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Genetic studies on the genus *Aquilegia* L.

I. Crosses between *Aquilegia vulgaris* L. and *Aquilegia ecalcarata* Maxim

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From the standpoint of taxonomy the genus *Aquilegia* is very interesting. It belongs to the family of *Ranunculaceae,* one of the most primitive of the angiosperm families, and has a widespread distribution indicative of at least Middle Tertiary origin. In the latest monograph (Munz 1946) of this genus 67 species distributed throughout the temperate zone of Euroasia and North America are described. The species are mainly allopatric. The differences between them consist almost exclusively in the size, shape, proportion, and colour of sepals and petals. Cytologically the genus *Aquilegia* is uniform. The somatic chromosome number of all the species examined so far is $2n=14$. The chromosomes are small and on the whole very alike so that the genus is not very convenient for cytological investigations. With regard to geographic distribution, ecological habitats, and above all the structure of flowers the particular species are highly differentiated. The group as a whole is a very typical example of genera with weak or no genetic barriers between the particular species. It has long been known that *Aquilegia* species are easily crossed. Anderson and Schafer (1931) and Skalińska (1928a, 1928b) demonstrated that in the genus a large proportion of F_1 hybrids, even from interspecific crosses between relatively distant species was at least partly fertile, which is an additional source of difficulties in the classification of these species. The genus *Aquilegia* is exceptional in that it combines an extreme morphological and ecological variability with a great facility for interspecific crosses. Differentiation in the genus tended to produce great morphological and ecological distinction without developing coenospecies (Clausen, Keck, and Hiesey 1945), so that the barriers between the particular units have a geographic or ecological rather than a genetic nature. This kind of barriers seems to result from narrow specialization in the structure of flowers adapted to various manners of pollination. From the results of investigations on the occurrence of interspecific crosses in nature and in horticultural conditions (Anderson and Schafer 1933) Grant (1942) reaches the conclusion that the main mechanism isolating the particular *Aquilegia* species consists in their adaptation to various pollinating agents and in a high degree of ecological differentiation. It seems therefore that, in spite of the considerable ability to produce fertile hybrids from artificial fertilization, cross pollination in natural conditions is relatively rare and so the differences between the species are maintained. Grant is of the opinion that the lack of sterility barriers is characteristic not only for the genus *Aquilegia,* but also for numerous other genera manifesting marked differentiation in the structure of flowers and a narrow adaptation to special pollinating agents, e.g. *Delphinium*, *Phaseolus*, *Antirrhinum* and orchids.

The genus *Aquilegia* can be divided into different groups of species with regard to the structure of flowers reflecting their adaptation to various pollinating agents. In all *Aquilegia* species the flowers have a corolla with the lower part of the petal forming a characteristic tube, tapering at the end and empty in the middle, that grows out of the lobe of the corolla. It is called the spur. The length of the spur differs in various species ranging from a few millimetres to 15 cm. Its shape can also vary from sharply curved through gently bent to straight. The colour of flowers in the particular species is white, blue, violet, purple, yellow, orange, and red. The flowers are either drooping or erect. According to Grant (1952) the genus *Aquilegia* can be divided into five groups or complexes that will now be described.

The *A. ecalcarata* group is probably the most primitive of all the groups in the whole genus. *A. ecalcarata* Maxim. is characterized by drooping purple flowers without spurs and with rather conspicuous petals. Pollination in this species is probably by insects with short mouth-parts, e.g. flies. The species grows in China and in Tibet. Its systematic position is still controversial. According to Drummond and Hutchinson (1920) *A. ecalcarata,* described for the first time in 1902 by Maximowicz, belongs to the separate genus *Semiaquilegia* forming as if a link between the genera *Aquilegia* and *Isopyrum*. The genus *Semiaquilegia* was set up by Makino (1902) who placed in it the species *Isopyrum adoxioides* described by De Candolle, because he believed that it was intermediate between *Isopyrum* and *Aquilegia* and thus deserved an independent generic name. Undoubtedly various traits in the structure of follicles and flowers, in particular the lack of spurs, relate *A. ecalcarata* to the genus *Isopyrum* or to the genus *Semiaquilegia* set up by Makino. Nevertheless, since *A. ecalcarata* gives fertile hybrids in crosses with various *Aqui-*

legia species and in view of the complete infertility of its crosses with *Isopyrum* (Skalińska 1958) it is more correct to include it to the genus *Aquilegia,* though the species must also be related with the genus *Isopyrum* because in cross pollination with *Isopyrum thalictroides* fertilization does take place. Anderson and Schafer (1931) called *A. ecalcarata* a living fossil. In their opinion it represents the original primitive form for the whole genus *Aquilegia* and the forms from which in the course of evolution the other species with various types of spurs adapted to various pollinating agens developed.

The second group, called by Grant the *A. vulgaris* group, covers many species growing over enormous areas stretching from East Asia to Western Europe and in North America. The group is characterized by drooping blue or purple flowers with relatively short curved spurs and rather long petals. The curved spurs, longer than in the first group, reflect the adaptation to pollination by insects with longer mouth-parts e.g. bumblebees and bees.

