Bull. Georg. Acad. Sci. 170, N1, 125-129; 2004

BOTANY

Relationships between Structure of the Reproductive Organs and the Life Cycle in the Family Gentianaceae

M. Akhalkatsi, G. Gvaladze, M. Gachechiladze

Institute of Botany, Georgian Academy of Sciences
Presented by Corr. Member of the Academy G. Nakhutsrishvili, March 2, 2004

ABSTRACT: *Gentiana angulosa, G. cruciata, G. pontica, G. pyrenaica, G. septemfida* and *Gentianopsis blepharophora* have anatropous ovules, reduced antipodals and prolonged seed maturation period (30-40 days). *Gentianella caucasea, G. germanica* (with hemitropous ovules) and *Swertia iberica* (with ana-campilotropous ovule) are characterized by proliferated antipodals and rapid seed development (less than 25 days). Proliferated antipodals accelerate embryogenesis by substituting for the function of the endosperm in the early stages and promote rapid seed development. The structural differences of ovule and embryo sac may determine duration of the life cycle, which may stipulate for development of a specific adaptation to the severe environmental conditions due to the shortening of a life cycle and origin of annual ephemers well adapted to the cold and arid climate of alpine environment.

Key words: antipodals, embryo sac, Gentianaceae, ovule.

Structure of reproductive organs, as usual, is genetically determined constant feature for each plant taxon. Characteristics, such as ovule and embryo sac type, peculiarities of embryogenesis and endosperm development, are similar for all representatives of a concrete family, genus, or species. In some cases, however, definite features vary within a taxon of higher hierarchy, such as family or genus, or even among individuals of a concrete species. In the family Gentianaceae, structure of reproductive organs reveals number of variable characters having a taxonomic value.

The Gentian family unifies more than 80 genera and approximately 1000 species (1). They are distributed almost on all continents and mainly occur in alpine ecotone. Most of them are herbaceous autotrophs. 8 genera of this family occur in Georgia - *Blackstonia, Centaurium, Comastoma, Gentiana, Gentianella, Gentianopsis, Lomatogonium* and *Swertia.* The biggest genus is *Gentiana* L., which, according to newest classification (2), is divided into 15 sections and contains 361 species world-wide. Genera *Gentianella* Moench and *Gentianopsis* Ma have been separated recently from the genus *Gentiana* (3, 4, 5) on the basis of a number of morphological, anatomical, karyological and molecular systematical data. However, the classification and phylogenesis of these genera are not finally determined and need further study. Among others, use of embryological and molecular biological approaches seems to be necessary.

According to some authors (6,7,8,9), embryological characters should be taken into consideration during taxonomic study of the family Gentianaceae. Particular attention should be paid to structural peculiarities of ovule and embryo sac. Ovule in most species of the family Gentianaceae is anatropous - *Gentiana* spp., *Gentianopsis* spp., but sometimes it might be orthotropous - *Halenia elliptica* (10)*, Cotylanthera tenuis*, *Voyriella parviflora*, *Voyria* spp. (11), hemitropous - *Comastoma tenellum*, *Gentianella* spp. (10), or ana-campylotropous - *Swertia* spp. (8,12). The number of cell layers in a single integument varies among species from 2 to 20. Megagametophyte develops according to *Polygonum* type in all Gentianaceae. However, the number of antipodals in the mature embryo sacs varies among species. There are three groups of species in the family Gentianaceae differing in antipodal structure (7,13): 1. antipodals uninucleate, ephemeral, degenerate before fertilization. They might be three, or 3-9; 2. antipodals always three, degenerate

after fertilization; 3. antipodals multinucleate, or with polyploid nuclei, hypertrophic, 3, 6, or more than 6.

