Article

Interaction between Floral Merism and Symmetry: Evidence from Fasciated Mutant of *Lupinus angustifolius* L. (Leguminosae)

Andrey A. Sinjushin * [ID]**, Ekaterina A. Bykova and Vladimir V. Choob**

Faculty of Biology, Lomonosov Moscow State University, Moscow 119234, Russia;
katebykova.90@mail.ru (E.A.B.); choob_v@mail.ru (V.V.C.)
* Correspondence: asinjushin@mail.ru; Tel.: +7-495-9395490

check for updates

Abstract: A significant diversity of flower structure in angiosperms is defined by few parameters, among which one may list floral merism and symmetry as the most important. However, observation of normal variation in different taxa indicates that high floral merism (more than six organs) is very rarely associated with a monosymmetry. Precise mechanisms underlying this tendency, as well as the mode of interaction between regulatory pathways of floral symmetry and merism, remain unidentified. In this work, we observed the floral ontogeny in normal plants of *Lupinus angustifolius* L. (Leguminosae), described the floral structure and its variations in fasciated mutant. Our results suggest that monosymmetry is regulated independently from the floral merism, so three types of petals differentiate properly even in flowers with an anomalously high number of organs. However, the adaxial floral domain is the most stable floral part, which is the least susceptible to the fluctuations of merism. This means that some genes responsible for the development of the adaxial flower domain may additionally stabilize it and hence prevent fluctuations of merism in the adaxial domain. The monosymmetry in Leguminosae is a character connected with pollination by insects and requires a precise interaction between all floral parts. That is why fluctuations, which impair this interaction and symmetry, may decrease the pollination efficiency.

Keywords: abaxial domain; adaxial domain; flag blossom; flower; Leguminosae; monosymmetry

1. Introduction

Variation of angiosperm flowers can be classified in accordance with few basic features. Among these features, one may list floral merism or merosity (a number of organs per whorl) and the type of symmetry. The main topic of the present paper deals with an interaction between these two features, both on an ontogenetic and evolutionary level.

In most cases, when discussing floral symmetry, one mainly implies a symmetry of a perianth, especially corolla, although in principle every flower whorl may have its own pattern of symmetry. A bilateral floral symmetry (monosymmetry) can be found in dimerous (Fumariaceae), trimerous (Commelinaceae, Orchidaceae, Pontederiaceae, Aristolochiaceae etc.), tetramerous (Onagraceae, Brassicaceae, Cleomaceae etc.), and especially pentamerous flowers (Leguminosae, Balsaminaceae, Lamiaceae etc.). The association of monosymmetry with a perianth merism higher than five is rare. For example, some representatives of Lythraceae (*Cuphea*, *Pleurophora*) and Resedaceae (*Reseda*) exhibit hexamerous monosymmetric flowers, and this list seems almost exhaustive, although short.

Our study aims to characterize the interaction between monosymmetry and variations in merism. Pentamerous monosymmetric flowers characterize many representatives of the third largest angiosperm family, Leguminosae or Fabaceae. Our earlier work indicated that polysymmetric

leguminous flowers are more prone to fluctuations of merism in comparison with monosymmetric ones [1]. This work focuses on the ontogeny of monosymmetry in the floral meristem (FM) with abnormally high merism. The questions are, (1) whether differences in flower development in fasciated mutant versus wild type plants exist, (2) whether different floral whorls are affected in a similar way, and (3) which floral domain (abaxial or adaxial) is more prone to fluctuations. An overall characterization of fasciated mutant plants beyond their floral phenotype is also briefly given.

2. Materials and Methods

A fasciated mutant line 5M2-253 of *Lupinus angustifolius* L. served as material for our study. This mutant was induced from the non-fasciated cultivar Nemchinovskii-846 (Nm-846) [2] which was used as a wild type sample. Plants were grown on an experimental plot at MSU Zvenigorod Biological Station (Western Moscow region) during summer seasons of 2016–2018. For scanning electron microscopy (SEM) studies, shoot apices and floral buds were collected at the preanthetic stage, fixed and stored in 70% ethanol, then dissected under a stereomicroscope and prepared for SEM according to the previously described protocol [3]. SEM images were taken with the microscope CamScan-S2 (Cambridge University, UK) in a Secondary Electron Image (SEI) regime with an accelerating voltage of 20 kV. All SEM works were carried out in the Interdepartmental laboratory of electronic microscopy of Biological Faculty of the M.V. Lomonosov Moscow State University. When preparing digital images for publication, they were processed using Corel PHOTO-PAINT 2017 (Corel Corporation, Ottawa, ON, Canada). In total, we obtained and analyzed 144 SEM images depicting 67 floral buds on different developmental stages, as well as apices. The counts of floral organs and of leaflets were made on fully open flowers and mature leaves, respectively.

Except for SEM, images were captured from an Olympus SZ61 stereomicroscope (Olympus Corp., Tokyo, Japan) using a UHCCD05000KPA camera (ToupTek Photonics, Zhejiang, China). All measurements were carried out in digital images using the program ImageJ 1.51k (National Institutes of Health, Bethesda, MD, USA).

