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**CURRENT PROBLEMS
OF DEVELOPMENTAL BIOLOGY**

Unusual Alternations of Floral Organs in *Paeonia*: Structure and Possible Mechanism of Formation

M. D. Logacheva, P. A. Prudkovsky, S. V. Efimov, and A. A. Penin
Moscow State University, Leninskie gory, Moscow, 119992 Russia
E-mail: alekseypenin@gmail.ru

Abstract—Morphological analysis of flowers was carried out in *Paeonia L.* cultivars. Some unusual alternations of floral organs were described: sepal–(petal–stamen) × n–carpel, where $2 \le n \le 4$ (appearance of an additional zone of petal and stamen formation in the medial flower part). The identity of floral organs was not affected in the flowers with this unusual alternation. It was shown on the basis of mathematical simulation of the genes responsible for flower development that these alternations may be determined by increased pool of stem cells, which may lead to delayed termination of flower development.

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Key words: ABC-model, *Paeonia*, stem cells, mathematical simulation of biological processes.

Despite significant morphological diversity of flowers, it has been proposed that the mechanisms controlling their development are very conservative (Soltis et al., 2002; Krizek and Fletcher, 2005). At present, a considerable progress has been achieved in their understanding. Based on studies of *Arabidopsis thaliana* (*L.*) Heynh. and *Antirrhinum majus* L. mutants with altered flower structure, a genetic model was developed for determination of the type of floral organ, ABC-model (Coen and Meyerowitz, 1991). According to this model, the genes that determine the type of floral organ are divided into three classes: A, B, and C. The activities of class A genes determines the development of sepals, those of class genes, carpels, while combination of their activities with those of class B genes determines the development of petals (A + B) and stamens (C + B). Expression of class A and C genes in the same regions of floral meristem and at the same time is impossible due to mutual negative regulation of the genes of these classes (Drews et al., 1991). This model described the development of a typical dicot flower including elements of four types: sepals, petals, stamens, and carpels. The ABC-model was later complemented by two classes of genes: (1) class D genes found in *Petunia hybrida* and responsible for the development of ovules (Colombo et al., 1995) and (2) class E genes whose defects lead to the development of only sepals in the flower (Pelaz et al., 2000).

Most plants used for studying the genetic control of flower development belong to the group “core eudicots” (higher dicotyledons) (APGII, 2003). Their flower usually consists of four types of organs arranged in a strictly determined order: sepals, petals, stamens, and carpels. It has been repeatedly proposed that all known flower types can be reduced to such an “ideal” flower type due to variation of several characters: presence/absence of certain elements, their number, shape, and coloration, presence/absence of their concrescence, etc (see, for example, Mendoza et al., 1998). Nevertheless, the structure of flowers cannot principally be reduced in some cases to such “ideal” flower. The present study was aimed at studying polymorphism of the flower structure in *Paeonia* cultivars with unusual, for dicotyledons, alternation of organs and analyzing them from the position of ABC-model.

MATERIALS AND METHODS

Studies were carried out on plants of *Paeonia lactiflora* Pall., cultivars from the collection of the Botanical Gardens, Moscow State University, based on this species (Arkadii Gaidar, Victoire de la Marne, Mary Brand, Auguste Dessert, Duc de Wellington, Mons. Martin Cahuzak, Sinbad, Marechal Bigot, and President Taft), and interspecific hybrid *P. lactiflora* Pall × *P. officinalis* L.—Robert W. Auten. *Paeonia* flowers are spiral or spirocyclic and, hence, cannot be described in terms of “whorls” conventionally used in formation of the ABC-model. We will use for description of *Paeonia* flowers a wider notion of the “zone of identical organs”, for which there is or appears to be no difference in the genetic control of development and which constitute a continuous file on the flower axis (Shestakov et al., 2005); the number of organs in this zone is not considered. In some cases, for example, for carpels, the zone may correspond to a circle. The structure of organs was analyzed using SEM and samples were prepared as described elsewhere (Ezhova et al., 2002).

The program realizing the mathematical model was written in Delphy 5.0 system and can work under Windows 98SE, Me, or NT5.X. It calculates the dynamics of functioning of the system of genes interacting according to the system of differential equations given in this paper. The program assumes regulation of the step of integration of differential equation, as well of the system parameters characterizing the gene states. The results of calculations can be expressed as graphs of concentration dependencies of the gene products as a function of time or as phase portraits of the system, i.e. dependence of concentrations of the product of one gene from those of another.

