SYMBIOTIC EFFECTIVENESS IN NODULATED RED CLOVER

II. A MAJOR GENE FOR INEFFECTIVENESS IN THE HOST

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1. INTRODUCTION

RESULTS reported in the preliminary paper of this series (Nutman, 1954) indicate that the sporadic ineffective response in clover inoculated with the normally effective bacterial strain A could in some cases be attributed to hereditary factors in the host. The simple proportions of effective and ineffective plants which were found to segregate in a number of families suggest simple modes of inheritance. This paper will be concerned with the subsequent breeding history of family 81, table 2 of the previous paper. This family, which was almost completely ineffective with strain A, was derived from two unrelated ineffective plants, referred to as P_1 and P_2 below.

All progeny tests were carried out under bacteriologically controlled conditions, as previously described. Each seed was planted singly in a test-tube on an agar slope inoculated either from the stock culture of strain A or from a subculture made of this strain after plating.

During each test, periodic observations were made on nodulation and growth, and at harvest, all plants were dry weighted (except those selected for further breeding) and observations made on nodulation. Visual scoring for symbiotic effectiveness in nitrogen fixation was carried out as described in the first paper ; viz. to the following grades : 0, completely ineffective ; 1, slightly effective ; 2, intermediate ; 3, incompletely effective ; 4, normally effective.

Selected plants were removed from tube culture and potted into soil and later cross-pollinated by hand, using the technique described by Williams (1925). Harvesting was done by hand and records were maintained of the numbers of florets pollinated and of the set obtained.

2. BREEDING EXPERIMENTS

The parent plants P_1 and P_2 were selected in 1941 from seed of different origin ; P_1 from the progeny of a naturally outcrossed red clover seedling and P_2 from a sample of late flowering Montgomeryshire red clover obtained from Messrs Sutton. Both were wholly

ineffective in their response with strain A and had large numbers of small nodules on their roots ; 68 for P_1 and 66 for P_2, compared with average numbers of nodules on effective counterparts of 20 and 28 respectively.

The results of breeding experiments with family 81 ($P_1 \times P_2$) and derivatives are set out in table 1 which shows the harvest grading of each family or group of families with strain A as inoculum throughout. In determining ratios of ineffective to effective plants in segregating families, half of the plants in grade 1 were scored as effectives and half as ineffectives (Nutman, 1954, p. 41). The data presented in this table are somewhat diverse because half-sister plants of P_1 were available for breeding tests. These comprised normally effective plants (S_1 and S_2) and un-nodulated resistant plants (R_1 and R_2) previously described (Nutman, 1949). Direct and reciprocal crosses are not separated in the table since no consistent maternal or paternal effects were found.

The results clearly indicate that the ineffective response in P_1 and P_2 is due to a simple Mendelian recessive, which is, however, subject to modification by other hereditary host influences.

Crosses among plants derived directly from P_1 and P_2, comprising a single self, two backcrosses and ten sib crosses, are shown in section 1 (fams. 81-104). These families are largely but not wholly ineffective ; the proportion of effectives is high in the backcrosses (approx. 1 : 1) and low in the sib crosses. The occurrence of an appreciable proportion of effectives in these families suggest a modification of the activity of the simple recessive, about which further data is given below.

The two half-sister resistant plants of P_1 (R_1 and R_2) with which P_1 and P_2 were crossed, did not form nodules, so that their response could not be determined. Genotypically, however, like their effective half-sisters, S_1 and S_2, the probability of their being heterozygous is high. This view is supported by the results of crosses between resistants and ineffectives : they segregated approximately equal numbers of effectives and ineffectives in two of the three families examined (fams. 105, 106), the third family (fam. 107) giving an excess of ineffectives (χ^2 3·68*).

This analysis is confirmed by the response of the progeny of crosses (i) among resistant outcross effectives (fams. 108-112), (ii) among effective segregants of families 105-106 (fams. 113-114), and (iii) between one of the effective segregants and its resistant parent (fam. 115). All of these gave one quarter ineffectives. The single cross between unlike segregants of family 105 (fam. 116) and the backcross of an ineffective to its resistant parent (fam. 117) also gave the anticipated equal numbers of effectives and ineffectives, the ineffective segregants breeding true (fams. 118, 119).

