THE GENETICS OF *AQUILEGIA VULGARIS*

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CONTENTS

1. Introduction

The common columbine, *Aquilegia vulgaris* L., has been in cultivation for centuries, and like many old garden plants, it shows great variation: a range of colours, doubleness, sepalody, colour pattern, albinism and variegation. Now some of these variant forms are no doubt derived from the original wild columbine and owe nothing to hybridity; but others may be derived from crossing-out to different species. The plant as it exists in gardens is a complex of uncertain history; and one of the objects of genetic study in such a case is to unravel the complex and discover to what source the components owe their origin. I shall use the name *A. vulgaris* to cover the whole group and shall return later to the question of interspecific relations.

The character differences described in the present paper are due to an independent factor pair **B**, **b** for blue/pink flower colour, and a linkage group consisting of two pairs and a set of three multiple allelomorphs:

C, **c**, tall/compact habit.

L, **l**, blue/lilac flowers.

A, $\mathbf{a}^{w}$, **a**, fully coloured/"white face"/fully white flowers.

2. Source of the material

The history of the stock begins in 1931 when a family 35/29, the progeny from selfing a light blue plant obtained from some cultivated source, was observed to be showing strong coupling of blue colour with tall habit and of lilac colour with compact habit. This family was used

in crosses with a tall and a dwarf white, bought as "nivea grandiflora" and "alba nana compacta" respectively. Later, other stocks were crossed in. 35/29 thus contributed the factors a^w, b, c and l, while the whites contributed a, which was at first thought to be an independent factor but later proved to be at the same locus as a^w.

The effect of the factors is as follows:

b and l alter the normal blue colour of the flowers. **BL** flowers are blue, **Bl** lilac, while **bL** and **bl** are pink and indistinguishable.

c gives plants about half the height of the normal **C**, with a tighter, bushier habit. Not all dwarfs are c. c flowers are raised above the horizontal at the time of opening, and turned vertically upwards when they have been open for a day or two. **C** flowers open nodding, and the stems only straighten up after flowering. **C**, c can be classified without difficulty on adult plants.

A plants have fully coloured flowers and leaves. a plants can be distinguished by the almost complete absence of anthocyanin in their stems and leaves. The flower is usually white or slightly tinged. The intermediate stage, a^w ("white face"), produces fully coloured leaves and sepals, but the petals, when in the normal spurred condition, have a white or creamy-white border about half an inch deep. White face is conspicuous on blue or lilac flowers, much less so on pink.

The colours in 35/29 were the lightest shades found on coloured-stemmed (**A** or a^w) plants. **BL** flowers were a pale sky blue, **Bl** a slaty mauve and **b** flowers a very pale cold pink, flushed with a little mauve on the backs. So pale is the pink that it was not realized until the 1933 families were examined that a^w could be classified, as it can with a little practice, on **b** plants. In 35/29 and the 1931 families, therefore, it is only scored on **B** plants; in later families on **b** as well.

On wholly spurless flowers, where the petals are all sepaloid, a^w is lost as it cannot be distinguished by eye from **A**. On intermediate-spurless flowers the a^w types may show a white central streak on the petals. The spurless (sepaloid) character, however, will not be further discussed here.

To return to a, it is possible to get very heavily tinged whites, in which the flower colour may simulate the light blues and pinks of the **A** and a^w series; but the abnormally light foliage is always in evidence. The heavily tinged whites correspond to the darkest, almost black, shades on the **A** stem. In the bulk of my material it is impossible to record **b** or **l** on a, but in some strains it may at least be possible to discriminate **B** and **b** (see Discussion, (*a*)).

Crosses between different whites and tinged whites in *vulgaris* have never given anything darker.

As **b** and **l** are lost on **a**, and **l** is lost on **b**, the most useful recessives are the dwarf white-faced purple, $a^wa^wBBccll$, and the corresponding pink extracted from purple, $a^wa^wbbccll$.

3. SINGLE-FACTOR RATIOS

The single-factor ratios of **b**, **c** and **l** are given in Table 1.

