Is the leguminous flower closed?

Andrey A. Sinjushin

Summary: A variety of gynoecium types in the legume family is briefly reviewed in terms of phyllotaxis and spatial patterning. All variations can be narrowed down to changes in sizes of floral meristem and time of its proliferation. Increase in floral meristem size results in the initiation of more carpels, so the phyllotaxis of floral apex changes from alternate to whorled. Differences between monomerous and polymerous gynoecium in legumes are quantitative and not connected with alternation in floral meristem determinacy.

Keywords: Fabaceae, floral development, monomerous gynoecium, zygomorphy, phyllotaxis

An outstanding variability of the flower, a distinguishing feature of angiosperms, concerns all floral domains including the gynoecium. The third largest angiosperm family, Fabaceae s.l. (= Leguminosae), has surprisingly uniform apocarpous gynoecia. With some rare exceptions, legumes produce flowers with a monomerous gynoecium which gives rise to a pod. Some species have a polymerous gynoecium which was earlier interpreted as a primitive state but now seems to evolve repeatedly as an evolutionary novelty (Sinjushin 2014; Paulino et al. 2014). Multicarpellate gynoecia may also arise in flowers of monocarpellate leguminous species as a result of mutations or other developmental distortions (Sinjushin 2014 and works cited there).

To date, detailed data on floral ontogeny of both multicarpellate wild species (Paulino et al. 2013, 2014) and mutants of normally monocarpellate taxa (Sinjushin 2014) are being accumulated.

When analyzing results of a careful comparative survey on floral development in different multicarpellate legumes, Paulino et al. (2014) concluded that the production of additional carpels in a leguminous flower is connected with a transition from a closed (determinate) to an open (indeterminate) state of floral meristem. These authors also pointed out that a polymerous gynoecium often accompanies a polymerous androecium and is absent from flowers with a typical papilionoid corolla with a specialized adaxial petal (flag).

This paper pursues a goal to briefly comment on these conclusions in order to render alternative interpretations of provided data.

Some features of genetic control of flower spatial patterning

Detailed studies on molecular mechanisms governing meristem activity in a model species *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) result in a self-consistent system. A meristematic state is maintained by expression of gene *WUSCHEL* (*WUS*) which is transcribed in a limited group of cells in both apical and floral meristem (AM and FM) (see Penin & Logacheva 2011 for review). Spatially *WUS*-expressing domain is limited by numerous genes which negatively regulate expression of *WUS*. Among them, the most well-known are *CLAVATA* (*CLV1*, *CLV2*, *CLV3*) and *FASCIATA* (*FAS1*, *FAS2*). If any of these regulators is mutated, an expression of

WUS loses negative control and is found in more AM cells, so AM enlarges – the phenomenon is called fasciation. In *A. thaliana*, the mutants with stem fasciation also bear features of flower fasciation which is most strikingly manifested in the gynoecium. Instead of two, more carpels are produced (PENIN & LOGACHEVA 2011). In many cases, an additional whorl of carpels is initiated within the first one resulting in two siliques, the second enclosed in the first one. It is not the case within legume family where all known fasciated mutants lack any signs of FM enlargement (see SINYUSHIN 2010 for details). Possibly it means that FM in this family has an additional negative regulation of *WUS* expression except for *CLV* and *FAS* in a stem. Possible candidates for this role are *CYCLOIDEA* (*CYC*)-like genes.

In *Antirrhinum majus* L. (Plantaginaceae), gene *CYC* defines a monosymmetric pattern of flower; *cyc* mutants produce a polysymmetric corolla (ALMEIDA et al. 1997). This gene was reported as suppressor of cell divisions in FM acting via the cyclin-dependent pathway (GAUDIN et al. 2000). Its orthologs were found in legumes where they have a similar expression pattern in FM obviously governing floral symmetry formation (FUKUDA et al. 2003).

As for regulation of FM sizes along a proximodistal axis, *WUS* is negatively regulated by *AGAMOUS* (*AG*) in *A. thaliana* (see PENIN & LOGACHEVA 2011 for review). This gene also promotes differentiation of organs' primordia into stamens and carpels. The *ag* mutants have a distal floral domain consisting only of petals which initiate a supernumerary quantity due to unlimited FM proliferation.

