Diversity and typification of ovules in flowering plants

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Summary: The ovules of flowering plants are characterized by a considerable diversity in the structure, especially in the form. To date, many authors emphasize 5 morphological types (orthotropous, anatropous, hemitropous, campylotropous and amphitropous), not including variants of the ovule position in the ovary. However, the characters by which the types of ovules can be identified are not always justified. This is especially true for campylotropous and amphitropous ovules.

Based on these features of the ovule morphogenesis, we suggest a new classification of morphological variants of the ovules, which includes 4 types and 4 subtypes: orthotropous, anatropous, hemitropous (hemi-anatropous and hemi-orthotropous subtypes), campylotropous (ortho-campylotropous and hemi-campylotropous subtypes).

The questions of the primitive morphological type of the ovule in flowering plants are discussed. We support the view that the original versions of ovules were orthotropous and anatropous ovules (at first with annulate integuments and then with raphe). However, the divergence of these two types probably evolved very early in evolution and their transformation was independent. All further transformations occurred through a series of intermediate stages (primarily variants of hemitropous and campylotropous ovules), and the correlative changes in the form and structure of the ovary, placentation, ovules and seeds, in the character of nutritive tissue and the size of the embryo have taken place.

Keywords: ovule types, classifications, gynoecium, placentation, diversity of ovules, trends of ovule transformations

The ovules are characterized by a considerable diversity in the shape, the degree of development and the structure. These are used to be analyzed properly from the viewpoint of different features such as the morphological type, the number of integuments and the genesis of the nucellus, integuments, chalaza, funiculus, obturator and a number of the embryonic structures. The study of the external and internal structure of the flowering plant ovules considering the possibility of using their structural characteristics for the purpose of systematics and phylogeny has been attracting the attention of embryologists since olden times (Davis 1966; Tobe & Raven 1985; Johri et al. 1992; Shamrov 2000b, 2008; Berg 2003; Matthews & Endress 2005; Kamelina 2009, 2011).

Differences in the ovule morphology were described for the first time by Mirbel (1829), who had distinguished the orthotropous, anatropous, campylotropous and amphitropous types. The latter one was considered as an intermediate variation between the anatropous and campylotropous ones because the contorted ovule possesses the same micropyle orientation as the anatropous one. For this very reason, the early researchers (Prantl 1881; Baillon 1882; Van Tieghem 1895; Wettstein 1903–1908) did not include the amphitropous type into their schemes graphically depicturing the ovules. Further, some other variations of ovules with transitional structure were proposed: the one residing between the anatropous and orthotropous ovules is hemi-anatropous (Agardh 1858) and one combining the features of anatropous, orthotropous and campylotropous ovules is hemi-campylotropous (Warming 1913).

However, the issues regarding the total number and the characteristics of ovule types are extremely disputable as well as are the criteria they are based on. The issues of morphological nature, origin and evolution of flowering plant ovules are disputed in the literature as well. These conditions became the very reason that urged us to appeal to criteria engaged for distinguishing the ovule morphological types, perform their verification and trace the directions of possible transformations of the flowering plant ovule.

The overall view of the ovule structure. First of all, the terms 'ovule' and 'seed' need to be clarified. The ovule is the organ of a seed plant, where the events of archesporium differentiation, megasporogenesis and embryo sac (megagametophyte) formation take place. After fertilization both the embryo (sporophyte of new generation) and the endosperm (storage nutritive tissue) arise. The complex transformations of the embryo, endosperm and surrounding tissues developing in conjunction with them result in the seed formation. That's why, likewise the principal difference in the characterization of the gynoecium (from initiation till pollination) and the fruit (from pollination till dissemination), all the processes occurring after fertilization don't characterize the ovule, but the forming seed that necessarily must be taken into account in pursuance of the research.

The main structural element of the ovule performing a reproductive function is the nucellus. Here, the reproductive cells arise passing the way from the archesporium to the megasporocyte and further to the embryo sac. Based on nucellus development and structure, the crassinucellate and tenuinucellate ovules can be distinguished. The archesporial cell in the crassinucellate ovule transforms into megasporocyte through a division separating the parietal cell; the nucellus is multilayered and persists for a long time after fertilization. In the tenuinucellate ovule the archesporial cell turns into the megasporocyte immediately; the nucellus is represented as the epidermal layer and gets disintegrated during megasporogenesis. The researchers often show simplistic approaches to treatment of the ovule types from viewpoint of nucellus genesis and they consider the presence (crassinucellate ovules) or absence (tenuinucellate ones) of the parietal tissue as the main criterion for distinguishing the ovule types. It was shown in numerous research works that the massiveness of the nucellus does not always coincide with the presence of the parietal tissue. It was suggested that an intermediate type of ovules does exist. Thus, Dahlgren (1927) distinguished the syndermal ovules, in which the parietal cells are absent, and the apodermal ones, where they are present. Davis (1966) considers the pseudocrassinucellate ovule as an independent type, in which the parietal tissue is absent, but a multi-layered nucellar cap is formed. A special epicrassinucellate ovule with a multilayered epidermis of nucellus and a multilayered parietal tissue is also distinguished (Teryokhin 1996). There are other variants of ovules as well: incompletely tenuinucellate, reduced tenuinucellate, weakly crassinucellate (Endress 2011). In our opinion (Shamrov 1997a, 1998, 2002a, 2008, 2015a), the entire diversity of ovules with the intermediate structure should be separated into an independent medionucellate type with a certain set of variations, depending on the combination of the elements of 'crassinucellarity' and 'tenuinucellarity'.

The nucellus of seed plants is surrounded by the integument or cover. The ovules of flowering plants are divided according to the number of integuments into bitegmic (with two integuments), unitegmic (with single integument) and ategmal (without integuments). In the integument the formation of various specialized structures occurs. One of them is the micropyle, a channel

formed at the tip of the ovule by one or both integuments for the passage of the pollen tube into the embryo sac. In the bitegmic ovules it can be formed by a single integument or by both (in the latter case the outer part called exostome and the inner one, i.e. endostome, can be distinguished). Depending on the structure of the integuments, the micropyle can be long or short, straight, zigzagged or U-shaped. In the tenuinucellate ovules the cells of the internal epidermis of the integument (of the inner integument in the bitegmic ovules) adjacent to the embryo sac transform as a rule into the integumentary tapetum or endothelium. In bitegmic orthotropous, some anatropous, hemitropous and campylotropous ovules the integuments develop as ring-shaped structures around the nucellus and are cup-shaped. In the majority of anatropous, some hemitropous and campylotropous ovules the outer integument or the single one often is asymmetrical or semi-annulate. In this case, the congenital fusion of the part of integument and funiculus occurs at the dorsal part of the ovule in course of development resulting in raphe formation (Shamrov 2000a, 2003, 2008).

The basal area of the ovule, in which the bases of the nucellus, integuments and funiculus are united, is referred to as chalaza. Chalaza belongs to the least studied elements, which may be due to the lack of a sharp border between itself and other parts of the ovule. Four types of chalaza can be distinguished: 1) pachychalaza or the massive one (comprises most of the ovule or seed as compared to the nucellus and integuments); 2) perichalaza or the peripheral one (the unidirectional growth of the chalaza in the region of the raphe and antiraphe occurs, resulting in replacement of the radial symmetry of the ovule and seed with the bilateral one); 3) mesochalaza or the intermediate one (the greater half of the formed ovule and the developing seed is occupied by the nucellus and the integument or just by the integument); 4) leptochalaza or the thin one (represented by a small group of cells that become destroyed during seed development) (Shamrov 1998, 2004, 2008).

The attachment of the ovule to the placenta is realized by means of a funiculus or a seedstalk. Depending on the characteristics of the funiculus structure and development, the ovules can be divided into 3 types: the funicular (ovule with funiculus), the afunicular (funiculus as a structure is absent) and sessile ones (funiculus is not morphologically apparent and the ovule is attached to the placenta by means of the basal part of the raphe). During ripening, a separation layer is formed between the seed itself and the funiculus (funicular seeds) or between the raphe and the placenta (sessile seeds) and subsequently a remnant (scar) called hilum appears (Shamrov 1998, 2004, 2008).

Thus, the ovule of flowering plants is a complex system and consists of a nucellus, an integument, a chalaza and a funiculus, forming the morphological axis of the ovule. This axis can be straight or curved and the elements of both versions of the axes can be represented in a single ovule type.

Possible ovule types. The ideas of researchers on the possible morphological types of ovule are based on various criteria (Shamrov 2008, 2015a). Initially, orthotropous, anatropous, campylotropous and amphitropous types were identified on the basis of the general criterion of the external structure: the shape of morphological axis and the position of the micropyle and the nucellus toward the placenta and funiculus (Mirbel 1829). With the accumulation of knowledge on the diversity of ovules, the classification was improved with some types being excluded, but at the same time new variants were introduced. Thus, the amphitropous ovule was excluded first, but then this type was restored (Goebel 1933) and the classification was supplemented with a

hemitropous ovule. By now, 5 types of ovules are distinguished (Goebel 1933; Maheshwari 1950; Savchenko 1973 and others).

According to Davis (1966), 266 out of 315 families, for which the morphological type of ovule is established, are dicotyledons. This characteristic is constant in 248 families. 204 families of them are characterized by anatropous ovules, 20 families by orthotropous ones, 13 by hemitropous ones and 11 by campylotropous and amphitropous ones. Different types were found in 67 families (differences at the level of the genus and even of the species).

Bocquet (1959) has presented orthotropous and anatropous ovules and their derivatives as two series based on the degree of the funiculus flexure and the related difference in length of the ovule conductive bundle. Taking into account the degree of curvature of the nucellus, in each series, along with the standard (ortho- and anatropous ovules), the ortho- and ana-campylotropous and ortho- and ana-amphitropous ovules are distinguished. Savchenko (1973) has supplemented the classification with the three types based on a greater degree of flexure of the funiculus: hypertropous, ortho- and ana-circinotropous. It's worth to note, that the term 'circinotropous ovule' was proposed by Archibald (1939).

