Cytogenetic studies on the genus *Aquilegia*

III. Inheritance of the traits distinguishing different complexes in the genus *Aquilegia*

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The genus *Aquilegia*, which belongs to the *Ranunculaceae*, one of the most primitive families in the *Angiospermae*, is itself in relation to other genera of this family very primitive. This is evidenced by such characters as radial flower structure, poor differentiation of the calyx and corolla, apocarpous styles, many ovules, spiral arrangement, entomophily etc.

According to the monograph of Muntz (1946) the genus has 67 species. They are all distributed mainly in old mesophytic plant associations of Asia, Europe and North America. In spite of the fact that in the sense of chronological time, *Aquilegia* is rather an old species, in the evolutionary sense it appears to be a very young genus. All the taxa investigated so far cytologically proved to be diploid. In nature no poliploids have ever been found. When crossing, even the most ecogeographically distant individuals it is possible to obtain hybrids. *Aquilegia* became a classical example of a genus in which internal crossing barriers do not exist or are very weak.

The vegetative parts (leaves, stems, root systems) in all the taxonomic units are very similar to each other. This similarity extends even to related genera such as *Isopyrum* and *Thalictrum*.

In spite of that many botanists have for a long time distinguished various *Aquilegia* species (Gerard 1597; De Candolle 1824; Baker 1878; Brühl 1893; Rapais 1909). Always the main diagnostic criteria were based on the flower parts, since flower morphology of this genus is distinctly differentiated. Various taxonomic units differ in flower colour, their position length and shape of sepals and petals (in particular the length and shape of the spurs), length of stamens, and their geographical ranges.

It needs to be stressed that most of the species are allopatric. Parallel with species descriptions in *Aquilegia* attempts have been made at arranging them in natural groups. (Baker 1878; Payson 1918; Boothman 1934; Grant 1952). The subdivision of the genus which has been used in this study and which appears to be least artificial is that

which was proposed by Grant (1952). It is based on some botanical characters dealing with flower structure and on the geographical distribution. Characters of the flower morphology are in *Aquilegia* particularly important, since they are adaptations to different types of pollinators.

The five complexes distinguished by Grant (*ecalcarata*, *vulgaris*, *alpina*, *canadensis*, *caerulea*) are in fact five different pollination systems. The taxonomic units belonging to one of the complexes are distinctly different in their morphology from taxa belonging to different complexes. Certain types of insects or birds visit only the types of taxa whose flower structure enables successful uptake of food, either nectar or pollen. As a result cross-pollination is only possible between such forms that have the same type of flower structure. Specific pollinators visit specific flower types, and are therefore the chief agents of isolation between different taxa. Thus according to Grant's classification mechanical isolation plays the relevant role in restricting a free exchange of genes.

The adaptation of species or species complexes (as in *Aquilegia*) depends on the combination of several phenotypic characters, which are in turn determined by a combination of genetic factors. The formation of new species, or of new taxonomic units on the species level, is basically a fixation of gene combinations that play a role in the adapting of the plant to a given set of environmental conditions. The chief agent of selection and fixation of new gene combinations in *Aquilegia* was, it appears, the pollinator.

It was the purpose of the study to investigate the inheritance in *Aquilegia* of several characters of adaptive value, and the mechanism of speciation in this genus. Thus crosses were made between different taxa representative of the species complexes mentioned above. In particular an analysis of the hybrids between *A. ecalcarata* (the only representative of the *ecalcarata* complex) which is considered as the most primitive species in the whole genus (Prażmo 1960) and representatives of the other complexes was stressed.

MATERIALS AND METHODS

Taxa used in the crosses

The *ecalcarata* complex.

A. ecalcarata (Fig. 1), the single representative of this complex, has small flowers, nodding, purple, with spurless petals. Instead of the spurs at the end of the petals there are small pockets with nectaries at the

Fig. 1. *A. ecalcarata*, Fig. 2. *A. vulgaris*, Fig. 3. *A. elegantula*, Fig. 4. *A. chrysantha*.

base. This species is pollinated by bees and flies with short tongues. *A. ecalcarata* originates from Asia. It grows in China (Grant 1952; Muntz 1946), in the eastern part of the Kansu province, in Amdo province, in the eastern part of Hupeh province, in south-eastern parts of Shechwan, and presumably also in the neighbouring Tibet. Everywhere it occurs on considerable altitudes (2500—3000 m).

The vulgaris complex.

The taxonomic units comprising the *vulgaris* complex are distributed over great areas of Eurasia (Muntz 1946; Grant 1952), from Japan — *A. flabellata* through Europe — *A. vulgaris* — till France and Spain.

A. vulgaris, from which the complex takes its name, has many subspecies and races occurring in nature as well as many cultivated forms and varieties e.g. *A. baicalensis*.

The flowers of the taxa within this complex are nodding with curved spurs of medium length, fairly long lamina, a concealed androecium, colour: blue, purplish-blue (*A. vulgaris*, *A. baicalensis*), bluish-white (*A. japonica*) or white (*A. flabellata*). All the taxa are pollinated by bees and bumble bees with long tongues (Müller 1883; Knuth 1888—1889; Grant 1951).

The representatives of this complex used in the crosses were:

A. vulgaris L., no. 32, from seeds provided by the Warsaw Botanic Garden (WOB) Fig. 2., no. 41 from seeds obtained from the Wrocław Botanic Garden (WrOB) and nr. 13 from seeds collected in a natural stand in the Białowieża Forest;

A. baicalensis Hort. — seeds from WOB;

A. flabellata var. *nana alba* Hort. — seed from WOB;

A. japonica Nakai Hara = *A. flabellata* Sieb. Zucc. — seeds from WrOB.

The alpina complex.

Taxa of this complex occur in the southern parts of the temperate zone of Eurasia (Grant 1952), mainly on considerable elevations.

The flowers of these taxa are very similar in shape and colour to those in the *vulgaris* complex. The basic difference is in the shape of the spurs, which are less curved. The species of the *alpina* complex are pollinated by bees and bumble bees (Müller 1883; Knuth 1909).

A. alpina L., which was grown from seeds obtained from the WOB, and which was used in the crosses as a representative of this complex, resembled closely the species of the *vulgaris* complex in the curvature of the spur, size of flowers and shape of leaves. However in view of the

results of the analysis of hybrids this taxon was treated as a representative of the alpina complex.

The canadensis complex.

This complex consists of many different types occurring in the wide expanses of North America (Payson 1918; Grant 1952).

Flowers of the taxa within the canadensis complex are nodding red or reddish-yellow, with a exerted androecium and rather short blades of the corolla segments, with straight spurs, medium in length and wide at the base. They are well adapted to the pollination by humming birds (Schneck 1901; Graenicher 1910; Pickens 1931; Grant 1952). The following types were used as representatives of this complex:

A. canadensis L.: no. 140 — seeds from WOB, no. 33 — seeds from a natural stand in North America, Gray Summit near St. Louis;

A. elegantula Green — seeds from WOB (Fig. 3);

A. formosa var. *truncata* Baker — seed was obtained from Rancho Santa Ana, California, North America.

The caerulea complex.

The taxonomic units comprising the fifth complex occur in the central southern Rockies and in the mountain regions of the Great Basin of North America (Grant 1952).

The flowers of taxa within this complex are erect, white, yellow (*A. chrysantha*, *A. longissima*) or bright yellow (*A. pubescens*), with long slender, and straight spurs, a long petal lamina and a somewhat exerted androecium. They are pollinated by moths and butterflies (Knut 1909; Gray 1883; Trelease 1883; Payson 1918).

For hybridization the following were used:

A. chrysantha Gray — seed obtained from WOB (Fig. 4);

A. longissima Gray — seed obtained from WOB (it appears that the taxon obtained under this name is a form of *A. chrysantha* with some introgressant genes from *A. longissima*, and not a pure *A. longissima*);

A. caerulea — seeds from a natural stand in the Cascade Canon Teton Mts., *A. pubescens* — seeds from a natural stand obtained from Rancho Santa Ana, California.

METHODS

When making a genetical analysis of the characters differentiating the studied taxa I have measured and determined the following traits:

1. Length of the spur.
2. Shape of the spur.
3. Position of the flowers.

4. Colour of the flowers.
5. Blooming time.
6. Length of corolla blade.
7. Length of the androecium.
8. Width of the petals.
9. Shape of the sepal, length/width ratio.
10. Follicle length.
11. Number of ovules per follicle.
12. Size of seeds.
13. Height of the plant.
14. Shape of leaves.
15. * Pollen fertility.
16. * Seed germination.

On fig. 5 is presented a schematic drawing of an *Aquilegia* flower, and all the measured elements are indicated.

The plant populations grown from the obtained seeds were self-fertilized under controlled conditions. The selfed progeny grew in experimental plots in Skierniewice. Having found that the populations of the individual parental taxa are satisfactorily uniform in respect of the characters under study, cross pollinations were performed.

Seeds of the parental taxa, F_1 , F_2 and backcrosses were sown in the greenhouse, in boxes with soil. Germination began after 2—3 weeks. Seedlings 4—6 week old were transplanted into flower pots, and later after 2—3 months outplanted into the ground. All the plants flowered in the second vegetation season.

