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Putting heads together: Developmental genetics of the Asteraceae capitulum

Vandana Gurung, Sarita Muñoz-Gómez and Daniel S. Jones

Abstract

Inflorescence architecture is highly variable across plant lineages yet is critical for facilitating reproductive success. The capitulum-type inflorescence of the Asteraceae is marked as a key morphological innovation that preceded the family's diversification and expansion. Despite its evolutionary significance, our understanding of capitulum development and evolution is limited. This review highlights our current perspective on capitulum evolution through the lens of both its molecular and developmental underpinnings. We attempt to summarize our understanding of the capitulum by focusing on two key characteristics: patterning (arrangement of florets on a capitulum) and floret identity specification. Note that these two features are interconnected such that the identity of florets depends on their position along the inflorescence axis. Phytohormones such as auxin seemingly determine both pattern progression and floret identity specification through unknown mechanisms. Floret morphology in a head is controlled by differential expression of floral symmetry genes regulating floret identity specification. We briefly summarize the applicability of the ABCE quartet model of flower development in regulating the floret organ identity of a capitulum in Asteraceae. Overall, there have been promising advancements in our understanding of capitula; however, comprehensive functional genetic analyses are necessary to fully dissect the molecular pathways and mechanisms involved in capitulum development.

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Capitulum, Patterning, Floret identity, Phytohormones, *CYCLOIDEA*, Auxin, ABCE model, Brassinosteroids, Abscisic acid.

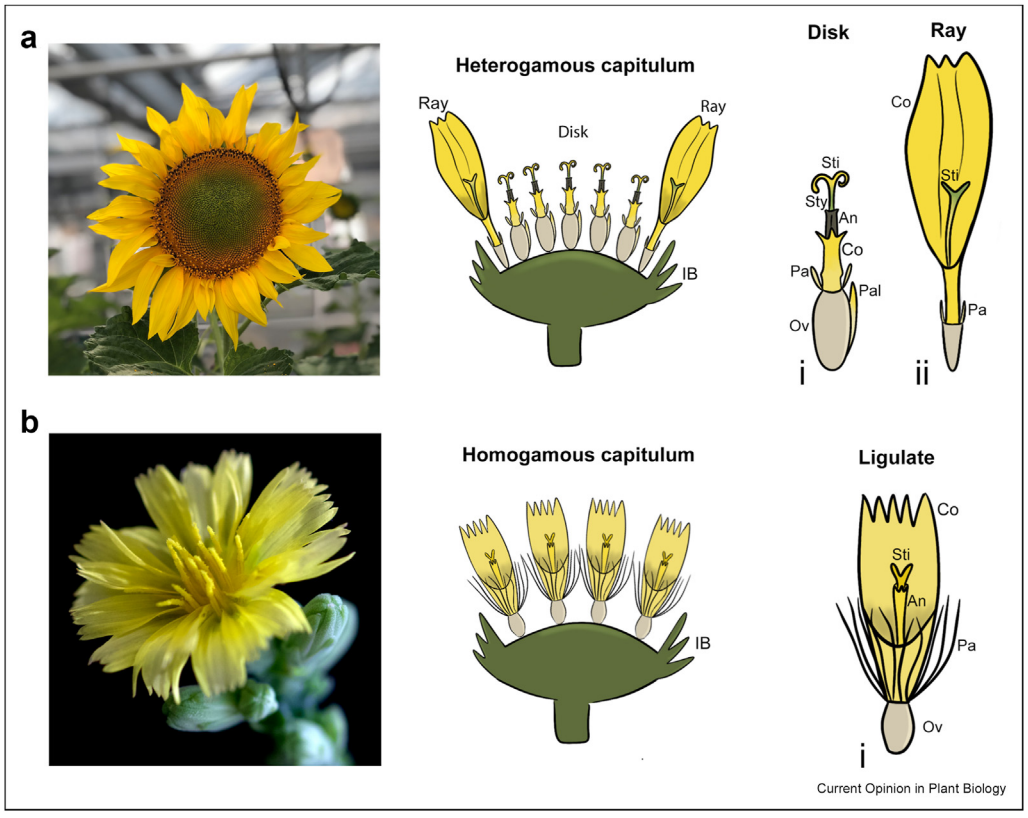
Introduction

The Asteraceae (sunflower family) is the most diverse group of flowering plants, accounting for around 10% (~25,000 species) of all known angiosperm species [1–4]. The family's success has been attributed to the evolution of the capitulum-type inflorescence, a highly specialized floral structure that superficially resembles a solitary flower but is comprised of multiple smaller flowers (florets) arranged in a disk-shaped cluster called a "head" [2,5] (Figure 1). The capitulum is considered a pseudanthium (false flower) that is enclosed by numerous layers of protective involucral bracts [5]. In addition to this shared inflorescence architecture, Asteraceae florets also have a unique modified calyx (outer whorl of floral organs) called pappus which often persists throughout fruit development to facilitate efficient seed dispersal [2], fused petals (corolla tube) and fused anthers (synanther tube) [6]. While these characteristics are shared, overall capitulum organization can vary widely across the family, attributed to the diversity in floret morphologies (specifically corolla symmetry and the presence/absence of reproductive whorls) and their arrangement. For instance, in the Heliantheae tribe, the capitula are typically radiate (a heterogamous head comprised of two floret forms; bilaterally symmetrical pistillate ray florets at the periphery and radially symmetrical disk florets at the center of the capitulum), *e.g.* *Helianthus annuus* (sunflower, Figures 1a and 5). In contrast, the Cichorieae tribe typically has ligulate capitula (a homogamous head comprised of only bilaterally symmetrical florets), *e.g.* *Lactuca sativa* (lettuce, Figures 1b and 5). Keeping this morphological diversity in mind is key to a comprehensive understanding of the genetics underlying capitulum development and evolution, especially when attempting to generalize development among such a large group of plants.

