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VARIATION IN WILD POPULATIONS OF *PAPAVER DUBIUM*

X. GENOTYPE-ENVIRONMENT INTERACTION ASSOCIATED WITH DIFFERENCES IN SOIL

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SUMMARY

The effect of soil nutrients on the expression of 11 metrical traits in 20 inbred lines of *Papaver dubium* was studied. Plants were raised on 16 different combinations of four fertilisers (Ca, N, P and K) and also on the experimental field. All four nutrients had a significant effect on all traits studied, but Ca had much the greatest single effect. All the nutrients enhanced rate of development. A linear relationship between genotype-environment interaction and environmental mean was found for all traits; in some cases a non-linear effect was also detected. Although all traits showed significant genotype-environment interaction, five proved to be fairly repeatable over environments and three fairly repeatable except on very poor soil. These results probably hold for the natural habitat, since the range of soil variation encountered by the species in nature is unlikely to exceed the range used in the present experiment. Three traits, including capsule number, showed poor repeatability; in such cases, relative fitnesses of different genotypes will probably change very markedly with changes in the natural environment. The fittest genotypes with respect to capsule number will be those showing marked phenotypic flexibility.

1. INTRODUCTION

GENETICAL variation for metrical characters within natural populations of *Papaver dubium* is very common (Lawrence, 1965, 1972; Gale and Arthur, 1972; Gale and Eaves, 1972). Any attempt to account for this variation must take account of possible genotype-environment interaction. Consider, for example, a component of fitness always under directional selection at the *phenotypic* level, capsule number; the plant with the highest number of capsules is the fittest. If, however, genotype *A* gives more capsules than genotype *B* in one environment, whereas the reverse holds in a different environment encountered by the same population (*e.g.* in a different year), directional selection at the *phenotypic* level could give rise to fluctuating selection at the *genotypic* level.

In practice, members of the same interbreeding population of *P. dubium* will encounter very different environments. The contrast between conditions in an arable field and the adjoining roadside verge is very marked, as is that between a disused railway track and the bank a few metres away. Variation over years in rainfall at critical periods of development implies, almost certainly, a marked fluctuation of the effects of the environment from one year to another.

Clearly, it is easiest to work with traits which show little genotype-environment interaction; since if repeatability over environments is poor, the population genetics of the trait will be very complicated. Moreover, we should like to make inferences from the performance of genotypes on the experimental field (where measurements are readily made) to performance in nature (where measurements are difficult). Such inferences are possible only for traits where genotype-environment interaction is minimal.

Although previous work (Gale and Arthur, 1972; Bradshaw, personal communication) on a small scale for a very small number of environments suggested that, for some traits at least, genotype-environment interaction does not lead to serious problems, a more detailed study involving a much more extensive series of environments roughly representing the wide heterogeneity of the natural habitat is necessary before any general conclusion can be reached about the importance of genotype-environment interaction in this species. In this paper we shall describe an investigation into the effects of difference of soil on the expression of 11 metrical characters in 20 inbred lines.

2. Materials and methods

The 20 inbred lines (S_8) used in the study were derived from five wild populations (Wellesbourne, Luddington, Welford, Blakedown and University Campus) in the West Midlands area. The lines were selected in such a way that for every character the two lowest and the two highest performing lines (on the experimental field) as well as intermediates were included, thus giving a wide range of variation. Plants were raised initially in the glasshouse and planted out at seven weeks after sowing. The major part of the experiment was carried out at Winterbourne, the experimental field of the Department of Botany, by courtesy of Professor J. G. Hawkes. The experimental area used there consists of 16 plots, comprising the 16 combinations of presence or absence of calcium (Ca), nitrogen (N), phosphorus (P) and potassium (K). These plots have been subjected to the same fertiliser treatments for over 30 years. These 16 combinations of fertiliser treatments are expected to represent a very heterogeneous set of soil conditions. Four sibs of each of the 20 lines were grown in each plot. In addition, four sibs from each line were raised on the experimental field of the Genetics Department (about half a mile from Winterbourne), which field represents the habitat used for most earlier poppy experiments. Thus we have 17 " environments " in all. Within each of the 17 environments, the 80 plants were individually allocated to positions at random. Plants on the experimental field were planted 1 foot apart; owing to shortage of space, this had to be reduced to 9 inches on the plots. In either locality plants were surrounded by guard plants and netted to avoid bird damage.