RESULTS AND DISCUSSION

Structure of P. lactiflora flowers (Fig. 1) The flower structure is characteristic for the family Paeoniceae: calyx of five sepals, corolla of 5–15 large milk-while petals, androecium of many stamens, and apocarpous gynoecium of 2–7 carpels. The order of floral organs does not differ from that of "ideal flower" (Fig. 1a)*.

Vegetative leaves of *P. lactiflora* are characterized by extended epidermal cells with smooth surface and convoluted anticlinal walls (Fig. 2a). The structure of sepal cells reflects gradual transition from leaves to sepals characteristic for this family: cells of the first sepal are similar to those of the leaf but they are less elongated and slight folds can be seen on their surface (Fig. 2b). Cells of the last (4th or 5th) sepals are spherical or rectangular with rounded angles and many folds on their surface (Fig. 2c). The petal cells are extended along the organ axis in rectangular or rhombic shape, their anticlinal cells are straight, frequent convoluted folds can be seen on their surface, which are oriented transversely or at an angle to the anticlinal walls (Fig. 2d). Cells of the staminal filaments are extended along the organ axis and their surface is covered by longitudinal folds (Fig. 2e). The surface of anther cells is also folded and the cells are rounded or almost square (Fig. 2f). Epidermal cells of the carpels are rectangular pr almost rounded, in the zone of ovary - smooth or small-folded and in the zone of style - with large folds (Fig. 2g). The stigma surface is covered by many glandular hairs (Fig. 2h).

Structure of flowers of P. lactiflora cultivars and hybrid *P. lactiflora* × *P. officinalis.* In all studied cultivars, the same types of floral organs are present as in the wild type, but the flower structure and alternation of organs are not typical for *P. lactiflora* and were not described for any other species of the family Paeoniaceae. Two main deviations in the structure of these flowers can be noted.

(1) Development of additional zones of petals and stamens (cv. Arkadii Gaidar, Victoire de la Marne, Mary Brand, Auguste Dessert, Duc de Wellington, etc.). In the simplest case, a flower is formed with the following alternation of organs: sepals, petals, stamens, second zone of petals, second zone of stamens, carpels (Fig. 1b). Carpels can sometimes not give rise to ovules and stigmatic tissue (Fig. 2i) but have their characteristic features, such as shape and surface of cells (Fig. 2j). The appearance and surface of cells of the first and second zone petals (Figs. 2k and 2l) and first and second zone stamens (Figs. 2m and 2n) do not differ. There may be more additional zones in the flower and, generally, the alternation of organs in such flowers can be described as follows: sepal–(petal–stamen) × *n*–carpel, where $2 \leq n \leq 4$.

When flowers are formed on lateral shoots with a lesser, than on the main shoot, number of organs (3 to 10 times), the number of alternations decreases (Figs. 3a and 3b) or flowers with normal alternation of organs are formed. Note that the same pattern is observed in the cases of unusual alternation of floral organs in some *Tulipa* sp. cultivars (Figs. 3c and 3d).

(2) Development of the zone of peripheral carpels after any zone of stamens in the preceding variant of alternation (cv. Robert W. Auten, Mons. Martin Cahuzak, Sinbad, Marechal Bigot, Old Tasteful, etc.). In the simplest case, the alternation of organs in such flowers is as follows: sepals, petals, stamens, peripheral carpels (Fig. 2o), second zone of petals, second zone of stamens, and carpels (Fig. 2l) terminating the flower axis (Fig. 1c). The surface of epidermal cells and stigmatic tissue of peripheral carpels (Figs. 2p and 2q) are similar to those in carpels terminating the flower axis (Figs. 2r–2t). In the case of increase in the number of additional zones of petals and stamens, peripheral carpels can arise after any zone of stamens.

Interpretation of structure of Paeonia flowers based on ABC-model. Normally, *P. lactiflora* forms a flower, which contains the same organs and in the same order, as the objects on which genetic control of the flower development was studied, and belongs to the same group: eudicots. Based on the hypothesis of common mechanisms underlying genetic control of the flower development (Soltis et al., 2002; Krizek and Fletcher, 2005), it can be proposed that the development of *P. lactiflora* flower is controlled in a similar way and can be described with the help of ABC-model (Fig. 1a). In flowers of *P. lactiflora* cultivars and hybrid *P. lactiflora* × *P. officinalis* with unusual alternation of organs, all types of organs are present, which are characteristic for the *P. lactiflora* flower. This means the functions of genes of ABC-classes controlling the organ type formation are not affected (Figs1b and 1c). Nevertheless, additional zones are formed in the flower, i.e. the function C-class genes for development termination may be affected. At present, two mechanisms of C-function realization are known. In the first case, characteristic for *A. thaliana* and *A. majus*, formation of the generative organs and termination of development are controlled by the same gene and both functions are affected simultaneously in mutants for this gene. For example, an *A. thaliana* mutant for gene *AGAMOUS* forms flowers that lack stamens and carpels and characterized by indeterminate growth (up to 70 organs develop, allele