The response of resistant plants which segregated in some of these families could not be recorded, since no nodules were formed. The

TABLE 1

Responses of families derived from the original ineffective selections P_1 and P_2 (Inoculated strain A only)

Family number	Cross type P_1 and P_2 original ineffective selections R_1 and R_2 resistant sisters of P_1 S_1 and S_2 effective sisters of P_1	Grading of parents 4 = effective 0 = ineffective	Grading of progeny					Observed corrected ratios	Expected ratios	χ^2
			0	1	2	3	4			
I. 81	$P_1 \times P_2$ †	0×0	26	1	...	...	...	...	...	...
92	P_1 self	0×0	1	...	...	...	...	...	...	...
93, 94	$P_1 \times (P_1 \times P_2)$	0×0	3	1	1	...	1	...	...	...
95-104	$(P_1 \times P_2) \times (P_1 \times P_2)$	0×0	97	3	1	1	4	...	...	...
II. 105-107	$P_1 \times R_1$, $P_1 \times R_2$, $P_2 \times R_2$	0×R	60	8	2	8	34	64 : 48	1 : 1	5·32
108-115	R_2 outcrosses *inter se*; $(P_1 \times R_1) \times (P_1 \times R_1)$; $R_2 \times (P_2 \times R_2)$	4×4 or R×4	25	11	5	16	75	30·5 : 101·5	1 : 3	2·96
116, 117	$(P_1 \times R_1) \times (P_1 \times R_1)$; $R_2 \times (P_2 \times R_2)$	4×0 or R×0	17	3	3	2	11	18·5 : 17·5	1 : 1	2·95
118, 119	$(P_1 \times R_1) \times (P_1 \times R_1)$	0×0	36	...	...	1	...	...	...	...
III. 120, 121	Outcrosses of S_1 and S_2	4×4	...	...	...	2	62	...	...	...
122-124	S_1 and S_2 outcrosses *inter se*; $S_1 \times S_2$	4×4	41	8	17	16	73	45 : 110	1 : 3	2·33
125-132	S_1 and S_2 outcrosses *inter se*	4×4	1	3	14	41	44	...	...	...
IV. 133-136	Outcrosses of P_1 and P_2	0×4	...	1	4	16	106	...	...	...
137-146	P_1 and P_2 outcrosses *inter se* (F_2)	4×4	26	1	7	30	71	26·5 : 108·5	1 : 3	15·75*
147, 148	Ineffective segregants from families 128 and 130 *inter se*	0×0	61	6	1	...	...	...	...	...
V. 149, 150	Effective recessive plant (from family 93) crossed with ineffective recessives of family 81 (see also table 6)	4×0	10	3	3	...	12	11·5 : 16·5	1 : 1	0·59

† Excluding expt 2 (see table 3). Significant deviations from expectation : * P = 0·05.
Yates correction applied to small families.

presence of resistants, however, caused no disturbance in the genetic ratios. The factors concerned in the inheritance of effectiveness and resistance may therefore be considered to be independent (see also Nutman, 1949 ; table 10, p. 277).

Section III lists crosses in which two effective half-sister plants of P_1 are involved. These, like their resistant sisters, are also evidently heterozygous for the ineffective gene. On outcrossing, wholly effective families are obtained (fams. 120, 121) whereas in F_2 and in the $S_1 \times S_2$ cross (fams. 122-124) the expected proportion (one-quarter) of families segregate ineffectives, the remainder being effective (fams. 125-132).

Section IV shows that the response of outcrosses of P_1 or P_2 to unrelated effectives (fams. 133-136) is wholly effective. The expected proportion of ineffectives did not, however, occur in all the families tested in F_2 (fams. 137-146). The values for χ^2 for the five groups of sib crosses into which these families could be divided were 3·26*, 1·59, 2·45, 1·03, 5·27*, a significant deficiency of ineffectives occurring in the last group and a significant excess in the first (consisting of one family). The ineffective segregants from two of these families bred nearly true to type (fams. 147-148).

In the material as a whole the evidence thus strongly favours the hypothesis of single gene control for ineffectiveness in the host. This gene will be designated i_1 and its dominant allele I_1.

