INHERITANCE IN *LOLIUM PERENNE* L. II. A SECOND PAIR OF LETHAL FACTORS.

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INTRODUCTION.

IN the first paper of this series[1] it was shown that the *Lolium perenne* plant, No. 43, is heterozygous for at least two pairs of factors. Its factorial constitution in respect of these has been represented by means of the symbols **LlYy**.

The **Ll** pair of factors is concerned with the survival or non-survival of green seedlings. Plants of factorial constitution **ll**, although not appreciably deficient in chlorophyll, only survive at most up to the early second-leaf stage, although they are vigorous in germination, and make progress until the second-leaf stage is closely approached.

The **Yy** pair is concerned with the appearance of chlorophyll-deficient seedlings.

The present paper deals with certain results obtained from another plant, No. 48, while results obtained by inter-crossing the two plants, 43 and 48, as far as lethal factors are concerned are also discussed.

PLANT 48.

This is a normal, full green, vigorous plant, selected, as in the case of plant 43, in the first instance as being promising from an economic point of view. Consequently, both when selfed and when inter-crossed with plant 43, only data which appeared to be of economic significance were at first collected, since the main idea at that time was merely to obtain a sufficient number of plants to proceed with a building up of a pedigree line.

Plant 48 proved to be relatively highly self-fertile, but the L_1 seedlings so produced were noticed to be of three different types—green, variegated and albino. A full record of such types has been kept throughout, but the study of variegation is admittedly difficult and the data are as yet not sufficiently complete for discussion.

As the work proceeded it was found that only a proportion of the

[1] *Journ. Gen.* XIX. p. 391.

green seedlings survived. The non-surviving green are at first vigorous and not appreciably, if at all, deficient in chlorophyll. As in the case of similar seedlings derived from plant 43, however, they never proceed far into the second-leaf stage, and when the two types of non-surviving green are grown together they cannot be distinguished from each other with any degree of certainty.

PLANT 48 SELF-POLLINATED.

One advantage of working on the perennial grasses is the fact that one and the same plant can be used over a number of seasons. For pot work, however, it is found necessary to rejuvenate the plant each year. This is done by breaking it up into plantlets of such a size that vigorous material may be available at flowering time[1]. In consequence of this breaking up of the original plant, plantlets have sometimes been given new identification numbers. The identification numbers 48, 49, 50 and 131 which may occur in this paper, therefore, refer to the different plantlets of the same original plant, No. 48.

In Table I are shown results obtained by selfing four different plantlets of plant 48. As will be the case throughout the paper, the actual figures for surviving and non-surviving green seedlings only are included.

In this table also the actual numbers are compared with those expected, calculated on a 3 : 1 basis.

TABLE I.

Surviving and non-surviving green seedlings obtained by self-pollinating plant 48.

Family	% germi- nation	Actual		Expected		Prob- able error	Devia- tion	Dev./P.E.
		Sur- viving	Non-sur- viving	Sur- viving	Non-sur- viving			
48/(1)	70·1	285	92	282·75	94·25	5·91	2·25	0·38
48(10)/(1)	85·4	357	109	349·50	116·50	6·30	7·50	1·19
49/(1)	84·3	583	188	578·25	192·75	8·11	4·75	0·58
49/(1)	53·0	114	42	117·00	39·00	3·65	3·00	0·82
		1339	431	1327·50	442·50	12·29	11·50	0·94

In each family taken independently and also in the aggregate for the four families the agreement between the numbers actually obtained and those arrived at by calculation is very close. In three of the families, however, there is a slight deficiency of non-surviving green.

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[1] If a plantlet is too big in comparison with the size of the pot, the flowering stems and inflorescences are appreciably weakened. The same thing would occur if a plant were not broken up and re-potted, owing to exhaustion of the soil in the pot.

L_1 PLANTS SELFED.

Results for surviving and non-surviving green seedlings have been obtained for six L_2 families. All these families were obtained by selfing L_1 plants. The actual results are given in Table II where a comparison is also made between the actual numbers and expectation on a 3 : 1 basis.

TABLE II.

L_2 results compared with expectation on 3 : 1 basis.

