GENETICS OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) COMPATIBILITY

III. THE FREQUENCY OF INCOMPATIBILITY *S* ALLELES IN TWO NON-PEDIGREE POPULATIONS OF RED CLOVER

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I. INTRODUCTION

The results of investigations to determine the number of allelomorphs governing self- and cross-sterility in red clover were presented in a preliminary report by R. D. Williams in 1939. Owing to circumstances brought about by the second World War and to the death of R. D. Williams in the autumn of 1943, the complete report was not published. As it was considered desirable that the full results of the investigations should be made available, the complete data have been collected for publication in this paper.

The evidence presented by Williams & Silow (1933) and Nijdam (1932) substantiated by more recent studies reported on p. 51 of this *Journal* has demonstrated conclusively that self- and cross-sterility in *Trifolium pratense* are determined by a series of multiple allelomorphs, S_1, S_2, S_3, S_4, etc., controlling the rate of pollen-tube growth in the stylar tissue. These allelomorphic factors act oppositionally, so that only pollen tubes bearing S factors which are different from those borne by the ovular parent are capable of traversing the full length of the style and effecting fertilization. As might be expected on the basis of this interpretation, apart from rare individuals carrying the self-fertility allelomorph S_f, all red clover plants are completely self-sterile or only very slightly pseudo-fertile. Cross-pollinations between plants of similar constitution (e.g. $S_1S_2 \times S_1S_2$) are also invariably fully sterile, while combinations of normal plants bearing different S factors are all fully fertile.

It was stated by Williams (1931) that true cross-incompatibility was seldom encountered in red clover when unrelated plants were mated. The cross-pollinations that have been effected since the publication of that paper in 1931 have fully substantiated the statement, and of the very small proportion of crosses which did prove cross-sterile, the majority were directly attributable to pathological disorders. The fact that the vast majority of non-pedigree red clover plants are cross-fertile suggests the existence of an extensive series of allelomorphic factors governing fertilization in the species.

Various investigators have already reported on the numbers of sterility allelomorphs involved in the following species: *Nicotiana Sanderae et alata*, *Prunus avium*, *Antirrhinum glutinosum*, *Oenothera organensis*, *Trifolium repens* and *T. pratense*. East & Yarnell (1929) reported the isolation of sixteen allelomorphs of the S factor—fifteen sterility alleles $S_1, S_2, \ldots, S_{15}$, and one self-fertility allele S_f—from material selected from *Nicotiana Sanderae* and *N. alata grandiflora*, whilst Crane & Brown (1937) have described cross-pollinations in *Prunus avium* which are interpreted on the basis of a minimum of nine different S alleles. Gruber (1932) claims to have isolated twenty-eight S alleles in a variety of *Antirrhinum glutinosum*, but it may be pointed out that the data on which his conclusions were based demonstrate the occurrence of no more than eight factors. In a natural population of *Oenothera organensis* estimated at about 500 plants, Emerson (1939) reported the presence of thirty-seven different alleles. In an investigation with two natural populations of *Trifolium repens*, Atwood (1944) reported that 73 and 80%, respectively, of the alleles contained in the two samples were different.

II. Object, material and methods

In a paper by Williams & Silow (1933) it was reported that twenty-one red clover plants selected from the Montgomery late-flowering strain were fully cross-fertile in all possible combinations. From this it was concluded on the oppositional factor hypothesis that there were at least seven different sterility allelomorphs operating in this material.

The investigation outlined below was undertaken with the object of determining the number of different sterility allelomorphs in a given number of non-pedigree plants selected at random from commercial populations of red clover. The tests were begun in 1932, and the first analysis was made on twenty-five plants from a population known as English late-flowering red clover. This was followed by a second analysis of twenty plants from another population commonly known as English broad red clover.