Eleven metrical traits were scored. The measurements of leaf number, plant height and plant diameter at 10 weeks (*i.e.* three weeks after planting) will be denoted LN10, H10 and D10 respectively. By this stage, differences between plots were very conspicuous. The traits scored at flowering time (day of opening of the first flower) were flowering time (FT) in days, flower diameter (FD), stem height (SHF), total height (HF) and number of buds (BF). In some cases (mainly on the limed plots and Genetics field) the potential first flower aborted. In such cases, the first flower actually to open

was taken as the first flower. All measurements were made in millimetres. Finally, when almost all plants had ceased growth in mid-September, the number of stigmatic rays on the three topmost capsules (SR), capsule number (CN) and final height (FH) were scored. Forty-seven plants had died before the time of scoring of these final characters. Most of the deaths occurred in calcium-deficient plots, where all plants suffered extensive damage from drought (table 1). Some other features of the plots, recorded in this table, will be discussed later.

TABLE 1

Deaths, abnormalities and other unusual features

Environments				Deaths	Very sick	Plants with single capsules	Plants flowering after (*a*) Day 40	Plants flowering after (*b*) Day 50	First flower aborted
Ca	N	P	K	0	0	0	12	0	24
Ca	N	P		2	1	0	6	0	2
Ca	N		K	1	0	0	7	0	20
Ca	N			1	0	0	17	1	4
Ca		P	K	1	0	0	14	4	12
Ca		P		5	5	0	15	3	7
Ca			K	0	0	0	18	5	14
Ca				5	0	0	15	4	8
	N	P	K	2	5	8	19	8	0
	N	P		4	2	4	18	9	0
	N		K	3	1	16	11	7	0
	N			10	8	17	31	16	2
		P	K	6	0	0	20	5	1
		P		0	0	0	21	6	2
			K	2	0	1	24	8	0
Control				2	0	0	26	8	0
Genetics field				2	0	0	14	0	13

3. RELATIVE IMPORTANCE OF DIFFERENT NUTRIENTS

Our data were analysed by the usual analysis of variance in a two-way classification involving replication within cells; both lines and environments were regarded as fixed (model 1). Full details of these (and other analyses discussed below) are available on request. For all 11 traits, between lines, between environments and lines × environments items were all highly significant. The environments sum of squares was then partitioned into items measuring the effects of Ca, N, P, K and all their possible interactions. Perhaps more important than the significance levels are the estimates of the magnitudes of the various effects. These are given, for main effects and first-order interactions, in table 2. For example, the Ca main effect, for a given character, is the mean performance of all plants receiving Ca less the mean performance of those not receiving Ca. The Ca × N interaction is the mean performance of plants receiving both Ca and N, less the mean of those receiving Ca but no N, less the mean of those receiving N but no Ca, plus the mean of those receiving neither Ca nor N. All effects tabulated are significant at the 5 per cent level, apart from the few non-significant results given in parentheses. We have not attempted to tabulate higher order interactions for the following reason. In that, for practical reasons, the various types

of plot could not be replicated, any differences between plots reflecting their physical position rather than soil content will tend to appear as a higher order interaction. In practice, these higher order interactions, although often statistically significant, were usually relatively small in magnitude. We may note in passing that while differences in position between plots may slightly bias our estimates of the effects of nutrients, they are of no consequence in relation to the main aim of the experiment, the study of genotype-environment interaction, for which it matters little whether the differences in our environments due to soil differences are slightly augmented by differences in position.

TABLE 2

Estimates of important nutrient effects

Characters	Ca	N	P	K	Ca×N	Ca×P	Ca×K	N×P	N×K	P×K
LN10	6·0	2·6	3·4	0·7	3·1	0·8	(0·1)	1·1	0·5	(0·4)
H10	−10·8	(−0·7)	3·0	2·5	8·8	(0·8)	1·4	1·4	(0·5)	1·2
D10	100·3	14·5	16·6	12·7	27·8	(1·6)	12·4	−8·4	−3·4	8·4
FT	−2·6	−1·2	−1·2	−1·2	−0·5	(0·4)	0·7	(0·2)	−0·7	(0·2)
FD	11·2	−4·5	1·8	(−0·5)	4·9	−2·3	1·4	(−0·3)	1·2	−1·2
SHF	57·4	(0·3)	(0·6)	24·2	24·7	(−1·8)	27·2	(2·6)	20·5	(3·0)
HF	212·6	−28·8	13·1	29·1	76·4	−21·7	35·9	(−4·8)	25·6	5·6
BF	5·9	1·2	0·8	1·9	2·5	(0·0)	2·0	(−0·2)	1·6	0·5
SR	1·9	−1·0	0·5	0·3	0·9	−0·6	(0·1)	(−0·15)	0·18	(−0·1)
CN	25·0	5·1	3·2	−1·8	16·2	(−0·6)	(−0·8)	(−0·1)	−1·1	1·5
FH	397·8	−43·7	28·6	11·5	174·7	−30·9	30·2	(−6·9)	19·9	15·1

