Cytogenetic studies on the genus *Aquilegia*

IV. Fertility relationships among the *Aquilegia* species

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In the genus *Aquilegia* different species complexes were distinguished (Grant 1952). Grant has established the division into complexes on the basis of flower morphology. Taxa belonging to one complex are characterised by a similar flower structure and are delimited from those belonging to other complexes due to their definite morphological distinctiveness. Between individual taxa of one complex there is a definite morphological continuity, and thus often one form substitutes for another on different, neighbouring ecogeographical regions (Payson 1918). Differences in flower structure are closely related to the type of pollinators that visit the various flowers. They are a reflection of the plants adaptation to the pollinators.

According to Grant (1949, 1950, 1952) *Aquilegia*, as well as *Antirrhinum*, *Delphinium*, *Asclepias* or *Ophrys*, represents a typical example of a genus with highly specialised flowers, in which the main role of an isolation barrier between taxa, or their complexes, is played by mechanical and ecological characteristics, where genetic barriers do not occur.

When the genetical analysis of the hybrids was described (Pražmo 1960, 1961, 1965) it was pointed out that, contrary to the generally assumed opinion (Payson 1918; Anderson 1931; Kappert 1944; Pelton 1958; Linnert 1961) not all taxa of *Aquilegia* cross equally readily and not all combinations will yield fertile hybrids.

As has been found, the division of *Aquilegia* into complexes, which maintain some degree of affinity in relation to each other, could be accomplished not only on the basis of morphological differences, but also on the basis of crossability and fertility of the hybrids.

On the basis of what is known about the pollination mechanism in *Aquilegia* (Grant 1951, 1952; Schneck 1901; Pickens 1931; Graenicher 1910), on the basis of the genetical analysis of the hybrids (Skalińska 1928, 1929, 1931, 1935; Pražmo 1960; 1961, 1963) and from the data about the ecogeographical distribution, it was possible to estimate the approximate degree of affinity between the different complexes.

As can be assumed *A. ecalcarata* is phylogenetically the oldest taxon. Taxa of the *vulgaris* and *alpina* complexes are its nearest relatives. Taxa of the complexes *canadensis* and *caerulea* are much more distant in affinity.

It was a purpose of this study to investigate crossability and fertility of hybrids in the first and later generations, for different taxa between complexes of species and thus estimate the degree of affinity between the delimited complexes.

MATERIALS AND METHODS

Taxa used for the crosses represented all the complexes distinguished within the genus *Aquilegia*. Most of them were obtained from the Warsaw and Wrocław botanical gardens. Each complex however was represented by at least one taxon coming from natural stands. Details concerning the origin of the different taxa are given in the paper published in 1965.

A. ecalcarata was used as a representative of the *ecalcarata* complex. The *vulgaris* complex was represented by three populations of *A. vulgaris*; *A. vulgaris* 32, *A. vulgaris* 13 and *A. vulgaris* var. *stellata* and also by *A. japonica*, *A. baicalensis*, and *A. flabellata*. *A. vulgaris* 13 originated from a natural stand in the Białowieża Forest in Poland.

The *alpina* complex was represented only by *A. alpina* (there is some doubt whether it is a representative of the pure *A. alpina* species, in view of its somewhat different leaves and slightly curved spurs.). The *canadensis* complex was represented by two populations of *A. canadensis*, one of which originated from a natural stand (33) in Gray Summit near St. Louis, USA, and by *A. elegantula* and *A. formosa*, also from a natural stand in North America (sent from Rancho Santa Ana, California).

A. pubescens, *A. caerulea*, *A. Chaplinei*, *A. chrysantha* and *A. longissima* represented the *caerulea* complex. *A. longissima* appears to be not a pure species but a form of *A. chrysantha* with a small amount of introgressant genes from *A. longissima*. *A. pubescens*, *A. Chaplinei* and *A. caerulea* originated from natural stands. The first two were obtained from Rancho Santa Ana, and the latter from the Cascade Canyon Teton Mts. Seeds of parental taxa, the F₁ and F₂ hybrids and of back-crosses were sown in the greenhouse into boxes with soil. Germination began after 2–3 weeks. Seedlings 4–6 weeks old were transplanted into flower pots and later, after 2–3 months were outplanted in the ground. All the plants flowered in the second vegetative season.

Crossings were made always under controlled conditions. Both for crossings and after self pollinations, the pollinations were repeated 2–3 times.

When estimating pollen fertility a 1% solution of acetocarmine and glycerine in a ratio 1:1 was used.

When analysing the pollen fertility in the parental forms and in the first generations of hybrids, pollen from 5 flowers was used. From each flower about 300 pollen grains were counted. In the second generation of hybrids and in the back-crosses about 200 pollen grains were counted from one flower. The fertility of an individual was estimated from two flowers. When analysing seed set in the parental forms and in the F₁ hybrids one follicle per infructescence and 10 fruits per individual were considered. In the F₂ and in the back-crosses 5 infructescences per indivi-

dual were taken, and from each of them one follicle were analysed, and then mean values were calculated.

For the study of the meiotic divisions in the pollen mother cells buds were fixed in Navashin's fixative. The preparations were made by the paraffin technique, cutting sections 5—6 μ thick and staining with gentian. This method was used only when analysing meiosis in *A. ecalcarata* and *A. vulgaris* 32, and in the F_1 hybrid between the two. In all the remaining taxa flower buds were fixed in Carnoy's fixative. Preparations were made as smears and were stained with 1% or 2% acetocarmine or with 1% propiocarmine.

EXPERIMENTAL PART

1. Cytological analysis

The progress of the meiotic divisions was observed in all forms of parental taxa as well as in the F_1 hybrids.