In a minority of families there are disturbing host genetic factors affecting the simple ratios of types (*viz.* fams. 93, 94, some of fams. 95-104, fam. 107, and possibly also fam. 146). These aberrations were further investigated by crossing ineffectives of family 81 with the anomalously effective plant of family 93 (fams. 149, 150). Equal numbers of effectives and ineffectives appeared in these families suggesting that the anomalously effective parent was itself homozygous for a modifying recessive factor (provisionally designated m_1) which prevents the development of the ineffective response in $i_1\,i_1$ plants. On this view $i_1\,i_1\,M_1\,M_1$ or $i_1\,i_1\,M_1\,m_1$ will be ineffective and $i_1\,i_1\,m_1\,m_1$ effective. Further work is required to test this hypothesis.

3. DESCRIPTION OF PLANTS HOMOZYGOUS FOR i_1

The response of $i_1\,i_1$ plants with the otherwise effective bacterial strain A is typically ineffective and cannot be distinguished phenotypically from the response of unselected red clover in symbiosis with ineffective strains of bacteria such as strains HKC or *f*12 described in the previous paper. In both, the nodules tend to be small and numerous.

In the absence of combined nitrogen in the root medium the plants remain dwarf and N-starved although they continue to nodulate freely for some time. No increase in dry weight takes place during the test (100 days) so that at the end of this period the average dry

weight of an ineffective plant will be 15 mgm. compared with about 80 mgm. for an effective plant. Plate 1 illustrates these differences ; fig. *a* shows appearance of the recessive $i_1\,i_1$, and fig. *b* the heterozygote ; both inoculated with strain A and segregating in the same family of plants (fam. 108).

4. BACTERIAL STRAIN SPECIFICITY RESPECTING i_1

Because of the similarity of the response of the i_1 line with strain A to that of unselected clover with ineffective strains of bacteria, it is of interest to examine the response of this line with bacterial strains which vary in their effectiveness with unselected clover. This was

TABLE 2

Response of the recessive line ($i_1\,i_1$) and unselected red clover with different strains of nodule bacteria

Bacterial strain	Recessive line						Unselected clover					
	Numbers of plants in each grade					Mean dry weight mgm.	Numbers of plants in each grade					Mean dry weight mgm.
	0	1	2	3	4		0	1	2	3	4	
Experiment 1												
A	12	...	...	...	...	...	...	1	...	1	12	...
2057	...	...	...	1	9	...	...	...	...	2	4	...
Haldon 1	...	...	1	...	7	...	1	...	...	...	3	...
Hannay 7	...	...	...	...	5	...	...	...	...	...	5	...
Clover C1	...	...	...	...	6	...	...	...	...	...	6	...
Experiment 2												
A	25	2	...	1	1	18·9	...	...	3	...	3	34·0
37	13	...	...	...	...	16·0	4	...	...	...	...	17·5
S4	4	4	3	2	...	20·1	...	...	1	2	3	23·7
42	2	1	1	2	...	22·5	8	2	2	...	2	22·0
39B	1	1	2	6	2	27·5	...	...	1	4	...	25·0
Sf2	...	...	...	4	3	36·9	...	...	...	1	5	37·4
91S2f	...	2	...	...	7	39·6	...	...	...	1	5	31·1
49	...	...	...	...	12	42·2	...	...	...	3	3	35·5

done in two experiments summarised in table 2 in terms of grading and plant dry weight. The results show that the ineffective response of the recessive line is quite specific to strain A. The response of the recessive line with other strains of bacteria of varying degrees of effectiveness with unselected clover is that appropriate to the strain. Within the limits of sampling error this relationship holds in detail for the eleven additional strains of bacteria studied ; the strains falling into the same order of increasing dry weight in experiment 2 with each kind of plant material.

5. BACTERIAL STRAIN VARIATION

Previous studies on the stability of strain A (Nutman, 1946) showed that mutations influencing its effectiveness with unselected host material occur very rarely but when they do so they may sometimes be recognised by the larger number of smaller nodules produced. Conversely a change from ineffectiveness to effectiveness was shown by the production of larger nodules with the variant strain. Using this criterion it has been possible to detect and isolate variants of strain A which respond effectively with the recessive line; details are set out in tables 3, 4, 5 and 6.

