The gynostemium: More than the sum of its parts with emerging floral complexities

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Abstract
Partial or complete floral organ fusion, which occurs in most angiosperm lineages, promotes integration of whorls leading to specialization and complexity. One of the most remarkable floral organ fusions occurs in the gynostemium, a highly specialized structure formed by the congenital fusion of the androecium and the upper portion of the gynoecium. Here we review the gynostemia evolution across flowering plants, the morphological requirements for the synorganization of the two fertile floral whorls, and the molecular basis most likely responsible for such intimate fusion process.

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Where do true gynostemia occur?

A true gynostemium (i.e. the congenital fusion between the stamens and the free styles and/or stigmas) has independently evolved only a few times across the angiosperms, once in the magnoliid genus *Aristolochia* (Aristolochiaceae) (Figure 1; [1]), and three times in the monocots, once in *Corsia* (Corsiaceae), once in *Pauridia* (Hypoxidaceae), and once in the lineage encompassing all Orchidaceae (Figure 2; [2]).

In *Aristolochia*, the gynostemium is a crown-like structure formed by 5 or 6 (rarely more) sessile extrorse anthers, which congenitally fuse to 3, 5, 6 or rarely more commissural stigmatic lobes (Figure 1m—t; [1,3,4]).

Whereas the number of anthers and stigmatic lobes is the same in most *Aristolochia* species (i.e. those of the subgenera *Aristolochia* and *Pararistolochia*), the six anthers are paired and fused to three stigmatic lobes in the species of subgenus *Siphisia*, which indicates that the fusion itself is independent from the number of primordia involved. In *Corsia*, the short gynostemium is the result of the fusion between the filaments of six fertile stamens (with extrorse anthers) and the style for about half of their length [2]. In *Pauridia*, the gynostemium occurs through the fusion of the three outer sterile stamens and the style, forming the so-called "stylar appendages" [2,5], whereas the inner fertile stamens attach to the perianth tube [2,5,6]. In all Orchidaceae, the gynostemium is formed by the staminal whorls and the stigmatic tips. However, there is considerable variation across subfamilies [7]: In the early diverging Apostasioideae, it is formed by the median outer stamen, two inner lateral stamens and the stigmas [8]; in Cypripedioideae, it is formed by the fusion of the two inner lateral stamens and the stigmas [7]; in the remaining subfamilies (Epidendroideae, Orchidoideae and Vanilloideae) the gynostemium is the result of the fusion of the single median outer stamen and the stigmas (Figure 2; [7,9—12]). At least in Orchidoideae and Epidendroideae, the two lateral outer whorl stamens often exist as sterile appendages in the gynostemium [13].

At first glance, the fusion of the floral fertile whorls would promote self-fertilization. However, asynchronous organ maturation (i.e. protandry or protogyny) appears to avoid autogamy except probably in *Pauridia* [14]. The flowers of *Aristolochia* are protogynous, where stigmas are receptive prior to anther dehiscence (Figure 1o, p; [1—3,15]). In *Corsia* and other Corsiaceae, flowers are protandrous [16]. Finally, with over 27.000 orchid species, it is hard to identify a single pattern for the timing of sexual organ maturation. Although self-pollination occurs in many orchids with simultaneous maturation of stamens and carpels [17,18], cross-pollination mediated by protogyny or proterandry is by far more frequent [19—21]. The orchid gynostemium often undergoes subtle position shifts over time, curving forward and backward. This could be a physical constraint to avoid immediate self-pollination, as pollen massulae (pollinia) mature spatially compartmentalized, away from the same flower stigmatic surface (Figure 2; [19,22]).

