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Diversifying floral organ identity

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Abstract

A fascinating component of floral morphological diversity is the evolution of novel floral organ identities. Perhaps the best-understood example of this is the evolutionary sterilization of stamens to yield staminodes, which have evolved independently numerous times across angiosperms and display a considerable range of morphologies. We are only beginning to understand how modifications of the ancestral stamen developmental program have produced staminodes, but investigating this phenomenon has the potential to help us understand both the origin of floral novelty and the evolution of genetic networks more broadly.

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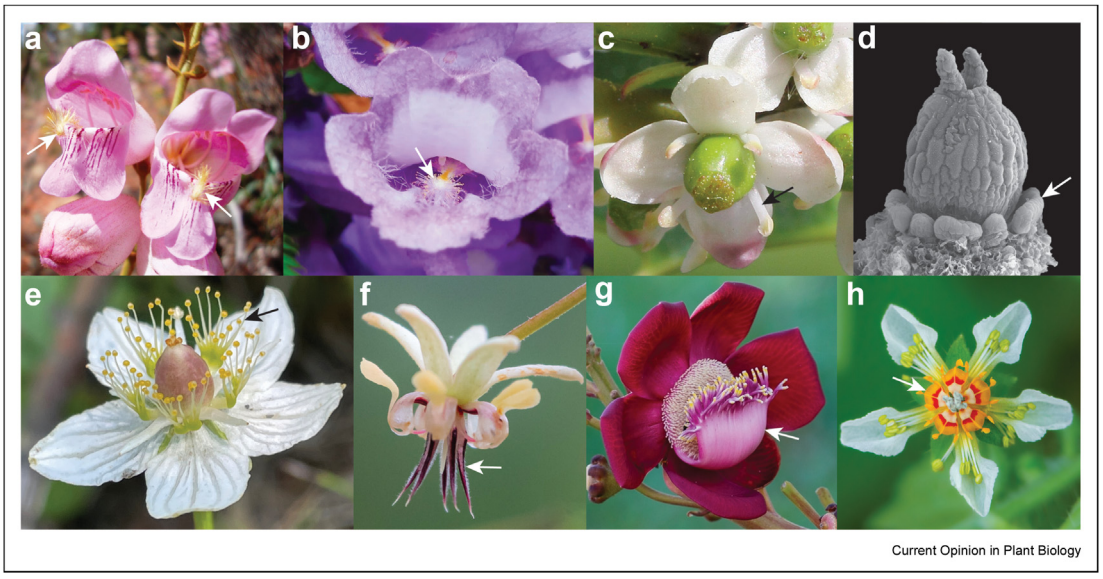
Introduction

Stamens, collectively termed the androecium, are a fundamental part of flowering plant reproduction as the production site of pollen. However, their evolutionary history is quite dynamic, and many aspects of stamens have been modified across angiosperms: their total number per flower, ranging from a single stamen in some members of Araceae [1] to upwards of 4000 in Cactaceae [2]; their polarity, where members of the ANA grade and Magnoliids often have laminar stamens, while eudicots and monocots have distinct, radialized stamen filaments that bear anthers with four-fold symmetry [3,4]; their fusion patterns, which range from fully free to partially or fully connate (such as in members of Fabaceae or Malvaceae [1]) to fully or partially adnate to the corolla (such as in members of Acanthaceae [5]); their elaboration and arrangement, such as the highly modified stamens in Melastomataceae [6]; and, in extreme cases, their fertility and primary function in pollen production, of which staminodes are the primary example.

Structures that are believed to result from the evolutionary sterilization of stamens are termed "staminodes" [7] and have been documented in at least one species in 32.5% of families across every major clade of angiosperms [8]. In some taxa, staminodes are retained as small, vestigial nubbins that serve no obvious purpose, but in many others, staminodes have evolved to serve an array of functions, including pollinator attraction or deception, or protection of developing ovaries, that are associated with diverse morphologies and are often integral traits used to systematically define entire clades (Figure 1; [8–10]). Staminodes routinely occur in one of three androecial configurations, each having distinct patterns of development and evolutionary histories [8]: (1) flowers that contain a whorl comprised of fertile stamens as well as one or more staminodes, which occur most frequently in flowers that have undergone the evolutionary transition from actinomorphy to zygomorphy [Figure 1a,b]; (2) flowers in which the androecium is wholly composed of staminodes due to their loss of all stamen function during their evolution of unisexuality [Figure 1c,d]; and (3) flowers that contain one or more full whorls of staminodes in addition to retaining a full whorl or whorls of functional stamens [Figure 1e,f]. In regard to Type 2 staminodes, which are studied in the context of sex determination, which is a broad and complex field of its own that would require a dedicated review (e.g. Ref. [11]), so we will focus on Types 1 and 3. It is also important to note that more rarely staminodes fall outside these three types, such as the staminodial hood seen in some members of Lecythidaceae [Figure 1g] and the corona-like ring of staminode complexes that alternate within the same whorl as fertile stamens in some members of Loasaceae [Figure 1h].