TABLE 3

Distribution of response in recessive line (family 81 only) inoculated with re-isolates of strain A

Origin of re-isolate	Name of re-isolate	Experiment	No. of plants in each grade				
			0	1	2	3	4
Stock culture controls . .	Strain A	1, Feb. 1943	10	...	...	...	...
		2, June 1943	1	...	...	1	4
		3, Nov. 1943	12	...	...	...	...
		4, Apl. 1944	4	1	...	...	...
Plant passage re-isolates—							
(i) From abnormally large nodules on ineffective plants	A2	2	...	...	1	1	1
	A3	2	...	...	...	1	2
(ii) From effective plants	A21	3	...	...	...	...	6
	A21	4	...	...	1	...	4
	A31	3	...	...	...	1	5
	A211	4	1	1	...	...	5
(iii) From small nodules on ineffective plants	A4	2	3	...	...	...	...
	A5	2	3	...	...	...	...
	A42	3	5	1	...	...	...
	A42	4	5	...	...	...	...
	A52	3	6	...	...	...	...
	A421	4	10	...	...	...	...

Re-isolates of the bacteria from nodules were numbered systematically, a digit being added to the parent strain number of re-isolation. Thus re-isolate A211 was taken from a nodule on a plant inoculated with re-isolate A21 which in turn derived from re-isolate A2 and strain A.

In experiments 1-4 (table 3) the recessive plant line used in tests of various re-isolates consisted of the original ineffective family $P_1 \times P_2$ (fam. 81, table 1) only. The response of unselected clover with each re-isolate was found to be normally effective and is not shown on the table.

At the first test of family 81 in February 1943, a wholly ineffective response was obtained, control clover plants giving the normal effective response. At harvest all the nodules on all the plants were measured with the results shown in table 4. Two of the plants on the recessive line had one large nodule each, whereas the remainder bore nodules of a small size only ; mean length about 0·6 mm. On unselected plants there were fewer small nodules below 1·0 mm. and a much higher proportion of nodules larger than 1·0 mm. ; the mean nodule length on individual plants ranged from 1·0 to 1·5 mm.

The two large nodules on plants 1 and 2 were of normal effective appearance and firm in texture and measured 2·2 and 3·6 mm. in

TABLE 4

Distribution of nodule length on plants of the recessive line and on unselected red clover

	Number nodules per plant	Frequency distribution (per cent.) of nodules in each size group			
		0·0-0·9 mm.	1-1·4 mm.	1·5-1·9 mm.	above 2 mm.
Recessive line—					
Plant 1 . . .	123	92·7	6·5	...	0·8
Plant 2 . . .	111	93·7	3·6	1·8	0·9
Mean remainder . .	105	99·4	0·6	...	...
Unselected clover . .	46	63·1	22·9	11·0	3·0

length and from these the re-isolates A2 and A3 were obtained. From small nodules on the same plants, each measuring 0·5 mm. in length, the re-isolates A4 and A5 were also obtained. These were examined in experiment 2 and showed that a dissociation of strain A had taken place, the large nodule isolates giving an effective response with the recessive line and the small nodule isolates retaining the parental effective response of strain A. At this test the stock culture (which was not plated before testing) responded, however, in a largely effective manner with the recessive line. This suggests that dissociation had in fact taken place in the stock culture some time previous to experiment 1, the dissociant form having increased relative to the parent form in the interval between experiments. This possibility was examined by plating strain A and picking single colonies which were then tested in experiment 3. This test showed that the plated stock culture had now retained its completely ineffective response (for this reason the response of the recessive line in test 2 is omitted from table 1 since bacterial strain variation is here also involved).

Subsequently strain A has been plated before carrying out each group of experiments.

