buniadeae, Sisymbrieae, Isatideae, Bivonaeeae, Biscutelleae, Turritideae, Erysimeae, Descurainieae, Alyssopsideae, and Smelowskieae, among others (Appel and Al-Shehbaz, 2003). The color arises from the accumulation of lipid-soluble carotenoids in the chromoplasts of epidermal cells or of yellow-colored aurone flavonoids (Davies *et al.*, 1998; Tanaka *et al.*, 2008). Color-based polymorphism in *Brassica napus* (Brassiceae), where some accessions have yellow flowers and others have white flowers, segregates as a single locus, and the white flower phenotype is dominant (Zhang *et al.*, 2015). The locus responsible is a homolog of *Carotenoid cleavage dioxygenase 4* (*CCD4*) and encodes an enzyme that breaks down the colored carotenoids into colorless volatile compounds. There are several *CCD4* loss-of-function alleles in different Brassica crops, suggesting that the transition from white to yellow flowers has occurred independently multiple times (Zhang *et al.*, 2015). In addition to their role in pollinator attraction, carotenoids perform essential roles in photosynthesis, abscisic acid and strigolactone synthesis, and volatile signaling (Nisar *et al.*, 2015; Sun *et al.*, 2018). The homolog responsible for floral color polymorphism (*C3.CCD4*) is one of four *CCD4* copies in the genome of *B. napus*, which is specifically expressed in the petals (Zhang *et al.*, 2015). The presence of several *CCD4* copies allowed for the evolution of novel traits, while other paralogs retained the essential ancestral function.

Parsimoniously, the transitions between white and pink/purple color and between white and yellow color require in the simplest case a single step, resulting from the modification of enzymes in the same chemical pathways. The presence of different color morphs in the same species demonstrates that such transitions are common (Majetic *et al.*, 2007; Dick *et al.*, 2011). Transitions between yellow and pink/purple, as observed in *Erysimum* (Gómez *et al.*, 2015), are rare and require at least two separate modifications, the loss of yellow pigments and the gain of anthocyanin accumulation. How coupling of these changes occurs without a white-flowered intermediate warrants further investigation.

Concluding remarks and future directions

The conserved floral organization throughout the phylogenetic breadth of Brassicaceae is interspersed with variation in almost every aspect of the floral morphology, with most of the diversity confined to floral construction and mode. Exceptional character states, such as monosymmetry, apetaly, stamen reduction, and organ fusion, have evolved apparently independently in several disparate lineages in the family, raising questions about the mechanistic basis of the repeatability of morphological evolution and the extent to which the developmental program can be modified. Examining such exceptional cases in floral morphology informs on the constraints that contribute to the morphological stasis in mustard flowers. Why is the floral plan of Brassicaceae more uniform compared with the floral diversity of their relatives in Capparaceae and Cleomaceae? Also, why is floral uniformity in Brassicaceae so prevalent when many vegetative characters, such as leaf shape (Nicotra *et al.*, 2011), vary considerably among species?

Addressing these questions requires a better understanding of the floral morphospace of Brassicacae through systematic ontogenetic studies of floral diversity through space and time and its developmental basis. Targeted genetic studies in experimentally tractable model taxa, enabled by comparative genomics in species that cannot be genetically manipulated in the lab, will provide mechanistic insight into the evolution of various character states and the genetic basis of morphological diversity. Modifying known developmental regulators to alter morphology, complemented by observations in the field, will offer novel insights into the adaptive significance of floral traits and their contribution to the interaction between plants and their mutualists. In addition to visual cues, such as floral color and geometry, the perception of a flower from a pollinator's standpoint depends on olfactory (Sas *et al.*, 2016), gustatory (Nepi *et al.*, 2018), and tactile (Whitney *et al.*, 2011) cues. These cues attract and orient the pollinators to provide a reliable transfer of gametes and offer additional dimensions for the study of floral diversity. Finally, the structural and developmental insight will provide ways to introduce valuable traits into crop species to increase pollination services and improve ecosystem resilience in the face of global anthropogenic challenges.

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References

Abraham MC, Metheetrairut C, Irish VF. 2013. Natural variation identifies multiple loci controlling petal shape and size in *Arabidopsis thaliana*. PLoS One **8**, e56743.