3. Results

3.1. Vegetative Morphology of Fasciated vs. Normal Plants

The fasciated mutant and the isogenic non-fasciated plants differ mainly in the size of the shoot apical meristem (SAM). From seedling (possibly even embryo) stage onwards, the SAM of the mutant becomes enlarged, flattened in a ridge-like mode and anomalously high, i.e., the SAM apex is more distant from the zone where leaf primordia become apparent (Figure 1A–D). In 10-days-old seedlings, maximum width of SAM averages ($\pm$standard deviation) 452.89 ± 122.99 μm in mutant ($n = 7$) and 150.81 ± 13.33 μm in control ($n = 6$). Growth of the fasciated SAM results in a flattened stem with longitudinal striation and an unusually high number of conducting elements (Figure 1H–I).

On the periphery of the enlarged SAM, leaf primordia initiate in irregular order (Figure 1C). As a result, on later stages, some nodes contain more than one leaf (Figure 1J), so phyllotaxis is irregular.

Due to shifts in phyllotaxis, it is often difficult to distinguish the borders between neighbor nodes in fasciated plants (Figure 1J). In such plants, leaves are often clustered in an asymmetric mode with few palmate leaves emerging on approximately the same level. That is why the comparison of the number of leaflets node by node between fasciated and non-fasciated plants is not quite informative. However, the average number of leaflets per palmate compound leaf in fasciated plants appears to be lower than in the wild-type cultivar (Figure 1K).
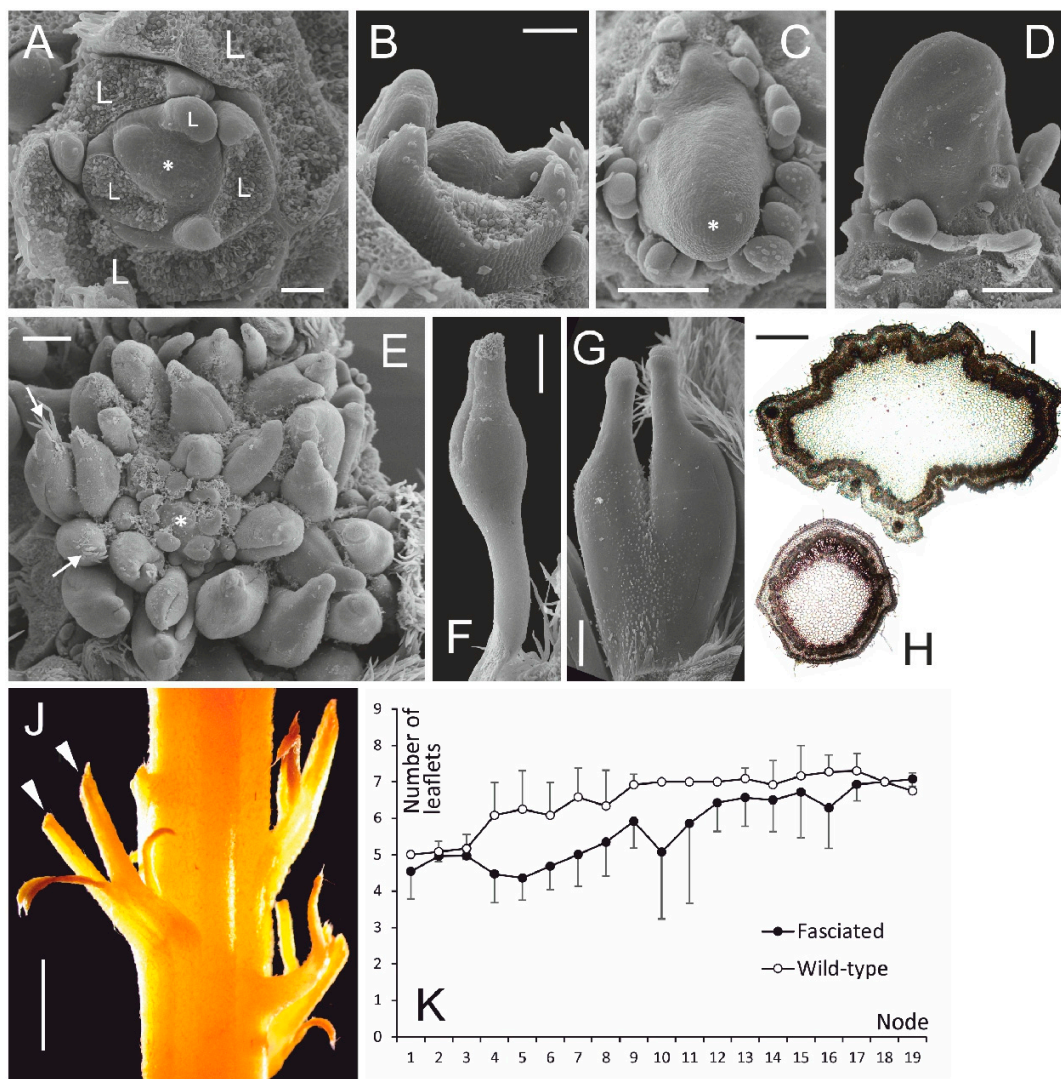
Figure 1. Details of vegetative morphology in normal and fasciated plants of *Lupinus angustifolius*. (**A**–**D**) Apical meristems of week-old seedlings: shoot apical meristem (SAM) of non-fasciated plant, apical (**A**) and side (**B**) views; SAM of fasciated mutant, apical (**C**) and side (**D**) views. (**E**), Apical meristem of fasciated plant (ca. one month after germination) producing numerous carpels (note long trichomes), some of which are underdeveloped (**F**) or fusing (**G**). (**H**,**I**) Cross-sections of fasciated (**H**) and non-fasciated (**I**) shoot (phloroglucinol staining). (**J**), Fasciated shoot (ethanol-fixed material) with two petioles (leaflets removed) in one node. (**K**), Distribution of leaves with different number of leaflets along fasciated versus wild-type shoots (average and standard deviation are presented). Arrowhead = leaf petiole; asterisk = apex; arrow = trichomes on ectopic carpels. Scale bars: 100 μm (**A**,**B**), 300 μm (**C**–**G**), 1 mm (**H**,**I**), 5 mm (**J**).