Structural peculiarities of reproductive organs may reflect not only phylogenetic relationships, but also be considered in connection with functional traits. Earlier studies (9,14) of the embryology, phenology and reproductive ecology of three alpine species of the family Gentianaceae have revealed relationships between structure of the ovule and the life history of the species. One of the investigated species - *Gentiana pyrenaica* is perennial herb, and the other two *Gentianella caucasea* and *G. germanica* are annual monocarpic ephemers. The structural differences have been identified as follows: *G. pyrenaica* has anatropous ovule, 8-10 layer thick integument and 3 reduced ephemeral antipodals. The both species of the genus *Gentianella* possess hemitropous ovule, 4-5 layer thick integument and 16 proliferated antipodals. Simultaneously, the differences have been found in synchronization of embryogenesis and endosperm development. In particular, the first division of the zygote in *G. pyrenaica* takes place after 128 endosperm nuclei have been formed. In *G. caucasea* and *G. germanica* the zygote already divides in the 8-nucleate stage of the endosperm. At the same time, in short-leaved monocarpic *G. caucasea* the time span for seed development amounts to 16-20 days (14), in *G. germanica* to 20-25 days (15). However, perennial *G. pyrenaica* requires about 40 days for seed formation under comparable climatic conditions to *G. caucasea* (15). It was suggested that rapid seed development in *Gentianella* species might be promoted by the proliferated antipodals, an apparently nutritive tissue, which may supply the embryo with nutrients and growth controlling substances before endosperm is formed. This may accelerate embryogenesis by substituting for the function of the endosperm in the early stages. Thus, it might be suggested that the structural differences among related genera may determine duration of the life cycle and in such a way stipulate for development of a specific adaptation mechanism for successful propagation of the species in a concrete environment.

On the basis of these suggestions, we have decided to conduct further embryological investigation of some species of the family Gentianaceae belonging to different genera. We have investigated embryology of 3 species for the first time - *Gentiana angulosa* M. Bieb. (= *G. verna* var. *angulosa* Kusn.), *G. pontica* Solotk. (= *G. verna* ssp. *pontica* (Soltok) Hayek) and *Gentianopsis blepharophora* (Bordz.) Galushko (=*Gentianopsis ciliata* (L.) Ma). We have collected additional data on 3 species investigated earlier by other authors - *G. cruciata* L. (= *Gentianopsis cruciata* (L.) ssp. *cruciata* Tutin; 13), *G. septemfida* Pall. (7) and *Swertia iberica* Fisch. & C.A. Mey (12). At the same time we have analyzed data on three species studied earlier *G. pyrenaica* L. (=*G. dshimilensis* C. Koch), *Gentianella caucasea* and *G. germanica* (9,14).

Embryological study has been conducted using traditional methods for light microscopy. Additionally, clearing fluid by J. Herr (16) has been used to investigate the whole fixed ovules. The Examination was carried out using light microscope Polivar, Reichert, Austria.

Comparative embryological investigation has revealed number of characters common for all investigated species. Anther is four-loculate. Mature pollen contains two cells. The gynoecia are superior, unilocular, bicarpellate (very rare tricarpellate) and paracarpous. They are terminated by a short style and a 2-lobed stigma. It is situated on a gynophore. Numerous ovules develop on the parietal placentae along the fused margins of the carpels. Vascular bundles reach chalaza, but do not enter ovule. The Ovule is tenuinucellar and unitegmic. Nucellus degenerates during the extension of the embryo sac. The archesporium is hypodermal, unicellular and functions directly as megaspore mother cell. Tetrad of megaspores is linear. *Polygonum* type of embryo sac develops from chalazal megaspore. The polar nuclei fuse before fertilization and secondary nucleus is located near the egg cell. The mature embryo sac consists of the three-celled egg apparatus, the central cell and different number of antipodals. Fertilization is porogamous. Triple fusion outstrips syngamy. Pronuclei fuse by pre-mitotic type. Endosperm is nuclear. Embryogenesis follows the *Solanad* type. Mature seed contains embryo at early torpedo stage, cellular endosperm and seed coat. The seed coat consists of a sculptured outer layer, derived from the epidermis of the ovule, and a transparent membranous envelope originated from the crushed cells of the inner layers of the integument. The sculpturing differs among species and has a taxonomic value.

Investigated species differ by some quantitative characters (Tab. 1), which usually are variable within the family Gentianaceae. Ovule type is anatropous in *G. angulosa, G. cruciata, G. pontica, G. pyrenaica, G. septemfida, Gentianopsis blepharophora,* hemitropous in *Gentianella caucasea, G. germanica,* and ana-campilotropous in *Swertia iberica*. Another variable characteristic is the number of cell layers in the integument. This feature is important systematic character, while integument layers form seed coat with a peculiar species specific surface texture having a taxonomic value. Ovule type and integument thickness determine seed shape and seed coat structures. The species with anatropous ovules have oblong seeds. Hemitropous ovules give rise to globose seeds. The seed coat structure display considerable variability within the genus *Gentiana* characterized by thick integument forming a reticulate seed coat with a species specific texture. The species of *Gentianella* and *Gentianopsis* with 4-5 cell layers of the integument develop seed coat without obvious sculpturing.