When characterizing the morphological type of ovule, some additional criteria were used, for example, its position in the ovary. Depending on the direction of the funiculus inflexion, the ascending, rising upward and descending seeds are identified (Agardh 1858; Sterling 1963). The epitropous and apotropous ovules are also distinguished and various criteria are used. The orientation of the micropyle relative to the funiculus and placenta: in apotropous the ovule the micropyle is turned towards the funiculus and placenta, whereas in the epitropous one the micropyle is turned in the opposite direction (Agardh 1858). The character of the funiculus flexing relative to the top or the base of the ovary: in epitropous ovule the funiculus is curved from bottom to top, whereas in apotropous one it is curved from the top downward (Warming 1913; Takhtajan 1948; Maheshwari 1950; Bocquet 1959). Other criteria include: 1) the orientation of the micropyle with respect to the upper end of the ovary: in epitropous ovule the micropyle is directed upward, whereas in the apotropous one it is directed downward (Sterling, 1963); 2) the coordination with the carpel flexure: in epitropous or antitropous types the ovule and carpel are flexing in opposite directions, whereas in apotropous or syntropous ones the ovule is flexing in the same direction as the carpel (Endress 2011). The pleurotropous ovules are also distinguished, in which the micropyle remains unturned and the funiculus does not curve (Andersson 1931; Takhtajan 1948; Maheshwari 1950).

The original, though still unclaimed, classification of the ovule types has been proposed by Korchagina (1969). She considers both the morphological characteristics of the ovule (5 criteria) and its position in the ovary cavity (2 criteria). The morphological criteria for distinguishing ovules include: 1) the mutual position of the ovule and funiculus (supra-funicular, non-reverted and sub-funicular ovules); 2) the angle of attachment of the ovule to the placenta (upright and inclined ovules); 3) the shape of the longitudinal axis (non-curved and curved ovules); 4) presence of the funiculus (non-funicular or sessile ovule and funicular one, including straight-funicular and flex-funicular ovules); 5) the occurrence of a definite growth of the integumentary, nucellar and funicular tissues (ovules with obturator, arillus, caruncle, etc.). Respectively, according to the position in the ovary the criteria are: 1) the orientation of the micropyle in relation to the base of the stylodium canal (ovules directed straight, sideward and backward); 2) position of

the ovule relative to the place of its attachment on the placenta (descending, horizontal and ascending ovules).

Characteristics, by which one can identify the ovule types, are not always well-argued. Without the study of a complete morphogenesis, particularly on a series of sections in different planes, it is often impossible to state whether the ovule in a particular taxon is anatropous, hemitropous or campylotropous. This is especially true for campylotropous and amphitropous ovules. Of all the options mentioned above, the most clearly distinguished are the anatropous and orthotropous ovules which, undoubtedly, are verified as independent types. The critical stage in the development of ovule of any type is the megasporogenesis, during which the significant changes in their morphogenesis take place. The anatropous ovules at this stage already exhibit the final shape: a straight morphological axis, in which the axis of the funiculus and the micropyle-chalaza axis (ovules with ring-shaped integuments) or the axis of the raphe and the micropyle-chalaza axis (ovules with raphe) lie at an angle of 180° to the placenta. Further, neither the ovule nor even the seed change their shape and remain completely reversed, so that the micropyle turned to the placenta and the hilum get located side by side. Let us illustrate the formation of anatropy by the example of some plants examined by us. In the representatives of family Nymphaeaceae, the ovules with the raphe formed in the basal and middle parts take an anatropous shape either at the beginning of meiosis (*Nymphaea alba*, *N. gigantea*, *Victoria amazonica* (Winter & Shamrov 1991; Shamrov & Winter 1991b)) or already at the stage of the sporogenous cell (*Nuphar lutea* (Shamrov & Winter 1991a)). Then, the latter species forms the so-called hypertropous ovule. Ovules with a complete raphe (the fusion of the funiculus and integument occurs along the entire length) are anatropous during the prophase of the first meiotic division in *Gagea stipitata* (Liliaceae) (Shamrov 1999b) (Fig. 1A–G) or at the stage of the tetrad of megaspores in *Gentiana lutea* and *G. cruciata* (Gentianaceae) (Shamrov 1988, 1990) (Fig. 2A–E).

The shape of orthotropous as well as of anatropous ovules develops already during megasporogenesis: the direct morphological axis (funiculus, chalaza and micropyle) is located at an angle of 90° to the placenta. In ovules, regardless of the number of integuments, the placenta retains a ring-shaped structure at all stages of morphogenesis, even in anatropous ones, in which the raphe is not formed. The orthotropous ovule and even the seed remain straight until the very end. The micropyle occurs located at the opposite pole in relation to the funiculus and the placenta (e.g. in *Hydrostemma longifolium* from family Barclayaceae) (Winter 1993). Studying the relationship between the orthotropous ovule and the type of gynoecium and its prevalence among dicotyledonous and monocotyledonous plants (Davis 1966; Takhtajan 1966, 1987; Kamelina 2009, 2011), one can conclude that the formation of a typical orthotropous ovule is more characteristic of plants with a syncarpous, less often pseudomonomerous gynoecium. Most of them possess bitegmic ovules: Araceae, Barclayaceae, Centrolepidaceae, Commelinaceae (*Commelina*, *Cyanotis*, etc.), Eriocaulaceae, Amaryllidaceae (*Crinum*), Hanguanaceae, Haemodoraceae (*Dilaris*, *Xiphidium*, etc.), Lemnaceae (*Lemna*, *Wolffia*), Mayacaceae, Platanaceae, Restionaceae, Ruscaceae, Saururaceae, Xyridaceae. Only some plants have been found to possess unitegmic ovules: Juglandaceae, Myricaceae.

Besides the anatropous and orthotropous ovules, the hemitropous ones are also distinguished, which are characterized by the features of the former or latter type. They can have both the ring-shaped integuments and raphe. In the second case, the funiculus approaches from the side, fuses
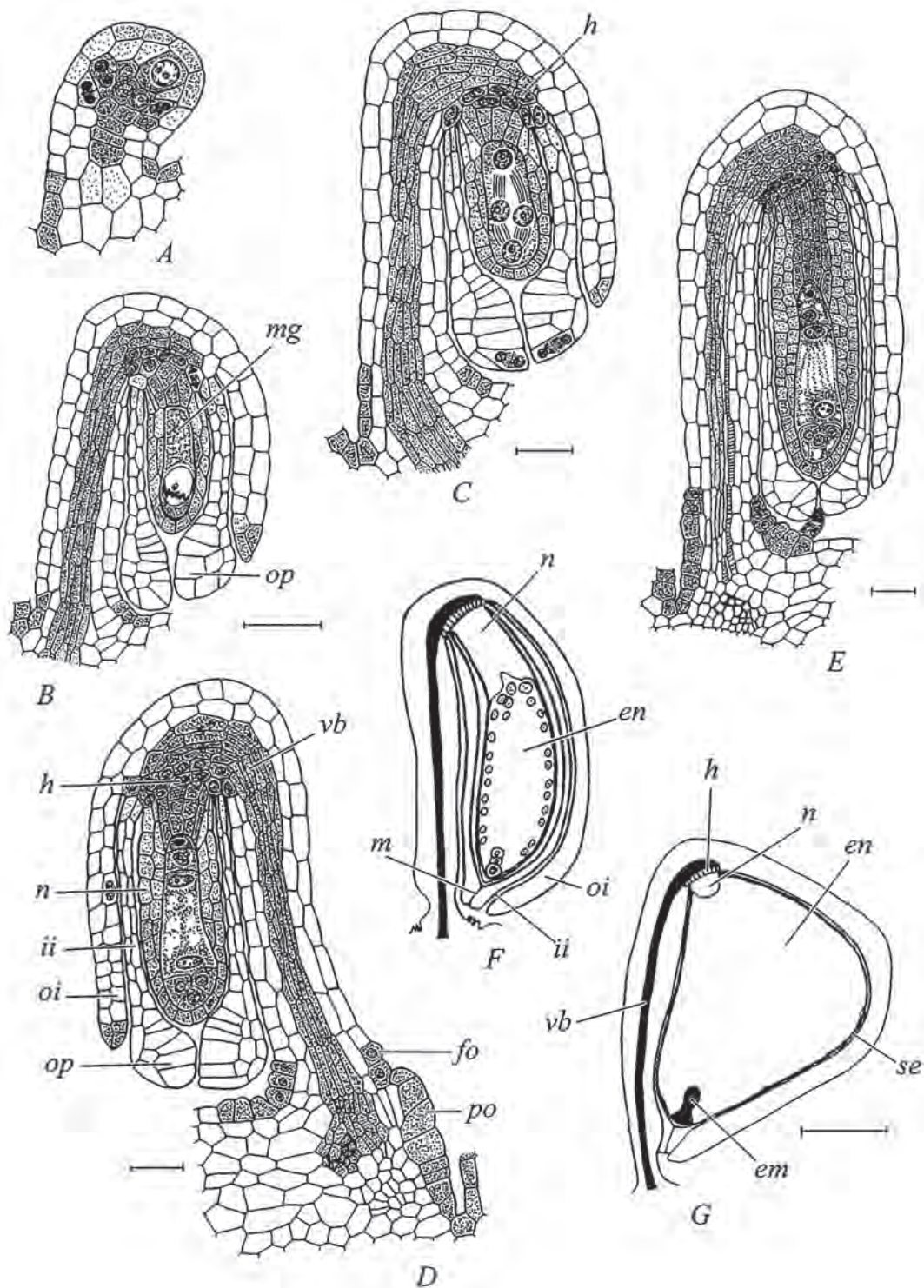
Figure 1. Transformation of anatropous bitegmic ovule into hemi-campylotropous seed in *Gagea stipitata* (Liliaceae). A – ovule before meiosis; B, C – stages of meiosis; D – formed embryo sac; E – ovule before fertilization; F, G – stages of hemi-campylotropous seed development. em – embryo, en – endosperm, fo – funicular obturator, h – hypostase, ii – inner integument, m – micropyle, mg – megasporocyte, n – nucellus, oi – outer integument, op – operculum, po – placentary obturator, se – seed coat, vb – vascular bundle. Scale bars = 20 μm (A–E), 100 μm (F, G).