3.2. Normal Flower Development and Structure in Lupinus angustifolius

The details of floral development in some species of *Lupinus* had been previously reported and illustrated [4,5]. However, in this paper we briefly describe a normal flower ontogeny of *L. angustifolius* (previously undescribed) to highlight differences between the wild-type and fasciated flowers.

On the inflorescence apex, bracts initiate in a helical order (Figure 2A), so the whole inflorescence comprises a simple open bracteous raceme. Soon after emergence, bract acquires a crescent shape with a wide base surrounding elliptic floral primordium (Figure 2B). The first appearing structures are two bracteoles in transversal position (Figure 2C), followed by five broad primordia of sepals. It is interesting to note, that calyx develops slightly faster on the abaxial side (Figure 2D). The inner

part of the floral meristem (FM) acquires a pentagonal shape, as five petal primordia initiate almost simultaneously (Figure 2E). At the same stage (or somewhat later), the innermost part of FM produces the dome-shaped carpel primordium (Figure 2E). Petal primordia are delayed in their growth in comparison with primordia of the outer stamen whorl (Figure 2F). Alongside with enlargement of outer staminal primordia, the carpel assumes a crescent shape with the future cleft facing towards the adaxial side (Figure 2F). The inner whorl stamens are the latest initiated flower organs, and the adaxial side staminal primordia appear at the very end of flower development (Figure 2F,G).
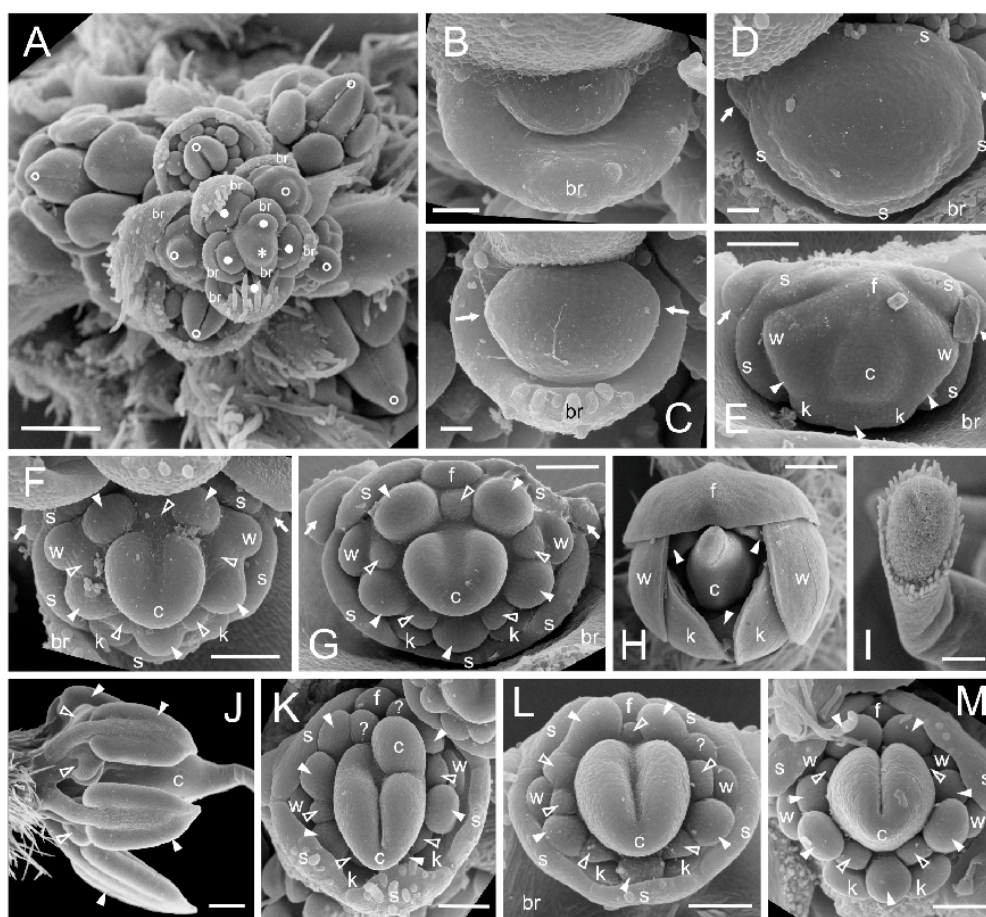

Figure 2. Early stages of floral development in normal (**A**–**J**) and fasciated (**K**–**M**) plants. (**A**), Inflorescence apex (asterisk) with initiating floral meristems (FMs) and developing floral buds, some of bracts and perianth of larger buds removed. (**B**), FM in an axil of bract. (**C**), Bracteoles become visible. (**D**), Sepal formation. (**E**), Initiation of petals, outer stamens and carpel. (**F**), Primordia of inner stamens become discernible. (**G**), Isolation of all floral primordia. (**H**), Young flower exhibiting a descending petal aestivation (calyx removed). (**I**), Stigma. (**J**), Androecium of preanthetic floral bud (perianth removed). (**K**–**M**), Developing fasciated flowers with supernumerary primordia of different organs, such as additional carpel (**K**), six stamens of inner whorl (**L**), and extra wing petals (**M**). Abaxial side is oriented downwards on all images. Circle = floral primordium; empty circle = floral bud; arrow = bracteole; arrowhead = outer whorl stamen (or its primordium); empty arrowhead = inner whorl stamen; br = bract; c = carpel; f = flag; k = keel petal; s = sepal; w = wing petal; ? = primordium of uncertain identity. Scale bars: 30 μm (**B**–**D**), 100 μm (**E**–**G**,**I**,**K**–**M**), 300 μm (**H**,**J**).

