

## Genetic Studies on the genus *Aquilegia* L.

### II. Crosses between *Aquilegia ecalcarata* Maxim. and *Aquilegia chrysantha* Gray

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This second paper on the experiments with the genus *Aquilegia* reports the results of genetic analyses of the crosses between *A. ecalcarata* and *A. chrysantha*. There are many reasons to assume that *A. ecalcarata* is a relic species closely resembling the primitive plants from which the whole genus *Aquilegia* originated (W. Pražmo 1960). *A. chrysantha* belongs to the *caerulea* complex (Grant 1952), which probably differentiated in North America and is pollinated by moths.

*A. chrysantha* has very long spurs and in this regard diametrically differs from *A. ecalcarata*, which has no spurs. Since the main trend in the differentiation of *Aquilegia* species has been towards morphological differences in the flower structure (Clausen, Keck, and Hiesey 1945) which reflect the adaptation of the particular species to the different modes of pollination (Grant 1952), this investigation has been primarily concerned with the analysis of the morphological traits of flowers.

#### MATERIAL AND METHODS

The plants of *A. ecalcarata* ( $P_e$ ) are 15 to 30 cm. high; they have triangular leaves and small purple flowers drooping on very slender peduncles (Fig. 1). The sepals of the calyx are, elliptically ovoid, and pointed at the tip. The petals of the corolla are narrow and elongated, rounded at the tip, spurless, and bent at the base into a bag-like process. The species comes from China.

The plants of *A. chrysantha* ( $P_{ch}$ ) are taller, 60 to 90 cm. high, with bright green triangular leaves and large, erect, pale yellow flowers on stiff peduncles (Fig. 2). The sepals of the calyx are ovate-lanceolate. The petals of the corolla are oblong-ovate, bluntly rounded at the tip, and parted. The spurs are very long (37 to 46 mm.), parted fairly wide at the base, gradually passing to a narrow tube slightly thicker at the end (the nectary). The stamens stand well above the petals of the corolla.

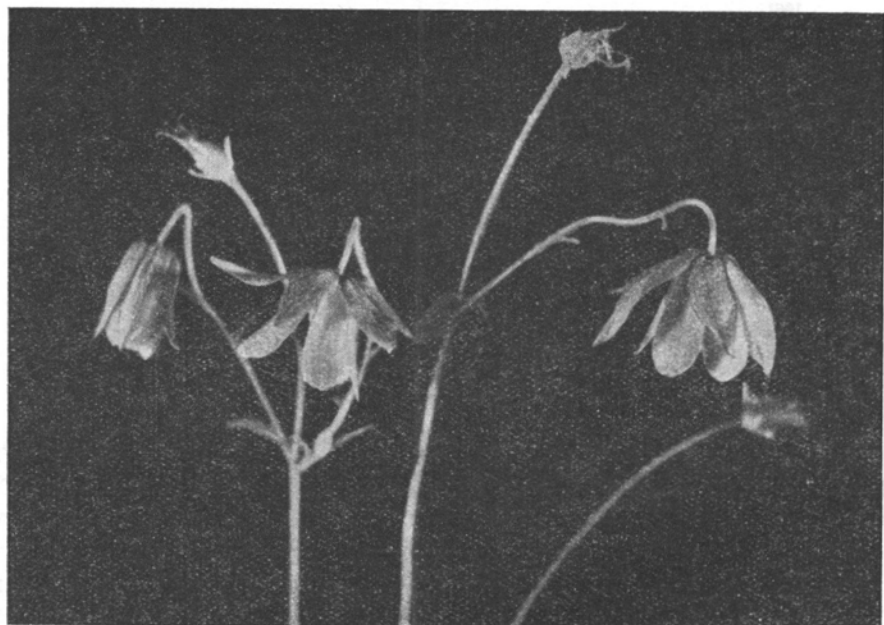


Fig. 1. *Aquilegia ecalcarata*

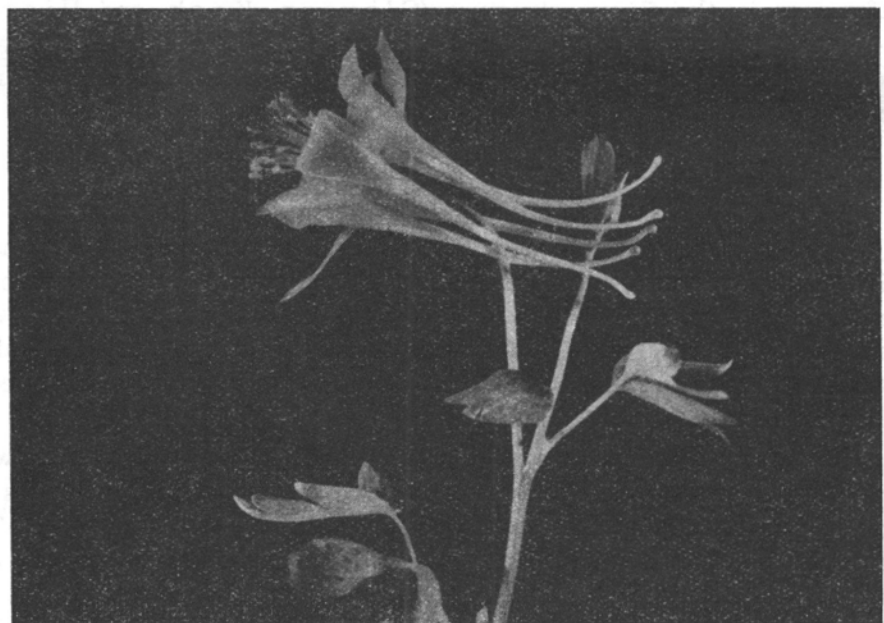


Fig. 2. *Aquilegia chrysantha*

*A. chrysantha* comes from the South-East of the United States of North America.