The third group called *A. alpina* resembles greatly the *A. vulgaris* group and differs from it only by the straight spurs. Its distribution area spreads from Japan through the whole Euroasian continent to Spain and is thus sympatric with the *vulgaris* complex. In so far as the system of pollination is concerned there seems to be no significant differences between the two groups. There are, thus no essential reasons why the two complexes of species should be distinguished as separate groups and it would be far more correct to join the *alpina* with the *vulgaris* complex.

The fourth and the fifth groups, composed of species native to North America, were called by Grant *A. canadensis* and *A. caerulea* respectively. Both groups have very wide distribution areas and include numerous species with many subspecies and varieties. In the *canadensis* group the drooping red-yellow flowers with not very long straight spurs and short petals are adapted to pollination by hummingbirds.

The species of the *caerulea* group have rather large usually yellow flowers with straight or gently bent long spurs. The complex became differentiated in North America and is adapted to pollination by moths.

In view of the above mentioned evidence it seems very likely that the *A. ecalcarata* species constitutes the primitive form for the whole genus, in which the adaptation to various types of pollinating agents developed along different lines. For this reason this species has been selected for crosses with other species belonging to the particular *Aquilegia* groups in order to investigate the genetic nature of the processes of specialization. If it is true that the general trend of evolution in this genus is towards the elongation of the spurs, then the *vulgaris* group seems to be most closely related to the *ecalcarata* group. Such a supposition is supported, moreover, by the geographic distribution of the two species. The present paper, the first in a series of reports, describes the results obtained by analysing hybrids of *A. vulgaris* x *A. ecalcarata*.

MATERIAL AND METHODS

Plants of two *Aquilegia* species growing in the Botanical Garden in Warsaw, i.e. *A. vulgaris* from Poland and *A. ecalcarata* from China, were self-fertilized in controlled conditions. The offsprings obtained from these "self-fertilized" seeds grew on experimental plots at Skierniewice and flowered on the second year after they had been sown out. The populations of both parental forms proved to be sufficiently homozygous for starting the crosses. *A. ecalcarata* (P_e) was a small plant with small drooping purple flowers, short follicles and no spurs. Its leaves were triangular and bilobate (fig. 3). *A. vulgaris* (P_v) had drooping blue flowers with distinctly curved spurs, rather conspicuous petals, and large follicles; the leaves were double, bilobate.

The setting of seeds when *A. vulgaris* was pollinated with pollen from *A. ecalcarata* and vice versa was almost normal. The germination rate was high and amounted to about 75 per cent. Similarly, numerous seeds, from which the F_2 generation grew out, were obtained without much difficulty from self-pollination of F_1 plants (it is better to repeat the operation more than once), though the fertility of F_1 was distinctly reduced. The seeds obtained from F_1 plants were mixed and sown out into boxes with soil. After germination every young plant was transferred into a separate pot and some weeks later planted out on the experimental plots. Backcrosses were also obtained without any difficulty. The germination rate of F_2 seeds was about 50 per cent.

All measurements of the height of plants, the length of spurs, pollen fertility etc. were made the same year on plants growing on neighbouring plots so as to obtain data of a high comparative value. Since the length of spurs differs according to the age of flowers (Kappert 1944) the spurs were measured, as far as possible, on flowers of the same age, i.e. the next day after the splitting of anthers. For pollen fertility examinations the preparations were stained with aceto-carmine.

For examinations of meiosis in pollen mother cells the buds were treated with Nawaschin's fixative according to Müntzing's modification. The preparations were embedded in paraffin wax, cut into sections 5—6 microns thick, and stained with gentian violet.

RESULTS

A. Cytological analysis

For analysing cytologically *A. vulgaris*, *A. ecalcarata* and F_1 hybrids 50 PMCs were examined from each of these groups of plants, in every one of the following stages of the first division: metaphase, anaphase, and telophase. In both parental species and in F_1 the haploid number of chromosomes was n = 7. In metaphase there were always only 7 bivalents. The segregation of chromosomes in anaphase was absolutely regular and no abnormalities were observed (fig. 1).

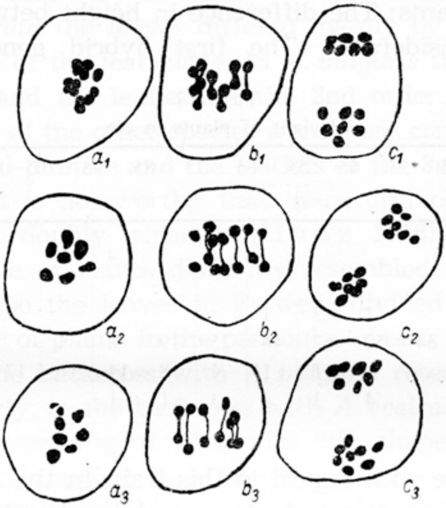

Fig. 1. Meiosis in PMCs of *A. vulgaris* (a_1, b_1, c_1), *A. ecalcarata* (a_2, b_2 c_2), and in the first hybrid generation *A. vulgaris* x *A. ecalcarata* (a_3, b_3, c_3), a_1, a_2, a_3 — first meta-anaphase; b_1, b_2, b_3 — first meta-anaphase; c_1, c_2, c_3 — first telophase