Twenty pollen mother cells were observed during the following stages of the first division: mataphase, metaanaphase, anaphase and telophase. All the original taxa proved to be diploids with a haploid number of chromosomes $n=7$ (Tabl. 1). Both in the pure taxa and in the first generations of hybrids there were in the meta-

Fig. 1. Meiosis in PMCs of *A. ecalcarata* (a_1 , b_1 , c_1); *A. canadensis* (a_2 , b_2 , c_2) and in the first hybrid generation *A. ecalcarata* $\times$ *A. canadensis* (a_3 , b_3 , c_3); a_1 , a_2 , a_3 — first metaphase, b_1 , b_2 , b_3 — first meta-anaphase, c_1 , c_2 , c_3 — first telophase

phase always 7 bivalents. Chromosome segregation in the anaphase was perfectly normal. No aberrations nor abnormalities were found (Fig. 1).

Table 1
Chromosome numbers in *Aquilegia*

Name of species	2n	Own data n	Source
<i>A. akitensis</i> Huth	14		Sakai 1935, Żukowa 1961
<i>A. alpina</i> L.	14	7	Levitsky 1931, Gregory 1941
<i>A. amaliae</i> Heldv.	14		Żukowa 1961
<i>A. atrata</i> Koch.	14		Mattick 1949
<i>A. atrovirens</i> M. Pop.	14		Żukowa 1961
<i>A. californica</i> A. Gr.	14		Skalińska 1929, Żukowa 1961
<i>A. canadensis</i> L.	14	7	Winge 1925, Gregory 1941
<i>A. caerulea</i> L.	14	7	Winge 1925, Żukowa 1961
<i>A. Chaplinei</i> St. ex Pay.			
<i>A. compacta</i>	14		Żukowa 1961
<i>A. chrysantha</i> A. Gr.	14	7	Skalińska 1928, Gregory 1941
<i>A. dichroa</i> Freyn	14		Gregory 1941, Żukowa 1961
<i>A. ecalcarata</i> Maxim.		7	
<i>A. Einseleana</i> Sch.	14		Gregory 1941, Żukowa 1961
<i>A. elegantula</i> Green		7	
<i>A. flabellata</i> Sieb. & Zucc. = <i>A. japonica</i>	14	7	Skalińska 1961
<i>A. flabellata</i> var. <i>nana</i> alba	14	7	Skalińska 1931
<i>A. formosa</i> Fisch.	14	7	Skalińska 1931
<i>A. formosa</i> var. <i>truncata</i>		7	
<i>A. fragrans</i> Benth	14		Żukowa 1961
<i>A. glandulosa</i> Fisch.	14		Sokołowska 1 Strielkova 1938, Gregory 1941
<i>A. glauca</i> Lindl.	14		Żukowa 1961
<i>A. Karelinae</i> Fedts.	14		Żukowa 1961
<i>A. longissima</i> Gray	14		Gregory 1941, Skalińska 1945
<i>A. olympica</i> Boiss.	14		Sokołowska 1 Strielkova 1940
<i>A. ottonis</i> Orph.	14		Żukowa 1961
<i>A. pyrenaica</i> DC.	14		Levitsky 1931, Żukowa 1961
<i>A. pubescens</i> Corv	14	7	Żukowa 1961
<i>A. sibirica</i> Lam.	14		Żukowa 1961
<i>A. skinneri</i> Kook	14		Gregory 1941
<i>A. vulgaris</i> L.	14	7	Winge 1925, Langlet 1927, Levitsky 1931, Gregory 1941, Skalińska 1928, Delay 1947 Lemos-Pereira 1948, Mattick 1949
- var. <i>hispanica</i> Borb.	14		Żukowa 1961
- var. <i>nivea</i>	14		Sokołowska 1 Strielkova 1940, Żukowa 1961
- var. <i>baicalensis</i>		7	
- var. <i>stellata</i>		7	

Chromosome numbers from Tischler (1950), Löve and Löve (1961) and Żukowa (1961).

2. Crossability

Crossability of two taxa can be estimated from the number of hybrid seeds obtained as a result of crossing these taxa, and comparing this number of seeds with the number of ovules potentially capable of being fertilised.

Number of well formed seeds as a percentage of the total number of available ovules was here used as a measure of crossability.

When studying seed set, ovules were found that were fertilized, but their development was inhibited at various stages. In pure taxa such abortive seeds were found very rarely, at the most in 6.1% and in a few cases even 0% (Table 2).

Table 2
Seed and pollen fertility in studied taxa of *Aquilegia*

Taxon	Seed setting %	Abortive seeds %	Seed germination %	Pollen fertility %
Complex <i>ecalcarata</i>				
<i>A. ecalcarata</i> - P _e	71.4	2.1	79.4	81.8
Complex <i>vulgaris</i>				
<i>A. vulgaris</i> 32 - P _{v32}	78.3	1.2	78.0	96.0
<i>A. vulgaris</i> 13 - P _{v13}	79.5	1.3	79.9	75.8
<i>A. vulgaris</i> var. <i>stellata</i> - P _{vs}	69.0	0	65.7	77.3
<i>A. japonica</i> - P _j	69.8	2.9	71.6	76.6
<i>A. baicalensis</i> - P _b	68.8	0	79.4	68.5
<i>A. flabellata</i> - P _f	77.5	4.9	85.8	90.5
Complex <i>alpina</i>				
<i>A. alpina</i> - P _a	72.5	0	81.3	75.9
Complex <i>canadensis</i>				
<i>A. canadensis</i> 140 - P _{c140}	70.2	1.1	71.4	82.5
<i>A. canadensis</i> 33 - P _{c33}	74.3	3.9	78.7	71.2
<i>A. elegantula</i> - P _{el}	62.6	2.8	68.9	98.5
<i>A. formosa</i> - P _{fo}	59.8	2.3	61.4	71.8
Complex <i>caerulea</i>				
<i>A. chrysantha</i> - P _{ch}	51.4	5.4	77.7	84.2
<i>A. longissima</i> - P _l	65.7	6.1	69.5	75.1
<i>A. pubescens</i> - P _p	59.2	3.1	68.8	69.8
<i>A. Chaplinei</i> - P _{Cha}	62.2	5.2	59.3	86.3
<i>A. caerulea</i> - P _{ca}	68.4	2.8	63.1	79.2