Table 1. *Single-factor ratios of* **b**, **c** *and* **l**

Genes	No. of families	Backcross X	Backcross x	χ^2 Deviation from 1 : 1	χ^2 Heterogeneity	D.F.	P
B, b	17	400	375	0·806		1	0·5–0·3
					12·979	16	0·7–0·5
C, c	18	483	456	0·342		1	0·7–0·5
					21·626	17	0·2–0·1
L, l	17	256	254	0·0078		1	0·95–0·9
					13·686	16	0·7–0·5
		F_2		3 : 1			
B, b	22	764	260	0·083		1	0·8–0·7
					19·656	21	0·7–0·5
C, c	17	491	135	3·909		1	0·05–0·02
					10·349	16	0·9–0·8
L, l	11	280	98	0·173		1	0·7–0·5
					8·096	10	0·7–0·5

In the **C, c** selfs one family contributes largely to the total deviation, which calculated on the other sixteen families has $P = 0{\cdot}2–0{\cdot}1$. The rest of the data give good agreement with expectation.

A is dominant over a^w and **a**, and a^w is dominant over **a**. In every case tested (five families, ninety-five plants in all) white crossed by white face and reciprocally has given nothing but white faces and whole whites in F_1.

If a^w and **a** were not allelomorphic, we should expect that some whites would give a fully coloured F_1 when crossed with white face, and that in the F_2 from a fully coloured plant and a white giving white face on crossing with the latter, some white faces would appear in F_2 or backcross. Actually one white face is recorded in the F_2 family 6/33, but this was probably a stray from the neighbouring family 7/33, where many such occurred. If we accept it at its face value it may be a mutant. The only alternative would be to suppose that the whites were **aa**, the white faces **A ww**, and that the two genes were very closely linked, giving 0·2 % of crossing-over. This is possible, but unlikely. The data on linkage fully bear out the hypothesis of multiple allelomorphism.

The single-factor ratios for **A**, $\mathbf{a^w}$, **a** are given in Table 2.

Table 2. *Single-factor ratios of* **A**, $\mathbf{a^w}$, **a** *in different types of mating*

Mating*	No. of families				χ^2 Deviation from 1 : 1	χ^2 Heterogeneity	D.F.	*P*
Aa × **aa** and reciprocal	4	**A** 141		**a** 147	0·1450		1	0·8–0·7
						0·3828	3	0·95–0·9
$\mathbf{Aa^w} \times \mathbf{a^wa^w}$	2	89		57	6·918		1	<0·01
						1·195	1	0·3–0·2
$\mathbf{a^wa^w} \times \mathbf{Aa^w}$	6	173		166	0·114		1	0·8–0·7
						7·363	5	0·2–0·1
$\mathbf{Aa^w}$ × **aa** and reciprocal	5	115		123	0·268		1	0·7–0·5
						0·550	4	0·98–0·95
		A	$\mathbf{a^w}$	**a**	2 : 1 : 1			
Aa × $\mathbf{a^wa}$ and reciprocal	2	48	21	37	5·774		2	0·1–0·05
						2·580	2	0·5–0·3
		X		**x**	3 : 1			
Aa selfed	15	476		136	5·318		1	0·05–0·02
						11·882	14	0·7–0·5
$\mathbf{a^wa}$ selfed	6	189		51	1·800		1	0·2–0·1
						1·826	5	0·9–0·8
$\mathbf{Aa^w}$ selfed	8	271		97	0·362		1	0·7–0·5
						10·271	7	0·2–0·1

* Reciprocals throughout are grouped where there is no significant difference between them.

There is on the whole a satisfactory agreement with expectation. In the cross $\mathbf{Aa^w} \times \mathbf{a^wa^w}$, where *P* is less than 0·01, most of the deviation is due to a family 14/34, segregating 44 : 33 ($\chi^2_{[1]} = 6{\cdot}528$).[1] The reciprocal group of six families shows no significant deviation; combination of the two groups would also reduce the deviation to a non-significant figure, but this is scarcely legitimate as the heterogeneity between the two groups of families tested by means of a 2 × 2 table gives $\chi^2_{[1]} = 4{\cdot}025$. In the **Aa** selfs there is a significant shortage of the recessive class, *P* falling between 0·05 and 0·02; the deviation here is largely due to a group of sister families, 21, 22, 24 and 26/31, unrelated to the main linkage and white lines (see above, Source of the material). If these four families are removed the remaining eleven are a very good fit.