B. The inheritance of traits distinguishing the two parental species

In the genetic analysis of the differences between *A. vulgaris* and *A. ecalcarata* the following traits were measured or estimated:

1. height of plants
2. shape of leaves
3. length of spurs
4. length of the petals of corolla
5. length of sepals of calyx
6. bredth of sepals of calyx

7 colour of flowers
8. length of follicles
9. number of seeds in follicle
10. size of seeds
11. pollen fertility
12. seed fertility.
Moreover, the correlations between some of these traits were examined.

1. Height of plants: The difference in height between *A. vulgaris* and *A. ecalcarata* is considerable. The first hybrid generation was almost exactly intermediate with regard to this trait. In the F_2 population of 264 individuals there were some plants resembling by their height either of the parental species (Table 1). However, the most numerous group consisted of plants the height of which was approximately the same as in F_1. Moreover, in F_2 there was a transgressive segregation. A marked transgression occurred also in the backcross with *A. vulgaris*. In the backcross with

Table 1
Height of plants in cm.

Class values	5		15		25		35		45		55		65		75		85		95		105		115		125		135	N	$\bar{x}$
Pv														1		1		4										6	85
Pe				6																								6	20
F_1								1		3		1																5	50
F_2		1		18		34		52		62		44		25		19		8		1								264	50
Pv × F_1										1		1		4		8		14		16		17		8		1		67	102
Pe × F_2		1				1		4		3		4		3		1		1										18	53

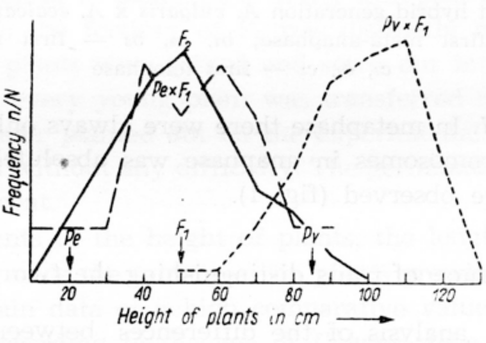

Fig. 2. Curves showing the variation of the height of plants in F_2 and in backcrosses of the hybrid *A. vulgaris* x *A. ecalcarata*

A. ecalcarata there was some transgression relatively to F_1 but the variation range coincided on the whole with the variation range in F_2 (Fig. 2).

2. Shape of leaves: So far as the vegetative parts are concerned the differences between *Aquilegia* species are very slight. In particular, the leaves of various species are very similar. For this reason the study of the variations in this trait in F_2 was rather difficult. There were no measurable criterions which would permit to divide the segregates into different classes. The variations were very definitely continuous. In the case of *A. vulgaris* and *A. ecalcarata* the leaves differed mainly by the degree of complexity and the size of the leaf blade. In *A. vulgaris* the leaves were large twice odd-pinnate and the leaflets of the 2nd order were tripartite and bilobate. The leaves of the other species were more compound and smaller, they were thrice odd-pinnate and the leaflets of the 3rd order were tripartite and bilobate. It is noteworthy that in natural conditions the leaves of *A. ecalcarata* are doubly tripartite (Munz 1946). In the first hybrid generation the shape of leaves distinctly resembled the ecalcarata type. With regard to shape the leaves in F_2 were divided into 5 classes only (fig. 3). The number of plants in the particular classes is shown in Table 2.

The leaves of the backcross with *A. vulgaris* resembled the leaves of this species. Similarly, in the backcross with *A. ecalcarata* the leaves were of the ecalcarata type. Fig. 3 illustrates the shape of leaves in both parental species. in F_1, and in F_2.

Table 2
Inheritance of leaf's shape

Class	I	II	III	IV	V	N
Pv, Pe, F_1	Pe		F_1		Pv	
F_2	6	12	84	201	27	303
Pv × F_1			9	41	26	76
Pe × F_1	10	5	3			18

3. Length of spurs: The presence or the lack of spurs is the most essential trait distinguishing the two species. The data in Table 3 show the average length of spurs in the two parental species and in F_1 as well as the variation range for this trait in F_2 and in backcrosses. The first hybrid generations was more closely related to *A. vulgaris,* a fact observed already by Anderson and Schafer (1931). The second generation consisted of 202 plants of which 50 had flowers without spurs. This shows very clearly that segregation was monogenic according to the ratio 3:1 (the anticipated numbers were 50,5:151,5). All of the remaining 152 plants had spurs the length of which varied continuously ranging from 3 to 21 mm (fig. 4). In F_2, besides plants of the parental types, individuals