The parent plants for the crosses were obtained by self-pollination of individuals of both species.

The pollination of *A. ecalcarata* by pollen of *A. chrysantha* produced well developed follicles containing seeds that germinated very well. In spite of several attempts all reciprocal crosses were unsuccessful. The first hybrid generation of 22 plants was uniform with regard to the traits considered in the investigation. All the hybrid individuals began flowering simultaneously with *A. ecalcarata* (between May 17 and 20). *A. chrysantha* began flowering a fortnight later (May 29 to June 2).

After seven or eight days when the hybrids were in full blossom they were self-pollinated or inter-pollinated so as to obtain seeds for the  $F_2$  generation. The setting of seeds in isolating bags without additional pollination was 4.3 per cent and when additional pollination was repeated several times the setting of seeds increased to 8 per cent. The total number of collected seeds was 2097. The seeds were sown out into boxes in the spring of the following year. After four to five weeks 630, i.e. 30 per cent, of the seeds germinated. At the stage of two leaves the seedlings were transferred into pots and three months later they were planted out into experimental plots. A certain number of seedlings (21 per cent) perished soon after germination.

For pollen fertility counts pollen grains were stained with 1 per cent aceto-carmin. Buds collected for examinations of meiosis in pollen mother cells were fixed in Carnoy's fixative. The preparations were made with the smear technique and stained with 1 per cent aceto-carmin or 1 per cent propio-carmin. The colours of flowers were determined according to the Horticultural Colour Charts I and II.

## RESULTS

### Cytological Analysis

*A. ecalcarata*, *A. chrysantha*, and the first generation of crosses all have the haploid chromosome number  $n=7$ . From each of the three groups of plants 20 PMCs were examined in the stages of metaphase, meta-anaphase, and late anaphase of the first division. In the metaphase of the first meiotic division there were always seven bivalents. The segregation of chromosomes in anaphase was always undisturbed (Fig. 3).

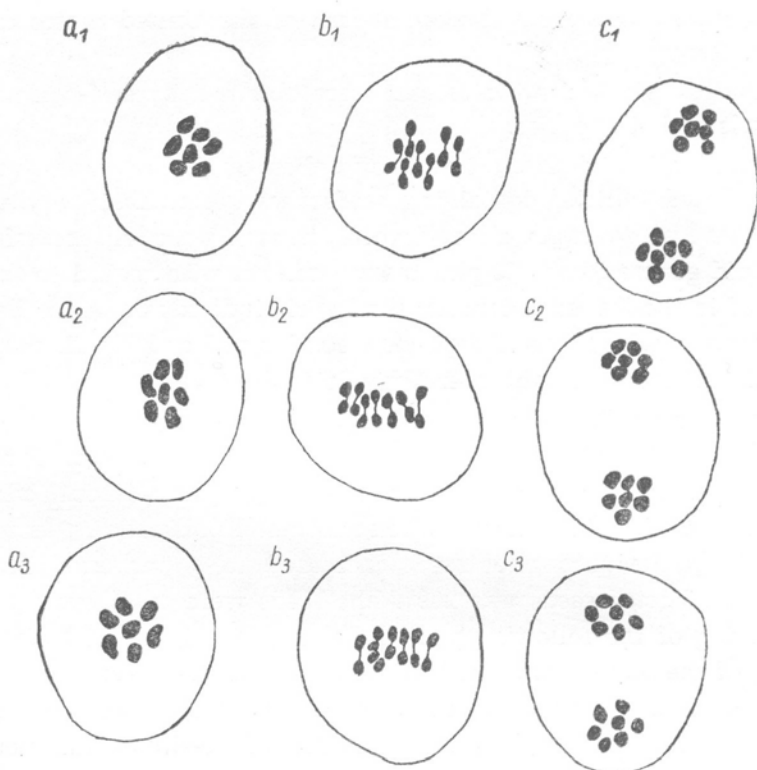


Fig. 3. Meiosis in PMCs of *A. ecalcarata* ( $a_1, b_1, c_1$ ), *A. chrysantha* ( $a_2, b_2, c_2$ ), and the first hybrid generation of the cross *A. ecalcarata*  $\times$  *A. chrysantha* ( $a_3, b_3, c_3$ ):  $a_1, a_2, a_3$  — first metaphase,  $b_1, b_2, b_3$  — first meta-anaphase,  $c_1, c_2, c_3$  — first telophase

The inheritance of traits distinguishing the two parental species

The following traits were considered in the genetic analysis of the crosses of *A. ecalcarata* with *A. chrysantha*:

- 1) height of plants,
- 2) shape of leaves,
- 3) length of spurs,
- 4) length of petals of the corolla,
- 5) width of petals of the corolla,
- 6) length of sepals of the calyx,
- 7) width of sepals of the calyx,
- 8) colour of flowers,
- 9) growth habit of flowers,
- 10) length of follicles,
- 11) number of ovules in follicle,



- 12) seed fertility,
- 13) size of seeds,
- 14) pollen fertility.

The correlation between some of these traits was also studied.