Number and structure of antipodals vary among investigated species. Three reduced antipodals are characteristic for G. pyrenaica and G. pontica. 3 to 6 ephemeral antipode cells occur in G. *angulosa, G. cruciata* and *G. septemfida*. 9 antipodals degenerating before fertilization are observed in *Gentianopsis blepharophora.* Proliferated antipodal tissue with 9-16 cells are found in *Gentianella caucasea, G. germanica* and *Swertia iberica.* It is noteworthy, that all species with ephemeral antipodals possess anatropous ovules and species with proliferated antipodals – hemitropous or ana-campilitropous. The antipodal nucleus shows different ploidy level among studied species. Typical haploid nucleus occurs in *G. angulosa, G. pontica, G. pyrenaica, G. septemfida* and *Gentianopsis blepharophora. G. cruciata* has 6 antipodals with one diploid nucleus each. Proliferated antipodal cells of *Gentianella caucasea, G. germanica* and *Swertia iberica* possess one or more polyploid nuclei.

Definite synchronization between embryogenesis and endosperm development have been found. Endosperm cellularizaton has begun after formation of 128 endosperm nuclei, i.e. after 7 synchronous mitotic divisions, almost in all investigated species, except of *G. germanica*, in which cell wall formation starts at 8-nucleate stage. However, the first division of the zygote took place at different stages of endosperm development. In all species with ephemeral antipodals, the zygote starts division simultaneously with endosperm cellularization at 128-nuclate stage. In the species with proliferated antipodals, the zygote divides after formation of only 8 endosperm nuclei.

Table 1. Life form (P – perennial; A – annual, B – biennial), ovule type (A – anatropous, H – hemitropous, AC – anacampilotropous), the numbers of integument cell layers and antipodals, ploidy of antipodal nucleus (n- haploid, 2n – diploid, pn – polyploid), synchronization of embryogenesis and endosperm development and time span of seed development in 9 species of the family Gentianaceae.

N	Species	Life form	Ovule type	Number of integument cell layers	Number of antipodals	Ploidy of antipodal nucleus	Number of endosperm nuclei at cellularization	Number of endosperm nuclei at first division of the zygote	time span of seed development (days)
1.	*Gentiana angulosa*	P	A	5-8	3-6	n	128	128	> 40
2.	*G. cruciata*	P	A	6-10	6	2n	128	128	> 30
3.	*G. pontica*	P	A	9-10	3	n	128	128	> 40
4.	*G. pyrenaica*	P	A	8-10	3	n	128	128	> 30
5.	*G. septemfida*	P	A	8-10	3,4,6	n	128	128	> 30
6.	*Gentianella caucasea*	A	H	4-5	9-16	pn	128	8	<20
7.	*G. germanica*	A	H	4-5	16	pn	8	8	<25
8.	*Gentianopsis blepharophora*	B	A	4-5	9	n	128	128	>30
9.	*Swertia iberica*	P	AC	8-9	9-16	pn	128	8	<25

The structural differences mentioned above show definite correlation with the duration of the life cycle of the investigated species. Namely, the specific reproductive period, i.e. time span between fertilization and seed dispersal, varies from three to six weeks among species with different structure of antipodals. Species with proliferated antipodals are characterized by rapid seed development, e.g. *G. caucasea* needs only 16-20 days (9) for maturing of the seeds, *G. germanica* – 20-25 days (15), *Swertia iberica* - approximately 25 days. Other species with ephemeral antipodals have much prolonged specific reproductive period. According to our opinion, rapid seed development might be promoted by proliferated antipodals, which play an active role in supplying of the embryo with nutrients in the early stages of endosperm development and accelerates embryogenesis. This is similar to a number of angiosperms, in which antipodals play an active role in supplying the embryo sac with nutrients and growth controlling substances (17).

Evolution of the family Gentianaceae (18) is related with climate change during continental alternations at the end of Jurassic period and in Paleogene, when climate became cold and arid. The survival of the tertiary gentians adapted to more humid and warm climate became possible due to development of a new morphological type with xeromorphic characters. It is supposed (18) that a number of structural changes took place - the choripetalous corolla became sympetalous, leaves and stigma lobes narrowed, sympodial main stem have changed to monopodial, etc. These characters allowed successful adaptation of the new morphotypes to cold and arid climate. They occur in different combinations in modern gentian species and have a taxonomic value for taxonomists. We suppose that one more mechanism of adaptation to the severe environmental conditions might be shortening of a life cycle and origin of annual ephemers, which are well adapted to the cold and arid climate of alpine environment. This suggestion is true for species in which the combination of both characters – annual life form and proliferated antipodals occur. In *Swertia iberica* proliferated antipodals ensure rapid seed development, however, they are perennial plants flowering in late summer. *Gentiana prostata* is an annual herb, but with three reduced antipodals and long specific reproductive period (19). All these data confirm our earlier suggestion (9,14) that structural peculiarities of ovule and megagametophyte of the Gentian family reveal clear relationships with the life cycle determining successful reproduction and secure an establishment of a species in a concrete habitat.

