Wulfenia

Finis coronat axem: Terminal inflorescences in tribe Fabeae (Fabaceae: Faboideae)

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Summary: The comparison of inflorescence symmetry was conducted for wild-type plants of *Pisum sativum* and *Vicia faba* together with mutants which have a distorted inflorescence structure: *det* of pea and *ti* of broad bean with terminal flower-bearing racemes, *fa* of pea with an expressed stem fasciation and double pea mutants *fa det* with the many-flowered terminal raceme. The pendulum symmetry of the whole shoot is demonstrated for indeterminate plants of pea and broad bean. The rare spiral symmetry of an inflorescence is also demonstrated. The changes in symmetry of a partial inflorescence when altering its position from lateral to terminal are described. The role of dominance of a terminal raceme in evolution of leguminous inflorescence is hypothesized.

Keywords: inflorescence symmetry, *Pisum*, *Vicia*, terminal inflorescence

The whole variability of inflorescence structure in Fabaceae can be divided into two basic groundplans. In some legumes, the main shoot terminates with an inflorescence, i.e. the flowers are in axils of phyllomes direct on a first order axis (determinate). This flower-bearing part of a main shoot is designated as main florescence. It is often preceded by similar inflorescences of second order, either having a sterile portion (long paracladia) or lacking it (short paracladia, see Weberling 1989 for terminology). Other species produce a shoot which keeps growing during the whole life of a plant (or the whole season in case of perennials) and all flower-bearing axes (paracladia) arise from axils of leaves on this primary (main) shoot. Such inflorescences are referred to as indeterminate or truncated (cf. Prenner et al. 2009). The second type is often interpreted as derived from the first one (e.g., Kuznetsova 1998; Akulova et al. 2000). In most cases, the mode of an inflorescence proliferation, either determinate or indeterminate, is taxon-specific. However, a switch between these two types may occur in some cases. Sell (1980, cited from Weberling 1989) mentions *Lespedeza bicolor* which sometimes fails to produce a terminal inflorescence after initiation of series of lateral ones. The cases of anomalous (untypical for a habitual ontogeny) inflorescence terminalization are easier to unambiguously detect and have significant importance for both the understanding of the regulation of inflorescence development and agriculture.

The tribe Fabeae Rchb. includes five genera (*Lathyrus* L., *Vicia* L., *Pisum* L., *Lens* Mill. and *Vavilovia* Fed.) (Schaefer et al. 2012). Among these, many species represent the most ancient and important crops (garden pea, lentil, broad bean etc.). Normally all species of Fabeae are characterized by an indeterminate growth. The flowers are formed on axillary (subtended with leaves of same morphology as in the vegetative zone) racemose partial inflorescences (short paracladia sensu Weberling 1989). The morphology of these paracladia is diverse within a tribe. They are usually bractless; bracts, if present, are reduced to spinules (*Lathyrus* p.p., *Vicia* p.p.). Leafy bracts are rare (*L. japonicus* Willd.). In certain species of different genera the axillary racemes are reduced to a single flower, sometimes on a very short stalk (*Vicia sativa* L., *Vavilovia formosa* (Stev.) Fed., *Pisum fulvum* Sibth. & Sm. etc.). Repjev et al. (1999) describe some of

vetches formerly placed in genus *Orobus* (e.g., *Vicia fauriei* Franch.) as having axillary panicles (not racemes) with large paired bracts. It is therefore possible that the axillary simple racemes in most Fabeae comprise a result of a reduction from a compound raceme or panicle, although this hypothesis needs further confirmation.

The shoot of Fabeae is normally characterized by a distichous phyllotaxis. When emerging from a seed, the seedling is incurved with the first scale-like leaf always placed on the convex side of the shoot. The position of the first leaf is already defined in the embryo and presets the arrangement of all subsequent leaves (Sinjushin & Akopian 2011). These normally develop in a plane. However, a mode of arrangement of axillary inflorescences clearly distinguishes one side of a shoot from another adding a dorsiventral asymmetry (pendulum symmetry, reviewed in Prenner 2013). A dorsiventral mode of partial inflorescence structure and ontogeny was also recorded in members of Loteae (reviewed in Sokoloff et al. 2007) as well as in Trifolieae, Abreae, Galegeae s.l. (*Astragalus* p.p.) and some other tribes (Naghiloo et al. 2012; Prenner 2013, etc.).

An agricultural usage of different Fabeae meets with some difficulties. One of them is that most of them have a prolonged vegetation period when axillary inflorescences initiate one by one. The same plant may have completely mature pods in the basal part of an inflorescence, while the apical part of a shoot continues flowering. As a result, the actual seed productivity is much lower than the potential one. A breeding process is hence aimed at the production of new cultivars with a shortened flowering period. This became possible after an identification of mutations which cause determinate growth. In pea, a mutation *determinate* (*det*) was described which causes formation of terminal inflorescence (Makasheva & Drozd 1987). Different alleles of the gene *DET* have been repeatedly obtained, and *DET* was characterized as a pea ortholog of *TERMINAL FLOWER1* (*TFL1*) of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (Foucher et al. 2003). The precise investigation of an ontogeny of *det* mutants led to the conclusion that the main axis itself remains indeterminate, i.e. no true terminal flower is formed (Singer et al. 1990). Since the determinate mutant was obtained, numerous pea cultivars with a determinate growth habit were bred (e.g., 'Pervenec' and 'Atlant' in Russia) which produce pods more or less simultaneously and hence are easier to harvest. Except for an outstanding agricultural value, determinate forms of pea are of significant scientific interest, as their ontogeny is untypical for *Pisum* and the whole tribe.

Phenotypically similar forms were also described in broad bean, *Vicia faba*. Two *terminal inflorescence* mutations, *ti-1* and *ti-2*, were induced which cause cessation of apical growth via conversion of a stem meristem into an inflorescence meristem (Sjödin 1971). An introduction of determinate growth habit into genotypes of cultivated bean resulted in the breeding of few cultivars with shortened flowering period ('Ticol', 'Piccolo', 'Topless' etc.). Later it was confirmed that determinate growth in *V. faba* is caused by a mutation in a gene homologous to *TFL1* of *A. thaliana* (Avila et al. 2006).

The genes *Dt* and *cd* determining formation of a terminal inflorescence were identified in chickpea, *Cicer arietinum* L. (van Rheenen et al. 1994). These genes exhibit an epistatic interaction and determinate plants are also characterized by a reduced number of leaflets and floral abnormalities (female sterility).

Apart from the listed examples, the mutants *terminal inflorescence* (*ti* and *ti-s*) with terminalized raceme were described in *Medicago sativa* L. (Dzyubenko & Dzyubenko 1994). The polymorphism in a growth habit, either indeterminate or determinate, was recorded in wild-growing populations

of *M. lupulina* L. (Weberling 1989; original observations). One may conclude that a genetic background conditioning an ontogenetic switch between different inflorescence types persists in the evolution of legumes, even of those which are normally characterized by an indeterminate growth.

The aim of this study is to investigate inflorescence morphology of determinate mutants of *Pisum sativum* and *Vicia faba* compared with normal plants. Special attention is paid to changes in inflorescence symmetry which accompany the terminalization of flower-bearing racemes. Together with the model legume *Lotus japonicus* (Regel) K. Larsen, *P. sativum* and *V. faba* are objects for developmental genetics which provides a possibility to study (onto)genetic control of inflorescence symmetry.

Materials and methods

The following plant genotypes were used: garden pea (*Pisum sativum* ssp. *sativum*) cv. 'Rosacrone' (fasciated indeterminate plants with genotype *fa*); the dwarf pea line 'DTR' with a determinate growth and many-flowered partial inflorescences (genotype *det fn fna*, considering the hypothesis of Lamprecht (1947) on genetic control of flower number per inflorescence); the line 'DTR(m)' bearing numerous morphological mutations together with *det*; the fasciated line 'Lupinoid' with a determinate growth (*fa det*); different lines and cultivars with an indeterminate growth pattern from the collection of Genetics Dept. of M. V. Lomonosov Moscow State University (Moscow, Russia). Except pea, two broad bean cultivars were studied, viz. 'Topless' (*ti*) and 'Chyornye Russkiye' (*TI*). All plants were grown in the greenhouse of the Biological Faculty of Moscow State University (temperature +25±5°C, long-day light) and on an experimental plot of S. N. Skadovskii Zvenigorod Biological Station (Western Moscow Distr., Russia). For scanning electron microscopy (SEM), shoot apices were fixed in 70% ethanol, then dissected and prepared in accordance to a protocol described by Sinjushin & Demidenko (2010).