In mature flowers, the calyx consists of three separate lobes, with two adaxial free sepals and three abaxial sepals fused in a solitary lobe (Figure 3A). In this abaxial element, only the venation indicates that it originates from three initially independent primordia. The corolla is typically papilionaceous ("flag blossom"), monosymmetric, with three types of petals: one adaxial flag (vexillum), two lateral

wings, and two abaxial petals with medial and distal portions fused in a keel (Figure 3B–D). While the flag is monosymmetric, the wings and the keel petals are asymmetric (Figure 3B–D). Moreover, the wings differ from the other petals by the characteristic epidermal micromorphology, viz. ridge-like sculpture associated with entomophily (Figure 3C,E). Petals are pale blue to lilac, with keel petals usually white with a dark beak. In bud, the flag covers all other petals, while keel is the innermost part hidden under wings (=descending petal aestivation; Figure 2H).

All ten stamens fuse in their base (monadelphous androecium) (Figure 3F) but different whorls can be clearly distinguished (Figures 2J and 3F). The stamens of the outer whorl have long anthers and comparatively short filaments. On the contrary, the inner whorl stamens bear small subglobose anthers and their filaments elongate significantly before anthesis (Figures 2J and 3F).
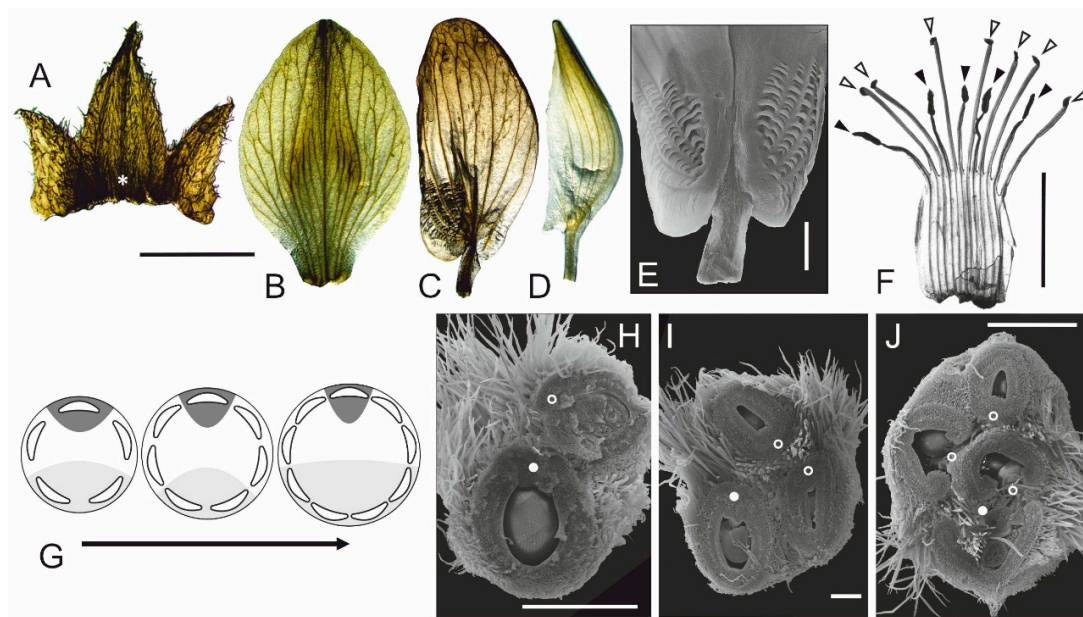

Figure 3. Morphology of mature normal (**A–D**) and fasciated (**E,H–J**) flowers. Objects on **A–D** and **F** are ethanol-fixed material. (**A**), Calyx (dissected between adaxial sepals). (**B**), Flag. (**C**), Wing (adaxial side to the left; note epidermal folds on the adaxial side). (**D**), One of two keel petals (adaxial side to the left). (**E**), A proximal portion of abnormal symmetric lateral petal with the epidermal folds characteristic for wings but on each side of midvein. (**F**), Androecium of fasciated flower with supernumerary stamens (dissected on the adaxial side). (**G**), Schematic representation of changes of corolla differentiation following increase of merism (see text for discussion). Dark grey = adaxial area prepatterned for flag petals, light grey = abaxial area of keel differentiation, white = lateral area of future wings. (**H–J**), Cross-sections of gynoecia of fasciated flowers with two (**H**), three (**I**), and four carpels (**J**). Abaxial side is oriented downwards on **H–J**. Note that three carpels on J are with unfused margins. Asterisk = abaxial lobe of calyx; arrowhead = outer whorl stamen; empty arrowhead = inner whorl stamen; circle = cleft of adaxial carpel; empty circle = cleft of carpel other than adaxial one. Scale bars: 300 μm (**I**), 1 mm (**E,H,J**), 5 mm (**A–D,F**).