The stability of the variant strains A2 and A3 was further examined by successive re-isolations at random from effective plants to give the plant passage isolates A21, A31 and A211. The responses of these new re-isolates were tested on the same family of recessive line plants in experiments 3 and 4, and were found to be completely effective. The effective response of the A2 substrain was thus retained during plant passage and in subculture outside the plant. At each experiment re-isolates were also taken at random from small nodules on ineffective plants. These re-isolates, numbered A42, A52, A421 in the table,

TABLE 5

Distribution of response in various recessive line families inoculated with strain A and re-isolates A211 and A421

Experiments and dates	Plant families used in tests	Distributions with														
		Strain A					Re-isolate A211					Re-isolate A421				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
5 and 6, 8/46-4/48	Backcrosses fams. 93, 94	3	1	1	...	1	...	...	1	2	5	15	...	1	2	2
7-13, 1/45-7/52	Sib crosses fams. 95, 104	60	1	1	...	3	2	4	7	...	18	49	6	6	4	4
14, 15, 2/52-7/52	Recombined fam. 147	21	...	...	...	...	22	1	1	...	...	19	4	1	...	..
16, 1/53	Sib crosses fams. 95, 96	7	...	...	...	...	...	1	2	3	13	...	...	...	...	..
,,	Recombined fam. 147	5	...	...	...	...	4	5	1	...	...	5	...	...	...	..
,,	fam. 148	5	...	...	...	...	...	...	1	2	5	4	...	...	...	..

gave rise to wholly ineffective responses in family 81 and were thus indistinguishable from strain A.

Further tests of some of these re-isolates were carried out with plant material derived from family 81. These are shown in table 5. In experiments 5 and 6 backcross families were used (fams. 93, 94, table 1). In experiments 7-13 and part of 16, tests were made of sib crosses (fams. 95-104, table 1), and in experiments 14, 15 and part of 16, families were tested in which the homozygote $i_1\ i_1$ was recombined after outcrossing (fams. 147, 148, table 1).

The results of these further tests differed from those found for family 81. With backcross families the stock culture and A421 gave rise to small proportions of effective plants instead of responding wholly ineffectively. This incompletely ineffective response, however, has been noted already for these backcrosses in table 1, and was attributed to the action of modifying factors in the host ; the aberrant effectives

are probably due here to the same cause. Later experiments (7-13) undertaken with sib families in which the effect of modifying factors are less marked showed that no further change in A421 had taken place, this strain continuing to produce a largely ineffective response and the substrain A211 a largely effective response.

In experiments 14 and 15 tests were carried out on a reconstituted recessive line following outcrossing (fam. 147, table 1), and this gave completely ineffective responses throughout even with substrain A211. This change in the response of recessive line with A211 was unlikely to be due to a reversion in A211 since experiment 14 was carried out before experiments 12 and 13, and may be attributed to the use of a recombined recessive in place of one derived directly from the original parent plants. A subsequent test (expt. 16) showed that no further change had taken place in strain A211 which continued to give an effective response with the sib families 95, 96, and an ineffective response with the recombined recessive family 147, as in the previous experiment.

A closely related recombined recessive, family 148, tested at the same time gave, however, effective symbiosis with substrain A211. This family was derived from a single outcross whereas in family 147 the recessive i_1 gene was recombined from two outcrosses.

It would therefore appear that the interactions between bacteria and host may involve (i) a factor common to all A re-isolates reacting with i_1 gene, and (ii) a factor specific to strain A211 interacting with another host factor (or factors) which is lost to the i_1 line by outcrossing.

A number of further experiments were carried out in which isolations were made from large and small nodules appearing on plants of the recessive line, all inoculated with A421 or derivatives; the results are summarised in table 6. All tests of re-isolates were made with direct derivative families of the original ineffective cross without outcrossing (fams. 81-104 only).

Isolations were taken from large and small nodules on plants of three kinds; (i) ineffectively responding plants showing a markedly discontinuous distribution of nodule size, *viz.* plants with one or two large nodules and very many small ones and no nodules of intermediate size, (ii*a*) ineffective plants on which there were a number of large nodules and nodules of medium size scattered at random on the root system as well as small ones, (ii*b*) ineffective plants which produced larger nodules on the younger parts of the root; and (iii) effectively responding plants of the recessive line with normal effective nodulation. The ineffective plants from which these isolations were made were from families 81-104 and the effectives from families 94-104.

Although no isolate was obtained similar to A211 in giving a completely effective response with the recessive line, a number of those isolated were of appreciably greater effectiveness than strain A

or the parental isolate A421 tested at the same time (table 5, experiments 7-13): *viz.* re-isolates A421641, A421643, A4211, A4212, A4219, A421, 10, A421, 11 and A421, 12. These gave rise to about equal numbers of effective and ineffective symbiosis, but their greater

TABLE 6

Effectiveness of further re-isolates of strain A with plants homozygous for i_1

Re-isolates taken from nodules on plants of families 81-104 and tested on the same families. Details given only for experiments in which re-isolates differed from strain A or from parallel re-isolates.