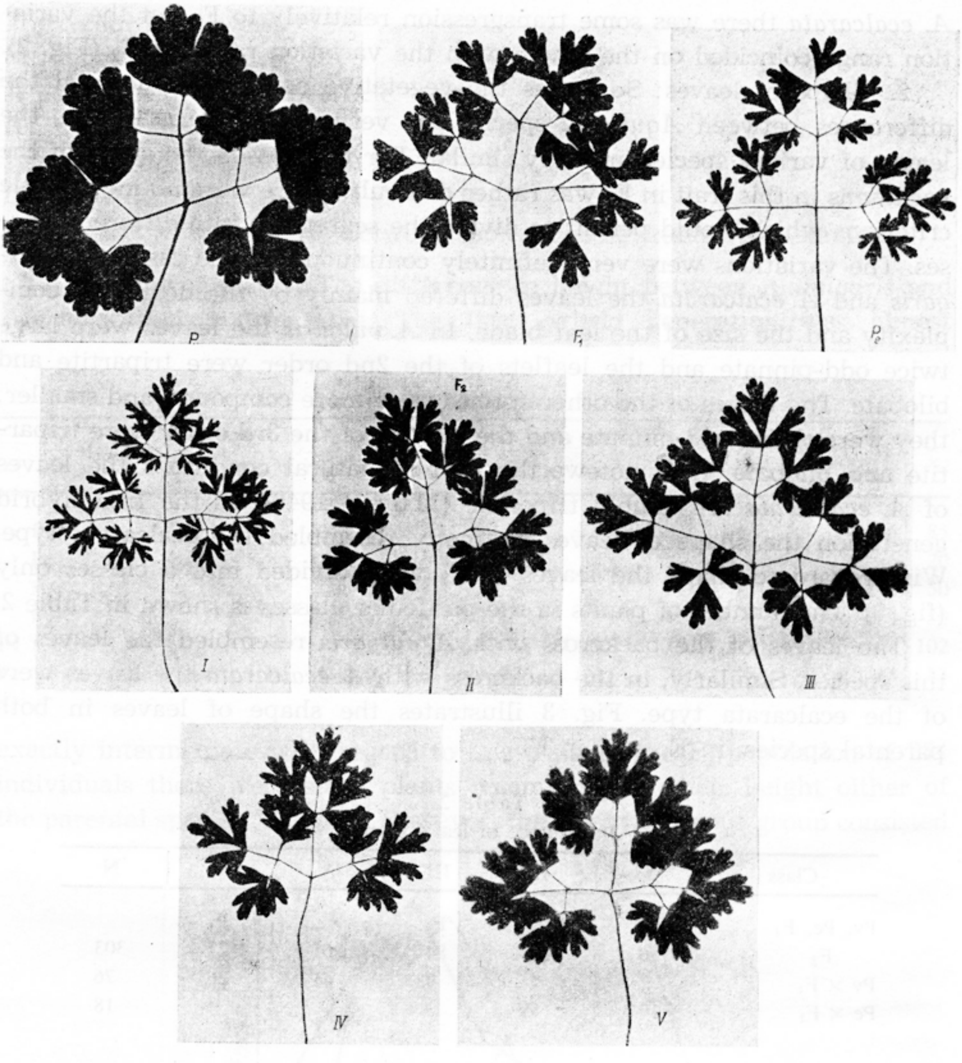

Fig. 3. Basal leaves of *A. vulgaris* — Pv, *A. ecalcarata* — Pe, F_1 and F_2 grouped in five classes

reproducing the trait in the intermediate form were the most numerous. All the plants in the backcross with *A. vulgaris* had spurs and in the backcross with *A. ecalcarata* out of 18 plants 11 had no spurs. This segregation approached the anticipated ratio 1:1, however, in view of the small number of individuals the Mendelian ratio could not be established accurately. It seems that the results of both backcrosses confirm the monogenic character of this trait.

Table 3

Length of spurs in mm.

Medium class value	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	N	$\bar{x}$	$\pm s\bar{x}$	t
Pv																	1	6	2	8	2		1			20	18,3	0,91	2,84
Pe	20																												
F_1														2	2	4	7	4	1							20	15,5	0,28	2,84
F_2	50			1	2	4	4	2	4	6	11	7	17	22	26	22	9	10	4			1				202	16,0*	0,38	2,54
$Pv \times F_1$														2	2	2	4	7	13	11	8	12	3	2	1	67	18,7	0,082	2,66
$Pe \times F_1$	11		1		2	1	1	1	1																	18	5,1*	0,692	3, 9

* Mean $\bar{x}$ for F_2 with $N = 202 - 50 = 152$, for $P_e \times F_1$ with $N = 18 - 11 = 7$