The diagrams were produced using CorelDRAW X3 v.13 (Corel Corporation). When describing and depicting the structure of an inflorescence, the following designations were used: in a determinate inflorescence, the terminal raceme was marked as T, the subterminal as T–1, and the number of any lateral raceme was described in relation to the terminal (T) one. In all indeterminate inflorescences, the count was made beginning from the node of floral initiation (N), as N+1, N+2 etc.

Results

Normal (indeterminate) inflorescence

Both *Pisum sativum* and *Vicia faba* produce flowers on axillary racemes subtended by leaves with normal morphology, i.e. the subtending leaves are not morphologically distinguishable from those in the more basal and vegetative part of the shoot. Two principal differences between *P. sativum* and *V. faba* are obvious. First, the flowers are borne on long (pea) versus very short (bean) stalks. In bean, the first flower is attached slightly above the level of a node, while in pea the distance from the node to the level of the first flower's attachment is much longer than the size of the flower itself. Second, the leaves in pea bear opposed leaflets. In contrary, leaflets in the leaf of *V. faba* are arranged in alternate manner, so one of the leaflets is always closer to the node than the other (Fig. 1 F). One may easily distinguish the proximal leaflet, while in pea usually only the proximal
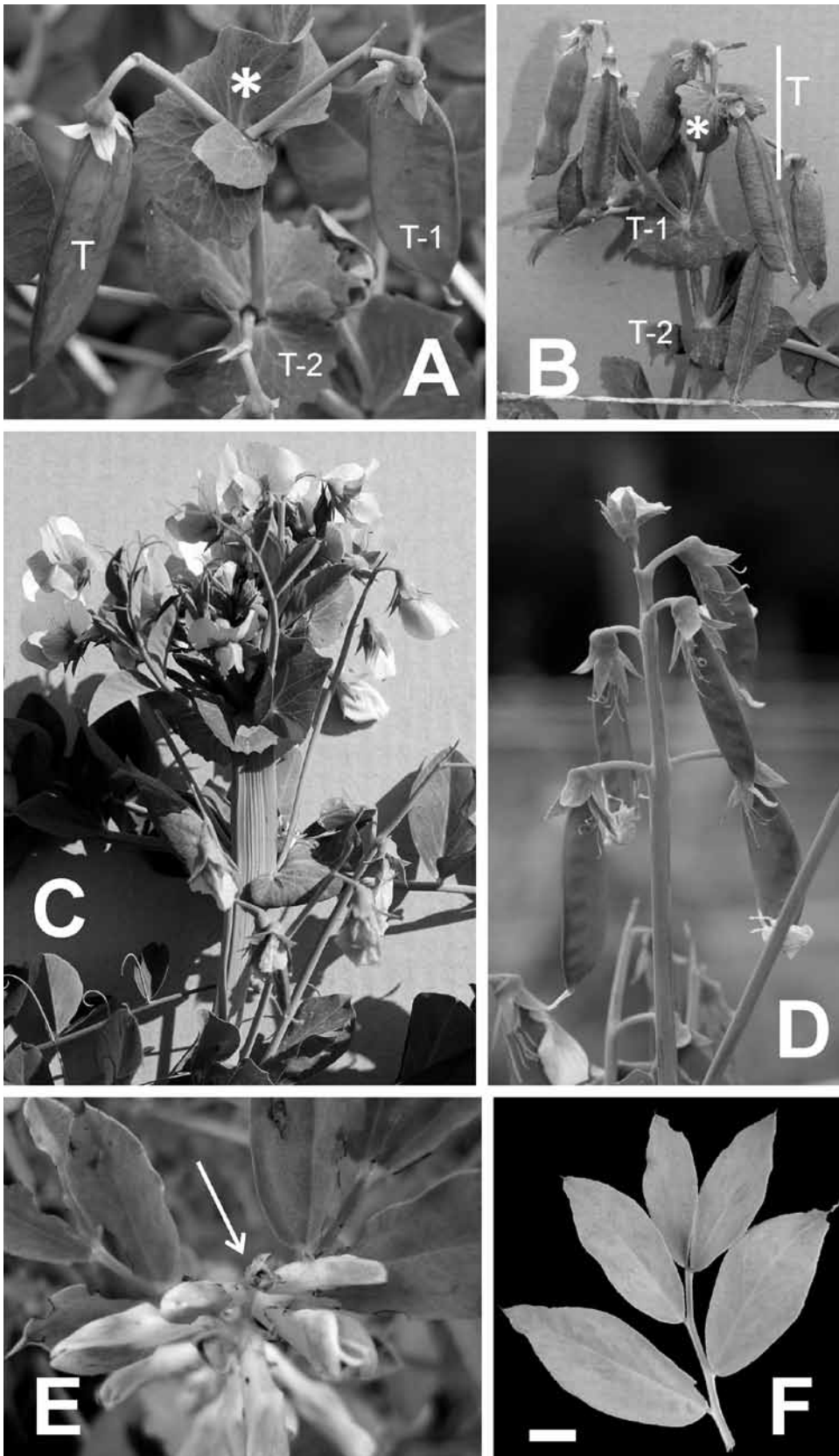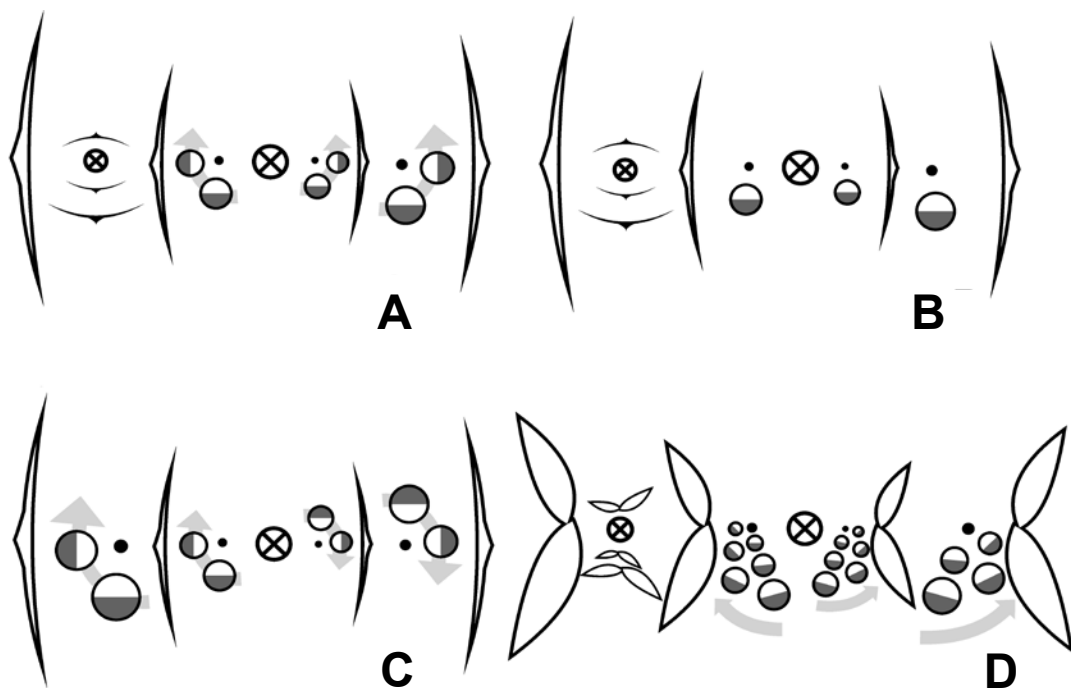
Figure 2. Diagrams of normal plants of *P. sativum* (A–C) and *V. faba* (D). The axillary racemes can follow the pendulum symmetry (A, D), be reduced to a single flower (B) or form a spiral (C). The direction of turn is indicated with grey arrows. White arc = leaf; crossed circle = indeterminate stem; black circle = flower-bearing axis with limited growth; half-tone circle = flower (grey half corresponds to abaxial part, white one to adaxial part). In D and other diagrams of *V. faba*, the designation of leaf does not reflect the actual number of leaflets, but only an asymmetry of the leaf basis.

pair of leaflets can be marked. In this connection, the position of leaves along a shoot is usually regular in *V. faba*: when looking from the adaxial side, all leaves have the most proximal leaflet either on the left side of the rachis or on the right side (Fig. 2 D). No cases of alternations were recorded in indeterminate bean plants, although they were found in *ti* mutants (see below). For convenience, the side of a shoot with proximal leaflets is referred to as dorsal and the opposite side as ventral. The vegetative second-order shoots (paracladia) which reproduce the structure of the main shoot arise in axils of leaves below N. The first leaf of these paracladia always forms towards the dorsal side of a main shoot (Fig. 2 D). The adaxial side of paracladia becomes dorsal in both species (Fig. 2 A, D).

