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Experimental Study of Floral Morphogenesis I. Study of Developmental Possibilities of Floral Primordia in *Campanula rapunculoides* L. and *Veronica austriaca* L. subsp. *austriaca*

Experimentální studie květní morfogeneze
I. Studie vývojových možností květních základů
Campanula rapunculoides* L. a *Veronica austriaca* L. subsp. *austriaca

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Abstract — Spraying plants with maleic hydrazide solution in the early stages of inflorescence differentiation results in a number of floral abnormalities. Their study permits to follow the possibilities of floral primordia development, the metamorphosis of floral members, as well as their phylogenetic origin. The acropetal succession of the development of floral primordia in racemose inflorescence is the reason why the maleic hydrazide effect affects floral primordia in different stages of their development and gives rise to metamorphoses of different quality. The more original and primitive forms arise from altogether undifferentiated primordia on the inflorescence apex. The more developed and more complex structures originate from primordia in more advanced stages at the base of inflorescence. An inhibitory intervention in the differentiation of the actinomorphic primordia in *Campanula rapunculoides* L. brings about a greater number of zygomorphic flowers and floral members reductions. A stimulative intervention in the differentiation of zygomorphic primordia in *Veronica austriaca* L. subsp. *austriaca* results in the origin of actinomorphic flowers, whose floral number resembles the theoretical number of the family *Scrophulariaceae*. The results obtained corroborate the theory of phylogenetic recapitulation.

The floral morphology of *Zea mays* L. helps us to realize that the meristematic tissues of the reproductive organs are endowed with numerous potential possibilities of growth and development. A considerable number of these developmental possibilities, however, are affected by regulation processes, so that finally only those of them get actualized which give rise to forms characterizing the respective species and variety (Spraque 1955). Judging from this point of view, we may see in the final structure of any flower the outcome of just a small portion of the potentional development of floral primordia.

If the normal succession of the differentiation processes of floral primordia gets disturbed, it usually results in the origination of abnormalities. The seemingly incidental abnormalities are not a freak of nature but they mirror to a great extent the laws of evolutionary processes, as Čelakovský 1896, Velenovský 1910 and Vuillemin 1926 already pointed out, and they can impart important pieces of knowledge about the metamorphosis of floral members. Domin comes in 1923 to the conclusion that when studying abnormalities minutely we do not find, in fact, any essential difference between an abnormal form and a normal one.

Even though the occurrence of abnormalities is in nature a rather rare phenomenon, Daumann's works 1964 show that having investigated a large quantity of material we can make up of the spontaneous abnormalities even a continuous succession demonstrating the metamorphoses of floral members as well as their origin. But apart from the spontaneous abnormalities, it is possible to call forth such phenomena also by experiment (Klebs 1906). Here, however, success depends on detailed knowledge of differentiation processes of floral primordia and on the ability of determining a suitable intervention in a suitable developmental-stage. Deliberately produced abnormalities not only mirror the laws of development of floral members, but also enable us to follow the interventions in different developmental stages of floral primordia. Research which adheres to these principles can obtain remarkable results. Dostál (1960) managed to produce by simple surgical interventions from non-differentiated primordia atavistic forms that resembled ancestral predecessors of the present forms. The pinnate leaves of *Aesculus hippocastanum* L. show that this species is connected with the older and more primitive family *Sapindaceae*, while the ternate leaves of *Pisum sativum* L. betray its appurtenance to the family *Fabaceae*.

The aim of our work was to disturb by suitable chemical interventions normal succession of differentiation processes of floral primordia in racemose inflorescence and thus to call forth a number of abnormalities. By studying the latter we wished to obtain more precise knowledge of the potential capabilities of floral primordia, of the metamorphosis of floral members and of the possibility of their phylogenetic origin. To accomplish these interventions we made use of maleic hydrazide solution (MH) spraying the plants with it at the onset of inflorescence differentiation after regular weekly intervals. In the first series of experiments we produced changes in the course of differentiation of the actinomorphic primordia in *Campanula rapunculoides* L., while the second series was reserved for studying varying effect of such interventions on the differentiation of zygomorphic primordia in *Veronica austriaca* L.