4. Linkage of **c**, **l** and **a**

The locus of **A**, $\mathbf{a^w}$, **a** has proved to be linked with **C**, **c** and **L**, **l**. The backcross data are summarized in Table 3.

[1] The number of degrees of freedom is given in square brackets as a subscript to χ^2.

Table 3. *Linkage of* cl, la *and* ca

Mating	No. of families	XY	Xy	xY	xy	χ^2 Deviation	χ^2 Heterogeneity	D.F.	P
$\frac{CL}{cl} \times$ ccll and reciprocal	10	200	12	6	196	345·16		1	<0·01
							1·16	9	<0·99
$\frac{LA}{la^w} \times$ lla^{w}a^w and reciprocal	4	99	10	16	84	117·99		1	<0·01
							1·05	3	0·8–0·7
$\frac{La^w}{lA} \times$ lla^{w}a^w and reciprocal	3	11	44	55	7	55·71		1	<0·01
							0·55	2	0·8–0·7
		LA	lA	La^w	la^w	ba	ba^w	a	
$\frac{La^w}{la}$Bb $\times \frac{lA}{la}$ and reciprocal	2	(17 | 19)	14	1	(12 | 5 | 32)	11·26		1	<0·01
										0·18	1	0·7–0·5
		XY	Xy	xY	xy				
$\frac{CA}{ca} \times$ ccaa and reciprocal	3	110	21	16	110	130·3		1	<0·01
							0·37	2	0·9–0·8
$\frac{CA}{ca^w} \times$ cca^{w}a^w	2	41	5	7	33	44·6		1	<0·01
							1·44	1	0·3–0·2
cca^{w}a$^w \times \frac{Ca^w}{cA}$	2	11	45	67	14	55·24		1	0·01
							0·03	1	0·9–0·8

Table 4 gives additional linkage data from selfed families.

Table 4. *Selfed families involving linkage of* cl, la *and* ca

Constitution of parent	No. of families	Segregation			
		CL	Cl	cL	cl
CL.cl	3	107	2	3	24
		LA	lA	Ab	a
LA.la	1	45	1	(22	15)
		LA	lA	La^w	la^w
LA.la^w	1	23	1	3	2
		LA	lA	Ab	a
La.lABb	2	36	18	(14	23)
La.lABB[1]	3	79	35	—	(38)
		LA	lA	La^w	la^w
La^w.lA	2	36	22	27	1
		CA	Ca	cA	ca
CA.ca	1	13	0	1	3
		CA	Ca^w	cA	ca^w
CA.ca^w	2	50	4	4	10
		CA	Ca	cA	ca
Ca.cA	7	120	61	62	2
		CA	Ca^w	cA	ca^w
Ca^w.cA	1	18	9	9	1

[1] One selfed family plus two families from sister plants crossed inter se.

Table 5 gives the figures obtained from families with all three linked factors segregating.

Table 5. *Linkage of* cla[1]

	CLA.cla^w × cla^w 5 families		CLa^w.clA × cla^w 3 families		Total
Non-recombination	CLA	98	CLa^w	40	272
	cla^w	79	clA	55	
Single recombination in region 1	Cla^w	6	ClA	0	11
	cLA	1	cLa^w	4	
Single recombination in region 2	CLa^w	8	CLA	11	40
	clA	14	cla	7	
Double recombination	ClA	1	Cla^w	0	3
	cLa^w	2	cLA	0	
	Total	209		117	326

The order of the factors is therefore cla.

Recombination in region 1 $= \frac{14}{326} = 0{\cdot}0429$.

Recombination in region 2 $= \frac{43}{326} = 0{\cdot}1319$.

Coincidence value $= \dfrac{326 \times 3}{14 \times 43} = 1{\cdot}62$, which shows no evidence of interference.

