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# Unstable Genotypes [and Discussion]

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# Unstable genotypes

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Unstable genotypes are normally recognized in cultivated plants by the appearance of coloured spots or flecking which are easily seen and scarcely affected by the environment. Unstable genes giving variation in characters such as plant mass, height, yield and earliness are unlikely to be recognized because normally it would be virtually impossible to distinguish such variation from environmental variation and other genetic variation normally ascribed to segregating genes, without deliberate search and detailed analysis. Continuous variation may contain a host of instabilities, or their more stable products, which could have had their origins in the many normal processes of differentiation. It is not known how easily heritable changes can be induced by the environment in plant species in general, but in flax and *Nicotiana rustica* environmentally induced changes giving large relatively stable differences in plant mass, height and flowering time provide a means of studying the behaviour of unstable genotypes affecting these characters. Crosses between environmentally induced flax types and varieties show that the phenotypic differences are not essentially any different from, and could be dispersed unnoticed among, the rest of the genetic and environmental variation. They also show additivity, dominance and gene interaction, and they can be the cause of asymmetric response to selection and inbreeding depression.

## 1. Major gene differences

Unstable genotypes are well known in cultivated plants. They are due to genetic changes occurring in cells at frequencies well above the frequencies that one normally associates with gene mutations and are confined, as far as one can judge, to a particular chromosome region for any one character showing the particular instability. Genetic changes occurring in unstable genotypes are not due to classical gene mutations, deletions or base changes, but to changes in gene regulation, which may be reversible or maintained for indefinite periods. They are, however, frequently referred to as mutations, and the factors concerned as highly mutable genes.

The presence of an unstable gene is often first revealed by the occurrence of a mosaic of cells, or of colour flecking of a tissue. For example, in *Antirrhinum majus* (Harrison & Fincham 1964) an inactivated anthocyanin gene, $Pal \rightarrow pal^{rec}$ gives white flowers instead of red. But $pal^{rec}$ is unstable and is frequently reactivated, $pal^{rec} \rightarrow Pal$. Whenever this occurs, red spots appear on the white petals, so that the instability of this allele is easily recognized and the frequency of reactivation assessed. Such changes may or may not be an aberrant form of differentiation, but they are of particular interest because the reactivation, or other change in gene regulation, can be transmitted by the gametes to the next generation and when this occurs new types arise.

The process of inactivation or reactivation is generally held to be due to changes in heterochromatization (see, for example, Hagemann & Snoad 1971), to the movement of controlling elements (see, for example, McClintock 1965; Peterson 1976), or to changes in the number of

repeated DNA sequences of some kind (see, for example; Brink *et al.* 1968; Ritossa 1970), but little is known of what controls or regulates the activating or inactivating process. Furthermore, unstable genotypes frequently show a range of activity, perhaps a stepwise change from one level to another. Paramutation (Brink 1960) is a particular form of instability arising when two different alleles are brought together in the heterozygote. One allele, or chromosome segment, apparently induces a change in the activity of, or paramutates, its homologue, a change that may persist, or eventually disappear, in later generations.

Most studies on unstable genotypes have been on the instability of major genes, i.e. genes giving phenotypic differences that are easily seen and which are almost unaffected by the environment, which is of course why they were detected in the first place. Table 1 gives examples of characters scored in *Antirrhinum majus* (Harrison & Fincham 1964), maize (Peterson 1976; Brink 1960), soybean (Peterson & Weber 1969) and tomato (Hagemann & Snoad 1971). Not only are the phenotypes clear cut, but once recognized as being due to unstable genes their stepwise, or range of, activity can often be measured.

TABLE 1. CHARACTERS SCORED IN THE DETECTION AND ANALYSIS OF UNSTABLE GENOPTYES IN SOME CULTIVATED PLANTS

| | locus | character |
|---|---|---|
| *Antirrhinum* | *Pal* | red spots on white petals |
| maize | $A_2$–$E_n$ | coloured spots on seeds |
| maize | *R* | mottled pigmentation on seeds |
| soybean | $Y^m$ | yellow patches on leaves |
| tomato | *Sulf* | yellow leaves |

## 2. CONTINUOUS VARIATION

Unstable genes can also affect continuously varying characters such as plant mass, height, yield and earliness, characters of central importance to plant breeders, although there is no direct evidence that they or their more stable products are widespread. But nor is there evidence that they are not. Unstable genes giving variation in these characters are unlikely to be recognized because normally it would be virtually impossible to distinguish their variation from environmental variation and other genetic variation normally ascribed to the segregation of genes without deliberate search and detailed analysis. Observed continuous variation may be partly due to a multitude of genetic instabilities, or their more stable products, which could have their origin in the many processes of differentiation which are part of the normal growth and development of the individual. Paramutation could easily supply a pseudo-Mendelian framework for heterozygous but homogeneous $F_1$ plants with release of variation due to gene instability, rather than due to segregation, in the $F_2$ and later generations. At issue is how widespread these instabilities are and, in the present context, how important they are in plant breeding.

