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Effects of stem fasciation on inflorescence and flower morphology in legumes

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Summary: A comparison of floral and inflorescence morphology was conducted between normal and fasciated accessions of *Pisum sativum* and *Lupinus angustifolius*. Pea plants with a fasciation of the main stem exhibited fewer flowers per axillary inflorescence and in some cases fewer ovules per carpel. Flowers of fasciated peas of different origin appeared normal. Oppositely, fasciated mutant of lupine had anomalously increased number of organs in most or all whorls. Differences between regulation of meristem activity in two leguminous species are being discussed with the involvement of literature data.

Keywords: Leguminosae, flower, inflorescence, zygomorphy, fasciation, meristem, *Pisum*, *Lupinus*

Plant morphogenesis is connected with the existence of groups of cells which proliferate during the whole life, i.e. meristems. All aerial parts of a plant organism originate from a stem apical meristem (SAM) which results from plumule of embryo. Genetic control of SAM activity is most precisely dissected in the model plant species, *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). A number of genes negatively regulate SAM sizes; in case of mutations, SAM increases abnormally, a phenomenon commonly known as fasciation (White 1948, Choob & Sinyushin 2012). Numerous mutations cause stem fasciation in *A. thaliana*, the best known being *clavata* (*clv1*, *clv2*, *clv3*) and *fasciata* (*fas1*, *fas2*; see Choob & Sinyushin 2012 for review). As teratological reviews indicate, fasciation affects different organs in distinct taxa (White 1948). Although genetic control of fasciation lies beyond the given survey, some associations in developmental anomalies are of special interest in non-model species, as they may shed some light upon coordinated evolution of different plant organs. Results of the search for pleiotropy of fasciation in two legume species of outstanding practical value, a garden pea and a narrow-leaved lupine, are presented in this paper.

Materials and methods

Mutants and initial cultivars presented in Table 1 were used for comparison. Plants were grown on experimental plot on a territory of S.N. Skadovskii Zvenigorod Biological Station (Western Moscow District, Russia) during summer of 2016. Most of the measurements and counts were performed in field or on freshly collected material. Floral buds of *Lupinus* were fixed in 70% ethanol and dissected under stereomicroscope in laboratory. Number of examined plants of every accession ranged from 13 to 24. At least 35 flowers of each genotype were examined for morphological differences.

Measurements of petal sizes were carried out on digital photo images by means of the program Meazure 2.0.0.158 (C Thing Software, US). Data were statistically treated with Statistica 7 software package (StatSoft Inc., US).

Table 1. Characteristics of plant accessions used in the survey.

Fasciated line (genotype)	Isogenic precursor (genotype)	Mode of induction	Reference
Pisum sativum			
'Shtambovyi' (*fas*)	cv. 'Nemchinovskii 766' (N 766, *FAS*)	Ethylmethane sulphonate (EMS)	Sinjushin & Gostimsky 2006
Wt12185 (*fa2*)	cv. 'Kaliski' (*FA2*)	Fast neutrons + N-nitroso-N-ethyl urea (NEU)	Święcicki 2001
P64 (*sym28*)	cv. 'Frisson' (*SYM28*)	EMS	Sagan & Duc 1996
K301 (*nod4*)	cv. 'Ramonskii 77' (*NOD4*)	NEU	Sidorova & Uzhintseva 1992
Lupinus angustifolius			
$5M_2$-253	cv. 'Nemchinovskii 846' (N 846, genotype not defined)	Gamma rays (^{60}Co)	Konovalov et al. 1989

Results

Pisum sativum. The garden pea is characterized by a compound racemose inflorescence. The main shoot proliferates unlimitedly during whole vegetation period and produces frondose leaves subtending short bractless axillary racemes usually of one or two flowers. No bracteoles are present. Flowers are of papilionaceous morphology with five fused sepals (the abaxial one is longer than others), five petals (flag, two wings and two fused abaxial petals forming a keel), diadelphous androecium with ten stamens in two whorls (stamen opposed to flag is free, other nine fused producing a tube) and a single carpel.

Unfortunately, an accession registered as cv. 'Kaliski' appeared dwarf and hence not isogenic to the line Wt12185. It was seemingly denoted erroneously, so no comparisons were performed in this pair. However, line Wt12185 was precisely studied regarding floral morphology.