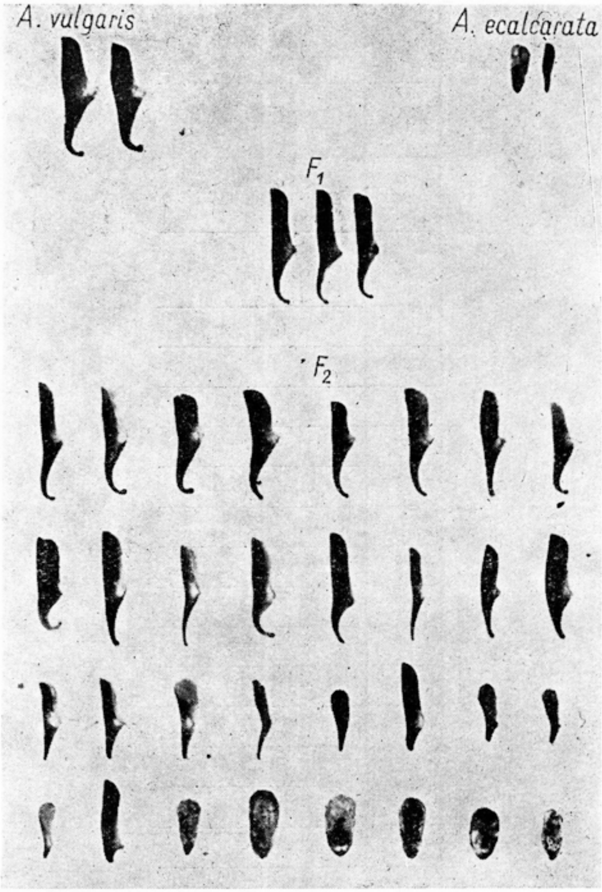

Fig. 4. Photograph showing the petals of the corolla in the parental species, F_1 and F_2. Note the continuous character of the variation in the length of the spured F_2 plants

4. The length of the petals of the corolla: In both parental species the length of the petals was almost the same. Table 4 illustrates the variation range for this trait in *A. vulgaris*, *A. ecalcarata*, F_1, F_2, and in backcrosses.

The two species must have numerous polymerous genes determining the length of the petal, which was indicated by the considerable transgression in F_2.

5. and 6. The length and breadth of sepals: The behaviour of these traits is shown in Table 5.

The shapes of sepals in the two species differed markedly. To illustrate the differences of shape an index was calculated by dividing the length of sepals by their breadth. The frequency distribution of the values of this index for both parental species, F_1, F_2, and the backcrosses is shown in Table 6.

Table 4
Length of corollas blade in mm.

Medium class value	8	9	10	11	12	13	14	15	16	17	18	19	20	21	N	$\bar{x}$	$\pm s_{\bar{x}}$	t
Pv					1	1	5	13							20	14,4	0,16	2,84
Pe				1		2	7	7	3						20	14,3	0,23	2,84
F_1								5	1	11	3				20	16,5	0,19	2,84
F_2	1	1	5	7	19	36	36	33	34	13	10	4	3		202	14,4	0,15	2,57
Pv × F_1			1		6	22	10	15	10	3					67	12,0	0,31	2,66
Pe × F_1								4	1	2	5	2	2	2	18	13,8	1,07	2,87

In the first hybrid generation the trait had an intermediate value while in the second generation it manifested a typical transgressive segregation. The transgression was very distinct which means that the sepal shape, similarly as the length of the petal, was inherited polygenically.

Table 5
Length and breadth of sepals in mm.

	length			breadth			t
	$\bar{x}$	$\pm s_{\bar{x}}$	N	$\bar{x}$	$\pm s_{\bar{x}}$	N	
A. vulgaris	28,0	1,184	20	12,98	0,25	20	2,84
A. ecalcarata	15,5	0,21	20	5,78	0,16	20	2,86
F_1	22,4	0,38	20	9,00	0,29	20	2,84

7. The colour of flowers: The flowers of *A. vulgaris* are violet-blue. In the colour chart according to which the colours were determined (Horticultural Colour Chart I, II) this colour is defined as Campanula violet 37 (corolla) and Aster violet 38 (calyx). *A. ecalcarata* flowers are purple. The colour of the corolla is Lilac purple-o31/2 and of the calyx Pansy violet o33. The colour of the flowers of F_1 plants was intermediate — colour of calyx was Aster violet 38 and of the corolla — Pansy violet o33. Microscopic examinations of the petals indicated that in both species the colour was produced by anthocyanin contained in epidermal and mesophyll cells. Usually anthocyanin was dissolved in cell sap. Besides anthocyanin no other pigment was found. In the F_2 generation, consisting of 262 individuals, 6 had flowers of the same colour as in *A. vulgaris,* 49 had flowers as in *A. ecalcarata*, and the remaining 207 individuals had flowers appro-

Table 6

The values of the coefficient of shape of the sepals

Class value	1,6	1,7	1,8	1,9	2,0	2,1	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	3,0	3,1	3,2	3,3	3,4	3,5	3,6	3,7	3,8	3,9	4,0	N	$\bar{x}$	$\pm s_{\bar{x}}$	t
Pv				1		6	8	2		1	1															20	2,2	0,036	2,84
Pe						2		1		1	1	1	6	3	2				1	1						20	2,7	0,08	2,84
F_1						1	2	3	4	4	2		3	2												20	2,4	0,006	2,84
F_2			2	3	6	9	12	10	25	22	25	18	16	11	14	8	7	6		3		2		2	1	202	2,6	0,03	2,57
Pv × F_1		2	7	13	8	8	9	12	4	1	1	1	1													67	2,1	0,03	2,66
Pe × F_1						3			3	3	2	2	2	1												18	2,4	0,06	2,87

aching in colour F_1 plants, but diverging in the directions of both parental species.