When crossing *A. ecalcarata*, the representative of the *ecalcarata* complex with representatives of the *vulgaris* and *alpina* complexes, and in the reciprocal crosses it was found that the seed set was almost normal. In most of the crosses it was above 55%, usually around 60% and maximally 68%. Seed set in pure taxa of these complexes varied from 69% to 78%.

Seed set in crosses between *A. ecalcarata* and representatives of the *canadensis* complex (including the reciprocal crosses) was somewhat lower. It varied from 35% to 39%.

When crossing *A. ecalcarata* with taxa of the *caerulea* complex lowest seed set was obtained after pollinating *A. chrysantha* with *A. ecalcarata* pollen. For several seasons it was not possible to obtain normal well formed seeds. Follicles

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Table 3
Compatibility and fertility in interspecific pollinations

Crosses	Compatibility		Fertility				
	seed setting	seed germi- nation	seeds from free polli- nation	seeds from control- led polli- nation	abor- tive seeds	seed germi- nation	pollen
	%	%	%	%	%	%	%
1	2	3	4	5	6	7	8
Complex ecalcarata x vulgaris							
P _e x P _{v32}	68.2	77.0	25.8	20.2	0	77.0	71.6
P _{v32} x P _e	65.5	73.6	19.0	18.9	6.0	65.4	41.6
P _e x P _{v13}	68.8	83.9	24.2	21.8	2.4	79.5	69.2
P _{v13} x P _e	61.4	76.6	23.1	19.6	1.3	78.2	72.1
P _e x P _b	42.1	76.6	18.3	12.8	0	68.0	50.4
P _b x P _e	57.7	65.3	16.3	9.9	0	68.9	48.0
P _e x P _f	50.8	75.4	9.7	10.3	3.8	47.5	39.5
P _f x P _e	60.6	58.8	8.1	9.7	2.1	42.7	44.7
P _e x P _j	60.0	67.2	36.6	23.8	1.1	71.3	59.4
P _j x P _e	52.6	59.8	25.8	21.3	2.1	68.4	55.2
P _{vs} x P _e	62.4	71.2	25.3	24.4	3.2	69.5	49.9
Complex ecalcarata x alpina							
P _e x P _a	52.3	60.1	16.8	17.4	0	53.0	69.0
P _a x P _e	57.0	58.2	21.7	18.2	0	63.4	68.7
Complex ecalcarata x canadensis							
P _e x P _{c140}	32.5	48.3	11.0	8.3	0	28.6	35.6
P _{c140} x P _e	29.1	37.0	12.0	7.0	0.4	25.9	39.6
P _e x P _{c33}	35.6	50.1	11.8	6.3	4.2	34.9	37.2
P _{c33} x P _e	39.2	51.0	11.4	7.2	0.7	33.6	36.3
P _e x P _{e1}	37.3	31.4	9.5	3.0	0	21.2	31.0
P _e x P _{fo}	30.5	29.6	8.8	6.8	2.1	23.1	32.8
P _{fo} x P _e	24.9	31.7	4.9	3.1	1.9	20.2	28.3
Complex ecalcarata x caerulea							
P _e x P _{ch}	28.1	41.2	4.3	6.3	2.0	35.1	68.0
P _{ch} x P _e	1.3	15.0	3.6	2.9	4.2	23.4	57.0
P _e x P _l	26.5	45.4	2.8	2.0	0.4	28.4	33.7
P _l x P _e	23.4	31.5	4.7	1.6	0	22.0	31.5

continue Table 3

1	2	3	4	5	6	7	8
$P_{Cha} \times P_e$	12.3	8.2	2.1	0	-	-	30.2
$P_p \times P_e$	3.4	5.8	-	-	-	-	-
Complex vulgaris $\times$ canadensis							
$P_{v32} \times P_{c140}$	60.2	63.2	21.1		5.3		41.2
$P_{v13} \times P_{c140}$	55.8	59.6	20.7		3.8		43.6
$P_{v32} \times P_{fo}$	14.8	36.8	24.3		5.3		41.2
$P_{v32} \times P_{el}$	47.8	63.1	22.2	19.5	3.9		46.1
$P_{c33} \times P_j$	54.3	61.2	22.0		0		45.3
$P_{el} \times P_f$	44.5	60.6	28.1	21.0	0	55.7	58.0
$P_j \times P_{fo}$	44.3	64.3	37.6		2.2		58.3
$P_{fo} \times P_j$	41.4	54.7	34.2		6.4		59.3
$P_{c140} \times P_f$	48.4	65.0	35.4	38.2	0	49.8	56.0
$P_f \times P_{c140}$	49.6	39.9	9.1	0	4.2		0
$P_f \times P_{el}$	42.2	43.6	14.0	0	2.6		0
Complex vulgaris $\times$ caerulea							
$P_f \times P_l$	35.5	34.6	18.3	-	4.5		47.6
$P_l \times P_{v32}$	35.0	40.0	18.1	22.5	3.4	-	47.0
$P_{v32} \times P_{ch}$	25.7	28.9	19.8		1.9		49.5
$P_f \times P_{ch}$	38.4	40.1	-	-	-	-	-
$P_{ch} \times P_f$	38.5	37.2	-	-	-	-	-
Complex canadensis $\times$ caerulea							
$P_{ch} \times P_{c140}$	67.4	78.5	58.3		2.6		66.1
$P_{c140} \times P_p$	68.4	81.6	74.4		0		82.1
$P_{c140} \times P_l$	67.7	80.0	68.4		1.4		65.2
$P_{ca} \times P_{c140}$	62.3	75.4	71.5		2.4		70.0