When analyzing the early stages of inflorescence ontogeny using SEM, it is evident that the leaves in *V. faba* are not initiated in a flat plane but somewhat shifted to the ventral side (Fig. 5 B, C; see below). In contrast, inflorescences are shifted to the dorsal side. This asymmetry persists in a mature inflorescence (Fig. 1 E).

Figure 1. The phenotypes of studied forms. A – The inflorescence of *det* mutant of pea (early fruiting stage). The T–1 raceme develops in an axil of a reduced bracteous leaf (asterisk). B – Inflorescence of many-flowered *det* pea line 'DTR' (late fruiting stage). A bract (asterisk) precedes the first flower of T raceme. C – The fasciated (*fa*) plant of pea, cv. 'Rosacrone'. D – The terminal many-flowered raceme of *det fa* double mutant (line 'Lupinoid' of pea). E – The terminal raceme of *ti* mutant of broad bean (cv. 'Topless'), apical view. The shoot is terminated with a flower-like aberrant structure (arrow). The ventral (bearing leaves) and dorsal (flower-bearing) surfaces of the shoot are visible. F – Asymmetry of leaf basis in broad bean (cv. 'Topless'). Scale bar: F = 1 cm. Photographs A–D: Fedor A. Konovalov.

A strict regularity was found in the arrangement of flowers along the axis of an axillary raceme. In pea, flowers (usually two) are attached on different levels and form a reduced spiral. The divergence angle between them comprises about 90°. In most of the examined plants, the direction of torsion was alternate in consecutive nodes (pendulum symmetry): if a divergence angle in raceme N was ca. 90° (counterclockwise), then an angle in N+1 comprised –90° (clockwise), 90° in N+2 and so on (Fig. 2 A). Only one plant (the hybrid of F_2 cross between lines 'Lupinoid' and 'Ras-tip' without any features of fasciation or determinate growth) was found which had no alternation in divergence angles (Fig. 2 C). In this plant, the floral arrangement of the entire annual shoot represents a spiral.

The partial (axillary) inflorescences of *V. faba* bear a variable number of flowers (3.7±1.0; average ± standard deviation is presented) and the basal racemes usually produce less flowers than the upper ones. Flowers are organized in zigzag manner in two rows and in each pair one flower is placed higher than the other (Figs 2 D; 4 C). The flower arrangement can be hence described as modified spiral rather than whorled. No accurate measurements of divergence angle were made, but it was always less than 90°. The direction of torsion in this spiral also follows the same dorsiventral habit as in pea (Fig. 2 A, D). In broad bean, the ontogeny of two characters follows a dorsiventral pattern, viz. the position of the proximal leaflet and the divergence angle in axillary racemes (Fig. 2 D).

Determinate inflorescence

Pisum sativum. Normally, pea plants which are homozygous at *det* (line 'DTR') produce two lateral racemes and then their growth terminates via the formation of an apical raceme (Fig. 1 A, B). The average number of flowers is unequal in these three partial inflorescences: 2.1±0.5 in T, 4.2±1.0 in T–1 and 4.4±0.6 in T–2. The flowers of racemes T and T–2 open simultaneously, while T–1 is somewhat retarded.

When examining the flower arrangement of determinate forms of pea (*det* mutants), the following tendency is observed. The position of the first flower in the terminal inflorescence is not on the dorsal side of the shoot as in all axillary racemes, but in an axil of the next leaf, i.e. in the same plane as the upper flowers of lateral inflorescences (Fig. 3). The subtending leaf itself is either reduced (i.e. bracteous), with a single leaflet and a pair of stipules or it consists of one or two reduced stipules or it is completely absent. The second flower of the terminal inflorescence is attached higher than the first one. The angle between them comprises ca. 90° (rarely more). The direction of torsion in the terminal inflorescence (T) coincides with one of T–2: if a turn in T–2 is counterclockwise and in T–1 clockwise, then in T a turn is counterclockwise and vice versa (Fig. 3 A, B). Even if the torsion of axillary racemes is the same along the whole shoot (the spiral arrangement without dorsiventral asymmetry, see above), the torsion of T also coincides with T–2 (Fig. 3 C).

The morphology of the T–1 inflorescence is the most unusual. A subtending leaf is often bracteous (divergent from a normal pinnate leaf of preceding nodes) and two flowers sometimes fuse producing a single structure with an increased number of organs on the common receptacle which sometimes give rise to twin pods.

The axillary racemes are often themselves terminated with terminal flower-like structures (TFLS) which are never observed to produce pods and which usually shed. The morphology of such
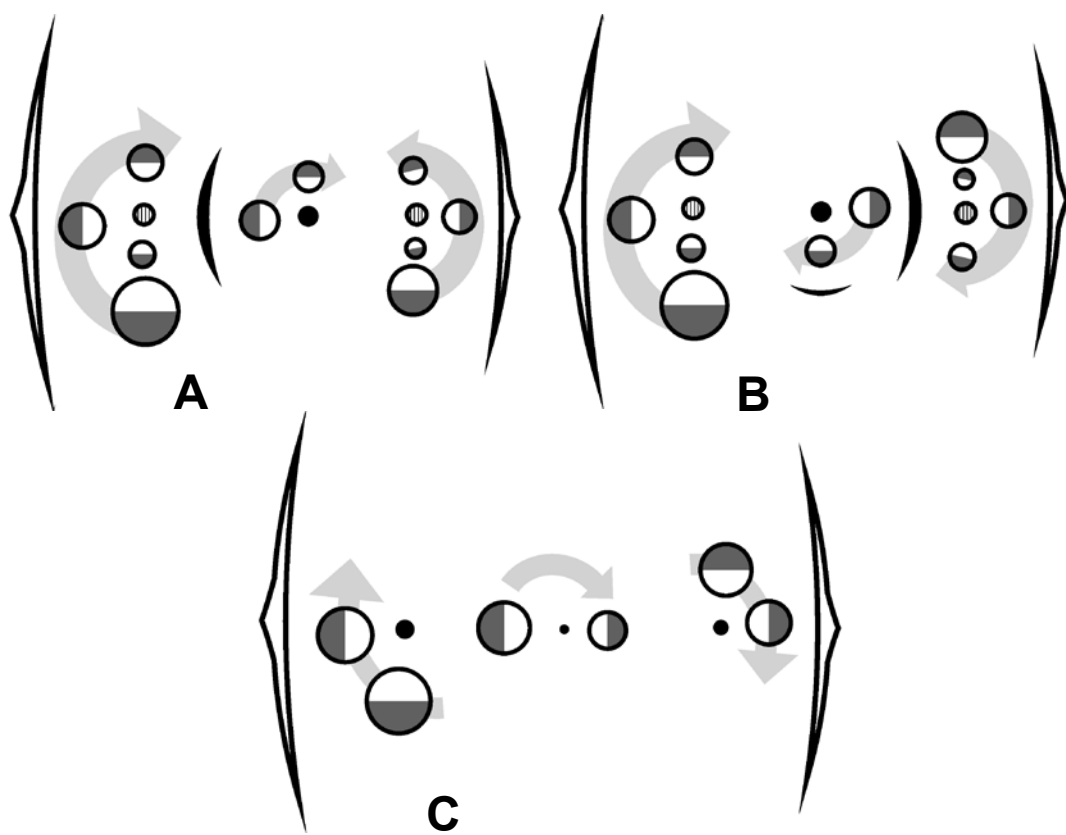
Figure 3. The polymorphism of inflorescences in determinate (*det*) plants of pea with either pendulum (A) or spiral (B, C) symmetry (diagrams). A, B – Plants of 'DTR' line with many-flowered axillary racemes. C – The recombinant *det* plant. Hatched circle = terminal flower-like structure; black arc = bract; see Fig. 2 for further designations.

structures was more precisely described in an earlier paper (Sɪɴᴊᴜsʜɪɴ 2011), so they are not presented in detail here. Only in few cases the main shoot is also terminated with a TFLS. Some flowers on lateral racemes of mutants *det* are accompanied by paired green bracts or a single bract which was not placed under the flower itself but in lateral position. Such bracts were not detected in axillary inflorescences of a line 'DTR(m)' which bears some other morphological mutations including reduced stipules (mutation *stipules reduced, st*).