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* Color variants of Figs. 1, 3, and 4 are available on-line: www.mccme.ru/~mlog/articles/paeonia.

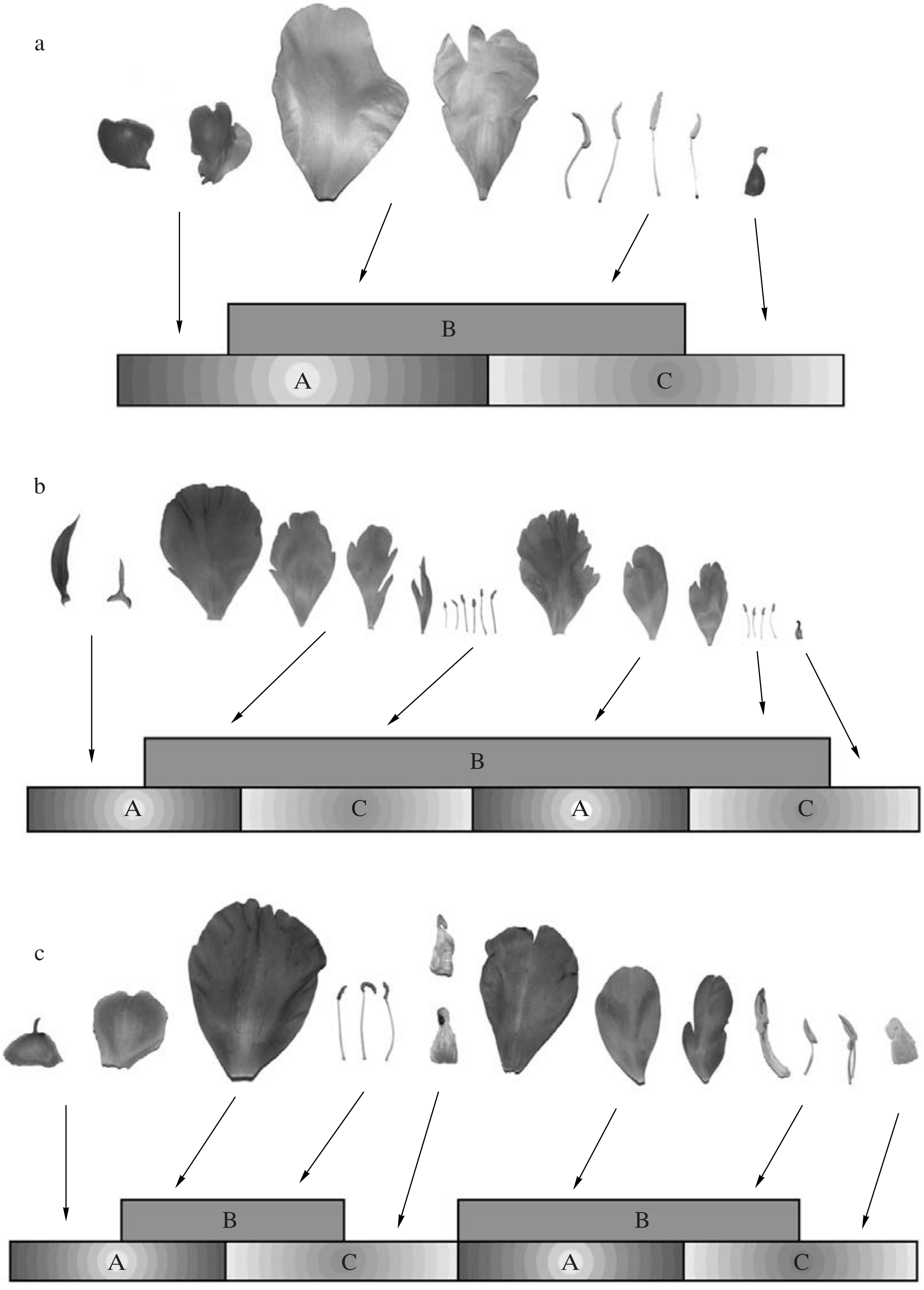

Fig. 1. Sequence of positions of floral organs and their interpretation based on ABC-model: (a) *Paeonia lactiflora*, (b) cv. Victoire de Marne, (c) cv. President Taft.