Hybridisation was always performed under controlled conditions (tomofan isolation bags). Both when crossing and self-pollinating the pollinations were repeated 2—3 times.

All the measurements in respect of one cross (of the parental taxa, the F_1 , and F_2) as for example the height of the plants, length and width of the sepals and petals, the pollen fertility and seed germination, were made in the same vegetative season, in order to obtain comparable results.

From each individual of the parental form 10 flowers were measured, and in the F_2 and the backcross 5 flowers.

Since the length of the spur varies depending on the age of the flower (Kappert 1944), the flowers measured were as far as possible in the same stage of development, namely one day after the bursting of the first anthers.

* Interspecific crossability and hybrid fertility will be discussed in the next paper.

Fig. 5. Scheme of *Aquilegia* flower measurements.

A — androecium; B — sepal; C — lamina; D — spur; E — petal; l — length; s — width.

The height of the plant was measured as the height of the tallest flowering shoot.

The colour of the flower was estimated according to the Horticultural Colour Chart vols. I and II.

THE EXPERIMENTAL PART

Genetical analysis of the hybrids

1. Presence or absence of the spurs

One of the more important characters discriminating between the studied taxonomic units is the presence or absence of the spurs.

A. ecalcarata — a primitive taxon has spurless petals. They differ from sepals in that they have at their bases small pockets with nectaries.

A. vulgaris, *A. baicalensis*, *A. flabellata* var. *nana alba* and *A. japonica* have spurs, as well as all the other taxa of the *Aquilegia* genus which belong to the remaining complexes.

Having crossed *A. ecalcarata* with each of the taxa belonging to the *vulgaris* complex, the F_1 , F_2 and F_3 populations obtained were analysed for this character.

Flowers of the first generation were all spurred. The second hybrid generation segregated into spurred and spurless plants in a 3:1 ratio respectively. Table 1 presents the obtained and expected numerical values and the calculated χ^2 and p .

It can be assumed that the absence of the spur in *A. ecalcarata* is a recessive character in respect to its presence in the taxa of the *vulgaris* complex. Denoting the allele for spur absence as a , the *A. ecalcarata* genotype will be aa . The genotype of taxa in the *vulgaris* complex will be AA . The first generation of hybrids will thus be heterozygotes denoted by Aa . In the F_2 the genotypes Aa and AA are not distinguishable phenotypically.

When crossing the F_1 — Aa — hybrids with the recessive homozygote aa a population was obtained which always segregated into spurless and spurred individuals in a 1:1 ratio (Table 2).

The backcross population with the AA homozygote consisted solely of spurred individuals. The backcross with *A. vulgaris* ($P_v \times F_1$) yielded 67 plants, all with spurred flowers. All the individuals of the backcross with *A. baicalensis* ($P_b \times F_1$), 72 in all, were also spurred. The same was found in the 83 individuals obtained from the backcross with *A. flabellata* ($P_f \times F_1$).

Analysing the segregation of this character in the several F_3 populations obtained from self-pollinations of the F_2 individuals, it was found that:

- 1) From spurless plants only spurless progeny was obtained.
- 2) Some of the spurred plants did not give a segregating progeny, but only plants with spurred flowers.

Table 1

F ₂ hybrid populations	Spurs		Expected ratio	Chi ²	p	Mendelian ratio
	present	absent				
<i>A. ecalcarata</i> × <i>A. vulgaris</i>	201	85	214.5 : 71.5	3.3985	0.2 — 0.05	3:1
<i>A. vulgaris</i> × <i>A. ecalcarata</i>	152	50	151.5 : 50.5	0.0066	0.95 — 0.8	3:1
<i>A. ecalcarata</i> × <i>A. vulgaris</i>	20	4	18.0 : 6.0	0.88	0.5 — 0.2	3:1
<i>A. ecalcarata</i> × <i>A. baicalensis</i>	205	61	199.5 : 66.5	0.6064	0.5 — 0.2	3:1
<i>A. baicalensis</i> × <i>A. ecalcarata</i>	244	80	243 : 81	0.01645	0.95 — 0.8	3:1
<i>A. ecalcarata</i> × <i>A. flabellata</i>	15	6	15.75 : 5.25	0.01427	0.95 — 0.8	3:1
<i>A. flabellata</i> × <i>A. ecalcarata</i>	182	44	169.5 : 56.5	3.6868	0.2 — 0.05	3:1
Total	1019	330	1011.75:337.25	0.1610	0.8 — 0.5	3:1

Table 2

Back-cross populations	Spurs		Expected ratio	Chi ²	p	Mendelian ratio
	present	absent				
<i>A. ecalcarata</i> × (<i>A. vulgaris</i> × <i>A. ecalcarata</i>)	22	24	23 : 23	0.0868	0.8 — 0.5	1:1
<i>A. ecalcarata</i> × (<i>A. baicalensis</i> × <i>A. ecalcarata</i>)	44	58	51 : 51	1.9214	0.2 — 0.05	1:1
<i>A. ecalcarata</i> × (<i>A. ecalcarata</i> × <i>A. flabellata</i>)	105	88	96.5 : 96.5	1.497	0.5 — 0.2	1:1
Total	171	170	170.5:170.5	0.00292	0.99 — 0.95	1:1

3) Some of the spurred plants yielded a progeny segregating into plants with spurred and spurless flowers in a 3:1 ratio respectively (this is presented in Table 3).

Table 3

F ₃ hybrid populations	Spurs		Expected ratio	Chi ²	p	Mendelian ratio
	present	absent				
42/47	20	8	21 : 7	1.904	0.2—0.05	3:1
42/32	48	11	44.25:14.75	1.27	0.5—0.2	3:1
39/44	33	9	31.5 :10.5	0.0929	0.8—0.5	3:1
69/169	22	5	20.25: 6.75	0.6044	0.5—0.2	3:1
69/171	19	4	17.25: 5.75	0.709	0.5—0.2	3:1
Total	142	37	134.25:44.75	1.7893	0.2—0.05	3:1

The obtained results permit the conclusion, that the presence of a spur in the columbines of the vulgaris complex is a monogenetic character. Also, according to Clausen (unpublished, see Grant 1950) the difference between spurred and spurless forms of *A. vulgaris* is determined by one gene.

A. alpina was the only representative of the alpina complex, thus it is difficult to generalise the obtained result and claim that all the taxa in this complex display a different type of inheritance of the spur.

The obtained F₂ population of the *A. alpina* × *A. ecalcarata* cross yielded plants with and without spurs in the ratio 192:14. This is nearest to the Mendelian ratio of 15:1, where the theoretical ratio should be 183,13:12,87. In this case Chi² is 2,956 with p between 0,2 and 0,5. Thus the deviation is still within the limits of probability. In the back-cross to *A. alpina*, which gave 103 individuals, all the plants were with spurs. This leads to the conclusion that the absence of the spur is determined by the presence of two recessive genes *aabb*. For the occurrence of the spur the presence of only one of the dominant genes is sufficient. The conclusion is supported by the results of crossing *A. ecalcarata* with the taxa of the caerulea complex.

The representatives of the caerulea complex used in the crosses with *A. ecalcarata* were *A. chrysantha*, *A. longissima*, *A. pubescens* and *A. caerulea*. An analysis of the F₂ hybrid generation with respect to the presence or absence of the spur was made on the hybrids *A. ecalcarata* × *A. chrysantha* and *A. ecalcarata* × *A. longissima*.

Of the 284 F₂ plants from the first cross only 14 had spurless flowers. This corresponds to the theoretical ratio of 266,25:17,75 (Chi² = 0,2252 with a p between 0,8 and 0,5) and fits the 15:1 Mendelian ratio.

In the backcross with *A. ecalcarata* 1/4 of the individuals had spurless flowers (9 with spurs and 5 without).

The F_2 with *A. longissima* yielded only 12 individuals. One of them had spurless flowers. Among the 26 individuals of a backcross to *A. ecalcarata* 7 were spurless, whereas all the backcrosses to *A. longissima* gave plants with spurred flowers ($N=6$).

Also 9 progenies of the F_3 generation obtained from self-pollination of 9 F_2 hybrids between *A. ecalcarata* and *A. chrysantha* were analysed for the presence or absence of the spur.

F_2 individuals with spurless flowers gave in the progeny (F_3) only spurless individuals. There were two such populations. Three of the studied populations gave no segregation, but only individuals with spurred flowers. The segregation ratios obtained from the remaining F_3 populations are shown in Table 4 and 5.