Evolutionary origins of the Asteraceae capitulum

While floral morphology and organization vary, the capitulum-type inflorescence is highly conserved across the family suggesting a single evolutionary origin. How the Asteraceae capitulum evolved remains a debated question; however, two major hypotheses have been proposed. The earliest interpretations suggest that the capitulum is derived from an elongated inflorescence, an indeterminate raceme, or a determinate cyme [5,7]. Supporting this hypothesis, Pozner et al. suggested that

Figure 1

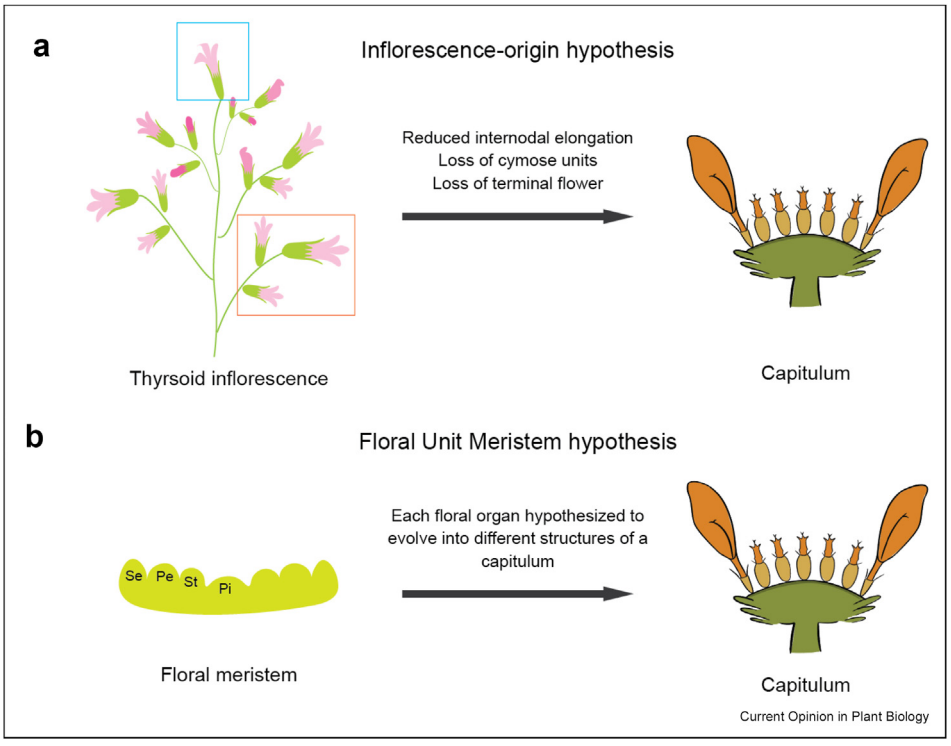


Representative diversity in capitula of Asteraceae. (**a**) Sunflower (*H. annuus,* Heliantheae) exhibit heterogamous capitulum, composed of disk florets with tubular corolla tubes formed by five fused petals (i), and true rays formed by three fused petals (ii). (**b**) In lettuce (*Lactuca sativa*, Cichorieae), capitula are homogamous and carry only ligulate florets that result from the fusion of five petals (i). **An**, Anther; **Co**, Corolla; **IB**; Involucral Bracts; **Ov**, ovary; **Pa**, Pappus; **Pal**; Pales; **Sty**, Style; **Sti**, Stigma.

a capitulum evolved from a thyrsoid inflorescence (a primary determinate racemose axis with basal lateral cymose branches) [8], via a sequential process of reduced internodal elongation, loss of basal cymose unites and terminal flower (Figure 2a) [9]. Examination of inflorescence development in the Calyceraceae, sister to the Asteraceae, indicates that both families likely share an ancestral cephalioid inflorescence form (a condensed thyrsoid inflorescence). More recently, Claßen-Bockhoff and Bull-Hereñu proposed that capitula develop from floral unit meristems (FUMs) and may not be considered true inflorescence meristems based on ontogenetic and histological similarity with floral meristems (Figure 2b) [10]. These similarities include a "naked" stage of development where the meristem expands in size without producing organs, and organ initiation occurs as fractionating subunits in contrast to acropetal development for flower formation. This hypothesis suggests that the evolution of the Asteraceae capitulum is linked to changes in meristem regulation of a thyrsoid terminal flower, indicating a floral meristem origin of the capitulum.

Recent genetic studies have been particularly focused on gathering molecular evidence to support either of these evolutionary hypotheses. To test a floral meristem origin of the capitulum, the flower identity genes *UNUSUAL FLORAL ORGANS* (*UFO*) and *LEAFY* (*LFY*) were functionally analyzed in the Asteraceae developmental model system, *Gerbera hybrida* (Mutisieae; Figure 5). These genes have been implicated to play a role in the establishment of inflorescence meristem identity in *G. hybrida* based on their expression pattern and functional analysis [11]. Transcriptional knockdown of *G. hybrida LFY (GhLFY*) resulted in continued meristematic activity at the center, forming floral primordia in a random manner at the center of the capitulum. Overexpression of *G. hybrida UFO* (*GhUFO*) caused the formation of whorls of floral organs instead of floral primordia at the center of the capitulum suggesting its function in determining the floral fate of capitula [11]. Severe suppression of both genes resulted in the conversion of floral organs to bract-like structures indicating its function in floret specification, which is consistent with their expression in floral primordia during early

