Evolutionary details of anthocyanin-coloring formation of corolla of flowering plants

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Abstract. The assumption about the connection of species evolutionary advancement with the flowering period and corolla anthocyanin and white coloring was checked on 85 species of concrete flora of the city of Chisinau and 147 species of potential flora of Moldova. It was established that with flowering period increase the families evolutionary advancement increases, the proportion of species with white and violet-blue corolla decreases, while the proportion of species with rose-purple corolla increases.
Key words: corolla, anthocyanin coloring, white coloring, evolutionary advancement, flowering periods.

Introduction. While studying the demographical situation of flowering plants, Illichevsky (1938) has paid attention that "the general way of flowering in flora in main features repeats the history of its development: at the beginning of the vegetation period the primitive types of flowers (flowers with superior ovary, with two rounds of androecium) dominate; at the end of summer higher types get sharply prevail, such as the flowers with inferior ovary, sympetalous ones, those with one round of stamen, etc. intensively bloom; as well as flora "creation wreath" – the most highly organized thistle family - absolutely dominates at the end of summer".

There are similar reasonings about the correspondence of flower coloring and species evolutionary advancement in works of Blagoveschensky (1966a, b). The author compares various anthocyanin colorings of flowering plants corolla between themselves, that are caused by delphinidine, cyanidine and pelargonidine, of which the first is considered as more evolutionally advanced pigment.

For Moscow region flora Nasimovich (1993) has stated, that the more evolutionally advanced the family is, the less white-flower species it includes. For example, in Moscow region white-flower species make: in Caryophyllaceae family 74%; in Rosaceae family 51%. There are many of them (from 16 to 33%) in Brassicaceae, Liliaceae, Orchidaceae, Ranunculaceae, Fabaceae families too. But in such families as Geraniaceae, Boraginaceae, Campanulaceae wild-growing white-flower species are completely absent. There are few of them (from 10 to 15%) in families of Scrophulariaceae, Violaceae, Lamiaceae, Asteraceae families. Thus, white flowers are more typical for the species, that are less evolutionally advanced.

Three main classes of anthocyanin pigments of the angiospermous plants (pelargonidine, cyanidine and delphinidine) differ first of all in the number of hydroxyl groups in a beta-ring: pelargonidines have the largest number, cyanidines have one hydroxyl group more while delphinidines even one hydroxyl group more (Britton 1980; Andersen & Jordheim 2006; Shoeva 2013). Depending on different endogenic and exogenous factors, delphinidine anthocyanins tend to be violet, blue or purple, cyanidine ones become blue, purple-red or red, while pelargonidine ones almost always are red or orange. Hopkins & Rausher (2012) established, that evolutionary transitions from colored corolla to white-flower ones are connected with the loss of function mutations, while the transitions from blue-violet to red-orange colors are caused by the anthocyanin synthesis switch from more hydroxylated to less hydroxylated. Thus, if flora phylogenesis is repeated in the phytocenosis ontogenesis, so the evolutionary transitions between the plants qualitative characteristics have to be repeated, in particular the evolutionary transitions between the flowering plants anthocyanin corolla coloring. The aim of the presented paper is to verify this assumption.

Material and Method. The main object of the study (2005-2014) was the plant cover of C layer in Chisinau city, Moldova. The species of plants were determined at the Department of Botany, Ecology and Forestry of the Chisinau State University. Flowering periods were established by several sources (Negru 2005, 2006, 2007; Ciocarlan 2000; Geideman 1986; Asseeva & Tikhomirov 1964). Releves of stationary plots was carried out according to the classical method of Rabotnov (1987). Corolla coloring was evaluated based on six-point scale, where 1 is light blue, 2 – blue, 3 – violet, 4 – rose, 5 – crimson, 6 – purple. In the tables white corolla color is marked by “0”, and corollas of yellow shades were designated by the letter “y” and were not taken into consideration during the analysis. The statistical analysis was made according to standard algorithms within “Excel” program range.