Although the morphological development and evolutionary diversity of staminodes have been well characterized [see 8,9 for thorough documentation], we are in the early phases of determining how genetic modifications to the stamen developmental program have given rise to these many examples of novel organ identities. In this review, we highlight what is known about the

Figure 1



A sampling of staminode diversity. **(a)** *Penstemon palmeri*, Type 1 staminode (iNaturalist user mustardlypig). **(b)** *Jacaranda mimosifolia*, Type 1 staminode (iNaturalist user joas_df). **(c)** *Ilex aquifolium*, Type 2 staminodes (Holger Casselman). **(d)** *Paronychia minima*, Type 2 staminodes (A. Appleton). **(e)** *Parnassia palustris*, Type 3 staminodes (iNaturalist user zanethebrain). **(f)** *Theobroma cacao*, Type 3 staminodes (iNaturalist user lesbectrotters). **(g)** *Couroupita guianensis*, staminodes outside of type categorization (iNaturalist user ronaldo_g_lebowski). **(h)** *Loasa triloba*, staminodes outside of type categorization (iNaturalist user niboldus). Arrows point to staminodes.

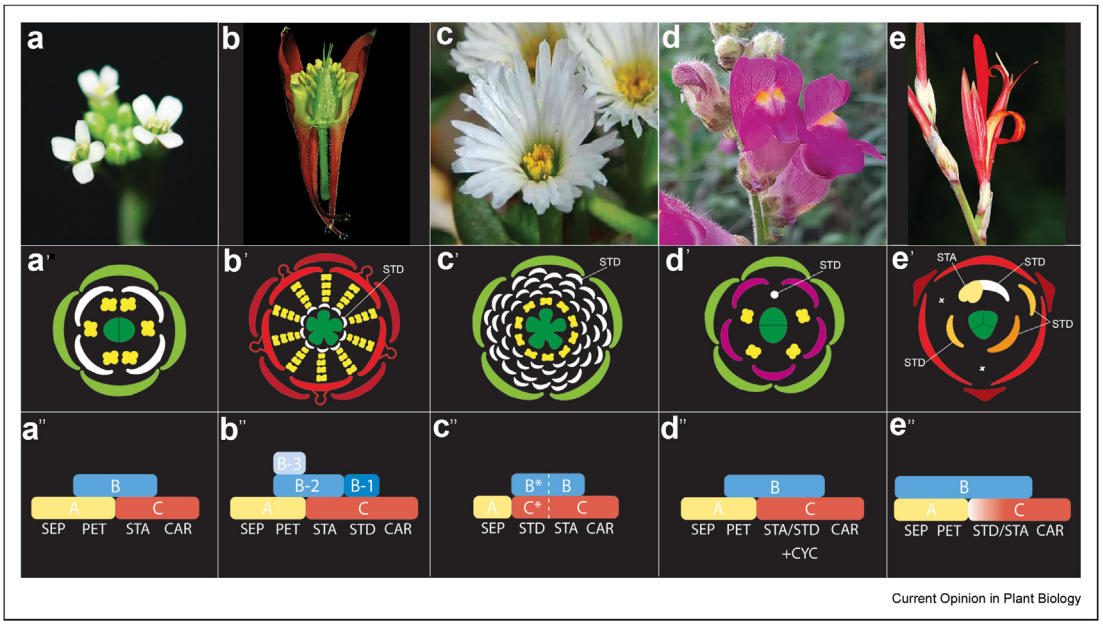
genetic underpinnings of staminode evolution and development and touch on what is yet to be discovered about these common yet understudied structures.

Staminode identity

The developmental basis of floral organ identity has been studied for decades, and can be predicted by the generally well-conserved ABC model of floral organ identity, in which classes of MADS-box transcription factors act in a combinatorial fashion to produce four floral organ types (Figure 2a; [12,13]). In this model, the A-class gene expression alone codes for sepals; A- and B-class genes together code for petals; B- and C-class genes together code for stamens, and C-class genes alone code for carpels [13]. Staminodes, as derivatives of stamens, likely express some combination of B- and C-class genes, but it is possible that modification of the B + C identity program has occurred in many different ways.