Figure 2



Proposed hypotheses for capitulum origin. **a)** Inflorescence-origin hypothesis suggests that a capitulum is evolutionarily derived from a thyrsoid inflorescence. **b)** Floral unit meristem hypothesis proposes a floral meristem origin of capitulum. **Se**, Sepal; **Pe**, Petal; **St**, Stamen; **Pi**, Pistil. Terminal flower in blue box. Cymose unit in orange box.

stages of development. Additionally, *GhLFY* is also suspected to play a role in ray floret development as its suppression resulted in advanced organ initiation and branching of floret primordia to produce additional primordia. This pattern of development was found to be similar to the development of branched cymose units of Calyceraceae, a sister lineage to Asteraceae [9,11]. This study suggests that a capitulum meristem is analogous to a floral meristem. However, the pleiotropic effects of *GhLFY* on ray floret identity indicate that rays may have arisen from the peripheral cymose branches of Calyceraceae inflorescence through the gain of floral identity [11,12], which by extension suggests that a capitulum is derived from an inflorescence. In sunflower, the *missing flowers* (*mf*) mutant was reported to exhibit loss of axillary meristems and ray florets, and a considerable reduction in disk florets count [13]. The underlying mutation was found to be in *H. annuus REGULATOR OF AXILLARY MERISTEM FORMATION-LIKE* gene (*HaROXL*) and its transient silencing via VIGS (Virus-induced gene silencing) resulted in reduced ray and disk florets production, mimicking the mutant phenotype [14]. Because the gene seemingly affects axillary meristem and ray floret fate, it can be speculated that rays are derived from shoots developing from the axillary meristem. Thus, depending on interpretation, both hypotheses are supported by different studies, further complicating our understanding of Asteraceae capitulum evolution. Therefore, it is important to develop functional genetics tools to decipher the molecular basis of capitulum formation and organization. This review takes a dive into the architecture of a typical capitulum, while focusing on two important features-pattern formation and floret identity specification.

Patterning during capitulum development

Capitula have multiple levels of pattern formation during development: 1) head patterning (*i.e.*, typically spiral arrangement of florets across the head) and 2) floret patterning (*i.e.*, florets are often organized into peripheral and central floret forms, typically with unique identities and morphologies). Floret initiation follows a spiral phyllotactic arrangement, wherein the florets appear at a 137.5° divergence angle between two consecutive floret initials [15]. These florets are formed along curved lines known as parastiches (*para*; beside, *stiches*; row) that follow clockwise and counter-clockwise patterns in a Fibonacci series progression [15]. In a Fibonacci series, the summation of two preceding numbers forms the third number: 1, 1, 2, 3, 5, 8, 13, 21, 34 …, and spirals of heads follow the consecutive numbers of this series [4,16,17]. Spiral arrangements, albeit very common in plants, are especially intriguing in Asteraceae species where florets are positioned on a

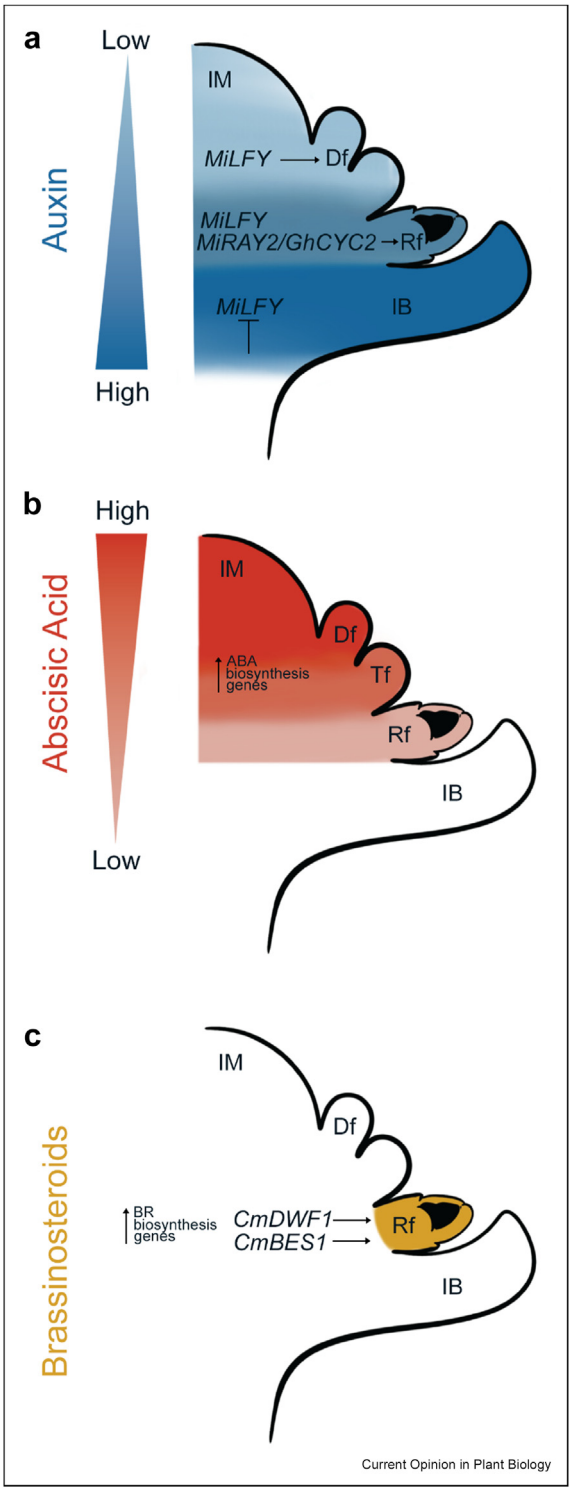
wide, flat receptacle in a high-order Fibonacci progression (*e.g.* sunflower display 89 clockwise and 144 counter-clockwise spirals [15,16]). Head patterning likely requires the precise regulation of many genes induced by the movement of a diffusible signal (*e.g.* a morphogen) [18,19]. Phytohormones are suitable candidates for this function considering their highly conserved role in regulating organogenesis and pattern formation across plants [18]. Other candidates include small peptide hormones, transcription factors, and mobile small RNAs, all of which have demonstrated roles in patterning during plant development [20,21].