Finally, spatial patterning of floral structures is ruled by the same mechanisms as in the vegetative stem, i.e. follows the phyllotaxis principles which are precisely studied and verified by modeling (ALEXEEV et al. 2005). Initiation of a novel lateral primordium on AM or FM is associated with origin of auxin flow through it and an inhibitory zone around it. No more primordia can be initiated within this zone, so the next phyllome primordium arises on a maximum offset from the previous after enough meristem volume has been accumulated. The resulting spatial arrangement of organs depends on both meristem size and area of inhibitory zone around every newly initiated primordium. If the meristem is comparatively small, phyllomes would readily be arranged in an alternate way with torsion angle beginning from 180° (small meristem) and tending to 137,5° (larger meristem with comparatively small inhibiting zones). In the largest meristems, a whorled phyllotaxis can be realized with a number of organs per whorl increasing with meristem enlargement. The latter scenario is more typical for flowers with whorled organ arrangement.

Formation of additional carpels in terms of phyllotaxis

When discussing the structure of flower with a (pseudo)monomerous gynoecium, one of the main questions is whether a single carpel occupies true terminal position or arises laterally. In legumes, a single carpel is interpreted as a final derivative of floral meristem being truly terminal (TUCKER 1987) although this topic is debatable (LEINFELLNER 1969).

The idea of true terminal position of a single conduplicate carpel seems untenable as such carpel has clearly demarked abaxial and adaxial surfaces hence exhibiting clear dorsoventral polarity typical for organ of foliar origin. As a leaf itself cannot be terminal on a stem, a conduplicate carpel cannot terminate the main axis, i.e. arise in true terminal flower. Although rare, such flowers can be found among legumes, such as well-known *Gleditsia triacanthos* L. and *Gymnocladus*

dioicus (L.) K. Koch. (Weberling 1989). This constructional limitation seems sufficient to discard the idea of true terminal origin of carpel in leguminous flower.

Although a monocarpellate flower seems the initial evolutionary state in the whole Fabaceae s.l. family, it is most probable that ancestors of the whole order Fabales possessed a multicarpellate flower. That is the case of non-leguminous families nested within the same order, i.e. Polygalaceae, Surianaceae and Quillajaceae (APG 2009). If it's accepted that a primary structural plan for flower of Fabales is pentamerous and pentacyclic, one may expect that a gynoecium was also pentamerous (G_5), possibly apocarpous. In a lineage which gave rise to Fabaceae s.l., only a single carpel remained (G_1). This has exactly the expected cleft orientation which might be in an abaxial carpel in a pentamerous gynoecium. It is true for a flower with a median sagittal petal on the adaxial side, i.e. not for mimosoid legumes (Tucker 2003).

It is possible to explain the transition from G_5 to G_1 in terms of spatial regulation of FM patterning. If it is supposed that gynoecium of Fabales was initially spiral rather than whorled (which is not true at least for Surianaceae and Quillajaceae with a simultaneous initiation of carpels, see Bello et al. 2007), than such evolutionary switch could be achieved via shortening of FM from a distal portion – possibly via *AG* activation. After such abbreviation, only the first carpel was able to initiate which persisted as the contemporary gynoecium of a leguminous flower.

Alternatively, one may propose that a striking decrease of FM size occurs in the ontogeny of such flower after spatial patterning of the inner staminal whorl. In this case, the fourth floral whorl initiates as pentamerous, but then FM diminishes letting only a single organ initiate. Cases, when a merosity is unequal in different flower whorls, are not rare (e.g., $G_{(3)}$ in flowers with a pentamerous perianth in Hypericaceae or Cucurbitaceae). This may be explained exclusively by changes in FM sizes during initiation of different floral domains – or, more strictly, in ratio between FM size and area of inhibition zones of primordia of different domains. In terms of phyllotaxis, an androecial domain in leguminous flower is characterized by a whorled arrangement of phyllomes (stamens) while the fifth has an alternate one. Interestingly, cases of monomerous gynoecium can hardly be explained within the concept of two zones of floral spatial patterning, acropetal and basipetal (Choob & Penin 2004, Penin & Logacheva 2011). Floral apex remains open, as it produces no terminal organs.