ag-1). The greatest amount of the gene product is necessary for the floral meristem termination and a lesser one for determination of the organ type. It is also possible that different regions of the protein are involved in the realization of these functions (Sieburth et al., 1995). In the second case, C-class functions are divided among different genes. Thus, two *AG* homologs are known in the maize: *ZAG1* and *ZMM2*, which are expressed in that region of floral meristem, where later generative organs arise. However, when the function of them (*ZAG1*) is lost, the development of generative organs is not affected, while the flower determination is lost

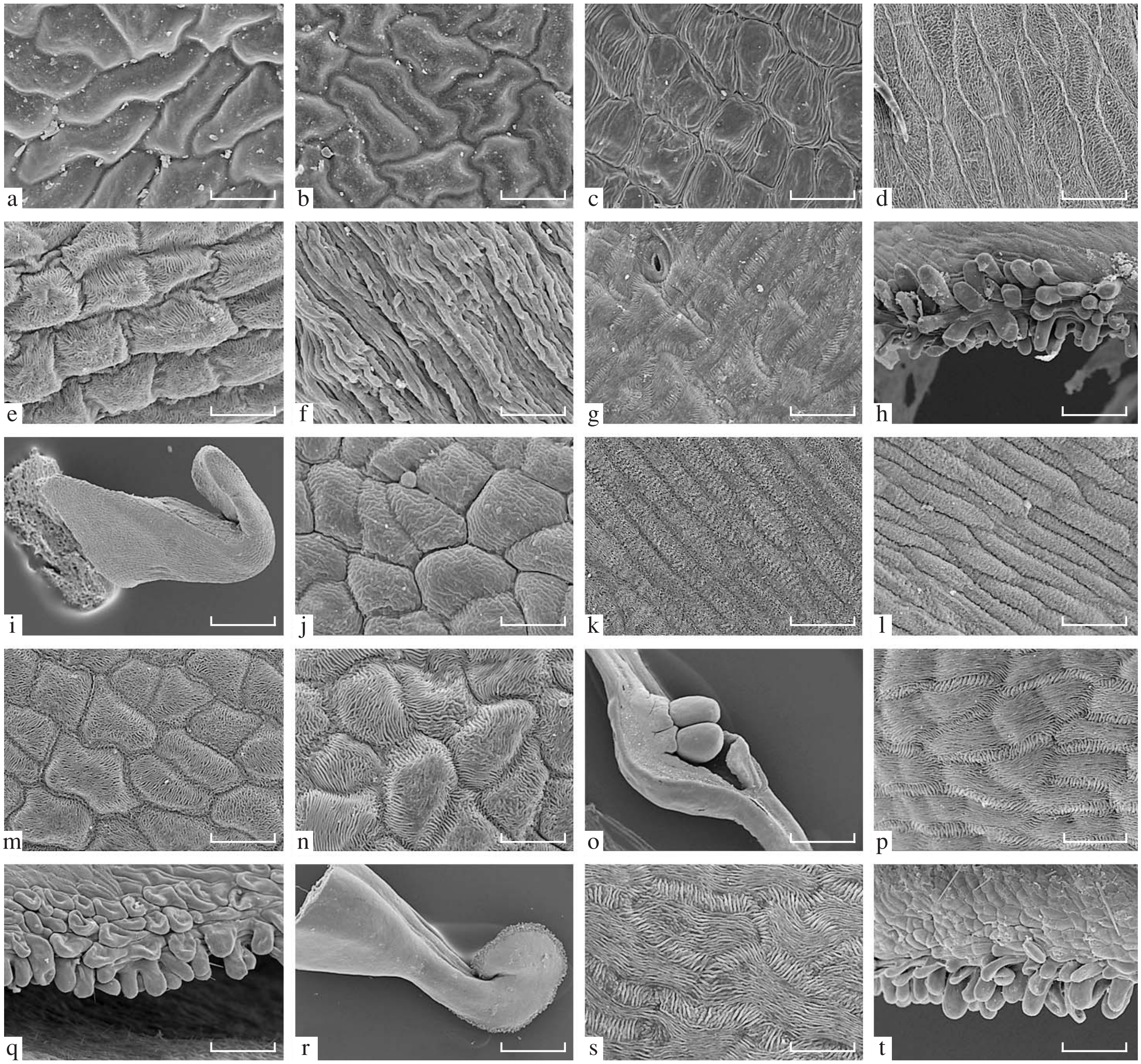

Fig. 2. Structure of floral organs in *Paeonia lactiflora* and cvs. Arkadii Gaidar and President Taft. (a–h) *Paeonia lactiflora*; surface of cells: (a) vegetative leaf, (b) first sepal, (c) fifth sepal, (d) petal, (e) staminal filament, (f) anther, (g) carpel, (h) stigmatal tissue. (i–n) cv. Arkadii Gaidar, terminal carpel: (i) general appearance; (j) cell surface, (k) petals of the first zone, (l) petals of the second zone, (m) stamens of the first zone, (n) stamens of the second zone. (o–u) cv. President Taft, peripheral carpel: (o) general appearance, (p) cell surface, (q) stigmatal tissue, central carpel, (r) general appearance, (s) cell surface in the zone of style, (t) stigmatal tissue. Scale: 40 (a, f, g, m, n, p), 60 (b, c, o, t), 80 (d, u), 30 (e, p, t), 100 (h), 300 (i), 15 (l), and 1000 μm (o, s).

(Mena et al., 1996). A similar mechanism of reversions from the generative organs to leaves is observed in *Impatiens balsamina*. In this species, an *AG* homolog (*IbAG*) is expressed in the meristem of such reverting flowers at the same level as in the normal flowers, but does not prouide for termination, which may be caused by another factor acting independently from *IbAG* (Ordidge et al., 2005). If the defects in *Paeonia* flowers were related to the first type deviations, an indeterminate axis growth would be observed and in the case of the second type deviations, not only carpels would be present at the axis end. Since the flowers of *Paeonia* cultivars are always terminalized by carpels, the function of C-class genes ensuring termination is not affected.

Thus, the number of the zones of expression of the A-, B-, and C-class genes changes in the *Paeonia* flowers without alterations of their functions and, as a result, two types of processes can be identified, which are not observed in the normal flowers: (1) alternation of the zones with expression of A- and C-class genes (Figs. 1b and 1c) and (2) decreased activity of B-class