The phytohormone auxin has been established as a key highly conserved regulator of organogenesis and pattern formation in the shoot across plants. Auxin efflux carriers, such as PINFORMED1 (PIN1) family proteins, establish floral primordia positioning by transporting auxin from the apex to incipient primordia sites, and auxin signaling reporters (*e.g.* the synthetic DR5 promoter) are often used as one of the first markers of lateral organ initiation during inflorescence development [22,23]. To reveal the role of auxin in head patterning in Asteraceae, *DR5rev::3XVENUS-N7* (a synthetic auxin response reporter) lines were analyzed in *G. hybrida* [16]. During the early stages of capitulum formation, involucral bract primordia appeared in an almost circular pattern at the periphery. New auxin maxima then intercalated equidistant to its neighboring incipient primordial sites and moved laterally to its older neighbor, forming a zigzag pattern that resulted in the creation of long and short gaps between the incipient primordia. This developmental pattern repeats itself forming a spiral lattice that moves centripetally and eventually consumes the undifferentiated dividing region at the center. It seems that a fine tuning between cellular division and differentiation at the meristem is crucial for this pattern continuation [16]. *CLAVATA3* (*CLV3*), a member of the *CLAVATA3/EMBRYO-SURROUNDING REGION* (*CLE*) peptide hormone family, has been widely studied for its role in regulating shoot and inflorescence stem cell proliferation [24]. In *G. hybrida,* the *GhCLV3* expression domain expands as the capitulum meristem expands. *GhCLV3* expression then contracts with the progression of organ initiation across the developing head, suggesting that *GhCLV3* may position the morphogenetically competent zone at the center of the capitulum meristem [16]. Recent independent studies showed that *CLV* signaling affects auxin output and thus flower primordia outgrowth in *Arabidopsis thaliana*, possibly via *YUCCA* (involved in auxin biosynthesis) regulation in response to environmental conditions [25,26]. It is, therefore, worth investigating whether peptide hormones such as *CLV3* interact with auxin to define head patterning in the Asteraceae.

Early studies in sunflower revealed that after inflicting physical wounds on a developing capitulum, the Fibonacci progression of head patterning was disrupted [27]. By creating a circular wound at the center of the developing head, the central region of the receptacle was physically disconnected from the periphery preventing transport across the wound site. Wounding re-initiated the formation of involucral bracts in both the central and peripheral regions, followed by the formation of ray or disk florets. While this altered the typical Fibonacci progression, the developmental order of organ initiation was re-established and remained conserved, suggesting that the signal for floret initiation and specification is either autonomously generated at sites of incipient primordia or is mobilized through more internal tissues (*e.g.*, vasculature), hinting at the long-distance transport of these signals. A recent study observed auxin signaling dynamics during head repatterning after wounding in *G. hybrida* using auxin reporter lines (*DR5rev::3xVENUS-N7*) [17]. Confocal microscopy revealed that auxin signaling maxima appeared a few cell layers away from the wound edge and moved centripetally. The appearance of auxin maxima and differentiation of both involucral bracts and floret primordia, despite wound infliction, suggest that auxin maintains the developmental order of these structures at the receptacle. Examining localization and function of native PIN proteins, auxin efflux carriers, or auxin biosynthetic genes (*e.g. YUCCA*) would provide more insight into this patterning mechanism.

Phytohormones have also been shown to play significant roles in floret patterning and identity establishment (Figure 3). Transcriptomic and metabolomic profiling in *Argyranthemum frutescens* (Anthemideae; Figure 5) revealed that elevated expression of auxin biosynthesis and auxin transport genes is correlated with ray floret initiation as opposed to trans (intermediate), and disk florets (Figure 3a) [28]. This same study showed that in contrast to auxin, the phytohormone abscisic acid (ABA) may regulate an opposing pattern as ABA-related biosynthesis genes are upregulated specifically in *A. frutescens* disk florets (Figure 3b). Mechanistic evidence explaining ABA's role in capitulum patterning is, however, lacking. Recently, Brassinosteroids (BRs) were also implicated as playing a role in ray floret identity specification in *Chrysanthemum morifolium* (Anthemideae; Figure 3c and 5) [29]. Several BR-biosynthesis and signaling-related genes such as *DWARF1* (*DWF1*), *BRASSINOSTEROID INSENSITIVE1-like2* (*BRL2*), *HERCULES Receptor Kinase 1* (*HERK1*), and *AP2/ETHYLENE RESPONSIVE FACTOR TINY* (*AP2/ERF TINY*) and class IV Homeodomain Zipper transcription factors (*HD-ZIPs*) were found to be downregulated in a mutant *C. morifolium* with increased numbers of disk florets per head [29]. RNAi knockdown of *CmDWF1* in the *C. morifolium* cultivar '1581' resulted in capitula with increases in the number of disk florets relative to ray florets [29]. Exogenous application of the BR inhibitor, Brassinazole, caused a similar increase in disk floret