Figures in parentheses are non-significant at the 5 per cent level.

Considering first the plots only, it is evident from the results that calcium had much the largest single effect on the expression of all characters. Though it is very difficult to separate the effects of calcium *per se*, soil *p*H and other correlated factors associated with both acid and calcareous soil, it was evident from our results that calcium deficient plots provided very poor growth conditions for the plants (see also table 1).

Nitrogen, phosphorus and potassium also had significant effects on most of the characters, although these were much smaller than the calcium effect. With occasional exceptions, all four nutrients when present increased mean performance of morphological traits and accelerated flowering. Data on LN10, D10 and FT indicates that all nutrients speeded up growth and development when present (results on height are best ignored here, in view of the complicated pattern of development shown by this character). Among the interaction effects, Ca × N is much the most important. Clearly, we have secured a wide range of environments, giving ample opportunity for manifestation of genotype-environment interaction, if present.

Finally, we consider briefly growth on the experimental field. The comparison orthogonal to those given in table 2 is " genetics field versus all other environments ", but this is not particularly helpful. In that the field has been limed on several past occasions, it would be expected that performance on the field would be roughly comparable with that on the limed plots, and this in fact occurred. The most interesting comparison is between

mean performance on the field and mean performance on the CaNPK plot, which turned out to be:

LN10 – 4·0, H10 – 9·6, D10 – 52·7, FT 0·9, FD 3·1, SHF – 41·3, HF – 77·8, BF 3·5, SR 0·4, CN 41·5, FH 23·9.

All of these are statistically significant. The results indicate the complexity of the action of the environment; vegetative growth was slower on the field earlier in the season, yet final height was greater. Reproductive growth, represented by BF and CN was greater on the field.

4. Statistical analysis of genotype-environment interaction

Two methods are available for the analysis of genotype-environment interaction. In the first, which is a simple extension of the method used earlier for *P. dubium* (Gale and Arthur, 1972), we take all possible pairwise

Table 3

Summary of results of correlation analysis between different environments

	Number of correlation coefficients within range				
Characters	0-0·2 or negative†	0·2-0·4	0·4-0·6	0·6-0·8	0·8-1·0
LN10	35	21	21	36	23
H10	0	0	3	38	95
D10	0	0	0	37	99
FT	0	0	0	0	136
FD	1	2	38	64	31
SHF	9	7	18	41	61
HF	12	3	19	43	59
BF	25	38	30	34	9
SR	0	4	28	82	22
CN	47	38	21	27	3
FH	10	23	27	44	32

Correlations above 0·44 are significant at 5 per cent level.
† Negative values were never significant.

combinations of environments and for every such pair calculate correlation coefficients between line performances, character by character. Although in earlier work, rank correlations were calculated, we have decided that the usual product-moment correlations are more appropriate; if we have several lines of roughly similar performance, quite minor changes of performance with environment can produce a substantial change in ranking, so that the rank correlation coefficient is oversensitive to genotype-environment interaction which is too small to be of much biological importance. Results are summarised in table 3. It will be seen that for H10, D10 and FT, correlations were generally high, indicating good repeatability of performance over environments. The same applies, to a reduced extent, to FD and SR. Thus, for these characters, genotype-environment interaction is probably not a serious problem.