Vicia faba. The plants of cv. 'Topless' produce three to four lateral (partial) inflorescences before terminating with an apical raceme. The number of flowers is unequal in different positions: 5.1±1.1 in T, 2.8±1.5 in T–1, 4.2±1.2 in T–2, 4.5±0.8 in T–3, and 4.7±1.1 in T–4. However, not all flowers give rise to mature pods and some of them fade. The subterminal (T–1) raceme sometimes fails to flower and senesces. The axillary inflorescences usually end with a sterile process (stub), but may also be terminated with flower-like structures which always fade without producing fertile pods, as in pea. In some cases, the main shoot also ends with an aberrant flower, so the whole annual shoot may become monoaxial sensu Nᴏᴛᴏᴠ & Kᴜsɴᴇᴛzᴏᴠᴀ (2004).

The transition to apical raceme also has a striking effect on inflorescence symmetry. With a single exception, all terminal racemes of cv. 'Topless' (*ti* homozygotes) tend to follow distichous organotaxis typical for the vegetative shoot, but not for an axillary raceme (Fig. 4A, B). Flowers of T were either in the same plane with leaves of a main shoot or had a divergent angle obviously
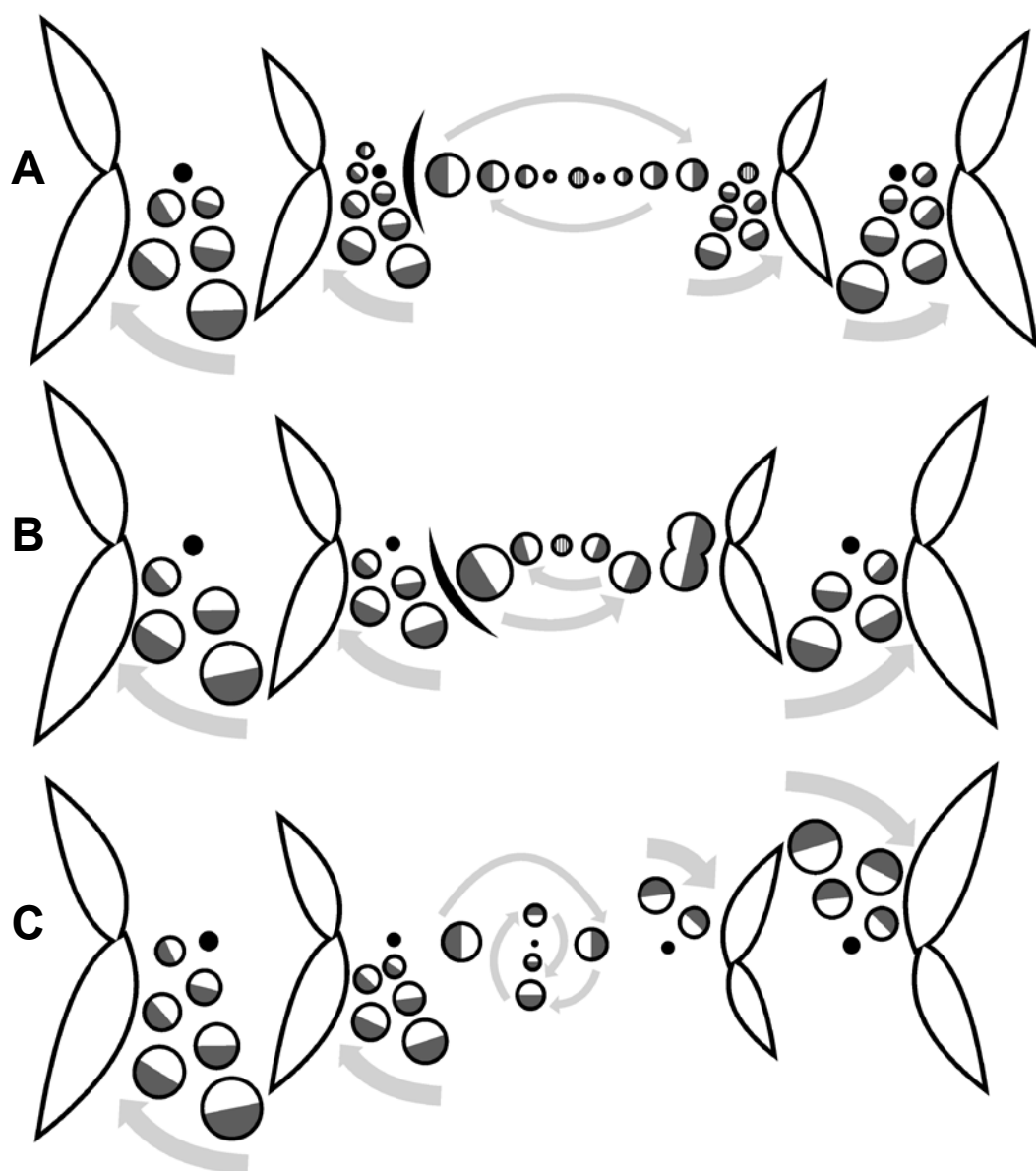
Figure 4. The polymorphism of inflorescences in determinate (*ti*) plants of *V. faba* (diagrams). A – The distichous plane of a terminal raceme. B – The divergence angle between flowers of a terminal raceme tends to 180° but the torsion is obscure. C – The spiral symmetry of short paracladia together with spirodistichous organotaxis in the terminal part. Fused circle (B) = fused flowers of the same node. See Figs 2–3 for other designations.

exceeding 90° and gradually tending to 180° (Fig. 4 A, B). Among all specimens observed, only one was found, which had a diverse symmetry on a terminal raceme: the arrangement of flowers in T was spirodistichous rather than simply distichous (Fig. 4 C). Another queerness accompanying this phenomenon is that the lateral inflorescences of this unusual plant follow a spiral organotaxis, i.e. the torsion is the same (clockwise) in all axillary racemes (Fig. 4 C).

The T–1 axillary structure sometimes combines some features of a two-flowered raceme and a solitary flower, i.e. represents fused flowers as in *det* mutants of pea.
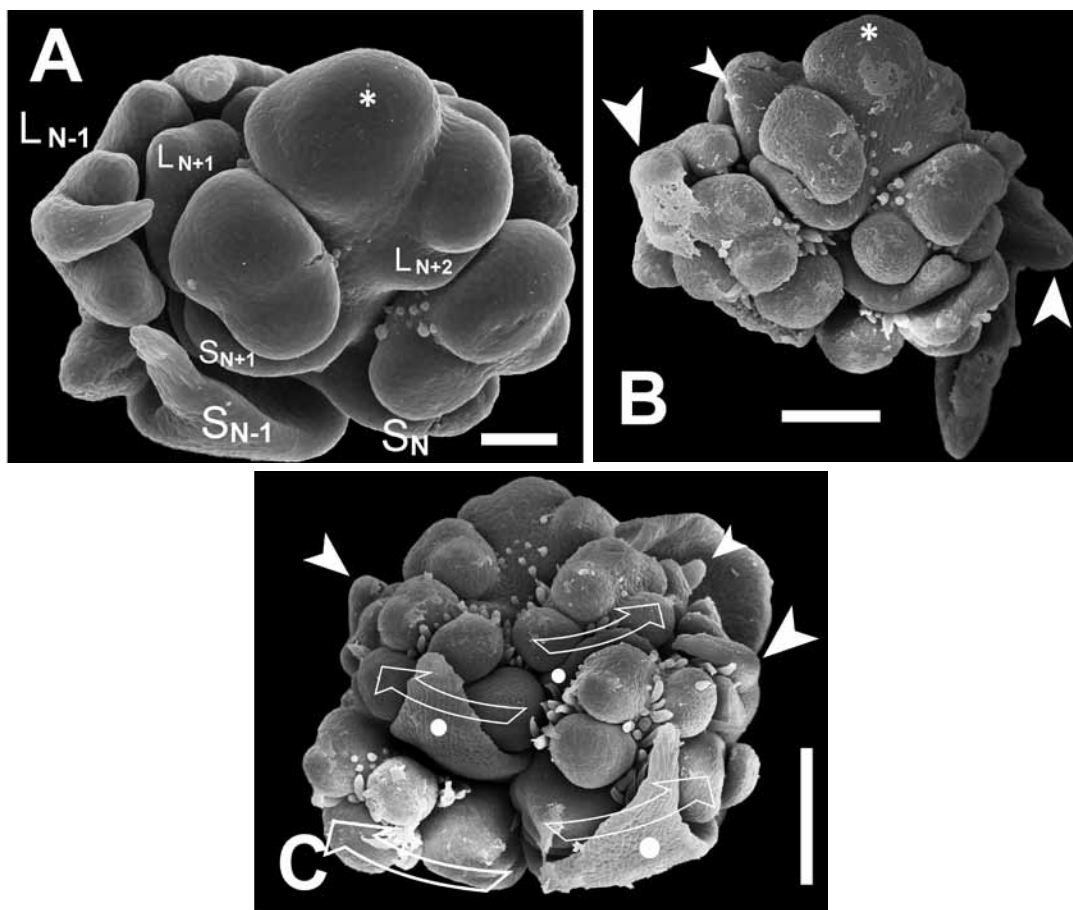
Figure 5. SEM images of early developmental stages of *Vicia faba* cv. 'Topless'. A – Three lateral racemes have been initiated, but SAM (asterisk) remains undifferentiated (apical view). B – Four lateral racemes have been initiated; flowers begin differentiation (apical view). The mutual position of axillary inflorescences and subtending leaves is clearly visible. C – The beginning of terminal raceme differentiation (lateral view from the dorsal side). Flowers of four axillary racemes almost completely separated. Arrowheads indicate the apices of leaf rachises. Dots = stipules on dorsal side; asterisk = undifferentiated SAM; S = stipule; L = leaf (lower indices clarify the position of node). Scale bars: A, B = 100 μm; C = 300 μm.