Figure 2. Developmental stages of anatropous unitegmic ovule and seed in *Gentiana cruciata* (Gentianaceae). A–C – during megasporogenesis; D – before fertilization; E – mature seed. em – embryo, en – endosperm, es – embryo sac, h – hypostase, iin – integument initials, mg – megasporocyte, n – nucellus, sc –seed coat, vb – vascular bundle. Scale bars = 20 μm (A–C), 200 μm (D, E).

with the integument in the basal and middle areas, therefore the remaining ring-shaped apical part of the integument can be equal to the raphal one. In the ovules, the integuments, nucellus and chalaza lie on a straight axis parallel to the placenta, but at an angle of 45° to the funiculus, i.e. the total convolution of the ovule is 135° relatively to the placenta. Based on the position of the structures of the ovule chalazal region and on the shape of the axis of the funiculus or raphe at their transition to the common straight morphological axis, we proposed to distinguish two subtypes in the hemitropous type. One of them is a hemi-anatropous subtype, in which the axis in the ovule, when passing in a funiculus or a raphe, changes the angle of inclination relatively to the placenta as a result of the displacement of the chalaza, the hypostase and the conducting bundle into the hemi-position. This is a typical version of hemitropous ovule (Davis 1966; Bouman 1984). It is often confused with an anatropous ovule, if the latter is not studied in the dorsiventral but in the bilateral plane. The second subtype is the hemi-orthotropous one. A variation of such ovule was described in *Polygonum affine* (Shamrov & Anisimova 2015). In this case, the axis in the ovule curves through the funiculus or raphe toward the chalazal region retaining the ortho-position. Due to the predominant growth of the micropyle and middle parts on the antiraphal side of the ovule as compared to the raphal side in the great part of the integuments and nucellus, the morphological axis running parallel to the placenta remains straight, therefore the micropyle does not approach the placenta. Such ovules are presumed to belong to representatives of families Ceratophyllaceae, Chloranthaceae, Circaeasteraceae, Cymodoceae, Hydrocharitaceae, Lardizabalaceae, Poaceae, Polygonaceae, Potamogetonaceae, Ruppiaceae, Zannichelliaceae, Zosteraceae, for which previously various types of ovules have been reported, mainly the orthotropous ones. The ovules and seeds therein occupy a lateral position in the ovary, they are pendant and the micropyle that does not approach the placenta is often turned to the base of the ovary. Gynoecium in these plants is predominantly polymerous-apocarpous or pseudomonomerous, rarely monomerous-apocarpous, therefore the indehiscent single-seeded fruitlets or, in some cases, single-seeded fruits often form (Takhtajan 1966, 1987).

We have already noted that the type of ovule and its position in the ovary can change in the course of development, therefore, likewise most authors, we believe it is right to determine the type of ovule at the stage of the mature embryo sac before pollination. However, a number of researchers, including Goebel (1933), who has established the hemitropous and amphitropous variants in the status of types, characterize the ovules not before pollination, but during the early stages of embryogenesis, i.e. in the period of seed formation. And though many authors included the amphitropous ovule in the classification of ovule types with somewhat unclear criteria, a discussion about how campylotropous and amphitropous ovules can be distinguished is continuing until now.

A viewpoint exists that amphitropous ovules differ from campylotropous ones by the presence of a 'basal body' (Bocquet 1959; Bocquet & Bersier 1960; Savchenko 1973). It should be noted that the 'basal body' can be of different morphological nature, and it is described not only in amphitropous ovules, but in campylotropous ones as well. Other researchers do not use the concept of 'amphitropous ovule' in their studies and qualify any kind of ovules with curved embryo sac as campylotropous ones (Corner 1976; Bouman & Boesewinkel 1991; Bouman 1992). Along with the true campylotropous ovule, the obcampylotropous one can also be distinguished, in which the antiraphe is the more developed part compared to the raphe (Corner 1976).

The original data on the morphogenesis of ovule and seed in *Capsella bursa-pastoris* and *Arabidopsis thaliana* (Brassicaceae) suggest that the 'amphitropous ovule' is just a developmental stage of the campylotropous seed after fertilization (Shamrov 2002b, 2007). In this regard, we came to the conclusion that the amphitropous ovule type should be recognized as non-existent (Shamrov 2008). This viewpoint is confirmed by the fact that in the literature the forming seeds at the stages of early embryogenesis are typically referred to as amphitropous ones, rather than the ovules.

On the basis of the analysis of the results of long-term studies and available literature data, it can be concluded that the ovule types in some studied taxa require a reinvestigation, especially for those plants in which the ovules are located in the longitudinal plane of the ovary. That is why the ratio of different types among the flowering plants can change.

We would like to draw attention to the fact that campylotropous ovules are characterized by the presence of a straight axis passing through the funiculus or raphe, but the biggest part of the morphological axis is curved. Their development begins before pollination, but finishes at the time of dissemination. First of all, we should note that the signs of campylotropy begin to appear, as a rule, during the period of megasporogenesis. As we have already noted, at this time the orthotropous and anatropous ovules already take their final shape, and in hemitropous ones a turning begins relatively to the placenta. In contrast to these types, in campylotropous ovules the changes in the manner of growth occur at the antiraphal side, resulting in asymmetry. As a result, the morphological axis of the ovule gets curved. After this, and even before fertilization, the embryo sac, nucellus and chalaza can become curved. Thus, with regard to the category of ovules in question, the information about campylotropous variants before pollination is incomplete, and it would be correct to characterize the phenomenon of campylotropy through the system of ovule-seed. Campylotropous ovules and seeds show a great variety, which is manifested in the peculiarities of morphogenesis, as well as in the degree of curving of the morphological axis. Considering these differences, two subtypes can be distinguished in the campylotropous type: ortho-campylotropous and hemi-campylotropous. In each subtype, there are various variants of ovules and seeds, based on the degree of the axis flexure (the axis is wide arc-shaped or narrow horseshoe-shaped), the correspondence between the shape of the embryo sac and that of the emerging endosperm and embryo (the shape may be reproduced completely or partially), the pattern of accumulation of reserve nutrients in the seed (in the endosperm and perisperm or in the embryo).

As a model representative of the ortho-campylotropous subtype, the morphogenesis of the ovule of *Capsella bursa-pastoris* (Brassicaceae) can be considered (Shamrov 2002b). The bitegmic ovule in this plant develops first as an orthotropous one, in which both integuments are ring-shaped. But, unlike an orthotropous ovule, even during the primordium formation (at the stage of the archesporial cell), its turning relatively to the placenta begins (Fig. 3A–H). Further, the turn of the ovule increases, but the ring-shaped structure of the integuments persists, whereas the raphe is not formed. At the stage of the tetrad of megaspores, when the ovules are still located in the transversal plane of the ovary, the activation of cell divisions occurs at the base of the outer integument and at the outer region of the chalaza on the dorsal side. In the outer integument and the chalaza, the number of layers increases from two to three. At the same time, the number of divisions increases in the apical and middle parts of both integuments, which turn out to be more extensive as compared to the integuments on the ventral side. Chalaza and integuments, especially the outer one, become asymmetric, which leads to the curving of the morphological
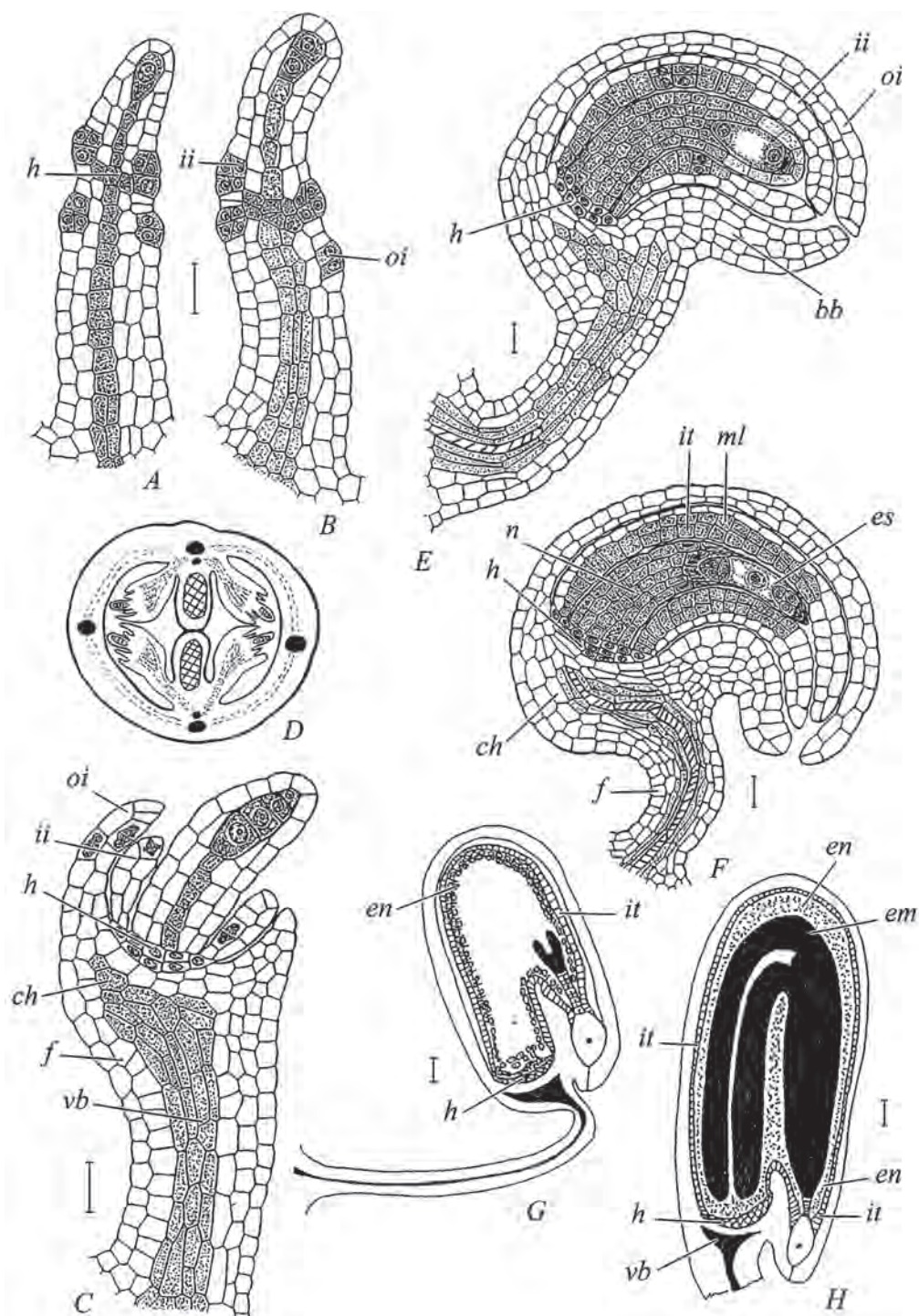
Figure 3. Developmental stages of ortho-campylotropous bitegmic ovule and seed in *Capsella bursa-pastoris* (Brassicaceae). A–D – ovules (on transverse sections of ovary) during stages of megasporocyte (A), dyad of cells (B) and tetrad of megaspores (C, D); E, F – ovules (on longitudinal sections of ovary) during the stages of binuclear (E) and mature (F) embryo sacs; G – stage of heart-shaped embryo; H – mature seed. bb – basal body, ch – chalaza, em – embryo, en – endosperm, es – embryo sac, f – funiculus, h – hypostase, ii – inner integument, it – integumentary tapetum, ml – middle layer, n – nucellus, oi – outer integument, vb – vascular bundle. Scale bars = 10 µm (A–F), 50 µm (D, G, H).