The counts of Table 2 show that fasciation does not alter flowering time (i.e. number of sterile nodes) among most of the studied lines. Only plants of line K301 (*nod4*) exhibit significantly fewer sterile nodes than in control accession. Fasciated plants usually have shorter shoots compared to control cultivars due to shortening of upper internodes thus resulting in a 'false umbel' phenotype.

All three fasciated mutants produce significantly fewer flowers per axillary raceme than initial cultivars (Table 2). Two accessions, K301 and 'Shtambovyi', developed anomalous flowers terminating axillary racemes which were described more detailed earlier (Sinjushin 2011). These structures usually bore fewer floral organs than normal flowers and never produced any pods.

No floral abnormalities were observed in fasciated mutants of pea and initial cultivars. One flower of line K301 produced an additional sepal on the abaxial side. However, similar anomalies were previously recorded in non-fasciated cv. 'Viola' (data not shown), thus suggesting that such deviations are not assigned to fasciation.

Lupinus angustifolius. As a member of Galegeae, another tribe than pea, *Lupinus* has distinct inflorescence and floral morphology (Fig. 1 B). Flowers develop directly on the main shoot (simple raceme) in axils of bracts which have been abscised before anthesis. After the main shoot finishes flowering, numerous paracladia arise in axils of leaves in the upper part of the first-order shoot. Flowers are papilionaceous, with small caducous bracteoles seen on calyx. The calyx has

Table 2. Quantitative parameters of studied plant accessions (average ± standard deviation). Statistically significant differences between fasciated mutant and isogenic cultivar are marked with asterisk (Mann-Whitney test; * $p < 0,05$; ** $p < 0,01$).

Accession	Node of flowering initiation	No. of flowers per axillary raceme	Flag length, cm	Flag width, cm	No. of ovules
N 766	13.89 ± 0.32	1.60 ± 0.50	1.42 ± 0.10	2.15 ± 0.26	6.04 ± 0.46
'Shtambovyi'	13.90 ± 0.79	1.17 ± 0.38**	1.58 ± 0.13	2.05 ± 0.29	6.29 ± 0.63
'Frisson'	14.47 ± 0.51	1.81 ± 0.39	no data	no data	8.43 ± 0.68
P64	14.78 ± 0.90	1.59 ± 0.56*	1.44 ± 0.08	1.82 ± 0.16	8.10 ± 0.70
'Ramonskii 77'	15.69 ± 1.03	1.65 ± 0.48	1.54 ± 0.01	2.01 ± 0.08	5.89 ± 0.93
K301	14.71 ± 0.59**	1.30 ± 0.46**	1.57 ± 0.06	1.96 ± 0.13	4.75 ± 0.91*
L. angustifolius N 846	18.53 ± 1.18	no data	1.15 ± 0.09	0.75 ± 0.07	4.91 ± 0.64
L. angustifolius $5M_2$-253	no data	no data	1.25 ± 0.09*	0.81 ± 0.08	4.92 ± 0.40

five sepals, three abaxial sepals are fused in a single tricuspid lamina. Petals are of the same three types as in pea. The androecium is monadelphous. Stamens of outer whorl have shorter filaments and large anthers, while stamens of inner whorl have longer filaments and very small anthers. The gynoecium consists of a single carpel.

When examining morphology of fasciated mutant of *L. angustifolius*, an increase of number of floral parts was recorded in some flowers, while others remained normal. Additional organs were inserted in all whorls including gynoecium which might become di- or trimerous (Fig. 1 A). Supernumerary petals were usually differentiated normally; in most cases, the development of additional wings was observed (Fig. 1 A). Keel petals often remained unfused and other petals might have atypical differentiation, some stamens had features of petalization with petal-like excrescences (Fig. 1 A).

Due to stem fasciation, a precise count of node of flowering initiation in fasciated lupines was complicated, so this parameter was not scored.

Discussion

Variability of abnormalities accompanying fasciation in plants

When examining literature on plant teratology, one may conclude that two types of developmental anomalies are referred to as fasciation: (i) gradual increase of apex (as in fasciated mutants) and (ii) fusion of few normally independent organs. Both phenomena result in development of ridge-like enlarged meristem and deformed flattened organs (stems, inflorescences, flowers). In the latter case, distortions in one structure (e.g. stem) have no influence on organs of the same series (other stems) or other type (e.g. flowers). Oppositely, mutations in genes regulating stem apical meristem (SAM) activity usually lead to a gradual increase of a single growing point and may affect organs of different type.