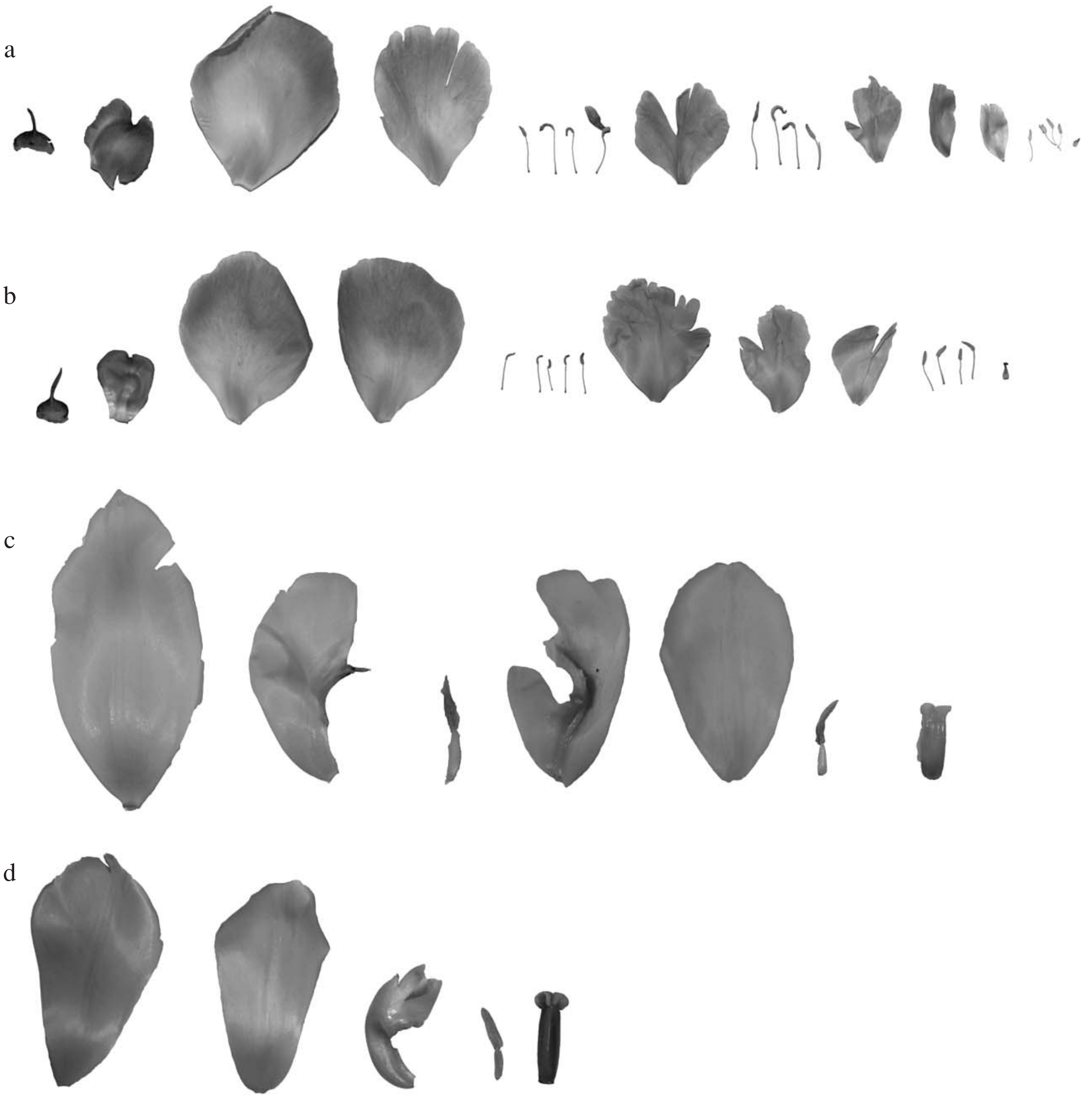

Fig. 3. Sequence of organ positioning in flowers: *Paeonia lactiflora* (cv. Auguste Dessert) on the main (a) and lateral (b) shoots and *Tulipa* sp. (cv. Monte Carlo) on the main (c) and lateral (d) shoots.

genes (Fig. 1c) which can occur after any additional zone of stamens.

Modeling of succession of A and C functions. Let us consider mechanisms which may lead to alternation of A and C along the flower axis without affecting the functioning of these genes. In mathematical models describing the functioning of a genetic network that controls the flower development, such a state is characterized when changes (increase and decrease) in the amount of products of A- and C-class genes occur synchronously as a result of mutual inhibition of these genes (Mendoza et al., 1999; Galimzyanov and Churaev, 2001; Churaev and Galimzyanov, 2001). When the parameters are deviated, this state passes to a regime with predominance of the product of one of these genes. It can be proposed that alternation of A and C functions arises as a result of functioning of such a regime in the flower apex.

Let us consider this system of genes. We will use the model (Coutinho et al., 2006), according to which the effect of the product of each gene is discrete: the product of gene C inhibits expression of gene A, if its concentration exceeds a certain threshold value ($C > T_C$) and, similarly, the product of gene A inhibits expression of gene C, if $A > T_A$. In addition, we will take into account that the effect may take place with a certain delay: correspondingly τ_C and τ_A for genes C and A. Approximations related to the