Table 4

F ₃ hybrid populations	Spurs		Expected ratio	Chi ²	p	Mendelian ratio
	present	absent				
68/42	36	10	34.5 :11.5	0.2608	0.8 —0.5	3:1
68/49	5	2	5.25: 1.75	0.0357	0.95—0.8	3:1
Total	41	12	39.75:13.25	0.05109	0.95—0.8	3:1

Table 5

F ₃ hybrid populations	Spurs		Expected ratio	Chi ²	p	Mendelian ratio
	present	absent				
68/60	25	2	25.3:1.7	0.5645	0.5—0.2	15:1
68/179	30	1	29.1:1.9	0.4538	0.5	15:1
Total	55	3	54.4:3.6	0.1066	0.8—0.5	15:1

The genotype of *A. chrysantha* and *A. longissima* may be represented as *AABB* (the same notation should be applied also to *A. alpina*). All the segregants in the F_2 , in which at least one of the two alleles is dominant appear with spurred flowers. Only individuals with an *aabb* genotype are spurless, and there is only 1/16 of the total population with such a genotype. A backcross with a recessive homozygote segregates according to the 3:1 expected ratio. The F_3 populations which did not segregate in respect of the discussed character came from F_2 plants whose genotype could have been *AABB*, *AABb*, *AaBB*, *AAbb*, and *aaBB*. The

F₃ populations in which all the individuals had spurless flowers originated from individuals with an *aabb* genotype.

The taxa of the *canadensis* complex with which *A. ecalcarata* was crossed and the F₂ population was analysed were *A. canadensis* and *A. elegantula*. The first hybrid generation of both reciprocal crosses gave plants with spurred flowers. In Table 6 are presented the segregation ratios obtained in the F₂, which correspond to the Mendelian ratio of 3:1. Crossing the heterozygous F₁ hybrid with a recessive homozygote of *A. ecalcarata* a progeny was obtained segregating in the ratio 52:32, which resembles the Mendelian ratio 1:1 (theoretically 42:42, $\chi^2 = 4.6$ with a *p* between 0.5 and 0.01). All the individuals of the back-cross to *A. canadensis*, there were 26 of them, had spurred flowers.

On the basis of an analysis of the hybrids it was concluded that the taxa of the *canadensis* complex have a genotypic constitution of the type *AAbb* or *aaBB*.

Table 6

F ₂ hybrid populations	Spurs		Expected ratio	Chi ²	<i>p</i>	Mendelian ratio
	pre-sent	ab-sent				
<i>A. ecalcarata</i> × <i>A. canadensis</i>	90	20	82.5:27.5	2.726	0.2 —0.05	3:1
<i>A. canadensis</i> × <i>A. ecalcarata</i>	63	15	58.5:19.5	1.384	0.5 —0.2	3:1
<i>A. canadensis</i> 33 × <i>A. ecalcarata</i>	15	6	15.75:5.25	0.0142	0.95—0.8	3:1
<i>A. ecalcarata</i> × <i>A. elegantula</i>	24	12	27:9	1.3000	0.5 —0.2	3:1
Total	192	53	183.15:61.95	1.4804	0.5 —0.2	3:1

It can be inferred from the above data that formation of the spur is determined monogenetically. Thus it can be assumed, that the gene controlling flowers without spurs has mutated. If the genotype of *A. ecalcarata* be denoted as *aabb*, and that of taxa of the *vulgaris* complex as *AAbb* it becomes interesting to establish whether in the American taxa of the *canadensis* complex it is the same gene *a* which has mutated into an *A* allele causing the occurrence of spurs, or whether it is the gene *b*. With this in view the F₂ progeny of the *A. canadensis* × *A. flabellata* and *A. elegantula* × *A. flabellata* was analysed. In both the populations, numbering N₁ = 92 and N₂ = 61 plants respectively, none were found with spurless flowers. The genotype of both these taxa was analogous to the *A. vulgaris* type, and taxa of that complex, namely *AAbb*. Otherwise in the studied F₂ progenies a segregation in the ratio

15:1 would have been obtained. Skalińska (1929) when crossing *A. californica* and *A. flabellata* also did not find in the analysed F_2 ($N=81$ plants) individuals with spurless flowers. Taxa of the *canadensis* and *vulgaris* complexes have presumably the same gene determining the occurrence of the spurs.

In *A. alpina*, *A. chrysantha* and *A. longissima* a further gene has mutated, the presence of which in the heterozygous or homozygous state plays no basic role in the formation of the spurs. Possibly its action is somehow related with the length of the spurs, which in these taxa is large.

2. Shape of the spurs

Length of the spurs and their shape (Fig. 6) — particularly the degree of their curvature — play a very important role in the choice

Fig. 6. Spurs of different *Aquilegia* species.

a — *A. ecalcarata*; b — *A. vulgaris*; c — *A. flabellata*; d — *A. japonica*;
e — *A. alpina*; f — *A. canadensis*; g — *A. elegantula*; h — *A. formosa*;
i — *A. pubescens*; j — *A. caerulea*; k — *A. chrysantha*; l — *A. longissima*.

of the pollinator. Mouth parts of different types of pollinators: *Diptera*, humming birds, aligned according to their length and shape correspond surprisingly well to the length and shape of the spurs of the different taxonomic units of *Aquilegia*.

Taxa of the *canadensis* and *caerulea* complexes have flowers with straight spurs. Taxa of the complexes *vulgaris* and *alpina*, with few exceptions have curved or hooked spurs. The degree of curvature in different forms is very variable. Kappert (1944) measured the curvature as the radius of a circle an arc of which is represented by the spur. That author has also pointed out that depending on the age of the flower the shape of the spur somewhat changes. In flowers that have

Table 7

F ₂ hybrid populations	Spurs			Expected ratio	Chi ²	p	Mendelian ratio
	absent	curved or hooked	straight				
<i>A. ecalcarata</i> × <i>A. baicalensis</i>	25	53	25	25 : 58.5:19.5	2.068	0.5 — 0.2	4:9:3
<i>A. baicalensis</i> × <i>A. ecalcarata</i>	23	34	15	18 : 40.5:13.5	2.586	0.5 — 0.2	4:9:3
<i>A. flabellata</i> × <i>A. ecalcarata</i>	23	73	30	31.2: 70.3:23.5	4.048	0.2 — 0.05	4:9:3
Total	71	160	70	74.2:169.3:56.3	3.868	0.2 — 0.05	
<i>A. alpina</i> × <i>A. ecalcarata</i>	14	120	72	13 :144 :48	15.46		4:45:15
<i>A. canadensis</i> × <i>A. flabellata</i>	—	95	30	93.75:31.25	0.0666	0.8 — 0.5	3:1
<i>A. elegantula</i> × <i>A. flabellata</i>	—	37	14	38.25:12.75	1.2658	0.5 — 0.2	3:1
<i>A. californica</i> × <i>A. flabellata</i> *	—	54	27	60.75:20.15	3.000	0.5 — 0.2	3:1
<i>A. flabellata</i> × <i>A. chrysantha</i> *	—	29	9	28.5 : 9.5	0.035	0.95—0.8	3:1
Total		215	80	221.25:73.75	0.7055	0.5 — 0.2	3:1

* Data from Skalińska (1929).

just opened, the spurs are more strongly curved. When determining the shape of the spurs I have used flowers, as far as possible, of the same age, i.e. the next day after the splitting of first anthers. No accurate measurements were made, only the spurs were classified into four groups; straight, curved, slightly hooked and strongly hooked.

Taxa with curved or hooked spurs, when crossed with taxa that have straight spurs (and reciprocally) always yielded in the F_1 plants that have spurs to some extent curved or hooked. Generally the degree of curvature is less than in the parental form.

The spurless *A. ecalcarata* crossed with forms that have straight spurs gives in the F_1 a progeny with straight spurs. However when it is crossed with forms that have curved or hooked spurs it yields an F_1 progeny with spurs that are curved, but to a lesser degree.

The trait for curved spur appears to dominate incompletely the trait for a straight spur.

In the crosses *A. canadensis* $\times$ *A. flabellata*, *A. elegantula* $\times$ *A. flabellata* and *A. longissima* $\times$ *A. vulgaris*, the hybrid F_1 generation had more strongly curved spurs than the reciprocal crosses.

A similar phenomenon was observed by Skalińska (1929) in the F_1 hybrids of the reciprocal crosses between *A. californica* and *A. flabellata*. On the other hand the hybrids between *A. chrysantha* and *A. vulgaris* are definitely matroclinal with respect to this character, which was also noted by Skalińska in her work done in 1928. A similar matroclinal inheritance she has noted (1929) in the hybrids between *A. flabellata* and *A. chrysantha*. It can be assumed that the degree of curvature of the spur in some hybrids is dependent on the interaction between the paternal genotype and the maternal cytoplasm.

Table 7 presents the obtained and expected numerical ratios in the F_2 . The segregation ratios indicate that the trait for spur straightness is monogenic, which, it appears, is confirmed by the segregations obtained in the back-crosses (Table 8).

On the basis of the above tables and other data it can be concluded that *A. ecalcarata* contains factors determining a straight spur shape (v). The allele controlling this trait is recessive with respect of the allele (V). determining a curved spur. This symbol was proposed by Skalińska (1928) in her work dealing with the hybrids between *A. vulgaris* and *A. chrysantha*.

3. Position of flowers

Also the position of flowers, a character playing a very important role in pollination, proved to be determined by one pair of alleles. Of all the studied taxa only *A. chrysantha* and *A. longissima* have erect flowers.