Name of re-isolate	Date of isolation	Size of nodule	Numbers of plants in each response grade				
			0	1	2	3	4
A . . .	...	...	127	5	2	1	5
I. *Re-isolates from nodules of two distinct sizes on ineffective plants*							
A421641 . .	9/48	large	10	4	7	4	4
A421642 . .	,,	,,	22	....	...	...	...
A421643 . .	,,	small	6	1	2	4	3
Negative results with 12 re-isolates . . .			60	3	2	3	1
IIa. *Re-isolates from large nodules on younger roots of ineffective plants*							
Negative results with 2 re-isolates . . .			9	4	...	...	...
IIb. *Re-isolates from ineffective plants with nodules of all sizes*							
Negative results with 5 re-isolates . . .			18	4	5	...	...
III. *Re-isolates from nodules on effective plants*							
A4211 . .	8/46	large	3	1	...	...	2
A4212 . .	,,	,,	4	...	...	...	2
A4219 . .	4/47	,,	3	3	3	1	...
A421, 10 . .	,,	,,	1	2	3	2	...
A4213 . .	8/46	small	15	...	...	...	1
A4214 . .	,,	,,	6	...	...	...	...
A421, 11 . .	4/47	,,	1	...	6	...	2
A421, 12 . .	,,	,,	3	1	1	2	1
Negative results with 4 re-isolates . . .			33	4	1	...	...

effectiveness was not related in any consistent way to the size of nodule from which they were isolated (with the possible exceptions of A421, 11 and A421, 12) or to the host's response. Thus, while the large nodule isolate A421641 was of greater effectiveness than A, so also was the small nodule isolate A421643, whereas the companion

large nodule isolate A421642 was wholly ineffective. The absence of any exclusive influence of host response on bacterial strain variation is shown by the scattered distribution of the variant strains in the table, though they are most frequent among isolates from effective plants.

TABLE 7

Effectiveness of A substrains on segregating lines of host and on homozygous i_1 lines after outcrossing

Family type	Distribution of response with strains indicated														
	A					A421, 9 and A421, 10					A421, 11 and A421, 12				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
(i) Anomalous effectives in recessive line × ineffective parent: fams. 149, 150	10	3	3	...	12	...	1	...	...	6	...	1	1	1	4
	A					A421					A421643				
(ii) Sib crosses of F_1 effectives after outcrossing															
Group 1, fams. 137 to 142	15	...	3	11	55	23	1	1	16	37	1	5	6	22	42
Group 2, fams. 143, 146	2	...	1	11	14	8	4	3	7	9	5	6	6	8	6
	A					A421					A421643				
(iii) Sib crosses of F_2 ineffectives after outcrossing															
fam. 147 (derived from group 1 above)	20	1	...	...	...	21	2	...	...	...	17	3	1	...	1
fam. 148 (derived from group 2 above)	5	...	...	...	...	5	...	...	...	...	1	1	2	1	1

Material of the recessive line which had not been outcrossed was becoming exhausted at this stage, so that for the further examination of these isolates recourse was made to segregating families and to recombined recessives after outcrossing. The segregating families were of two kinds; those in which the effective response was due to the modifying factor m_1 (fams. 149, 150) and F_2 segregating families after outcrossing (fams. 137-146). The results of these tests are shown in table 7. They confirm the greater effectiveness of the substrains A421, 9, 10, 11, and 12 and A421643 compared with the ancestral strains A and A421.

With strain A each group of families gave the expected proportions of effectives and ineffectives ; those in section (i) segregating 1 : 1 and those in the section (ii) 1 : 3 (approx.) and those in the third section breeding true for ineffectiveness. With the new isolates, however, these ratios no longer held for some of the families, *viz.* for those in section (i) and the first group of sib crosses in section (ii) ; all of which responded in a largely effective manner. Group 2 families in section (ii) and the homozygote family 147 of section (iii), however, were not more effective with A421643 than with A or A421. These differences between families suggest that other host factors may also be concerned in the restoration of the effective response in the recessive line. The families in group 2, section (ii) (fams. 143-146) had already been noted above in table 1 to give rise to a *deficiency of ineffectives* with strain A which is statistically significant.