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axis (Fig. 3A, D). During the formation of the embryo sac, the number of layers at the base of the outer integument and the outer region of the chalaza increases to four, the micropyle gets formed (the exostome formed by the cells of the outer integument is more elongated as compared to the endostome), and in the middle part of the outer integument on the ventral side a basal body forms with a larger number of layers (5–6 in contrast to 4 at the dorsal side) (Fig. 3E, F). Massiveness of the outer region of the chalaza increases after fertilization (up to 6–7 layers), and the arc-shaped flexure of the axis becomes horseshoe-shaped (Fig. 3G, H).

The 1st variant is characterized by an arc-shaped morphological axis in the ovule and a horseshoe-shaped one in the seed; the embryo in the mature seed often occupies almost the whole of its cavity and reproduces the horseshoe shape of the flexure; reserve nutrients accumulate mainly in the embryo (Alismataceae, Cneoraceae, Brassicaceae, Nyctaginaceae, Portulacaceae, Resedaceae, some Alliaceae) (Figs 3A–H; 4A–D). In the 2nd variant, the embryo is almost straight, has a small size and all the reserve substances accumulate in the endosperm (Dilleniaceae). In both variants, the ovules and seeds are characterized by a long funiculus. The structures of the chalazal region appear first in the hemi-position, but later, in the course of increasing the degree of flexure, they shift again to the ortho-position in the developing seed. The 3rd variant is characterized by persistence of the arc-shaped flexure of the ovule and seed; embryos have different sizes and degrees of flexure; the structures of the chalazal region remain in the initial ortho-position (probably in some Euphorbiaceae). Such a structure of ortho-campylotropous ovules and seeds could be caused by changes in the structure of the funiculus. In some plants, the funiculus disappears and its functions begin to be performed by placental tissues, which, forming peculiar placental emergences or directly connecting with chalaza, form placento-chalaza (afunicular ovules).

The establishing of hemi-campylotropy can be illustrated by the picture of ovule morphogenesis in *Swertia iberica* (Gentianaceae) (Shamrov 1991). In most representatives of this family, the ovules are anatropous, unitegmic and are characterized by the formation of a complete raphe. The early developmental stages prior to the beginning of meiosis are similar (Fig. 5 A–F; compare with Fig. 2A, B). But when the megasporogenesis is getting completed, the asymmetric chalaza is formed in *S. iberica* ovules on the raphal side due to intensive cell division in the subepidermis (Fig. 5G, H; compare with Fig. 2C), which coordinately causes lengthening of the integument in the apical part, flexure of the micropyle and the embryo sac (mainly at the poles). As a result, the ovules become hemi-campylotropous and after fertilization become located in the longitudinal plane of the ovary, displacing in rows (Fig. 5I–K). In the seed of this species, as well as in other members of the family Gentianaceae, the embryo is straight, small in size, therefore all reserve nutrients accumulate in the abundant endosperm.

In the hemi-campylotropous subtype, these three variants can also be distinguished. The 4th variant is the arc-shaped flexure of the morphological axis, which is inherent in both ovule and seed; in the mature seed, its cavity is almost completely occupied by the embryo, which reproduces the arc shape of the seed flexure and the reserve nutrients accumulate mainly in it (Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Melastomataceae, Molluginaceae, Sapindaceae, Tetragoniaceae, Tovariaceae, some Zygophyllaceae, etc.). In the 5th variant, the correspondence between the flexure of the structures of the ovule and the embryo does not always appear; the embryo can remain small, straight or slightly curved, and following the
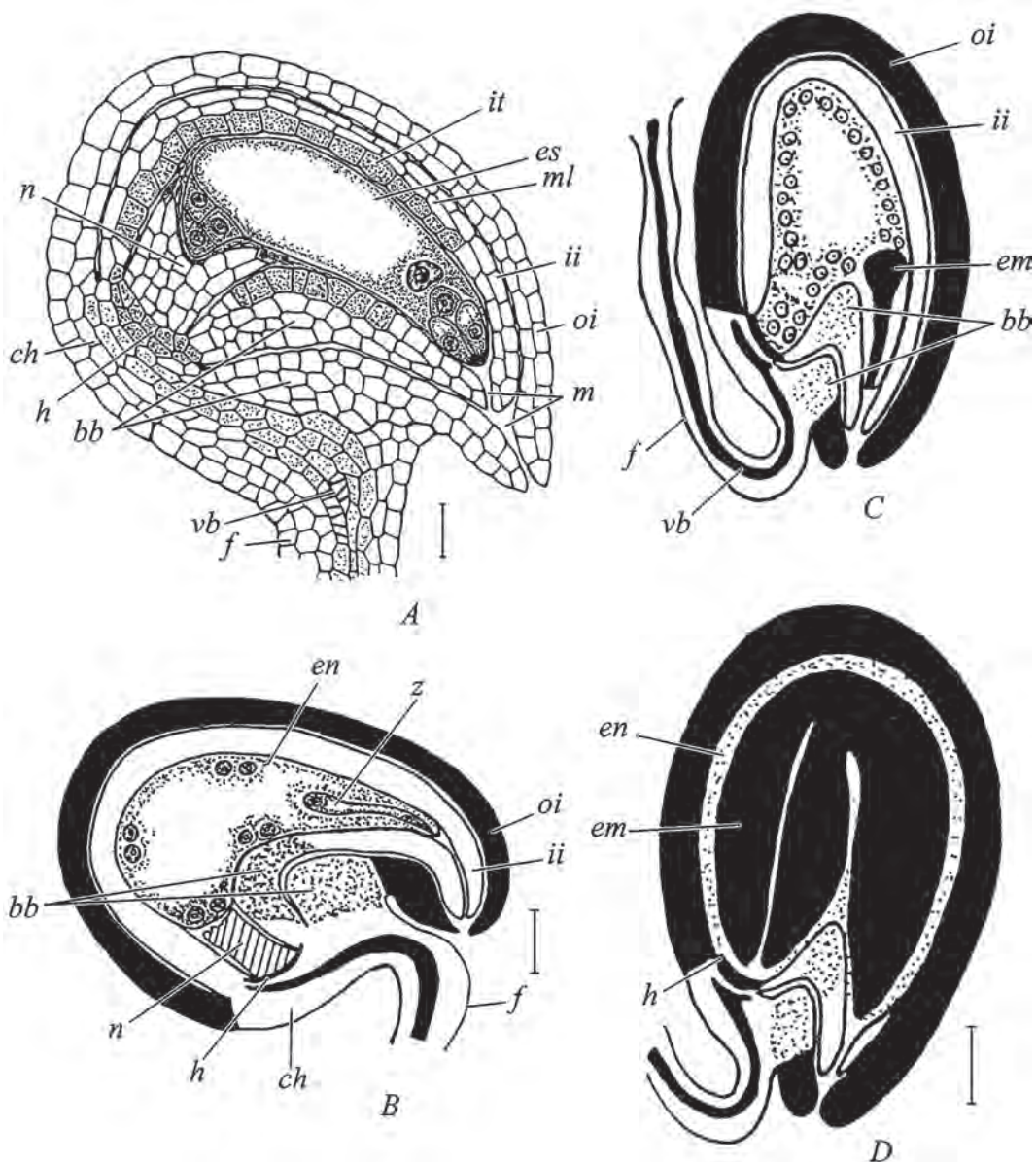
Figure 4. Structure of ortho-campylotropous bitegmic ovule and seed in *Arabidopsis thaliana* (Brassicaceae). A – ovule before fertilization; B – forming ovule at zygote stage, C – globular embryo stage; D – mature seed. bb – basal body, ch – chalaza, em – embryo, en – endosperm, es – embryo sac, f – funiculus, h – hypostase, ii – inner integument, it – integumentary tapetum, m – micropyle, ml – middle layer, n – nucellus, oi – outer integument, vb – vascular bundle, z – zygote. Scale bars = 10 µm (A), 20 µm (B), 50 µm (C, D).