An initial problem is the separation of variation due to unstable genes from that due to the environment, and from continuous genetic variation of the kind considered to be based on Mendelian inheritance. In some cases it is possible that they may be picked up by biometrical methods. Another way is to start with homozygous and homogeneous plants and induce heritable changes in gene regulation by growing the plants in different environments, as has been done with flax (Durrant 1962, 1971) and *Nicotiana rustica* (Hill 1965). These changes need not be regarded as exceptional because for example in *Antirrhinum* (Harrison & Fincham




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1964) and in maize (Peterson 1958) the instability of major genes is markedly influenced by the environment, particularly temperature. Unstable genes have also been induced by chemical mutagens. J. Begum obtained unstable major genes in flax by treating the seed with ethylmethanesulphonate, and at least one other mutation affecting plant mass is probably due to a regulatory change of some kind. Paramutants at the *R* locus in maize are highly sensitive to chemical mutagens (Axtell & Brink 1967).

In so far as the products of unstable genotypes affecting yield can be stabilized and maintained indefinitely, they are of potential interest to the plant breeder, but we would want to know more about their behaviour on crossing. Here we can turn to some crosses made with flax plants in which inherited changes have been environmentally induced.

### 3. Environmentally induced changes in flax

Relatively permanent heritable changes have been induced in two varieties of flax by growing the plants with additional nutrients, mainly different fertilizer combinations, and at different temperatures. Because heritable changes are environmentally induced in them, the two varieties are called plastic varieties. Table 2 shows some of the characteristics of two types of plants, the large genotroph (L) and the small genotroph (S), induced in the plastic variety Stormont Cirrus (Durrant 1962, 1971; Evans *et al.* 1966; Durrant & Nicholas 1970; Timmis & Ingle 1973; Cullis 1976). The L and S genotrophs breed true in most environments, like two genetically distinct types, and only in some circumstances do they reveal the nature of their origin. For example, when L and S are crossed, thus bringing together their two different levels of gene regulation, the $F_1$ is genetically unstable, i.e. an overtly unstable genotype has been generated by crossing, with release of genetic variation.

Table 2. Large and small genotrophs induced from the flax variety Stormont Cirrus

(Mean values over years; DNA in arbitrary units.)

| | large (L) | small (S) |
|---|---|---|
| plant weight/g | 47 | 13 |
| plant height/cm | 87 | 68 |
| total nuclear DNA/pg | 115 | 100 |
| number of rRNA genes | 2914 | 1801 |
| capsules | hairless | hairy |

It has been convenient in the past to recognize two classes of genotrophs, the plastic type, like the original Stormont Cirrus plants in which heritable changes can be induced by the environment, and the stable type, like the L and S genotrophs, in which heritable changes are not subsequently easily induced. But these are relative terms, because plants of the Stormont Cirrus variety appear to have gradually lost their plasticity in some characters over some ten generations in the environments in which they have been grown, although phenotypically they do not appear to be different. On the other hand, L and S genotrophs possess a certain degree of plasticity. For example, L plants are normally maintained by growing them each year in a greenhouse for at least the first 5 weeks from sowing. If, instead, the plants are grown out of doors each generation (figure 1) the amount of nuclear DNA drops each year (Durrant & Jones 1971; Joarder *et al.* 1975), until after five generations out of doors they have lost about 15% of their total nuclear DNA, at which point they contain about the same amount of DNA




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as the small genotroph, S. During this period there is no change in the L phenotype, but in the sixth generation the L plants are liable to change suddenly to approximately the S phenotype. It is reasonable to suppose that other, undetected, changes occur in the genetic material over the years, which could eventually have important effects on the phenotype. The gradual loss of plasticity of Stormont Cirrus plants in some characters over several generations may be due to similar causes.

Crosses of L and S respectively to Stormont Cirrus variety (SC) from which they were induced, give approximately additive effects. Measurements on the fourth generation by Y. Al-Saheal in figure 2*a* show that the difference in DNA between the two sets of crosses, 4.8 units, is about half the difference between the L and S parents, as would be expected if the contributions of both parents in each case, L and SC, S and SC, were strictly additive. The DNA amounts in the crosses have, however, dropped a little from the mid-parent values, which may be due to the environment or to a gradual shift towards the lower parent in each case. Plant mass and height in these crosses behave similarly.