An advantage of the correlation approach is that environments giving rise to " anomalous " results are readily identified. In the case of SHF, HF

and FH it turned out that the bulk of the low correlations were found when *one* environment lacked calcium but was supplied with nitrogen, *i.e.* performance on the $Ca^{-}N^{+}$ environments differed markedly from that on all others. For example, in the case of SHF, the 16 correlations lying between 0 and 0·4 were all cases where one environment was $Ca^{-}N^{+}$ and the other environment was not; the same applied to 16 out of the 18 correlations lying between 0·4 and 0·6. For HF the corresponding figures are all 15 cases between 0 and 0·4 and 16 out of 19 cases between 0·4 and 0·6. For FH figures are 30 out of 33 in 0-0·4 and 15 out of 27 in 0·4-0·6. For both HF and FH correlations were particularly low when one plot had N only.

TABLE 4

Summary of results of regression analysis for 20 lines

	LN10	H10	D10	FT	FD	SHF	HF	BF	SR	CN	FH
Number of significant regression mean squares	20	18	20	16	20	15	20	20	20	20	20
Number of significant remainder mean squares	3	12	6	10	5	9	2	6	3	3	4
r^2 values (in per cent)											
(i) More than 80%	14	3	20	1	9	7	20	13	8	19	20
(ii) Between 80 and 40%	6	11	0	8	11	6	0	7	11	1	0
(iii) Below 40%	0	6	0	11	0	7	0	0	1	0	0

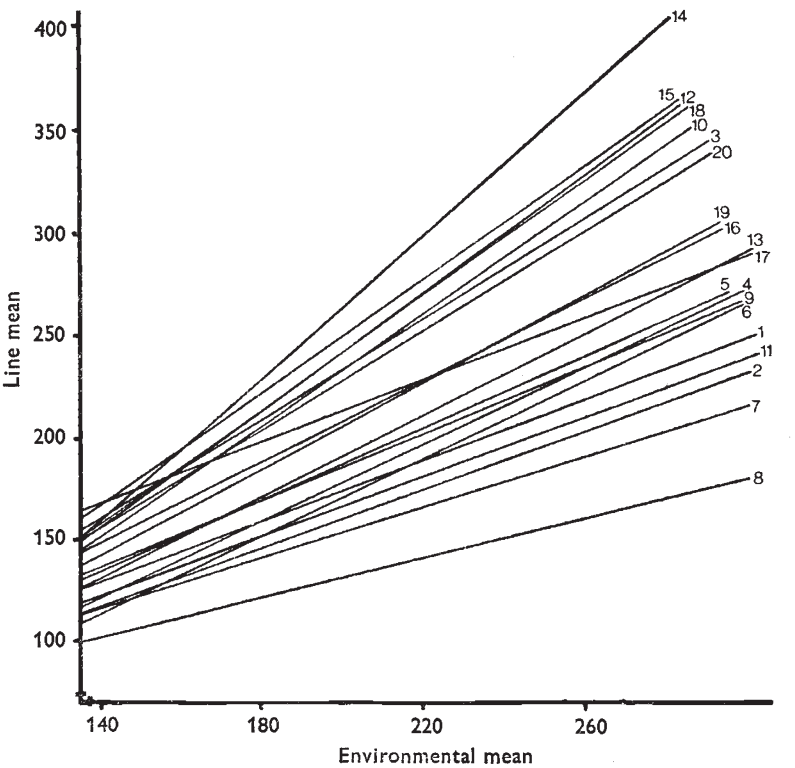

FIG. 1.—Diameter at 10 weeks: regression of inbred line means on environmental mean.

Analogous considerations apply to LN10, where most of the low correlations occurred when one plot was Ca^+ and the other Ca^-.

The correlations for BF and CN were decidedly irregular with no clear pattern emerging; the highest correlations were usually found when both environments were Ca^+ although in some cases low correlations appeared even here. Many correlations were very low. Results for these two characters illustrate the limitations of the correlation approach; as we shall see below, substantial progress towards elucidating the situation can be made by using a different approach.

In this second procedure, we regress (for every character) the performance of every line in the different environments against the corresponding overall environmental mean (Yates and Cochran, 1938; Finlay and Wilkinson, 1963; Perkins and Jinks, 1968). One obvious advantage of this method is that aberrant inbred lines are easily spotted. The results of regression analyses of variance are summarised in table 4. We also give

$$r^2 = \frac{\text{Regression } SS}{\text{Regression } SS + \text{Remainder } SS}.$$

this being the proportion of variation in inbred line means accounted for by the regression. It will be seen that for H10, FT and SHF remainder mean squares are often significant and r^2 values often low, so that the linear regressions do not adequately summarise the data in these cases. For the remaining characters results were as follows. D10, FD and SR regression lines showed rather little crossing, except for lines that were adjacent; this is illustrated in the graph for D10, given in fig. 1. In this graph, line 17 is somewhat aberrant, but other lines preserve their relative position rather well. We thus confirm our earlier conclusion that genotype-environment interaction is not of major importance for these three characters.