Material and Methods

For our experiments we have used the plants *Campanula rapunculoides* L. and *Veronica austriaca* L. subsp. *austriaca*. Both are grown in the flower-beds in the physiological garden at the J. E. Purkyně University, Brno. The young budding plants displaying the commencement of differentiation of floral primordia we sprayed regulary every week with the solution of maleic hydrazide. We protected the plants from rain with bell jars. Maleic hydrazide, by exercing an inhibiting effect and disturbing the regularity of growth, resulted in a number of floral abnormalities. The spray of *Veronica austriaca* L. subsp. *austriaca* with stronger concentrations brought about in the year of spraying (1964) a vegetative growth of stems. The stems bore flowers first in the following year (1965) and showed a number of abnormalities, associated with stimulation effect.

We used a binocular microscope to follow the acropetal succession of the floral primordia development in racemose inflorescence. The accelerated growth of the abaxial floral members of *Veronica austriaca* L. subsp. *austriaca* was compared with the content of the endogenous, growth regulating substances in adaxial and abaxial halves of floral primordia. For cutting the primordia we have employed a micromanipulator, while for testing the content of the growth substances agar plates and the *Avena* coleoptile curvature test according to Söding (1952) was used.

Experiments and Results

The differentiation of floral members of the primordia in *Campanula rapunculoides* L. progresses evenly and symmetrically. In the initial differentiation stages five small elevations appear, which turn into actinomorphic bell-shaped corolla. Theoretically we assume that the corolla originated through coalescence of five separate petals, and we find indication thereof in the existence of the five main veins running through the coalesced part of corolla and terminating in the separate corolla lobes.

Spraying the plants *Campanula rapunculoides* with 0,1 % solution of maleic hydrazide disturbed the normal succession of differentiation processes and called forth a whole scale of abnormalities (Plate XXIV). The most frequent occurrence was the coalescence of two upper corolla lobes resulting in the origination of zygomorphic pentamerous or tetramerous flowers. Further fusion or coalescence of corolla lobes gave rise to tetramerous or trimerous flowers, both actinomorphic and zygomorphic. The origin of zygomorphic flowers had as a rule for its concurring phenomenon the formation of dimerous pistils.

Table 1. Morphogenic effect of maleic hydrazide on floral primordia of *Campanula rapunculoides* L.

	Floral formulas					Number of flowers
Control	☿ *	K5	C(5)	A5	$G(\bar{3})$	400
Maleic hydrazide 0,1%	☿ ↓	K5	C(5)	A5	$G(\bar{3})$	29
	☿ *	K4	C(4)	A4	$G(\bar{3})$	13
	☿ ↓	K4	C(4)	A4	$G(\bar{2})$	21
	☿ *	K3	C(3)	A3	$G(\bar{2})$	5
	☿ ↓	K3	C(3)	A3	$G(\bar{2})$	26
	♀ ↓	K2	C2	A0vel2	$G(\bar{2})$	9

The extent of the induced changes was in a striking correlation with the acropetal succession in the development of floral primordia in racemose inflorescence. Whereas at the base of inflorescence there originated flowers with only slight deviations in structure, in the center or in apical position one could find flowers with ancestral signs (separate petals). To get a good survey we have arranged the abnormalities in a table with floral formulas (Table 1). The MH effect was in all cases inhibitive and it contributed to the development of the zygomorphic flowers, which again implied a reduction of floral members. The zygomorphic flowers are four times as numerous as the actinomorphic ones. It is noteworthy that the floral number in the structure of abnormal flowers was the same in each case with a few exceptions relating to gynoecium.

When compared to the above-described species, the floral differentiation of *Veronica austriaca* L. subsp. *austriaca* is characterized with a quicker growth and development of the abaxial parts of floral primordia. In the bract axil we can observe a quicker development of those floral members which in maturity form the lower half of the flower. The differentiation of the upper stamens in transverse position is fast and exceeds in speed the growth of the inward floral members. The primordia of the other petals bear no visible traces of other abortive stamens. The pistil is the last to undergo differentiation. From Table 2 one may assume that the greater quantity of growth substances in the abaxial half of floral primordia cooperates in producing zygomorphic flowers. Although the obtained values are only relative and keep within the limits of biological variability they, neverthless, indicate that the level of the growth substances distinctly varies in floral primordia already. Maleic hydrazide produces changes in the auxin gradient in floral primordia. In adult normal flowers of control plants the unstability of the number of sepals attracts interest, especially in relation to the locality of inflorescence. At the base the flowers have five sepals, in the center the fifth sepal tends gradually to decrease, while in apical position we find flowers with four sepals only.