Figure 3



Diagrammatic representation of phytohormone patterning during capitulum development. Suite of genes involved in differentiation of structures of a head, compiled from multiple independent studies in Asteraceae. (**a**) Auxin concentration gradient across a capitulum. High auxin concentration is necessary for involucral bract development. Auxin concentration lowers as the gradient moves acropetally on the wide receptacle, aiding in the establishment of ray and disk florets. (**b**) Abscisic acid (ABA) concentration patterns are opposite to auxin, moving from low to high in an acropetal manner, with the highest concentration needed for disk floret differentiation, followed by trans and ray florets. (**c**) Brassinosteroids (BR) specify ray floret identity. ***GhCYC2,*** *Gerbera hybrida CYCLOIDEA2*; ***CmDWF1***, *Chrysanthemum morifolium DWARF1*; ***CmBES1***, *C. morifolium BRI-EMS SUPPRESSOR 1*; ***MiRAY2***, *Matricaria inodora RAY2;* ***MiLFY,*** *M. inodora LEAFY*. **IM,** Inflorescence meristem; **IB**, Involucral Bracts; **Df,** Disk florets; **Tf,** Trans florets, **Rf**, Ray florets.

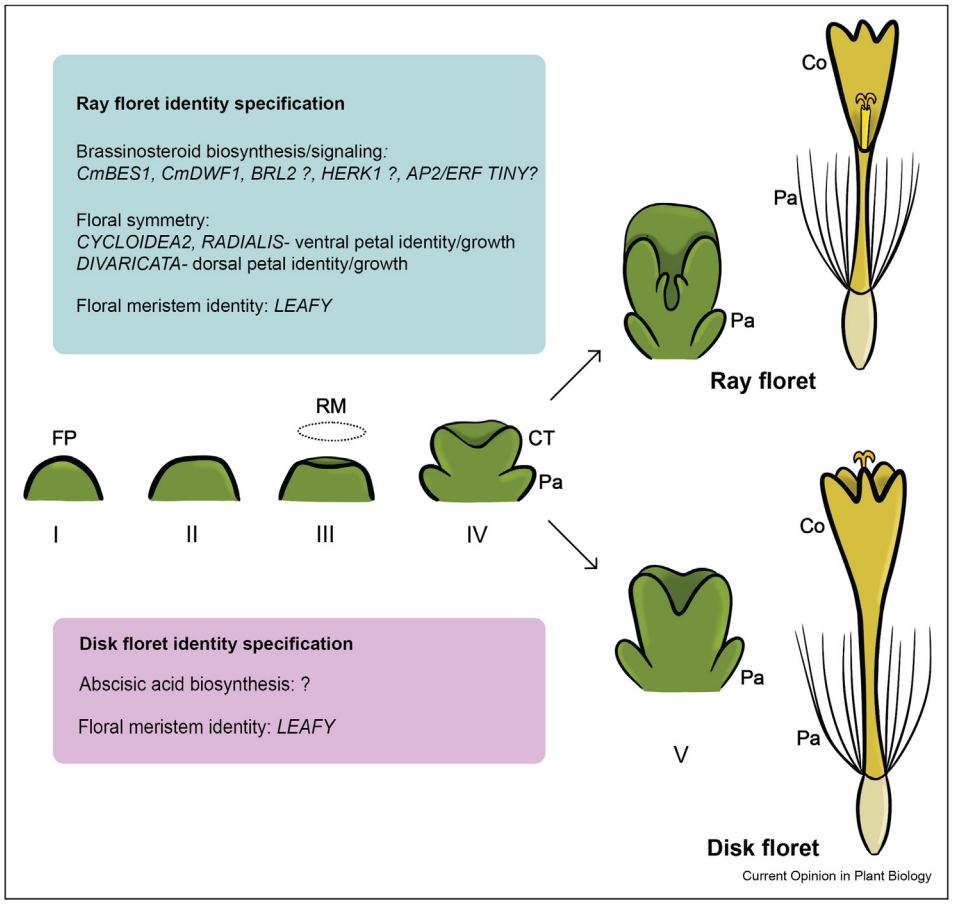
numbers (per capitulum) [29]. An independent study on *C. morifolium* showed that an overexpression of the BR transcription factor, *BRI1-EMS-SUPPRESSOR 1* (*CmBES1*), resulted in an increased number of ray florets and a higher degree of petal fusion in ray florets [29,30]. The fusion phenotype is consistent with the known function of BR in suppressing organ boundaries via activation of a BR-transcription factor, (*BRASSINAZOLE-RESISTANT 1*) *BZR1* that represses organ boundary genes such as *CUP SHAPED COTYLEDON* (*CUC*) in *Arabidopsis thaliana* [31]. Evidence from these studies suggests that BR-signaling promotes ray identity, possibly through organ boundary regulation, and that reduced BR-signaling may result in disk floret formation (Figure 3c). In plants, BRs affect a variety of developmental processes such as floral transition, flower development, meristem determinacy, shoot growth, and stomatal differentiation in diverse species [32–35]. It is worth further exploring the role of BR signaling in regulating floret identity in a complex capitulum inflorescence. In addition to BRs, auxin gradients in developing inflorescence meristems of two Asteraceae species (*Senecio vulgaris*; Senecioneae tribe and *Matricaria inodora;* Anthemideae tribe, Figure 5) were found to influence the formation of involucral bracts, ray florets, and disk florets in a concentration-dependent manner [36] (Figure 3a). High concentrations of auxin serve as a developmental cue for bract development, while medium and the lowest levels of auxin are associated with ray and disk floret differentiation, respectively. Additionally, exogenous auxin application resulted in homeotic conversion of florets to bracts in *M. inodora* [36]. In *M. inodora*, *LEAFY* (*MiLFY*) is suppressed at the sites of highest auxin concentration promoting bract differentiation. Lower auxin concentration (to a specific level) subsequently promotes *M. inodora RAY2* (*MiRAY2*; an ortholog of *S. vulgaris RAY2* and *GhCYC2*) expression resulting in the formation of ray florets while a further decrease in auxin at the farthest end of the maxima induces *MiLFY,* ultimately promoting disk and ray floret production. In *G. hybrida*, *GhLFY* was mainly shown to affect ray floret identity as well as inflorescence meristem specification [11]. Taken together, these studies demonstrate that floret identity specification and patterning in capitula is associated with complex regulation of phytohormone production, transport, and signaling. Additionally, cross-talk between phytohormone pathways frequently occurs in plants. Auxin and BRs both modulate *Auxin Response Factors* (*ARFs*) post-translationally, indicating some interdependency