Table 7
Length of the follicles in mm

Medium class value	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	N	$\bar{x}$	$\pm s_{\bar{x}}$	t
A. vulgaris														1		5	3	10	1	20	23	0,84	2,84
A. ecalcarata	4	2	6	6	2															20	9	0,43	2,84
F_1					2	8	8	2												20	12,5	0,48	2,84

8. Length of follicles: In *A. ecalcarata* the follicles are shorter than in *A. vulgaris*. The data in Tables 7 and 8 show this trait in the parental species and in F_2. The first generation resembled more *A. ecalcarata* than the other parent. In F_2 segregation was rather pronounced; in the majority of plants the follicle length resembled the length of this organ in F_1 and *A. ecalcarata* plants, but there was not one F_2 plant in which the follicle length even approached the length of follicles of the other parental species. This means that the trait, similarly as the preceeding ones, was determined by several polymerous genes.

Table 8
Length of follicles in mm

Medium class value	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	N	$\bar{x}$	$\pm s_{\bar{x}}$	t
P_v, P_e, F_1			P_e			F_1											P_v						
F_2		2	11	16	31	32	30	30	10	8	6	1	1							178	12,4	0,15	2,57

9. Number of seeds in follicle: Together with measurements of the follicle length counts were made of the number of well developed seeds, of unfertilized ovules, and of ovules that had been fertilized but did not develop. The total of these counts was considered as the „number of seeds in a follicle". It was found that in shorter follicles of *A. ecalcarata* the number of seeds was smaller. The manner in which this trait was segregated in F_2 as well as its mean values in F_1 and in both parental species are shown in Table 9.

In F_1 individuals the trait was expressed intermediately, while in F_2 its range of variation was wide, so that extreme F_2 individuals had seed numbers either higher than *A. vulgaris* or lower than *A. ecalcarata*. This

Table 9

Number of seeds per follicle

Class value	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	N	$\bar{x}$	$\pm s_{\bar{x}}$	t
A. vulgaris																				P_v					
A. ecalcarata			P_e																						
F_1										F_1															
F_2	2	5	9	9	14	20	13	21	23	13	15	9	12	5	2	2	1	1	1	1	1	170	25	0,3	2,57

Table 10

Mean length, breadth and size of seeds in parental species, F_1 and F_2

	Length				Breadth				Size			
	$\bar{x}$	$\pm s_{\bar{x}}$	N	t	$\bar{x}$	$\pm s_{\bar{x}}$	N	t	$\bar{x}$	$\pm s_{\bar{x}}$	N	t
A. vulgaris	6,9	0,06	20	2,84	4,0	0,16	20	2,84	28,1	0,42	20	2,84
A. ecalcarata	4,3	0,2	20	2,84	2,6	0,07	20	2,84	11,3	0,28	20	2,84
F_1	6,00	0,07	20	2,84	3,2	0,06	20	2,84	19,2	0,40	20	2,84
F_2	6,00	0,03	415	2,57	3,1	0,03	415	2,57	18,3	0,37	83	2,57

Table 11

Size of seeds (length x breadth) in 83 F_2 plants

Medium class value	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	N	$\bar{x}$	$\pm s_{\bar{x}}$	t
P_v, P_e, F_1		P_e								F_1									P_v				
F_2					3	9	6	11	13	16	12	6	4	1	2					83	18,3	0,37	2,63

result can be explained by assuming that the trait is determined by many different polymerous genes.

10. Size of seeds: Since the size of seeds differed in the two species the manner in which this trait was inherited was investigated. The length and the breadth of the seeds was not measured in millimetres but in the divisions of a scale [1] (Table 10). The majority of the results are given as the product of these values. The distribution of variations in the size of seeds is shown in Table 11.

Figure 5 shows the polygons of variation in the size of seeds in *A. vulgaris* and *A. ecalcarata* as well as in the first and second filial generations of the hybrids between these two species. The segregation in F_2 was of the so-called intermediate type, i.e. there were no individuals reproducing either of the parental species. The size of seeds was probably determined by rather numerous polymerous genes.