	% germi-nation	Actual		Expected		Prob-able error	Devia-tion	Dev./P.E.
Family		Sur-viving	Non-sur-viving	Sur-viving	Non-sur-viving			
49/(2)30/	60·2	72	34	79·5	26·5	3·01	7·5	2·49
49/(2)38/	76·0	288	124	309·0	103·0	5·93	21·0	3·37
48/(2)1/	76·5	145	55	150·0	50·0	4·13	5·0	1·21
48/(2)4/	70·8	364	124	366·0	122·0	6·45	2·0	0·31
		869	337	904·5	301·5	10·14	35·5	3·50
49/(2)26/	77·6	14	0	10·5	3·5	1·09	3·5	3·21
49/(2)28/	69·7	9	0	6·75	2·25	0·87	2·25	2·59
		23	0	17·25	5·75	1·40	5·75	4·11

These results fall naturally into two groups. In four of the families a proportion of the green seedlings failed to survive, but the agreement with expectation on a 3 : 1 basis is very good only in the case of one family. In each family there is excess of non-surviving green which causes the Dev./P.E. result for the four families taken together to be of such a magnitude as to suggest a significant deviation. When the families are considered individually, however, only in one case is this figure more than 3.

It must be borne in mind also that in L_2 the seedlings are generally very weak so that it is probable that some proportion of the non-surviving have perished simply through lack of vigour. In such a case a deviation in this direction is to be expected rather than otherwise.

In two families, however, none of the green seedlings perished. In both cases, the number of green plants obtained was very small, so that taken individually, this fact is hardly significant. When the two families are taken together, however, the deviation naturally becomes more pronounced.

The significance of the figures for these two families is increased by the fact that loss of vigour should here be operative to the same extent as in the other families, while actually no seedlings were lost.

These L_2 results, therefore, on the whole support the evidence

obtained from the L_1 families in indicating that survival or non-survival of green seedlings is dependent upon a single pair of factors.

L_2 PLANTS SELFED.

Unfortunately, L_3 results have been obtained only from L_2 plants other than those included in Table II.

Altogether, twelve L_3 families have been obtained. These are all derivatives of the two L_1 plants 48/(1)21 and 48/(1)22. Each of these was selfed, and the two were also inter-crossed, but at this time a full record of the behaviour of the green seedlings was not being kept. There is, therefore, no direct L_2 evidence to show the constitution of these L_1 plants in respect of surviving and non-surviving green seedlings.

The L_3 results are shown in Table III where some of them are also compared with expectation on a 3 : 1 basis. The families are here grouped according to parentage.

TABLE III.

L_3 results: surviving and non-surviving green seedlings.

Family	% germi- nation	Actual		Expected		Prob- able error	Devia- tion	Dev./p.e.
		Sur- viving	Non-sur- viving	Sur- viving	Non-sur- viving			
48/(3)21/28/	51·8	87	1					
48/(3)21/27/	75·4	171	0					
48/(3)21/29/	58·3	48	24	54·00	18·00	2·48	6·00	2·42
48/(3)21/31/	59·8	28	9	27·75	9·25	1·78	0·25	0·14
48/(3)21 × 22/29/	68·1	275	0					
48/(3)21 × 22/26/	59·2	90	42	99·00	33·00	3·35	9·00	2·69
48/(3)21 × 22/28/	74·5	221	78	224·25	74·75	5·05	3·25	0·64
48/(3)21 × 22/36/	71·0	410	124	400·50	133·50	6·75	9·50	1·41
48/(3)22/8/	50·0	4	0					
48/(3)22/15/	72·0	15	0					
48/(3)22/11/	70·3	223	5					
48/(3)22/13/	57·9	107	3					

In the L_3 families derived directly from the L_1 plant 48/(1)21, two show segregation into surviving and non-surviving green seedlings, while two show no such segregation.

In two of the L_3 families derived directly from the other L_1 plant, 48/(1)22, a very small proportion of the green seedlings failed to survive, due undoubtedly to lack of vigour, and at least three families are clearly of the non-segregating type. The number of families is too small to allow for anything but a suggestion that the L_1 plant 48/(1)22 was homozygous for the absence of the lethal factor.