Institute of Botany, Georgian Academy of Sciences
Kojori road 1, 0105 Tbilisi, Georgia
Head of the Laboratory of Plant Reproduction Research
E-mail: akhalkatsim@yahoo.com

REFERENCES

1. A. L. Takhtajan. Systema Magnoliophytorum. L., Nauka, 1987. (in Russian).
2. T-N. Ho, S-H. Liu, The infrageneric classification of *Gentiana* (Gentianaceae). Bull. Brit. Mus. Nat. Hist. (Botany) 20, 1990, 168-192.
3. Y.C. Ma. *Gentianopsis* – a new genus of Chinese Gentianaceae. Acta Phytotaxonomica. 1, 1951, 5-19. pls. I-V.
4. C. Faverger. Quelques aspects de l'evolution et de la phylogenie dans la familie des Gentianaceae. Atti dei Seminari di Fitochemica sulle piante continenti principi amari. 1985, 21-55.
5. Y-M. Yuan, P. Küpfer. Molecular phylogenetics of the subtribe *Gentianinae* (Gentianaceae) inferred from the sequences of internal transcribed spacers (ITSs) of nuclear ribosomal DNA. Plant Syst. Evol. 196, 1995, 207-226.
6. D.H. Maheshwari. Embryological studies in Gentianaceae (*Gentianoideae* and *Menyanthoideae*). Proc. Indian Acad. Sci. 56, 1962, 195-216.
7. Shamrov I.I. The family Gentianaceae In: Comparative Embryology of flowering plants. T.B. Batygina, M.S. Jakovlev (Eds), Leningrad, Nauka, 1987, 137-145. (in Russian)

8. I.I. Shamrov. Ovule development and significance of its features for Gentianaceae systematics. Opera Bot. Belg. 7, 1996, 113-118.
9. M. Akhalkatsi, J. Wagner. Comparative embryology of three Gentianaceae species from the Central Caucasus and the European Alps. Pl. Syst. Evol. 204, 1997, 39-48.
10. K.A.H. Stolt. Zur Embryologie der Gentianaceen und Menyanthaceen. Kgl. Svensk Vet.-Akad. Handl. 61, 1921, 1-56.
11. E. Öhler. Entwiklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentianaceen. Planta 3, 1927, 641-733.
12. I.I. Shamrov. The ovule of *Swertia iberica* (Gentianaceae): structural and functional aspects. Phytomorphology 41, 1991, 213-229.
13. I.I. Shamrov. Development of the ovule and structural peculiarities of the embryo sac of representatives of the family Gentianaceae. Bot. Zhurn**. 72,** 2, 1988, 213-222. (in Russian).
14. M. Akhalkatsi, J. Wagner. Reproductive phenology and seed development of *Gentianella caucasea* in different habitats in the Central Caucasus. Flora 191, 1996, 161-168.
15. J. Wagner, M. Achalkazi, S. Mayr. Anwendung quantitativ embryologischer Methoden in Entwicklungsbiologie und Reproduktionsökologie der Pflanzen. Anz. österr. Akad. Wiss., Math.-Naturwiss. Kl. 131, 1995, 7-18.
16. J. Herr. A new clearing-squash technique for the study of ovule development in angiosperms. Amer. J. Bot. 58, 1971, 785-790.
17. M.T.M. Willemse, J.L. Went van. The female gametophyte. In: Embryology of Angiosperms. B.M. Johri (ed.), Springer Verlag, Berlin-NY. 1984.
18. V.V. Zuev. Ecological and morphological evolution in the tribes of *Gentianeae* and *Swertieae* Griseb. of the Gentian family (Gentianaceae Juss.) on the example of *Siberian* species). Bull Mosk. obsh. ispitat. prirody. otd. biol. 97, 2, 1992, 131-143. (in Russian).
19. K.A.H. Stolt. Über die Embryologie von *Gentiana prostrata* und die Antipoden der Gentianaceen. Bot.Not. 1927, 225-242.