The early stages of an inflorescence development in *det* mutants of pea were precisely documented in the work of Singer et al. (1990), so in this study more attention was paid to determinate mutants of *V. faba*. As in indeterminate forms, the meristem of an axillary inflorescence initiates asymmetrically in relation to the subtending leaf: the future inflorescence is covered only with a stipule of the subtending leaf, but not with the leaf itself and looks somewhat 'forced out' of the axil (Fig. 5 C). The flowers on axillary racemes initiate in a zigzag-like sequence which is then reflected in the arrangement of flowers and the order of flowering (Fig. 5 C).

The inflorescences of fasciated peas

Together with raceme terminalization, the influence of fasciation on inflorescence symmetry was also studied. Different fasciated genotypes of pea are known, which are all characterized with a progressive enlargement of the shoot apical meristem (SAM). This abnormality results in distortions of phyllotaxis: few leaves develop on a single node, often with axillary racemes
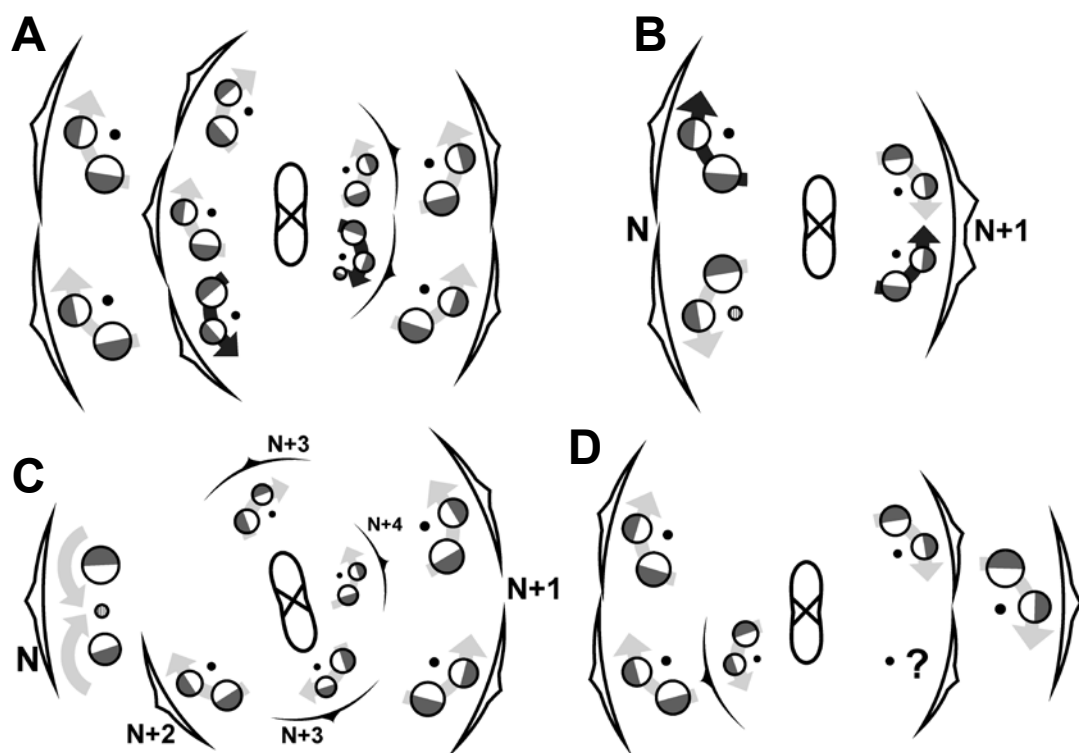
Figure 6. The polymorphism of inflorescences in fasciated (*fa*) plants of pea (diagrams). A – 'Semiwhorls' of leaves keep a distichous phyllotaxis, the whole shoot tends to have dorsiventral (pendulum) symmetry. The deviating torsions are marked with dark grey arrows. B – Alterations of the torsion of axillary racemes in a same node: racemes in node N are turned outward of the node, while in N+1 they are turned inward. C – Non-distichous phyllotaxis in a strongly fasciated plant with pendulum symmetry. D – The spiral torsion of axillary racemes. The raceme marked with '?' senesced before description became possible. See Figs 2–3 for designations.

which in their turn can fuse along their length or remain free (Sinjushin & Gostimsky 2006). Symmetry of such anomalous inflorescences has not been analyzed previously, so an effort was made to uncover the basic principles of spatial flowers' arrangement.

The fasciated pea plants are all remarkable with more or less anomalous phyllotaxis. In an upper part of a shoot, two or more leaves of a node develop as unilateral 'semiwhorls' (Fig. 6A). These 'semiwhorls' continue following distichous arrangement along the shoot, but in uppermost part of a stem leaves or their groups deviate from distichous regularity (Fig. 6C, D). The upper internodes usually fail to elongate, so the axillary racemes become clustered in an apical part of the stem thus producing a corymb-like inflorescence (Fig. 1C).

In most cases, partial inflorescences on fasciated shoots remain two-flowered and a divergence angle can be estimated in modified spirals. With some exceptions, which seem to have no specificity, this angle also comprises ca. 90° as in non-fasciated plants. The partial inflorescences possess no features of fasciation which only influences vegetative shoots in pea (first- and second-order vegetative stems). The arrangement of axillary racemes along the fasciated shoot may follow three patterns: (1) the alternating torsion of racemes in successive nodes results in dorsiventral asymmetry of an inflorescence (Fig. 6A); (2) the constant mode of torsion of racemes in successive nodes leads to the formation of a spiral inflorescence (Fig. 6D); (3) the alternation of direction
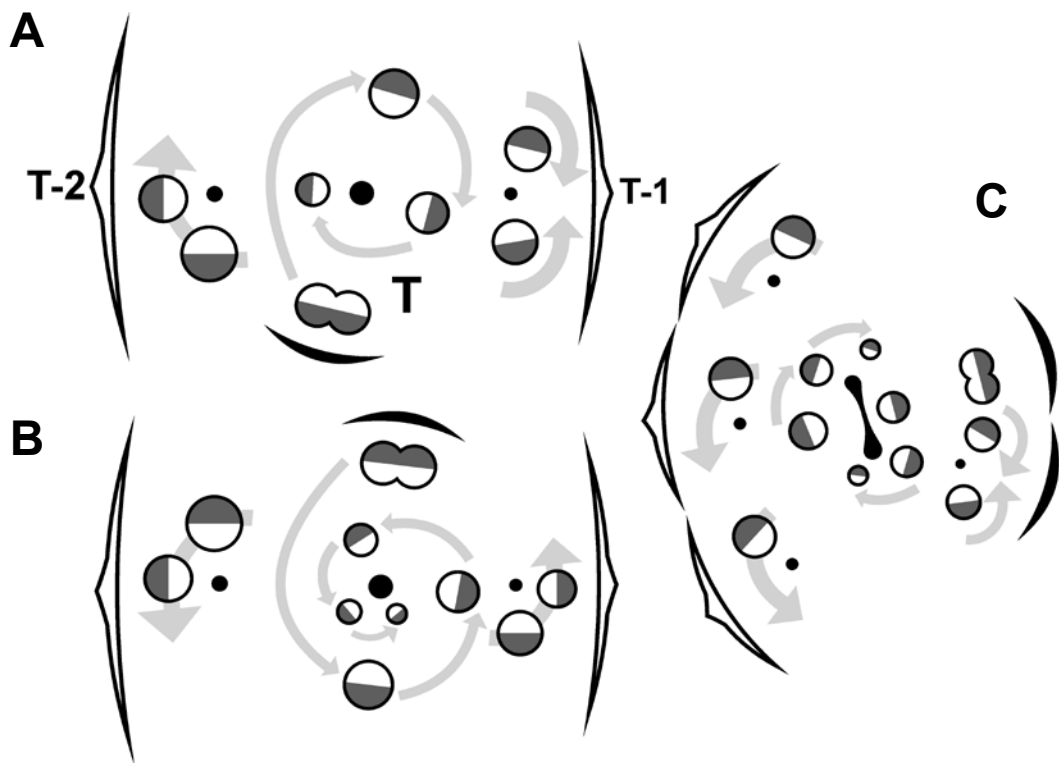
Figure 7. The variability of terminal structures in fasciated determinate mutants (*fa det*) of pea. See Figs 2–4 for designations.