From L_2 plants resulting from the inter-crossing of the two L_1 plants, three segregating families and one non-segregating family were obtained.

The results are therefore consistent with the assumption that plant 48/(1)21 was heterozygous, while 48/(1)22 was homozygous. As in the case of the segregating L_2 families there is a rather persistent excess of non-surviving green seedlings, but this excess is irregular and in no L_3 family does the Dev./P.E. figure exceed 2·7. It is, therefore, at most, only of doubtful significance.

When the five segregating L_3 families, with a total of 1074 seedlings, are taken together, however, the deviation from expectation on a 3 : 1 basis is only 8·5, while the probable error is 9·57. This gives a Dev./P.E. figure of only 0·89.

The rather persistent excess of non-surviving green seedlings suggests the possibility that two pairs of linked factors may be involved. If from the L_2 and L_3 families we select four of those showing a considerable excess of non-surviving green (families 49/(2)30/, 49/(2)38/, 48/(3)21/29/ and 48/(3)21×22/26/) we get a total of 498 surviving : 224 non-surviving green. This agrees very closely with expectation with linkage (coupling phase) of two pairs of factors, supposing the cross-over value to be about 13 per cent.

The L_1 results, however, show no indication of the presence of two such pairs of factors, and the results for the other families are very irregular.

When, further, all the results for the L_1, L_2 and L_3 segregating families are considered together, we get a total of 3005 surviving to 1045 non-surviving green seedlings, while the expectation on a 3 : 1 basis would be 3037·5 to 1012·5. There is still an excess of non-surviving green with a Dev./P.E. figure of 1·75, which is somewhat high but of very doubtful significance.

It must be borne in mind that the "non-surviving" class includes all those green seedlings that perished from whatever cause. In eight non-segregating families with a total of 639 seedlings, nine died back. In a total of 4050 seedlings (the total for all segregating families) the equivalent would be 57, which more than covers the excess of non-surviving green.

If only the L_2 and L_3 families are considered (since there is no evidence of such loss in L_1 families) the equivalent figure is 32, while the actual excess of non-surviving green is 44. In spite, therefore, of the fact that the excess of non-surviving green in some families may suggest that two pairs of linked factors are involved, the evidence reviewed as a whole is very strongly in favour of the hypothesis that segregation is throughout monohybrid in type, with, in some cases, excess of non-surviving green caused by the death of weak, but genetically surviving seedlings.

PLANTS 43 AND 48 INTER-CROSSED.

It has now been shown that plant 48 is heterozygous for one, and probably only for one pair of factors concerned with the appearance of non-surviving green seedlings. These seedlings are indistinguishable from the non-surviving green derived from plant 43.

When the two plants were inter-crossed, 118 F_1 seedlings were obtained from 126 seeds. These seedlings were all full green and none of them failed to survive.

It is, therefore, evident that the appearance of non-surviving green seedlings in the two lines is not dependent upon the same pair of factors. Since the effect in the two cases is very similar, and the factors concerned in the case of plant 43 have already been indicated by means of the symbols Ll, those of plant 48 may be designated $L_1 l_1$. The formula representing plant 43 will therefore be $LlL_1 L_1$ and that for plant 48, $LLL_1 l_1$.

F_2 RESULTS.

The F_1 plants obtainable (with independent segregation and re-combination) when two such plants are inter-crossed are of the four types $LLL_1 L_1$, $LlL_1 L_1$, $LLL_1 l_1$ and $LlL_1 l_1$ in equal numbers. Since ll and $l_1 l_1$ seedlings are indistinguishable, F_2 families obtained by selfing F_1 plants cannot show whether such plants are $LlL_1 L_1$ or $LLL_1 l_1$. In F_2 therefore three types of families should be obtained in the ratio:

1 consisting of surviving green seedlings only.
2 consisting of surviving and non-surviving green in the ratio 3 : 1.
1 consisting of surviving and non-surviving green in the ratio 9 : 7.