Results and Discussion. The evaluation of gamma diversity of concrete C layer flora of Chisinau demonstrated the presence of 178 species of herbaceous plants from 42 families. The most widely represented are five of them: Asteraceae (37 species), Poaceae (19 species), Fabaceae (16 species), Brassicaceae (11 species), Lamiaceae (8 species). The visual analysis of corolla coloring in this sample has shown four color groups: violet-blue (13 species), white (34 species), yellow-orange (41 species), purple-rose (34 species) and the groups with reduced corolla and/or corolla with coloring that was difficult to determinate (53 species). In this paper we analyze the species with supposed anthocyanin corolla coloring (light blue, blue, violet, rose, crimson, purple), as well as the group of white-flower plants (Table 1).

To check the assumption, that “the general way of flora flowering in main features repeats the history of its development”, we compared “T”, that is the order of the families evolutionary advancement according to the phylogenetic system of flowering plants classification made by Takhtadzhian (1966), and the order of the specific flora flowering on the plots studied in Chisinau. Having ranged the presented plants sample (85 species) into groups with 5 families in each, we have got 5 classes of families with averaged flowering periods (Figure 1). The trend line demonstrates the increase of the families evolutionary advancement with the increase of the average number of the month, in which flowering begins. Thus, if for the first five families the average number of the month, in which flowering begins, is equal to 4.89 ± 0.26 (May), for the second, the third and the fourth family classes it is 5.16 ± 0.14 and 5.18 ± 0.17 respectively, so for the last five families this index increases for about a month and makes 5.73 ± 0.13 (June).

Then before analyzing the plants species with flowers colored in anthocyanin colors we would like to check up the following assumption: does the proportion of white-flower plant species change with the change of the families evolutionary advancement? For this purpose, we have counted the proportion of white-flower species in each of the five above mentioned ones (Figure 2). The relative (referring to the anthocyanin-colored species) reduction of white-flower species proportion depending on the increase of the families evolutionary advancement was stated. In the first two classes of the families the proportion of white-flower species makes more than half of the species composition (57.14%); in the last two classes this index is approximately half lower (28.35%). Thus, our data, received in the investigation of the concret flora of Chisinau, have partially proved the results of Nasimovich (1994), based on the specific flora of Moscow region. The only difference was that in this comparison he took into consideration all found species of flowering plants, while we considered only anthocyanin-colored ones. The transitions between blue and purple flowers are caused by presence of pelargonidine, cyanidine and delphinidine pigments in flowers corolla.

In seven cases of the contrast transitions from blue to red coloring the reduction of anthocyanin hydroxylation was noticed. Thus, at least in two taxons of the angiospermous plants the evolutionary transitions from blue to red flowers are highly correlated with the pigment class change. It was assumed, that the pigment class change is caused by the inactivation of one or more branches of this way.

After the withdrawal of the species with white corolla coloring and the representatives of monocotyledonous ones (*Connvalaria maialis* L. and *Allium rotundum* L.) from the general list of the specific flora of Chisinau, the other species were divided into four classes with different evolutionary advancement. The comparative analysis of 25 families of dicotyledonous flower plants with different evolutionary advancement has shown the insignificant tendency to corolla anthocyanin coloring from blue (2.4) to rose

(3.7) (Figure 3). But due to non-equilibrium of the evolutionary classes (the first class includes 4 families and 5 species, but the four class includes 4 families and 20 species) this assumption requires more volume material studying.