The recombination values were calculated from the combined data by the method of maximum likelihood, and a χ^2 appropriate for the detection of heterogeneity between the various bodies of data concerned in each recombination value was calculated in the manner described by Mather (1938, § 18). These values are given below.

cl	$4{\cdot}286 \pm 0{\cdot}867\,\%$	$\chi^2_{[1]} = 0{\cdot}018$, $P = 0{\cdot}9$–$0{\cdot}8$
la	$13{\cdot}095 \pm 1{\cdot}695\,\%$	$\chi^2_{[6]} = 13{\cdot}608$, $P = 0{\cdot}05$–$0{\cdot}02$
ca	$15{\cdot}235 \pm 1{\cdot}468\,\%$	$\chi^2_{[3]} = 2{\cdot}836$, $P = 0{\cdot}5$–$0{\cdot}3$

These values confirm the order cla arrived at from the three-point data. In the la figures, where the χ^2 is on the margin of significance, most of the heterogeneity is contributed by one family (26/31, $\chi^2 = 10{\cdot}28$), which contained a single purple plant. I suspect that this plant was a stray and that the family was in fact not heterozygous for l. The remaining seventeen families covered by the la data give a good fit. If 26/31 was wrongly included the recombination percentage would rise to approximately 13·6.

5. Discussion

(*a*) *Comparison with the results of Kristofferson*

The only other genetic analysis of *Aquilegia* known to me is that of Kristofferson. He postulates a factor B for blue, another factor R for

[1] These data are included in Tables 3 and 4.

red; **BR** by interaction gives dark blue, **br** is white. This does not appear to square with my results; but I suspect he was working with a deeply pigmented strain where the **a** plants show a heavy tinge (see p. 340). Our classes would then correspond as follows:

Kristofferson		Present scheme	
BR	dark blue	**AB**	blue
Br	light blue	**aB**	white, blue tinge
bR	red	**Ab**	red
br	white	**ab**	white, pink tinge?

My symbol **B**, **b** therefore agrees with Kristofferson's, that is, we both use it to distinguish blue from not-blue. His **R**, however, I have had to discard as it cuts across my **A**, **a** classification. Since in material homozygous for full colour I can self a blue and get a 3 to 1 segregation for blue and red, it is clear that no second factor for red need be invoked. One must postulate the presence, in Kristofferson's material, of an intensifier which will allow **B**, **b** to be separated on **a** plants. Such an intensifier, as stated above, appears to be present in some of my material.

Kristofferson also worked with a character "white margin". It bred as follows:

Dark blue self × white

F_1	18 dark blue self	17 dark blue, white margin
F_2	(3 plants tested) 63 self, 20 white margin	Bred true for white margin

He does not state where, if at all, the whites reappeared; but says: "The effect of this factor is seen in the dark blue and red types, and only these have been used in determining the ratio of the segregation." Now in a similar cross with my material, that is, a blue self × white giving blue selfs and white faces, the F_1 blue selfs would segregate blue selfs to white as 3 : 1, and the F_1 blues with white face would segregate white face to white as 3 : 1. It is possible that when Kristofferson says his white margins breed true, he is ignoring the segregated whites; but it seems impossible to reconcile the breeding behaviour of his tested F_1 blue selfs, which are explicitly stated to have given 63 whole colour to 20 white margin, with my scheme where they would have given no white faces. One must conclude that assuming Kristofferson's account to be correct, his factor **c** is not the same as my $\mathbf{a}^{w}$.

(*b*) *Doubleness of the flower*

Various crosses were made to test the relations between doubleness and the linkage group. In family 15/37 there was complete linkage of doubleness with tallness (27 tall double, 10 compact single). On the

other hand in families 7/37 and 8/37, where the doubleness was from the same source as in 15/37, doubleness was independent of white face (segregation 80 : 29 : 22 : 6, $\chi^2_{[1]}=0{\cdot}293$, $P=0{\cdot}7$–$0{\cdot}5$). It seems possible therefore that the parent of the family 15/37 contained an inversion, which would account for such aberrant behaviour.

The inheritance of doubleness, which is somewhat complex, will be reported upon later.