Similarly as in the first set of experiments also here the racemose type of inflorescence enabled us to affect floral primordia in different stages of development. Spraying plants with different

Table 2. Amount of growth substances in floral primordia of *Veronica austriaca* L. subsp. *austriaca* subjected according to SÖDING to *Avena* coleoptile curvature test. Average of 10 measurements

	control plants	Plants sprayed with maleic hydrazide
Adaxial half of floral primordia	0	4
Abaxial half of floral primordia	8	3

Table 3. Morphogenic effect of maleic hydrazide on floral primordia of *Veronica austriaca* L. subsp. *austriaca*

Concentration of maleic hydrazide	Floral formulas					Number of flowers	
						1964	1965
0.025% — 0.075%	☿	*	K(8)	[C(8)A3vel4]	G(2)+(3)	2	6
	☿	↓	K(7)	[C(7)A3]	G(2)+(1)	8	14
	☿	*	K(6)	[C(6)A4]	G(2)	1	2
	☿	↓	K(5)	[C(5)A3vel4]	G(2)	14	50
Control	☿	↓	K(4vel5)	[C(4)A2]	G(2)	800	1000
0.1% — 0.2%	☿	↓	K(4)	[C(4)A1]	G(2)	9	20
	♀	↓	K(4)	C(4)	G(2)	12	
	♀	*	K(3)	C(3)	G(2)	19	
	♀	⊕	K2	C2	G(2)	31	

maleic hydrazide concentrations in the year 1964 resulted in a number of abnormalities (Plate XXV) not only in the same year but also in the following one. In 1964 abnormalities consisting of the reduction of floral members predominated. On the contrary, in the following year we could observe flowers with greater number of floral members. As to inflorescence itself, we found that the maleic hydrazide effect corresponds to the toxicity curve. When average concentration was applied, there appeared at the base of inflorescence first two or three flowers with a reduced number of floral members, next one or two normal flowers and, finally, two or three flowers with an increased number of floral members. The total number of occurring abnormalities is to be found in Table 3, which not only gives the numeral occurrence of each of them in the first and the second year respectively, but also their dependence on MH concentration. The Table shows that the number of perianth whorls is to a certain extent a characteristic feature of the floral structure as a whole. The coalescence or doubling of sepals in the single flowers can be understood better when following the same processes occurring with corolla lobes. The most frequent is the doubling of the lower middle petal and the origin of the zygomorphic pentamerous flowers. We also encountered quite frequently zygomorphic flowers with doubled transverse petals, which means that heptamerous flowers came into being. Occasionally there occurred a split of a coalesced upper petal in the median position, which again gave rise to actinomorphic hexamerous or octamerous flowers. Reductions of floral members, called forth by higher concentrations in the first year, brought into being actinomorphic pistillate trimerous flowers. When reduction occurred even in the upper petals, there originated a dimerous pistillate flower with separate sepals and separate petals. Variability in the number of stamens was substantially higher. The occurrence of three or four stamens in the flower was frequent. The fifth upper stamen in the middle was quite an isolated case, and in its place we could observe a staminode of a corollalike colour. Of all the floral members pistil was least liable to changes. Only in some cases two syncarpous pistils originated, the number of carpels in one case being $G(\underline{2}) + (\underline{1})$ and in the other case $G(\underline{2}) + (\underline{3})$. The ovaries contained a lower number of seeds, whose power of germinating was only 2%.