embryo sac, the shape of the ovule and seed flexure is often reproduced by the endosperm in which the reserve substances accumulate (Buddlejaceae, Canellaceae, Ericaceae, Fumariaceae, Hemerocallidaceae, Hypecoaceae, Papaveraceae, Schisandraceae, perhaps, some Araliaceae, Menispermaceae, Phyllonomaceae, Umbelliferae, Winteraceae). In the 6th variant, the ovule is almost anatropous during pollination and it is characterized by the formation of a complete raphe. But already at this stage, the signs of campylotropy, namely the asymmetry in the structure of the nucellus, integuments, hypostase and chalaza in the base of the ovule and the flexure of
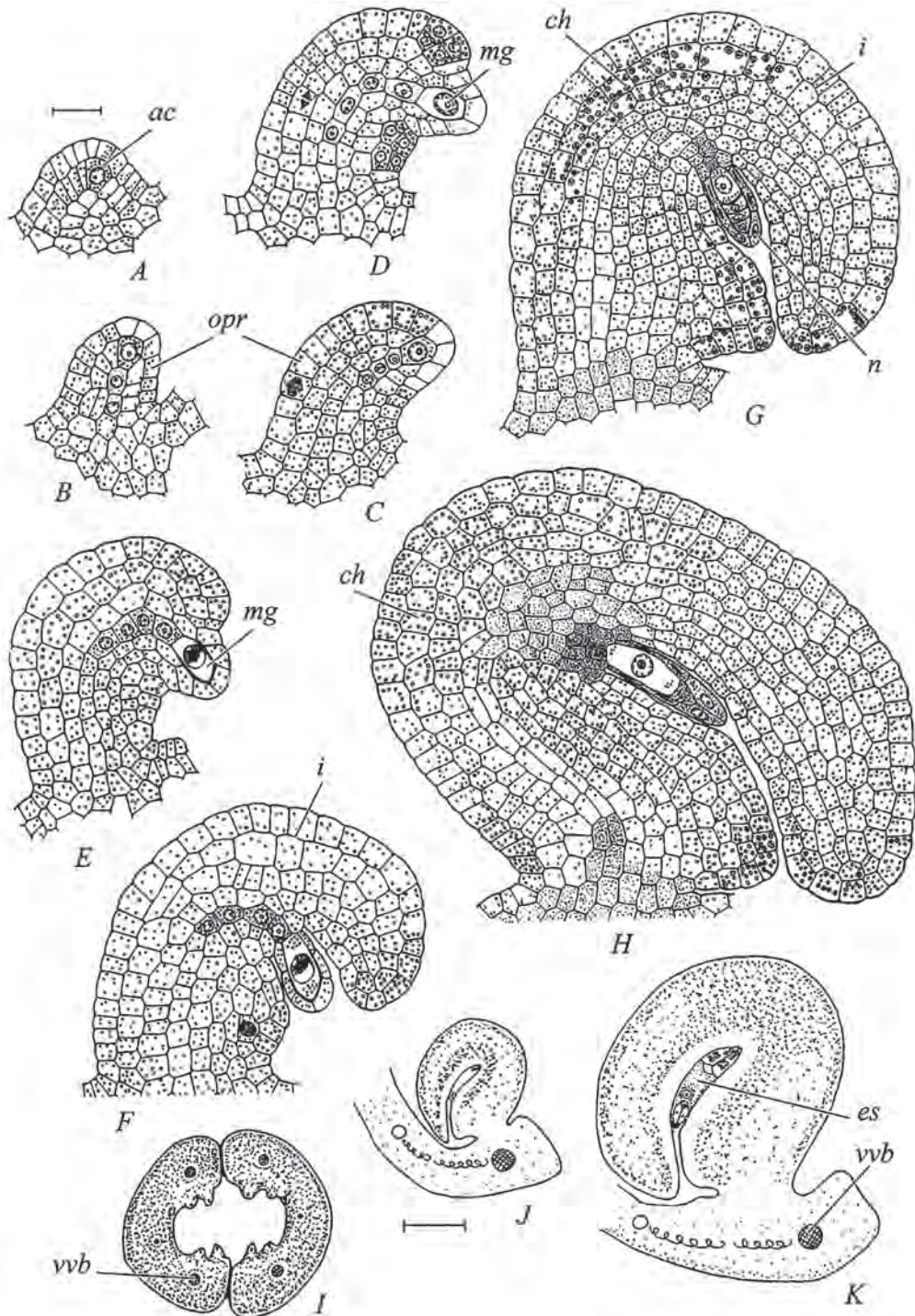
Figure 5. Developmental stages of hemi-campylotropous unitegmic ovule in *Swertia iberica* (Gentianaceae). A–C, I – archesporial cell; D–G – megasporocyte before (D) and during (E, F) meiosis; G – tetrad of megaspores; H – functional megaspore of tetrad; J – 4-nuclear embryo sac; K – ovule before fertilization. I, J, K – starch accumulation is symbolically shown by dots (compact – high level, thinned out – low level); A–H – deposit of carbohydrates in the cells is revealed (starch as grains and dextrines as dot-like structures). ac – archesporial cell, ch – chalaza, es – embryo sac, i – integument, mg – megasporocyte, n – nucellus, opr – ovular primordium, vvb – ventral vascular bundle. Scale bars = 20 μm (A–H), 200 μm (I, J, K).

the morphological axis, can be revealed in it (Fig. 1A–D). After fertilization, the arc-shaped flexure increases, especially in the micropylar and chalazal areas. The more intensive growth of the integuments on the anti-raphal side begins. The reserve substances accumulate in the endosperm, where a special endospermal cavity forms, which indicates the phenomenon of the underdevelopment of the embryo (Fig. 1E–G). The latter one is small and slightly curved. During cancellation of the dormancy and before germination, the length of the embryo increases several times (*Tulipa tarda* (Kravkina & Koteeva 2005); *Fritillaria pallidiflora* (Pozdova et al. 2008)), therefore the embryo reproduces the shape of the arcuate flexure of the seed (some Liliaceae, perhaps Alliaceae, Araliaceae, Umbelliferae).

New classification of the ovule types. In our opinion, the entire variety of the morphological types of ovules based on the external morphology (without taking into account the position in the ovary, the orientation of the micropyle relative to the poles of the latter, etc.) can be reduced to four ovule types. However, unlike the previously proposed classification (Shamrov 2007, 2008), the new one includes 2 more subtypes in the hemitropous type, taking into account the results of the more recently published studies and reflecting the main variants within each type. Previously (Shamrov 1999a), when discussing the topography of the nucellus, we proposed to distinguish 3 zones with respect to the micropyle and chalaza: the micropylar (above the embryo sac), the medium (encompasses the embryo sac sideward) and the chalazal (below the level of the embryo sac). Based on these ideas, we considered it possible to distinguish three main parts in the ovule: the micropylar (the integuments and the nucellus at the level of the upper boundary of the embryo sac), the chalazal (the chalaza with a conducting bundle starting from the funiculus, as well as the hypostase, integuments and nucellus down to the lower boundary of the embryo sac) and medium part (integuments and nucellus within the boundaries of the embryo sac). During the turning of the ovule, the change in the angle of the morphological axis toward the placenta and funiculus, or curving the axis, occurs in the region of the hypostase, where the bases of the nucellus, integuments and chalaza are located. We suppose that during the transformation of the ovules, a gradual shift of such structures of the chalazal part as hypostase, chalaza and conducting bundle, from ortho- (orthotropous, hemi-orthotropous, ortho-campylotropous) to hemi- (hemi-anatropous, hemi-campylotropous) and further to ana- (anatropous) position occurred. It should be emphasized that, in our opinion, the ana-campylotropous as well as the ana-amphitropous ovules do not exist. In this case, during the displacement of the structures from the hemi- to the ana-position, all parts of the ana-amphitropous and ana-campylotropous ovules will gradually become located on the same axis, whereas the micropyle will be located near the placenta, i.e. the ovule will become anatropous. In addition, as we have already noted, the 'amphitropous ovule' is only a developmental stage of the campylotropous seed after fertilization.

The following criteria are used as a basis for distinguishing types and subtypes: the shape of the morphological (longitudinal) axis of the ovule and the complex of its defining elements; the mutual relative position of various elements during the development process; correlation of different parts of the ovule. Within the type or subtype, the ovules can have a different structure. This especially applies to integument and funiculus.

The 1st type is **orthotropous**. The ovule is characterized by the axis in the form of a straight line passing through all its parts (micropylar, medium and chalazal); the poles of the axis do not change their position (the micropyle lies opposite to the chalaza and is located at an angle of 90°

toward the placenta), chalaza, hypostase and a conducting bundle in the ortho-position (bitegmic and unitegmic, funicular and afunicular) (Fig. 6A, Aa).

The 2nd type is **anatropous**. The ovule is characterized by the straight morphological axis on which the funiculus or the raphe is located. The axis changes the angle of inclination toward the placenta
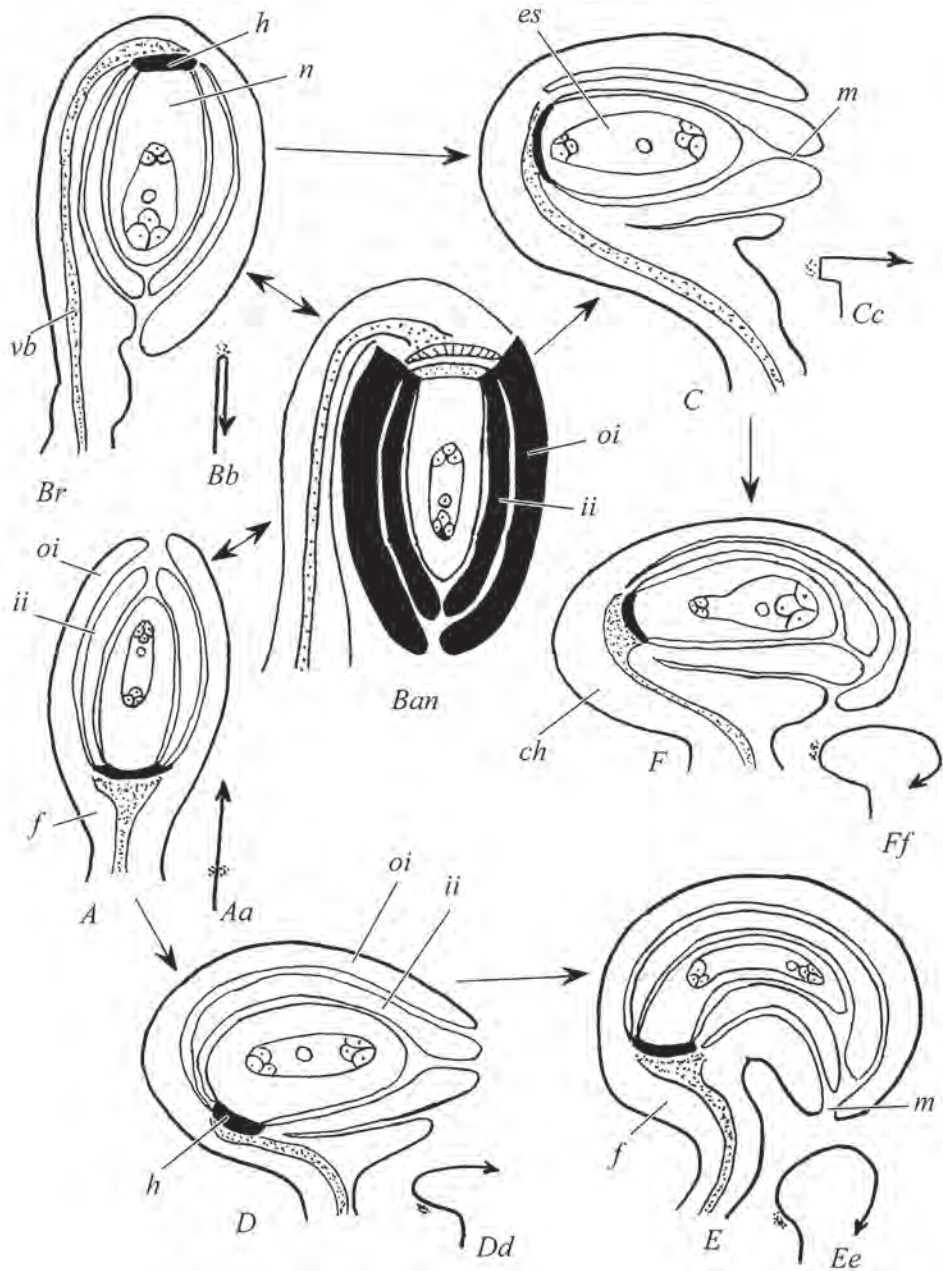

Figure 6. Types and subtypes of the ovules and possible trends of their transformations in flowering plants. A–F – types and subtypes: A – orthotropous; Ban (annulate ovule), Br (ovule with raphe) – anatropous; C, D – hemitropous (C – hemi-anatropous, D – hemi-orthotropous); E, F – campylotropous (E – ortho-campylotropous, F – hemi-campylotropous); Aa–Ff – form of morphological axis in ovule (hypostase location, where the angle change of axis relatively to placenta or its curve occurs, is shown by dots). ch – chalaza, es – embryo sac, f – funiculus, h – hypostase, ii – inner integument, m – micropyle, n – nucellus, oi – outer integument, vb – vascular bundle.

by 90°, as a result of which the greater deal of the elements (integuments, nucellus, chalaza) occur to be located parallel to the funiculus; that's why an inversion of the axis poles occurs (turning by 180°), so that the micropyle occurs to be located near the hilum and placenta, whereas the chalaza, the hypostase and the conducting bundle shift to an ana-position (bitegmic with ring-shaped integuments or with raphe, unitegmic with raphe, funicular including circinotropous, and also sessile, afunicular) (Fig. 6 Ban, Br).