between the two phytohormones. Interactions between phytohormone pathways can thus provide plants the ability to regulate diverse developmental processes [37].

Dimorphic floret development

Asteraceae florets harbor incredible diversity in their morphology, development, and reproductive abilities. For instance, corolla (petals) in Asteraceae florets display variation in symmetry, degree of fusion, and pigmentation. In many species, this morphological diversity is even represented within the same capitulum (*e.g.*, the iconic heterogamous capitulum of the sunflower; Figure 1a). While the mature corolla of florets is morphologically distinct, i.e., rays are bilaterally symmetrical and disks are radially symmetrical, their early ontogeny is similar (Figure 4). Because the most apparent difference between these florets is in their corolla symmetry, genes associated with specifying symmetry during development are thought to be associated with floret identity establishment. One such gene family is the *TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTORS* (*TCP*) family of transcription factors, specifically *CYCLOIDEA* (*CYC*) that has been widely studied in the establishment of dorsoventral asymmetry in *Antirrhinum majus* (Snapdragon; order Lamiales) [38]. Expansion of the *CYCLOIDEA* gene family is possibly connected with the morphological complexity within Asteraceae [39,40]. In Asteraceae species, such as *S. vulgaris*, *M. inodora*, *G. hybrida*, and *H. annuus*, *CYC* homologs, specifically the *CYC2* subclade, specify ray floret identity [36,39,41,42]. The *CYC2* subclade has six paralogs (*CYC2a-e/g*) that regulate multiple aspects of corolla development in ray florets, *e.g. CYC2c* and *CYC2d* regulate ray floret identity [39,43,44], whereas *CYC2e* and *CYC2b* determine length of ray/ligule corollas (Figure 4) [41,43,45].

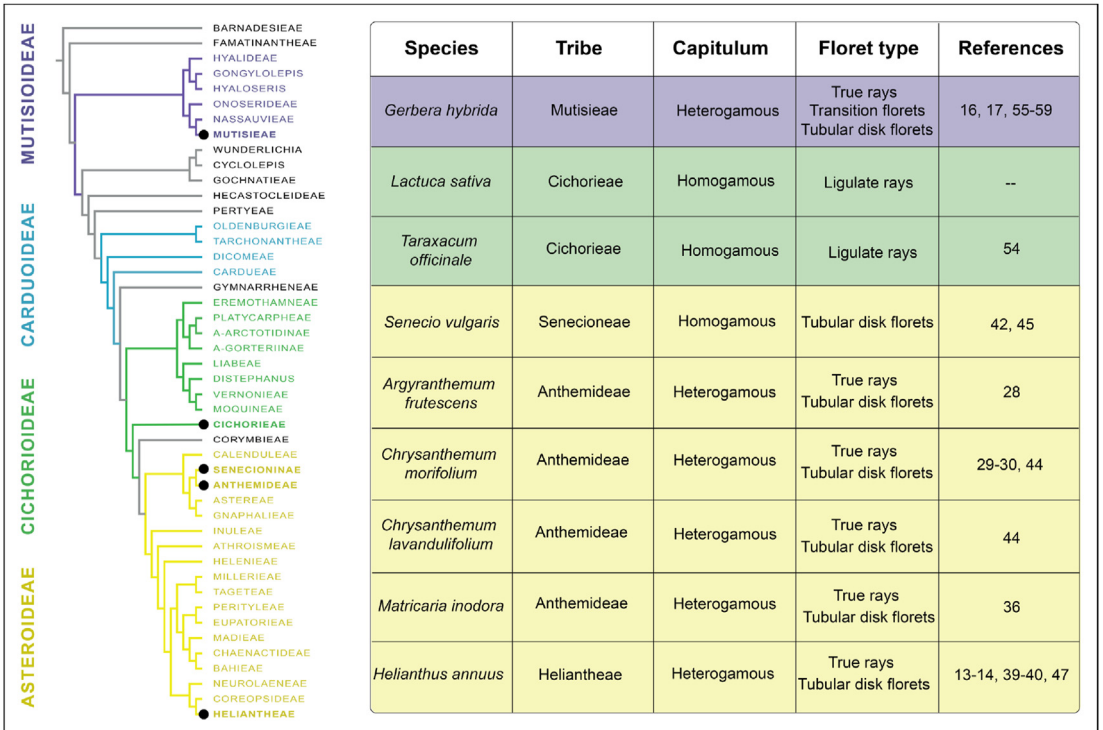
Figure 4



Lateral diagrammatic view of florets development in a heterogamous capitulum. Roman numerals mark the stages of corolla development. I) depicts floret primordia establishment, II) Early establishment of ring primordia, III) marks the initiation of ring meristem at the rim, IV) Upward growth of the fused corolla forming an incipient corolla tube and pappus emergence, and V) shows the differences in disk and ray florets; disk florets exhibit unified growth of the entire fused corolla tube, whereas rays display differential growth of three fused petal primordia that form the ray and the remaining two petal primordia stall their growth. Genes potentially involved in ray (blue) versus disk (pink) floret distinction are highlighted in the box. **Co**, Corolla; **CT**, Corolla Tube; **FP**, Floret Primordia; **Pa**, Pappus; **RM**, Ring Meristem. "**?**" indicates no supporting evidence yet.