Table 1

Concrete C level flora of Chisinau with anthocyanin and whit corolla color

N	*Species*	*T*	*C*	*F*	*N*	*Species*	*T*	*C*	*F*
1	*Consolida regalis* S. F. Gray	1	3	5.0	44	*Conium maculatum* L.	15	0	5.5
2	*Papaver dubium* L.	2	0	4.7	45	*Bryonia alba* L.	16	0	5.5
3	*Fumaria officinalis* L.	3	3	5.0	46	*Galium aparine* L.	17	0	5.0
4	*Silene moldavica* (Klok.) Sourkova	4	0	5.2	47	*Vinca minor* L.	18	2	4.7
5	*Stellaria media* (L.) Vill.	4	0	3.7	48	*Convolvulus arvense* L.	19	0	5.2
6	*Saponaria officinalis* L.	4	0	6.0	49	*Calystegia sepium* (L.) R.Br.	19	0	5.7
7	*Anagallis foemina* Mill	5	2	4.5	50	*Echium vulgare* L.	20	2	5.5
8	*Viola mirabilis* L.	6	1	4.5	51	*Miosotys arvensis* L. (Hill.)	20	1	4.5
9	*Viola odorata* L.	6	3	4.7	52	*Veronica chamaedris* L.	21	2	4.5
10	*Capsella bursa pastoris* (L.) Medik	7	0	4.0	53	*Veronica spicata* L.	21	3	5.5
11	*Alliaria petiolata* (Bieb) Cavara et Crande	7	0	4.5	54	*Ajuga genevensis* L.	22	2	5.5
12	*Crambe tatarica* Sebeok.	7	0	4.5	55	*Glechoma hederaceae* L.	22	2	4.0
13	*Arabidopsis thaliana* (L.) Heynh.	7	0	4.0	56	*Salvia nemorosa* L.	22	3	6.0
14	*Berteroa incana* (L.) DC	7	0	5.0	57	*Ballota nigra* L.	22	5	5.5
15	*Lepidium draba* (L.) Desv.	7	0	4.5	58	*Mentha piperita*	22	4	6.5
16	*Armoracea rusticana* Gaerth., Mey et Scherb	7	0	5.5	59	*Leonorus cardiaca* L.	22	0	6.5
17	*Lavatera thuringiaca* L.	8	4	6.2	60	*Lamium purpureum* L.	22	6	3.7
18	*Althea officinalis* L.	8	0	6.5	61	*Solanum dulcamara* L.	23	3	5.5
19	*Hibiscus trionum* L.	8	0	6.0	62	*Solanum nigrum* L.	23	0	6.0
20	*Urtica dioica* L.	9	0	6.0	63	*Datura stramonium* L.	23	0	6.0
21	*Lythrum salicaria*	10	5	6.2	64	*Campanula persicifolia* L.	24	2	6.0
22	*Vicia craca* L.	11	3	5.5	65	*Cicorium intubus* L.	25	2	5.7
23	*Vicia angustifolia* Reichard	11	5	5.0	66	*Lactuca tatarica* (L.) C. A. Mey	25	2	5.0
24	*Vicia villosa* Roth.	11	3	5.0	67	*Cardus acanthoides* L.	25	5	6.0
25	*Viciatetrasperma* (L.) Moench	11	1	5.0	68	*Cardus nutans* L.	25	5	5.7
26	*Trifolium pratense* L.	11	5	5.7	69	*Cardus hamulosus* Ehrh	25	6	5.2
27	*Trifolium fragiferum*	11	5	5.0	70	*Arctium lappa* L.	25	6	6.5
28	*Trifolium montanum*	11	0	5.0	71	*Lathyrus tuberosus* L.	25	5	5.5
29	*Coronilla varia L.*	11	4	6.0	72	*Cyrsium palustre* (L.) Scop.	25	5	6.5
30	*Melilotus albus Medic*	11	0	6.0	73	*Cyrsium arvense* (L.) Scop	25	4	6.5
31	*Linum austriacum* L.	12	1	4.7	74	*Xeranthemum annuum* L.	25	3	6.5
32	*Geranium robertianum* L.	13	5	5.0	75	*Onopordum acanthium* L.	25	5	6.5
33	*Geranium pratense* L.	13	2	6.0	76	*Centaurea pseudomaculosa* Dobrokz	25	5	6.0
34	*Polygonum aviculare* L.	14	0	5.5	77	*Galinsoga parviflora* Cav	25	0	6.5
35	*Polygonum hidropiper* L.	14	0	6.5	78	*Achillea milefolium* L.	25	0	6.0
36	*Polygonum persicaris* L.	14	0	7.0	79	*Erigeron annuus* (L.) Pers.	25	0	6.0
37	*Rumex conglomerates* Murray	14	0	6.5	80	*Tripleurospermum inodorum* Sch. Bip.	25	0	5.0
38	*Fallopia convolvulus* (L.) A. Love	14	0	5.5	81	*Centaurea difussa* Lam.	25	0	6.0
39	*Conyza canadensis* L.	15	0	6.5	82	*Chamomilla recutita* (L.) Rauschert	25	0	4.5
40	*Daucus carota* L.	15	0	5.5	83	*Crepis pannonica* (Jacg.) C. Koch	25	0	6.5
41	*Heracleum sibiricum* L.	15	0	6.0	84	*Connvalaria maialis* L.	26	0	4.5
42	*Anthriscus sylvestris* (L.) Hoffm	15	0	5.2	85	*Allium rotundum* L.	27	6	6.7
43	*Caucalis platycarpos* L.	15	0	5.5					

Comments: C – color, F - flowering periods, T - order of the families evolutionary advancement.