Al-Shehbaz IA. 2001. A review of gamosepaly in the Brassicaceae and a revision of *Desideria,* with a critical evaluation of related genera. Annals of the Missouri Botanical Garden **87**, 549–563.

Al-Shehbaz IA. 2012. A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon **61**, 931–954.

Appel O, Al-Shehbaz IA. 2003. Cruciferae. In: Kubitzki K, Bayer C, eds. The families and genera of vascular plants, Vol. **5**. Berlin: Springer Verlag, 75–174.

Arber A. 1931. Studies in floral morphology. I. On some structural features of the cruciferous flower. New Phytologist **30**, 11–41.

Avino M, Kramer EM, Donohue K, Hammel AJ, Hall JC. 2012. Understanding the basis of a novel fruit type in Brassicaceae: conservation and deviation in expression patterns of six genes. EvoDevo **3**, 20.

Bani B, Adiguzel N. 2006. A new genus record for Turkey: *Asperuginoides* Rauschert. International Journal of Botany **2**, 440–442.

Bayat S, Schranz ME, Roalson EH, Hall JC. 2018. Lessons from Cleomaceae, the sister of crucifers. Trends in Plant Science **23**, 808–821.

Beilstein MA, Al-Shehbaz IA, Kellogg EA. 2006. Brassicaceae phylogeny and trichome evolution. American Journal of Botany **93**, 607–619.

Bell AD. 2008. Plant form: an illustrated guide to flowering plant morphology. Portland, OR: Timber Press.

Berger Y, Harpaz-Saad S, Brand A, Melnik H, Sirding N, Alvarez JP, Zinder M, Samach A, Eshed Y, Ori N. 2009. The NAC-domain transcription factor GOBLET specifies leaflet boundaries in compound tomato leaves. Development **136**, 823–832.

Blein T, Pulido A, Vialette-Guiraud A, Nikovics K, Morin H, Hay A, Johansen IE, Tsiantis M, Laufs P. 2008. A conserved molecular framework for compound leaf development. Science **322**, 1835–1839.

Bosch JA, Heo K, Sliwinski MK, Baum DA. 2008. An exploration of *LEAFY* expression in independent evolutionary origins of rosette flowering in Brassicaceae. American Journal of Botany **95**, 286–293.

Bowman JL. 1994. Arabidopsis: an atlas of morphology and development. New York: Springer-Verlag.

Bowman JL. 2006. Molecules and morphology: comparative development genetics of the Brassicaceae. Plant Systematics and Evolution **259**, 199–215.

Bowman JL, Brüggemann H, Lee JY, Mummenhoff K. 1999. Evolutionary changes in floral structure within *Lepidium* L. (Brassicaceae). International Journal of Plant Sciences **160**, 917–929.

Bowman JL, Smyth DR. 1998. Patterns of petal and stamen reduction in Australian species of *Lepidium* L. (Brassicaceae). International Journal of Plant Sciences **159**, 65–74.

BrassiBase. 2019. Tools and biological resources for Brassicaceae character and trait studies. https://brassibase.cos.uni-heidelberg.de. Accessed 12 February 2019.

Busch A, Horn S, Mühlhausen A, Mummenhoff K, Zachgo S. 2012. Corolla monosymmetry: evolution of a morphological novelty in the Brassicaceae family. Molecular Biology and Evolution **29**, 1241–1254.

Busch A, Horn S, Zachgo S. 2014. Differential transcriptome analysis reveals insight into monosymmetric corolla development of the crucifer *Iberis amara*. BMC Plant Biology **14**, 285.

Busch A, Zachgo S. 2007. Control of corolla monosymmetry in the Brassicaceae *Iberis amara*. Proceedings of the National Academy of Sciences, USA **104**, 16714–16719.

Cardinal-McTeague WM, Sytsma KJ, Hall JC. 2016. Biogeography and diversification of Brassicales: a 103million year tale. Molecular Phylogenetics and Evolution **99**, 204–224.

Causier B, Schwarz-Sommer Z, Davies B. 2010. Floral organ identity: 20 years of ABCs. Seminars in Cell & Developmental Biology **21**, 73–79.

Cheng Y, Zhao Y. 2007. A role for auxin in floral development. Journal of Integrative Plant Biology **49**, 99–104.