Table 8

Backcross populations	Spurs			Expected ratio	Chi ²	p	Mendelian ratio
	absent	curved or hooked	straight				
<i>A. baicalensis</i> × (<i>A. ecalcarata</i> × <i>A. baicalensis</i>)	58	20	24	51 :25.5 :25.5	2.242	0.5—0.2	2:1:1
<i>A. ecalcarata</i> × (<i>A. ecalcarata</i> × <i>A. flabellata</i>)	42	32	29	51.5:27.75:25.75	3.296	0.2—0.05	2:1:1
Total	100	52	53	102.5:53.25:51.25	0.698	0.8—0.5	2:1:1

Table 9

F ₂ hybrid populations	Number of plants	Colour groups					Chi ²	p	Mendelian ratio
		I	II	III	IV	V			
<i>A. canadensis</i> × <i>A. flabellata</i>	obtained expected	65 48.9	19 16.3	17 14.3	3 5.5	15 21.7	7 7.3	0.05—0.01	27:9:9:3:12:4
<i>A. elegantula</i> × <i>A. flabellata</i>	obtained expected	42 33.75	10 11.25	8 18.25	3 3.75	12 15	4 15	0.2 —0.05	27:9:9:3:12:4

A larger F_2 progeny was only obtained in the cross *A. ecalcarata* $\times$ *A. chrysantha*. The character for an erect flower (*d*) is recessive in respect of the character for nodding flower (*D*). According to the usual notation *A. ecalcarata* will be *DD*, *A. chrysantha* *dd* and the F_1 *Dd*. The segregation in the F_2 into 209 nodding flowered plants and 66 plants with erect flowers and in the back-cross with nodding flowered plant a segregation into 20 and 15 plants with nodding and erect flowers respectively, suggest simple Mendelian ratios of 3:1 and 1:1. The theoretical values 206,25:68,75 ($\chi^2 = 0,1466$ with $p = 0,5$) indicate accidental deviations.

4. Colour of flowers

In the genus *Aquilegia* it is possible to identify five basic colour types; purple, violet or blue, red, yellow and white. These colours have in the different taxa various intensities, tints and shades. Flowers of some taxa are uniformly coloured whereas in other they are bicoloured. Petals, sepals and spurs of one flower may have a different colouring. In some taxa the blades of petals have their margins of a different colour, or possibly the same colour but in a lighter shade. All the tints of purple, violet, blue and red are dependent on the presence of anthocyanins dissolved in the cell sap. The yellow colour is caused by yellow anthoxanthin present in chromoplasts. These occur both in the epidermal cells and in the mesophyll.

On the basis of colour analysis in the taxa and hybrids I have studied it can be concluded that the colours have complicated relationships.

Individual taxa demonstrate distinct differences in the shade of flower pigmentation, which results in differences between various F_1 progenies. Particularly in the F_2 between taxa belonging to the vulgaris complex and *A. ecalcarata*, the exact delimitation of classes was not possible. The variation of the pigmentation was definitively continuous in nature. On the basis of results obtained it does not appear possible to determine the number or kind of factors responsible for different shades of purple, blue and violet. Double or triple self-pollination of the original plants in order to obtain as far as possible homozygous material, was probably insufficient in respect of flower colour. Presumably the individuals used as parental forms could in later generations segregate according to the shade of flower pigmentation.

In describing my results I have followed Skalińska (1928, 1931, 1935) in assuming the existence of the following genes for colour:

- gene *R* — which regulates the synthesis of anthocyanin,
- gene *F* — which modifies the red pigment into bluishviolet,
- gene *Y* — which regulates the existence of yellow chromoplasts,

gene *C* — a basic gene, which together with gene *R* causes the formation of anthocyanin in the form of a red coloured cell sap.

When crossing the purple *A. ecalcarata* with the white *A. flabellata*, it was found that the flowers of F_1 hybrids contain anthocyanin. In the second generation there was a segregation into 205 plants with coloured flowers and 60 plants with white flowers. The expected segregation ratio was 198,75:66,25, with a $\chi^2 = 0,7861$ at p between 0,5 and 0,2. This agrees with the Mendelian ratio of 3:1. In a back-cross with *A. flabellata* the segregation was 40:45 (theoretical ratio 42,5:42,5, $\chi^2 = 0,2942$ with a p between 0,8 and 0,5) which agrees with the Mendelian ratio 1:1. In the back-cross with *A. ecalcarata*, there was no segregation. All the individuals ($N=100$) were coloured. From among the F_2 progeny 6 plants were selected, which were self fertilized in order to obtain the F_3 . On examining the segregation ratios in these populations the presumed genotypes of the F_2 plants were derived and the genotypes of the phenotypically distinguished F_3 segregants were estimated. Three F_2 plants with white flowers, and presumed *ccyyRRFF* genotypes gave F_3 progenies exclusively with white flowers. One plant with violet flowers gave an F_3 progeny with pigmented flowers. Its genotype, and the genotype of its progeny was probably *CCyyRRFF*. Two plants, with pigmented, but purplishviolet flowers, gave a progeny segregated into plants with coloured and white flowers in the ratios 16:4 and 21:5. The expected ratios were 15:5 and 19,5:6,5 respectively ($\chi^2 = 0,20$ and 1,94 with a p between 0,5—0,8 and 0,2—0,5) which agrees with the Mendelian ratio 3:1. The genotypes of these F_2 plants were presumably *CcyyRRFF*, and of the F_3 segregants *CcyyRRFF* and *ccyyRRFF*.

The obtained segregation ratios support the suggestion that *A. flabellata* is a homozygous recessive in respect of the basic gene *C*. With respect to other factors determining flower colour in this taxon it has to be assumed that it is a dominant homozygote for *R* and *F*. This is supported by the result of crossing *A. flabellata* with the yellow flowered *A. longissima*. F_1 of this cross has flowers with a violet tint.

When crossing the yellowish-red *A. canadensis* and *A. elegantula* with the white *A. flabellata* the F_1 progeny obtained had bicoloured yellow-violet flowers. Following Skalińska's interpretation of the factors controlling flower colour it can be assumed that *A. canadensis* and *A. elegantula* have factors *C*, *R* and *Y*, but do not have the factor *F*, which is indicated by the absence of a bluish tint in the cell sap. Since *A. flabellata* has an *ccyyRRFF* genotype, the F_1 of both the hybrids should be *CcYyRRFf*. The flower colour of the F_1 hybrids confirms this conclusion, it is yellow-violet as a result of the introduction by *A. flabellata* of the *F* factor. In the F_2 a clear segregation was observed.

The segregants were classified into 6 groups with the following flower colours and probable genetic constitutions.

I. Flowers with anthocyanin, violet in tint, and with the yellow pigment.

The pigmentation of these flowers was presumably dependent on an interaction between the *Y* factor homozygous or heterozygous with the homozygous *R*, *F* and *C* factors. The genotype of such plants would be *C(c) Y(y) RR F(f)*.

II. Plants with violet flowers without any trace of a yellow pigment. Their colour is the result of the interaction between the factors *C*, *R*. The genotype would be *C(c) yy RR F(f)*.

III. Plants with pinkish-yellow or pinkish-reddish-yellow flowers. The genotype would be *C(c) Yy RR ff*.

IV. Plants with pinkish flowers, containing only anthocyanins but no chromoplasts. The presumable genotype is *C(c)yyRRff*.

V. Plants with yellow or yellowish-white flowers, with a genotype *cc Y(y) RR (f)(f)*. Here the factor *Y* determines the colour. The presence of factor *R* or possibly factor *F* is not observable in view of the homozygotic state of the factor *c*.

VI. Plants with white flowers. These are recessive homozygotes with respect to the factors *C* and *Y*. The factors *R* and *F* are present, but the latter can be recessive. Such a genotype will be *cc yy RR F(f)*.

The obtained numerical ratios of segregants in the designated 6 groups and the theoretical expectations, together with the corresponding genotypes are presented in table 9. The presented data support the conclusion that the genotype of *A. canadensis* and *A. elegantula* is *CCYYRRff*.

When crossing *A. ecalcarata* with *A. canadensis* (and the reciprocal cross) and *A. ecalcarata* with *A. elegantula* the *F*₁ progeny obtained had bicoloured flowers, yellow-violet, which indicates that *A. ecalcarata* introduces the *F* factor into the hybrids. In the *F*₂ progenies of these crosses no individuals were found with white flowers. This indicates that both forms introduce into the cross the *C* gene.