The 3rd type is **hemitropous** (the axis micropyle–chalaza is straight and parallel to the placenta). Within the hemitropous type, two subtypes are distinguished: the **hemi-anatropous**, in which a straight morphological axis in the ovule, being represented by a funiculus or a raphe, changes the angle of inclination toward the placenta as a result of the displacement of the chalaza, the hypostase and the conducting bundle into the hemi-position. Most of the elements (integuments, nucellus, chalaza) lie likewise in anatropous ovule, but at an angle of 45° toward the placenta, i.e. the turning of the ovule occurs up to 135° toward the placenta (bitegmic with ring-shaped integuments or with raphe, unitegmic with raphe, funicular, sessile) (Fig. 6C, Cc). In the **hemi-orthotropous** subtype, the straight morphological axis in the ovule, passing through the funiculus or raphe, curves towards the structures of the chalazal area (raphe, chalaza, the base of the integuments, the hypostase and the conducting bundle) that keep the ortho-position. Due to the more extensive growth of the structures of the micropylar and medium parts on the antiraphal side of the ovule compared to the raphal one, the axis remains straight in a great part of the integuments and the nucellus, the micropyle does not approach the placenta, which leads to a change in the angle of rotation by 135° toward the placenta likewise it takes place in the hemi-anatropous ovule (bitegmic and unitegmic with raphe, funicular, sessile) (Fig. 6D, Dd).

The 4th type is **campylotropous**. The ovule is characterized by a straight axis passing through the funiculus or raphe; the greatest part of the morphological axis is curved. In the campylotropous type, two subtypes are distinguished: the **ortho-campylotropous** ovule, wherein the chalazal part (chalaza, hypostase and conducting bundle) is organized likewise in the orthotropous ovule, whereas the micropylar part (mostly micropyle as itself) likewise in the anatropous one (mostly funicular bitegmic, with raphe, perhaps bitegmic afunicular and sessile) (Fig. 6E, Ee) and the **hemi-campylotropous** ovule, wherein the chalaza, hypostase and the conducting bundle are in the hemi-position, and the micropylar part persists in the same state as in the anatropous ovule (bitegmic with ring-shaped integuments or with raphe, unitegmic with raphe, funicular and afunicular) (Fig. 6F, Ff).

Directions of possible transformation of flowering plant ovules. When studying ovules and characterizing the types and variants, it is necessary to take into account not only their characteristics, but also the structure of the ovary, namely its shape, the need for creating space for developing ovules (the presence in the ovary of not only fertile, but also sterile zones), peculiarities of placentation and formation of placental outgrowths, the degree of development of funiculus and conducting tissue and the type of obturator.

Ovary and ovules arose in the course of evolution as an integrated system that ensures the formation of a female gametophyte, pollination, fertilization and the emergence of a new sporophyte. Therefore, changes in one structure cause corresponding transformations of the other one (Shamrov 2006, 2012; Shamrov & Kotelnikova 2011). The main specificity of the gynoecium morphogenesis is the consistency between shape and size of the ovary, on the

one hand, and the number of forming ovules on the other. It is known that in some taxa, the reproductive strategies aim at the appearance of a large number of small ovules, whereas in others, the number of ovules in the ovary is reduced and their size is increased. Most important is the fact that both directions of the coordinated development of the ovary and ovules could proceed without additional mechanisms for creating a space for the accommodation of developing ovules and subsequent seeds. Since the number of initiated ovules can not always be actualized, their abortion is observed in the ovaries at different stages, leading to a decrease in the number of seeds in the fruit and the appearance of few-seeded and single-seeded fruits. In some plants, such as species of *Vicia*, the size of the ovary and fruit correlates with the total number of ovules and the number of aborted seeds. Species with small fruits (*V. hirsuta*, *V. pubescens*) are characterized by small seeds and low abortion, whereas in species with large fruits (*V. lutea, V. sativa*) an opposite dependence is observed (Ortega-Olivencia & Delesa 1997).

In a number of plants, the relationship between the structure of the ovary and the number of ovules is solved by the creation of an additional space. In this case, changes occur in the shape of the ovary, the structure of the placentas and ovules (structures of the chalazal and micropylar areas, as well as of the funiculus and obturator), the destruction of septa (in lysicarpous gynoecium), which occur at different times. Let us illustrate these statements with examples of development of ovules and seeds of various flowering plants.

In *Euphorbia palustris* (Euphorbiaceae), the syncarpous gynoecium consists of 3 carpels, with a single ovule per locule, which is located in the upper part of the ovary on a short funiculus and is turned with its micropyle to the placental obturator. The signs of campylotropy can be revealed yet before fertilization due to the predominance of growth of the structures of the antiraphal side. This applies especially to the outer integument, which becomes 8–10-layered in the apical part (here an intensive elongation of the integument is observed), whereas it is usually 4-layered in the rest. The outer integument overgrows the nucellar beak and forms an exostome turned toward the placental obturator. After fertilization, the exostome shifts toward the placenta. Gradually, this process involves an inner integument that completely overgrows the beak-shaped structure of the nucellus from the antiraphal side with the formation of an endostome, which results in acquiring a zigzag structure of the micropyle. The morphological axis of the seed turns out to be curved, and the seed itself becomes hemi-campylotropous, at that the flexure affects not only the micropylar, but also the medium and chalazal parts and the endosperm. The embryo also slightly curves and increases in size, occupying about ⅔ of the seed (Titova et al. 2015).

In *Paeonia lactiflora* (Paeonaceae), 15–20 ovules begin to develop in each (of 3–5) carpel of the polymerous-apocarpous gynoecium. Yet, before fertilization, the anomalous ovules can be revealed based on the set of marker signs, which later degenerate, and only 5–6 seeds are formed in the mature fruitlet (follicle). The formation of space in the ovary for the development of fertile seeds is facilitated by certain features of the structure of ovules, which are sessile, lacking the funiculus proper and attaching to a greatly expanded placental outgrowth by the aid of the basal part of the raphe. This causes their turning to be more pronounced than in the hemi-anatropous ovule, but not reaching 180°, and the rotation into the anatropous position is not completed (Shamrov 1997b).

In the ovary of the representatives of family Menispermaceae (apocarpous gynoecium consists of 1–6 or more carpels), usually 2 ovules begin to develop, one of which (usually the lower one)

degenerates then. The mature seed occupies the entire ovary space in the longitudinal direction (Ortiz 2012). Similar phenomena also occur in a number of other plants. In the species of the family Umbelliferae, two ovules located at different levels initiate in each locule of the 2-locular ovary (Aleksandrov & Pervuchina 1952; Kordyum 1967). As shown by a study performed by Kordyum (1967), initially (differentiation of archesporium) the upper ovule is turned towards the apex of the ovary and the lower one towards the base of the ovary. Both ovules at the early stages develop in the same way. However, with the entry of the megasporocyte into meiosis, the upper ovule stops its development and the funiculus does not form in it, but only the remnants of the integument and nucellus consisting of the lignified cells can be found. Almost the entire cavity of the ovary is occupied by the lower ovule, in which a turn occurs into the anatropous position, whereas its micropyle becomes turned upwards. The same processes occur in the 3–4-locular ovary of the representatives of the family Araliaceae. In each locule, only the lowermost of 1–3 ovules is formed, whereas the upper part of the ovary retains the aberrant ovule (Singh 1954). The presence of two ovules in each carpel, of which only the lower one develops, is noted in the family Alismataceae (Johri 1935). Many basal dicot plants are characterized by the formation of aberrant ovules with a tendency to reduce their number in the ovary or locule from four (Daphniphyllaceae) or two (Proteales, Ranunculales, Saxifragales) to one. In the families Altingiaceae and Hamamelidaceae the additional ovules appear in the ovary, but they do not develop (Endress & Igersheim 1999).

In *Cerasus vulgaris* (Rosaceae), gynoecium is not monomerous-apocarpous, as it is usually indicated in literature, but syncarpous (pseudomonomerous) and consists of two carpels, differing in size, structure and reproductive capacity. Fertile carpel comprises ⅔ of coenocarpous gynoecium, therefore the ovary of sterile carpel does not form its own locule, but fuses with the ovary of the fertile carpel, which causes the formation of a more massive wall of the ovary in the fusion area, and the ovary itself becomes derivatively unilocular. The sterile carpel is actually devoid of placenta, with the exception of the upper part of the ovary, where their remnants persist without any signs of formation of ovules. In the fertile carpel, the ovules arise on two corner placentas in the upper and lower parts of the ovary. On each placenta two ovules differentiate, however, in the upper part they stop their development at the stage of primordium. Two ovules of different sizes form at the base of the ovary before fertilization, but only one of them transforms into a seed (Shamrov & Yandovka 2008). In the paracarpous (consisting of 3 carpels) gynoecium of *Polygonum affine* (Polygonaceae), only the single hemi-orthotropous ovule develops on just a single placenta of the paracarpous suture. This ovule lies laterally and occupies the entire space of the ovary (Shamrov & Anisimova 2015).

All these phenomena are most pronouncedly reflected in the coenocarpous gynoecium with a large number of developing ovules and seeds. In the paracarpous gynoecium of *Capsella bursa-pastoris* (Brassicaceae), the campylotropous ovules and seeds, depending on the length of the funiculus, occupy various positions in the forming fruit (silique) compressed in the bilateral plane (Shamrov 2002b). At the early stages, the ovules are located in the transversal plane, but already during the embryo sac development they begin to curve, therefore they are displaced in rows: those with the short funiculus turn out to be located near the septum, and those with the longer funiculus appear near the ovary wall. The same situation persists in the fruit.