If FM sizes and duration of proliferation vary, a leguminous flower can become multicarpellate. It is noteworthy that in bicarpellate flowers of Fabaceae the second carpel is initiated later. It is of smaller size and often fails to develop fully. This is the case of anomalous *Cassia fistula* L. described by Paulino et al. (2014), *Albizia lophanta* Benth. (Sattler 1973) and possibly *bi-pistil* mutants of *Medicago truncatula* Gaertn. (Nair et al. 2008). A similar phenomenon was recorded in *Cerasus vulgaris* Mill. by Shamrov & Yandovka (2008). If two carpels initiate one after another in a normally monocarpellate flower, the resulting gynoecium is of G_{1+1} rather than G_2 type. Such flower is more 'open' than normal G_1, as FM proliferates for longer time but both carpels are lateral and occupy the successive nodes. If this proliferation would continue, a flower might produce more carpels which are all arranged in an alternate way (Fig. 1D). This case was not recorded in legumes but is typical for G_∞ gynoecia, like in Rosaceae or Ranunculaceae. Flowers of *Myosurus* L. comprise one of the most striking examples of time-expanded FM proliferation which results in a very elongate receptacle bearing numerous carpels. Paulino et al. (2014) also
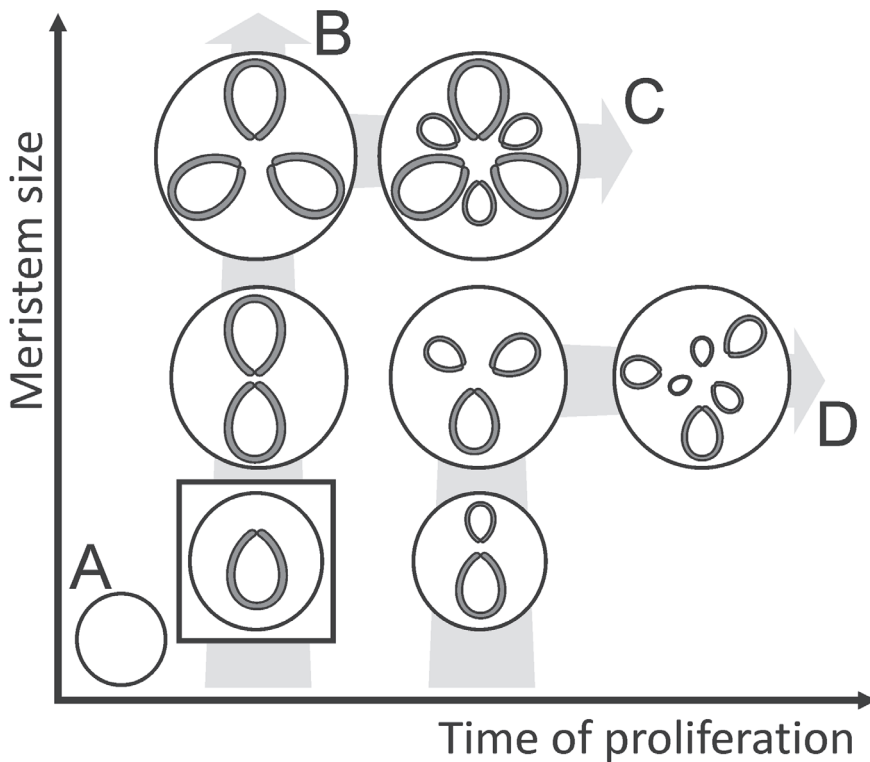
Figure 1. Variations in apocarpous gynoecium caused by changes in FM size and time of proliferation. See text for explanation. A typical gynoecium of leguminous flower is enframed.

provide some intriguing examples of multicarpellate leguminous flowers in which carpels are initiated centripetally, but not always belong to a single whorl (*Inga* p.p.). The hypothetical evolution of gynoecium in Fabales ($G_5 \rightarrow G_1 \rightarrow G_n$) possibly represents the case of variation in gynoecium structure via merosity changes discussed by SOKOLOFF (2015)

An enlargement of FM in gynoecial domain may cause initiation of carpels in a whorl. A merosity of this whorl depends on FM size (Fig. 1B). The first stage is to produce two carpels on the same node (true G_2 case). For example, flowers of *Alchemilla vulgaris* s.l. (Rosaceae) normally have a single carpel but sometimes can produce two equally developed carpels oriented with their clefts towards the centre of the receptacle (GLAZUNOVA 1986).