1. Height of plants. The average height of *A. ecalcarata* was 19.7 cm.  $\pm$  0.51 and of *A. chrysantha* 74.0 cm.  $\pm$  2.04. As is to be seen the difference was considerable. The height of the first hybrid generation was intermediate:  $\bar{x}$  = 55.7 cm.  $\pm$  1.19. In the second hybrid generation

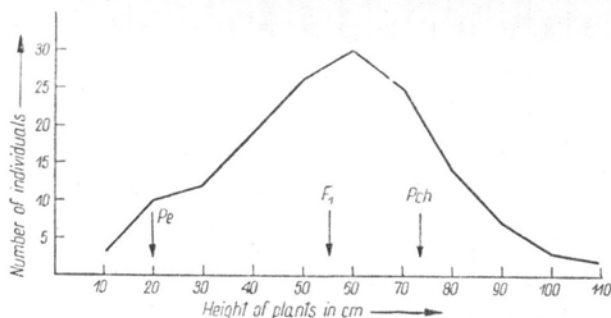


Fig. 4. Curve showing the variation of the height of plants in  $F_2$  ( $N = 152$ ) of the hybrid *A. ecalcarata*  $\times$  *A. chrysantha*

of 152 individuals there were plants approaching the height of either of the parental species, but plants of approximately the same height as in  $F_1$  predominated (Fig. 4).

2. Shape of leaves. The leaves in *A. ecalcarata* and *A. chrysantha* were very similar. In both parental species the leaves were thrice odd-pinnate and the leaflets of the 1st and 2nd order were tripartite and bilobate. As is to be seen in fig. 5 the leaves of *A. ecalcarata* were more deeply incised. The first hybrid generation had leaves almost exactly identical with *A. chrysantha* (Fig. 5). Owing to this in  $F_2$  only two classes

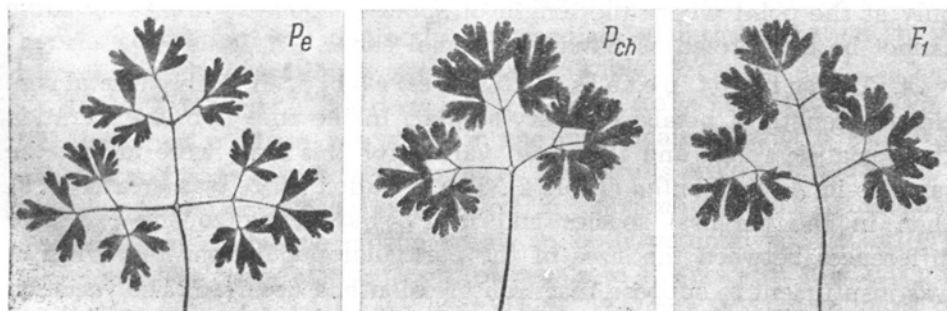


Fig. 5. Basal leaves of *A. ecalcarata* —  $P_e$ , of *A. chrysantha* —  $P_{ch}$ , and of  $F_1$  plants

were distinguishable and even so with difficulty. In one class were the plants with leaves resembling *A. ecalcarata* and in the other the plants with leaves like in *A. chrysantha* and  $F_1$ . Of the 310  $F_2$  plants 62 were of the first class and 248 of the second.

Table 1  
Length of spurs in mm.

Medium class value	0	2	5	8	11	14	17	20	23	26	29	32	35	38	41	44	$\bar{x}$	$\pm s_{\bar{x}}$	N
$P_e$	20																		20
$P_{eh}$														5	7	8	41.4	0.56	20
$F_1$								5	11	4							22.8	0.35	20
$F_2$	17	35	24	16	20	36	31	34	41	13	12	5					15.2*	0.51	284

\* Mean  $\bar{x}$  for  $F_2$  with  $N=284-17=267$

3. Length of spurs. The most important trait distinguishing the two species was the presence or the absence of the spur and also the length of the spur. Table 1 lists the length of spurs in *A. chrysantha* and in  $F_1$  as well as the variation range of this trait in  $F_2$ . With regard to the length of spurs the first hybrid generation was intermediate. Among the 284 individuals in  $F_2$  there were 17 with spurless flowers and 267 plants had flowers with spurs of various length. Fig. 6 shows the petals (the spur with the petal) arranged in the decreasing order of spur lengths from 33 mm. to 0 mm. The ratio 17 to 267 corresponds to the Mendelian ratio of 1 to 15 which shows that there can be two dominant genes responsible for the presence of spurs in *Aquilegia chrysantha*. The distribution of the variation of the spur length in  $F_2$  (Table 1) markedly departs from the usual relations. The number of individuals in the particular classes is very much the same and the drop of the curve begins only at the point where the length of spurs is about 26 mm. This curve cannot be interpreted as having a normal shape.

4. Length of petals of the corolla. In both parental species the length of petals was very similar. In the first hybrid generation the variation range and the mean values for this trait were almost the same as in *A. chrysantha* (Table 2). The variation range was greater in  $F_2$  than in the parental species and in  $F_1$ , but owing to the excessive differences between the sizes of the particular populations the evidence was insufficient to suppose that transgression had occurred. The variation in  $F_2$  was continuous, which could indicate the presence of polymeric genes.