Given that MADS-box transcription factors tend to function in entire whorls, Type 3 staminode identity, in which flowers have evolved one or more full whorls of staminodes, in addition to retaining functional stamens, are perhaps the best candidates for a simple modification of B or C gene expression, and we have two such examples. The flowers of the lower eudicot *Aquilegia* (Ranunculaceae) contain two whorls of papery, interlocking staminodes nested within many whorls of fertile stamens, yielding flowers with five distinct organ identities (Figure 2b; [14]). In *Aquilegia*, ancient paralogs of the B-class gene *APETALA3 (AP3)* have recently diversified in function: *AqAP3-1* is neofunctionalized to control staminode identity, while the subfunctionalized copies *AqAP3-2* and *AqAP3-3* are respectively responsible for fertile stamen and petal identity (Figure 2b; [15,16]). In an example that is simultaneously more evolutionarily complex and more morphologically simple, *Delosperma napiforme* (Aizoaceae) represents a lineage that ancestrally lost its petals but then re-evolved petaloid organs from the outer stamens to reestablish four organ identities (Figure 2c; [17]). In these flowers, the fertile stamens and petaloid staminodes both express B- and C-class homologs early in development, but this expression is only transient in the outer staminodes, while it is maintained throughout the development of fertile stamens (Figure 2c; [17]). Although we would greatly benefit from an investigation of further examples of Type 3 staminodes, these two cases already reveal a variety of evolutionary trajectories. It seems intuitive that *Aquilegia* has taken advantage of gene duplication to intercalate a fifth identity between the fertile stamens and carpels, but it is interesting that *Delosperma* has re-evolved petaloid organs without relying on this mechanism. In both examples, we see regulatory evolution acting upstream of the MADS box genes to produce greater spatial and temporal complexity. It remains to be determined whether these

Figure 2



The standard ABC pattern of *A. thaliana* compared to four examples of staminode identity. Each column corresponds to a single species with the top row showing a flower, the second row showing a simplified floral diagram, and the third row showing a simplified representation of the floral organ identity program. **(a)** *Arabidopsis thaliana* (Andrea Appleton). **(b)** *Aquilegia* × *coerulea* 'Kirigami' (Colin Teo; diagram and schematic adapted from Sharma and Kramer 2013 and Min et al., 2022). **(c)** *Delosperma napiforme* (Succulent Alley; schematic adapted from Brockington et al., 2011). **(d)** *Antirrhinum majus* (iNaturalist user josep_sais_brucet). **(e)** *Canna indica* (C. Gracie, NYBG Steere Herbarium; diagram adapted from Miao et al., 2014). SEP = sepal; PET = petal; STA = stamen; STD = staminode; CAR = carpel. In panel b″, B-1, B-2, and B-3 indicate paralogs of the B-class gene *APETALA3*. In panel c″, * indicates transient gene expression. In panel e″, the gradient of color in the C domain represents a gradient of C gene expression.

changes are in cis- or trans-to the loci themselves and, perhaps more intriguingly, how the regulatory delimitation of the distinct staminode whorls has been achieved in each taxon.

Staminodes and floral symmetry

Of course, differential expression of MADS-box genes between stamens and staminodes is not the only developmental divergence between the two organs. Floral symmetry is a critical aspect of floral architecture and may have impacts on organ morphology without changes to the expression of the organ identity genes. In this regard, the Type 1 staminode present in *Antirrhinum majus* (Plantaginaceae) has been well-studied. The flowers of *A. majus* are bilaterally symmetrical with four fertile stamens and a single, highly reduced dorsal staminode (Figure 2d; [18,20]). In *A. majus*, MADS-box genes are not differentially expressed between stamens and staminodes, but rather, another genetic pathway acts in parallel to the identity module. Floral zygomorphy in eudicots is typically controlled by the convergent recruitment of *CYCLOIDEA (CYC)* homologs [18,19], and in *A. majus*, *CYC* has been found to function additively with B- and C-class genes to transform the single, dorsal stamen into a staminode (Figure 2d; [20]). Notably, this aspect of *CYC* function is specific to its interaction with stamen identity as a homeotic transformation of the dorsal stamen into other identities restores organ development [21]. In the close relative *Mohavea confertiflora*, which has staminodes in both dorsal and lateral positions, *CYC* expression is coincidently expanded laterally, suggesting that this CYC + B + C = staminode program can be executed in any androecial position [22].