Figure 1
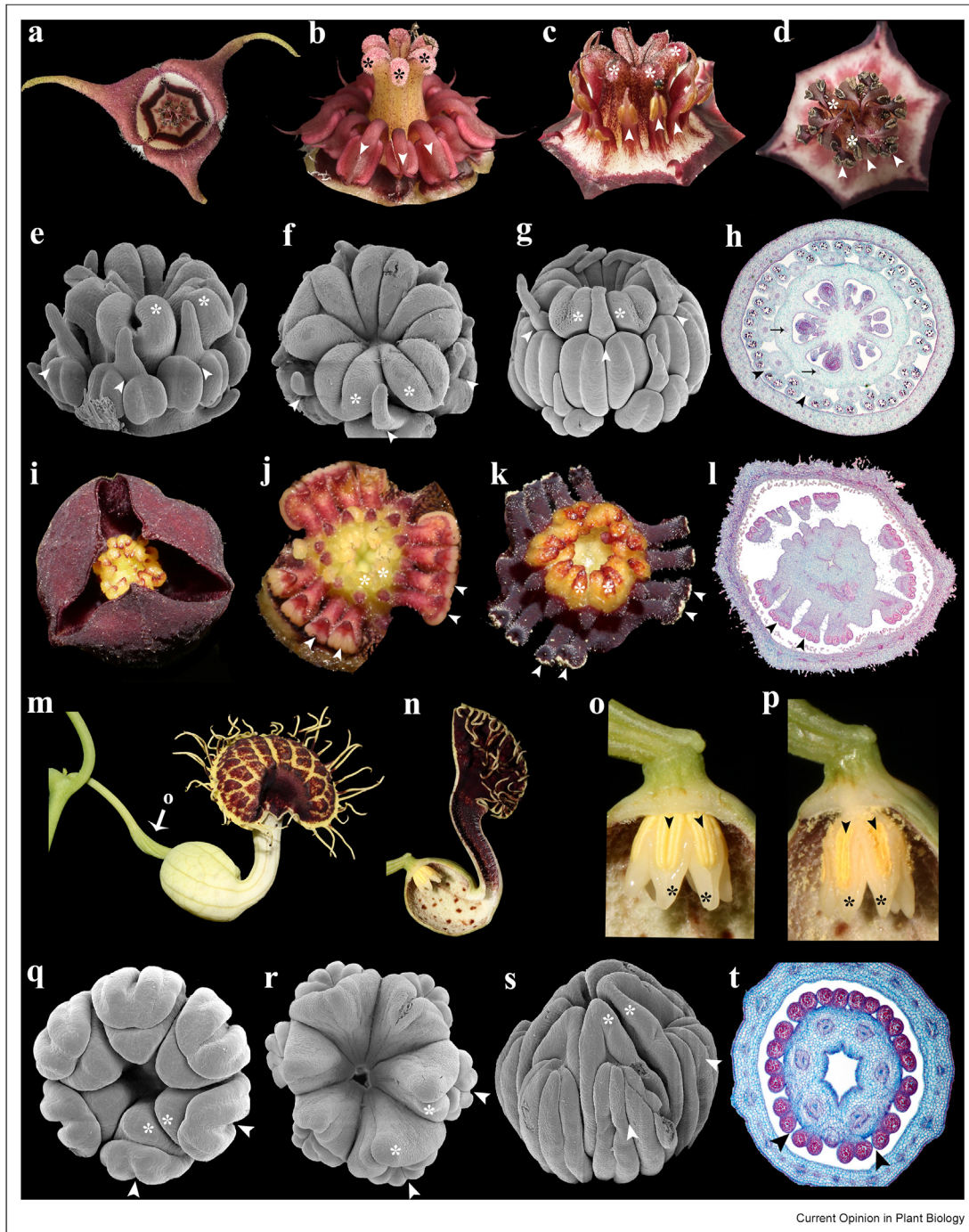
Floral morphology of representative Aristolochiaceae. **(a–h)** *Asarum canadense*; **(a)** frontal view of a flower in anthesis; **(b–d)** successive female and male floral phases. **(e–g)** successive stages in young floral buds showing free stamens which are not fused to the adjacent stigmas; . **(h)** transverse section of a floral bud; note incipient stamen/carpel fusion; **(i–l)** *Thottea siliquosa*; **(i)** frontal view of a flower at anthesis. **(j–k)** successive female **(j)** and male **(k)** floral phases; note incipient fusion of the base of stamens with stigmatic lobes; **(l)** transverse section of a flower; **(m–t)** *Aristolochia fimbriata*; **(m)** frontal view of a flower at anthesis; **(n)** sagittal section of flower; note the position of the gynostemium at the base of the utricle; **(o–p)** successive female **(o)** and male **(p)** phases of gynostemium **(q–s)** successive stages of a forming gynostemium showing six stamens fused to six commissural stigmatic lobes; **(t)** transverse section of a floral bud. Arrowheads point to stamens; asterisks point to stigmatic (commissural) lobes. Arrows indicate the medial carpellary vascular bundle. Abbreviation: O, inferior ovary.