of *A. chrysantha* contained about 30% of fertilized ovules, these however were inhibited at some stage of development. Seeds were wrinkled, dry, brown and did not germinate after sowing. Finally a few good seeds were obtained, which after computation turned out to represent a seed set of 1,3%. A similar result was obtained in the cross *A. pubescens* $\times$ *A. ecalcarata*, namely 3,4%. Generally the seed set in crosses between *A. ecalcarata* and representatives of the *caerulea* complex was distinctly lower than in crosses between *A. ecalcarata* and representatives of other complexes (it varied from 1,3% to 28% Table 3).

When crossing taxa belonging to the different recognised, complexes except *A. ecalcarata*, it was found that an almost normal ability to produce sound seed

was obtained when taxa of the *canadensis* complex were crossed with those of the *caerulea* complex (and reciprocally). The crosses between taxa of the *vulgaris* complex and taxa of the *caerulea* complex (and reciprocally) have yielded an about half normal seed set, in the order of 30% or so. A relatively high crossability was found in the crosses between taxa of the *vulgaris* and *canadensis* complexes (including reciprocals). Seed set in these crosses varied from 42% to 60%, generally however under 50%. Thus it was somewhat lower than in crosses between *A. ecalcarata* and taxa of the *vulgaris* complex (Table 3).

To supplement the crossability estimates in Table 3 are presented the data on the percentage of germination of the seed obtained from interspecific crosses. Comparing Table 2 with Table 3 it can be seen the germination of seed of the pure species is in most cases better than the germination of seeds from interspecific crosses. Hybrid seeds obtained from the crosses between *A. ecalcarata* and representatives of the *vulgaris* and *alpina* complexes, and also those between the *vulgaris* and *canadensis* complexes germinated well, in fact only slightly less than the seed from pure species. The hybrid seed from the crosses between the *canadensis* and *caerulea* complexes germinated very well, just as the pure forms. A definite lowering of germination capacity was however observed in crosses between *A. ecalcarata* and the representatives of the *canadensis* complex (31% to 51%), between *A. ecalcarata* and the representatives of the *caerulea* complex, and between the representatives of the *vulgaris* and *canadensis* complexes (Table 3).

3. Fertility and vigour of the hybrids

a. Seed set in the hybrids

The ability to form sound seed by the first generation hybrids was estimated by the same method as was used in the estimation of crossability. In Table 3 data are presented on the seed set in different F_1 populations exposed to open pollination, pollinated under controlled conditions, percentage of seeds inhibited in development after free pollination and the germination capacity of seeds from selfpollination, (that is seeds obtained under controlled pollination). The germination capacity of seeds from the hybrids is similar to the germination of seeds obtained from the interspecific crosses.

Seeds of hybrids between *A. ecalcarata* and taxa of the *vulgaris* complex germinated better than seeds of hybrids between *A. ecalcarata* and taxa of the *canadensis* and *caerulea* complexes. It can be clearly seen that in all F_1 hybrids there is a lowering of seed set and the germination capacity also was lowered.

The percentage of seeds obtained as a result of free pollination is somewhat greater than after selfpollination. A highest seed set was observed in hybrids between *A. ecalcarata* and the taxa of the *vulgaris* and *alpina* complexes. F_1 hybrids between *A. ecalcarata* and taxa of the *canadensis* complexes demonstrate a very much lower seed set, and those between *A. ecalcarata* and representatives of the *caerulea* complex an almost complete sterility (Table 3). The percentage of fertilized ovules which

were inhibited in their development is in the F_1 generation almost the same as in the parental forms. It varies in different crosses from 0% to 6,4%.

After crossing taxa belonging to different complexes (with the exception of *ecalcarata*) it turned out that the greatest seed set was found in the F_1 hybrids between representatives of the *caerulea* and *canadensis* complexes. Here the seed set is almost the same as after selfpollination of pure forms. Hybrids between the *vulgaris* and *canadensis* complexes have a seed set similar to that in the hybrids between *A. ecalcarata* and representatives of the *vulgaris* and *alpina* complexes, and therefore somewhat lower. The seed set of F_1 hybrids between representatives of the *vulgaris* and *caerulea* complexes is similar to that between *A. ecalcarata* and the *canadensis* complex, namely about 19% (Table 3).

Also it was found that the hybrids between *A. flabellata* as the maternal parent and *A. canadensis* or *A. elegantula*, and also between *A. Chaplinei* and *A. ecalcarata*

Fig. 2. Curves showing the variation of the seed fertility in F_2 *A. flabellata* $\times$ *A. ecalcarata* ($N=60$).

Broken line — seeds inhibited in development in per cent. Continuous line — seeds well developed in per cent.

were sterile after selfpollination. After free pollination the seed set was very depressed, particularly in the last of the three crosses mentioned, however it was possible to obtain some seed. (Table 3).

To summarize, the F_1 hybrids between the representatives of the *canadensis* and *caerulea* complexes had much higher seed set than hybrids between the taxa representing *vulgaris* and *canadensis* or *vulgaris* and *caerulea* complexes.