In *Arabidopsis thaliana*, numerous mutations simultaneously cause stem fasciation and flower malformations (usually an increase of floral organ number; see Choob & Sinyushin (2012)

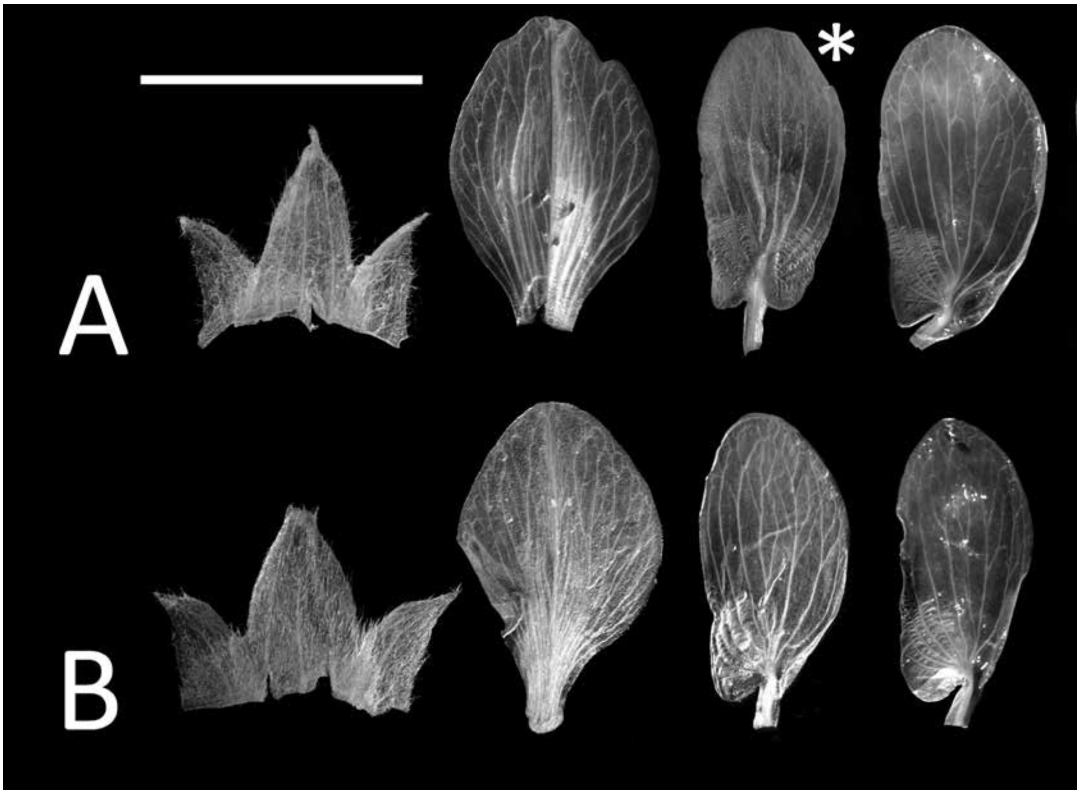


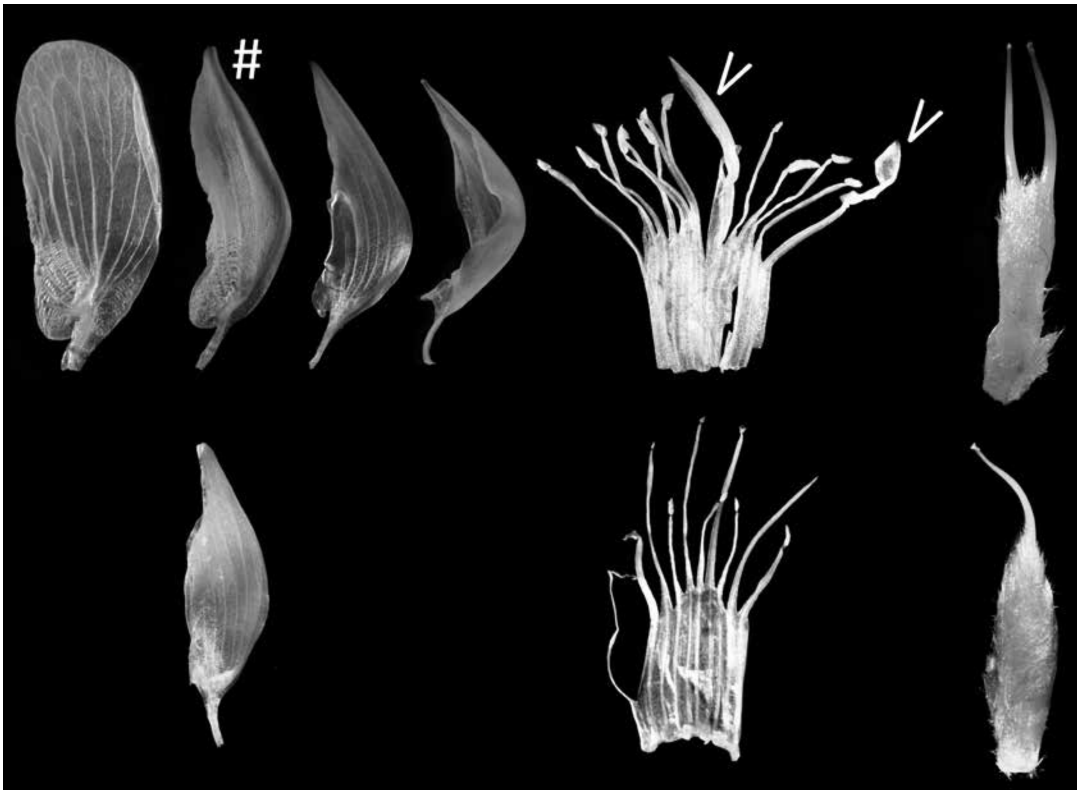
Figure 1. Floral phenotypes of *Lupinus angustifolius*, fasciated mutant 5M2-253 (A) and non-fasciated cv. 'Nemchinosvskii' 846 (B). Calices are dissected along adaxial side. * = anomalous symmetric wing; # = keel-like petal with wing-like epidermis in a proximal part; arrowheads = petaloid excrescences on stamens. Scale bar = 1 cm.

for review). However, this rule is not general. It was proposed earlier (Choob & Sinyushin 2012) that shoot fasciation is not followed by flower fasciation in known legume mutants. In cultivated strawberries, the flower is usually fasciated, while stem seems normal; the same is true for tomatoes. One may conclude that stem fasciation and flower fasciation are separable traits in different taxa. Whereas sizes of apical (AM) and floral (FM) meristems are governed by the same molecular mechanism (Choob & Sinyushin 2012), some additional regulators probably exist for different meristems in distinct taxa.

This variability exhibits no obvious links with inflorescence morphology. Flower fasciation is recorded in *Arabidopsis* with its simple racemose inflorescence, in buckwheat (*Fagopyrum esculentum* Moench; Sakharov 1986) and tobacco (*Nicotiana tabacum* L.; White 