In a number of representatives of the family Gentianaceae (*Swertia*, *Gentiana*), in contrast to other species characterized by a paracarpous gynoecium, the ventral margins of the two carpels

are not wrapped inside the ovary, but they tightly join and then fuse. As a result, the ovary size increases (in the bilateral plane) in the region of the suture. The sutures become very short and do not turn inward but outward. This causes a shift of the sites of placenta differentiation from the sutures to the lamina of the carpel (the so-called false-laminar placentation), so the ovules form on both the inner (at the edge of the suture near the ventral bundles) and the outer (facing the dorsal bundles) placentas. The number of ovule rows in each carpel increases as compared to *Capsella bursa-pastoris* (2 rows) and counts 4 rows in *Gentiana lutea* and *Swertia iberica* and 10 rows in *Gentiana cruciata*. Ovules on the placentas develop asynchronously, the first ones form on the inner placentas. However, in *G. cruciata* anatropous ovules, regardless of the shape of the placentas, lie in the transverse plane of the ovary, and in *G. lutea* some differences in their position are revealed. In *G. lutea*, ovules on the inner placentas are found along the entire length of the ovary, whereas on the outer ones they develop only in its lower part (Shamrov 2013). It should be noted that in the middle part of the ovary, the ovules on the inner placentas have different sizes, they shift and alternate (large and small) in the rows (Fig. 7A–J).

In the syncarpous gynoecium of *Juncus filiformis* (Juncaceae), the anatropous ovules in different parts of the ovary have a different length of the funiculus (Shamrov et al. 2012). In the lower part, the ovules with short funiculi are arranged in a single row at the inner placentas in the transverse plane. In the middle part, 2 more rows of ovules appear on the outer placenta, and the additional ovules are aligned in the longitudinal plane, whereas the original ones stay in the transverse plane, but the length of their funiculi increases. In the upper part of the ovary, there are only additional ovules in the longitudinal plane, and their number gets reduced by half at the top.

In *Dioscorea nipponica* (Dioscoreaceae) (Torshilova et al. 2003), the size of the locules in the 3-locular ovary of the syncarpous gynoecium in some cases decreases, and with a weak manifestation of the funiculus proper and the lack of sufficient space in the ovary locule for performing rotation, a flexure of morphological axis occurs instead, which leads to the formation of a hemi-campylotropous ovule instead of an anatropous one. A detailed investigation of 10 species of the section *Stenophora* of genus *Dioscorea* (Titova & Torshilova 2015) showed that the studied species form 3 groups: in the first group the seeds are typically hemi-campylotropous (*D. balcanica*, *D. caucasica*, *D. deltoidea*, *D. deltoidea* var. *orbiculata*, *D. villosa*), in the second one they are slightly hemi-campylotropous (*D. nipponica*, *D. tokoro*), and in the third one the seeds combine signs of the first two groups (*D. collettii*, *D. septemloba*, *D. gracilima*, *D. tenuipes*).

In *Canarium pimela* (Burseraceae) (Shamrov 2015b), one of the four carpels in syncarpous gynoecium is sterile and the ovary is 3-locular. The placenta becomes functioning only at one ventral margin, therefore the epitropous hemi-campylotropous ovules (one ovule per locule, although the second ovule can form in one of the locules, but its development ceases at an early stage) are arranged with offset, occupying different levels of the ovary due to the funiculus flexure.

In the lysicarpous gynoecium (various representatives of the family Caryophyllaceae (Shamrov & Kotelnikova 2011; Kotelnikova & Shamrov 2012) and *Pinguicula vulgaris*, Lentibulariaceae (Titova 2012)), the ovules and seeds, depending on their topography in the ovary, differ in structure and degree of flexure of the structures providing campylotropy, as well as in the degree of the funiculus manifestation. In *Pinguicula vulgaris*, mature seeds are almost anatropous in the upper part of the ovary, hemi-campylotropous in the middle and campylotropous without the possibility of determining the hemi- or ortho-position in the lower ones. In species of
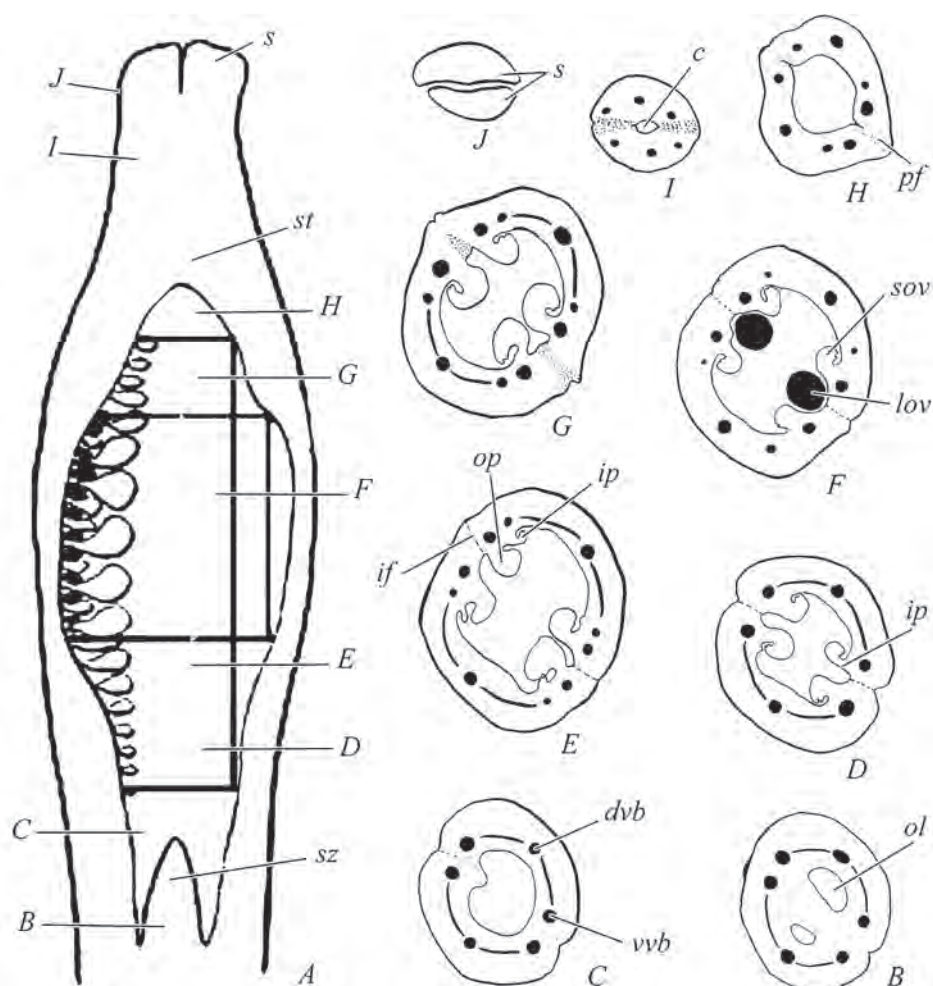
Figure 7. Paracarpous gynoecium structure and arrangement of ovules within ovary in *Gentiana lutea* (Gentianaceae). A – longitudinal section of gynoecium; B–J – gynoecium structure (series of transverse sections): synascidiate (B) and hemisymplicate (C–H) zones; I – style; J – stigma lobes. B, C, H – sterile zones; D–G – fertile zones, in which ovules are located on both at inner (D, G) and outer (E) placentas, in the middle part of the ovary asynchronous development of the ovules at inner placentas is revealed (small and large ovules) (F). c – canal, dvb – dorsal vascular bundle, if – imperfect fusing, ip – inner placenta, lov – large ovule, ol – ovary locule, op – outer placenta, pf – perfect fusing, s – sigma, sov – small ovule, sz – synascidiate zone, st – style, vvb – ventral vascular bundle.

Caryophyllaceae, the spaces being devoid of placenta form in the upper and lower parts of the ovary. These sterile areas are getting occupied by the ovules of the nearest fertile areas, which is accompanied by a change in the position of the ovules. At the base of the ovary the latter ones are located on the short funiculi in the transversal plane. In the middle part of the ovary, the funiculi become longer and the ovules become to be arranged in the longitudinal plane. At the top of the ovary, the ovules have the largest sizes and long funiculi, their number decreases and they are oriented in the longitudinal plane.

The gynoecium of *Delosperma tradescantioides* (Aizoaceae) is a special case. Especially the pattern of placentation shows characters of two gynoecium types (syncarpous and paracarpous). In this species the ovules are located on the central-angular (axile) and, mainly, parietal placentas. Forming hemi-campylotropous ovules are located in various sterile zones of the ovary: in the lower

part on the central-angular placentas with short funiculi, in the upper part in special pockets on the parietal placentas with long funiculi (Shamrov 2014).

Studies of ovule are always inextricably associated with issues of its origin. By now, there are three theories explaining the nature of ovule: axial, foliar and *sui generis* theory. As we have already noted, the seeds of flowering plants are characterized by a considerable variety in genesis and structure. There are several hypotheses (theories) explaining the origin of the integument and the nucellus in seed plants on the basis of data on the structure of reproductive structures in sporiparous plants: the indusial, synangial and telomic theories, the conception of the nucellus modification and the theory of double integument. These hypotheses can not be considered as alternatives but as equals, because the establishing of ovule structures in different taxa of flowering plants could occur in independent ways (see Shamrov 2006, 2008 for a complete list of literature sources on this issue).

The issue of the primacy of the particular morphological type of flowering plant ovule is disputed. We have already stated that, according to Davis (1966), the largest number of flowering plant families are characterized by anatropous ovules. That is why many researchers consider the anatropous ovule to be the primary one, taking into account not only the wide prevalence, but also its presence in many primitive flowering plants. The orthotropous ovule is inherent in most gymnosperms, so some authors consider it as the ancestral one for flowering plants. Some reserchers believe that both types could be initial, and the possibility of arising anatropous ovule from the orthotropous one is not ruled out.

Earlier, we supported the idea that basic ovules could be orthotropous (Shamrov 2006, 2008). However, the analysis of data on the peculiarities of ovule morphogenesis combined with the results on the structure and development of the gynoecium allowed us to make some adjustments to the characteristics of basic ovule of flowering plants. First of all, all known variants of ovules pass through the stage of straight-directed structure in their development, especially during the formation of primordium on the placenta. It can be stated for sure that the ovules of two types (orthotropous and anatropous) are predetermined for further development during the period of megasporogenesis, i.e. their ways of morphogenesis at this time are already diversified. The shape of these ovules stays almost unchanged not only before pollination, but even during their transformation into seeds. It can be assumed that the divergence of these two types occurred probably very early in evolution. On the other hand, similar features appear in the morphogenesis of orthotropous and anatropous ovules. Thus, the orthotropous ovules are characterized by radial symmetry and ring-shaped integuments (the examples of the families in which they are found are mentioned above). Among the group of anatropous ovules, there are also variants with ring-shaped integuments, which are characteristic of representatives of a number of primitive angiosperms: Amborellaceae, Chloranthaceae, Gomortegaceae (Endress & Igersheim 1997; Heo et al. 2004), Canellaceae, Degeneriaceae, Himantandraceae, Illiciaceae, Myristicaceae (Igersheim & Endress 1997).