introduction of discretization and delay are necessary since actually, the system is much more complex, but the work of a long chain of bonds can be described as a first approximation by one bond, which, however, is delayed and has a threshold pattern. This approach does not allow precise modeling of a real gene network, but makes it possible to demonstrate the main qualitative features of its work. With these approximations, the system may be described by a system of two differential equations.

$$\dot{C} = \begin{cases} -C(t-\tau_C), & A(t-\tau_A) > T_A \\ 1 - C(t-\tau_C), & A(t-\tau_A) < T_A, \end{cases}$$

$$\dot{A} = \begin{cases} -A(t-\tau_A), & \mathrm{C}(t-\tau_C) > T_C \\ 1 - A(t-\tau_A), & \mathrm{C}(t-\tau_C) < T_C. \end{cases}$$

This system has two stable points (C = 0; A = 1) and (C = 1; A = 0). Practically all starting conditions lead the system to one of these points. The solution dividing these two zones of starting conditions is exceptional. It represents synchronous fluctuations of concentrations of the products of both genes around their threshold values (Fig. 4a)[**]. In the case of a small deviation of the parameters, transition to one of stable points takes place (Fig. 4b). This would mean for a plant that only one gene is active during the entire period of plant development and this does not occur in reality. Therefore, this model is insufficient for description of a situation, in which concentrations of the products of two genes are fluctuated in antiphase. The presence of additional genes, whose products regulate the level of expression of class A and/or C genes, is necessary for such fluctuations to arise.