An analysis of seed set in F_2 hybrids, one parent of which was always *A. ecalcarata*, have shown that the variation was continuous (Fig. 2.) The lower was the seed set in the F_1 the greater proportion of F_2 individuals had a seed set higher than the F_1 . For example in the hybrids *A. ecalcarata* $\times$ *A. vulgaris* (mean seed set for F_1 was 20,2%) 19,6% of the F_2 individuals had higher than in F_1 seed set. On the other hand in the hybrid between *A. ecalcarata* and *A. canadensis* and the hybrid between *A. ecalcarata* and *A. chrysantha*, which both had in the F_1 a seed set of 8,3%, respectively 38% and 86,2% of the F_2 progeny had a better seed set.

In all the F_2 populations studied (except crosses including *A. alpina*) completely

sterile individuals were found (Table 4). It has to be emphasised that a part of the F_2 individuals perished immediately after germination, even though the seedlings generally looked normal. In a few cases, in different crosses, a very poor rooting system was observed in the seedlings.

Table 4
Seed fertility from free pollination in F_1 , F_2 and backcrosses in %

Crosses	Mean for F_1	Number of plants	Range in F_2	Most numerous class in F_2	Range in bc	Most numerous class in bc
Complex <i>ecalcarata</i> × <i>vulgaris</i>						
<i>A.ecalcarata</i> × <i>A.vulgaris</i>	25.8	107	0-75.2	15-20		
<i>A.vulgaris</i> × <i>A.ecalcarata</i>	19.0	160	0-77.4	15-20		
<i>A.ecalcarata</i> × F_1		46			1.3-85.6	40-45
<i>A.vulgaris</i> × F_1		67			5.4-88.6	45-50
<i>A.flabellata</i> × <i>A.ecalcarata</i>	8.1	175	0-91.6	20-25		
<i>A.ecalcarata</i> × F_1		100			1-91.7	30-35
<i>A.flabellata</i> × F_1		75			2.7-89.0	35-40
Complex <i>ecalcarata</i> × <i>alpina</i>						
<i>A.ecalcarata</i> × <i>A.alpina</i>	16.8	50	8.6-95.5	35-40		
<i>A.alpina</i> × <i>A.ecalcarata</i>	21.7	55	8.0-92.4	35-40		
<i>A.alpina</i> × F_1		100			15.2-96.4	50-55
Complex <i>ecalcarata</i> × <i>canadensis</i>						
<i>A.ecalcarata</i> × <i>A.canadensis</i>	11.0	50	0-49.8	10-15		
<i>A.canadensis</i> × <i>A.ecalcarata</i>	12.0	50	0-38.6	10-15		
<i>A.ecalcarata</i> × F_1		50			1.2-75.6	40-45
Complex <i>ecalcarata</i> × <i>caerulea</i>						
<i>A.ecalcarata</i> × <i>A.chrysanthia</i>	4.3	151	0-68.6	10-15		
F_1 × <i>A.ecalcarata</i>		13			5.2-48.7	15-20
F_1 × <i>A.chrysanthia</i>		35			15.6-49.8	35-40

On the other hand no completely sterile individuals were to be found in backcrosses. Back-crosses with *A. ecalcarata* had a very small part of the progeny with a seed set from 1% to 5%, whereas in the back-cross to the other parental form no individuals were found with a seed set lower than 5% (Table 4). Generally in the populations of back-crosses a distinct increase of seed-set is observable. Most of the plants had a seed set greater than 15%. In various populations of back-crosses the most numerous class of individuals had a seed set above 30% (with only one exception, vide Table 4).

Number of normally developed seeds and those inhibited in their development differed considerably in different individuals of the F_2 generation. Variation of these characters is continuous (Fig. 2). In the first hybrid generation, in spite of the much lowered seed set in some combinations, no seeds were found which were inhibited in development, and if so then a very low percentage (Table 2). For example in the cross *A. flabellata* × *A. ecalcarata* in the F_1 the seed set was 8.1% but there were only 2.1% of seed inhibited in development. In the F_2 of these hybrids, in the individuals where the seed set ranged from 10%—20% there was a minimum of 25% of seed inhibited in development (Fig. 3). To summarize the seed set in the F_2 of

hybrids, as well as in the back-crosses, varied continuously. The range of variation the F_2 was wide, from 0% to 96%. In the back-crosses there is a definite increase in the seed set.

b. Pollen fertility

The populations of the original taxa did not differ substantially in the fertility of pollen, which was generally high. In Table 2 mean values of pollen fertility are presented for the parental taxa, and in Table 3 mean values for the F_1 hybrids. In the first hybrid generation of different crosses a lowering of pollen fertility was noticed.

The plants considered as sterile, produced either pollen which was totally degenerated, and did not stain with acetocarmine (such plants were often found in the analysed individuals of F_2) or anthers that were dried up, flattened and did not burst.

Such was the case in the F_1 hybrids between *A. flabellata* and *A. canadensis* and between *A. flabellata* and *A. elegantula*. Anthers of these hybrids if observed when the flowers were still in the buds appeared to be normal. However, the pollen did

Fig. 3. Table of correlation between number of seeds well developed and number of seeds inhibited in development in F_2 *A. flabellata* $\times$ *A. ecalcarata*

not stain (there was very little of it), it was distinctly shrunk and had thick cell walls. After blooming the anthers did not open, they were shrivelled and flattened. In both the hybrids meiosis was normal, as far as one could judge on the basis of 10 pollen mother cells in which divisions were found. It is possible that the sterility of the F_1 hybrids from these crosses was caused by a disharmony between paternal genes (in this case some factors characteristic of the *canadensis* complex) and the cytoplasm of *A. flabellata*. The F_1 of the reciprocal crosses had generally a rather high pollen fertility, about 60%.