Chuang CF, Running MP, Williams RW, Meyerowitz EM. 1999. The *PERIANTHIA* gene encodes a bZIP protein involved in the determination of floral organ number in *Arabidopsis thaliana*. Genes & Development **13**, 334–344.

Coen E, Kennaway R, Whitewoods C. 2017. On genes and form. Development **144**, 4203–4213.

Dick CA, Buenrostro J, Butler T, Carlson ML, Kliebenstein DJ, Whittall JB. 2011. Arctic mustard flower color polymorphism controlled by petal-specific downregulation at the threshold of the anthocyanin biosynthetic pathway. PLoS One **6**, e18230.

Davies KM, Bloor SJ, Spiller GB, Deroles SC. 1998. Production of yellow color in flowers: redirection of flavonoid biosynthesis in Petunia. The Plant Journal **13**, 259–266.

Dinneny JR, Yadegari R, Fischer RL, Yanofsky MF, Weigel D. 2004. The role of *JAGGED* in shaping lateral organs. Development **131**, 1101–1110.

Edger PP, Hall JC, Harkess A, *et al*. 2018. Brassicales phylogeny inferred from 72 plastid genes: a reanalysis of the phylogenetic localization of two paleopolyploid events and origin of novel chemical defenses. American Journal of Botany **105**, 463–469.

Edger PP, Heidel-Fischer HM, Bekaert M, *et al*. 2015. The butterfly plant arms-race escalated by gene and genome duplications. Proceedings of the National Academy of Sciences, USA **112**, 8362–8366.

Eldridge T, Łangowski Ł, Stacey N, *et al*. 2016. Fruit shape diversity in the Brassicaceae is generated by varying patterns of anisotropy. Development **143**, 3394–3406.

Endress PK. 1992. Evolution and floral diversity: the phylogenetic surroundings of Arabidopsis and Antirrhinum. International Journal of Plant Sciences **153**, S106–S122.

Endress PK. 1994. Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge University Press.

Endress PK. 2006. Angiosperm floral evolution: morphological developmental framework. Advances in Botanical Research **44**, 1–61.

Endress PK. 2010*a*. Floral structure and trends of evolution in eudicots and their major subclades. Annals of the Missouri Botanical Garden **97**, 541–583.

Endress PK. 2010*b*. Disentangling confusions in inflorescence morphology: patterns and diversity of reproductive shoot ramification in angiosperms. Journal of Systematics and Evolution **48**, 225–239.

Endress PK. 2016. Development and evolution of extreme synorganization in angiosperm flowers and diversity: a comparison of Apocynaceae and Orchidaceae. Annals of Botany **117**, 749–767.

Franzke A, Lysak MA, Al-Shehbaz IA, Koch MA, Mummenhoff K. 2011. Cabbage family affairs: the evolutionary history of Brassicaceae. Trends in Plant Science **16**, 108–116.

Fujikura U, Jing R, Hanada A, Takebayashi Y, Sakakibara H, Yamaguchi S, Kappel C, Lenhard M. 2018. Variation in splicing efficiency underlies morphological evolution in *Capsella*. Developmental Cell **44**, 192–203.e5.

Galstyan A, Hay A. 2018. Snap, crack and pop of explosive fruit. Current Opinion in Genetics and Developement **69**, 863–878.

Gómez JM, Perfectti F, Lorite J. 2015. The role of pollinators in floral diversification in a clade of generalist flowers. Evolution **69**, 863–878.

Gómez JM, Torices R, Lorite J, Klingenberg CP, Perfectti F. 2016. The role of pollinators in the evolution of corolla shape variation, disparity and integration in a highly diversified plant family with a conserved floral bauplan. Annals of Botany **117**, 889–904.

Guo Y-L, Bechsgaard JS, Slotte T0, Neuffer B, Lascoux M, Weigel D, Schierup MH. 2009. Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. Proceedings of the National Academy of Sciences, USA **106**, 5246–5251.

Hameister S, Neuffer B, Bleeker W. 2009. Genetic differentiation and reproductive isolation of a naturally occurring floral homeotic mutant within a wild-type population of *Capsella bursa-pastoris* (Brassicaceae). Molecular Ecology **18**, 2659–2667.