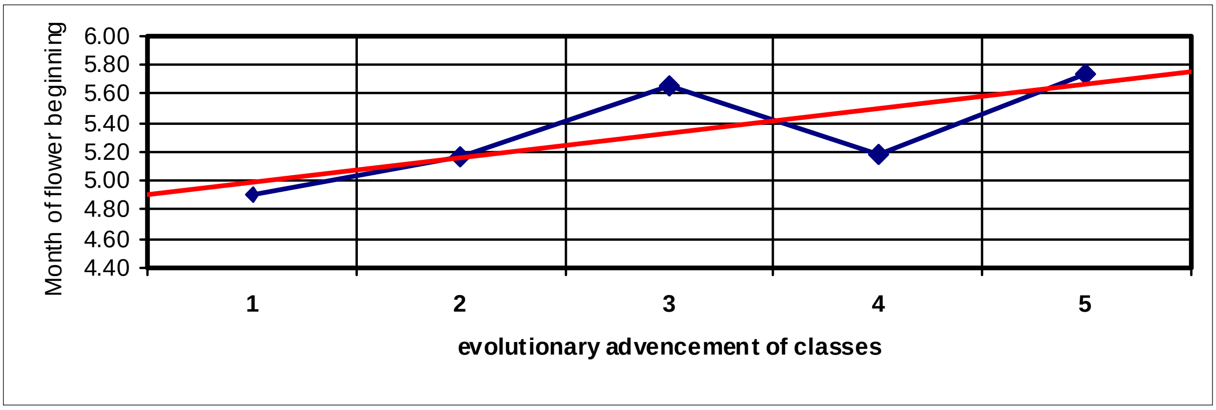

Figure 1. Distribution of classes of dicotyledonous flowering plants with different evolutionary advancement on flowering time scale (1-5 is the species evolutionary class).

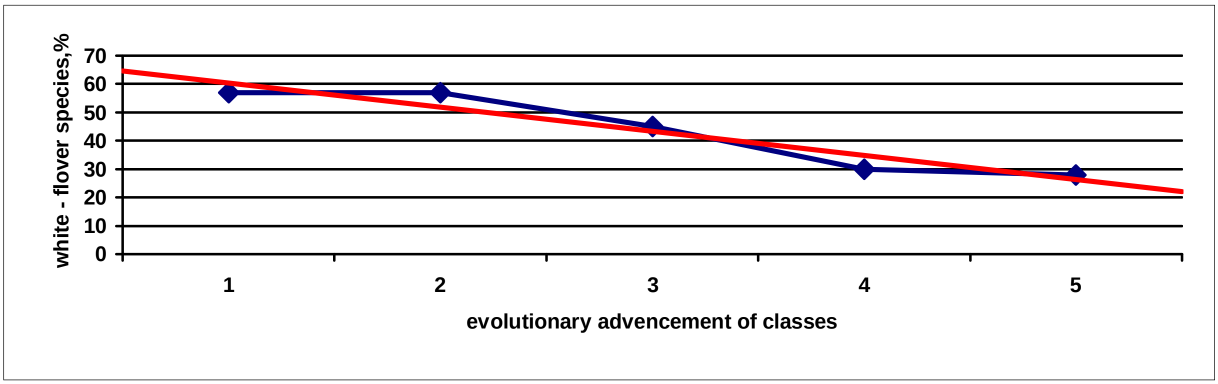

Figure 2. White-flower species proportion in classes of dicotyledonous flower plants with different evolutionary advancement (1–5 are the species evolutionary classes).