Floral asymmetry is a less common phenomenon but one that has also been shown to have a direct correlation with organ identity [23]. The most thoroughly studied example in this context is *Canna indica* (Zingiberaceae), which represents an extreme expression of the overall trend in this order to sterilize stamens (discussed in Ref. [23]). *Canna* demonstrates several forms of floral asymmetry and does not fit neatly into the Type 1–3 categories described above (Figure 2; [23]). The androecium of *C. indica* is complex, having evolved multiple petaloid staminodes as well as a highly modified single remaining fertile stamen that contains only a half an anther born on a petaloid filament (Figure 2e; [24]). The androecium is organized into two whorls: the outer whorl contains one petaloid staminode, while the inner whorl contains three morphologically distinct petaloid staminodes. The first of these is similar to the

organ in the outer whorl, but the other two are differentiated into an enlarged organ termed the labellum and the petaloid, fertile half-stamen (Figure 2e; [25]). The genetic basis of these unique organ types is still being explored, but expression of the B-class gene *GLOBOSA (GLO)* and C-class gene *AGAMOUS* (*AG*) appears to be much higher in the fertile portion of the half-stamen than the sterile staminode tissue [23]. This suggests a dosage model in which higher expression is required for the development of the fertile component. Further, unlike the case of zygomorphy in *A. majus*, it appears that whatever pathway controls floral asymmetry in *C. indica* is acting upstream of the floral organ identity gene expression. This parallels other instances of floral zygomorphy in monocots, most notably the orchids, which similarly exhibit differential expression of floral MADS box genes along their dorsal/ventral axis [26].

Staminode elaboration

Developmental processes downstream of the establishment of organ identity and floral symmetry, including but not limited to fusion, color, texture, and aspects of organ polarity, can dramatically influence the morphology of staminodes. In this regard, it is important to consider the divergence of staminode morphogenesis relative to that of the stamens from which they are derived. A typical eudicot and monocot stamen is composed of a narrow filament that bears a terminal anther with two theca containing two pollen sacs each. Studies in rice and *Arabidopsis* have found that this morphology requires a dramatic reorganization of typical lateral organ polarity [27], such that the filament is radialized (abaxialized in rice, adaxialized in *Arabidopsis*), while the anther has four axes of adaxial/abaxial identity juxtaposition, which drives the outgrowth of the theca [4]. Studies in *Aquilegia* and *C. indica* have found that the evolution of staminode identity has gone hand in hand with reversals of the canonical stamen polarity patterns.

The staminodes in *Aquilegia* present as a papery sheath that encloses the carpels and are thought to be equivalent to laterally expanded stamen filaments (Figure 2b; [14]). Meaders et al. [14] found that the YABBY transcription factor *FILAMENTOUS FLOWER (FIL)* is initially expressed from the base to the tip of the abaxial surface of both stamen and staminode primordia, but in the stamen, it becomes localized to the anther region and is lost from the adaxialized, radial filament. In the staminodes, *FIL* expression persists across the entire abaxial surface throughout development, which reestablishes a balanced abaxial/adaxial axis and promotes lateral outgrowth. A similar phenomenon is observed in Zingiberaceae, where YABBY genes are overexpressed in abaxialized, radial stamen filaments, but a balanced abaxial—adaxial axis is reestablished in laminar staminodes [28]. However, a more complex pattern is observed in the chimeric half-stamen of *C. indica*, where adaxial identity is present as a small patch in the half-anther, but both adaxial and abaxial identities are observed in the laminar petaloid appendage [24]. Thus, it appears that the loss of fertile stamen identity in these disparate staminodes is closely associated with reversion to the typical lateral organ pattern of abaxial/adaxial identity, which facilitates their elaboration as laminar structures.

As seen in Figure 1, laminar expansion is only the beginning of the elaborations observed in diverse staminodes. In *Aquilegia*, the papery staminodes are associated with an asymmetric pattern of lignification as well as marginal curling that facilitates lateral adhesion, both of which may be related to a protective function. The genetic basis of color, texture, branching, bending, curling, and other features of staminodes present across angiosperms are yet to be discovered.

Concluding thoughts and future considerations

The evolution of new floral organ identity programs has occurred in many different times across the angiosperms, commonly using stamens as a starting point. From the examples discussed here, we see that evolution can take diverse trajectories to yield staminodes. In regard to identity, modifications can occur upstream of the B/C loci, as well as at the level of these genes themselves or in parallel. Beyond mere sterilization, a critical component of staminode elaboration in some taxa appears to be re-establishment of organ abaxial/adaxial polarity in order to promote lateral expansion. Detailed, comparative studies in closely related taxa, ideally with distinct conformations of staminodes, could provide greater insight into the regulatory mechanisms underlying staminode evolution and diversification, especially in nonmodel species.

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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