1948) having axillary cymose inflorescences. Possibly, a better grouping factor is flower symmetry, as monosymmetric flowers seem to be more stable regarding their merism. The given survey was conducted in order to uncover correlations between fasciation of SAM and other meristem types in a group with monosymmetric flowers, like Leguminosae.

Pleiotropic effect of shoot fasciation in legumes

A stem fasciation in pea and lupine was described earlier regarding a primary distortion, i.e. an increase and flattening of SAM which results in a band-like stem with supernumerary vascular bundles and abnormal phyllotaxis (Rubets & Shumova 2003; Sinjushin & Gostimsky 2006).



Among examined non-allelic mutants of pea, only line K301 (*nod4*) exhibited a somewhat earlier flowering time, i.e. lower position of node of flowering initiation (Table 2). Effect of fasciation on flowering time was recorded earlier in *Vigna unguiculata* (L.) Walp. (Mahna et al. 1990) and *Lathyrus sativus* L. (Waghmare et al. 2001), but in these cases fasciated mutants flowered and reached maturity later than wild-type plants. Delayed maturity was also recorded in a fasciated mutant of *Cicer arietinum* L. (Gaur & Gour 1999). Two fasciated mutants of *Glycine max* (L.) Merr. flowered earlier (Tang & Skorupska 1997) or later (Albertsen et al. 1983) than non-fasciated cultivars. Fasciation had no influence on time of flowering and reaching maturity in *Lens culinaris* Medik. (Tyagi & Gupta 1991).