of racemes' turns of the same node (Fig. 6 B). The tendencies (1) and (2) are the same as in non-fasciated plants (see above) while the phenomenon (3) is specific for fasciated inflorescences. If few leaves are produced on the same node, racemes in their axils may either be turned in the same way (both (counter)clockwise) or in different directions. In the latter case, both racemes can be turned inward or outward of the node (Fig. 6 B). In some cases, partial inflorescences of the same node may fuse. As a result, three-flowered bilaterally symmetric racemes arise and resemble dichasia, but have diverse origin and the order of their flowering remains acropetal (node N on Fig. 6 C).

Determinate fasciated inflorescences in pea

In double mutants *fa det*, a many-flowered terminal raceme arises (Sɪɴᴊᴜsʜɪɴ & Gᴏsᴛɪᴍsᴋʏ 2006) (Fig. 1 D). The meristem of the fasciated shoot is completely converted into a terminal flower-bearing axis. The number of flowers in a terminal axis usually exceeds one in *det* mutants: 4.3±1.4 in 'Lupinoid' (*fa det*) versus 1.4±0.5 in *det* recombinants from F_2 of cross 'Lupinoid' × 'Ras-tip' (*FA DET*).

The symmetry of such inflorescences was also studied. The zone of axillary racemes did not differ significantly from fasciated indeterminate pea plants (see above), i.e. either with dorsiventral pendulum symmetry or with spiral, sometimes without regular patterning (Fig. 7). The terminal raceme never exhibits a distichous organotaxis which is typical for the non-fasciated determinate forms of pea and broad bean. The arrangement of flowers is spiral instead, the first flower often deviating from the position expected for the leaf next to T–1 (Fig. 7 A, B). Many of such structures bear flowers arranged in spirodistichous mode (Fig. 7 A).

Discussion

The symmetry of normal (indeterminate) shoots of *Pisum sativum* and *Vicia faba*

As distinct from a variation in morphology of wild-growing species collected in their natural habitats, the observed polymorphism does not deal with natural selection. The studied mutants were once isolated and then reproduced in germplasm collections. This is why the unusual ontogeny had never been under a pressure of adaptive selection and nothing is known about its adaptive value. It is simply a distortion of developmental mechanics which, however, makes it possible to understand, how this mechanics normally works. Except this, almost all studied specimens belong to genetically uniform lines and the observed phenotypic variation within a line reflects the ontogenetic plasticity of the same genotype. To date, the inflorescence symmetry has almost completely remained beyond the scope of geneticists working with pea (few exceptions exist, like LAMPRECHT 1967).

When examining normal (indeterminate) inflorescences of pea, one can state that the position of the first flower in the two-flowered axillary raceme coincides with the position of the first frondose leaf on second-order vegetative shoots (Fig. 2 A, B, D). Both of these phyllomes are turned towards the dorsal side of a first-order axis. This shows that the first flower in partial inflorescences in pea is subtended by the first leaf on the inflorescence axis (i.e. prophyll), although this prophyll itself normally remains cryptic. It is easy to conclude that two flowers in partial inflorescences of pea are subtended by cryptic (present only on the level of gene expression) prophylls. This idea coincides with a hypothesis of KUZNETSOVA (1998) who stated that reduction to a two-flowered state in legumes is stable and widespread as these two flowers are subtended by prophylls. It is noteworthy that most of peas and their closest relatives (some of *P. sativum* ssp. *sativum* accessions and other subspecies, *P. fulvum*, *Vavilovia formosa*) have partial inflorescences reduced to a single flower. The number of flowers per axillary raceme was proposed to be controlled by two genes (LAMPRECHT 1947) or even one gene (SINGER et al. 1999). But these facts do not devalue the idea on fertile axils of prophylls in pea.

Together with a pendulum symmetry of inflorescence typical for Fabeae, some cases of spiral symmetry were described in both pea and broad bean. The symmetry seems to be independent from the presence of a terminal raceme. It also has non-genetic character (as both types were found in a genetically uniform sample), at least in the studied material. However, a decision between two possible symmetry plans, either pendulum or spiral, is not made on occasion. The spiral arrangement remains rare (at least not as often as the pendulum type) and a mechanism of switching between them is yet to be discovered.

In *V. faba*, pendulum symmetry affects not only partial inflorescences, but it is also observed in leaves in which the proximal portion is asymmetric (Fig. 1 F). This phenomenon is not unique for *V. faba* but can also be observed in *V. sylvatica* L. and in some oroboid vetchlings (e.g., *Lathyrus vernus* (L.) Bernh., data not shown). In these species, an angle of torsion of an axillary raceme strongly correlates with the position of the most proximal leaflet. The existence of spiral symmetry in inflorescences is predictable in these species (although seemingly not documented). The pea accessions with alternate arrangement of leaflets are of certain interest for studies on the inflorescence symmetry.

The structure of the terminal inflorescence in determinate mutants

In addition to the length of the stalk and leaf symmetry (see above), one more difference between pea and broad bean can be found. It deals with the relative size of the terminal inflorescence. As original counts indicate, a terminal axis in pea bears fewer flowers than the preceding lateral ones. Oppositely, the terminal inflorescence in *V. faba* is the largest, while the subterminal one (T–1) is the smallest among all laterals, often failing to flower. A similar difference was described by Akulova et al. (2000) for two species of *Anthyllis* (Loteae) which have different proportions of the terminal part of the inflorescence. In *A. aurea* Turrill the terminal racemose unit is larger than the lateral ones, whereas the opposite tendency can be observed in *A. barba-jovis* L. (Akulova et al. 2000). A terminal raceme is a rare feature in Loteae and the listed species deviate from other members of *Anthyllis* in having biaxial inflorescences. The relative proportions of terminal and subterminal racemes may depend on the ratio of sizes of SAM parts appointed for development of these racemes. If a signal on terminal raceme formation arises late enough, the subterminal partial inflorescence is formed completely. In this case, the remnants of SAM are being appointed to form a terminal raceme. If the SAM is predisposed to convert into a flower-bearing axis early in development, the most part of this meristem forms a terminal raceme and the subterminal one is produced residually.

More than that, the subterminal structures may be suppressed by the terminal inflorescence. In both studied species, structures of hybrid flower-to-inflorescence nature arise on a border between a terminal raceme and zone of short paracladia. When zones of lateral racemes and terminal inflorescence overlap (presumably on level of gene expression), leaves tend to be bracteous, flowers of presumptive two-flowered inflorescences fail to separate and the whole node bears organs of an intermediate morphology. Except for morphology, the phyllotaxis may serve as an indicator of such hybridization. As Akulova et al. (2000) pointed out, *A. aurea* (unlike *A. barba-jovis*) produces basal bracts of the terminal inflorescence in positions which are more typical for the frondose leaves of a vegetative zone. In *det* mutants of pea, the first flower of the terminal raceme always arises in the axil of a (cryptic) bract which follows the distichous pattern of preceding frondose leaves (Fig. 3). However, in most cases the second flower of a terminal raceme is on its proper position if analyzed without reference to its position (angle between 1st and 2nd flowers comprises ±90°, as in axillary racemes). The torsion is completely predictable and fully proper for this raceme as it is in an axillary position (i.e. coincides with the one of T–2).