Altogether, nineteen F_1 plants have been tested by selfing. Of these, five gave families of one, two, three, eight and eleven plants each, respectively. The results for one of these plants are included with other F_2 results in Table IV, since for this particular plant, other evidence, to be given below, has been obtained.

The results for families giving eleven plants or less cannot be relied upon.

Of the green seedlings obtained in two of the F_2 families none failed to survive, while in a third, only one out of twenty-two died. It would, therefore, appear that these three families were obtained from plants which were homozygous non-lethal.

Family 179/(2)32 occupies an anomalous position. The actual ratio is 404 surviving to 37 non-surviving green seedlings, whereas expectation

TABLE IV.

F_2 families: actual number of surviving and non-surviving green seedlings obtained by selfing F_1 plants.

| | Seedlings | |
Family	Surviving	Non-surviving
112/(2)28/	2	0
179/(2)35/	80	0
179/(2)38/	21	1
179/(2)39/	48	0
179/(2)32/	404	37
112/(2)29/	266	76
112/(2)33/	360	125
179/(2)29/	67	19
179/(2)33/	43	14
179/(2)31/	71	36
112/(2)8/	255	224
179/(2)28/	294	259
179/(2)34/	23	18
179/(2)36/	32	33
112/(2)4/	828	860

would be approximately 331 : 110 on a 3 : 1 basis and 413 : 28 calculated on a 15 : 1 basis. There is a distinctly closer agreement with the latter than with the former.

The F_1 parent of this family has not been tested except in this instance, so that no further direct evidence as to its constitution is available. A definite 15 : 1 ratio has, however, not elsewhere been found in connection with these particular characters.

This family is also very large in comparison with other "179" families obtained under the same conditions[1]. This suggests possible contamination by foreign pollen. With selfing in the open, employing dense fabric covers, there are two possible ways in which contamination may occur. In the first place, a cover may become slightly defective and the defect not be detected. In the second place, covering may be too long delayed. The importance of such sources of contamination has long been fully recognised, so that every possible precaution is taken to guard against them. That contamination from either of these sources may very rarely happen is, however, not impossible.

If such contamination had occurred in the case of plant 179/(1)32 (and this is supported by results for types of seedlings other than green), the tendency would be to decrease the proportion of non-surviving green

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[1] Families 179/(2)28/ and 29/ together with all the "112" families were obtained from seed produced under greenhouse conditions and from repeated selfing. As far as possible, selfing in connection with genetical work is always conducted under these conditions, but the number of plants selfed each season is far in excess of the greenhouse space available.

seedlings. It would, therefore, appear that this family actually represents a 3 : 1 ratio, since it is highly improbable that such an amount of contamination should have occurred as to reduce the proportion of nonsurviving green from the 193 expected on a 9 : 7 segregation basis to the 37 actually obtained.

In the next group, there are four families, three of which, as shown in Table V, agree very closely with expectation calculated on a 3 : 1 basis. In the fourth family there is an excess of non-surviving green seedlings, which is, however, probably not significant.

TABLE V.

Results for four F_2 families compared with expectation calculated on a 3 : 1 basis.

Family	Actual		Expected		Probable error	Deviation	Dev./P.E.
	Surviving	Non-surviving	Surviving	Non-surviving			
112/(2)29/	266	76	256·50	85·50	4·54	9·50	2·09
112/(2)33/	360	125	363·75	121·25	6·43	3·75	0·58
179/(2)29/	67	19	64·50	21·50	2·71	2·50	0·92
179/(2)33/	43	14	42·75	14·25	2·21	0·25	0·11
	736	234	727·50	242·50	9·10	8·50	0·93

When the aggregate for the four families is taken the agreement with expectation is very good, so that these four families may be regarded as definitely showing monohybrid segregation.

The next family, 179/(2)31/, again occupies a rather anomalous position with 71 surviving and 36 non-surviving seedlings. The expectation in this case, calculated on a 3 : 1 basis, would be 80·25 : 26·75. This gives a Dev./P.E. figure of 3·06. Calculated on a 9 : 7 basis, on the other hand, the expectation would be 60·2 : 46·8 which gives a Dev./P.E. figure of 3·12. In the absence of other evidence, therefore, it is impossible to determine whether this family shows a monohybrid or a dihybrid type of segregation.