It is interesting to note, that in all the studied populations there is an excess of plants with violet, unicoloured flowers in relation to a lower than expected number of red flowered plants. It is also necessary to note that the *F*₂ segregants with a red flower colour did not have their pigmentation of exactly the same shade as the parental forms but more dirty-red, or even purple-red. It is possible that as a result of the interaction of some modifying factors, introduced by both the parents, or perhaps as a result of the interaction of the paternal and maternal

Table 10

F ₂ hybrid populations	N	Colour groups		Expected ratio	Chi ²	p	Mendelian ratio
		I	II				
<i>A. ecalcarata</i> × <i>A. canadensis</i>	59	46	13	44.25:14.75	0.276	0.8—0.5	3:1
<i>A. canadensis</i> × <i>A. ecalcarata</i>	58	50	8	43.5 :14.5 ₁	3.884	0.05—0.01	3:1
<i>A. ecalcarata</i> × <i>A. elegantula</i>	40	27	13	30 :10	1.200	0.5—0.2	3:1
Total	157	123	34	117.5 :39.25	0.934	0.5—0.2	3:1

Table 11

F ₂ hybrid population	Number of plants	Colour groups				Chi ²	p	Mendelian ratio
		I	II	III	IV			
<i>A. ecalcarata</i> × <i>A. chrysanthia</i>	obtained	127	58	56	34	3.58	0.5—0.2	27:16:12:9
	expected	116	68.7	51	38.7			

genomes, the red colour of the cell sap is modified into one that is violet or purplish-red.

This could also explain why in the backcross (*A. ecalcarata* $\times$ *A. elegantula*) $\times$ *A. elegantula*, no plants were found with reddish-yellow flowers, but only those whose flowers were violet-purplish-yellow. Thus the analysed F_2 generation was classified on the basis of colour only into 2 groups:

I. Plants with anthocyanin and chromoplasts, with probable genotypes $CC Y(y) RR F(f)$ or $CC Y(y) RR ff$.

II. Plants only with anthocyanin with a genotype $CC yy RR F(f)$ or $CC yy RR ff$.

Table 10 illustrates the obtained segregation in the F_2 .

Correspondingly in the back-cross with *A. ecalcarata* one would expect to obtain:

I. 1/2 of the plants with bicoloured flowers, containing both anthocyanin and chromoplasts $CC Y(y) RR Ff$.

II. 1/2 of the plants whose colour is determined only by anthocyanin — $CC yy RR F(f)$.

The segregation obtained agreed with the expected. Out of 83 plants from this progeny 39 belonged to group I and 44 group II (theoretically 41,5:41,5, $\chi^2 = 0,3$).

When crossing taxa of the vulgaris or ecalcarata complex with the taxa of the caerulea complex as for example *A. ecalcarata* $\times$ *A. chrysanthia*, *A. ecalcarata* $\times$ *A. longissima* or *A. longissima* $\times$ *A. vulgaris*, an F_1 progeny is obtained that is bicoloured, containing both anthocyanins and the yellow chromoplasts.

Since in the rather large F_2 progeny of the cross *A. ecalcarata* $\times$ *A. chrysanthia*, consisting of 256 individuals, not a single plant had white flowers, it can be assumed that the taxa of the caerulea complex are dominant homozygotes with respect to the basic colour factor C.

When analysing in detail the above mentioned F_2 progeny with respect to colour (Pražmo 1961) I have found that there are three pairs of alleles segregating here: Y/y , R/r , and F/f . In the F_2 four basic types of colour pigmentation were obtained, which presumably had the following genotypes:

I. $CC Y(y) R(r) F(f)$ — Plants with bicoloured flowers similar to F_1 with anthocyanins violet in tint and with yellow chromoplasts.

II. $CC Y(y) rr ff$ — Plants with yellow or creamy-yellow flowers (a colour caused by the presence of chromoplasts).

III. $CC Y(y) R(r) ff$ — Plants with bicoloured flowers, pinkish-yellow, due to the presence of anthocyanins in the red from, and chromoplasts.

IV. *CC yy R(r) F(f)* — Plants with unicoloured flowers, violet or purplish-violet. The colour is determined by the presence of anthocyanins.

In Table 11 are presented the numerical data on the segregation into these 4 groups and the expected ratios.

In the progenies obtained from backcrosses segregations agreeing with the expected were also obtained. The backcross with *A. ecalcarata* consisted of only 14 individuals. Of these 9 had bicoloured flowers indicating presence of both anthocyanins and yellow chromoplasts. The genotype of such plants could be *CC Yy R(r) F(f)*. The remaining 5 plants had unicoloured flowers with an anthocyanin in the violet-purplish or purple form. The genotype of these plants was presumably *CC yy R(r) F(f)*.

The backcross progeny to *A. chrysanth*a was somewhat more numerous, it consisted of 36 individuals. The following phenotypes were observed: 16 individuals with yellow flowers and a probable *CCYYrrff* genotype, 10 individuals with bicoloured flowers, violet and yellow (anthocyanin and chromoplasts) with a *CC Y(y) R(r) F(f)* genotype, and 10 individuals with bicoloured flowers, red and yellow (anthocyanin in the red form and chromoplasts) with the *CC Y(y) R(r) ff* genotype. The numerical ratios obtained agreed with the theoretical 1/2:1/4:1/4. The theoretical segregation should have been 18:9:9.

An analysis of the segregation ratios in five F_3 progenies of this cross enabled the deciphering of the probable F_2 genotypes from which the F_3 progenies were obtained.

Two of the F_3 progenies studied consisted entirely of plants with yellow flowers (the genotypes of the F_2 plants and their progenies were all *CC YY rr (f)(f)*).

One plant with violet-yellow flowers (*CCYyRRFF*) gave in the F_3 a segregation into 50 plants with violet-yellow flowers and 24 with violet flowers (theoretical expectation was 55,5:18,8, $\chi^2 = 2,17$, p between 0,2 and 0,05) which agreed with the expected 3:1 ratio.

A violet plant with indications of the yellow pigment on the petals (genotype *CCYyRRFf*) segregated into 14 violet-yellow flowered plants [*CC Y(y) RR F(f)*]: 6 reddish-yellow flowered plants [*CC Y(y) RR ff*]: 4 violet-purple flowered plants [*CC yy RR F(f)*]. There were no red plants with the genotype *CCyyRRff*. The theoretical ratio should have been 13,5:4,5:4,5:1,5, $\chi^2 = 2,0735$, with a p between 0,8 and 0,5. The obtained result agrees with the Mendelian ratio 9:3:3:1.

A plant with violet-yellow flowers, genotype *CCYYRrFf*, gave in the F_3 a segregation into 55 violet-yellow flowered plants [*CCYYRrFf*]: 12 yellow [*CCYYrrff*]: 17 reddish-yellow [*CCYYRRff*]. The theoretical

ratio should have been 47,25:21,0:15,75, $\chi^2 = 5,220$ with a p between 0,2 and 0,05. The ratio obtained agrees with the Mendelian ratio 9:4:3.

The obtained results once again support the suggestion concerning the genotype of *A. chrysantha* and *A. ecalcarata* with respect to flower pigmentation.

The F_2 progeny of *A. longissima* $\times$ *A. ecalcarata* consisted of only 12 plants. Thus it is not possible to take into account the numerical segregation ratios. However, the existence in the F_2 of all the expected colour types confirms the assumption that the genotype of *A. longissima* is the same as that of *A. chrysantha*, namely CCYYrrff.

The backcross (*A. ecalcarata* $\times$ *A. longissima*) $\times$ *A. longissima*, which yielded 36 individuals segregated in the ratio:

8 plants with bicoloured flowers, violet-purplish and yellow, with a probable CC Yy R(r) F(f) — genotype. Such plants should constitute 1/4 of the progeny, i.e. 9.

12 plants with yellow flowers and the genotype CCYYrrff. There should be 1/2 of such plants and therefore 18.

16 plants with bicoloured flowers and a probable CC y(y) R(r) ff genotype. There should be 1/4 of such plants and therefore 9.

The $\chi^2 = 7,51$, with a p between 0,01 and 0,05, which indicates that the deviation from the expected ratio is not significant. The backcross progeny with *A. ecalcarata* was also satisfactorily in agreement with the expected ratio of 1/2 of the plants with bicoloured flowers, similar to the F_1 — CC Yy R(r) F(f), and 1/2 of the plants unicoloured, without the yellow pigment and a CC yy R(r) F(f) genotype. The ratio obtained was 12:14, while the theoretical one was 13:13. $\chi^2 = 0,1538$ with a p between 0,5 and 0,8.

The results obtained permit the conclusion that all the taxa of the caerulea complex, with yellow coloured flowers have the same genetic constitution. This assumption is further supported by the fact that the F_1 hybrids between the taxa belonging to the *canadensis* and *caerulea* complexes never show any tendency to change the pigmentation of the cell sap from red to violet or purple-violet, which one would expect only when the factor *F* is introduced into the hybrid.

The studies on flower colour indicate that there exists an interaction between four pairs of allelomorphs, each of which is inherited independently.

It appears that the taxa belonging to the same complexes contain the same alleles of the genes *C*, *R*, *Y*, and *F*. On the other hand it is obvious that besides the above mentioned four pairs of alleles, other factors are also operating, possibly modifiers or inhibitors, which have not been studied here. This is evidenced by the variation in shade of the

colours between the taxa belonging to the same complexes, and the difficult to resolve differences in the distribution of the pigments in the perianth (paler petals, white petal margins etc.).