In this connection it is significant that each of the families 137-142 in group 1 of section (ii) of table 7 was derived from a pair of different outcrosses, whereas those in group 2 were in each case derived from a single outcross. Families 143-146 would therefore be genetically less heterogeneous than families 137-142. The different behaviour of these families with respect to substrain A421643 may be due to a factor contributed by the common outcross grandparent.

The largely effective response of the recombined family 148 with substrain A421643 and the failure to confirm a higher level of effectiveness in family 147 with this strain exactly parallels the results already found with these families for A211 (table 5). The effective response of i_1 plants with these strains depends upon the same combination of host factors.

The data is not yet extensive enough to determine the nature of these supplementary host factors, but the relatively simple proportions of effectives and ineffectives often found with these mutant strains (*e.g.* in tables 3 and 6) suggest that simple host factors may be concerned. It is, at any rate, clear that the modification of the ineffective host's response occasioned by specific mutation in the bacteria is not concerned solely with the primary gene i_1.

6. DISCUSSION

The investigation of a Mendelian recessive (i_1) associated with a completely ineffective response in clover inoculated with an otherwise effective strain of bacteria (strain A) has revealed a number of host-bacterial interactions of some interest.

With effective strains of bacteria other than strain A, the recessive i_1 line gives a normally effective response which is indistinguishable from the response of unselected material with these strains. The specific host-bacterial interaction leading to ineffectiveness in the i_1 line with strain A can be represented as $i_1\, i_1$/A where A denotes the bacterial strain factor involved (contributed by strain A), factors

from other strains (*e.g.* B) interacting with i_1 to give an effective symbiosis ($i_1\, i_1$/B).

There is also evidence from breeding experiments (table 1) that a simple modifying host factor (m_1) affects the response with the parent bacterial strain A so that the association of host and bacteria represented by $i_1\, i_1$, $m_1\, m_1$/A is effective, whereas $i_1\, i_1$, $M_1\, m_1$/A and $i_1\, i_1$, $M_1\, M_1$/A retain the ineffective response.

Two kinds of bacterial variant have been described. The first of these (substrain A211, table 3) restores the response of symbiosis of the constitution $i_1\, i_1$/A to complete effectiveness, resembling in phenotypic effect the combination of factors $i_1\, i_1$/B above, although it is associated here with variation in the bacterial strain. This change in effectiveness, which may be a result of a simple mutation in the bacteria (*e.g.* from A to C) appears also to be influenced by inherited factors in the host since it may no longer be detected with reconstituted i_1 ineffectives following outcross (table 5, family 147). Denoting these modifying host factors by N, the restored ineffective condition may be due to the following factor combination : $i_1\, i_1$, N/C.

The second type of bacterial variation indicated by the results given in table 6 is associated with the restoration of the effective response in a proportion of recessive plants only. This result again suggests that modifying plant factors O are concerned in such a way that the factor combination $i_1\, i_1$, O/D is effective.

These results can therefore all be ascribed to the combined influence of a primary gene and of modifying factors in the host interacting with hereditable bacterial factors. It is possible that each factor or group of factors in the plant interact with corresponding factors in the bacteria.

This tentative hypothesis is intended only as a pointer to future work. There is very little data on which to interrelate these factors or to determine whether they are simple or complex, dominant or recessive. The available evidence is not at variance with the view that they may be simple.

Nor is it intended, on the basis of these results, to discuss the mechanism of the genetic control of effectiveness in symbiosis. From some of these results an attempt has been made to elaborate a dynamic scheme for these interactions (Nutman, 1951), this will be revised at a later stage in these studies.

7. SUMMARY

1. Clover plants with a simple recessive host factor, i_1, show a completely ineffective response when inoculated with the normally effective bacterial strain A.

2. Plants homozygous for i_1 give an effective response with other effective strains of bacteria unrelated to strain A as well as with a stable variant of strain A (substrain A211).

3. Other bacterial variants of strain A were isolated which give rise to mixed responses in i_1 homozygotes.

4. A recessive suppressor m_1, is also inferred which restores $i_1 i_1$ plants to complete effectiveness with strain A.

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