A *CYC2e* homolog in *G. hybrida*, *GhCYC2*, has ray floret (bilaterally symmetrical) specific expression and its overexpression promotes petal expansion and disrupted stamen development in disk florets, making them more ray-like [41]. In *H. annuus*, *HaCYC2c* and *HaCYC2d* are primarily expressed in ray florets [39,40,46]. Mutations in *HaCYC2c* have been identified in the two sunflower mutants *double-flowered* (*dbl*) and *tubular-rayed* (*tub*). *Tub* mutants carry a transposon insertion in the coding region of *HaCYC2c* that results in the disruption of its function and formation of radial ray florets. In contrast, the *dbl* mutation causes elevated expression of *HaCYC2c* resulting in disk to ray floret transitions [39]. Similarly, a mutation in *HaCYC2c* underlies the *turf* (*tubular-ray floret*) mutant phenotype in sunflower where ray florets are radially symmetrical and have both functional male and female reproductive structures [47]. In an independent study, *C. morifolium CYC2c* (*CmCYC2c)* was highly expressed in the ray florets as opposed to other structures of the capitulum and its overexpression in *Chrysanthemum lavandulifolium* (Anthemideae; Figure 5) promoted corolla growth and an increased ray floret count [44]. Overexpression of *C. lavandulifolium CYC2d* (*ClCYC2d*) prevents ray floret growth [43]. The *CYC2* orthologs *RAY1* and *RAY2* in *S. vulgaris* also exhibit ray floret-specific expression patterns in both non-radiate (tubular disk florets only) and radiate (ray and tubular disk florets) forms, with a much stronger expression in non-radiate than radiate [42]. It was further confirmed that an overexpression of *SvRAY1* prevents ray formation in radiate backgrounds, which is consistent with higher expression levels of *SvRAY1* in the non-radiate form of *S. vulgaris*. *SvRAY2* is mostly related to promoting ventral (abaxial petals that form rays) identity in ray florets [42]. Because *CYC* plays an important role in floret identity establishment, other symmetry-related genes have been implicated to function in determining the floret identity in Asteraceae. In a separate study, orthologs for floral symmetry genes from *A. majus* i.e., *AmCYC* (*A. majus CYCLOIDEA*), *AmRAD* (*A. majus RADIALIS*) and *AmDIV* (*A. majus DIVARICATA*) [48] were studied in *S. vulgaris*. Expression of *SvRAY1/2/3*, *SvRAD* (*S. vulgaris RADIALIS*), *SvDIV1B* (*S. vulgaris DIVARICATA*) are restricted to all five petals of ray florets specifically, potentially establishing the bauplan (blueprint) for bilateral symmetry [45]. It was proposed that as petals emerged, *SvRAY3* and *SvRAD* remained in the ventral domain, and *SvDIV1b* expression was restricted to dorsal domains, allowing asymmetric ventral corolla elongation [45]. It is interesting that

Figure 5



Species	Tribe	Capitulum	Floret type	References
Gerbera hybrida	Mutisieae	Heterogamous	True rays Transition florets Tubular disk florets	16, 17, 55-59
Lactuca sativa	Cichorieae	Homogamous	Ligulate rays	--
Taraxacum officinale	Cichorieae	Homogamous	Ligulate rays	54
Senecio vulgaris	Senecioneae	Homogamous	Tubular disk florets	42, 45
Argyranthemum frutescens	Anthemideae	Heterogamous	True rays Tubular disk florets	28
Chrysanthemum morifolium	Anthemideae	Heterogamous	True rays Tubular disk florets	29-30, 44
Chrysanthemum lavandulifolium	Anthemideae	Heterogamous	True rays Tubular disk florets	44
Matricaria inodora	Anthemideae	Heterogamous	True rays Tubular disk florets	36
Helianthus annuus	Heliantheae	Heterogamous	True rays Tubular disk florets	13-14, 39-40, 47

Current Opinion in Plant Biology

A simplified tree illustrating the phylogenetic relationships among Asteraceae subfamilies and tribes. Tabulated list of species reviewed for reader's convenience. Color code depicts the sub-families that the species belongs to. Purple, Mutisioideae; Green, Cichorioideae; Yellow, Asterioideae. Numbers in the reference column correspond to the order in the bibliography. Tree generously provided by Mauricio Bonifacino (Universidad de La Republica Uruguay).

SvRAY3 and *SvRAD* control ventral petal identity in *S. vulgaris* as opposed to *AmCYC* and *AmRAD* which control dorsal petal identity in *A. majus* [38,48,49]. This is suggestive that floral symmetry genes are potentially re-wired in ray florets of a capitulum. Functional validation is needed to support this observation.