Above has been presented the genetic basis for the control of characters most important in the adaptation of *Aquilegia* species and in their taxonomic diagnosis. It was found that they are determined by one or only a few pairs of allelomorphs. This is a particularly significant conclusion because generally a simple Mendelian type of segregation is rare when dealing with characters that differentiate between taxonomic units (ecotypes or ecospecies) Clausen (1958). It is a widely applicable principle that characters differentiating this type of taxonomic units are conditioned by many genes.

All the remaining characters that were analysed, and which differentiate the studied taxa of the genus *Aquilegia* have shown a polygenic type of inheritance.

These characters were: height of the plants, or rather the length of their floral shoots, shape of the leaves, length of the spurs, degree of their curvature, dimensions of the petals and sepals, length of the stamens, length of the follicles, number of ovules in the follicles, size of the seed, seed fertility, fertility of the pollen, and the time of appearance of the first flowers.

In the first generations of the hybrids, these characters are usually intermediate, sometimes however they are more similar to one or the other parent. For example as regards the length of the spurs, the F_1 progeny usually has spurs more akin to those of the parent that had longer spurs.

As regards the shape of the spur, the F_1 hybrid progeny in some of the crosses has spurs with a curvature more akin to that parent which had more curved spurs, e.g.: in crosses *A. elegantula* $\times$ *A. flabellata*, *A. canadensis* $\times$ *A. flabellata* and *A. longissima* $\times$ *A. vulgaris* F_1 hybrids have spur curvatures more like the male parent.

The length of the androecium in the F_1 is often less than the arithmetic mean between the lengths of the parental androecia.

Time of flowering of the F_1 hybrids is always more close to that parent which flowers earlier.

The length of the follicle in the F_1 hybrids was in all the studied F_1 hybrids more akin to the parent with the shorter follicle. This is particularly evident in these hybrids which have *A. ecalcarata* as one of the parents (Fig. 7). However, the number of ovules is always strictly intermediate between the numbers characteristic of the parents.

The length of the floral shoots was in most of the studied crosses intermediate between that of the parents. In such crosses where the differences in plant height were not great, as for example between

Fig. 7. Variation in the length of the follicles in F_2 generation of *A. flabellata* (P_f) $\times$ *A. ecalcarata* (P_e). Black colour — medium values for parents and F_1 hybrids.

Fig. 8. Curves showing the variation of the height of plants in F_1 , F_2 and backcrosses of the hybrid between *A. ecalcarata* and *A. flabellata*.

A. ecalcarata and *A. flabellata*, or between *A. ecalcarata* and *A. elegantula* it was found that the F_1 progenies, including reciprocal crosses, were taller and more luxurious than the parental populations (Fig. 8).

Fig. 9. Petals of the parental species, F_1 and F_2 generations. Note the continuous character of the variation in the length of the spurred F_2 plants.

Leaves in the F_1 hybrid progenies of all the crosses in which *A. ecalcarata* was one of the parents were always intermediate and there were no differences between reciprocal crosses.

In the second generations of these hybrids all these characters segregate within the range of variability of the parental forms, sometimes however it is possible to note transgressions. The variation has a distinctly continuous pattern (Fig. 9). This information indicates that the characters mentioned are inherited in a polygenic manner.

Besides polygenes presumably also modifier genes and inhibitor genes are operating. This is indicated by the result of the cross between *A. ecalcarata* and *A. chrysanthia*. In the F_2 the range of variation in the length

of spur demonstrated a certain irregularity, was substantially displaced towards one of the parental forms. This deviates from the normal situation, where only two distribution patterns are expected, either the F_2 progeny segregates into two more or less equal groups of individuals in which the studied character approaches the two extremes, or else none of the individuals approximates either of the parental forms. Such a situation arises when the character in question is regulated by a very large number of polymeric factors and thus there is a very small probability of individuals similar to either of the parents occurring. It could be assumed that in the case encountered there exists a process of elimination of some genotypes, particularly since the fertility of pollen and germination of seed was low, if it were not for the fact that the segregations with respect of other characters were perfectly normal. As a result the most probable explanation of the abnormal distribution lies in the presence of factors within the *A. ecalcarata* genotype that inhibit the action of *A. chrysantha* factors causing spur elongation. A similar action of inhibitor and modifier genes was often reported in many interspecific and intervarietal hybrids (Clausen and Hiesey 1958 in *Potentilla*, Kruszevska 1961 in *Mirabilis*).

AN ATTEMPT TO EXPLAIN THE MECHANISM OF SPECIATION IN *AQUILEGIA*

Among the studied taxa of *Aquilegia*, *A. ecalcarata* appears to be the most primitive. In such characters as spur absence, short follicles, feeble but flexible shoots, *A. ecalcarata* is similar to *Isopyrum*. A further proof of affinity between these two plants is to be found in the fact that it was possible to fertilize *A. ecalcarata* with *Isopyrum thalictroides* pollen (Skalińska 1958) even though the zygote degenerated at the stage of a few cells.

A. ecalcarata was first described by Maximowicz in 1889 and included in the genus *Aquilegia*.

In 1902 Makino has created a new genus *Semiaquilegia* in which he has placed one species of *Isopyrum*, *Isopyrum adoxioides* D.C. and called it *Semiaquilegia adoxioides*. According to the author this species was sufficiently different from the remaining to merit a separate genus.

Drummond and Hutchinson in 1920 have included in the generic status *Semiaquilegia* Makino also the species *Isopyrum Henryi* Oliv. calling it *Semiaquilegia Henryi*, and *A. ecalcarata* Maxim. as *Semiaquilegia simulatrix* Drumm. and Hutch., and also they have described for the first time a new species *Semiaquilegia Castwoodinae*. They have also created a new genus *Paraquilegia*, in which they have included four

species earlier considered as belonging to the genus *Isopyrum*. According to Drummond and Hutchinson this genus is the more primitive one, and the precursor for the genera *Semiaquilegia*, *Aquilegia* and *Isopyrum*.

In the present study it was assumed that *A. ecalcarata* (according to Drummond and Hutchinson *Semiaquilegia simulatrix*) belongs to the genus *Aquilegia*, if for no other reason simply because the presence of spurs is the formal most important criterion for the inclusion in this genus, not to mention the ease with which hybrids are obtained when crossing *A. ecalcarata* with other taxa of the genus *Aquilegia*.

The inception of spurs in this primitive taxon has originated the "new type" of columbine. As has been demonstrated on the basis of an analysis of the hybrids, the formation of a spur is determined monogenetically. A mutation, with a large genotypic effect has presumably caused the evolution, by a single step of a new flower type, at once distinctly isolated from the rest of the population as a result of a different pollination mechanism. The presence of a spur has become a successful barrier eliminating the possibility of interbreeding of the new form with the original population. Only insects with sufficiently long mouth parts could extract nectar from the nectaries found at the bottom of the long spurs. Bees with sufficiently long tongues were presumably the insects to which the spur proved to be adapted. These bees have limited their visits only to this type of new flowers. In this way an interdependence developed between the flower and the insect, which was the basis for the action of selection mechanisms and further adaptation.

Another mutation with a distinct phenotypic effect, playing no doubt an important role in the selection of pollinators, was that which regulated the shape of the spurs. As has been shown the determining whether the spur is straight or curved is monogenic. It has therefore developed as a result of a single mutation.

Also it appears that in the process of differentiation within the genus *Aquilegia* of considerable consequence was the "large" mutation concerning the positioning of the flower, nodding or erect, and the few mutations that have determined the flower pigmentation.

Purple, blue and violet flowers (such are the colours of the columbines within the *ecalcarata* and *vulgaris* complexes) are pollinated by flies, bees and bumble bees, which distinguish these colours. Red colour is not "seen" by them and as a result they do not react to it (Grant 1949, 1950a, 1951, 1953; Frisch 1914).

Red colour is distinguished by birds which react to a whole series of shades around this part of the spectrum. Columbines with reddish-

-yellow flower colour belong to the canadensis complex and grow in North America, where they are pollinated by humming birds.

White and yellow colour is distinguished by moths (this is related to the fact that they visit the flowers at night). Such is the colour of columbines within the caerulea complex. Flowers of these taxa are erect. The evolution in North America of several taxa, among which it is possible to distinguish two pollination mechanisms, different from those found in Eurasia, was presumably the result of very few mutations with large phenotypic effects concerning flower pigmentation and positioning, and a whole series of small mutations selected and fixed by the process of evolution.

The differences between the Eurasiatic taxa belonging to the vulgaris complex appear to be controlled by a whole series of polygenes. Characters conditioned by polygenes have evolved as a result of many mutations, with small phenotypic effects.

The evolutionary role of the so called "large mutations", which cause a visible phenotypic effect, in comparison with the mutations giving only small effects, has for a long time been controversial.

Small mutations were first described by Baur (1924) in *Antirrhinum majus* and related species, where they occur very commonly. In several different types of experiments as for example those of East (1935) on *Nicotiana rustica*, Lindström (1941) on tomatoes, Dobshansky (1953) on *Drosophila*, it has been shown that the occurrence of mutations with small morphological effects is common, both in the plant and in the animal kingdoms. The universality of this phenomenon is evidenced by the fact that most of the differences between different taxonomic units, at all levels, are controlled by multiple genes. Harland (1936), Mather (1943) and many other investigators believe that in the formation of differences between species and therefore in the process of speciation, the greatest role is played by "small" mutations.