Some class C genes are activated by the products of the genes responsible for maintenance of the meristem proliferative activity. For example, the *A. thaliana* gene *AG* is activated by the product of gene *WUSCHEL* (*WUS*) responsible for the maintenance of proliferative activity of the shoot meristems. In turn, the product of gene *AG* inhibits expression of gene *WUS*, thus leading to termination of the flower development (Lohmann et al., 2001). Negative regulation of the expression of class A genes is realized with the help of microRNAs which does not allow the functional product synthesis even in the presence of *AP2* RNA. Expression of the genes encoding these microRNAs is regulated by other genes involved in development of the generative organs, rather than by *AG* directly (Chen, 2004).

Let us consider a system of three genes. We will add an F class gene (similar to *WUS* by functions), whose product regulates positively the level of C gene expression, which inhibits, in turn, the gene F expression. Correspondingly, three new parameters are added to the system: thresholds T_{C2} and T_F, which determine the conditions of inhibition of gene F expression by the product of gene C and activation of gene C expression by the product of gene F, as well as τ_F - the time of delay of the effect of gene F product. In such a system of two genes with one positive and one negative feedback and without account for gene A, the appearance of a limit cycle is possible, which is similar to the known Lotka-Volterra mathematical model or "predator-prey" model (Coutinho et al., 2006). Changes in gene concentrations will now be described by a system of three differential equations:

$$\dot{C} = \begin{cases} F(t-\tau_F), & F(t-\tau_F) > T_F \oplus A(t-\tau_A) < T_A \\ F(t-\tau_F) - C(t-\tau_C), & \text{in all other cases} \end{cases}$$

$$\dot{A} = \begin{cases} -A(t-\tau_A), & C(t-\tau_C) > T_C \\ 1 - A(t-\tau_A), & C(t-\tau_C) < T_C. \end{cases}$$

$$\dot{F} = b \times \begin{cases} -F(t-\tau_F), & C(t-\tau_C) > T_{C2} \\ 1 - F(t-\tau_F), & C(t-\tau_C) < T_{C2}. \end{cases}$$

Coefficient *b* in this system sets the rate of changes in concentration of gene F product. Unlike the system of two genes, there are no stable points—a limit cycle based on F and C interaction and modified by the product of gene A is formed instead of them. As a result, stable fluctuations of concentrations of gene C and A products arise in antiphase (Fig. 4c), which remain stable even when the parameters characterizing gene activities are deviated by no less than 20% (Fig. 4d). Alternation of organs may arise as a result of such interaction, when the activities of class F genes are not finally inhibited after activation of class A genes, but the cycle returns to the initial state due to decreased activities of class F genes leading to decreased activities of class C genes.

The absence of final inhibition class F genes in the flowers of *Paeonia* cultivars during formation of additional zones of organs may be due to a significant increase of meristem (pool of stem cells) in the flowers whose development is controlled by class F genes. The presence of such changes is corroborated by an increased number of floral organs in *Paeonia lactiflora* cultivars, as compared to that in the wild type plants. When the pool of stem cells on lateral shoots decreases, as follows from significantly decreased number of floral organs formed on them, as compared to the main shoot, the number of alternations decreases (Figs. 3a and 3b) or flowers with normal alternation of organs are formed.

The second phenomenon observed in *Paeonia* flowers, decreased activities of class B genes after some additional zones of stamens leading to the formation of peripheral carpels, can also be related to gene interactions according to the "predator-prey" type. Thus, gene *SUPERMAN* (*SUP*) in *A. thaliana* prevents the transport of class B gene products to the zone of formation of carpels. This gene is activated in the presence of activities of class B and C genes as the boundary between stamens and carpels. In *Paeonia* flowers with inactive class B genes, the gene orthologous to *SUP* becomes inactive, which allows de novo activation of class B genes. The appearance of carpels only after some zones of stamens appears to be explained by that the activities of

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[**] The program with realization of this and following model is available on-line: www.mccme.ru/~mlog/articles/paeonia.