In addition to floral symmetry, the organization of different floral whorls varies within a capitulum and among capitula of different species. Studies have explored the applicability of the widely studied ABCE model [50–53] of flower development in Asteraceae. Orthologs for ABCE genes have been identified in Asteraceae that seemingly affect floral organ specification. The outermost whorl of pappus, modified calyx (sepals) in Asteraceae florets, is considered a key innovation [5,54]. To provide molecular evidence for the sepal-related origins of pappus, Vijverberg et al. showed that sepals-specific A-class gene in *Taraxacum officinale* (Cichorieae, Figure 5), *Tof-APETALA1*(*TofAP1*), exclusively showed higher expression levels in pappus than any other floral tissue of the capitulum [54]. Pappus identity was also found to be associated with *GERBERA REGULATOR OF CAPITULUM DEVELOPMENT* (*GRCD;* a *SEPALLATA* ortholog) expression and a loss in *GRCD3* was correlated with the conversion of pappus to bract-like structures in intermediate *GRCD4/5* RNAi lines [55]. The homeotic conversion of traits indicates that A-class function is conserved in establishing sepal identity in Asteraceae. Orthologs of MADS box genes identified in *G. hybrida* have been shown to share similar expression patterns as in *Arabidopsis*. Overexpression of *GERBERA AGAMOUS like 1/2* (*GAGA1/2*), a putative C-class ortholog, resulted in conversion of otherwise pigmented petals of *G. hybrida* florets to unpigmented stamen-like organs, while the identity of pappus remained intact [56]. On the contrary, a reduced expression of *GAGA1/2* resulted in formation of petal-like organs in place of stamens and conversion of carpels to a green structure with mixed identity of carpels and pappus. Furthermore, extra whorls of pappus and corolla were observed at the center suggesting that *GAGA1/2* not only functions as a C-class gene, but also effects the determinacy of the floral meristem [56]. Similarly, homeotic conversions were also observed when B-class orthologs (*GGLO; Gerbera GLOBOSA* and *GDEF; Gerbera DEFICIENS*) were downregulated in *G. hybrida*, further confirming the utility of the ABCE model in intepreting floret development in Asteraceae [56,57]. Reduced expression of *GGLO* caused second whorl organs to form bract-like structures, whereas no significant effects were seen in the third whorl [56]. However, co-suppression lines in a separate study showed stamen conversion to carpel-like structures, suggesting that *GGLO* function is mediated by *GDEF* [57]. In accordance with E-class genes, the *G. hybrida* orthologs *GRCD1-8* have been characterized to play role in establishing organ identity in Asteraceae. There are 8 copies of *GRCDs* in Asteraceae that supposedly display whorl-specific sub-functionalization [55]. Because *GRCD1* downregulation mimics phenotypic effects of *GAGA1/2* downregulation in the third whorl specifically and because they share similar expression pattern, it was suggested that *GRCD1* participates with *GAGA1/2* to determine stamen identity [58]. *GRCD2* and 7 affect carpel identity specifically, wherein their down-regulation result in the formation of pigmented petal-like organs and green bract-like structures, respectively, in place of carpels in stronger lines, resembling *GAGA1/2* downregulation [55,59]. Moreover, *GRCD2* possibly with its paralog *GRCD*7 affected meristem determinacy where its reduced expression maintained the undifferentiated state of meristem for a relatively longer period, producing more florets than usual [55,59], suggesting pleiotropic effects of *SEPALLATA* -like genes in Asteraceae. In addition, *GRCD4/5* affects petal development during different stages of development, as shown by differential effects of downregulation during petal development [55]. Severe reduction in *GRCD4/5* expression levels results in maintenance of undifferentiated inflorescence meristem at the center and all floral organs were converted to leaves [55], which is consistent with the phenotype of Arabidopsis quadruple *sep* mutants [52]. These studies are suggestive of ABCE model's applicability during floret organ specification in Asteraceae; however, targeted reverse genetics approach may not be sufficient to fully elucidate floral regulatory networks. Pleiotropic effects of overexpression and downregulation of the above-mentioned genes are insufficient to describe their precise function during floral organ specification. More functional genetic approaches (*e.g.*, mutagenesis screens, targeted mutagenesis via CRISPR, etc.), generating and characterizing stable knockouts of floral homeotic genes in Asteraceae, are required to discover novel developmental regulators of floret organ identity in this diverse family.

Conclusion

The huge success of Asteraceae is associated with the evolution of a capitulum. Despite their considerable representation in angiosperms, our fundamental understanding of Asteraceae capitulum development and its evolutionary origin remains unresolved. Deciphering the molecular basis of capitulum development and organization requires identifying mechanisms regulating the establishment of florets and their identity specification. Recent studies show that phytohormones such as auxin not only affect floret establishment in spirals, but they possibly regulate genes that specify floret identities and contributes to overall capitulum architecture. These phytohormones could potentially interact with floral symmetry genes and other morphogens, such as peptide hormones, to govern the position of different floret types across the inflorescence. Through functional genetics, these interactions can be established to unravel

the underlying mechanisms involved in capitulum formation. Two major challenges that lay ahead are; 1) finding model systems that represent the broad diversity of the Asteraceae and 2) developing functional genomic tools in these model systems to generalize molecular mechanisms regulating different capitulum morphologies across the family. Once overcome, we feel that current knowledge gaps in our understanding of the origin of the Asteraceae capitulum can be filled, revealing novel insights into broader molecular/genetic mechanisms that regulate development across this important, highly diverse group of plants.

CRediT authorship contribution statement

Vandana Gurung: Conceptualization, Data curation, Writing - original draft, Visualization, Supervision. **Sarita Muñoz-Gómez:** Conceptualization, Visualization. **Daniel S. Jones:** Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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