Except for the example given above and the cross between *A. ecalcarata* and *A. vulgaris* the pollen fertility in the reciprocal crosses did not differ to any extent. From Table 3 it can be seen that the pollen fertility in the F_1 between *A. ecalcarata* and taxa of the *vulgaris* and *alpina* complexes is somewhat higher than in the F_1

between *A. ecalcarata* and representatives of the *canadensis* and *caerulea* complexes. F_1 between taxa of the *caerulea* and *canadensis* complexes and representatives of the *vulgaris* complex is partially sterile whereas hybrids between representatives of the *canadensis* and *caerulea* complexes are fertile.

An analysis of pollen fertility in the studied F_2 , in the back-crosses (Fig. 4) and in some of the F_3 has shown that the variation is continuous, and its distribution

Fig. 4. Curves showing the variation of pollen fertility in F_2 *A. ecalcarata* (P_e) $\times$ *A. chrysantha* (P_{eh}) — continuous line; in back cross with *A. ecalcarata* — dotted line; in backcross with *A. chrysantha* — broken line

is generally normal. Most numerous are individuals with a pollen fertility similar to the F_1 or somewhat higher. The range of variation is narrower in the back-crosses than in the F_2 . In these populations there are no sterile plants (Table 5).

In all the F_3 plants studied, *A. ecalcarata* $\times$ *A. vulgaris* — 4 populations, *A. flabellata* $\times$ *A. ecalcarata* — 3 populations and *A. ecalcarata* $\times$ *A. chrysantha* — 3 populations, there was a segregation. A part of the individuals has a fertility, lower, than the parental forms, while a part had a higher pollen fertility. However, individuals with a higher pollen fertility dominated.

DISCUSSION OF THE RESULTS

According to the opinions of numerous authors (Payson 1918, Anderson 1931; Grant 1952 and others) in the genus *Aquilegia*, all the taxa, in spite of considerable morphological differentiation and geographic distribution hybridize equally readily and produce fertile progeny.

Table 5
 Pollen fertility in F_1 , F_2 and backcrosses

Crosses	Mean for P_1	Number of plants	Range in F_2	Most numerous class in F_2	Range in bc	Most numerous class in bc
Complex ecalcarata × vulgaris						
A.ecalcarata × A.vulgaris	71.6	209	5.6-98.4	65-70		
A.vulgaris × A.ecalcarata	41.6	160	0-95.4	40-45		
A.ecalcarata × F_1		43			18.0-95.1	70-75
A.vulgaris × F_1		67			31.6-98.8	75-80
A.ecalcarata × A.baicalensis	50.4	103	13.5-97.1	55-60		
A.baicalensis × A.ecalcarata	48.0	69	0-86.5	50-55		
A.ecalcarata × F_1		102			9.8-92.2	70-75
A.baicalensis × F_1		72			4.2-93.4	70-75
A.flabellata × A.ecalcarata	44.7	148	0-96.7	50-55		
A.ecalcarata × F_1		83			9.5-85.4	70-75
A.flabellata × F_1		102			36.2-93.3	80-85
Complex ecalcarata × alpina						
A.ecalcarata × A.alpina	69.0	49	16.2-97.1	60-65		
A.alpina × A.ecalcarata	68.7	158	0-86.7	60-65		
A.alpina × F_1		97			35.6-98.3	80-85
Complex ecalcarata × canadensis						
A.ecalcarata × A.canadensis	35.6	86	12.4-82.1	40-45		
A.canadensis × A.ecalcarata	39.6	52	11.6-86.9	45-50		
A.ecalcarata × F_1		58			32.4-88.6	65-70
A.ecalcarata × A.elegantula	31.0	31	12.3-97.5	40-45		
A.elegantula × F_1		42			26.5-91.3	40-45
Complex ecalcarata × caerulea						
A.ecalcarata × A.chrysantha	68.0	222	0-96.1	50-55		
F_1 × A.ecalcarata		12			13.7-85.4	45-50
F_1 × A.chrysantha		35			12.4-96.4	50-55
A.ecalcarata × A.longissima	33.7	12	0-64.7	50-55		
F_1 × A.ecalcarata		26			8.3-96.7	70-75
F_1 × A.longissima		37			12.9-97.8	75-80
Complex canadensis × vulgaris						
A.elegantula × A.flabellata	58.0	45	0-89.8	70-75		
A.canadensis × A.flabellata	56.0	85	17.5-93.5	70-75		

As has been found in these studies and as is known from literature (Table 1) all the taxa have the same number of chromosomes, $n=7$, and the whole genus is diploid. This fact however does not explain the ease of obtaining hybrids between individual taxa. Instances are known of readily obtainable hybrids between polyploid species with different chromosome numbers.

For example when tetraploid *Geum montanum* from the sub-genus *Oreogeum* is crossed with duodecaploidal *G. pyrenaicum* from the sub-genus *Eugeum*, hybrids are just as readily obtained as between two hexaploidal species (Gajewski 1957).

Fig. 5. Sheme of the phylogenetic relationship between studied species complexes in the genus *Aquilegia*

In the genus *Fragaria* hybrids are readily obtained in all possible interspecific combinations in spite of differences in chromosome numbers, in morphological characters and in the geographical distribution (East and Mangelsdorf 1927).

On the other hand in some genera species with the same chromosome numbers cross with difficulty and sometimes even it is impossible to obtain hybrids.

In the genus *Nigella*, whose species have the same chromosome numbers $n=6$, and the chromosomes are morphologically very similar it has not been possible to obtain interspecific hybrids (Reinders 1942).

The ease with which it has been possible to obtain hybrids between *Aquilegia* species has been primarily demonstrated on the basis of crosses performed under conditions of artificial pollination (Skalinska 1928a, 1928b, 1929, 1931, 1935; Anderson and Schafer 1931; Kappert 1944; Linnert 1961).