Figure 2
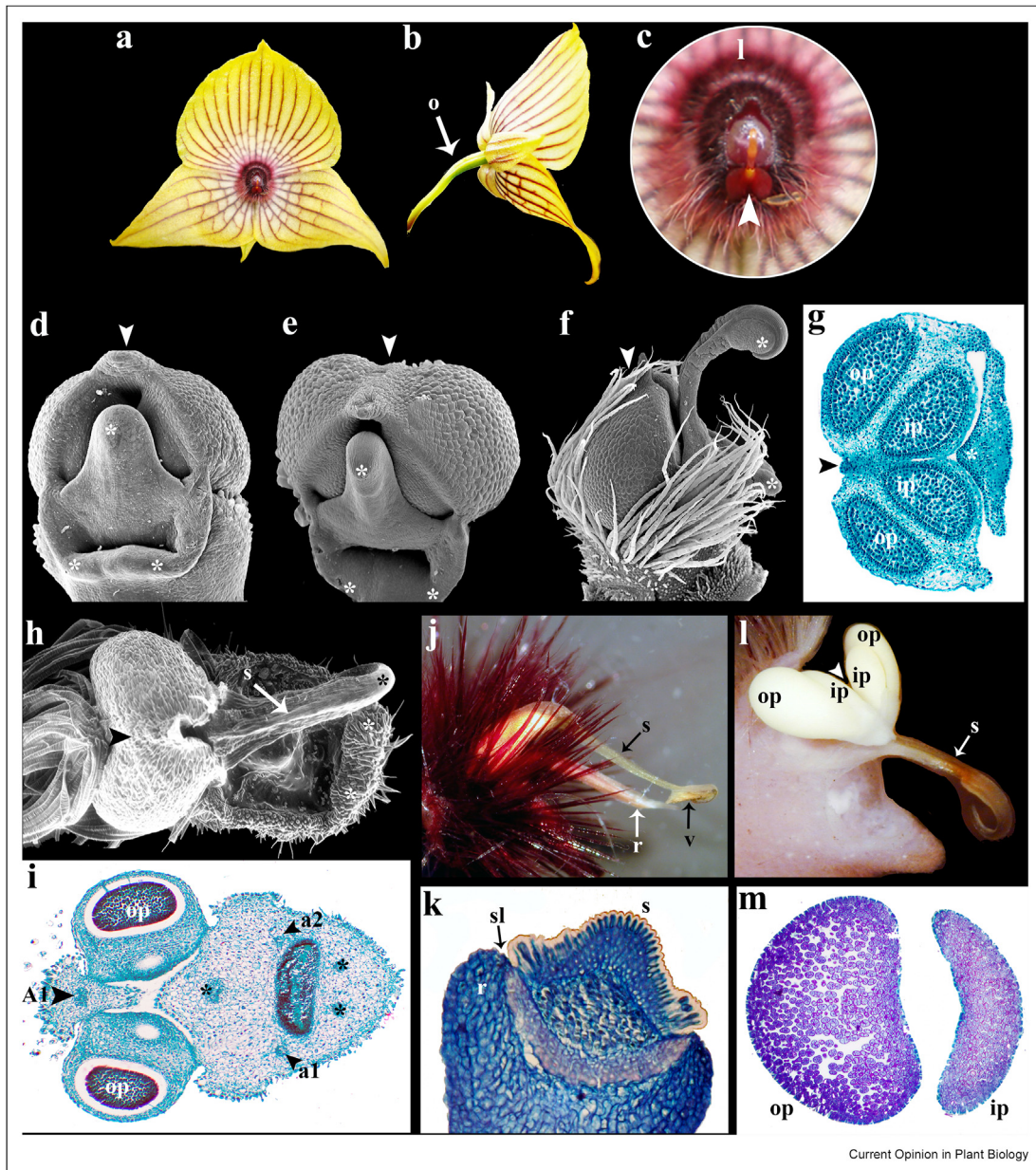
Telipogon nervosus (Orchidaceae: Epidendroideae) as an example of the most frequent type of gynostemium formed by a single fertile outer stamen and three stigmas. **(a)** frontal and **(b)** lateral view of a flower at anthesis, **(c)** detail of the gynostemium opposite the labellum, **(d–f)** successive stages of young floral buds showing stamens and stigmas congenitally fused, **(g)** Transverse section of the fertile stamen with four pollinia and the medial stigmatic lobe (to the right). **(h)** mature gynostemium; **(i)** transverse section of a mature gynostemium below the level of the stigmatic surface; **(j–m)** mature pollinarium; **(j)** lateral view of a mature pollinarium prior to anther cap dehiscence; **(k)** transverse section of the medial stigmatic tip (rostellum) and the stipe forming tissues and separation layer; **(l)** mature pollinarium ready for dispersal (bristles removed); **(m)** transverse section of pollen massulae from a single theca as dispersed. Arrowheads point to stamens; asterisks point to stigmatic tips. Abbreviations: A1, medial outer stamen; a1, a2, lateral inner stamens; l, labellum; ip, inner pollinia; o, inferior ovary; op, outer pollinia; r, rostellum; s, stipe; sl, separation layer; v, viscidium.

Outside magnoliids and Monocots, adnation of stamens and stigmas has been previously reported and referred to as a "column" or even a "gynostemium" in members of the Stylidoideae (Stylidiaceae: Asterales). However, no true congenital fusion between stamens and stigmas occurs in this family [23], which is reasonable as the proterandrous flowers undergo secondary pollen presentation that requires displacement of the style/stigma free from the androecial-derived tube [24].

What are the requirements to form a gynostemium?

The gynostemium is a homoplasious trait, although no reversals seem to occur once it is evolutionarily fixed. Nevertheless, in all cases several structural conditions are required for the formation of a gynostemium: 1) high floral synorganization; 2) perianth organs partially or completely fused, or tightly appressed to stamens and stigmas; 3) sessile anthers; and 4) inferior ovary. We will examine in detail the gynostemium in the two taxa in which it has been evolutionarily fixed in all the species, namely, the genus *Aristolochia* (with ca. 650 spp.) and the family Orchidaceae (with over 27.000 spp.)