A contrasting picture is exhibited by LN10, HF and to some extent FH; in these cases, illustrated in the graph for LN10 (fig. 2) crossing of regression lines is very marked. Thus, for LN10, inbred line 15 with the greatest number of leaves in a " good " environment is one line from the bottom in a poor environment. Again we confirm our conclusion that for these three characters, genotype-environment interaction is very marked (but see Section 5).

Finally, we consider BF and CN which gave very similar graphs; the graph for CN is given in fig. 3, from which it is apparent that the lines, very close together in poor environments, spread out rapidly as the environment improves. The low correlations between environments for these characters are thus seen to be due, in part, to the purely statistical phenomenon of " attenuation ". In the case of BF, it turned out that significant differences between inbred lines could still be detected even for the poorest environments. In such a case we may justifiably compare the rankings of inbred lines in good and poor environments. These showed very little agreement; thus BF is really not repeatable over environments. For CN we were unable to detect any significant difference between inbred lines in the poorest environments. Thus our results are consistent with the notion that in very poor environments all inbred lines perform equally for CN. Perhaps, however, a very large-scale experiment would reveal such a significant difference between inbred lines, possibly accompanied by poor agreement between ranking of lines over good and bad environments. Even if the latter turns out to be the case, it follows from fig. 3 that average relative fitness of a genotype will be

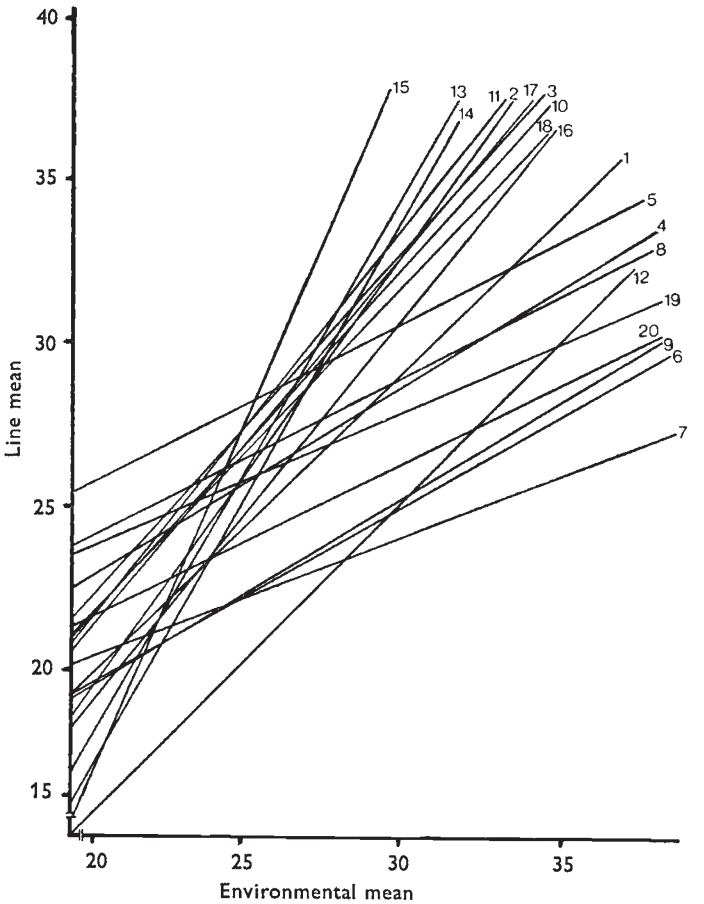

FIG. 2.—Leaf number at 10 weeks: regression of inbred line means on environmental mean.

dominated by its performance under favourable conditions, unless these are very rarely encountered by the population. With this latter proviso, we conclude that the fittest genotypes with regard to CN will be those particularly sensitive to differences in the physical environment, able to produce a marked increase in capsule number during a period when the environment improves, a striking example of phenotypic flexibility. We should perhaps note that plants with as many as 50 capsules can, on occasion, be found growing in the wild.