The non-pedigree plants used in these tests were each crossed with a plant known to be homozygous for the sterility factors (see this *Journal*, p. 54). The progenies obtained from these crosses were classified by means of appropriate sib tests into their respective genotypes. All the F_1 plants obtained as a result of these crosses would be expected to possess one common sterility factor derived from the homozygous parent as well as an unknown factor derived from the non-pedigree parent. In order to ascertain that the constitution of these progeny genotypes agreed with expectation they were back-crossed with the homozygous parent. All the genotypes were finally cross-pollinated in all possible combinations in order to test the identity of the unknown factor derived from the non-pedigree parent.

Where it was necessary to perform a large number of crosses over several seasons involving one particular plant, clones of the material were prepared and renewed each autumn to ensure an adequate supply of flowers for crossing. The plants were pollinated by hand without previous emasculation, and a minimum of fifty-five and an average of sixty-five florets were pollinated in each test. All the pollinations were effected in glasshouses which were protected against contamination by insect pollinators. The sterile combinations were retested several times during two, and sometimes three, successive seasons.

The seeds were threshed by hand and classified as good, fair, and poor, representing, respectively, plump and well developed, under-developed and shrivelled, and very small non-viable seeds.

III. THE NUMBER OF S ALLELES IN TWENTY-FIVE ENGLISH LATE-FLOWERING RED CLOVER PLANTS

It has been previously stated that these twenty-five plants were chosen at random from a variety commonly known as English late-flowering red clover. Experience on crossing red clover plants has shown that plants selected at random from large populations prove to be reciprocally fully fertile, and it is concluded on the oppositional factor hypothesis that such individuals all possess different sterility allelomorphs. It is therefore tentatively assumed that the above twenty-five plants each possessed a different pair of S factors, and they have been designated by the genetic constitution S_xS_y—x and y ranging from 5 to 10.

(1) $S_xS_y \times S_3S_3$

Each of the twenty-five plants (Aa 2100(1) to Aa 2100(25)) was used as pistillate parent with 604(2) 3Ma (1P). This particular plant has been shown (see p. 57 of this *Journal*) to be of the genetic constitution S_3S_3. The results of these crosses are set out in Table 1.

Table 1. *Percentage seed setting obtained by crossing twenty-five unrelated red clover plants Aa* 2100(1) *to* (25) ♀ *by* 604(2) 3*Ma* (1*P*) ♂—S_3S_3

♀ parents	Florets pollinated	Seed set: Good	Seed set: Fair	Seed set: Total	Percentage set
Aa 2100(1)	75	43	1	44	58·6
,, (2)	47	12	0	12	25·5
,, (3)	70	54	1	55	78·6
,, (4)	53	30	3	33	62·3
,, (5)	51	33	1	34	66·7
,, (6)	62	72	0	72	116·1
,, (7)	72	42	0	42	58·3
,, (8)	108	41	1	42	38·8
,, (9)	73	67	0	67	91·8
,, (10)	71	63	2	65	91·5
,, (11)	71	55	0	55	77·5
,, (12)	112	101	2	103	92·0
,, (13)	56	48	0	48	85·7
,, (14)	74	67	3	70	94·6
,, (15)	87	77	3	80	92·0
,, (16)	54	44	0	44	81·5
,, (17)	99	0	10	10	10·1
,, (18)	62	44	0	44	71·0
,, (19)	56	54	4	58	103·6
,, (20)	61	43	0	43	70·5
,, (21)	59	45	6	51	86·4
,, (22)	58	51	0	51	87·9
,, (23)	69	31	0	31	45·0
,, (24)	63	16	8	24	38·1
,, (25)	71	32	0	32	45·1

It will be noted that the seed set in twenty-four of the above crosses ranges from 26 to 116%, demonstrating that S_3 pollen was fully functional on the styles of twenty-four out of twenty-five of the female parents. Plant Aa 2100(17) produced only ten poorly developed seeds which failed to germinate, and this might be interpreted as indicating that Aa 2100(17) carried an S_3 factor. Owing to the fact that no further investigations were carried out on this plant, no definite pronouncement can be made regarding its sterility constitution.