Hameister S, Nutt O, Theissen G, Neuffer B. 2013. Mapping a floral trait in Shepherd purse—'stamenoid petals' in natural populations of *Capsella bursa-pastoris* (L.) Medik. Flora **208**, 641–647.

Hanzawa Y, Money T, Bradley D. 2005. A single amino acid converts a repressor to an activator of flowering. Proceedings of the National Academy of Sciences, USA **102**, 7748–7753.

Hay A, Tsiantis M. 2016. *Cardamine hirsuta*: a comparative view. Current Opinion in Genetics & Development **39**, 1–7.

Hay AS, Pieper B, Cooke E, *et al*. 2014. *Cardamine hirsuta*: a versatile genetic system for comparative studies. The Plant Journal **78**, 1–15.

Heenan PB, Mitchell AD, McLenachan PA, Lockhart PJ, de Lange PJ. 2007. Natural variation and conservation of *Lepidium sisymbrioides* Hook.f. and *L. solandri* Kirk (Brassicaceae) in South Island, New Zealand, based on morphological and DNA sequence data. New Zealand Journal of Botany **45**, 237–264.

Hervieux N, Dumond M, Sapala A, Routier-Kierzkowska A-L, Kierzkowski D, Roeder AHK, Smith RS, Boudaoud A, Hamant O. 2016. A mechanical feedback restricts sepal growth and shape in *Arabidopsis*. Current Biology **26**, P1019–1028.

Hileman LC. 2014. Trends in floral symmetry evolution revealed through phylogenetic and developmental genetic advances. Philosophical Transactions of the Royal Society B: Biological Sciences **369**, 20130348.

Hintz M, Bartholmes C, Nutt P, Ziermann J, Hameister S, Neuffer B, Theissen G. 2006. Catching a 'hopeful monster': shepherd's purse (*Capsella bursa-pastoris*) as a model system to study the evolution of flower development. Journal of Experimental Botany **57**, 3531–3542.

Horovitz A, Cohen Y. 1972. Ultraviolet reflectance characteristics in flowers of Cruciferae. American Journal of Botany **59**, 706–713.

Huang CH, Sun R, Hu Y, *et al*. 2016. Resolution of brassicaceae phylogeny using nuclear genes uncovers nested radiations and supports convergent morphological evolution. Molecular Biology and Evolution **33**, 394–412.

Irish V. 2017. The ABC model of floral development. Current Biology **27**, R853–R909.

Koenig D, Weigel D. 2015. Beyond the thale: comparative genomics and genetics of *Arabidopsis* relatives. Nature Reviews. Genetics **16**, 285–298.

Koes RE, Verweij CW, Quattrocchio F. 2005. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends in Plant Sciences **5**, 236–242.

Lampugnani ER, Kilinc A, Smyth DR. 2013. Auxin controls petal initiation in *Arabidopsis*. Development **140**, 185–194.

Łangowski Ł, Stacey N, Østergaard L. 2016. Diversification of fruit shape in the Brassicaceae family. Plant Reproduction **29**, 149–163.

Larsson E, Franks RG, Sundberg E. 2013. Auxin and the *Arabidopsis thaliana* gynoecium. Journal of Experimental Botany **64**, 2619–2627.

Lee J-Y, Mummenhoff K, Bowman JL. 2002. Allopolyploidization and evolution of species with reduced floral structures in *Lepidium* L. (Brassicaceae). Proceedings of the National Academy of Sciences, USA **99**, 16835–16840.

Lenser T, Graeber K, Cevik ÖS, *et al*. 2016. Developmental control and plasticity of fruit and seed dimorphism in *Aethionema arabicum*. Plant Physiology **172**, 1691–1707.

Li M, Sack FD. 2014. Myrosin idioblast cell fate and development are regulated by the Arabidopsis transcription factor FAMA, the auxin pathway, and vesicular trafficking. The Plant Cell **26**, 4053–4066.

Li N, Liu Z, Wang Z, *et al*. 2018. *STERILE APETALA* modulates the stability of a repressor protein complex to control organ size in *Arabidopsis thaliana*. PLoS Genetics **14**, e1007218.