(c) *Interspecific relations*

The typical *Aquilegia vulgaris*, like all the western European columbines, has whole-coloured flowers; the bicolor form is exceptional, and as far as I know is only found in the cultivated plexus. A number of Asiatic and American species, however, normally have bicolor flowers in which the spurred petal is wholly or partly white. It is possible that the white-face form in the common columbine arose independently by mutation; but from what we know of the genetics of garden plants (e.g. *Verbena*, Beale, 1940) it seems more likely that it was introduced from a bicolor species. So far as is known, *A. vulgaris* crosses with every other species of *Aquilegia* (Anderson & Schafer, 1931), and in most cases the hybrids are highly fertile. Subsequent selection for the robust *vulgaris* types might well result, after a few generations, in a plant indistinguishable from the true *vulgaris* except in the flower-pattern character.

If white arose by mutation in *A. vulgaris*, and white face was brought in from another species, the normal linkage behaviour suggests that there have been no great structural changes in the chromosome in question during species formation.

If the white face character has been brought in by a cross, a possible parent would be the Japanese species *A. flabellata*. This was introduced to Europe in the eighties. The original diagnosis says "Sepala coerulea, petala apice flavescentia" (Siebold & Zuccarini, 1846), and this corresponds with the figure in the *Alpine Plants of Japan* (Miyoshi & Makino, 1907), where the sepals are blue and the petals which show are white. Pallid forms are also known, see *Rev. Horticole*, 1887, p. 548, and 1896, p. 109 ("fleurs blanches, légèrement lanées de rose lilacé") and *Curtis's Bot. Mag.* 1911, t. 8354 (flowers white). *A. flabellata* is a reputed parent of *Aquilegia* × *Helenae* Hort.

A plant received as *Aquilegia* × *Helenae*, but indistinguishable from *A. flabellata*, having a blue flower with a white edge, was used in some of my early crosses and segregated "white face" of the usual type in F_2. However, white *vulgaris* crossed with a very pale, almost white, *flabellata*

gave blue flowers with white face. This indicates that the white of the *flabellata* used is not at the same locus as that of the *vulgaris*. Either the white face of *flabellata* may be entirely distinct from that of *vulgaris*; or white face and white in *flabellata* may be controlled by two factors, the first of which is allelomorphic with the white of *vulgaris* while the second is independent. It is possible that the very pale *flabellata* used was not a true white, but carried a factor for dilution rather than for albinism. In this case it would not be surprising to find this factor at a different locus from the white of *vulgaris*. Unfortunately the original material has been lost, and the question cannot be settled on the data available.

6. SUMMARY

1. Four sets of factors are described in *Aquilegia vulgaris*:

B, **b**, blue/pink flower colour.

C, **c**, tall/compact habit.

L, **l**, blue/purple flower colour.

A, $\mathbf{a^w}$, **a**, whole colour/white face/white flower.

2. **cla** are linked in that order, the recombination percentages being:

cl $4{\cdot}286 \pm 0{\cdot}867$.

la $13{\cdot}095 \pm 1{\cdot}695$.

ca $15{\cdot}235 \pm 1{\cdot}468$.

3. **B**, **b** is independent of the linkage group. Doubleness also is normally independent of it, but is completely linked with **c** in one family, a cytological abnormality being suspected.

Dr Edgar Anderson and Prof. J. B. S. Haldane have taken part in the analysis of the *Aquilegia* experiments. I am obliged to Dr K. Mather for help in the statistical treatment and to Miss M. S. Campbell of the Natural History Museum for a note on the bibliography of *A. flabellata*.

REFERENCES

ANDERSON, E. & SCHAFER, B. (1931). "Species hybrids in *Aquilegia*." *Ann. Bot., Lond.*, 45, 639–46.

BEALE, G. H. (1940). "The genetics of *Verbena*, I." *J. Genet.* 40, 337–58.

Curtis's Bot. Mag. (1911), t. 8354.

KRISTOFFERSON, K. B. (1922). "Studies on Mendelian factors in *Aquilegia vulgaris*." *Hereditas, Lund.*, 3, 178–90.

MATHER, K. (1938). *The Measurement of Linkage in Heredity*. London: Methuen.

MIYOSHI, M. & MAKINO, T. (1907). *Alpine Plants of Japan*, Part II.

Rev. Horticole (1887), p. 548; (1896), p. 109.

SIEBOLD, P. F. v. & ZUCCARINI, J. G. (1846). *Florae japonicae familiae naturales*; in *Abh. Akad. Münch.* IV$_{2}$, II (1846), 183.