The cross-fertility shown in these crosses is interesting, as it gives an indication of the frequency of the S_3 gene. Assuming the possibility of Aa 2100(17) being heterozygous for S_3, it is clear that of the fifty sterility allelomorphs present in the twenty-five female parents utilized in the above crosses, S_3 occurred only once. The forty-eight alleles present in the female parents of the twenty-four fertile crosses possessed no inhibitory action on pollen from 604(2) 3Ma (1P), and it is, therefore, concluded that these were all distinct from the S allele which has been designated as S_3. The frequency of the S_3 gene cannot be regarded as exceptional, as the data presented below demonstrate that the other S factors in red clover possess equally low frequencies.

(2) *Classification of F_1 families into S_3S_x and S_3S_y genotypes*

Since the male parent (604(2) 3Ma (1P)) was homozygous for the sterility factors and of the genetic constitution S_3S_3, the F_1 progeny of each of the twenty-four fertile crosses would be expected to consist of two sterility genotypes, S_3S_x and S_3S_y, in the ratio of 1 : 1. For the purpose of ascertaining the presence of these groups, six plants were tested from each of twenty-two F_1 families, while seventy and eighty-nine plants, respectively, were subjected to tests in the remaining two F_1 families. These tests were effected by means of tester sibs, and the plants were classified as S_3S_x or S_3S_y on the basis of their fertility relationships with the testers. As a check on the constitution of the F_1 plants, two inter-fertile (1 S_3S_x and 1 S_3S_y) tester sibs were invariably used for testing each family, and in all cases the results obtained by the initial pollinations with tester sib 1 were corroborated by the results obtained with tester sib 2. The sister plants which were sterile with no. 1 tester sib were fertile with no. 2 tester sib, and vice versa.

The results of these tests are set out in Tables 2 and 3.

Table 2. *The number of plants belonging to the S_3S_x and S_3S_y genotypes in each of the twenty-four F_1 families*

Family no.	No. of S_3S_x plants	No. of S_3S_y plants	Family no.	No. of S_3S_x plants	No. of S_3S_y plants
1	3	3	13	2	4
2	2	4	14	2	4
3	2	4	15	3	3
4	4	2	16	5	1
5	3	3	18	3	3
6	42	28	19	1	5
7	5	1	20	3	3
8	4	2	21	4	2
9	5	1	22	1	5
10	4	2	23	5	1
11	2	4	24	3	2
12	44	45	25	2	4

Total = 154 S_3S_x : 136 S_3S_y. ($\chi^2 = 1{\cdot}12$; $P = 0{\cdot}30$–$0{\cdot}20$.)

No difficulty was experienced in distinguishing between compatible and incompatible crosses in these tests, and from Table 3 it will be noted that the compatible crosses gave an average seed set of 77·9%. Of the 120 incompatible crosses, on the other hand, 115 yielded a seed set of from 0 to 5%, the other five crosses yielding between 5 and 10%. The average seed set for all the incompatible matings was 0·84%, being the result of pseudo-fertilization.

Table 3. *Frequency table showing fertility of the crosses depicted in Table 2*

	Percentage fertility. Class limits											
	0–5	5–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–100+
No. of crosses	115	5		1	2	5	14	22	29	39	21	13
	Sterile			Fertile								

Average percentage seed set in sterile crosses = 0·84. Average percentage seed set in fertile crosses = 77·9.

Table 2 demonstrates that each of the twenty-four F_1 families consisted of two inter-fertile, intra-sterile genetic groups, S_3S_x and S_3S_y. The total distribution of 154 S_3S_x : 136 S_3S_y agrees closely with expectation on the basis of a 1 : 1 ratio. F_1 family 12, of which eighty-nine plants were tested, gave a perfect fit with a 1 : 1 ratio, while family 6, of which seventy plants were tested, although not exhibiting such a close agreement with equality as the former family, cannot be regarded as deviating significantly from expectation. The calculated value of χ^2 for the segregation in family 6 is 2·80 and P is 0·10–0·05.