Liu N, Sliwinski MK, Correa R, Baum DA. 2011. Possible contributions of *TERMINAL FLOWER 1* to the evolution of rosette flowering in Leavenworthia (Brassicaceae). New Phytologist **189**, 616–628.

Long J, Barton MK. 2000. Initiation of axillary and floral meristems in *Arabidopsis*. Developmental Biology **218**, 341–353.

Lopez L, Wolf EM, Pires JC, Edger PP, Koch MA. 2017. Molecular resources from transcriptomes in the Brassicaceae family. Frontiers in Plant Science **8**, 1488.

Luo D, Carpenter R, Vincent C, Copsey L, Coen E. 1996. Origin of floral asymmetry in *Antirrhinum*. Nature **383**, 794–799.

Maier AT, Stehling-Sun S, Offenburger SL, Lohmann JU. 2011. The bZIP transcription factor *PERIANTHIA*: a multifunctional hub for meristem control. Frontiers in Plant Science **2**, 79.

Majetic CJ, Raguso RA, Tonsor SJ, Ashman TL. 2007. Flower color–flower scent associations in polymorphic *Hesperis matronalis* (Brassicaceae). Phytochemistry **68**, 865–874.

Mandáková T, Hloušková P, German DA, Lysak MA. 2017*a*. Monophyletic origin and evolution of the largest crucifer genomes. Plant Physiology **174**, 2062–2071.

Mandáková T, Li Z, Barker MS, Lysak MA. 2017*b*. Diverse genome organization following 13 independent mesopolyploid events in Brassicaceae contrasts with convergent patterns of gene retention. The Plant Journal **91**, 3–21.

Marsch-Martínez N, Ramos-Cruz D, Reyes-Olalde JI, Lozano-Sotomayor P, Zúñiga-Mayo VM, de Folter S. 2012. The role of cytokinin during Arabidopsis gynoecia and fruit morphogenesis and patterning. The Plant Journal **174**, 2062–2071.

Matthews ML, Endress PK. 2006. Floral structure and systematics in four orders of rosids including a broad survey of floral mucilage cells. Plant Systematics and Evolution **260**, 199–221.

Matsuhashi S, Sakai S, Kudoh H. 2012. Temperature-dependent fluctuation of stamen number in *Cardamine hirsuta* (Brassicaceae). International Journal of Plant Sciences **173**, 391–398.

Maugarny A, Gonçalves B, Arnaud N, Laufs P. 2015. CUC transcription factors: to the meristem and beyond. In: Gonzalez DH, ed. Plant transcription factors: evolutionary, structural and functional aspects. Cambridge, MA: Academic Press, 229–247.

Méndez M, Gómez JM. 2006. Phenotypic gender in *Hormathophylla spinosa* (Brassicaceae), a perfect hermaphrodite with tetradynamous flowers, is variable. Plant Systematics and Evolution **262**, 225–237.

Merxmüller H, Leins P. 1967. Die verwandtschaftsbeziehungen der kreuzblütler und mohngewächse. Botanische Jahrbücher für Systematik **86**, 113–129.

Meyer HM, Teles J, Formosa-Jordan P, Refahi Y, San-Bento R, Ingram G, Jonsson H, Locke JCW, Roeder AHK. 2017. Fluctuations of the transcription factor ATML1 generate the pattern of giant cells in the *Arabidopsis* sepal. eLife **6**, e19131.

Meyerowitz EM, Bowman JL, Brockman LL, Drews GN, Jack T, Sieburth LE, Weigel D. 1991. A genetic and molecular model for flower development in *Arabidopsis thaliana*. Development Supplement **1**, 157–167.

Monniaux M, Pieper B, Hay A. 2016. Stochastic variation in *Cardamine hirsuta* petal number. Annals of Botany **117**, 881–887.

Monniaux M, Pieper B, McKim SM, Routier-Kierzkowska A-L, Kierzkowski D, Smith RS, Hay A. 2018. The role of *APETALA1* in petal number robustness. eLife **7**, e39399.

Mühlhausen A, Lenser T, Mummenhoff K, Theißen G. 2013. Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes. The Plant Journal **73**, 824–835.

Müller CJ, Larsson E, Spíchal L, Sundberg E. 2017. Cytokinin–auxin crosstalk in the gynoecial primordium ensures correct domain patterning. Plant Physiology **175**, 1144–1157.