In nature hybrids are very rarely found, since most of the species are allopatric. Only in very few instances, where two species meet in the same ecological niche

hybrids can be observed (Anderson 1931; Clausen, Keck and Hiesey 1945; Grant 1952; Pelton 1958).

Numerous crosses I have performed under controlled conditions indicate, that not all taxa of *Aquilegia* cross just as readily. The lowest ability to set sound seed was observed in the crosses between *A. ecalcarata* and taxa of the *caerulea* complex, followed by crosses between *A. ecalcarata* and taxa of the *canadensis* complex. The highest ability to set seed was found in the combinations between *A. ecalcarata* and taxa of the *vulgaris* complex, and also between taxa of the *canadensis* and *caerulea* complexes. A lowered ability to set seed has also been demonstrated when crossing taxa of the *vulgaris* complex with taxa of the *canadensis* and *caerulea* complexes (Table 3).

In the light of the results obtained the view of Grant (1949, 1950, 1952) that in *Aquilegia* there are no distinct genetic barriers isolating individuals species does not seem to be substantiated. Admittedly both under controlled conditions and in nature hybrid seed is obtainable, but the results of this study demonstrate, that depending on the combination of taxa that are crossed, the ability to obtain hybrids differ considerably.

For example, when *A. ecalcarata* was crossed with species of the *vulgaris* complex, the percentage of sound seeds obtained varied from 51% to 68%. When crossing *A. ecalcarata* with species of the *canadensis* complex the percentage of sound seeds varied from 29% to 40% and with the taxa of the *caerulea* complex from 1,3% to 31%.

In some instances the lowering of seed set indicated an incompatibility of maternal cytoplasm with the paternal genotype. When crossing *A. chrysantha* with *A. ecalcarata* it was shown that 30% of the ovules were fertilized but inhibited in development. There were only 1,3% of normally developed seeds. In the reciprocal cross the ability to produce sound seeds was 28%. In the remaining instances the abnormal development of the seeds could have been caused both by the lethality of the zygotes and by an abnormal development of the endosperm, which could only be ascertained by means of embryological studies. Skalińska (1958) after crossing *A. ecalcarata* with *Isopyrum thalictroides* has demonstrated an inability of the hybrid zygote to develop and its death at the stage of a few cells.

The percentage of seed germination is markedly lower in the F_1 than in the parental taxa.

The possibility of obtaining partially fertile hybrids between taxa indicates a relatively close phylogenetic affinity between these taxa. However, this correlation cannot be reversed, i.e. the impossibility of obtaining hybrids is not always a proof of the absence of affinity.

Taking into consideration the crossability and the percentage of germinating seeds some suggestions can be made concerning the degree of affinity, if not between individual taxa, than at least between complexes of taxa. Closest to *A. ecalcarata* appear to be the *vulgaris* and *alpina* complexes. The *canadensis* and *caerulea* comple-

xes are related less. The *canadensis* complex is closer to *caerulea* complex than either to the *vulgaris* or to the *alpina* complexes.

There are different ways of classifying the phenomena of sterility in hybrids. The classification proposed by Dobzhansky (1951) seems most convincing. He distinguishes between gene sterility and chromosomal sterility.

The gene sterility is not associated with the haploid nor the diploid state. It is the result of lack of coordination between the genes inherited from parents. This sterility manifests itself as abnormalities in the development of sex organs, which prevents meiosis, or in aberrations at the gene level in the meiotic process itself.

Chromosome sterility is the result of the chromosomes from the two parents being nonhomologous.

Stebbins (1951) claims, that sterile hybrids are often found in which the conjugation and separation of chromosomes is apparently normal. This type of sterility is, according to Stebbins associated with heterozygosity of the parental chromosomes with respect to small structural differences. When these differences are small, the chromosomes conjugate even though they are not completely homologous. Conjugation and crossing-over between partially homologous chromosomes, which differ only in small translocations or inversions leads in effect to the formation of gametes with duplications and deficiencies. Such gametes are lethal. Stebbins calls this phenomenon (1958) "cryptic structural hybridity". The boundary between gene and chromosomal sterility is not always easy to determine.

The course of meiosis in the studied species of *Aquilegia* and in the F_1 was always regular. This was reported by Skalińska (1935) in the cross between *A. chrysantha* $\times$ *A. flabellata*. Linnert (1961) has conducted a very careful analysis of bivalents at the pachytene stage in some species and F_1 hybrids of *Aquilegia*. She has determined the degree of chromosome conjugation in the following species and hybrids: *A. vulgaris*, *A. glandulosa*, *A. canadensis*, *A. buergeriana*, *A. longissima*, *A. ecalcarata* and in the F_1 hybrids *A. glandulosa* $\times$ *A. ecalcarata*, *A. buergeriana* $\times$ *A. canadensis* and *A. longissima* $\times$ *A. glandulosa*. In all cases she has established a complete homology of chromosomes.

The present studies on the course of meiosis in different taxa and several F_1 hybrids of *Aquilegia* have completely confirmed its regularity and the absence of univalents. Chromosome conjugation appeared always to be complete.

These observations were made on the first metaphase, which is a stage during which it is not possible to notice even larger structural differences between partially homologous chromosomes.

Partial sterility of interspecific hybrids of *Aquilegia* manifested itself in the following way:

1. Lowered pollen fertility and a lower seed set particularly in the first hybrid generations. For example the pollen fertility in the F_1 between *A. ecalcarata* and *A. vulgaris* was about 70%, and the seed set was about 20%. In the F_1 between *A. ecalcarata* and *A. canadensis* pollen fertility was 36% and seed set was 8%. In the F_1 between *A. longissima* and *A. vulgaris* pollen fertility was 47% and seed set

was 18% etc. (Table 3). The pollen fertility in different parental taxa varied from 70% to 99% and the seed set varied from 50% to 80%.