(3) *Back-crossing S_3S_x and S_3S_y genotypes to the S_3S_3 parent*

One plant of each of the forty-eight genotypes (24 S_3S_x and 24 S_3S_y) was isolated on the basis of the tests described in the preceding section, and reciprocally back-crossed to the S_3S_3 parent, 604(2) 3Ma (1P). As an additional check on the constitution of these forty-eight plants each was tested for self-fertility. On the basis of the constitution of the parents used in these crosses ($S_xS_y \times S_3S_3$), each F_1 plant should have possessed an S_3 factor, and hence would be expected to be fertile as male, but sterile as female in back-crosses involving the homozygous S_3S_3 parent.

The results obtained from selfing and back-crossing are shown in Table 4.

Table 4 shows that all the plants used in these tests were cross-fertile as male but cross-sterile as female with the S_3S_3 parent. The results demonstrate the presence of an S_3 factor as well as an S_x or S_y factor in all the F_1 progeny plants tested. Unfortunately, a few crosses were inadvertently omitted, but the results are sufficiently complete to conclude that each of these F_1 progeny plants contained one unknown S factor—the other being an S_3 factor derived from 604(2) 3Ma (1P).

(4) *Tests of identity of forty-eight S_x and S_y alleles*

In order to determine the actual number of similar alleles carried by the twenty-four original English late-flowering red clover plants which yielded F_1 families, representative plants from each of forty-eight S_3S_x or S_3S_y genotypes were inter-crossed in all possible combinations. For the purpose of these tests one sister plant was selected from each of the forty-eight genotypic classes. All the crosses which were obviously fertile were performed in only one direction, while those which gave a seed set of less than 30% were repeated in both directions. All the incompatible combinations were repeated twice or three times in different seasons. The results of these tests are set out in Tables 5–7.

It will be seen from the results presented in Table 6 that the differences in percentage seed set between the fertile and sterile combinations were quite clearly defined. The percentage seed set in the sterile crosses ranged from 0 to 6·3, while in the fertile crosses

Table 4. *The percentage fertility obtained from selfing and back-crossing one S_3S_x and one S_3S_y plant from each of the twenty-four F_1 families*

(*Note.* $1x$ and $1y$ in Table 4, and all subsequent tables, represent S_3S_x and S_3S_y plants from F_1 family 1, while $2x$ and $2y$ represent S_3S_x and S_3S_y plants in family 2, etc.)