Four other families, as shown in Table VI, give a fair agreement with expectation on a 9 : 7 basis. In none of the families taken individually

TABLE VI.

Results for four F_2 families compared with expectation calculated on a 9 : 7 basis.

Family	Actual		Expected		Probable error	Deviation	Dev./P.E.
	Surviving	Non-surviving	Surviving	Non-surviving			
112/(2)8	255	224	269·4	209·6	7·32	14·4	1·97
179/(2)28	294	259	311·1	241·9	7·83	17·1	2·18
179/(2)34	23	18	23·1	17·9	2·14	0·1	0·05
179/(2)36	32	33	36·6	28·4	2·71	4·6	1·70
	604	534	640·2	497·8	11·29	36·2	3·20

is the deviation such as to be considered significant, but owing to the
fact that in the three largest families it is in the direction of excess
of non-surviving green, the Dev./P.E. figure for all families together
reaches 3·20.

It is quite possible that this excess is due to the death of weak,
genetically-surviving seedlings, but it also suggests a possible linkage
(repulsion phase) between the two pairs of factors with a certain amount
of crossing over. Whether this is the case or not would be difficult to
prove conclusively, owing to some inevitable confusion between the
non-survival of true "lethal" and weak "non-lethal" seedlings, while
back-crossing with recessives is obviously impossible.

A single family, 112/(2)4/ consisted of 828 surviving and 860 non-
surviving green seedlings. This result is the aggregate for a number of
results with which the individual results agree fairly closely. It is,
therefore, evident that the result is not abnormal, but it is also obvious
that it does not agree with expectation calculated on a 9 : 7 basis, there
being a great excess of non-surviving seedlings. Such a linkage as that
suggested by the results already given might partially account for this
excess. There would still remain, however, a considerable excess of
non-surviving green.

In any case there is a clear indication that the F_1 plant concerned
is heterozygous for two pairs of factors, so that in this respect it falls into
line with the other four plants which give a segregation of 9 : 7 in F_2.

Of the fourteen plants which gave apparently reliable results,
therefore, three have been found to be homozygous non-lethal, five gave
monohybrid segregation, and five were heterozygous for two pairs of
factors, while one other family was segregating, but whether it was
heterozygous for one or for two pairs of factors could not be decided.

With fifteen plants, the expectation would be 3·5 LLL_1L_1, 7 LlL_1L_1
+1 LLL_1l_1 and 3·5 LlL_1l_1. The numbers actually obtained are either
3 : 5 : 6 or 3 : 6 : 5, so that agreement is as near as might be expected
with the number of plants tested.

F_1 PLANTS BACK-CROSSED TO PARENTS AND SIDE-CROSSED TO SISTER PLANTS.

Some of the F_1 plants tested by selfing have been further tested by
back-crossing to their parents and by side-crossing to sister plants. The
results are given in Table VII. In this table also, where the families
are fairly large, and there is evidence of segregation, the actual results
are compared with expectation on a 3 : 1 basis.

TABLE VII.

F_1 plants back-crossed to parents and side-crossed to sister plants.

Family	Parents	Actual		Expected		Prob-able error	Devia-tion	Dev./P.E.
		Sur-viving	Non-sur-viving	Sur-viving	Non-sur-viving			
183/(1)	112/(1)23 × 43	28	1					
185/(1)	49 × 112/(1)23	7	2					
186/(1)	112/(1)23 × 48/(1)22	25	0					
303/(1)	112/(1)28 × 43	38	0					
311/(1)	49 × 112/(1)28	4	0					
318/(1)	112/(1)28 × 112/(1)8	27	0					
319/(1)	112/(1)8 × 112/(1)28	3	0					
320/(1)	112/(1)28 × 112/(1)29	33	2					
321/(1)	112/(1)28 × 112/(1)33	7	0					
312/(1)	112/(1)29 × 49	97	1					
305/(1)	112/(1)33 × 43	90	0					
301/(1)	112/(1)4 × 43	51	19	52·50	17·50	2·44	1·50	0·61
309/(1)	112/(1)4 × 49	77	26	77·25	25·75	2·96	0·25	0·08
302/(1)	43 × 112/(1)8	52	22	55·50	18·50	2·51	3·50	1·39
310/(1)	112/(1)8 × 49	41	8	36·75	12·25	2·04	4·25	2·08
317/(1)	112/(1)29 × 112/(1)4	58	14	54·00	18·00	2·48	4·00	1·61
304/(1)	112/(1)29 × 43	31	11	31·50	10·50	1·89	0·50	0·26
313/(1)	112/(1)33 × 49	57	19	57·00	19·00	2·55	0·00	0·00
307/(1)	179/(1)28 × 43	36	17	39·75	13·25	2·13	3·75	1·76
Total for eight segregating families		403	136	404·25	134·75	6·78	1·25	0·18