Mummenhoff K, Al-Shehbaz IA, Bakker FT, Linder HP, Mühlhausen A. 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of southern Africa. Annals of the Missouri Botanical Garden **92**, 400–424.

Mummenhoff K, Polster A, Mühlhausen A, Theissen G. 2009. *Lepidium* as a model system for studying the evolution of fruit development in Brassicaceae. Journal of Experimental Botany **60**, 1503–1513.

Nepi M, Grasso DA, Mancuso S. 2018. Nectar in plant–insect mutualistic relationships: from food reward to partner manipulation. Frontiers in Plant Science **9**, 1063.

Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H. 2011. The evolution and functional significance of leaf shape in the angiosperms. Functional Plant Biology **38**, 535–552.

Nikolov LA, Shushkov PS, Nevado B, Gan X, Al-Shehbaz IA, Filatov D, Bailey CD, Tsiantis M. 2019. Resolving the backbone of the Brassicaceae phylogeny for investigating trait diversity. New Phytologist (in press).

Nikolov LA, Tsiantis M. 2017. Using mustard genomes to explore the genetic basis of evolutionary change. Current Opinion in Plant Biology **36**, 119–128.

Nisar N, Li L, Lu S, Khin NC, Pogson BJ. 2015. Carotenoid metabolism in plants. Molecular Plant **8**, 68–82.

Nutt P, Ziermann J, Hintz M, Neuffer B, Theissen G. 2006. Capsella as a model system to study the evolutionary relevance of floral homeotic mutants. Plant Systematics and Evolution **259**, 217–235.

Ohno CK, Reddy GV, Heisler MG, Meyerowitz EM. 2004. The *Arabidopsis JAGGED* gene encodes a zinc finger protein that promotes leaf tissue development. Development **131**, 1111–1122.

Ó'Maoiléidigh DS, Graciet E, Wellmer F. 2014. Gene networks controlling *Arabidopsis thaliana* flower development. New Phytologist **201**, 16–30.

Ó'Maoiléidigh DS, Stewart D, Zheng B, Coupland G, Wellmer F. 2018. Floral homeotic proteins modulate the genetic program for leaf development to suppress trichome formation in flowers. Development **201**, 16–30.

Pieper B, Monniaux M, Hay A. 2016. The genetic architecture of petal number in *Cardamine hirsuta*. New Phytologist **209**, 395–406.

Provart NJ, Alonso J, Assmann SM, *et al*. 2016. 50 years of *Arabidopsis* research: highlights and future directions. New Phytologist **209**, 921–944.

Prunet N, Yang W, Das P, Meyerowitz EM, Jack TP. 2017. *SUPERMAN* prevents class B gene expression and promotes stem cell termination in the fourth whorl of *Arabidopsis thaliana* flowers. Proceedings of the National Academy of Sciences, USA **114**, 7166–7171.

Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E. 2007. Evolution and development of inflorescence architectures. Science **316**, 1452–1456.

Rollins RC. 1993. The Cruciferae of Continental North America. Stanford, CA: Stanford University Press.

Ronse de Craene LP. 2010. Floral diagrams: an aid to understanding floral morphology and evolution. Cambridge: Cambridge University Press.

Sas C, Müller F, Kappel C, Kent TV, Wright SI, Hilker M, Lenhard M. 2016. Repeated inactivation of the first committed enzyme underlies the loss of benzaldehyde emission after the selfing transition in *Capsella*. Current Biology **26**, 3313–3319.

Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T. 2000. The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell **100**, 635–644.

Shu G, Amaral W, Hileman LC, Baum DA. 2000. LEAFY and the evolution of rosette flowering in violet cress (*Jonopsidium acaule*, Brassicaceae). American Journal of Botany **87**, 634–641.

Sicard A, Kappel C, Lee YW, Wozniak NJ, Marona C, Stinchcombe JR, Wright SI, Lenhard M. 2016. Standing genetic variation in a tissue-specific enhancer underlies selfing-syndrome evolution in *Capsella*. Proceedings of the National Academy of Sciences, USA **113**, 13911–13916.

Sicard A, Lenhard M. 2011. The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. Annals of Botany **107**, 1433–1443.