	Selfing			Back-crossing on to 604(2) 3Ma (1P)—S_3S_3					
				S_3S_3 ♂			S_3S_3 ♀		
S_3S_x and S_3S_y plant no.	No. of florets	No. of seeds	Percentage set	No. of florets	No. of seeds	Percentage set	No. of florets	No. of seeds	Percentage set
$1x$	43	2	4·7	37	0	0	43	17	39·5
$1y$	57	0	0	50	0	0	50	36	72·0
$2x$	61	2	3·3	52	0	0	52	28	53·8
$2y$	76	1	1·3	50	0	0	49	30	61·2
$3x$	53	0	0	39	2	5·1	59	33	55·9
$3y$	58	3	5·2	36	0	0	44	35	79·5
$4x$	52	0	0	45	0	0	50	15	30·0
$4y$	65	0	0	46	0	0	49	23	46·9
$5x$	61	0	0	59	2	3·4	33	18	54·5
$5y$	58	0	0	55	0	0	54	27	50·0
$6x$	69	0	0	52	0	0	56	27	48·2
$6y$	59	0	0	41	0	0	62	35	56·4
$7x$	49	0	0	41	0	0	50	43	86·0
$7y$	75	0	0	62	1	1·6	44	26	59·1
$8x$	57	0	0	40	0	0	45	13	28·9
$8y$	37	0	0	37	2	5·4	42	21	50·0
$9x$	67	2	3·0	50	0	0	45	38	84·4
$9y$	62	0	0	45	1	2·2	44	34	77·3
$10x$	54	0	0	49	3	6·1	55	35	63·6
$10y$	55	0	0	52	2	3·8	51	56	109·8
$11x$	38	0	0	50	0	0	46	36	78·3
$11y$	41	1	2·4	37	4	10·9	55	39	70·9
$12x$	45	2	4·4	46	4	8·7	50	49	98·0
$12y$	43	0	0	35	2	5·7	35	14	40·0
$13x$	51	5	9·8	32	3	9·4	60	36	60·0
$13y$	72	1	1·4		Not tested		36	31	86·1
$14x$	63	0	0	72	1	1·4	44	33	75·0
$14y$	57	0	0		Not tested		49	28	57·1
$15x$	54	1	1·8		Not tested		33	25	75·7
$15y$	56	0	0	62	4	6·4	85	25	29·4
$16x$	79	0	0	58	0	0	53	45	84·9
$16y$	79	0	0	54	0	0	36	41	113·9
$18x$	55	2	3·6	61	0	0	55	26	47·3
$18y$	33	0	0	50	0	0	53	22	41·5
$19x$	37	0	0	52	1	1·9	51	37	72·5
$19y$	51	1	1·9	49	0	0	49	39	79·6
$20x$	57	0	0	36	0	0	47	48	102·1
$20y$	73	0	0	90	0	0	43	40	93·0
$21x$	84	0	0	90	0	0	41	36	87·8
$21y$	69	0	0	39	0	0	45	29	64·4
$22x$	80	0	0	75	0	0	51	41	80·4
$22y$	41	0	0	46	0	0	51	26	50·9
$23x$	46	0	0	31	0	0	37	18	48·7
$23y$	74	0	0	32	0	0	44	25	56·8
$24x$	94	1	1·1	50	3	6	46	43	93·5
$24y$	51	0	0	40	2	5	36	35	97·2
$25x$	62	0	0	42	0	0	35	28	80·0
$25y$	66	0	0		Not tested			Not tested	

the range extended from 20·7 to 172·5. Though these results indicate that certain plants were more highly cross fertile than others in compatible pollinations, it is probable that external conditions were in the main largely responsible for the differences in percentage fertility exhibited by the fertile combinations. As stated, all fertile crosses yielding a seed set of less than 30 % were repeated in both directions, and the results from these repeat crosses were invariably very different from the first cross. Instances were met with where crosses which yielded a seed set of less than 30 % in one season gave over 80 % in the second season. As will be seen from Table 6, only five crosses of the 1121 fertile crosses did not exceed the 30 % seed-set mark, and even these were quite easily differentiated from the sterile combinations which were fully sterile or only very slightly pseudo-fertile.

Table 6. *Frequency table showing fertility of the crosses depicted in Table* 5

Percentage fertility. Class limits	0–2	2–4	4–6	6–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–100+
No. of crosses	6		1			5	13	24	49	76	126	211	259	358
	Sterile					Fertile								

Table 7. *The percentage fertility of the seven incompatible cross-pollinations shown in Table* 5

Cross	♀ plant in cross	No. of florets pollinated	Seed set	Percentage set
$1x \times 13x$	$1x$	552	12	2·2
	$13x$	528	56	10·6
$3x \times 7x$	$3x$	369	0	0·0
	$7x$	345	9	2·6
$3y \times 9y$	$3y$	378	6	1·6
	$9y$	297	4	1·3
$4x \times 20x$	$4x$	349	0	0·0
	$20x$	285	0	0·0
$7y \times 18y$	$7y$	224	7	3·1
	$18y$	301	6	1·9
$12x \times 18x$	$12x$	168	4	2·4
	$18x$	186	1	0·5
$25y \times 20y$	$25y$	160	0	0·0
	$20y$	279	3	1·1