Interestingly, axillary racemes in all fasciated mutants of pea produced significantly fewer flowers than in isogenic, non-fasciated accessions (Table 2). Axes of these racemes had no features of fasciation. In *Lotus japonicus* (Regel) K. Larsen with silenced ortholog of gene *CLAVATA3*, fasciation of the main shoot was associated with an increased number of flowers per axillary umbel (Okamoto et al. 2011). Tyagi & Gupta (1991) reported an increase of number of 'pods per bunch' in lentil, but it is unclear whether these counts consider a single axillary inflorescence or all inflorescences in a node. As stated previously, such variations confirm that in different clades (even within the same family) meristems of different orders are governed by different sets of regulators.

Unfortunately, most of the listed mutations in legumes remain unidentified on molecular level thus complicating conclusions on evolution of regulatory network controlling meristem activity in different taxa. In *Arabidopsis*, numerous genes are known which, when mutated, cause stem fasciation. Most of these mutations also provoke flower fasciation, but sometimes have an opposed effect. For example, mutant *tonsoku* has a stem fasciation and reduced number of floral organs

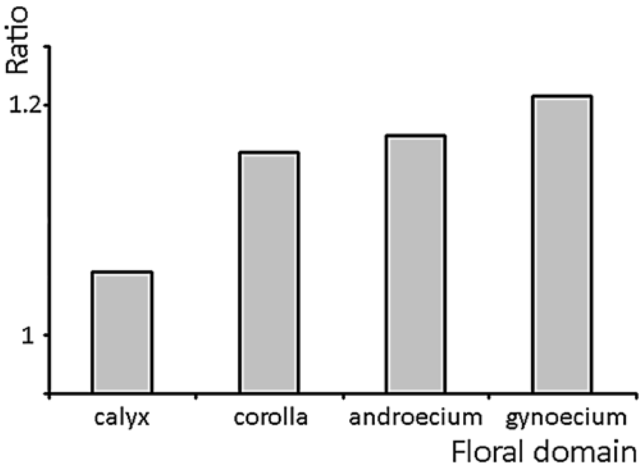


Figure 2. Ratio between organ number in fasciated : non-fasciated flowers of *Lupinus*.

(Suzuki et al. 2004). A stem fasciation can be associated with different floral abnormalities in *A. thaliana*, so one may expect a similar polymorphism in Leguminosae.

Shoot fasciation is accompanied by floral anomalies in *Lupinus*, but not in *Pisum*

Anomalous 'fused' flowers with an increased number of carpels were recorded in some species of *Lupinus*, where they sometimes arise spontaneously, i.e. in non-fasciated plants (Atabekova 1959). However, in these species they develop rarely and sporadically, while in the fasciated mutant of *L. angustifolius* most of the flowers were abnormal. The observed complex of anomalies can be interpreted as flower fasciation showing a clear character: organs in every whorl (in acropetal direction) are affected more than in preceding (more basipetal) whorl (Fig. 2; see Choob & Sinyushin 2012). Gynoecium is most strikingly changed, producing 2–3 carpels instead of one.

More detailed studies on floral ontogeny in fasciated mutant of *L. angustifolius* are needed, but the observed accession combines shoot and flower fasciation what is untypical for legumes. Previously, Albertsen et al. (1983) reported numerous anomalies in flowers of fasciated soybean mutants which seem to result from true fasciation, as 95% of studied flowers were affected. An increased number of floral organs accompanied fasciation in *Vigna radiata* (L.) R. Wilczek (Dwivedi & Singh 1990). Tyagi & Gupta (1991) pointed at the fact that fasciation in lentil had no effect on the flower or fruit phenotype, while another fasciated mutant of the same species appeared sterile (Sharma & Sharma 1983). Investigations on flower development of fasciated accessions of *Lupinus* may shed some light on the problem of evolution of a polymerous gynoecium in Leguminosae, which is in some cases interpreted as derived from a monomerous state (Sinjushin 2014).

Absence of developmental abnormalities in flowers of fasciated pea lines points to the existence of additional negative regulators of FM size besides those which negatively regulate SAM size. In *Arabidopsis*, FM and SAM size are controlled by the same set of genes, so stem fasciation is accompanied by floral anomalies. Mutation in the line of studied *Lupinus* remains unidentified, but a single gene seems to control sizes of FM and SAM. This observation widens our understanding of interaction of different regulatory pathways in angiosperms. For example, the earlier proposition that flowers in fasciated legume mutants are always normal (Choob & Sinyushin 2012) needs adjustment now.

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