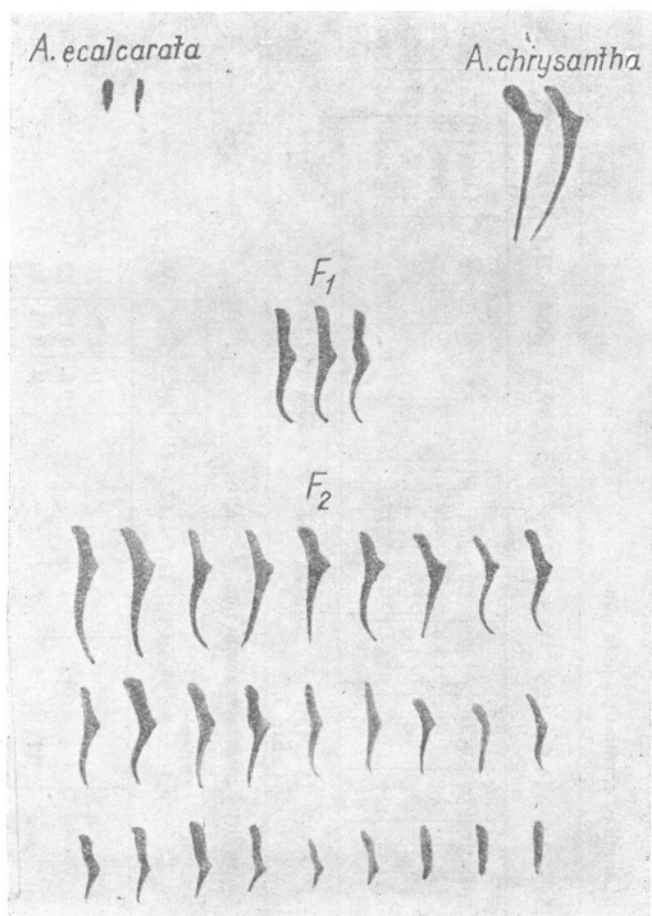


Fig. 6. Photographs of the petals of the corolla in the parental species, in  $F_1$  and in  $F_2$

5. Width of petals of the corolla. The petals of *A. chrysantha* were much wider than of *A. ecalcarata*. In this regard the first hybrid generation was distinctly intermediate and the variation in the second hybrid generation was continuous (Table 3).

6 and 7. Length and width of sepals of the calyx. The variation of these two traits in the investigated plants is shown in tables 4 and 5. As is to be seen so far as these traits are concerned the first hybrid generation clearly resembled *A. chrysantha*. The same data indicate that the shape of the sepals in both parental species differed distinctly. The ratio of the length of sepals to their width was accepted as the index reflecting the shape of sepals. The data in Table 6 show the variation range of the shape of sepals in *A. ecalcarata*, *A. chrysantha*,  $F_1$ ,

Table 2

Length of corollas blade in mm.

Class value	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	$\bar{x}$	$\pm S_{\bar{x}}$	N
$P_e$							1		2	7	7	3						14,4	0,23	20
$P_{ch}$								1	1	2	7	5	2	2	1			15,75	0,34	20
$F_1$								2	2	2	8	7		1				15,2	0,25	20
$F_2$	2	4	10	10	22	25	45	39	42	42	28	26	17	7	3	3	1	13,1	0,34	284

Table 3

Breadth of corollas blade in mm.

Class value	3,5	4,5	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	$\bar{x}$	$\pm S_{\bar{x}}$	N
$P_e$				3	8	8	1					7,0	0,19	20
$P_{ch}$							5	14	1			9,8	0,12	20
$F_1$					2	3	11	3	1			8,9	0,21	20
$F_2$	5	19	43	54	73	56	23	9	1	1		7,7	0,09	284

Table 4

Length of sepals in mm.

Class value	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	22,5	23,5	24,5	25,5	26,5	$\bar{x}$	$\pm S_{\bar{x}}$	N
P <sub>e</sub>								3	8	5	2	2	2	4	4	4	1					15,6	0,39	20
P <sub>ch</sub>										2	2	3	4									19,5	0,50	20
F <sub>1</sub>										1	3	4	3	2	6	1						19,2	0,39	20
F <sub>2</sub>	3	2	2	6	9	10	14	10	15	22	20	23	24	40	29	26	13	11	4	1	2	18,4	0,71	284

Table 5

Breadth of sepals in mm.

Class value	2,5	3,5	4,5	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	$\bar{x}$	$\pm s_{\bar{x}}$	N
$P_e$		1		6	9	4										5.8	0.16	20
$P_{eh}$					1	3	5	1	5	4					1	9.0	0.46	20
$F_1$						5	6	4	5							8.0	0.27	20
$F_2$	3	22	34	45	46	53	46	46	22	7	5	1				7.3	0.11	284

Table 6

The values of the coefficient of shape of the sepals (1/b)

Class value	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	22,5	23,5	24,5	25,5	26,5	27,5	28,5	29,5	30,5	31,5	32,5	33,5	34,5	$\bar{x} \pm$	$s_{\bar{x}}$	N
$P_e$								2		1		3	1	4	4	2	1				2	2.71	0.08	20
$P_{eh}$	1	1	1	1	1	3	4	1	1	1	1	1	1	2	1	1						2.20	0.35	20
$F_1$		1	1	2	2	2	1	1	3	1	1	1	3	1		3						2.32	0.30	20
$F_2$	2		3	5	8	12	23	30	33	29	35	34	25	15	10	4	8	2	2	4		2.45	0.67	284

and  $F_2$ . The shape of sepals in the hybrid generation was intermediate and in  $F_2$  the range of variation did not extend beyond the parental types.

8. Colour of flowers. *A. ecalcarata* had purple flowers. According to the Horticultural Colour Chart the corolla of this species was lilac purple 031/2 and of the calyx pansy violet 055. The colour of these flowers was caused by anthocyanin present in the cell sap. The flowers of *A. chrysantha* were yellow. The colour of both petals and sepals was aureolin 3/1 and of the spurs barrium yellow 503/2. The colour of the flowers was due to the presence of chromoplasts. The first hybrid generation was uniform and had flowers of two colours. The sepals of the calyx and the spurs were lilac purple 131/1; the petals of the corolla were mimosa yellow 602/1 over 2/3 of their surface on the inside and the outside along the margins and lilac purple 031/2 on the outside. With regard to the colour of flowers the 275 individuals in  $F_2$  were divided into four classes.

The first class was composed of plants with flowers coloured similarly as in  $F_1$ . The colour of the sepals and spurs in these plants was always of various shades of purple or violet. The petals had different colour patterns: 1) 25 individuals had petals all yellow; 2) 33 individuals had petals purple (various shades of purple) on the outside and all yellow or yellow over 1/3 or 2/3 of the surface on the inside; 3) 69 individuals had petals partly yellow (e.g. over 1/3 of the surface) both on the outside and the inside. Altogether there were 127 plants with flowers coloured similarly as in  $F_1$ .