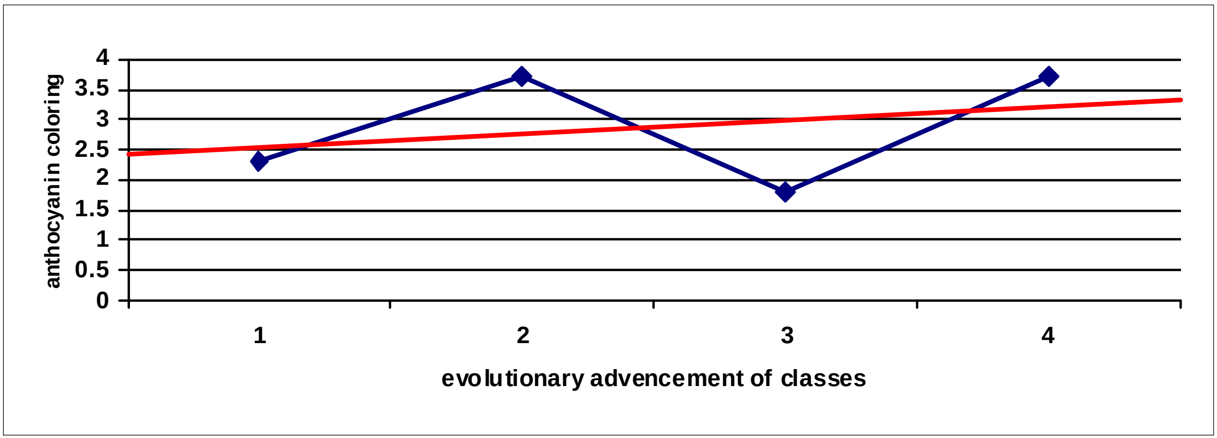

Figure 3. Arrangement of dicotyledonous flower plants species with anthocyanine-colored corolla on the scale of the families evolutionary advancement (1-4 is the species evolutionary classes).

Rausher (2008) while studying the transitions between the anthocyanin coloring of the angiospermous plants of *Penstemon* and *Ipomoea* genera, made a conclusion, that no one case of the synthesis switching from cyanidine to pelargonidine is known without flower coloring change from blue to red.

To simplify the studying of corolla anthocianin coloring dependency on the species evolutionary advancement on the level of genus taxons, we have chosen three flower plants genera (Table 2), that were different in their evolutionary advancement and that were characterized by the species composition enough for comparison: *Viola* (the 1-st class), *Vicia* (the 2-nd class), *Centaurea* (the 4-rd class). The anthocyanin coloring shades, valued basing on the conditional scale from 0 (blue) to 6 (red), were arranged in such an order, in which they are changed at hydroxylation reduction. The mosaic corolla coloring was estimated by the most hydroxylated shade, as in this case the maximum level of hydroxylation does not reduce, simply the intermediate (less hydroxylated) products are also laid in flower tissues. Non-anthocyanin species (with white and yellow carolla) were not taken into consideration, as their anthocyanin way was inactivated in the very beginning of the biochemical chain, and we cannot consider the state of the genes, responsible for the subsequent reactions, including hydroxylation.

Table 2

Genera *Viola, Vicia* and *Centaurea* representation in potential flora of Moldova and their distribution according to corolla coloring and flowering periods (based on Geideman, 1986)