As currently circumscribed, the perianth-bearing Piperales include the families Asaraceae, Aristolochiaceae, Hydnoraceae and Lactoridaceae [25]. Only species in the genus *Aristolochia* exhibit a gynostemium, contrasting with the free stamens and stigmas found in *Asarum*, *Lactoris*, *Hydnora*, *Prosopanche*, and *Saruma* (Figure 1a–h). A transitional stage occurs in the genus *Thottea*, which exhibits an incipient proximal fusion between stamens and stigmas (Figure 1i–l). All perianth bearing Piperales possess an inferior or semi-inferior ovary and highly synorganized flowers with a uniseriate perianth composed of sepals (Figure 1; [1,3,4,26]), with the noticeable exception of the monospecific *Saruma*. However, only the *Aristolochia* perianth shows complete fusion and bilateral symmetry versus partial fusion and radial symmetry in the remaining genera (Figure 1m). Finally, pollination in *Aristolochia* is by far the most specialized as insects (mostly flies) are attracted to the kettle-like shaped trap flowers. Here, they get temporarily sequestered during the female phase of the gynostemium, allowing pollen deposition carried by the insect from another flower. Once pollination has been secured, the gynostemium enters the male phase, when anther dehiscence occurs allowing new pollen to be deposited on the insect (Figure 1m–p; [15,27]).

Conversely, a gynostemium is thought to have been in place in the ancestor of Orchidaceae [2,8]. Orchids are sister to all other Asparagales [28], yet similar congenital fusions or even transitional stages are not found elsewhere in the order, perhaps with the exception of *Pauridia* [2]. The orchid gynostemium is a unique and extraordinarily complex pollination apparatus that incorporates fertile stamens, lateral staminodia, and a strongly zygomorphic upper region of the gynoecium. The median stigma lobe grows faster and usually protrudes above and further out, while the two lateral stigmas remain below (Figure 2; [29–32]). Among the three stigmas, a broad receptive disc is formed for the landing of pollinia (Figure 2). The most exquisite elaboration occurs in monandrous orchids, in which the single fertile stamen is fused to the medial stigmatic lobe, and together, they protrude forming the rostellum,

a structure responsible for the formation of the pollinarium. The latter is a compound pollen dispersal unit that corresponds (from proximal to distal) to the four (or 2, or 8) pollinia per flower, their common stalk (stipe or caudicle), and their sticky pad (viscidium), which directly attaches to the pollinator (Figure 2; [30–32]).

## What do we know about the identity of the gynostemium parts?

As the gynostemium is the result of congenital fusion between stamens and stigmas that crown an inferior ovary, two sets of genes can help to better comprehend how such fusion occurs without compromising organ identity. On one hand, given that fusion of primordia from adjacent floral whorls distorts boundaries among organs, boundary gene regulation might be critical to assess gynostemia development. On the other hand, floral organ identity and gynoecium patterning genes could help to identify the androecial *versus* the gynoecial identity in this compound structure. We will analyze both sets of genes below.

Stamen and carpel patterning genes

The ABCE model of floral organ identity genes, identified primarily in the model *Arabidopsis thaliana*, establishes that a specific expression and interaction of MADS-box transcription factors controls the identity of each of the four floral whorls. Specifically, the combination of B, C, and E class genes results in stamen identity, while the C and E class gene combination promotes carpel identity [33–37]. The expression of all floral organ identity MADS-box *Aristolochia* homologs has been carefully assessed in *Aristolochia fimbriata*. In this species, the expression of the B-class homologs *PISTILLATA* (*PI*) and *APETALA3* (*AP3*) is, in fact, restricted to the staminal region of the gynostemium; however, *AfimPI* is transiently expressed also early on in the young stigmatic tips (Figure 3; [3]). The expression of *AGAMOUS* (*AG*) is only restricted to the ovary, and does not reach the stigmatic region of the gynostemium (Figure 3; [4]). This already points to an alternative genetic control of the stigmatic lobe formation, suggesting that they could correspond either to massive filaments, or to *de novo* structures that do not share genetic identity with either true stamens or carpels. An additional scenario emerges from the possibility that *in situ* RNA hybridization is less efficient in highly vacuolated lipid-rich or sugar-rich tissues, and perhaps, alternative experiments should be used to establish gene transcription throughout the gynostemium.

In *Arabidopsis*, downstream of *AGAMOUS* (*AG*), other genes are turned on to shape the specific carpel regions and tissues. *SPATULA* (*SPT*), a bHLH transcription factor, and *CRABS CLAW* (*CRC*), a YABBY homolog, are key factors to establish basal-apical patterning in the gynoecium [38–40]. *CRC* is a direct target of *AG* and