Differences between the anatropous ovules with ring-shaped integuments and the orthotropous ones only concern the angle of rotation and the position of the micropyle towards the placenta. Therefore, it is possible that at the first stages of the evolution the ovules had ring-shaped integuments and were formed within the two trends depending on the structure of the ovary and the conditions for the development of all initiated ovules (Fig. 6A, Ban). On the basis of

anatropous ovules with ring-shaped integuments, the anatropous ovules could form, in which a raphe was formed as a result of the congenital fusion of the funiculus and outer integument (Fig. 6A, Br). In a number of primitive families, both ring-shaped and semi-ring structures of the outer integument were found: Calycanthaceae, Hernandiaceae, Monimiaceae (Endress & Igersheim 1997), Aristolochiaceae, Nymphaeaceae (Igersheim & Endress 1998). In *Butomus umbellatus* (Butomaceae), an orthotropous ovule with ring-shaped outer integument can be formed instead of the characteristic anatropous ovule with raphe (Igersheim et al. 2001).

It can be shown for two species of the family Juncaginaceae that the ovule at early stages is orthotropous. However, in *Lilaea subulata* (Campbell 1898) it becomes hemi-anatropous, retaining the ring-shaped structure of the integuments, whereas in *Triglochin palustre* (Nikiticheva & Proskurina 1990) an anatropous ovule with raphe forms. Because in representatives of family Juncaginaceae often just only 3 of 6 carpels of the gynoecium reach the mature state, in some carpels the ovules may have ring-shaped integuments, and in others the raphe forms in the ovules, which was actually revealed (Nikiticheva & Proskurina 1990). This is evidenced by data on the structure of ovules in the family Ixerbaceae: as a rule, of two ovules arising in each locule of the ovary, the upper one, possessing the ring-shaped outer integument, degenerates, and the lower one, possessing the raphe, transforms into a seed (Matthews & Endress 2005). We would like to draw attention to the fact that the transformation of the outer integument from the ring-shaped to the semi-ring one can not be traced during the morphogenesis of the ovule, since the raphe arises during the congenital fusion. To resolve an issue of the final organization of ovule, it is necessary to take into account a number of peculiarities of the formation of both carpels as parts of the gynoecium and ovules in the ovary (their number and position in space, the presence of deviations).

Molecular genetic studies on the morphology of ovule in *Arabidopsis thaliana* revealed a bunch of abnormalities, the analysis of which can shed light on the structure of basic ovules (Robinson-Beers et al. 1992; Gaiser et al. 1995; Schneitz et al. 1995; Angenent & Colombo 1996, Gross-Hardt et al. 2002; Endress 2011). First of all, it was concluded that the independent developmental programs for each integument take place. In *ant* mutants, the ovules are completely devoid of integuments and resemble the sporangiophores of the seed plant ancestors. Studies have shown that although the gene *ANT* is engaged in the initiation of integuments, the latter ones are never formed. The products of this gene are involved mainly in the formation of the ovule primordium, as well as the primordium of any other organ (leaf, flower, flower elements). The *WUS* gene acts similarly in *wus* mutants. In *A. thaliana* mutant *ino*, only the inner integument arises, initiation of the outer integument occurs, but it stops development, therefore the ovule structure resembles the structure of the gymnosperm ovules with the single integument. In mutants *bel1*, *bel2* and *bel3*, the inner integument is not initiated and is not formed, and the outer one develops as an abnormal bell-shaped structure. The mutant *sup* is characterized by the change in the course of the development of ovules, which instead of campylotropous become orthotropous and hemitropous. Therefore the integuments appear to be ring-shaped and form the elongated tubule-shaped structures with radial symmetry. In structure they resemble the ovules of some primitive extant and fossil angiosperms. It should be noted that in the studied mutants, as a rule, there is no flexure of the morphological axis in the ovules, although their turning relatively to the placenta occurs.

Data of the study of *A. thaliana* mutant plants are surprisingly in tune with the results on the structure of ovules in parasitic species of the order Santalales. In these plants, the structural evolution proceeded, probably, from differentiated to undifferentiated ovules, and then to their complete disappearing (Shamrov et al. 2001). Thus, the ovules of the *ant* mutant are completely devoid of integuments, resembling by the structure the undifferentiated ovules (represented in fact by the ovule primordia) of some parasitic plants of the order Santalales: *Schoepfia pringlei, Ximenia americana, Olax stricta, Anacalosa puberula, Arceuthobium douglasii, Viscum album* (Teryokhin 1977). In this order, there are also some plants with so-called ategmic ovules. By the example of *Santalum album*, it was shown that in the hemi-orthotropous pachychalazal ovule of this species only the initials of the integument and the nucellar cap differentiate (Shamrov et al. 2001). This assumption was confirmed in molecular-genetic studies. By comparing the expression of *ANT* and *BEL1* genes in *Arabidopsis* and representatives of order Santalales concluded that ategmal ovules emerged, probably when merging integuments with nucellus, or nucellus acquired properties that limited the development of integuments in ancestral species (Brown et al. 2010).

Also, plants are found, in which the ovules are not formed, but the complex of cells arises in the placenta that perform the function of the nucellus with the development of sporogenous cells, megaspores, embryo sacs and embryos (*Loranthus europaeus* (Nikiticheva & Anisimova 1987)). Similar results were obtained in the study of the ovule of mutant *bel3*. During megasporogenesis, the integument-like structure becomes lobed and the lobes gradually go one beyond another and in some cases they form a carpel-like structure (Modrusan et al. 1994). At later developmental stages, the blades partly get flattened and branch dichotomously, forming the carpel by expense of the axes. At its edges the structures appear in the terminal position in which the archesporial cells and the megasporocytes differentiate as if they were in separate nucelluses. This knowledge formed the basis of a new hypothesis of the ovule origin, proposed by Herr (1995).

All these facts allow us to support the opinion of some authors (Bocquet 1959; Bocquet & Bersier 1960) that both types could be the ancestral ones for other variants of ovules. However, the question arises as to the transition from orthotropous ovules to ortho-campylotropous and ortho-amphitropous ones and from anatropous to ana-campylotropous and ana-amphitropous ones. Accepting the campylotropous variants as derived modifications of anatropous ovules, a number of researchers (Goebel 1933; Bouman 1984; Bouman & Boesewinkel 1991) supposed that they arose repeatedly and evolved several ways in the course of evolution.

In our opinion, the basic orthotropous and anatropous ovules were bitegmic with ring-shaped integuments, crassinucellate, with a well-manifested funiculus. Their further transformations proceeded in different directions. Earlier, we assumed that these transformations could proceed at least within the three lines of development, depending on the structure of the outer integument, the degree of development of the funiculus and the position of various elements of the ovule during development. The funicular, sessile and afunicular ovules were distinguished (Shamrov 2006, 2008). Based on the complex of available data, we believe that the transformations occurred through series of intermediate states (first of all variants of hemitropous and campylotropous ovules) (Fig. 6C–F), therefore the changes arose correlatively in the shape and structure of the ovary, placentation, ovule and seed as well as of the embryo sac, endosperm and especially the embryo (the degree of its differentiation, size, ability to accumulate reserve nutrients). Thus, in the representatives of the family Potamogetonaceae, the ovule uses to be defined as orthotropous (Davis 1966) or ortho-campylotropous (Kamelina 1990) before fertilization and

after fertilization as amphitropous (Kamelina 1990). An analysis of the aforementioned studies indicates that the formed ovule is hemi-orthotropous. It occupies a lateral position in the ovary, the micropyle does not approach the placenta, and the embryo sac is straight. At the stage of the 2-celled embryo, the axis of the forming endosperm slightly curves, and after the formation of the globular embryo the overall axis of the seed curves as well. Keeping the structures of the chalazal region in the ortho-position, the seed becomes ortho-campylotropous and a large curved embryo occupies most of its volume. Thus, the formation of campylotropy in these plants occurs later (not before fertilization during the ovule development, but at the stage of the 2-celled embryo in the forming seed), although the structure of the seed corresponds to the 1st variant described by us earlier. Furthermore, the ring-shaped structure of the outer integument is clearly visible at all stages.

On the basis of the data given, we suppose that the number of taxa with campylotropous ovules needs to be corrected and it is obviously greater than it has been considered until now. On the other hand, when characterizing campylotropous ovules and seeds, one must consider first of all the shape of the flexure of the integuments, including the micropyle as well as that of the nucellus and the chalaza, but not just only the embryo sac and the embryo. All the above arguments don't allow us to agree completely with the existing opinion, according to which the formation of campylotropy has a different phylogenetic and adaptive value as compared to anatropy, namely, in the period of embryo development and not at the stage of the embryo sac (Bouman & Boesewinkel 1991; Bouman 1992). In our opinion, this is correct only for campylotropous seeds with a large curved embryo occupying almost the entire cavity of them. However, the arising of such campylotropous ovules and seeds was caused not only by the establishing conditions within the seed to form a large differentiated embryo, in which the main reserve nutrients needed for germination became accumulated. At the same time, the traits of campylotropy were gradually shifted to the early developmental stages, mainly to the time of megasporogenesis (variant 1 on the basis of orthotropous and hemi-orthotropous ovules and variant 4 on the basis of anatropous and hemi-anatropous ones). The features of campylotropous seeds can be formed later, nearly before fertilization (on the basis of anatropous ovules upon in the establishment of the post-development phenomenon, when a large curved embryo forms after breakdown of dormancy – variant 6), or even at the early embryogenesis stages, as it was noted upon the transformation of the hemi-orthotropous ovules into the ortho-campylotropous seeds in the species of Potamogetonaceae. The second acquisition accompanying campylotropy was the more rational arrangement of the forming seeds in the ovary. As to the ovules and seeds, in which no correspondence in the flexure of the ovule and embryo structures could be observed (variants 2, 3 and 5), their establishing mainly proceeded towards the optimal arrangement of the ovules in the ovary, which was accompanied by a change in shape from an erect to a curved one, as a result of which the developing ovules occupy less space on the placentas. There was no increase in the size of the embryo and the reserve nutrients accumulated mainly in the endosperm.

Acknowledgements

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