The gynoecium consists of a single carpel with a densely pubescent ovary and glabrous style terminated by a papillose stigma (Figure 2I). Four to five ovules develop in the ovary. A carpel cleft is always oriented towards the adaxial side.

The monosymmetry of mature flower is therefore defined by calyx shape, three types of petals, slightly zygomorphic stamen tube (filaments including their fused parts are longer on abaxial side), and adaxial position of a single carpel's cleft. The floral formula for normal flower of *L. angustifolius* is B Bt $K_{[(2):(3)]}\downarrow$ $C1^{flag}:2^{wings}:(2)^{keel}\downarrow$ $A(5+5)\downarrow$ $\underline{G}1\downarrow$ Vm4-5 (format according to [6]).

3.3. Floral Structure in Fasciated Mutant

During two seasons of field observations, we recorded the following phenomenon. After initiating a series of vegetative nodes with palmate leaves, SAM almost completely converts to numerous carpels, while a central part of SAM might remain undifferentiated (Figure 1E). These ectopic carpels develop in different degrees, sometimes producing stigmata and ovules, although some of these carpels seem abortive (Figure 1F) or have unfused margins. They may also bear long trichomes (Figure 1E) which are characteristic for leaves and bracts. Many of these carpels are oriented with their clefts towards the center of the SAM but their arrangement is overall chaotic (Figure 1E). Some of these carpels fuse with each other producing paracarpous constructions (Figure 1G). The subsequent growth and initiation of flowers occurs on lateral branches or some lateral SAM portions that have unclear relations with the main SAM.

In general, the floral development in the fasciated mutant is similar to the ontogeny of normal flowers. The most significant difference is in the number of initiated primordia rather than in the order of their emergence. Only a part (in our counts, 38 of 54 mature flowers) of all the flowers in fasciated plants exhibit distinct morphological deviations from the typical structure.

In fasciated mutant, different floral whorls were affected with unequal frequencies (Table 1). It is difficult to estimate a precise number of calyx elements in mature lupine flower, so calyx was excluded from the counts. Extra petals are common. We recorded supernumerary flag only once, but the development of supernumerary wings is widespread, as well as the production of more than two keel petals (Table 1). Sometimes extra wings also fuse with their claws. Only two or none of few abaxial petals fuse giving rise to a keel. In most cases, it is easy to distinguish between different petal types but sometimes petals differentiate irregularly. For example, transitional wing-to-keel petals may appear, with epidermal ridge-like features similar to the wings but with acute terminus, as in keel petals (see Figure 1 in [7]). Wing petals may be atypically symmetric in shape (Figure 3E). In few flowers, keel petal is inserted in a calyx whorl and exhibits sepaloid features.

Table 1. Percentage of increase of the certain organ number, counted in 35 abnormal flowers. Calyx was not scored due to difficulties in recognition of sepals in mature flowers.

Type of Superfluous Organ(s)	Percent of Anomalous Flowers
Adaxial petal (flag)	2.9%
Lateral petals (wings)	68.6%
Abaxial petals (keel)	5.7%
Outer staminal whorl	54.3%
Inner staminal whorl	85.7%
Gynoecium	20.0%

4. Discussion

4.1. Phenotype of Fasciated Mutant of L. angustifolius Results from Superfluous Proliferation of Meristems

A phenomenon of fasciation drew researchers' attention for centuries and was recorded in numerous plant families [8]. Studies in the model plant *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) provided a detailed overview of molecular and cellular mechanisms underlying this developmental abnormality [9]. In general context, heritable fasciation is a result of the complete loss or insufficient negative control of meristem size. Multiple genes involved in meristem size control have been discovered in *A. thaliana* [9]. Mutations in these genes often result in fasciation, i.e., the function of negative control of SAM size is shared between many genes. In *A. thaliana*, the same genes govern sizes of both SAM and FM, so in most cases shoot fasciation is correlated to the increase of floral merism. However, it is not a uniform rule, as many other plant species may have heritable forms of either shoot or floral fasciation. Although floral anomalies were recorded in few other fasciated mutants of legumes [7], the discussed mutant of *L. angustifolius* is the first with flower morphology

characterized in details. In many legume species, floral merism seems to be under SAM-independent control, thus flowers in fasciated mutants are normal [7]. Nevertheless, in *L. angustifolius* a single mutation affects both the size of SAM and the floral merism. While in fasciated mutants of many other legume species (such as pea or lentils, see [7] for review) flowers remain normal, there are some cases when a single mutation causes both stem fasciation and floral anomalies, including extra flower organs, as in mung bean or soybean [7]. One may hypothesize that in legumes the system of SAM sizes control is as complex as in *A. thaliana*, and some genes may be expressed both in SAM and FM, while others are active in SAM only. Thus, some of mutations causing shoot fasciation may also affect floral ontogeny.

An unusual feature of fasciated mutant of lupine is connected with changes in leaf morphology (Figure 1K). As it was noted by White [8] (p. 328), leaves are usually modified by fasciation through their relation to the main axis. On a periphery of SAM, regions competent for organogenesis arise. These regions were called blastozones [10]. When SAM enlarges due to fasciation, two ways are possible. The first way is the initiation of additional blastozones (this results in altered phyllotaxis accompanied by normal leaf morphology). The second way is to increase the size of blastozones, but their number and position would be comparatively the same as in normal SAM. This way leads to the development of several (one, two, or more) leaves from a single blastozone. It was earlier concluded that in legumes (pea and lupine) the second scenario realizes [11]. As a result, two or more leaves appear instead of one (Figure 1J).