Figure 3
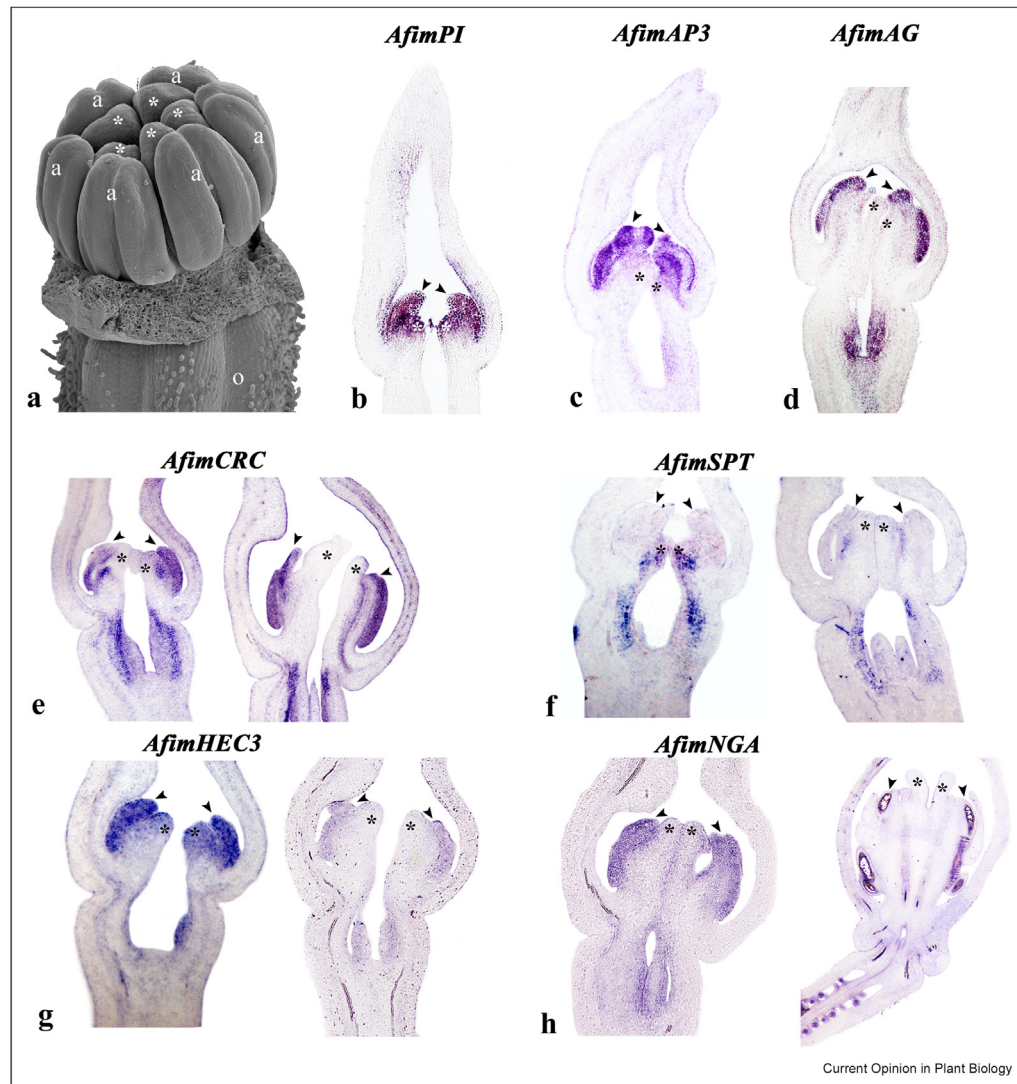
Summary of gene expression of B and C class MADS box organ identity genes and gynoecium patterning genes in *Aristolochia fimbriata*. **(a)** SEM micrograph of young gynostemium and portion of the inferior ovary as reference for figs. **b–h**. **(b–h)** *in situ* hybridization expression patterns of *PISTILLATA* (*PI*) **(b)**, *APETALA3* (*AP3*) **(c)**, *AGAMOUS* (*AG*) **(d)**, *CRABS CLAW* (*CRC*) **(e)**, *SPATULA* (*SPT*) **(f)**, *HECATE 3* (*HEC3*) **(g)**, and *NGATHA* (*NGA*) **(h)**. Arrowheads point to stamens; asterisks point to the stigmatic (commissural) lobes. Abbreviations: a, anthers; o, inferior ovary.

controls carpel growth, style development and apical closure [38—40]. *SPT* is critical for style and stigma formation and to the development of the medial tissue of the gynoecium [41]. At later stages in *Arabidopsis* gynoecium patterning, *HECATE* (*HEC*) is essential for style development and the subsequent formation of the transmitting tissue [42]. Finally, *NGATHA* (*NGA*) genes, belonging to the RAV clade of the B3-domain transcription factor family, also contribute to style and stigma development and proper carpel fusion [43,44]. Expression of carpel patterning homologs in *A. fimbriata* points to broader roles of *AfimCRC*, *AfimHEC*, *AfimNGA* and *AfimSPT* in stamen development, ovary wall identity, and style patterning ([45]; Figure 3). However, only *AfimHEC*, *AfimNGA* and *AfimSPT*, are transiently expressed in forming stigmatic lobes, but their expression is lost in fully formed gynostemia [45]. The data suggest that stigmas in gynostemia do not retain the expression of canonical gynoecium patterning genes ([45]; Figure 3).