N	*Viola*	*C*	*F*	*Vicia*	*C*	*F*	*Centaurea*	*C*	*F*
1	*V. alba*	0	3-4	*V. hirsuta*	0	5-6	*C. thirkei*	0-6	5
2	*V. jordanii*	0	3-5	*V. grandiflora*	0	5-7	*C. diffusa*	0-j	6-9
3	*V. arvensis*	0	4-9	*V. tetrasperma*	1	5-6	*C. angelescui*	1-2	5-6
4	*V. nemausensis*	0	3-5	*V. pisiformis*	1	5-6	*C. cyanus*	2	5-10
5	*V. mirabilis*	1	4-5	*V. villosa*	3	5-8	*C. jacea*	3	6-10
6	*V. palustris*	1	4-5	*V. biennis*	3	5-6	*C. rhenana*	4	6-10
7	*V. collina*	1	4-5	*V. dumetorum*	3-1	5-6	*C. marschaliana*	4-5	5-6
8	*V. tanaitica*	1	4-5	*V. sylvatica*	3-1	5-6	*C. pseudomaculosa*	5	6-8
9	*V. elatior*	1	4-5	*V. cracca*	3-2	5-8	*C. bibersteinii*	5	6-9
10	*V. kitaibeliana*	1	4-7	*V. lathyroides*	3-4	5-6	*C. caprina*	5	6-8
11	*V. suavis*	2	4-5	*V. cassubica*	3-0-5	5	*C. pannonica*	5	6-8
12	*V. hirta*	2	4-5	*V. striata*	3-6	5	*C. substituta*	5	7-8
13	*V. riviniana*	2	4-6	*V. peregrina*	5	5	*C. trinervia*	5	6-8
14	*V. odorata*	3	4-5	*V. pannonica*	5	5-7	*C. trichocephala*	5	6-9
15	*V. ambiqua*	3	4-5	*V. tenuifolia*	5	5-8	*C. pseudophrygia*	5	7-9
16	*V. reichenbachiana*	3	4-5	*V. sepium*	5	5-6	*C. stenolepis*	5	8-10
17	*V. canina*	3	4-6	*V. angustifolia*	5-6	5-6	*C. adressa*	5	6-9
18	*V. pumila*	3	4-6	*V. sativa*	5-6	5-6	*C. apiculata*	5	6-9
19	*V. persicifolia*	1-0-j	5-6				*C. stereophylla*	5	6-10
20	*V. matutina*	2-1-0-j	5-9				*C. iberica*	5	6-9
21	*V. tricolor*	3-1-0-j	6-8				*C. scabiosa*	6	6-10
22							*C. lavrencoana*	6	6-8
23							*C. solstitiales*	j	5-10
24							*C. adamii*	j	5-9
25							*C. orientalis*	j	6-10

Comments: C – color, F - flowering periods.

In *Viola-Vicia-Centaurea* row (Tables 3) a definite shift towards later flowering and less hydroxylated coloring is observed. As this genera order coincides with traditional understanding of their evolutionary advancement (Takhtadzhian 1980), it is possible to assume, that younger genera tend to less hydroxylated coloring, take later seasonal niche and concede the earlier season to more primitive genera.

Table 3

Comparison of *Viola, Vicia, Centaurea* genera basing on anthocyanin coloring and flowering periods

Genus	*Coloration in conventional units*			*Flowering periods*		
	Mean	*No of species*	*Difference for Student, t*	*Mean*	*No of species*	*Difference for Student, t*
Viola	1.83±0.27	18	2.77**	4.05±0.14	22	6.37***
Vicia	3.19±0.41	16		5.05±0.06	18	
Centaurea	4.62±0.27	21	2.31**	5.92±0.14	25	6.10***

Comments: ** $p < 0.01$; *** $p < 0.001$.

In order to make a valid conclusion, we added the genders from the potential flora of Moldova (Geideman 1986) to the list of genera *Viola, Vicia, Centaurea*, being compared.

Thus, the first group of the most evolutionary advanced genera included genera as follows: *Viola* – 18 species, *Clematis* – 4 species, *Pulsatilla* – 3 species, *Nigella* – 4 species, *Fumaria* – 4 species and *Lythrum* – 3 species. The second group included *Vicia* genus - 16 species, *Geranium* – 14 species and *Veronica* – 27 species. The third, the most evolutionary advanced group, was represented by *Centaurea* genus – 21 species, *Cirsium* – 13 species and *Salvia* – 10 species. Due to the species small number of the first group genus, the average value of corolla coloring and flowering periods was found not for each gender separately, but for the sum of all genera (except *Viola* genus). The results, given in Figures 4 and 5, confirm the conclusions, made in comparison of *Viola, Vicia, Centaurea* genera, based on anthocyanin coloring and flowering periods.