Table 5 shows the percentage fertility obtained from the 1128 crosses involved in these tests. From these results it will be noted that seven of the crosses were sterile or only slightly pseudo-fertile, and the details of the pollinations performed in these crosses are summarized in Table 7. Since each genotype carried only one unknown S_x or S_y allelomorph, plants in which the unknown alleles were similar should be cross-sterile, while all those bearing dissimilar S_x or S_y alleles should be cross-fertile. On the basis of the fertility relationships detailed above, it is concluded that all the S factors involved were different, with the exception of the following:

S_x plant $1 = S_x$ plant 13
S_x ,, $3 = S_x$,, 7
S_y ,, $3 = S_y$,, 9
S_x ,, $4 = S_x$,, 20
S_y ,, $7 = S_y$,, 18
S_x ,, $12 = S_x$,, 18
S_y ,, $20 = S_y$,, 25

It is thus evident that of the forty-eight sterility allelomorphs carried by the original twenty-four English late-flowering red clover plants, forty-one were different—thirty-four being represented only once and seven represented twice.

IV. The number of S alleles in twenty English broad red clover plants

This investigation on a random selection of twenty plants of English broad red clover was undertaken to confirm the surprising results obtained with the twenty-five English late-flowering red clover plants. Since these twenty plants were presumably unrelated, it was tentatively assumed that they all possessed different S alleles.

(1) $S_xS_y \times S_1S_1$

As a first step in determining the number of different S alleles carried by the twenty English broad red clover plants, each individual was crossed with the homozygous S_1S_1 plant,* 628(2) 2Ma (2P). The results of seed setting in these crosses are set out in Table 8.

Table 8. *The results of crossing twenty unrelated plants of English broad red clover, S_xS_y ♂, with* 628(2) 2Ma (2P), S_1S_1 ♀

Plant no.	Florets pollinated	Seed set	Percentage set	Plant no.	Florets pollinated	Seed set	Percentage set
1	58	47	81·0	11	46	22	47·8
2	53	16	30·2	12	47	36	76·6
3	71	24	33·8	13	35	6	17·1
4	59	22	37·3	14	16	9	56·2
5	54	22	40·7	15	52	29	55·8
6	43	11	25·6	16	38	26	68·5
7	41	14	34·1	17	33	26	78·8
8	46	6	13·0	18	35	20	57·1
9	53	51	96·2	19	54	22	40·7
10	40	26	65·0	20	38	22	57·9

It will be noted from Table 8 that all the crosses with the exception of nos. 8 and 13 gave a percentage seed set ranging from 25·6 to 96·2. Nos. 8 and 13 gave a seed set of only 13·0 and 17·1 %, respectively, but while this must be regarded as a low seed set in fertile crosses of red clover, the number of florets pollinated (forty-six in no. 8 and thirty-five in no. 13) was not sufficient to enable one to draw any conclusion as to the precise fertility relationships of these plants with 628(2) 2Ma (2P).

(2) *Classification of F_1 families into S_1S_x and S_1S_y genotypes*

Unfortunately it was possible to raise only one F_1 plant from each of families 13 and 17. Twenty plants from family 1 and four to six plants from the remaining seventeen F_1 families were selected and classified on the basis of their fertility reaction with tester sibs into S_1S_x or S_1S_y classes. The number of tester sibs used in these tests varied from one to three in different families. All plants which were sterile with no. 1 tester and fertile with no. 2 tester have been assigned the constitution S_1S_x, while all those which were fertile with no. 1 tester and sterile with no. 2 tester were designated as S_1S_y. The distinction between fertile and sterile crosses was in all cases quite definite—the average seed setting being 60·4 % for the fertile combinations and 0·8 % for the sterile combinations.

The results of these tests are set out in Table 9.

On reference to Table 9 it will be seen that each of the eighteen F_1 progenies consisted of two approximately equal-sized genotypic groups S_1S_x and S_1S_y, as was expected from the genetic constitution of the parent plants.

* For details of the genetic constitution of 628(2) 2Ma (2P), see this *Journal*, p. 54.