5. RELEVANCE OF OUR RESULTS TO THE SITUATION IN NATURE

Although poppies will grow in a wide range of situations in nature, it is very unlikely that this range exceeds that covered in our present experiment; certainly the range that we found in plant size is comparable with that found in nature. Some indication that we have indeed exceeded the natural range comes from the data on flowering time given in table 1. The proportion of plants flowering after day 50 was, on some plots (particularly $Ca^{-}N^{+}$) very much larger than we have found for comparable plants growing under

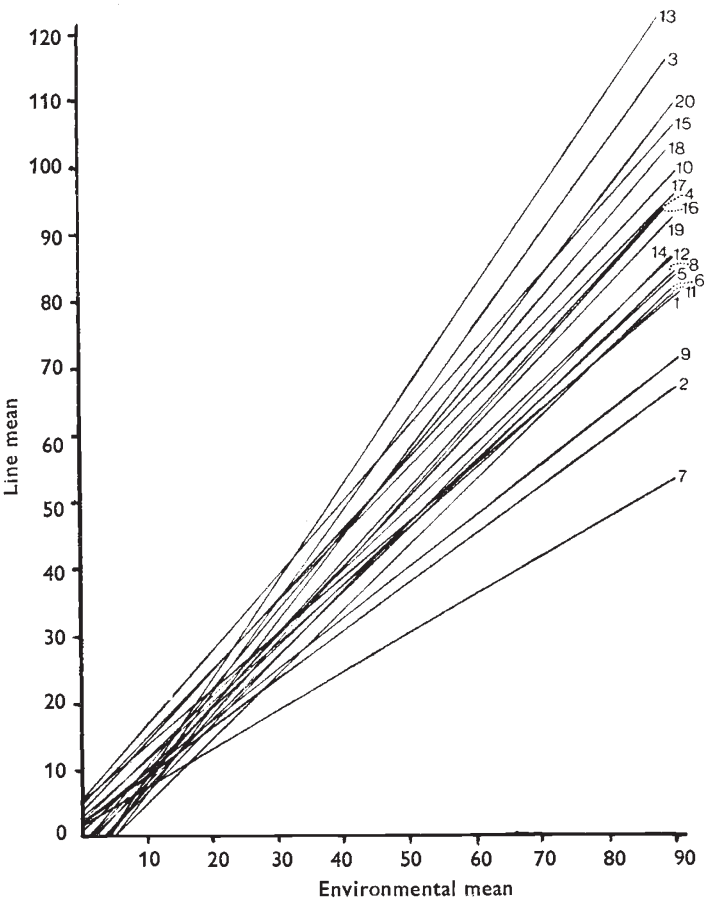

FIG. 3.—Capsule number: regression of inbred line means on environmental mean.

natural conditions (Gale, Rana and Lawrence, 1974; Bumstead, personal communication). We should note that early flowering plants began flowering at about the usual time (2nd June) so that the unusually large proportion after day 50 does represent an expansion in range rather than just a displacement.

These very late flowering plants produced no viable seed. Capsule number also gave an indication that the range of our environments was more extreme than in nature. In this case, line means ranged from 1·0 on the N only plot to 135·0 on the Genetics field, the latter being well above the value we have found for any plant from these populations in nature.

We can conclude with a high degree of confidence, therefore, that for characters H10, D10, FT, FD and SR, repeatability should be high over types of soil occurring in the wild, *i.e.* although plants react very markedly to changes in the environment, different genotypes do so in much the same way in respect to these traits. For SHF, HF and FH, where repeatability was good except on the very poor $Ca^{-}N^{+}$ soil, the situation is less certain. As is apparent from figures given in table 1, this very poor soil was on the margin for supporting the life of *P. dubium*; perhaps, therefore, comparably poor soil

would give very low viability for the species in the wild. If so, the lack of repeatability here is irrelevant. For LN, BF and CN, however, there is no escaping the conclusion that genotype-environment interaction must be taken into account when assessing how selection might act on these characters.

Our results indicate that measurements on the experimental field should give a reliable guide to performance of genotypes in the wild for characters, H10, D10, FT, FD, SR and for SHF, HF, FH on good soil, at least if factors other than soil type are roughly comparable. An obvious complication is the effect of the wide range of germination times found in nature, in contrast to the present experiment, where seeds were treated so as to produce near-simultaneous germination. This problem is being investigated.

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