2. Lowered pollen fertility and seed set in the F_2 and F_3 . In a number of F_2 hybrids analysed in this respect some individuals had a lower pollen fertility and seed set than the mean for the F_1 . For example in the F_2 between *A. baicalensis* $\times$ and *A. ecalcarata* 1,5% of the progeny had completely sterile pollen, and about 30% had a pollen fertility lower than mean for the F_1 . In F_2 of *A. ecalcarata* $\times$ *A. canadensis* 8% of the individuals did not produce any seed at all and 24% of the individuals had a seed set lower than the mean for the F_1 .

3. Frequent inhibition of the development of fertilized seed in the F_2 . In all the individuals of the F_2 generations seeds were found, which had been fertilized but inhibited at different stages of development (Fig. 3) as well as normal seeds. A similar phenomenon was reported by Skalińska (1928) in the F_2 hybrids between *A. vulgaris* and *A. chrysantha*.

4. Lowered germinability of F_1 and F_2 seeds.

5. Segregation in the F_2 , F_3 and back-crosses with respect to seed set and pollen fertility.

Thus we are dealing here with a degeneration of microspores, megaspores as well as zygotes, which can be caused by numerous gene factors and by the so called cryptic structural hybridity.

The lowered fertility of the *Aquilegia* hybrids is not associated with their constitutional weakness. On the contrary some F_1 hybrids of both reciprocal crosses between *A. ecalcarata* $\times$ *A. flabellata*, *A. ecalcarata* $\times$ *A. elegantula*, *A. canadensis* $\times$ *A. elegantula*, *A. canadensis* $\times$ *A. flabellata* and *A. vulgaris* $\times$ *A. formosa* are much taller and more luxuriant than the parental populations. A similar vigour of hybrids has been found by Anderson (according to Munz 1946) in the F_1 between *A. vulgaris* and *A. canadensis* and by Skalińska (1928) in the F_1 hybrid between *A. vulgaris* and *A. chrysantha* and between *A. chrysantha* and *A. flabellata* (1935).

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SUMMARY

Assuming that *A. ecalcarata* is the taxon most akin to the original form from which presumably the whole genus of *Aquilegia* has developed, it was crossed with representatives of distinguished species complexes.

Also taxa belonging to different complexes (except *ecalcarata*) were crossed with each other. Taking into consideration the crossability, the seed set and pollen fertility in the first and later generations of the hybrids some suggestions were made concerning the affinity between the different taxa. The results concerning fertility of hybrids, their seed set and pollen fertility, indicate the same grouping of taxa belonging to the studied complexes, into related units as could be concluded from their crossability.

The closest relatives of *A. ecalcarata* are the taxa belonging to the *vulgaris* and *alpina* complexes, then the taxa of the *canadensis* complex which are less related with *A. ecalcarata*. Still further related to *A. ecalcarata* are the taxa of the *caerulea* complex. The taxa of the *canadensis* complex appear to be more related to the taxa of the *caerulea* complex than to the taxa of the *vulgaris* complex.

The results obtained concerning the degree of affinity between the studied taxa confirm the suggestions presented in the previous paper (Prażmo 1965) based on phylogenetic relationships between the complexes as could be deduced from a genetic analysis, the methods of pollination and the geographical distribution. A scheme diagram of phylogenetic affinity between the studied taxa, on the basis of the data obtained, is presented in Fig. 5.

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Cytogenetyczne badania rodzaju *Aquilegia*

IV Stopień pokrewieństwa między gatunkami

Streszczenie

Zakładając, że *A. ecalcarata* jest taksonem najbardziej zbliżonym do wyjściowej, pierwotnej formy, z której powstał prawdopodobnie cały rodzaj *Aquilegia*, takson ten krzyżowano z przedstawicielami wyróżnionych kompleksów gatunkowych. Krzyżowano ponadto taksony należące do poszczególnych kompleksów (z wyjątkiem *ecalcarata*) między sobą. Biorąc pod uwagę zdolność do krzyżowania, płodność nasion i pyłku pierwszych i dalszych pokoleń wysunięto pewne przypuszczenia co do stopnia pokrewieństwa łączącego badane taksony. Wyniki dotyczące płodności mieszańców — płodności nasion i pyłku — wskazują na takie samo uszeregowanie taksonów należących do badanych kompleksów pod względem pokrewieństwa, jakie wynika ze zdolności do krzyżowania.

Najbliższymi *A. ecalcarata* wydają się taksony należące do kompleksu *vulgaris* i *alpina*, a mianowicie: *A. vulgaris*, *A. baicalensis*, *A. flabellata* i *A. alpina*. Dalej spokrewnione z *A. ecalcarata* wydają się taksony kompleksu *canadensis*: *A. canadensis*, *A. elegantula* i *A. formosa*, a jeszcze dalej taksony kompleksu *caerulea*: *A. pubescens*, *A. Chaplinei*, *A. chrysantha*, *A. longissima*. Taksony kompleksu *canadensis* wydają się bliżej spokrewnione z taksonami kompleksu *caerulea* niż z taksonami kompleksu *vulgaris*.

Otrzymane wyniki co do stopnia pokrewieństwa między badanymi taksonami potwierdzają sugestie wysunięte w poprzedniej pracy (Prażmo 1965) co do pokrewieństwa filogenetycznego między wyróżnionymi kompleksami, a oparte na analizie genetycznej, systemach zapylania i rozmieszczeniu geograficznym. Schemat pokrewieństwa filogenetycznego między badanymi taksonami, oparty na uzyskanych danych przedstawiono na fig. 5.