If FM continues proliferation after initiation of gynoecium, an additional whorl of carpels can arise within the first one (Fig. 1C). This process is illustrated by fasciated mutants of *A. thaliana* (see above). In ultimate cases of male flowers of dioecious species, FM might become unavailable for initiation of even one single carpel (Fig. 1A). However, in legumes the unisexuality is usually achieved as a result of preferential development of either androecium or gynoecium while the other domain is suppressed after initiation (TUCKER 2003).

As it has been discussed previously, some other ways to achieve multicarpelly exist (SINJUSHIN 2014). These are fusion of two independently patterned FMs (as possibly in *Swartzia*) and homeotic replacement of stamens with carpels. All these cases can be illustrated with developmental mutants and terata of *Pisum sativum* L. (SINJUSHIN 2014).

Polymerous gynoecium and perianth symmetry

As Paulino et al. (2014) noted, a floral multicarpelly is absent from legumes with typical flag blossoms. These authors proposed that this phenomenon deals with an intimate orchestration of all floral parts in a specialized flag blossom of most papilionoids – a state hardly achievable in a multicarpellate flower. In this connection, production of supernumerary carpels would impair the perfect mechanism of pollen presentation.

In addition to this valid proposition, one may point at the fact that almost all groups of legumes with polymerous gynoecia are restricted to clades which possess floral morphology distinct from one of highly specialized papilionoids. The vast majority of known species with a normal multicarpelly belongs to groups with either a monosymmetric perianth or with a blossom which acts in a special way (as in swartzioids) – not like in taxa with a 'typical' papilionoid perianth. From regulatory point of view, it is easier to initiate more carpel primordia in a flower where FM size is not restricted by differential *CYC* expression (see above). Absence of *CYC* activity would result in a polysymmetric perianth.

There is at least one intriguing example of multicarpellate papilionoid legume, viz. *Thermopsis turcica* Kit Tan, Vural & Küçük. It belongs to a genus with a typical monosymmetric papilionoid flower and possesses all features of floral zygomorphy including fused keel petals and a discernible flag (Uysal et al. 2014). No data on pollination strategy of this species exist; it was only identified as self-compatible (Tekdal & Çetiner 2014). Although a polymerous gynoecium in this genus is of recent origin, it is associated with a typical flag blossom. Details of pollination biology of this species are yet to be discovered.

Conclusion

During recent decades, we received a possibility to uncover deep molecular mechanisms which underlie developmental and evolutionary events instead of simply hypothesizing an existence of such events. Application of data obtained from studies on mutants of model objects to non-model species enables us to interpret the existing morphological data more fully. Changes which led to the origin of a polymerous gynoecium in leguminous flower can be interpreted in terms of apical meristem activity, sometimes even proposing candidate regulatory genes.

In this connection, a leguminous flower is always 'open' as a single carpel is lateral on FM. Production of additional carpels does not change its 'openness'. Such event means only that phyllotaxis of a gynoecium changed from alternate to whorled, but all carpels still occupy a single node. A flower with additional carpels within the first whorl (*Inga* p.p.) results from prolonged proliferation of FM, but it changes nothing in the structural plan of flower. Production of more than one carpel in a leguminous flower is not a significant change in floral architecture – this difference is only quantitative as in flowers of the same inflorescence which differ in their merosity.

Acknowledgements

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Address of the author:

Dr Andrey A. Sinjushin
Biological Faculty
Moscow State University
Moscow 119234
Russian Federation
E-mail: asinjushin@mail.ru