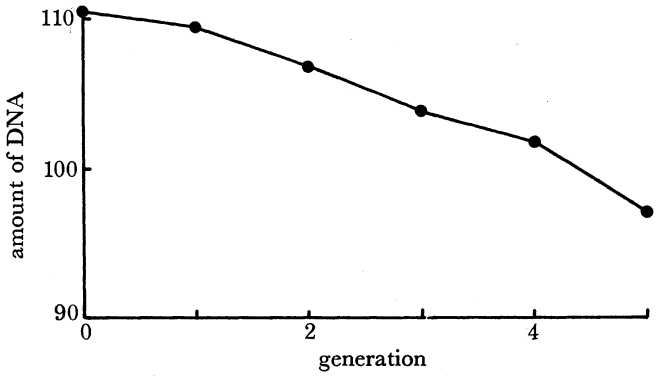


FIGURE 1. Decrease in amount of DNA (arbitrary units) over five generations in the L genotroph of the flax variety Stormont Cirrus when grown out of doors.

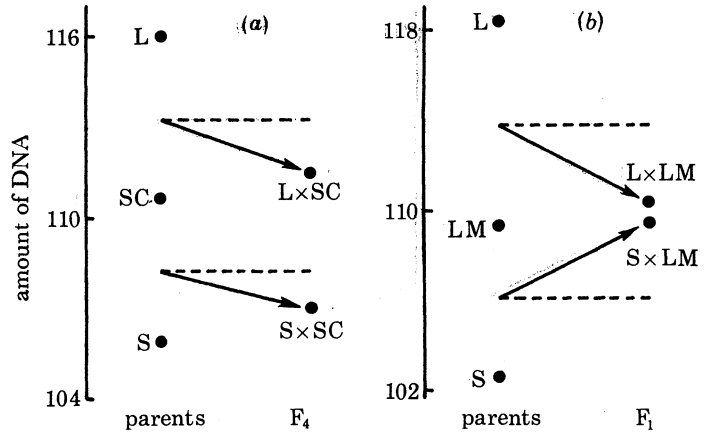


FIGURE 2. (*a*) Amounts of DNA (arbitrary units) in parents and $F_4$ generation of reciprocal crosses between L and Stormont Cirrus (L × SC), and S and Stormont Cirrus (S × SC). Broken lines show mid-parent values. (*b*) Amounts of DNA in parents and $F_1$ generation of reciprocal crosses between L and Liral Monarch (L × LM), and S and Liral Monarch (S × LM).




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### 4. Outcrosses with the flax genotrophs

Crosses of L and S with other varieties appear to fall into two groups. Either the other variety appears to revert, or cancel out, the heritable changes that originally occurred in the induction of L and S, or the other variety itself could have heritable changes induced in it by L and S (Durrant 1972).

The flax variety Liral Monarch (LM) has about the same, intermediate amount of DNA as Stormont Cirrus, and about the same, intermediate plant mass. When L and S are reciprocally crossed to it, the difference in amount of DNA between the two sets of crosses virtually disappears. Figure 2*b* shows that the amounts of DNA, measured by O. I. Joarder, are practically the same in the $F_1$ of the crosses between L and LM, and S and LM, 103.2 and 102.3 units, as in Liral Monarch itself, or in Stormont Cirrus. If the amounts were additive, contributions of

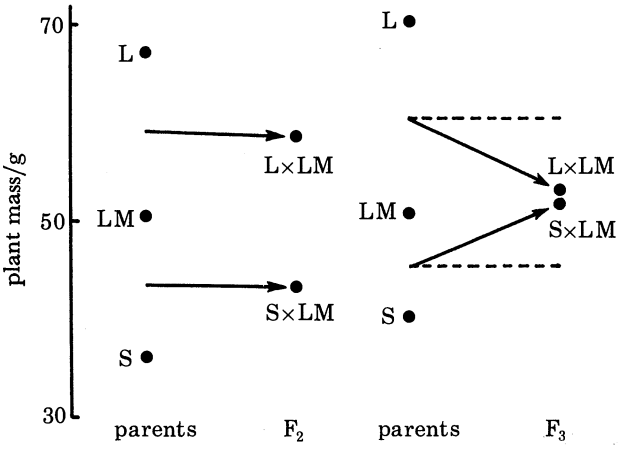


Figure 3. Plant masses of parents and $F_2$ and $F_3$ generations of reciprocal crosses between L and Liral Monarch (L × LM), and S and Liral Monarch (S × LM) grown in 1971.