Sicard A, Stacey N, Hermann K, Dessoly J, Neuffer B, Bäurle I, Lenhard M. 2011. Genetics, evolution, and adaptive significance of the selfing syndrome in the genus *Capsella*. The Plant Cell **23**, 3156–3171.

Simonini S, Stephenson P, Østergaard L. 2018. A molecular framework controlling style morphology in Brassicaceae. Development **145**, dev158105.

Sliwinski MK, Bosch JA, Yoon HS, Balthazar Mv, Baum DA. 2007. The role of two *LEAFY* paralogs from *Idahoa scapigera* (Brassicaceae) in the evolution of a derived plant architecture. The Plant Journal **51**, 211–219.

Sliwinski MK, White MA, Maizel A, Weigel D, Baum DA. 2006. Evolutionary divergence of LFY function in the mustards *Arabidopsis thaliana* and *Leavenworthia crassa*. Plant Molecular Biology **62**, 279–289.

Smith SD. 2016. Pleiotropy and the evolution of floral integration. New Phytologist **209**, 80–85.

Smyth DR, Bowman JL, Meyerowitz EM. 1990. Early flower development in Arabidopsis. The Plant Cell **2**, 755–767.

Soza VL, Le Huynh V, Di Stilio VS. 2014. Pattern and process in the evolution of the sole dioecious member of Brassicaceae. EvoDevo **5**, 42.

Spencer V, Kim M. 2018. Re'CYC'ling molecular regulators in the evolution and development of flower symmetry. Seminars in Cell & Developmental Biology **79**, 16–26.

Stewart D, Graciet E, Wellmer F. 2016. Molecular and regulatory mechanisms controlling floral organ development. FEBS Journal **283**, 1823–1830.

Sun T, Yuan H, Cao H, Yazdani M, Tadmor Y, Li L. 2018. Carotenoid metabolism in plants: the role of plastids. Molecular Plant **11**, 58–74.

Tanaka Y, Sasaki N, Ohmiya A. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The Plant Journal **54**, 733–749.

Theissen G. 2010. Homeosis of the angiosperm flower: studies on three candidate cases of saltational evolution. Paleodiversity **3** (Supplement**),** 131–139.

Theissen G, Melzer R. 2016. Robust views on plasticity and biodiversity. Annals of Botany **117**, 693–697.

Theissen G, Rümpler F. 2018. Evolution of floral organ identity. In: Nuno de la Rosa L, Müller G, eds. Evolutionary developmental biology. Cham: Springer.

Weberling F. 1989. Morphology of flowers and inflorescences. Cambridge: Cambridge University Press, 1–17.

Wellmer F. 2017. Floral development. In: Thomas B, Murphy DJ,Murray BG, eds. Encyclopedia of applied plant sciences, 2nd edn, Vol. **2**, Cambridge, MA: Academic Press, 269–274.

Wessinger CA, Rausher MD. 2012. Lessons from flower colour evolution on targets of selection. Journal of Experimental Botany **63**, 5741–5749.

Whipple CJ, Hall DH, DeBlasio S, Taguchi-Shiobara F, Schmidt RJ, Jackson DP. 2010. A conserved mechanism of bract suppression in the grass family. The Plant Cell **22**, 565–578.

Whitney HM, Bennett KM, Dorling M, Sandbach L, Prince D, Chittka L, Glover BJ. 2011. Why do so many petals have conical epidermal cells? Annals of Botany **108**, 609–616.

Yoon HS, Baum DA. 2004. Transgenic study of parallelism in plant morphological evolution. Proceedings of the National Academy of Sciences, USA **101**, 6524–6529.

Yuan YW, Byers KJ, Bradshaw HD Jr. 2013. The genetic control of flower-pollinator specificity. Current Opinion in Plant Biology **16**, 422–428.

Zhang B, Liu C, Wang Y, Yao X, Wang F, Wu J, King GJ, Liu K. 2015. Disruption of a *CAROTENOID CLEAVAGE DIOXYGENASE 4* gene converts flower colour from white to yellow in Brassica species. New Phytologist **206**, 1513–1526.

Zúñiga-Mayo VM, Gómez-Felipe A, Herrera-Ubaldo H, de Folter S. 2019. Gynoecium development: networks in Arabidopsis and beyond. Journal of Experimental Botany **70** (in press).