The second class was composed of plants with yellow flowers resembling *A. chrysantha*. The colour in various individuals was of different intensity from pale yellow to dark yellow. The sepals and the spurs always were somewhat darker than the petals. The number of these plants was 46. This class also comprised 12 plants with pale creamy or cream-white flowers.

The third class was composed of plants with flowers all pink or having pink sepals and yellow petals. The intensity and the shade of these colours varied. The class consisted of 56 plants.

The fourth class comprised individuals in which the colour of the flowers was determined by the presence of anthocyanin only. The sepals and the petals of the flowers in these plants were of various shades and intensities of purple or violet. There were 34 plants in this group. These results are analysed in the discussion further on.

9. Growth habit of flowers. *A. ecalcarata* had drooping flowers, whereas in *A. chrysantha* they were distinctly erect (fig. 1 and 2). In the first hybrid generation all the plants had drooping flowers, and in the second hybrid generation consisting of 275 individuals there were

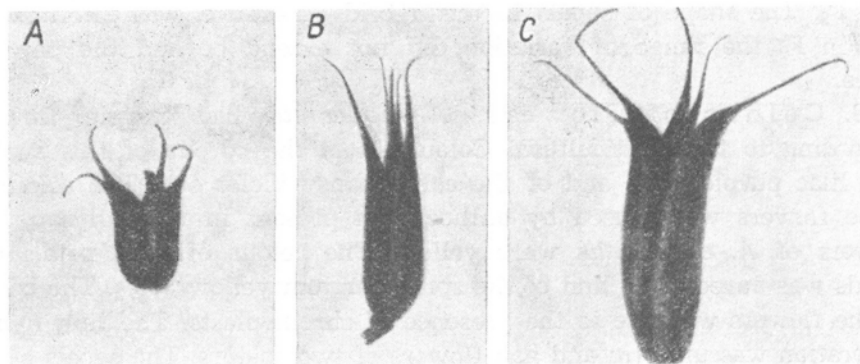


Fig. 7. Photographs of the follicles in the parental species and in  $F_1$ . A — *A. ecalcarata*; B —  $F_1$  generation of *A. ecalcarata*  $\times$  *A. chrysantha*; C — *A. chrysantha*

209 plants with drooping flowers and 66 with erect flowers. This means that the segregation was monogenic according to the ratio 3:1 (the anticipated numbers were 206.25 : 68.75).

10. Length of follicles. The two parental species differed greatly by the length of their follicles (Fig. 7). In *A. ecalcarata* the length of follicles was 9.0 mm.  $\pm$  0.29 and in *A. chrysantha* 25.4 mm.  $\pm$  0.87. With regard to this trait the first hybrid generation resembled *A. ecalcarata* the mean length of follicles in  $F_1$  plants being 12.8 mm.  $\pm$  0.32. Figure 8 shows the frequency distribution in the second hybrid generation of 150 individuals. The curve has a very regular shape with the peak at 12 mm. and slightly shifted towards *A. ecalcarata*. In view of the continuous nature of the variation the presence of polymeric genes can also in this case be assumed.

11. Number of ovules in follicles. The counts of the mean number of ovules in follicles included the number of unfertilized ovules, of ovules fertilized but undeveloped, and of normally developed seeds. In *A. ecalcarata* the number of ovules per follicle was 19.2, in *A. chrysantha* 40.3, and in the first hybrid generation 24.95. Figure 9 shows the variation of this trait in the  $F_2$  generation composed of 150 individuals. The peak of the curve is shifted towards *A. ecalcarata*. The curve has both arm sloping regularly and almost symmetrically. The continuous nature of the variation seems to be indicative of the polygenic mechanism in the heredity of ovule numbers.

12. Seed fertility. The counts for seed fertility were made on follicles obtained from free-pollinated flowers. For calculating seed fertility the ratio of well developed seeds to the total number of ovules in a follicle was determined. The proportion of well developed seeds in



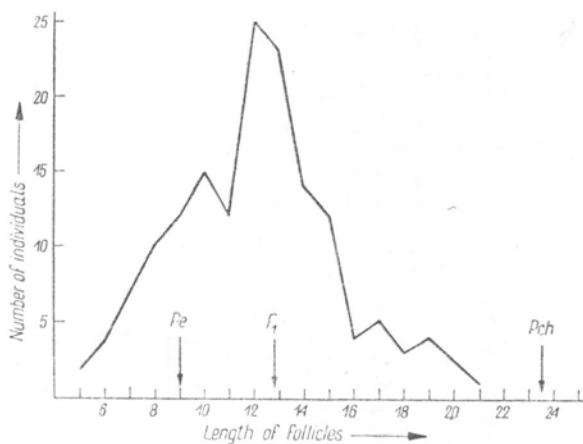


Fig. 8. Frequency distribution curves showing the length of follicles in 150 plants of the  $F_2$  generation of *A. ecalcarata*  $\times$  *A. chrysantha*

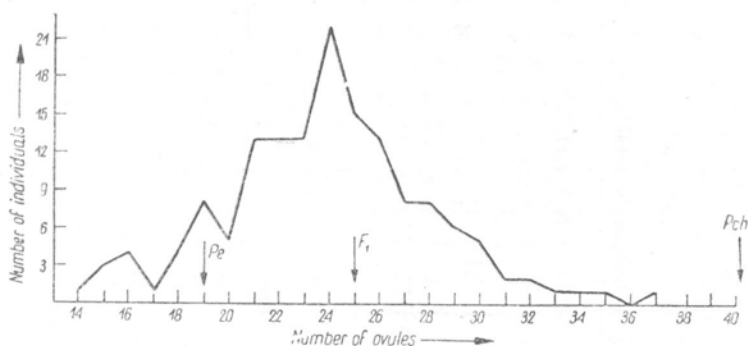


Fig. 9. Curve showing the variation in the number of ovules in the  $F_2$  generation of the hybrid *A. ecalcarata*  $\times$  *A. chrysantha*