(3) *Back-crossing S_1S_x and S_1S_y genotypes to the S_1S_1 parent*

In order to confirm the presence of the S_1 factor in the progeny derived from the $S_xS_y \times S_1S_1$ crosses, plants selected from twenty-four S_1S_x and S_1S_y genotypic groups were back-crossed both as male and female to the homozygous S_1S_1 parent. Before attempting the analysis of identity of the S_x and S_y alleles it was further considered advisable to test each of the plants involved in the inter-group crosses shown in Table 12 for self-fertility. The results of back-crossing and selfing are shown in Tables 10 and 11, respectively.

Table 9. *Classification of F_1 progeny (S_1S_x or S_1S_y) resulting from crossing eighteen unrelated plants (S_xS_y) with a homozygous S_1S_1 plant*

F_1 family no.	No. of tester sibs used	No. of S_1S_x genotypes	No. of S_1S_y genotypes
1	1 and 3	12	8
2	1	3	2
3	1 and 2	2	3
4	1, 3 and 4	3	2
5	1	1	4
6	5 and 6	4	1
7	1 and 2	3	2
8	1 and 2	2	3
9	2	2	3
10	1	1	4
11	6	3	2
12	5 and 6	2	3
14	3 and 4	1	3
15	1, 2 and 3	5	1
16	1 and 2	3	2
18	5 and 6	3	2
19	1	1	4
20	4 and 6	2	3

Total: 53 S_1S_x : 52 S_1S_y.

Note. F_1 families 13 and 17 from which only one plant was raised have been omitted from the above table.

Table 10. *Results of back-crossing to the homozygous parent (S_1S_1) of representative plants of twenty-four genotypes derived from the $S_xS_y \times S_1S_1$ crosses*

	No. of genotypes											
Direction of cross ...	$1x$	$1y$	$2x$	$2y$	$3x$	$6y$	$7x$	$7y$	$8y$	$9x$	$10x$	$11x$
S_1S_x or S_1S_y ♀ × S_1S_1 ♂	0	0	0	0	⊕	0	0	0	0	0	0	0
S_1S_1 ♀ × S_1S_x or S_1S_y ♂	–	+	+	+	+	+	+	+	–	+	+	+
Direction of cross ...	$11y$	$12x$	$12y$	$13x$	$14x$	$15x$	$15y$	$16x$	$16y$	$17x$	$18x$	$20x$
S_1S_x or S_1S_y ♀ × S_1S_1 ♂	0	⊕	0	–	0	0	0	0	0	0	0	0
S_1S_1 ♀ × S_1S_x or S_1S_y ♂	+	+	–	+	+	–	+	–	+	+	–	+

0 = full sterility. + = full fertility. ⊕ = pseudo-fertility. – = not crossed.

Table 11. *Frequency table showing percentage pseudo-fertility of S_1S_x and S_1S_y plants derived from the crosses $S_xS_y \times S_1S_1$*

	Percentage fertility. Class limits							
	0	0–1	1–2	2–3	3–4	4–5	5–6	6–7
No. of plants	93	0	0	3	1	2	0	4
	7–8	8–9	9–10	10–12	12–14	14–16	16–18	
No. of plants	0	0	1	0	1	1	0	

Total no. of plants = 106.

As may be seen from Table 10, all the back-crosses where the homozygous S_1S_1 plant was used as male parent were sterile with the exception of two plants—$3x$ and $12x$—which exhibited pseudo-fertility to the extent of 5·0 and 3·3 %, respectively. When the crosses were performed in the opposite direction, full fertility resulted, the average seed setting for the fertile pollinations being 36·47 % of florets pollinated. Although the results presented in Table 10 are not quite complete, they serve to demonstrate the inhibitory action conditioned by the S_1 allele in the S_1S_x and S_1S_y plants when pollinated by S_1 pollen from the homozygous parent. In the crosses S_1S_1 ♀ × S_1S_x or S_1S_y ♂, the S_x or S_y allele promoted full fertility.