F_1 PLANT 112/(1)23.

A full record of the behaviour of the green seedlings obtained from selfing this plant was not obtained.

Back-crossed to its parent, plant 43, twenty-nine green seedlings were obtained, one of which died (Table VII, Fam. 183/(1)). From the back-cross to the other parent, plant 49, nine green seedlings were obtained, two of which died (Table VII, Fam. 185/(1)). In this case, death appeared to be due to lack of vigour rather than to "lethal" factors.

Three F_2 plants (obtained by selfing 112/(1)23) were again selfed. A single seedling obtained in one family survived. In the second family one out of six died, while in the third five out of thirty-three failed to survive, so that although in none of these cases could death be definitely stated to be due to the lethal factors, the results must be considered indefinite.

The L_1 plant 48/(1)22 has already been shown to be of the factorial constitution **LLL₁L₁**, so that the fact that all the seedlings obtained when this was crossed with the F_1 plant 112/(1)23 survived throws no light upon the question of the factorial constitution of the latter plant.

The L_1 plant 48/(1)21 has been shown to be of constitution **LLL₁l₁**.

When this was inter-crossed with plant 112/(1)23, at least thirty-five out of fifty-two green seedlings survived, but there is no record of the remaining seventeen.

Eight of these plants from cross 48/(1)21 × 112/(1)23 were selfed, with results as shown in Table VIII.

TABLE VIII.

Families obtained by selfing plants ex 48/(1)21 × 112/(1)23. Actual results compared with expectation on a 3 : 1 basis.

	Actual		Expected				
Family	Sur-viving	Non-sur-viving	Sur-viving	Non-sur-viving	Probable error	Deviation	Dev./P.E.
188/(2)26/	134	61	146·25	48·75	4·08	12·25	3·00
188/(2)27/	20	3	17·25	5·75	1·40	2·75	1·96
188/(2)29/	154	46	150·00	50·00	4·13	4·00	0·97
188/(2)30/	85	25	82·50	27·50	3·06	2·50	0·82
188/(2)31/	10	10	15·00	5·00	1·31	5·00	3·82
188/(2)32/	18	2	15·00	5·00	1·31	3·00	2·29
188/(2)33/	62	2	48·00	16·00	2·34	14·00	5·98
188/(2)35/	61	27	66·00	22·00	2·74	5·00	1·82

The number of plants in three of these families is too small to give very reliable results, but only in one of them is there any indication of the possibility of a 9 : 7 ratio.

In one of the other families there is a considerable excess of non-surviving green, but not such as to indicate a necessarily significant deviation from the 3 : 1 ratio. Three other families give a fairly good agreement with this ratio, while the remaining family, with two non-surviving green in sixty-four, is all but certainly non-segregating.

These results, therefore, while confirming the general hypothesis of the existence of lethal factors, do not give conclusive evidence as to the factorial constitution of the F_1 plant 112/(1)23, although in general the evidence appears to indicate that it is **LLL₁L₁**.

F_1 PLANT 112/(1)28.

Only two green seedlings were obtained as the result of selfing this plant. Both of these survived (Table IV).

From the back-cross to plant 43, thirty-eight green seedlings were secured, all of which survived. Similarly, all four green seedlings from the back-cross to the other parent also survived.