Boundary genes

Several genes are critical for the separation of organ primordia, and most of them maintain the boundaries between organs (reviewed in the study by Aida et al. [46]). We will revise only those whose mutation results

in fusion between the androecial and gynoecial whorls in the model *A. thaliana*. It is worth noting, however, that none of the mutant phenotypes characterized so far resembles a true gynostemium in *A. thaliana,* as expected, given that their flowers have a superior ovary.

Boundary genes that play critical roles in establishing free stamens and distinct androecial *versus* gynoecial whorls are the *CUP-SHAPED COTYLEDON* (*CUC*) homologs, *CUC1* and *CUC2* [47]. In *cuc1/cuc2* double mutants, some stamens resulted fused up to the anthers or at least halfway through the filaments, and in some cases, stamens were fused to carpels [47]. *SUPERMAN*, a C2H2-type Zinc finger transcriptional repressor, also reinforces the limit between stamens and carpels. *SUP* acts to repress B-class genes, and stamen identity in the fourth whorl, in fact, the *sup-1* mutants exhibit supernumerary stamens in the position of carpels [48]. Interestingly, the study of a *sup* allelic series indicates that *SUP* also functions in floral meristem termination by repressing the activity of *WUSCHEL* (*WUS*), and in establishing the male-female boundary specification mechanism [49]. The floral meristem maintenance homeostasis loops have a number of players repressing *WUS* [50], like the mobile small peptide *CLAVATA3* and its receptor *CLAVATA1* [50]. This is the most plausible reason to explain why *sup/clv* double mutants exhibit strong stamen-carpel fusion, with strong expansion of apical stigmatic tissues. These phenotypes closely resemble those occurring during the formation of a gynostemium, suggesting that in addition to fusion, gynostemium formation may require accompanying gynoecial apical expansion [49].

Finally, it is worth noting that C2H2 Zn-finger factors are very diverse, and that other members in the family, such as *RABBIT EARS* (*RBE*) and *KNUCKES* (*KNU*) have been linked to the formation of the petal−stamen boundaries and the carpel-placenta boundaries, respectively [51,52]. However, it is unknown how these genes have evolved during angiosperm evolution and how the genetic complements have changed in model taxa, such as *Arabidopsis*, as compared to non-core eudicots, such as our case studies Aristolochiaceae and Orchidaceae.

Future research avenues

Partial and total congenital stamen/carpel fusion is rare in angiosperms; however, once it arises, no reversals occur. Because profound morpho-anatomical specializations occur when a gynostemium is formed, it is unlikely that this compound structure is the result of the action of a single gene; rather, it would have to be caused by the coordinated action of various genes involved in identity, boundaries, ovary position, and the floral meristem termination pathway. Expression patterns of more candidate genes should be tested in selected species of Aristolochiaceae and Orchidaceae to assess their roles in

the development and evolution of such remarkable structure. In parallel, comparative transcriptomic analyses may shed light into new candidate genes underlying fusion and elaboration in the gynostemium. Emerging models, such as those in Aristolochiaceae and Orchidaceae, are key to test gene function and evolution of gynostemia in this remarkable floral convergence.

Declaration of competing interest

Authors declare that they do not have competing financial interests or personal relationships that have influenced the work reported here.

Data availability

All data is available under the creative commons agreement.

Glossary

Gynostegium complex structure as a result of post-genital fusion between stamens and stigmas

Gynostemium complex structure as a result of congenital fusion between stamens and stigmas

Protandry maturation and dehiscence of stamens prior to the receptive phase of the stigma in the same flower

Protogyny maturation and dehiscence of stamens after the receptive phase of the stigma in the same flower

Synorganization coordinate elaboration of floral whorls resulting in an atypical, yet highly efficient and evolutionarily fixed, floral bauplan. It involves symmetry shifts in one or more whorls, congenital or postgenital fusion events, heterotopy and/or secondary displacement of floral parts

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. González F, Stevenson DW: **Gynostemium development in** *Aristolochia* (**Aristolochiaceae**). *Bot. Jahrb. Syst.* 2000, **122**: 249−291.

2. Rudall PJ, Bateman RM: **Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium** * **and labellum of orchids and other lilioid monocots**. *Biol Rev* 2002, **77**:403−441.

A comprehensive review on the occurrence of gynostemia in flowering plants and the underlying conditions for congenital fusion that occur in different plant groups.

3. Pabón-Mora N, Suárez-Baron H, Ambrose BA, González F:
** **Flower development and perianth identity candidate genes in the basal angiosperm *Aristolochia fimbriata* (Piperales: Aristolochiaceae)**. *Front Plant Sci* 2015, **1095**:1−20.
Authors identified that canonical B class genes are exclusive of stamens in *Aristolochia*, a member of the Piperales (Magnoliids) and the *PI* homolog is only transiently expressed in the region that will form the stigmatic tips of the gynostemium

4. Suárez-Baron H, Pérez-Mesa P, Ambrose BA, González F,
** Pabón-Mora N: **Deep into the *Aristolochia* flower: expression of C, D and E-class genes in *Aristolochia fimbriata* (Aristolochiaceae)**. *J Exp Zool* 2016, **328**:55−71.
The *AG* homolog in *Aristolochia* is never expressed in the stigmatic tips of the gynostemium, which points to an alternative genetic network shaping distal gynoecia development or it can suggest that stigmatic tips are not gynoecium derived.