*A. ecalcarata* ranged 52 to 60 per cent, average 57 per cent, and in *A. chrysantha* 30 to 42 per cent, average 36 per cent. Undeveloped ovules were found in neither of the parental species, but in the hybrids the inhibited development of ovules seemed rather common. In  $F_1$  fertility

Table 7  
Seed fertility in % in  $F_2$

Class value	0	1	10	20	30	40	50	60	70	80	90	100	N
Number of plants	7	20	44	32	25	6	13	3					150

Table 8

Length of the seeds in mm

Class value	1,05	1,15	1,25	1,35	1,45	1,55	1,65	1,75	1,85	1,95	2,05	2,15	2,25	2,35	2,45	2,55	2,65	2,75	2,85	2,95	3,05	3,15	3,25	$\bar{x}$	$\pm s_{\bar{x}}$	N
$P_e$	4	7		5	4												4	5	1					1,30	0,035	20
$P_{ch}$																	4	5						2,79	0,04	20
$F_1$											1	9	7			3								2,34	0,027	20
$F_2$				1	2	1	5	9	9	22	23	21	24	15	4	3	2	2	2					2,20	0,34	145

Table 9

Breadth of the seeds in mm

Class value	0,55	0,65	0,75	0,85	0,95	1,05	1,15	1,25	1,35	1,45	1,55	1,65	$\bar{x}$	$\pm s_{\bar{x}}$	N
$P_e$	4			9	4	3							0,79	0,032	20
$P_{ch}$											10	1	1,45	0,020	20
$F_1$							13		9	7			1,26	0,021	20
$F_2$	1	1	2	9	8	32	39	28	16	8	1		1,27	0,560	145

dropped to 8.3 per cent; the variation of seed fertility in the  $F_2$  generation is shown in table 7. Individuals with seed fertility ranging 15 to 20 per cent were the most numerous. Some individuals in  $F_2$  were completely sterile, whereas others were more fertile than the parental plants.

Table 10  
Pollen fertility in % in  $F_2$

Class value	0	10	20	30	40	50	60	70	80	90	100	N
Number of plants	12	12	23	25	31	45	28	22	20	4		222

13. Size of seeds. Simultaneously to the counts of the well developed seeds the length and width of seeds was measured, since it was noticed that the size of seeds in the two parental species considerably differed. Tables 8 and 9 list the values of the variation range and the mean values for the length and width of seeds in *A. ecalcarata*, *A. chrysantha*,  $F_1$ , and  $F_2$ . In both instances the mean values in  $F_1$  approached the values for *A. chrysantha*. In  $F_2$  variations were continuous.

14. Pollen fertility. In the parental species *A. ecalcarata* and *A. chrysantha* pollen fertility was 87 and 83 per cent respectively. In the first hybrid generation pollen fertility ranged 54 to 77 per cent, average 62 per cent. As is to be seen the drop of pollen fertility in  $F_1$  was small. The variation of this trait in  $F_2$  is shown in table 10. Among the 222 individuals of the  $F_2$  generation 24 plants had pollen fertility above 80 per cent and many plants had a greatly reduced pollen fertility.

#### CORRELATIONS

The photograph of the petals of the corolla (fig 6) in *A. ecalcarata*, *A. chrysantha*, and the  $F_1$  and  $F_2$  crosses between these two species seemed to indicate that there was no direct correlation between the length of petals and the length of spurs. To check this supposition the correlation between these two traits was examined. The coefficient of correlation between the length of spurs and the length of petals was  $r = -0.1174$  in *A. chrysantha*,  $r = -0.0315$  in  $F_1$ , and  $r = -0.0789$  in  $F_2$ . These values indicate that there was no correlation whatever between the two traits (the size of the populations in the first two instances was  $n = 100$  and in  $F_2$   $n = 267$ ).

On the other hand, there was a significant correlation in  $F_2$  between pollen fertility and seed fertility. The coefficient  $r = 0.9306$  when

$n=103$ . This seems to indicate that the same factors were responsible for the drop of both pollen fertility as well as seed fertility.

*A. chrysantha* had more ovules in its long follicles than were contained in the short follicles of *A. ecalcarata*. Moreover, *A. chrysantha* had much larger seeds. For this reason the dependence between these two traits in  $F_2$  was calculated. The coefficient of correlation between the length of follicles and the size of seeds was  $r=0.4658$  when  $n=128$ .

In the  $F_2$  population of 128 individuals the coefficient of correlation between the follicle length and the ovule number was  $r=0.5239$ . The coefficient of correlation between the number of ovules and seed size was  $r=0.4324$  when  $n=128$ .

#### DISCUSSION OF RESULTS

The hybrids obtained in this investigation between *Aquilegia ecalcarata* and *A. chrysantha* have once again confirmed the opinion that in the genus *Aquilegia* interspecific crosses even between distantly related species and the progeny from such crosses can be obtained without major difficulties (Anderson and Schafer 1931). In  $F_1$  of the hybrids obtained in the course of this investigation seed fertility was strongly and pollen fertility somewhat reduced, but at the same time in meiosis there were no visible symptoms of disturbances. Moreover, a large proportion of seeds did not germinate and a part of the seedlings perished at an early stage. The zygotic sterility may result from the meeting and the mutual interaction of unharmonious sets of genetic factors. The same explanation is suggested by Skalińska (1928) for the sterility of some hybrids of *A. chrysantha* with various species from the *vulgaris* complex that she experimented with in her investigations. In spite of the elimination of a part of micro- and macrospores and of some zygotes in various stages of development a regular segregation of most of the investigated traits was observed in the second generation of the cross between *A. ecalcarata* and *A. chrysantha*. Such traits as the height of plants, the shape of leaves, the size of petals and sepals, the length of follicles, the number of ovules in follicles, the size of seeds, and pollen and seed fertility all manifested a continuous variation, which seems to indicate that in the case of these traits many factors were segregated.