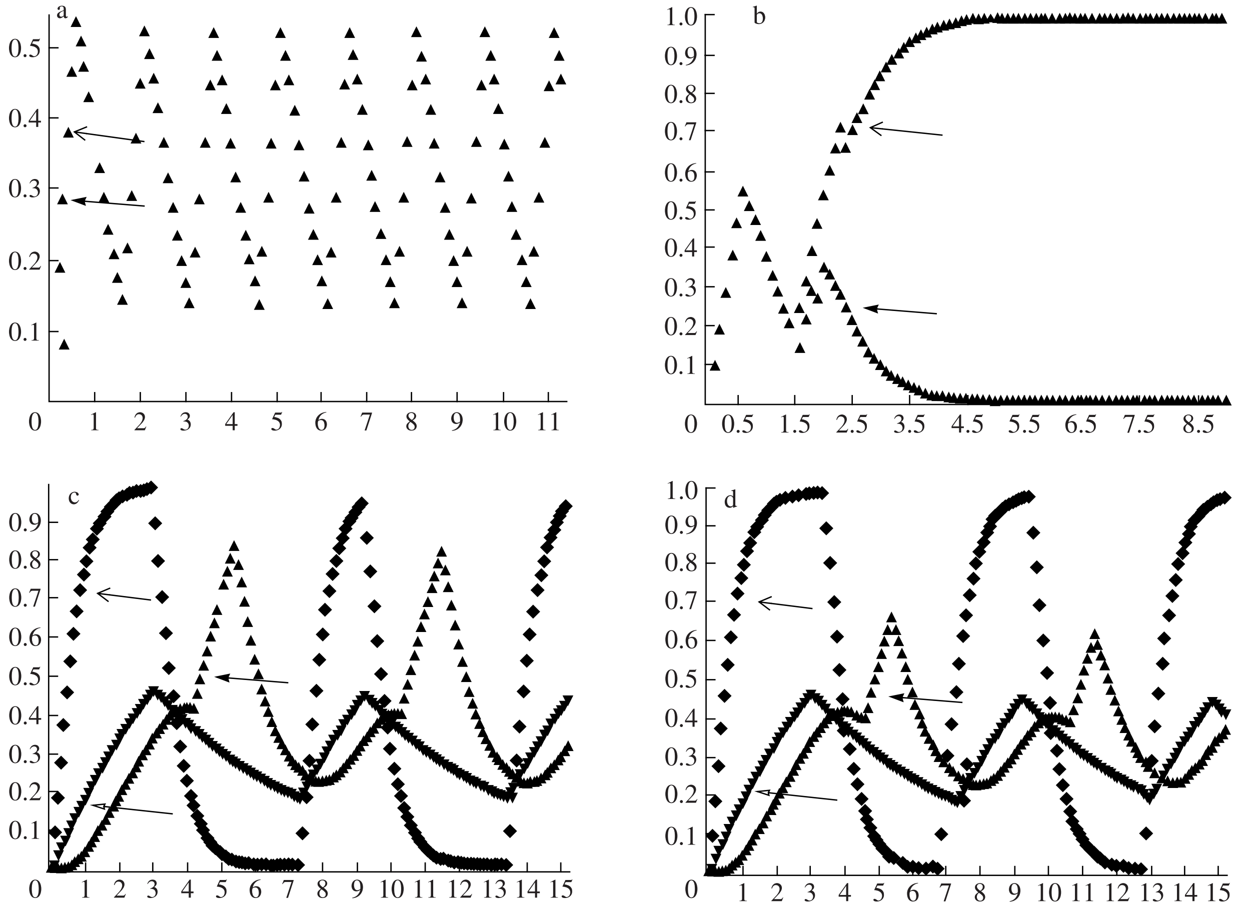

Fig. 4. The work of discrete model. Time-related dynamics of changes in concentrations of the products of genes is shown. Solutions for system of two genes: (a) upon equality of parameters (curves for A and C coincide), (b) at small deviations of parameters: solutions for system of three genes: (c) upon equality of parameters (d) at deviations of parameters. Classes of genes: (⟵) A, (◀—) C, (◁—) F. Abscissa: time, tacts of program work; ordinate: concentration of gene products, portions of maximum amount of molecules upon running model sets.

class C and/or B genes both always reach the level necessary for activation of *SUP* ortholog.

Thus, unusual alternation of floral organs in *Paeonia* cultivars is most likely related to an increased pool of stem cells, whose proliferation is not inhibited at once after activation of class C genes. As a result, several cycles of increased/decreased activities of class C genes take place and this cycle repeats several times as a function of the initial size of the pool of stem cells. Molecular studies are necessary to test the hypothesis about the appearance of such alternations.

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