However, the increase of leaflet number per single compound leaf does not accompany shoot fasciation, as it was earlier demonstrated for a garden pea, *Pisum sativum* L. [12]. An increased number of leaflets was reported for fasciated plants of *Trifolium pratense* L. [13], although this seems (at least partly) resulting from fusion of rachises of two leaves arising from the same blastozone.

As FMs initiate successively on the inflorescence apex (Figure 2A), they are all on different stages, so the direct comparison of FM sizes between normal and fasciated plants is very complicated. There are two possible explanations for increased number of floral organs (merism) in fasciated mutant of *L. angustifolius*. On the one hand, the increase of FM size may enable to initiate more primordia per whorl, as discussed by the theory of available space [14]. On the other hand, the primordia of certain floral organs may become smaller in mutant flowers, thus permitting more primordia to pack in each whorl. However, we suggest the first mechanism more preferable, as in fasciated mutants of other species, such as *A. thaliana* or rice, the increase of SAM size correlates with flower fasciation [15–17]. Stem and floral anomalies are parts of the pleiotropic manifestation of a monogenous recessive mutation in the discussed mutant of *L. angustifolius* [2]. It is difficult to hypothesize that the same mutation has such opposite effects (the increase of SAM size and the reduction of size of floral primordia), while in other species the association between genetic control of SAM and FM sizes is not rare. That is why we suppose it to be more reliable that both vegetative and floral phenotypes are resulted from atypical proliferation of SAM and FM, respectively. The floral merism therefore comprises an indirect measure of FM size, the latter being very hardly accessible to direct measurements.

In this connection, the fasciated mutant of *L. angustifolius* is an intriguing object, as two types of meristems (SAM and FM) may be under the same genetic control, i.e., a single gene defines proper sizes and geometry of meristems. Mutation of this gene leads to atypical proliferation of all these meristems and hence causes abnormal phenotype. As for quantitative differences in leaf morphology, they can be explained by the fact that enlarged (fasciated) leaf blastozone splits into several primordia of individual leaves, and these primordia may be of smaller size than those in non-fasciated plants. This means that the cells of a blastozone are shared between several primordia, which can give rise to leaves with fewer leaflets.

The phenomenon of fasciated SAM producing numerous carpels (Figure 1E) is unusual. These carpels most probably represent modified phyllomes, sometimes bear features of leaves or bracts, such as long trichomes (Figure 1E) or unfused margins. However, a similar unusual morphogenesis was recorded in fasciated mutants *clavata3* of *A. thaliana* after auxin transport inhibitor application [18].

Moreover, the true terminal derivatives of gynoecium, including carpels not belonging to a particular flower, were found among the so-called terminal flower-like structures in numerous monocots [19], as well as in some mutants of *A. thaliana* [20].

4.2. Different Floral Domains Are Affected with Unequal Frequencies

Table 1 represents data on frequencies of variation of different types of floral organs. The least stable floral part is the androecium, which is in agreement with previous observations in different leguminous species with both mono- and polysymmetric flowers [1]. Stamens are in many cases the last to initiate in leguminous flowers [21], including *Lupinus* (Figure 2E,F). Although a carpel is the most distal structure on the floral axis, it is initiated (and possibly prepatterned) prior to the androecium. The excess of FM is patterned for andoecium, and in many cases staminal pairs develop in places of single stamens (Figure 3F).

This phenomenon is also somewhat unexpected, as it could be assumed that a higher merism of androecium will result from initiation of more alternating solitary stamens in both whorl (e.g., A6 + 6 instead of A5 + 5; such flowers were also recorded but sparse). More often, a number of sites prepatterned for stamens remains the same (i.e., five per whorl) but every site may initiate two or three stamens instead of one. It is the so-called splitting or dédoublement of stamens (Figure 2K–L and Figure 3F), which is not unique among angiosperms [22] and may occur either sporadically or as a component of normal floral development. The situation in *Lupinus* flowers seems identical with patterning of leaves on a fasciated meristem, when pairs of leaves emerge instead of one (Figure 1J), i.e., a blastozone of corolla and androecium becomes enlarged and splits into two or more organ primordia. A similar duplication of stamens as well as unstable perianth merism was described in some caesalpinioid legumes, e.g., *Mendoravia* Capuron [23].

One more intriguing detail is that different petals are affected with unequal frequencies depending on their position (Table 1). This can only indicate that the zones of expression of genes responsible for petal differentiation are of different sizes (Figure 3G). Genes governing the lateral petal development (wings) have the largest area of expression, so supernumerary petal primordia are most abundant in this area. This results in the highest frequency of emergence of additional wings compared with other petal types. In contrast, the area of expression of gene(s) controlling flag differentiation is the smallest. The adaxial domain of FM is especially stable due to additional negative control of FM size by *CYCLOIDEA*-like genes (see [1] for discussion). That is why emergence of supernumerary flags is rare in flowers of fasciated *L. angustifolius*. Even in flowers of higher merism, the adaxial petal (flag) remains in the median position. Sometimes floral primordia may fall under control of two groups of genes; as a result, organs of intermediate shape appear, such as carpelloid or petaloid stamens, wing-to-keel petals etc. Such instability of floral structure in fasciated plants is seemingly incompatible with normal functioning of a monosymmetric flower with its perfect fit of all parts and hence should be inadaptive.