Side-crosses to the sister plants, 112/(1)8, 112/(1)29 and 112/(1)33, gave a total of seventy-two seedlings, only two of which failed to survive. Since the three sister plants with which the side-cross was made are

heterozygous, LlL_1l_1, LlL_1L_1 and LLL_1l_1 respectively, the evidence is conclusive that plant 112/(1)28 is of factorial constitution LLL_1L_1.

F_1 Plant 112/(1)4.

This plant when back-crossed to the parent plant 43 gave fifty-one surviving and nineteen non-surviving green seedlings, while from the back-cross to the other parent, the corresponding figures were seventy-seven and twenty-six. The agreement with expectation on a 3 : 1 basis is in both cases excellent. In the family obtained from the side-cross to plant 112/(1)29, the agreement is not quite so satisfactory, but the deviation is not significant.

When all three families are considered together the deviation from expectation on a 3 : 1 basis is only 2·25 in 245 plants, so that agreement is very close.

These results are both interesting and important in view of the fact that plant 112/(1)4 when selfed gave surviving and non-surviving green seedlings in the ratio 828 : 860. The present results would lead to the expectation of a clear 9 : 7 ratio, so that actually the excess of non-surviving green is distinctly large. Some of this excess would be covered supposing the two pairs of factors were linked (repulsion phase), but such linkage could not account for the whole of it.

The selfing results might suggest the interaction of a third pair of factors, but this suggestion is contradicted by the back- and side-crossing results, unless we assume that a new mutation has taken place.

Until further results have been obtained, therefore, the exact factorial constitution of the F_1 plant 112/(1)4 must remain doubtful, but at the same time it is quite evident both from selfing and from back- and side-crossing that it is heterozygous for the two pairs of factors concerned with the appearance of non-surviving green seedlings, and that its factorial constitution may be written LlL_1l_1.

F_1 Plant 112/(1)8.

The F_2 family obtained when this plant was selfed showed a fair agreement with expectation calculated on a 9 : 7 basis with some excess of non-surviving green seedlings.

Back-crossed to the parent, plant 43, there was again an excess of this type of seedling as compared with expectation on a 3 : 1 basis, while the back-cross to the other parent gave a deficiency of non-surviving green.

In no case, however, is the deviation from expectation of such a

magnitude as to be probably significant, and the factorial constitution of the F_1 plant may therefore be represented as LlL_1l_1.

F_1 PLANT 112/(1)29.

In the F_2 family obtained by selfing this plant, the agreement with expectation on a 3 : 1 basis was excellent.

Back-crossed to plant 43 and side-crossed to plant 112/(1)4 it again gave surviving and non-surviving green seedlings in ratios approximately in agreement with expectation on the same basis. Since 112/(1)4 is heterozygous for both pairs of factors, this indicates that plant 112/(1)29 is heterozygous only for one pair. This is confirmed by the results obtained by back-crossing to the parent, plant 49. In this case, only one out of ninety-eight green seedlings failed to survive.

The F_1 plant 112/(1)29 is therefore evidently of constitution LlL_1L_1.

F_1 PLANT 112(1)33.

This plant when selfed gave an F_2 family of 485 green seedlings in proportions of surviving and non-surviving very closely agreeing with expectation calculated on a 3 : 1 basis.

Back-crossed to the parent, plant 43, it gave no non-surviving green in a total of ninety seedlings, while when back-crossed to the other parent, the numbers were in exact agreement with expectation calculated on a 3 : 1 basis.

This F_1 plant is therefore only heterozygous for one pair of factors concerned with the appearance of non-surviving green seedlings and its factorial constitution is in this respect similar to that of its parent, plant 48, viz. LLL_1l_1.

F_1 PLANT 179/(1)28.

This plant has only been back-crossed to one of its parents, plant 43. From this cross, a slight excess of non-surviving green was obtained.

There was also an excess of this class of seedling when the plant was selfed, but in neither case was the excess of such a magnitude as to show a significant deviation.

Since the ratio from selfing was approximately 9 : 7, the factorial constitution of this plant is probably LlL_1l_1, but this has not been fully confirmed, as results from only one back-cross have been obtained.