Discussion

From the results obtained it is clear that experimental morphology may contribute towards the solution of some questions concerning metamorphoses of floral members and their phylogenetic origin. New ways of investigation open, at this cross-road, where arguments of comparative morphologists and taxonomists end on one hand and those of physiologists and biochemists on the other hand. It is sure that morphogenetic processes are closely connected with factors regulating growth. The papers of Resende (1953) and Heslop-Harrison (1958) point out that the changes of the gradient of endogenous auxin in meristematic tissues play a decisive part in the differentiation of floral primordia. These authors show that higher concentration of synthetic auxins inhibit the development of petals and stamens and promote the growth of sepals and pistils. It is true that the relation between maleic hydrazide and floral morphogenesis and physiological feminization of flowers is known (Naylor 1950 and Witwer 1954), but the way of its effect has not yet been understood. As we have shown in another study (Sladký 1966), MH affects also the level of growth substances in meristematic tissues of floral primordia. There is no doubt that the complexity of the problem is considerable (Ruhland 1965) and that it requires primarily a biochemical elucidation.

The disturbance of normal differentiation processes of floral primordia in various stages of their development discloses latent possibilities of development which floral primordia are endowed with. It is the acropetal character of the developmental processes that floral primordia undergo in racemose inflorescence which helps us precisely to determine the form of intervention. The primordia differentiation progresses gradually from the base to the apex, so that we can deal during inflorescence with a scale of different developmental stages and perform interventions of various intensity, for shorter or longer stretches of time, and repeat them if necessary. An intervention in the development of the youngest primordia in apical inflorescence produces ancestral forms, displaying characteristic features of the more primitive families (separate petals, apocarpous gynoecium, corolla coloured staminodes). An intervention in later stages of development at the base of inflorescence calls forth rather secondary changes, such as supplement of the characteristic features of the respective family (4—5 stamens, splitting of coalesced petals, actinomorphic flowers). It stands to reason that in the case of *Veronica*, whose normal design of floral structure already considerably differs from the theoretical floral diagram of the family *Scrophulariaceae*, the potential developmental possibilities are bound to be greater than in *Campanula*. The fact that ancestral forms originate from the youngest primordia, while the secondary forms from primordia in more advanced stages of development may be considered as corroboration of HAECKEL's biogenetic law, according to which an individual passes during its ontogeny through an abriged recapitulation of its phylogeny. Today we see in these various forms of abnormalities realized possibilities of the genetic pattern, in which the evolutionary process is embedded (GOTTSCHALK 1964).

Souhrn

Postřik rostlin roztoky maleinhydrazidu v době diferenciace květenství vyvolá řadu květních abnormit. Jejich studium dovoluje sledovat vývojové možnosti květních základů, metamorfozu květních částí i jejich fylogenetický původ. Akropetální charakter vývoje květních základů v racemosním květenství je příčinou, že účinek maleinhydrazidu postihl květní základy v různých fázích vývoje a vyvolal vznik abnormit různé kvality. Původnější a primitivnější tvary vznikají ze základů zcela nediferencovaných na apikále květenství. Odvozenější a složitější tvary vznikají ze základů vývojově pokročilejších na bazi květenství. Inhibiční zásah do diferenciace aktinomorfních základů *Campanula rapunculoides* L. vyvolá vznik většího počtu zygomorfních květů a redukci květních částí. Zásah stimulačního charakteru do diferenciace zygomorfních základů *Veronica austriaca* L. subs. *austriaca* vyvolá vznik květů aktinomorfních, které svým květním diagramem se blíží theoretickému diagramu čeledi *Scrophulariaceae*. Výsledky podporují koncepci fylogenetické rekapitulace.

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Explanations of the plates:

Tab. XXIV.: Scale of floral abnormalities induced by spraying plants of *Campanula rapunculoides* L. with solutions of maleic hydrazide during inflorescence differentiation. Normal flower above left.

Tab. XXV. Scale of floral abnormalities induced by spraying plants of *Veronica austriaca* L. subsp. *austriaca* with solutions of maleic hydrazide during inflorescence differentiation. Normal flower below left.