The correlation between determination of stem growth and formation of bracts (as in *det* mutants of pea but not in *ti* mutants of broad bean) still remains unclear. In *Arabidopsis*, mutations in *TFL1* cause both formation of a terminal flower and development of bracts (Penin 2008). The paired bracts of partial inflorescences in determinate (and some fasciated, see Sinjushin 2011) mutants of pea most probably represent the reduced stipules. This is also evident from the fact that in determinate mutants with reduced stipules (*det st*) bracts also fail to develop (see above). The bract suppression by *st* was previously demonstrated by Lamprecht & Mrkos (1950).

In broad bean, all flowers of a terminal raceme tend to follow a distichous pattern of the whole shoot. Organs of a hybrid nature sometimes arise in this species. Both facts are evidence for a weak delimitation between terminal inflorescence and zone of short paracladia, obviously weaker than in pea. The strongly reduced subterminal raceme also proves a fuzzy border between different zones of the shoot. The concept of three zones (terminal inflorescence, short paracladia
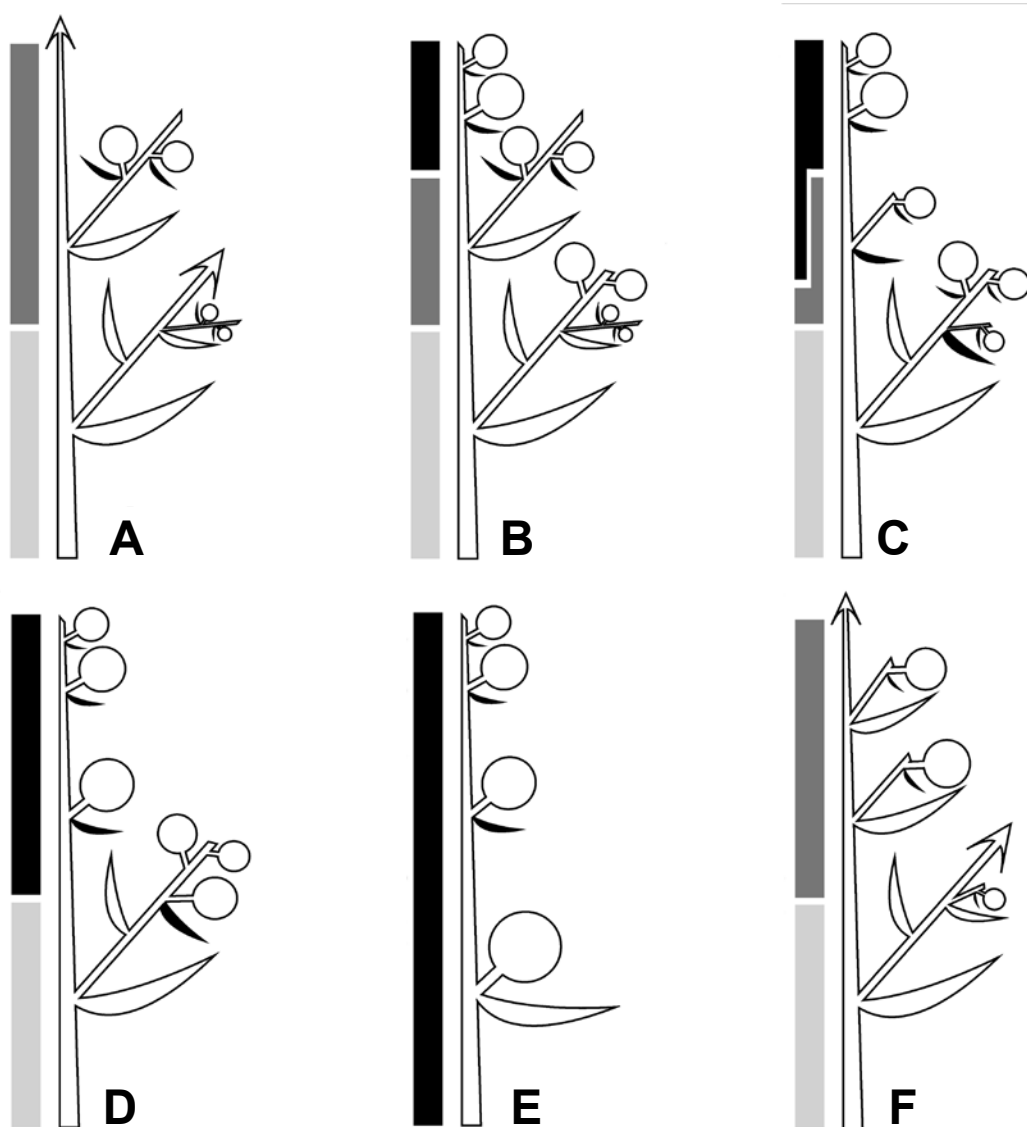
Figure 8. Three zones of a shoot and their contribution to an inflorescence structure. A – The indeterminate (triaxial or truncated) inflorescence including zones of long (grey) and short paracladia (dark grey). B – The biaxial inflorescence which includes a zone of a terminal racemose unit (black). C – The slip of the terminal zone basalwards leads to the suppression of short paracladia and dominance of the terminal raceme. D – Complete replacement of short paracladia with flowers of a terminal raceme, i.e. secondarily simplified inflorescence (as in *Lupinus*). E – No paracladia develop on a shoot (as in 'determinate' mutants of *Lupinus*, see text). F – The reduction of axillary racemes and their shortening (as in *Vicia* p.p.) result in a raceme which imitates a simple one (as in D).

and long paracladia) is sufficient for descriptions of most inflorescences observed in Fabaceae, in both normal plants and mutants (Fig. 8). The expansion of 'terminal' zone in basipetal direction explains loss of short paracladia in *Lupinus* (Fig. 8 D, see WEBERLING 1989 for discussion). Interestingly, in some mutants of lupines the long paracladia also become converted into flowers (e.g., KUNITSKAYA & ANOKHINA 2012). This may be explained by the further basipetal extension of the 'terminal' zone (Fig. 8 E). A complete lack of a terminal inflorescence seems to characterize almost the whole Inverted Repeat-Lacking Clade (IRLC) sensu WOJCIECHOWSKI et al. (2004).

However, some of IRLC members produce terminal inflorescences either normally (*Galega*, *Medicago*) or in mutants (*Pisum*, *Vicia*, *Cicer*) and both truncated and terminalized inflorescences persist in a variation of IRLC.

Finally, in some IRLC genera the axillary inflorescences become strongly reduced (e.g., *Vicia* p.p.), so the short paracladia appear as single flowers. In such cases the whole compound inflorescence gradually becomes 'secondary simple'. The result is the same as in case of terminalization of racemose unit with further suppression of axillary branching (Fig. 8 F). This phenomenon is out of the scope of this study and its biological significance still awaits examination.

Origin of pendulum symmetry in Fabeae

One of the most intriguing questions is: what developmental mechanism causes pendulum symmetry in a stem of vicioid legumes and many other tribes? Both inhibitory influences from SAM on an inflorescence and from a subtending leaf are possible explanations for an asymmetric initiation of an axillary raceme in an axil. Precise measurements are needed on a wider range of species, but the first flower seems to initiate opposite to the inflorescence-subtending leaf in broad bean (Fig. 5) and more or less equidistantly from subtending leaf and SAM in pea. The inhibitory effect of leaves is removed in the terminal inflorescence and a first leaf serves as bract in terminalized raceme. The partial inflorescences of *V. faba* bear flowers in two parallel rows which can easily be interpreted as modified distichous arrangement, as in the main shoot and vegetative axillary branches. Possibly such arrangement is derived from true distichy with the first prophyll of an axillary shoot opposite the subtending leaf. In terminal position, this distichy is restored when no inhibitory action happens. It is not a universal model because in some other vicioids the number of rows of flowers can exceed two (e.g., in *V. cracca* three rows can be traced). However, under the inhibitory effect on subtending leaves the displacement of one row occurs in axillary racemes. The dorsal side of the second-order vegetative shoot is also defined as the most distant from the subtending leaf. An indirect proof for a strong inhibitory action on leaves of second-order axes can be found in the symmetry plane of flowers which terminate axillary racemes in fasciated and determinate pea mutants (see SINJUSHIN (2011) for diagrams). These flowers develop with certain distortions but with an expressed abaxial-adaxial symmetry and only the adaxial side develops more or less constantly (SINJUSHIN 2011). Interestingly, the existence of such monosymmetric flowers terminating many-flowered racemes in line 'DTR' (Fig. 3A) contradicts the hypothesis of DEGTJAREVA & SOKOLOFF (2012) which says that in angiosperms monosymmetric flowers cannot be terminal on shoots bearing more than two (or three) phyllomes. In this connection, more precise analysis of flower symmetry is needed for determinate plants of *V. faba* bearing TFLS on a main shoot (Figs 1 E; 4 A, B).