The absence or presence of spurs, the growth habit of flowers, and the colour of flowers were controlled by a different hereditary mechanism. The results reported in this author's preceding paper (1960) led to the conclusion that in *A. vulgaris* only one gene was responsible for the formation of spurs. In the present experiments it was found that the absence of the spur in *A. ecalcarata* was recessive with regard to the presence of the spur in *A. chrysantha*, since all the  $F_1$  generation had

spurs. On the other hand, the segregation in  $F_2$  according to the ratio 15 : 1 (15 with : 1 without spurs) justifies the assumption that the formation of the spur in *A. chrysantha* was determined by two dominant genes. The question remains whether this genetic composition occurs in all the American *Aquilegia* species, or is characteristic only for *A. chrysantha* or the species from the *caerulea* complex.

The difference between the inheritance of the spur length in  $F_2$  of the cross *A. ecalcarata*  $\times$  *A. chrysantha* and in  $F_2$  of the cross *A. vulgaris*  $\times$  *A. ecalcarata* is very interesting. Although in the latter instance 3/4 of individuals with spurs manifested the normal polygenic segregation reflected by the spur-length variation curve of the usual shape, in the former instance 15/16 of individuals with spurs were divided in almost equal numbers between the particular spur-length classes (2 to 23 mm.). This means that in each case the inheritance of the spur length was controlled by a different mechanism. For the understanding of the still unexplained mechanism underlying the inheritance of the spur length in the cross *A. ecalcarata*  $\times$  *A. chrysantha* more experiments on the further generations and backcrosses are necessary.

If it is true that in the genus *Aquilegia* the morphology of flowers reflects adaptations to different animal pollinating agents (Grant 1952), then the growth habit and the colour of flowers must also have a great significance. The yellow colour and the erect position of flowers in *A. chrysantha* favour the pollination by the sphinx-moths, because the yellow colour is better visible at night. The information contained in Munz's monography (1946) seems to indicate that the type with drooping flowers predominates among the Eurasian species which are pollinated by flies and various bees. The American species of the *caerulea* complex pollinated by moths have erect flowers. The segregation in  $F_2$  of the cross *A. ecalcarata*  $\times$  *A. chrysantha* according to the ratio 3 : 1 (3 drooping : 1 erect) leads to the supposition that the transition from the drooping to the erect growth habit was accomplished in one mutation stage. The trait of the drooping flower was dominant with regard to the trait of the erect flower.

On the ground of investigations on the heredity of flower colour in diploid crosses between *A. flabellata nana* and *A. chrysantha* Skalińska (1935) assumed in *A. chrysantha* the basic factor *C* responsible for the occurrence of colour in flowers and the factor *Y* responsible for the yellow colour. In an earlier paper on the reciprocal crosses between *A. vulgaris* and *A. chrysantha* Skalińska (1928) had assumed the presence in *A. vulgaris* of the factor *R* responsible for the pink colour of the cell sap and of the factor *F* modifying the pink colour to violet.

Analysing the inheritance of the flower colour in the crosses between

*A. ecalcarata* and *A. chrysantha* this author has made the following assumptions:

*A. chrysantha* introduced into the cross the factor *C* responsible for the occurrence of colour and

the factor *Y* causing the yellow colour of flowers.

The factors *R* and *F* present according to Skalińska in *A. vulgaris* have been here attributed to *A. ecalcarata*, however, with the reservation that the factor *F* modified the pink colour of the cell sap to purple and that *A. ecalcarata* also introduced the factor *C* into the cross. Then

the genotype of *A. ecalcarata* might have been *CCyyRRFF*, and

the genotype of *A. chrysantha* might have been *CCYYrrff*.

The first hybrid generation with two-coloured flowers, i.e. with both anthocyanin and yellow chromoplasts, could comply with the general formula *CCYyRrFf*.

The intensity and the distribution of colours on the surface of sepals and petals in  $F_2$  can be disregarded, since these traits were undoubtedly controlled by other segregating genes; then the following basic types of flower colours could be distinguished:

a) Plants with distinctly two-coloured flowers having both the yellow pigment and anthocyanin. The colour shade of these flowers probably depended on the homozygous or heterozygous state of the factors *Y*, *R*, and *F* and could be e.g. purple-violet, violet-purple, dark purple, or light purple. Generally speaking the genotype of these plants might have been *CC Y(y)R(r)F(f)*. There were 127 plants of this type, whereas theoretically they should have constituted 27/64 of the whole population i.e. 116 plants.

b) Plants with yellow flowers (of various shades of yellow). The plants in this group had not the factor *R*, and they either had or had not the factors *Y* and *F*. This means that the plants doubly homozygous with regard to the recessive genes *y* and *r* and having the genotype *CCyyrr* were not white but pale creamy regardless of whether they had or had not the factor *F*. The number of plants with this phenotype was 12.