Zprávy o literatuře

E. Boureau:

Traité de Paléobotanique. Tome III. ***Sphenophyta-Noeggerathiophyta***

Masson et Cie, Paris 1964, 544 str., 436 obr., cena váz. 185 F

Pod hlavní redakcí ED. BOUREAUa, známého francouzského paleobotanika, začalo r. 1964 vycházet rozsáhle založené dílo podávající úplný soubor všech dosavadních znalostí z oboru paleobotaniky, plánované celkem do devíti svazků. Jeho smyslem je obohatit francouzskou odbornou literaturu o podobné, ba ještě podrobnější dílo, jakým jsou v anglické literatuře známé SEWARDovy Fossil plants (I—IV, 1898—1919) a Plant life through the ages (1931), spisy dnes již poněkud zastaralé, neboť od doby jejich zveřejnění uplynulo 30 až 50 let, během kterých paleobotanika vlivem některých úspěšných nových pracovních metod doznala velmi bouřlivý rozvoj. Jako první část z celého díla vyšel recensovaný svazek, věnovaný skupinám *Sphenophyta* a *Noeggerathiophyta*, napsaný samotným hlavním redaktorem ED. BOUREAUem. Jde o dvě z pěti jím celkem vymezených základních příbuzenských skupin (embranchements) pteridofytních (*Psilophyta, Lycophyta, Sphenophyta, Noeggerathiophyta, Filicophyta*). Pojednává o nich slohem velmi stručným, ale přitom do všech známých podrobností morfologických, anatomických i taxonomických. Zachycuje zde všechny dosud známé druhy z celého světa, s patřičnými poznámkami o nalezištích i stratigrafickém výskytu. Nalezneme tu i četné úvahy o jejich fylogenetických vztazích, o jejich ekologii i data paleogeografická. Skupinu *Sphenophyta* rozděluje do čtyř řádů (*Hyeniales, Pseudoborniales, Sphenophyllales* a *Equisetales*) a jedné pomocné skupiny typů primitivních a taxonomicky nejasných (většinou ze siluru a devonu). Významné je, že zde v rámci řádu *Equisetales* vhodným způsobem vyřešil též taxonomické postavení některých nálezů z permokarbonu oblastí angarsko-sibiřských a z mesozoika, vížících se na známé rody *Phyllotheca* a *Schizoneura*. K dosud běžně známým čeledím připojil čeledi *Sorocladaceae* (s angarsko-sibiřskými permokarbonskými rody *Sorocladus, Korethrophyllites, Corynophyllites*), *Neurophyllaceae* (s rodem *Neurophyllum* z permu v Koreji), *Phyllothecaceae* (s rody *Stellotheca, Annulina, Gamophyllites, Umbellophyllites, Raniganjia* a *Equisetina* — vesměs z permokarbonu angarsko-sibiřského nebo gondwánského, jakož i známý rod *Phyllotheca*) a *Schizoneuraceae* (s rody *Schizoneura* a *Paraschizoneura*). Rozlišuje tak v řádu *Equisetales* celkem 9 čeledí (*Archaeocalamitaceae, Autophyllitaceae, Apocalamitaceae, Calamitaceae, Sorocladaceae, Phyllothecaceae, Neurophyllaceae, Schizoneuraceae* a *Equisetaceae*). K starobylým nejasným typům silursko-devonským pak řadí též dost záhadné rody *Broeggeria* a *Haspia*, ač u nich dosud nebylo pozorováno ani přeslenité sestavení listů, ani článkovanost stonků. Druhou projednávanou základní skupinu *Noeggerathiophyta* dělí ve tři řády: *Noeggerathiales, Discinitales* a *Tingiales*, a to podle známých fruktifikačních šišticovitých souborů. Okruh svrchnokarbonských rhakopterid mezi nimi neuvádí, ač některá zjištění na jejich vztahy k těmto typům dost nasvědčují (vzácné nálezy fruktifikací!). Kniha je velmi bohatě ilustrována a obsahuje též velmi objemný seznam příslušné literatury z celého světa. Lze očekávat, že po vyjití ostatních svazků se toto dílo stane nepostradatelnou příručkou pro všechny pracovníky v paleobotanice a biostratigrafii a jistě též pro odborníky v nejrůznějších oborech botaniky, jmenovitě z hledisek taxonomických, morfogenetických a snad i geobotanických, asi tak jako dosud sloužily zmíněné spisy anglické.

F. Němejc

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