It appears however that the variability essential for the formation of higher taxonomic units can also arise by way of macromutations. These mutations, with drastic phenotypic effects, can change the adaptive traits of an organism, and therefore can presumably also change them accidentally into better ones. Some geneticist, Goldschmidt (1940) in particular, are in fact of the opinion that "large" mutations can so very much change the structure of an organism, or of its organs that this new individual could be classified into a different taxonomic unit than the rest of the individuals in the population from which it has arisen.

From studies on induced mutations it has been found, that there are large mutations which can radically change the structure of some organs. It needs, however, to be pointed out, that most of such changes

cause the emergence of traits that are, to a greater or a lesser extent, in disharmony with the rest of the features of the organism, which form a balanced whole, and therefore such mutant plants are quickly eliminated by means of natural selection.

There are also examples of large spontaneous mutations. For example the mutation of the gene "oxyloba" in *Malva parviflora* (Kristoffer-son 1926) or of the gene sphaerococcum in *Triticum aestivum* (Ellerton 1939; Sears 1947). Goldschmidt (1952, 1953) points out that in *Drosophila* there occur mutants with the wings reduced to thread-like organs (mutant known as tetraltera), and therefore basically wingless, or with two instead of the normal one pair of wings. This corresponds to changes, that in systematics are considered as differentiating not only genera, but even higher units.

Several examples of macromutations are known in *Antirrhinum*. The most interesting one is the transcendens mutation, which reduces the number of stamens from four to two. This mutant type lies beyond the normal range of *Antirrhinum* variability. It resembles in flower structure other genera of the *Scrophulariaceae* as *Mahavea* and *Veronica* (Stubbe 1952, 1959). Another mutant of *Antirrhinum majus*, known as „hemiradialis", has five instead of four anthers, and instead of a bilateral symmetry a semi-radial one. It resembles *Verbascum* in flower structure (Stubbe 1952, 1959). Still another mutant of *Antirrhinum majus* causes the formation of spurs in the flowers, thereby making them similar to the related genus *Linaria* (Schwanitz 1956). On the other hand in *Linaria* a mutant was found (called "gratioloides") with spurless flowers and radial symmetry instead of the normal bilateral flowers with spurs (Schwanitz and Schwanitz 1955).

The mutation of one gene in *A. vulgaris* into a mutant called compacta, with a pleiotropic effect (Anderson and Abbe 1933) causes a change of the whole form and many characters of the plant. This gene acts on all parts of the plant. It causes shorter and thicker stems, very much reduced internodes, with the flowers fixed on short, strong peduncles. All these effects are the result of premature secondary thickening of the cell walls.

It appears that mutations with large morphological effects can only become fixed in nature when they concern the latest stages of development in which an organ can become modified. When such a large mutation concerns an organ with a less specialized, more general structure, in a relatively late period of its ontogeny, the chances of desorganising its whole development are less. In this way a new stage of the evolutionary process can begin.

In primitive plants, related to *Isopyrum*, or to *A. ecalcarata*, having flowers with nectaries at the base of the petals, a single mutation could

have occurred that changed the development of the petal in such a fashion as to produce spurs, in which the nectaries were located. Most akin to such a spurless, primitive taxon, in which a spur could have appeared and became fixed, appears to be the Asiatic *A. ecalcarata*.

It can therefore be assumed, that *Aquilegia ecalcarata*, is related to the primitive, ancestral taxon, from which, by a process of a few macro-mutations and a whole series of small mutations with additive effects (under the influence of appropriate agents of selection) the genus *Aquilegia* arose.

On the basis of the studies here presented, it appears highly probable, that the genus *Aquilegia* had its origin in Asia. The original ancestral form, that gave a beginning to the whole genus, was presumably closely related to the presently living, relic species *A. ecalcarata*.

Studying the phylogenetical relationships of genera within the family *Polemoniaceae* and *Scrophulariaceae* Grant (1961) and Sprague (1962) conclude that the original pollination mechanism was adapted to pollination by bees. Phylogenetically younger species are pollinated by butterflies, moths and birds.

It appears that the evolution of the pollination mechanism in *Aquilegia* proceeded from the more primitive taxa of the *ecalcarata* and *vulgaris* complexes pollinated by flies and short-tongued bees, to a pollination by long-tongued bees and bumble bees in the taxa with longer spurs of the *vulgaris* and *alpina* complexes, then to a pollination by moths and butterflies in the *caerulea* complex, and finally to pollination by humming birds in the *canadensis* complex.

On the basis of what is known about the pollination systems, on the basis of an analysis of the progeny, and on the basis of the geographical distribution, some suggestions can be made about the degree of phylogenetic relationship between the complexes in the genus *Aquilegia*.

Closest to *A. ecalcarata*, that is the *ecalcarata* complex, appear to be the taxa of the *vulgaris* and *alpina* complexes. The *canadensis* and *caerulea* complexes contain taxa that seem to be phylogenetically younger and less related with the ancestral taxon.

SUMMARY

1. Most of the studied characters, such as: height of the plant, shape of the leaves, length of the spurs, degree of their curvature, dimensions of the petals and sepals, length of the androecium, length of the follicles, number of ovules, size of the seeds, time of flowering — demonstrate a continuous type of variation, sometimes transgressive, presumably caused by a segregation of polygenes. These characters have probably developed by way of many, small, additive mutations.

2. Such adaptive and diagnostic characters as the presence or absence of spurs, straight or curved spurs, positioning of the flowers, either nodding or erect, and the flower pigmentation are dependent on one or only few pairs of allelo-

morphs. These characters of apparently basic importance in the choice of pollinators, have originated as a result of single mutations, with big phenotypic effects.

3. If in the ancestral, spurless form of *Aquilegia* such a morphological change arose, as the introduction of a spur, this could have given the beginning to a new line of evolution. Such a single mutation has presumably caused the immediate formation of a flower type isolated from the rest of the population by its different pollination mechanism. The pollinators selective for the different mutations played in the evolution of the genus *Aquilegia* a decisive role.

4. The original ancestral form, which gave a beginning to the whole genus, was a form similar to the relic taxon *A. ecalcarata* now growing in Asia.

5. On the basis of knowledge about the evolution of the pollination mechanisms, the geographic distribution of the taxa of the genus *Aquilegia* and on the basis of the results of a progeny analysis, it can be assumed that from the distinguished complexes of species within this genus, the most primitive is the *ecalcarata* complex. Nearest to it in affinity appear to be the taxa of the *vulgaris* and *alpina* complexes, whereas the complexes *canadensis* and *caerulea* are much less related to it, but appear to be closely related to each other.

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REFERENCES

- Anderson E. and L. N. Abbe, 1933, A comparative anatomical study of mutant *Aquilegia*, Amer. Nat. 47:380—384.
- Baker, 1878, Gard. Chron. ser. 2, X, 19, 76. 111—203 (wg Munza 1946).
- Baur E., 1924, Untersuchungen über das Wesen, die Entstehung, und die Vererbung von Rassenunterscheiden bei *Antirrhinum majus*, Bibl. Genet. 4:1—17.
- Boothman, 1934, Gard. Chron. ser. 3, XCV, 12 (wg Munza 1946).
- Brühl P., 1893, Journ. Asiatic Soc. Bengal, LXII, 2, 270—324 (wg Munza 1946).
- Clausen J. and W. M. Hiesey, 1958, Experimental studies on the nature of species. IV. Genetic structure of ecological races, Carnegie Inst. Wash. Pub. 615:57—61.
- De Candolle, 1824, Prodr. I, 50—51 (wg Munza 1946).
- Dobzhansky T., 1953, Genetics and the origin of species, New York, Columbia University Press.
- Drummond I. R. and I. B. Hutchinson, 1920, A revision of *Isopyrum* and its nearer allies, Kew Bull. pp. 145—169.
- East E. M., 1935, Genetic reactions in *Nicotiana*. III. Dominance, Genetics, 20:443—451.
- Ellerton S., 1939, The origin and geographical distribution of *Triticum sphaerococcum* Perc. and its cytological behavior in crosses with *T. vulgare* Vill. Journ. Genet. 38:307—324.
- Frisch K., 1914, Der Farbensinn und Formensinn der Biene, Zool. Jahrb. Abt. 35:123—134.
- Gerard, 1597, Herbal (wg Munza 1946).
- Goldschmidt R., 1940, The material basis of evolution, New Haven, Yale Univ. Press pp. 436.
- Goldschmidt R., 1952, Homoeotic mutants and evolution, Acta Biotheoret. 10:87—104.