5. Thompson MF: **Studies in the Hypoxidaceae III. The genus *Pauridia***. *Bothalia* 1979, **12**:621−625.

6. Thompson MF: **Studies in the Hypoxidaceae. II. Floral morphology and anatomy**. *Bothalia* 1978, **12**:429−435.

7. Kurzweil H: **Floral ontogeny of orchids: a review**. *Beitr. Biol. Pflanzen* 1998, **71**:45−100.

8. Kocyan A, Endress PK: **Floral structure and development of *Apostasia* and *Neuwiedia* (Apostasioideae) and their relationships to other Orchidaceae**. *Int J Plant Sci* 2001, **162**:847−867.

9. Kurzweil H: **Developmental studies in orchid flowers I: Epidendroid and vandoid species**. *Nord J Bot* 1987, **7**:427−442.

10. Kurzweil H: **Developmental studies in orchid flowers II: Orchidoid species**. *Nord J Bot* 1987, **7**:443−451.

11. Kurzweil H: **Developmental studies in orchid flowers III: Neottioid species**. *Nord J Bot* 1988, **8**:271−282.

12. Kurzweil H: **Floral morphology and ontogeny in subtribe Satyriinae (Orchidaceae)**. *Flora* 1996, **191**:9−28.

13. Rudall PJ, Perl CD, Bateman RM: **Organ homologies in orchid flowers reinterpreted using the Musk Orchid as a model**. *PeerJ* 2013, **1**, e26.

14. Snijman DA: **A taxonomic revision of the genus *Pauridia* (Hypoxidaceae) in southern Africa**. *Phytotaxa* 2014, **182**:1−114.

15. González F, Pabón-Mora N: **Trickery flowers: the chemical mimicry of *Aristolochia* to accomplish deception to its pollinators**. *New Phyt.* 2015, **206**:10−13.

16. Neinhuis C, Ibisch PL: **Corsiaceae**. In *Families and genera of vascular plants. II. Flowering plants: monocotyledons: Lilianae*. Edited by Kubitzki K, Berlin: Springer-Verlag; 1998:198−201.

17. Van der Pijl L, Dodson CH: *Orchid flowers: their pollination and evolution*. University of Miami Press; 1966.

18. Arditi J: *Fundamentals of orchid biology*. Wiley; 1992:704.

19. Singer RB, Sazima M: **Flower morphology and pollination mechanism in three sympatric Goodyerinae orchids from Southeastern Brazil**. *Ann Bot* 2001, **88**:989−997.

20. Trujillo D, Franke T, Agerer R: **Evidence of protandry in *AA* Rchb.f. (Orchidaceae, Cranichideae)**. *Lankesteriana* 2011, **11**:368.

21. Tremblay RL, Pomales-Hernandez G, Méndez Cintrón M: **Flower phenology and sexual maturation: partial protandrous behavior in three species of orchids**. *Caribb J Sci* 2006, **42**:75−80.

22. Darwin C: *On the various contrivances by which British and foreign orchids are fertilized by insects, and on the good effects of intercrossing*. London: John Murray; 1862.

23. Erbar C: **Floral development of two species of *Stylidium* (Stylidiaceae) and some remarks on the systematic position of the family Stylidiaceae**. *Can J Bot* 1992, **70**:258−271.

24. Erbar C, Leins P: **Portioned pollen release and the syndromes of secondary pollen presentation in the *Campanulales-Asterales*-complex**. *Flora* 1995, **190**:323−338.

25. Jost M, Samain M, Marques I, Graham SW, Wanke S: **Discordant phylogenomic placement of Hydnoraceae and Lactoridaceae within Piperales using data from all three genomes**. *Front. Pl. Sci.* 2021, **12**.

26. González F, Stevenson DW: **Perianth development and systematics of *Aristolochia***. *Flora* 2000, **195**:370−391.

27. Oelschlägel B, Nuss M, Tschirnhaus M von, Pätzold C, Neinhuis C, Dötterl S, Wanke S: **The betrayed thief − the extraordinary strategy of *Aristolochia rotunda* to deceive its pollinators**. *New Phytol* 2015, **206**:342−351.

28. Seberg O, Petersen G, Davis JI, Pires JC, Stevenson DW, Chase MW, Fay MF, Devey DS, Jørgensen T, Sytsma KJ, Pillon Y: **Phylogeny of the Asparagales based on three plastid and two mitochondrial genes**. *Am J Bot* 2012, **99**:875−889.

29. Dressler RL: *The orchids: natural history and classification*. Cambridge, Massachussets: Harvard University Press; 1981.