Two observations are notable. First, even in case of atypical merism perianth differentiates properly producing three discernible types of petals. Second, abaxial and adaxial floral domains are affected with unequal frequencies, the adaxial one being the most stable. These two facts compose the key finding of our study, viz. that floral merism and symmetry are two factors interacting in a one-way mode. On the one hand, increase of merism accompanying stem fasciation does not interfere with normal differentiation of a monosymmetric corolla. On the other hand, genes controlling floral monosymmetry possibly are able to prevent the initiation of supernumerary petals on the adaxial side.

4.3. A Multicarpellate State in Fasciated Mutant Results from Proliferation of Floral Meristem (FM)

Floral development of *L. angustifolius* is similar to the one of *Thermopsis*, which is in consistency with their relation. Both genera are nested within a single Genistoids s.l. clade according to a molecular-based phylogenetic tree [24]. In earlier work, floral development in an unusual multicarpellate *T. turcica* Kit Tan, Vural & Küçük. was documented [3]. This species normally

develops three (rarely more or fewer) carpels per flower but its androecium remains typical papilionoid, composed of two pentamerous whorls. A paper [3] reported no variations in stamen number. For this work, 15 flowers of *T. turcica* (see [3] for details on origin of material) were additionally dissected, and their androecia appeared to be normal. Oppositely, androecium is the least stable part in fasciated flowers of *L. angustifolius*. The superfluous stamens often associate with a monomerous gynoecium. This indicates that polymerous (multicarpellate) gynoecia arise through different developmental mechanisms in these two genera. As it was hypothesized previously, three carpels in the flower of *T. turcica* may initiate in a sequential order along a spiral [3]. In *L. angustifolius*, an anomalous proliferation of FM gives a possibility to initiate more carpels, and their asynchronous development indicates that they arise on different levels, the first always being the abaxial one (Figure 2K). Possibly the initiation of several carpels instead of one in fasciated flowers of lupine results from both increase of FM size and its prolonged proliferation. Another factor to be discussed is the overall influence of fasciation on the meristem shape, which may appear the ridge-like (like SAM), providing some extra space to the central part of the FM.

This observation confirms the fact that some fasciated mutants are characterized by both increase of merism and prolonged proliferation of FM apex. As a result, a central part of FM remains undifferentiated even after initiation of a gynoecium and sometimes produces additional (inner) carpelloid structures [15–17].

The flower is widely postulated as a modified shoot (see [25] for review), and data from molecular genetics of floral development complement this suggestion [26]. That is why one may accept that carpels are phyllomes on the floral axis. In fasciated flowers of *Arabidopsis*, carpel number usually exceeds two but their arrangement remains more or less whorled. That is why a superfluous FM protrudes within carpel whorl, either remaining undifferentiated or giving rise to inner whorl(s) of carpels [16]. In contrast, the gynoecium of *Lupinus* consists of a single carpel, i.e., this phyllome is solitary in the upper node of the floral axis. If FM continues proliferation due to floral fasciation, more carpels can initiate but usually in a successive mode, so the resulting polymerous gynoecium is not whorled but spiral instead.

The emergence of additional carpels in *L. angustifolius*, which normally possesses a monocarpellate gynoecium, illustrates the idea of [27] on multiple and independent origin of a polymerous gynoecium in different lineages of Leguminosae. As highlighted in [27], the initiation of additional carpels can be associated with a polyandry or at least atypically high number of stamens compared with related monocarpellate taxa.

The supernumerary carpels, if emerge, are placed with their clefts more or less towards a center of receptacle and sometimes partly to the adaxial side (Figure 3H–J), i.e., similarly to the gynoecium of *T. turcica* [3].

5. Conclusions

Detailed ontogenetic and morphological analysis of flowers in fasciated mutant of lupine let us draw the following conclusions.

- The vegetative anomalies of fasciated mutant of *L. angustifolius* result from uncontrolled proliferation of SAM leading to initiation of superfluous structures, i.e., leaves and leaflets, asymmetric leaf arrangement on nodes and changes in phyllotaxis. As it was described by [2], the fasciated phenotype is caused by a mutation in a single gene. We hereby conclude that normal function of this gene is the negative regulation of SAM size. The causative mutation has a pleiotropic action leading both to SAM enlargement and to the increase of floral merism.
- Floral fasciation does not interfere with a control of monosymmetry, so in fasciated flowers all three types of petals differentiate properly.
- In fasciated flowers, different whorls are affected with unequal frequency. The least stable is the latest initiated inner whorl of the androecium.

- If judged by corolla, the lateral floral domains are the most susceptible to initiation of supernumerary organs with much higher frequency than the abaxial and adaxial domains. Thus, the petals positioned in (or close to) the medial plane of flower symmetry, are less prone to fluctuation. The stability of different corolla domains decreases in the following order: adaxial–abaxial–lateral.