From the results of selfing 106 S_1S_x and S_1S_y plants set out in Table 11 it is seen that ninety-three plants produced no seed, while ten of the pseudo-fertile plants gave a seed set of less than 7 %. The remaining three pseudo-fertile plants yielded from 9 to 15 % seed. Although the latter exhibited rather a high percentage of self-fertilization for red clover, there is no reason to doubt that they were genuine instances of pseudo-fertility.

(4) *Tests of identity of forty S_x and S_y alleles*

The thirty-eight S_x and S_y alleles (one S_x and one S_y from each of eighteen families and one S_x from each of two families) which had been isolated from F_1 families derived from crossing twenty English broad red clover plants with S_1S_1 were inter-pollinated with each other in all possible combinations. In addition, two other genotypes $21x$ and $22x$ were included in this series. Plant $21x$ was a sister plant of the homozygous S_1S_1 parent used in these crosses and had been previously shown to be heterozygous for the S_1 factor, while $22x$ was a plant selected from a family obtained by crossing the homozygous S_1S_1 plant with another homozygous S_2S_2 plant. The genetic constitution of $22x$ was therefore S_1S_2.

As in the case of the diallel crosses performed on the English late-flowering derivatives, most of the pollinations were performed in only one direction. All the reciprocal pollinations that were made, however, fully substantiated the results obtained in the opposite direction.

The results of these diallel pollinations expressed as percentage seed set are presented in Tables 12–14.

Table 13. *Frequency table showing fertility of the crosses depicted in Table* 12

Percentage fertility. Class limits	0–2	2–4	4–6	6–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100+
No. of crosses	2		1			6	28	36	89	115	153	172	102	76
	Sterile					Fertile								

Table 14. *Percentage fertility of the three incompatible pollinations shown in Table* 12

Genotypes	♀ plants in cross	No. of florets pollinated	Seed set	Percentage set
$1y \times 19x$	$1y$	205	0	0·0
	$19x$	149	0	0·0
$5x \times 6x$	$5x$	166	0	0·0
	$6x$	178	0	0·0
$11x \times 14y$	$11x$	199	6	3·0
	$14y$	305	22	7·2

Average fertility in incompatible crosses = 2·3 %.

Tables 12 and 13 demonstrate that 777 out of the 780 pollinations between the forty genotypes were fertile and yielded a seed set ranging from 21 to 164 %. Only three crosses proved to be incompatible, and as will be seen from Table 14 two of these ($1y \times 19x$ and $5x \times 6x$) were reciprocally cross-sterile, while the other ($11x \times 14y$) gave a seed set of

3·0 and 7·2% through pseudo-fertility when $11x$ and $14y$ were respectively used as females.

From these results it is concluded that the following S alleles were similar:

$$1y = 19x, \quad 5x = 6x, \quad 11x = 14y.$$

Of the forty unknown S allelomorphs present in this material, three were therefore represented twice, and thirty-four only once—the total number of different alleles being thirty-seven.

V. SUMMARY

1. Twenty-four out of twenty-five unrelated S_xS_y plants from a population known as English late-flowering red clover were cross-fertile as females with an S_3S_3 plant, 604(2)·3Ma (1P).

2. Forty-eight S_3S_x and S_3S_y genotypes were isolated from the twenty-four F_1 families obtained by crossing S_xS_y ♀ × S_3S_3 ♂.

3. Of the forty-eight S_x and S_y alleles, forty-one have been shown by their fertility relationships to be different.

4. Of twenty S_xS_y English broad red clover plants crossed as males with another homozygous S_1S_1 plant, 628(2) 2Ma (2P), eighteen were fully cross-fertile. The results obtained in the other two crosses were inconclusive.

5. Thirty-eight S_1S_x and S_1S_y genotypes were isolated from the F_1 families obtained from crossing twenty S_xS_y plants by S_1S_1.

6. In addition to these thirty-eight S_x and S_y alleles, two other alleles—an S_x (plant $21x$) and an S_2 (plant $22x$)—were included in the fertility tests. Thirty-seven out of a total of forty S_x and S_y alleles have been shown to be different.

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