- Goldschmidt R., 1953, Experiments with a homoeotic mutant, bearing on evolution, J. Exptl. Zool. 123:79—114.
- Graenicher S., 1910, On hummingbird flowers, Bull. Wisconsin Nat. Hist. Soc. (Milwaukee) 8:183—186.
- Grant V., 1949, Pollination systems as isolating mechanisms in Angiosperms. Evolution, 3:82—97.
- Grant V., 1950, The flower constancy of bees. Bot. Rev. 16:379—398.
- Grant V., 1951, The fertilization of flowers, Scientific American 184 (6).
- Grant V., 1952, Isolation and hybridization between *Aquilegia formosa* and *A. pubescens*, El Aliso 2(4):341—360.
- Grant V., Flowers, Encyclop. Americana. Vol. 11 and 12.
- Grant V., 1961, The diversity of pollination systems in the *Phlox* family, Recent Advances in Botany.
- Gray A., 1883, *Aquilegia longissima*, Bot. Gaz. 8:295.
- Harland S. C., 1936, The genetical conception of the species, Biol. Rev. 11:83—112.
- Kappert H., 1944, Untersuchungen über Plasmonwirkungen bei *Aquilegia* (Gynodioecie — Heterosis — Gestalt des Sporus), Flora Allgemeine Botanische Zeitung — Neue Folge Bd. 37, Der Ganzen Reiche 137 Bd.
- Knuth P., 1909, Handbook of flower pollination, Oxford.
- Kristofferson K., 1926, Species crosses in *Malva*, Hereditas 7:233—354.
- Kruszewska A., 1961, The heredity of specific traits in the hybrid *Mirabilis jalapa* × *Mirabilis longiflora*, Acta Soc. Bot. Pol. 30:3—4.
- Lindstrom E. W., 1941, Genetic stability of haploid, diploid and tetraploid genotypes in the tomato, Genetics 26:387—397.
- Makino T., 1902, Observations on the flora of Japan, Jap. Bot. Magazine. 16:119.
- Mather K., 1943, Polygenic inheritance and natural selection, Biol. Rev. 18:32—64.
- Munz P., 1946, *Aquilegia*. The cultivated and wild columbines, Gentes Herbarum 7:1—150.
- Müller H., 1883, The fertilization of flowers, London.
- Pickens A. L., 1931, Some flowers visited by birds, Condor 33:23—28.
- Payson E. B., 1918, The North American Species of *Aquilegia*, Contrib. U.S. Nat. Herb. 20:133—157.
- Pražmo W., 1960, Genetic studies on the genus *Aquilegia* L. I. Crosses between *Aquilegia vulgaris* L. and *Aquilegia ecalcarata* Maxim., Acta Soc. Bot. Pol. 29(1):423—442.
- Pražmo W., 1961, Genetic studies on the genus *Aquilegia* L. II. Crosses between *Aquilegia ecalcarata* Maxim. and *Aquilegia chrysantha* Gray, Acta Soc. Bot. Pol. 30(3):57—77.
- Rapais, 1909, Bot. Kozlem, Budapest 12:117—136.
- Schneck J., 1901, Notes on *Aquilegia canadensis* Linn. and *A. vulgaris* Linn., Bot. Gaz. 32:304—305.
- Schwanitz F., 1956, Grossmutationen, Umschau 56:45—48.
- Schwanitz F. and H. Schwanitz, 1955, Eine Grossmutation bei *Linaria maroccana* L. mut. *gratioloides*, Beitr. Biol. Pflanz. 31:473—497.
- Skalińska M., 1928, Études sur la stérilité partielle des hybrides du genre *Aquilegia*, Zeitschr. Ind. Abs. u. Vererbungsl., Suppl.: 1343—1372.
- Skalińska M., 1928a, Sur les causes d'une disjonction non-typique des hybrides du genre *Aquilegia*, Acta Soc. Bot. Pol. 5:141—173.
- Skalińska M., 1929, Das Problem des Nichterscheinens des väterlichen Typus in der Spaltung der partiell sterilen *Aquilegia* — Species — Bastarde, Acta Soc. Bot. Pol. 4(1):138—164.

- Skalińska M., 1931, A new case of unlike reciprocal hybrids in *Aquilegia*, Proc. 5th. Int. Bot. Cong. Cambridge: 250.
- Skalińska M., 1935, Cytogenetic investigations of an allotetraploid *Aquilegia*, Bull. Acad. Pol. Sci. et Lettres, Ser. B: 33—63.
- Skalińska M., 1958, Seed development after crosses of *Aquilegia* with *Isopyrum*, Studies in plant physiology. Nakladatelství Československé Akademie Ved, Praha.
- Sprague E. E., 1962, Pollination and evolution in *Pedicularis* (Scrophulariaceae). El Aliso 5(2):181—209.
- Stubbe H., 1952, Über einige theoretische und praktische Fragen der Mutationsforschung, Abhandl. sächs. Akad. Wiss. Leipzig, Math. natur. Kl. 47:3—23.
- Stubbe H., 1959, Considerations on the genetical and evolutionary aspects of some mutants of *Hordeum*, *Glicine*, *Lycopersicon* and *Antirrhinum*, Cold Spring Harbor Symposia Quant. Biol. 24:31—41.
- Stube H. and F. von Wettstein, 1941, Über die Bedeutung von Klein und Grossmutationen in der Evolution, Biol. Zentralbl. 61:265—297.
- Trelease Wn., 1883, *Aquilegia longissima*, Bot. Gaz. 8:319.

Cytogenetyczne badania nad rodzajem *Aquilegia*

III. Dziedziczenie cech wyróżniających kompleksy gatunkowe

Streszczenie

Celem pracy było zbadanie w rodzaju *Aquilegia* sposobu dziedziczenia się szeregu cech adaptacyjnych. W tym celu przeprowadzono krzyżowania między różnymi taksonami — przedstawicielami wyróżnionych kompleksów gatunkowych. Szczególny nacisk położono na analizę mieszańców między *A. ecalcarata*, który przyjęto za najbardziej pierwotny gatunek w całym rodzaju, a przedstawicielami innych kompleksów.

Większość badanych cech takich jak: wysokość roślin, kształt liści, długość ostróg, stopień ich zakrzywienia, wymiary płatków i działek, długość pręcikowia, długość mieszków, liczba zalążków, wielkość nasion, termin zakwitania wykazuje zmienność ciągłą, czasami transgresywną powodowaną prawdopodobnie segregacją poligenów.

Na ich tle szczególnie wyraźnie odcinają się cechy o dużym znaczeniu przystosowawczym, mające jednocześnie znaczenie diagnostyczne, takie jak: obecność lub brak ostróg, cecha ostroga prosta — zakrzywiona, pozycja kwiatów: zwisłe — wzniesione oraz barwa kwiatów.

Brak ostróg u *A. ecalcarata* jest recesywny w stosunku do obecności ostróg u innych taksonów. Obecność ostrogi jest cechą dominującą. F_2 między *A. ecalcarata* a taksonami kompleksu *vulgaris* i *canadensis* wykazywały rozszczepienie w stosunku 3:1. F_2 między *A. ecalcarata* a taksonami kompleksu *alpina* i *caerulea* wykazywały rozszczepienie w stosunku 15:1. Brak ostróg kontrolowany jest więc przez obecność dwóch genów recesywnych. Dla rozwoju ostrogi wystarczy obecność jednego genu dominującego.

Cecha ostroga prosta jest recesywna w stosunku do cechy ostroga zagięta czy zakrzywiona. Uwarunkowana jest ona monogenicznie, jak cecha kwiat wzniesiony, recesywna w stosunku do cechy kwiat zwisły. Podstawowa barwa kwiatów uzależniona jest od współdziałania czterech par alleli, które dziedziczą się niezależnie.

Na podstawie tego co wiadomo o systemach zapylania u *Aquilegia* oraz w oparciu o dane dotyczące rozmieszczenia geograficznego i analizy genetycznej potomstwa wysunięto pewne sugestie co do mechanizmu specjacji. Czynnikiem odgrywającym zasadniczą rolę w ewolucji *Aquilegia* były zapylacze selekcjonujące poszczególne mutacje. Większość cech różniących badane taksony uwarunkowana jest poligenicznie, powstawała więc prawdopodobnie na drodze wielu „drobnych“, kumulujących się mutacji. Cechy o zasadniczym znaczeniu przy doborze zapylaczy, powstała, jak można zakładać, w efekcie pojedynczych mutacji, tzw. „dużych“ mutacji o wyraźnym efekcie fenotypowym.

Jeśli u pierwotnej bezostrogowej formy *Aquilegia* nastąpiła taka zmiana morfologiczna jak powstanie ostrogi, mogło to dać początek nowej linii ewolucyjnej. Taka jednostkowa mutacja spowodowała prawdopodobnie powstanie nowego typu kwiatu od razu izolowanego od reszty populacji na skutek odrębnego mechanizmu zapylania. Zależność kwiat — owad była podstawą dla działania selekcji i dalszych adaptacji. Pierwotna forma wyjściowa, dająca początek całemu rodzajowi, była zapewne formą zbliżoną do obecnie żyjącego w Azji, relikтового taksonu *A. ecalcarata*.

Najbliższymi tego taksonu wydają się taksony kompleksów *vulgaris* i *alpina*, występujące w Eurazji. Znacznie dalej spokrewnione wydają się taksony północno-amerykańskich kompleksów *canadensis* i *caerulea*, które z kolei wydają się blisko spokrewnione ze sobą.