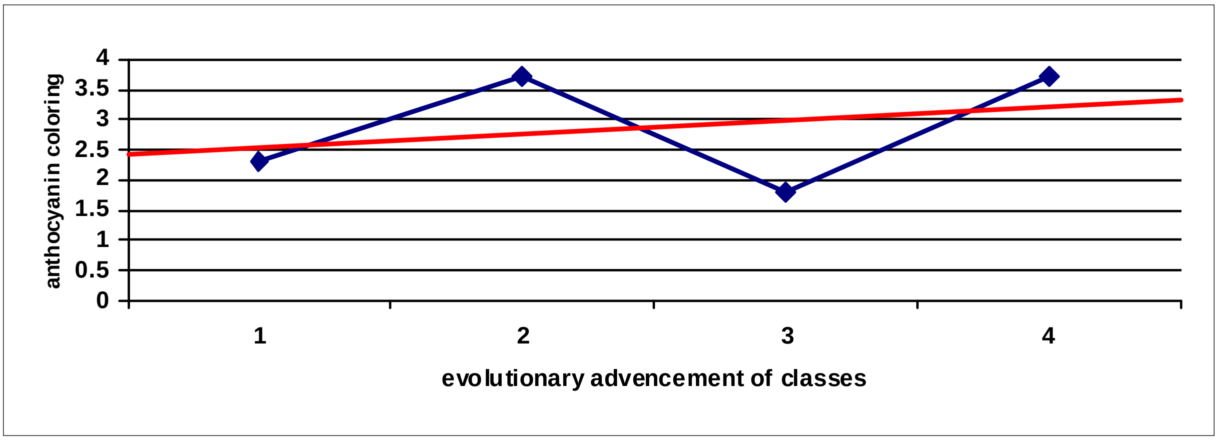

Figure 4. Distribution of genera with different evolutionary advancement (1, 2, 3) according to anthocyanin coloring type (1-6).

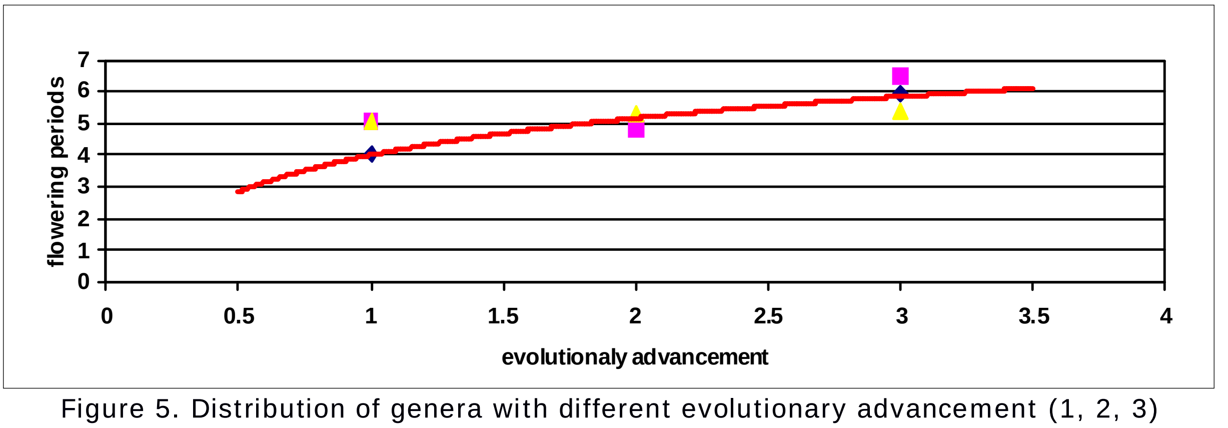

Figure 5. Distribution of genera with different evolutionary advancement (1, 2, 3) according to flowering periods.

Conclusions. The reduction of white-flower corolla species and the increase of anthocyanin-colored corolla species are observed with the increase of families evolutionary advancement.

For anthocyanin-colored corolla species the reduction of anthocyanin pigments average hydroxylation is recorded with the increase of the families evolutionary advancement, that is shown in the reduction of proportion of the species with light blue, blue and violet corolla, as well as in the proportion increase of the species with rose, crimson and purple corolla.

There is a connection between the species flowering periods and their evolutionary advancement, so with the flowering periods increase the families evolutionary advancement increases, the species proportion with white and violet-blue corolla reduces, and the species proportion with rose-purple corolla increases.

The seasonal convergence of flowering plants species was stated basing on corolla coloring.

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