<p style="text-align:center">F_3 RESULTS.</p>

Nine F_2 plants derived from the F_1 plant 112/(1)38 were selfed. A full record of the behaviour of the F_3 seedlings is not available, but as far as it goes, it shows that there was a distinct loss of green seedlings between germination and the time when the seedlings were planted out.

One of the F_2 plants gave no seed from selfing, while three others gave rise to families of one, ten and eleven plants each respectively. In each of the three families some seedlings failed to survive, but the total number of seedlings in each family was too small to show the type of segregation.

The results for the remaining five F_3 families are given in Table IX.

<p style="text-align:center">TABLE IX.</p>

<p style="text-align:center">Results for five F_3 families obtained by selfing F_2 plants, with a comparison of actual numbers with expectation calculated on a 9 : 7 basis.</p>

	Actual		Expected				
Family	Sur-viving	Non-sur-viving	Sur-viving	Non-sur-viving	Probable error	Deviation	Dev./P.E.
112/(3)38/1/	60	40	56·25	43·75	3·35	3·75	1·12
112/(3)38/8/	13	9	12·38	9·62	1·57	0·62	0·39
112/(3)38/10/	40	36	42·75	33·25	2·92	2·75	0·94
112/(3)38/12/	71	67	77·63	60·37	3·93	6·63	1·69
112/(3)38/14/	73	64	77·06	59·94	3·92	4·06	1·04
	257	216	266·07	206·93	7·28	9·07	1·24

These results are remarkable in the fact that whether the families be considered individually or in the aggregate there is throughout a satisfactory agreement with expectation calculated on a 9 : 7 basis.

The number of F_2 plants for which results were obtained is, however, too small to show that amongst F_2 plants generally there is necessarily a preponderance of double-heterozygotes. It may be purely accidental that these five plants should be of this type, but there is also a possibility that such plants are more highly self-fertile.

In any case, these results again show that from the two plants, 43 and 49, each heterozygous for one pair of factors, progeny plants are produced which are heterozygous for two pairs of factors concerned with the appearance of non-surviving green seedlings.

<p style="text-align:center">SUMMARY.</p>

Various plantlets of the original plant, number 48, gave when selfed a total number of 1770 green seedlings. Of these, 1339 survived while

431 perished at about the onset of the second-leaf stage. These numbers show but a slight deviation from expectation calculated on a 3 : 1 basis.

Results supporting the hypothesis that plant 48 is heterozygous for one, and only one pair of factors concerned with the survival or non-survival of green seedlings, were obtained from L_2 and L_3 families derived from plant 48, although in some cases there was an excess of non-surviving plants.

The non-surviving green seedlings derived from plant 48 are indistinguishable from those derived from plant 43, but when the two plants were inter-crossed, only surviving green seedlings were obtained in F_1.

In F_2, at least three types of families were obtained by selfing F_1 plants. Some families showed no segregation of surviving and non-surviving green seedlings; some gave segregation of the monohybrid type, while still a third group gave results agreeing with expectation calculated on a 9 : 7 basis. In one family, for some reason not yet definitely explained, non-surviving green seedlings occurred in considerable excess of expectation even on a 9 : 7 basis. Linkage of the two pairs of factors is suggested as a possible partial explanation.

When F_1 plants were back-crossed to their parents, results strongly supporting those obtained by selfing were obtained. Results obtained by inter-crossing F_1 plants were of the same kind.

A few F_3 families obtained all showed segregation in agreement with expectation calculated on a 9 : 7 basis. This, while supporting the general results in showing that two distinct pairs of factors are concerned, does not of necessity suggest any irregularity, owing to the small number of F_2 plants involved.

The factor pair derived from plant 43, and concerned with the appearance of non-surviving green seedlings, had previously been designated **Ll**. The pair now found in plant 48 has been represented by means of the symbol L_1l_1. Plants heterozygous for both pairs of factors and giving segregating families of the type 9 : 7 are therefore of factorial constitution LlL_1l_1. Non-surviving green seedlings may be llL_1L_1, LLl_1l_1, llL_1l_1, Lll_1l_1, or lll_1l_1.