On the whole, owing to the absence of the factor *R* the plants in this group had no anthocyanin in the flowers or only had traces of this pigment. The total number of the plants in this group was 58 (12 + 46). Theoretically they should have constituted 16/64 of the population, i.e. their number should have been 68.7.

c) Plants with pink or yellow-pink flowers and without the factor *F*. The general genotype of these plants was *CCY(y)R(r)ff* and their number was 56. Theoretically they should have constituted 12/64 of the whole  $F_2$  population, and thus their number should have been 51.6.

d) Plants with purple or violet-purple flowers and no yellow pigment. The general formula of these plants probably was *CCyyR(r)F(f)*. There

were 34 such plants. The theoretical proportion of this group in the  $F_2$  population should have been  $9/64$  i.e. 38.7 individuals.

As is to be seen the numerical ratio in the second hybrid generation was  $127 : 58 : 56 : 34$ , which agreed well with the theoretical ratio  $27 : 16 : 12 : 9$ . This justifies the assumption that there were three pairs of alleles ( $Y/y$ ,  $R/r$ ,  $F/f$ ) responsible for the colour of flowers in the hybrid in addition to the basic factor  $C$  controlling the occurrence of colours in flowers and introduced into the cross by both *A. ecalcarata* and *A. chrysantha*.

The  $X^2$  test showed that for  $X^2=3.844$  (for three degrees of freedom) the value of  $p$  was more than 0.25.

The hybrids between *A. vulgaris* and *A. chrysantha* investigated by Skalińska (1928) were matroclinal and in  $F_2$  there were no plants of the male-parent type. Neither were there any plants with two-coloured violet and yellow flowers. This means that in those hybrids the segregation was not typical. In the case of this investigation a similar effect was not observed and, in spite of the rather strongly reduced fertility and the high zygotic sterility, the segregation was normal.

The genetic analysis of the hybrids between *A. ecalcarata* and *A. chrysantha* clearly indicates that three out of the 14 investigated traits distinguishing these two "distant" species were inherited according to the Mendelian laws. The trait of the absence of the spur was probably controlled by two recessive genes and the spur developed even when only one dominant gene was present. The drooping or erect growth habit of flowers depended on one basic gene, whereas the colour of flowers was controlled by relatively few factors which segregated independently of each other but co-operated in their phenotypic manifestations. The other specific traits were controlled by the action of multiple genes which were not identified owing to their slight influence on the phenotype.

#### SUMMARY

1. The inheritance of specific traits in hybrids of *Aquilegia ecalcarata* with *Aquilegia chrysantha* was studied.

2. The haploid number of chromosomes in both species was  $n = 7$  and meiosis in the first hybrid generation proceeded without visible disturbances.

3. In the first hybrid generation both the pollen fertility and the seed fertility was reduced; the drop in the latter case being considerable. In  $F_1$  pollen fertility was 62 per cent and seed fertility 8 per cent.

4. In the second generation of the cross *A. ecalcarata*  $\times$  *A. chrysantha* the segregation of the following traits was continuous: the height of plants, the shape of leaves, the size of petals and sepals, the length of follicles, the number of ovules in follicles, the size of seeds, and pollen and seed fertility. The variation of these traits in  $F_2$  was continuous which seemed to indicate that many polymorous factors were being segregated.

5. The absence of the spur in *A. ecalcarata* was recessive with regard to the presence of the spur in *A. chrysantha*. The whole  $F_1$  generation was uniform in that it had spurs of intermediate length. In the second hybrid generation 1/16 of the total number of individuals had flowers without spurs and 15/16 of individuals had flowers with spurs. This means that the trait of the absence of spurs could have been controlled by the presence of two recessive genes. For the development of spurs only one dominant gene was necessary.

6. The trait of the erect flower in *A. chrysantha* was recessive with regard to the trait of the drooping flower in *A. ecalcarata*. The segregation in  $F_2$  was according to the ratio 3 : 1 (for three plants with drooping flowers there was one with erect flowers). This means that this trait was controlled monogenically.

7. The colour of flowers in the  $F_1$  hybrid generation was determined by the concurrence of the following factors:

factor C determining the presence of a colour,

factor Y determining the yellow colour (both these factors were introduced by *A. chrysantha*),

factor R determining the pink colour of the cell sap, and

factor F modifying the pink colour to purple (the last two factors as well as factor C were introduced by *A. ecalcarata*).

The first hybrid generation was uniform in that it had all the flowers of two colours: purple and yellow. As the result of the independent segregation of the factors listed above there were in the second hybrid generation four kinds of plants: 1) with two-coloured flowers (resembling  $F_1$ ), 2) with yellow or creamy flowers, 3) with pink or pink-yellow flowers, and 4) with purple flowers. The mutual proportions between the numbers of plants with the different kinds of flowers approached the ratio 27 : 16 : 12 : 9.

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## REFERENCES

- Anderson E., and Schafer B., 1931, Species hybrids in *Aquilegia*, Ann. Bot. 45: 639—646.
- Clausen J., D. D. Keck and W. M. Hiesey, 1945, Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autopolyploidy, with examples from the Madiinae, Carnegie Inst. Washington, Publ. No. 564: 174.
- Grant V., 1952, Isolation and hybridization between *Aquilegia formosa* and *A. pubescens*. El Aliso 2: 341—360.
- Munz P., 1946, *Aquilegia*. The cultivated and wild columbines, Gentes Herbarum 7: 1—150.
- Prażmo W., 1960, Genetic studies on the genus *Aquilegia* L. I. Crosses between *A. vulgaris* L. and *A. ecalcarata* Maxim. Acta Soc. Bot. Pol. 29 (1): 52—77.
- Skalińska M., 1928, Sur les causes d'une disjonction non typique des hybrides du genre *Aquilegia*. Acta Soc. Bot. Pol. Vol. V. Nr 2, str. 141—173.
- 1935, Cytogenetic investigations of an allotetraploid *Aquilegia*, Bulletin de l'Academie Polonaise des Sciences et des Lettres. Classe des Sciences Mathematiques et Naturelles. Serie B: Sciences Naturelles 1. 33—63.