30. Dressler RL: **Features of pollinaria and orchid classification**. *Lindleyana* 1986, **1**:25−30.

31. Dressler RL: **Rostellum and viscidium: divergent definitions**. *Lindleyana* 1989, **4**:48−49.

32. Rasmussen FN: **On the various contrivances by which pollinia are attached to viscidia**. *Lindleyana* 1986, **1**:21−32.

33. Coen ES, Meyerowitz EM: **The war of the whorls: genetic interactions controlling flower development**. *Nature* 1991, **353**:31−37.

34. Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky M: **B and C floral organ identity functions require *SEPALLATA* MADS-box genes**. *Nature* 2000, **105**:200−203.

35. Honma T, Goto K: **Complexes of MADS-box proteins are sufficient to convert leaves into floral organs**. *Nature* 2001, **409**:525−529.

36. Bowman JL, Moyroud E: **Reflections on the ABC model of flower development**. *Plant Cell* 2024, **36**:1334−1357.

37. Litt A, Kramer EM: **The ABC model and the diversification of floral organ identity**. *Sem. Cell Dev. Biol.* 2010, **21**:129−137.

38. Alvarez J, Smith DR: ***CRABS CLAW* and *SPATULA*, two Arabidopsis genes that control carpel development in parallel with *AGAMOUS***. *Development* 1999:2377−2386.

39. Alvarez J, Smith DR: ***CRABS CLAW* and *SPATULA* genes regulate growth and pattern formation during gynoecium development in *Arabidopsis thaliana***. *Int. J. Pl. Sci.* 2002, **163**:17−41.

40. Zuñiga-Mayo VM, Gómez-Felipe A, Herrera-Ubaldo H, DeFolter S: **Gynoecium development: networks in Arabidopsis and beyond**. *J Exp Bot* 2019, **70**:1447−1460.

41. Heisler MG, Atkinson A, Bylstra YH, Walsh R, Smyth DR: ***SPATULA*, a gene that controls development of carpel margin tissues in Arabidopsis, encodes a bHLH protein**. *Development* 2001, **128**:1089−1098.

42. Gremski K, Ditta GS, Yanofsky MF: **The HECATE genes regulate female reproductive tract development in *Arabidopsis thaliana***. *Development* 2007, **134**:3593−3601.

43. Alvarez JP, Goldshmidt A, Efroni I, Bowman JL, Eshed Y: **The *NGATHA* distal organ development genes are essential for style specification in *Arabidopsis***. *Plant Cell* 2009, **21**:1373−1393.

44. Trigueros M, Navarrete-Gómez M, Sato S, Christensen SK, Pelaz S, Weigel D, Yanofsky MF, Ferrándiz C: **The *NGATHA* genes direct style development in the Arabidopsis gynoecium**. *Plant Cell* 2009, **21**:1394−1409.

45. Pérez-Mesa P, Ortíz-Ramirez CI, González F, Ferrándiz C,
** Pabón-Mora N: **Expression of gynoecium patterning transcription factors in *Aristolochia fimbriata* (Aristolochiaceae)**

and their contribution to gynostemium development. *EvoDevo* 2020, **11**:4.

The authors studied the expression of all canonical gynoecium patterning genes in *Aristolochia fimbriata*, a species with gynostemium. The stigmatic tips rarely express a canonical gynoecium patterning gene, suggesting that they could be novel structures.

46. Specht CD, Howarth DG: **Adaptation in flower form: a**
  * **comparative evodevo approach**. *New Phyt* 2015, **206**:74−90.
A thorough review of organ boundary genetic regulation

47. Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M: **Genes involved in organ separation in *Arabidopsis*: an analysis of the cup-shaped cotyledon mutant**. *Plant Cell* 1997, **9**:841−857.

48. Sakai H, Medrano LJ, Meyerowitz EM: **Role of *SUPERMAN* in maintaining Arabidopsis floral whorl boundaries**. *Nature* 1995, **378**:199−203.

49. Breuil-Broyer S, Trehin C, Morel P, Boltz V, Sun B, Chambrier P,
** Ito T, Negrutiu I: **Analysis of the Arabidopsis superman allelic series and the interactions with other genes demonstrate developmental robustness and joint specification of male−female boundary, flower meristem termination and carpel compartmentalization**. *Ann Bot* 2016, **117**:905−923.
A detailed allelic dissection of *SUPERMAN* showing early and late roles for this transcription factor in carpel to stamen separation and floral meristem termination.

50. Prunet N, Morel P, Negrutiu I, Trehin C: **Time to stop: flower meristem termination**. *Plant Phys.* 2009, **150**:1764−1772.

51. Takeda S, Matsumoto N, Okada K: **RABBIT EARS, encoding a SUPERMAN-like zinc finger protein, regulates petal development in *Arabidopsis thaliana***. *Development* 2004, **131**:425−434.

52. Payne T, Johnson SD, Koltunow AM: **_KNUCKLES_ (_KNU_) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the Arabidopsis gynoecium**. *Development* 2004, **131**:3737−3749.