References

1. Sinjushin, A.A.; Karasyova, T.A. Stability of the floral structure in Leguminosae with flag versus non-flag blossom. *Wulfenia* **2017**, *24*, 1–10.
2. Konovalov, Y.B.; Klochko, N.A.; Anikeyeva, N.F. Induced mutant of narrow-leaved lupine. *Izvestiya TSKhA* **1989**, *6*, 185–188. (In Russian)
3. Sinjushin, A.A.; Tekdal, D.; Ciftci, C.; Cetiner, S. Floral development in *Thermopsis turcica*, an unusual multicarpellate papilionoid legume. *Plant Syst. Evol.* **2018**, *304*, 461–471. [CrossRef]
4. Tucker, S.C. Origin of symmetry in flowers. In *Contemporary Problems in Plant Anatomy*; White, R.A., Dickinson, W.C., Eds.; Academic Press Inc.: New York, NY, USA, 1984; pp. 351–396. ISBN 0-12-746620-7.
5. Paulino, J.V.; Mansano, V.F.; Prenner, G. Evidence for division of labor and division of function related to the pollen release in Papilionoideae (Leguminosae) with a heteromorphic androecium. *Int. J. Plant Sci.* **2016**, *177*, 590–607. [CrossRef]
6. Prenner, G.; Bateman, R.M.; Rudall, P.J. Floral formulae updated for routine inclusion in formal taxonomic descriptions. *Taxon* **2010**, *59*, 241–250. [CrossRef]
7. Sinjushin, A.A. Effects of stem fasciation on inflorescence and flower morphology in legumes. *Wulfenia* **2016**, *23*, 127–134.
8. White, O.E. Fasciation. *Bot. Rev.* **1948**, *14*, 319–358. [CrossRef]
9. Bowman, J.L.; Eshed, Y. Formation and maintenance of the shoot apical meristem. *Trends Plant Sci.* **2000**, *5*, 110–115. [CrossRef]
10. Hagemann, W.; Gleissberg, S. Organogenetic capacity of leaves: The significance of marginal blastozones in angiosperms. *Plant Syst. Evol.* **1996**, *199*, 121–152. [CrossRef]
11. Bykova, E.A.; Labunskaya, E.A.; Choob, V.V. Morphological changes in the structure of blastozones during fasciation of *Pisum sativum* L. and *Arabidopsis thaliana* (L.) Heynh. *Biol. Bull.* **2015**, *42*, 179–185. [CrossRef]
12. Bykova, E.A.; Labunskaya, E.A.; Sinjushin, A.A.; Choob, V.V. Changes in the structure of a compound leaf in fasciated mutants of pea (*Pisum sativum* L.). In Proceedings of the 9th International Conference on Ecological Morphology of Plants Dedicated to the Memory of I.G. and T.I. Serebryakovy. V. 1, Moscow, Russia, 10–13 December 2014; pp. 101–104.
13. Kajanus, B. Über einige vegetative Anomalien bei *Trifolium pratense* L. *Zeitschrift für induktive Abstammungs- und Vererbungslehre* **1913**, *9*, 111–133. [CrossRef]
14. Snow, M.; Snow, G.R.S. A theory of the regulation of phyllotaxis based on *Lupinus albus*. *Philos. Trans. R. Soc. Lond. B* **1962**, *244*, 483–513. [CrossRef]
15. Clark, S.E.; Running, M.P.; Meyerowitz, E.M. *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. *Development* **1993**, *119*, 397–418. [PubMed]

16. Clark, S.E.; Running, M.P.; Meyerowitz, E.M. *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. *Development* **1995**, *121*, 2057–2067.

17. Suzaki, T.; Sato, M.; Ashikari, M.; Miyoshi, M.; Nagato, Y.; Hirano, H.-Y. The gene *FLORAL ORGAN NUMBER1* regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1. *Development* **2004**, *131*, 5649–5657. [CrossRef] [PubMed]

18. Bykova, E.A.; Chergintsev, D.A.; Vlasova, T.A.; Choob, V.V. Effect of the auxin polar transport inhibitor on the morphogenesis of leaves and generative structures during fasciation in *Arabidopsis thaliana* (L.) Heynh. *Russ. J. Dev. Biol.* **2016**, *47*, 207–215. [CrossRef]

19. Sokoloff, D.; Rudall, P.J.; Remizowa, M. Flower-like terminal structures in racemose inflorescences: A tool in morphogenetic and evolutionary research. *J. Exp. Bot.* **2006**, *57*, 3517–3530. [CrossRef] [PubMed]

20. Penin, A.A.; Choob, V.V.; Ezhova, T.A. Basic principles of terminal flower formation. *Russ. J. Dev. Biol.* **2005**, *36*, 65–69. [CrossRef]

21. Tucker, S.C. Floral development in legumes. *Plant Physiol.* **2003**, *131*, 911–926. [CrossRef] [PubMed]

22. Ronse Decraene, L.P.; Smets, E.F. The morphological variation and systematic value of stamen pairs in the Magnoliatae. *Feddes Repert.* **1996**, *107*, 1–17. [CrossRef]

23. Zimmerman, E.; Prenner, G.; Bruneau, A. Floral ontogeny in Dialiinae (Caesalpinioideae: Cassieae), a study in organ loss and instability. *S. Afr. J. Bot.* **2013**, *89*, 188–209. [CrossRef]

24. Wojciechowski, M.F.; Lavin, M.; Sanderson, M.J. A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *Am. J. Bot.* **2004**, *91*, 1846–1862. [CrossRef] [PubMed]

25. Arber, A. The interpretation of the flower: A study of some aspects of morphological thought. *Biol. Rev.* **1937**, *12*, 157–184. [CrossRef]

26. Coen, E.S.; Meyerowitz, E.M. The war of the whorls: Genetic interactions controlling flower development. *Nature* **1991**, *353*, 31–37. [CrossRef] [PubMed]

27. Paulino, J.V.; Prenner, G.; Mansano, V.F.; Teixeira, S.P. Comparative development of rare cases of a polycarpellate gynoecium in an otherwise monocarpellate family, Leguminosae. *Am. J. Bot.* **2014**, *101*, 572–586. [CrossRef] [PubMed]