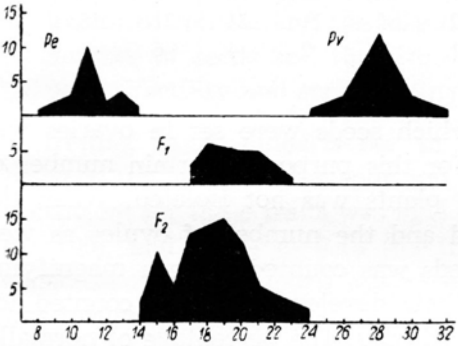

Fig. 5. Polygons of variations of seed size in *A. vulgaris*, *A. ecalcarata*, and in F_1 and F_2 generations of the hybrids between these two species. The figures along the x-axis indicate the size of seeds and along the y-axis the number of individuals

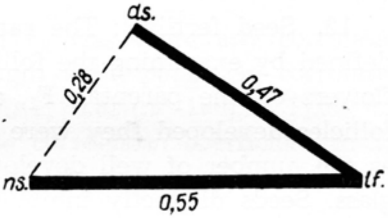

Fig. 6. Triangle illustrating the dependence between the length of follicles — lf, the number of seeds — ns, and the size of seeds — ds. Continuous lines mark the highly significant correlations, broken line the insignificant correlation

11. Pollen fertility: In the two parental species pollen fertility was absolutely normal: in *A. vulgaris* it amounted to 96 per cent and in *A. ecalcarata* to 82 per cent. In the first hybrid generation pollen fertility decreased markedly ranging in various preparation from 30 to 50 per cent, average about 42 per cent. In the majority of F_2 plants pollen fertility was the same as in F_1 or somewhat higher, but out of a total of 160 F_2 individuals only in 4 per cent pollen fertility was higher than 80 per cent (Table 12).

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[1] One division of the scale equalled 0,3 mm.

The plants from both backcrosses manifested a marked increase of fertility and in the majority of these plants pollen fertility was above 70 per cent.

Table 12

Pollen fertility in %

Class values	0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	N
A. vulgaris									1	5	6
A. ecalcarata								2	3	1	6
F_1				2	4						6
F_2	2	14	15	29	29	23	22	18	5	3	160
$P_v \times F_1$				1	2	2	11	24	21	16	67
$P_e \times F_1$		1			1	1	2	4	6		15

12. Seed fertility: The rate at which seeds were set in ovaries was defined by examining the follicles. For this purpose a certain number of flowers on the parental, F_1, and F_2 plants was not isolated. When the follicles developed they were opened and the number of ovules as well as the number of well developed seeds was counted using a magnifying glass. Seeds distinctly inhibited in their development were counted together with the completely undeveloped ones. The percentage of normally developed seeds in both parental species was about 75. Seed fertility in F_1 was strongly reduced and amounted only to 19 per cent. The range of variation of seed fertility in F_2 is shown in Table 13.

Table 13

Seed fertility in %

Class values	0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	N
A. vulgaris						2	2				4
A. ecalcarata					1		2				3
F_1		2	2								4
F_2	33	45	36	29	10	4	2	1			160

In the majority of F_2 plants seed fertility approached seed fertility in F_1. This means that pollen fertility was higher than seed fertility. There was not one plant in F_2 with seed fertility higher than pollen fertility.

Some of the plants in the second hybrid generation were almost completely sterile. The low proportion of developed pollen grains as well as the even lower proportion of fertilized and well developed seeds seem to indicate that in F_1 and more particularly in F_2, in spite of the almost normal course of meiosis, hybrid fertility depended on the co-operation of numerous genes segregating in F_2.

Correlations

The following correlations between various traits were examined:

length of spur and length of the petal of corolla
number of seeds and length of follicle
length of follicle and the size of seeds
number of seeds and the size of seeds
pollen fertility and seed fertility.

Neither in *A. vulgaris* nor in F_1 and F_2 was there any correlation between the length of the spur and the length of the petal. The correlation coefficient for these traits was in *A. vulgaris* $r=0.33$ ($n=100$), in F_1 $r=0.26$ ($n=100$), and in F_2 $r=0.18$ ($n=152$). The correlation coefficient in the backcross with *A. vulgaris* was $r=0.27$ i.e. also in this case the dependence was insignificant.

It was observed that the number of seeds in the long follicles of *A. vulgaris* was relatively high and that the seeds were larger. For this reason the correlation between these traits was examined in F_2.

The correlation coefficient between the length of follicles and the size of seeds was high, i.e. $r=0.477$ ($n=83$). A marked correlation was also noted between the number of the seeds and the follicle length $r=0.555$ ($n=83$). On the other hand there was no significant correlation between the number of seeds and their size ($r=0.28$). The interdependence of these three traits is illustrated by the triangle in fig. 6.

The correlation between pollen and seed fertility in F_2 plants was high. The correlation coefficient $r=0.888$ ($n=160$) seems to indicate that the degeneration of microspores, macrospores, or zygotes was caused by similar factors of a genic or chromosomal nature.

DISCUSSION

All the traits distinguishing the two *Aquilegia* species crossed in this investigation with the exception of the ability to form spurs, have proved to be determined polygenically and are inherited in a more or less continuous manner. The ability to form spurs is a trait of fundamental signifance. It is inherited monogenically and probably appeared as a result of one mutation.

The evolutional role in speciation of rare mutations having large morphological effects, as compared with mutations giving only small effects, has long been a problem of controversy. Some geneticists, as for instance Goldschmidt, believe that various taxonomic units appeared as a result of large mutations essentially altering the structure of an organism or of some organs. However, according to others opinions the large mutations have no major significance and the dominant role in speciation proceses is played by numerous mutations with small cumulative effects; this is indicated by that most of the differences between subspecies and species are segregated in hybrids in a complex fashion which seems to be caused by multiple factors (Tedin 1925, Müntzing 1930). The differences between traits determined by multiple factors must be due to the appearence and the stabilization in a population of numerous small mutations.