Conclusion

Three observations on the symmetry of a terminal raceme may be stated for two studied objects. (1) The divergence angle of a terminal structure tends towards the one typical for the preceding stem (zone of short paracladia), i.e. distichous. (2) The torsion usually follows the pattern expected for the axillary inflorescence (the same as in T–2) with very rare exceptions (Fig. 4 B). (3) The flower arrangement in a terminal raceme becomes spiral either if the whole stem has a spiral symmetry or if the SAM is enlarged, as in fasciated plants.

The *TERMINAL FLOWER1*-like genes (such as *DET* in pea and *VfTFL1* in broad bean) seem to be important for the evolution of the legume inflorescence according to Prusinkiewicz et al. (2007) and Benlloch et al. (2007). Earlier it was proposed that the function of these genes is connected with support of proliferation of axes; in case of mutation, axis of *n*-order is terminated with a structure of *n+1*-order (Sinjushin 2011). Surely, formation of a normal inflorescence results from an orchestration of few (but not numerous) genes (Singer et al. 1999, Benlloch et al. 2007). In legumes, some aspects of inflorescence evolution and ontogeny still remain poorly studied and understood. Among these, demarcation between different zones of a flower-bearing shoot and control of inflorescence symmetry are of special interest. The model leguminous species are a valuable tool in studies of these topics which need further and deeper examination.

Acknowledgements

References

Akulova Z. V., Kuznetsova T. V. & Sokoloff D. D. (2000): On the structure of inflorescences in the genus *Anthyllis* (*Papilionaceae*, *Loteae*). – Bot. Zhurn. **85**(1): 12–25. [In Russian]

Avila C. M., Nadal S., Moreno M. T. & Torres A. M. (2006): Development of a simple PCR-based marker for the determination of growth habit in *Vicia faba* L. using a candidate gene approach. – Mol. Breed. **17**(3): 185–190.

Benlloch R., Berbel A., Serrano-Mislata A. & Madueño F. (2007): Floral initiation and inflorescence architecture: a comparative view. – Ann. Bot. **100**(3): 659–676.

Degtjareva G. V. & Sokoloff D. D. (2012): Inflorescence morphology and flower development in *Pinguicula alpina* and *P. vulgaris* (Lentibulariaceae: Lamiales): monosymmetric flowers are always lateral and occurrence of early sympetaly. – Org. Divers. Evol. **12**(2): 99–111.

Dzyubenko N. T. & Dzyubenko E. K. (1994): Usage of *ti* mutants in a breeding of alfalfa. – Genetika **30** (Supplement): 41. [In Russian]

Foucher F., Morin J., Courtiade J., Cadioux S., Ellis N., Banfield M. J. & Rameau C. (2003): *DETERMINATE* and *LATE FLOWERING* are two *TERMINAL FLOWER1/CENTRORADIALIS* homologs that control two distinct phases of flowering initiation and development in pea. – Plant Cell **15**(11): 2742–2754.

Kunitskaya M. P. & Anokhina V. S. (2012): Genetic analysis of habit of branching determination in narrow-leaved lupine. – Vestnik BGU (Ser. 2) **2**: 46–49. [In Russian]

Kuznetsova T. V. (1998): Reduction in inflorescence: essence and role of reduction in evolution of modular organisms. – Zhurn. Obshch. Biol. **59**: 74–103. [In Russian]

Lamprecht H. (1947): The inheritance of the number of flowers per inflorescence and the origin of *Pisum*, illustrated by polymeric genes. – Agri Hort. Genet. **5**: 16–25.

Lamprecht H. (1967): Die *alternilateralis*-Mutante und die übrigen Infloreszenztypen von *Pisum*. – Phyton **12**: 266–277.

Lamprecht H. & Mrkos H. (1950): Die Vererbung des Vorblattes bei *Pisum* sowie die Koppelung des Gens *Br.* – Agri Hort. Genet. **8**: 153–162.

Makasheva R. Kh. & Drozd A. M. (1987): Determinate growth habit (*det*) in peas: isolation, symbolization and linkage. – Pisum Newslett. **19**: 31–32.

Naghiloo S., Dadpour M. R. & Movafeghi A. (2012): Floral ontogeny in *Astragalus compactus* (Leguminosae: Papilionoideae: Galegeae): variable occurrence of bracteoles and variable patterns of sepal initiation. – Planta **235**(4): 793–805.

Notov A A. & Kusnetzova T. V. (2004): Architectural units, axiality and their taxonomic implications in Alchemillinae. – Wulfenia **11**: 85–130.

Penin A. A. (2008): Bract reduction in Cruciferae: possible genetic mechanisms and evolution. – Wulfenia **15**: 63–73.

Prenner G., Vergara-Silva F. & Rudall P. J. (2009): The key role of morphology in modelling inflorescence architecture. – Trends Plant Sci. **14**: 302–309.

Prenner G. (**2013**): Papilionoid inflorescences revisited (Leguminosae-Papilionoideae). – Ann. Bot.: first published online December 12, 2012 doi:10.1093/aob/mcs258.

Prusinkiewicz P., Erasmus Y., Lane B., Harder L. D. & Coen E. (2007): Evolution and development of inflorescence architectures. – Science **316**: 1452–1456.

Repjev S. I., Stankevich A. K. & Leokene LV. (1999): Vetch. – In: Stankevich A. K. & Repjev S. I. [eds.]: Flora of cultivated plants 4 Part 2. – St.-Petersburg: SSC-VIR. 491 pp.

Schaefer H., Hechenleitner P., Santos-Guerra A., de Sequeira M. M., Pennington R. T., Kenicer G. & Carine M. A. (2012): Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. – BMC Evol. Biol. **12**: 250.

Sell Y. (1980): Physiological and phylogenetic significance of the direction of flowering in inflorescence complexes. – Flora **169**: 282–294.

Singer S. R., Hsiung L. P. & Huber S. C. (1990): Determinate (*det*) mutant of *Pisum sativum* (Leguminosae: Papilionoideae) exhibits an indeterminate growth pattern. – Amer. J. Bot. **77**: 1330–1335.

Singer S., Sollinger J., Maki S., Fishbach J., Short B., Reinke C., Fick J., Cox L., McCall A. & Mullen H. (1999): Inflorescence architecture: a developmental genetics approach. – Bot. Rev. **65**(4): 385–410.

Sinjushin A. A. (2011): On the role of genes *DETERMINATE*, *LATE FLOWERING* and *FASCIATA* in the morphogenesis of pea inflorescence. – Ratar. Povrt. / Field Veg. Crop Res. **48**: 313–320.

Sinjushin A. A. & Akopian J. A. (2011): On seedling structure in *Pisum* L., *Lathyrus* L. and *Vavilovia* Fed. (Fabeae: Fabaceae). – Wulfenia **18**: 81–93.

Sinjushin A. A. & Demidenko N. V. (2010): *Vavilovia formosa* (Fabeae, Fabaceae) on Meyen's 'panel with a multitude of lamps'. – Wulfenia **17**: 45–57.

Sinjushin A. A. & Gostimsky S. A. (2006): Fasciation in pea: basic principles of morphogenesis. – Russ. J. Developm. Biol. **37**(6): 375–381.

Sjödin J. (1971): Induced morphological variation in *Vicia faba* L. – Hereditas **67**(2): 155–180.

Sokoloff D. D., Degtjareva G. V., Endress P. K., Remizowa M. V., Samigullin T. H. & Valiejo-Roman C. M. (2007): Inflorescence and early flower development in Loteae (Leguminosae) in a phylogenetic and taxonomic context. – Int. J. Plant Sci. **168**(6): 801–833.

van Rheenen H. A., Pundir R. P. S. & Miranda J. H. (1994): Induction and inheritance of determinate growth habit in chickpea (*Cicer arietinum* L.). – Euphytica **78**(1–2): 137–141.

Weberling F. (1989): Structure and evolutionary tendencies of inflorescences in the Leguminosae. – Monogr. Syst. Bot. Missouri Bot. Gard. **29**: 35–58.

Wojciechowski M. F., Lavin M. & Sanderson M. J. (2004): A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. – Amer. J. Bot. **91**: 1846–1862.

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