It has now been demonstrated by induced mutations that large mutations are possible and can change very essentially the structure of some organs. Spontaneous large mutations have also been reported. Goldschmidt (1940) stresses that in *Drosophila melanogaster* there are mutants without wings or with four instead of the usual normally developed two wings. In systematics such differences are regarded as distinctive not only for genera but even for higher systematic units. Stubbe and Wettstein (1941) described mutations in *Antirrhinum majus* causing gross structural alterations in flowers. The most interesting of these mutations is the transcedens one in which the numer of stamens is reduced from four to two. The mutation resembles the tendency towards a reduced number of stamens apparent in numerous genera of the *Scrophulariaceae* family to which the genus *Antirrhinum* belongs. In this genus (Baur 1924), similarly as in many others, a mutant gene has also been identified which alters the bilabiate zygomorphic corolla normal for this genus into a radially symmetrical corolla characterstic for more primitive genera. Admittedly the above example is not the best that could be chosen to illustrate the evolutional significance of large mutations since the mutant is sterile. Owing to the structural alterations its flowers cannot be pollinated by the insects by which it is normally visited and the stamens are so arranged that the pollen cannot reach the stigmata. Atavistic mutations of this kind give rise to traits that are disharmonious with regard to the other traits of an organism so that such plants will be rapidly eliminated by selection. It seems that mutations can only be stabilized when they affect the development in the latest stage possible for modifying the mature structure in the direction of a selectively favourable shape. If such a large mutation affects an organ that is not highly specialized or one with a more general structure and if it takes place relatively late in the ontogenic development the probability of disorganizing the whole developmental process is smaller and the mutation may start a new specialization. Most probably both the genes with a large phenotypic effect as well the multiple ones, the "polygenes", with small effects have exerted in various plants an essential influence on the formation of new evolutional lines. The relative significance of these two factors has probably changed in various groups. Moreover, these two types of mutations do not represent strictly defined categories but are connected by a continuous series of mutations with intermediate effects.

In the light of the results obtained in this investigation it seems reasonable to assume that in the primitive forms related to *Isopyrum,* in which the nectaries were placed at the base of the petals of the corolla one mutation might have changed the developmental structure of flowers in such a way that spurs with the nectaries at their bottom appeared. A single mutation of this kind probably gave rise to a new type of flowers that from the very beginning were distinctly isolated from the remaining population by the different mechanism of pollination. At the same time the mutant could be adapted to other pollinating agents and thus an isolation barrier developed making less probable crosses with the primitive form from which it arose. This mutation might have started a new evolutional line for the genus *Aquilegia.* The other traits, characteristic for the two investigated species and determined by numerous polymerous and possibly modifying genes, have probably developed as a result of repeated gradual small mutations with an cumulative effect.

There is ample evidence to assum that *A. ecalcarata* is a relict form related to the primitive type original for the whole genus *Aquilegia,* while *A. vulgaris* is undoubtedly a derived though related form. The genetic analysis of the crosses between these two species shows that most of the traits distinguishing the parental forms developed through the accumulation of numerous mutations with small cumulative effects. This refers also to the length and shape of petals and spurs. However, the monogenic nature of the trait consisting in the ability to form spurs seems to indicate that the development of spurred species from plants without spurs might have been determined by one primitive mutation step. Further experiments with crosses between *A. ecalcarata* and species belonging to other groups mentioned at the beginning of this report will perhaps demonstrate in a more convincing manner the nature of speciation processes in the genus *Aquilegia.*

SUMMARY

1. The manner in which specific traits were inherited in the hybrids of *Aquilegia vulgaris* x *A. ecalcarata* were investigated.

2. It was found that both species had the haploid chromosome number n=7 and that in meiosis of F_1 hybrids the conjugation was absolutely normal.

3. F_1 hybrids were only partly fertile (pollen fertility = 42 per cent and seed fertility = 19 per cent).

4. In spite of the partial fertility of F_1 hybrids all the examined traits characterizing the parental species segregated regularly in F_2 and in backcrosses. The majority of the investigated traits, such as the height of plants, the shape of leaves, the length of spurs, the size of petals and sepals, the length of seed follicles, the number and the size of seeds, as well as pollen and seed fertility, manifested in F_2 and in backcrosses a continuous, sometimes transgressive variation reflecting the segregation of numerous polygenic factors.

5. The trait of the absence of spurs in *A. ecalcarata* was recessive with regard to the trait of the presence of spurs in *A. vulgaris,* the difference being monogenic. Of F_2 plants $^3/_4$ had spurs the length of which varied continuously, which means that the development of this organ was influenced not only by the main gene but also by numerous other genes modyfying the length of spurs.

6. In the discussion the part played by the gene determining the formation of spurs in the evolution of the genus *Aquilegia* from the primitive forms without spurs is considered.

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