Table 3. Amounts of apparent reversion in reciprocal crosses between L and Liral Monarch, and S and Liral Monarch in different generations and years

| | 1970 | 1971 | 1972 |
|---|---|---|---|
| $F_1$ | — | — | complete |
| $F_2$ | partial | none | complete |
| $F_3$ | — | complete | complete |
| $F_4$ | — | — | complete |

the parents to the $F_1$ values would be 106.4 and 99.3 units respectively. Reversion also appears to occur in plant mass but it is often progressive rather than immediate. For example, in figure 3, from work by O. I. Joarder, there is no reversion in an $F_2$ grown in 1971, but apparently complete reversion in an $F_3$ generation grown in the same year. The pattern over the years in table 3 shows that the plants must be grown in the right environment for reversion to occur. Evidently 1972 provided the right environment for reversion. Viewed in another way, crossing L or S with Liral Monarch generates plastic plants in which changes in plant mass can be induced in predetermined directions when they are grown in the right environments. L and S crosses with some other flax varieties have roughly similar plant mass patterns to Liral Monarch, whereas L and S crosses to the flax variety Hollandia tend to diverge in plant mass.

An important question in the present context is whether increased plant mass, or yield, can




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be obtained by using a plant such as the large genotroph L in a crossing programme. Linseed varieties have larger plant masses and seed yields than flax and normally they would not be crossed with flax types for improvement in seed yield unless it were to introduce some particular characteristic. The linseed variety Royal, for example, is about three times larger than Stormont Cirrus. The L and S genotrophs were, however, both reciprocally crossed to Royal and selection for high and low plant mass made over several years. The plant masses of the selection lines in figure 4 have been adjusted against the overall mean plant mass of the three parents, L, S and Royal, in each generation, and in figure 5 against Royal alone for comparison with it.

In the crosses between L and Royal, the plants respond well to high and low selection but they do not exceed the mass of Royal (figure 5). In fact, compared with Royal the high selection line responds once, in $F_3$, and thereafter stays fairly constant, practically the same as

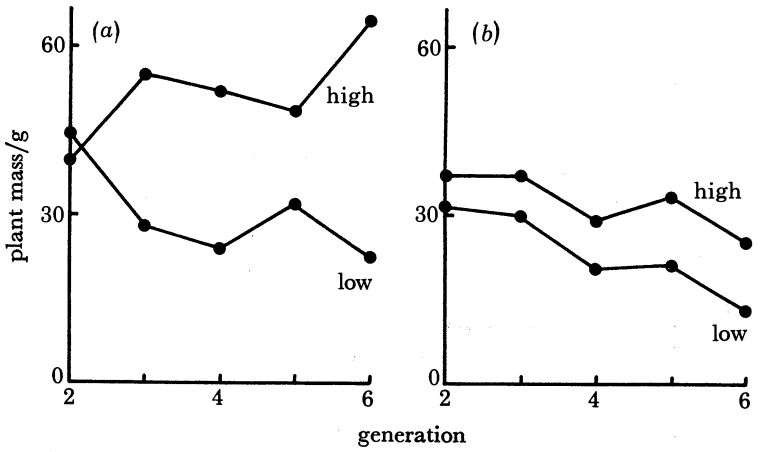


FIGURE 4. Plant masses of reciprocal crosses between (*a*) L and Royal and (*b*) S and Royal in high and low selection lines, adjusted against the standardized mean plant masses of the three parents.

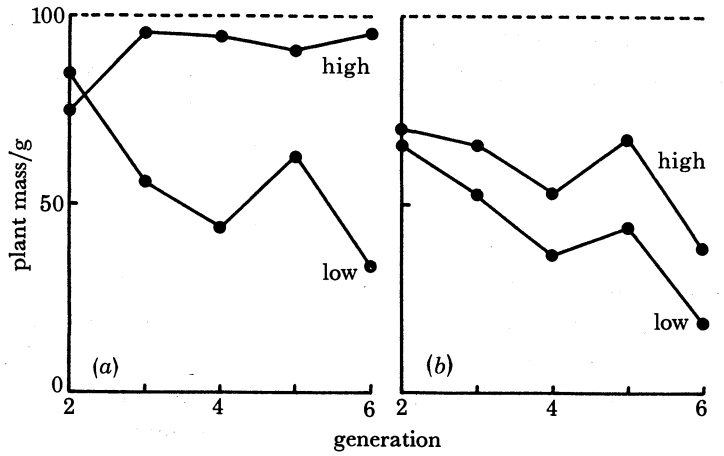


FIGURE 5. Plant masses of reciprocal crosses between (*a*) L and Royal and (*b*) S and Royal in high and low selection lines, adjusted against the standardized mean plant masses of Royal only (broken line).

Royal, as though a switch to the higher plant mass had occurred in that generation. It is possible the Royal plant mass might be exceeded were derivatives of crosses between L and S used instead of L, and $F_3$ backcrossed to Royal or recurrent selection employed. In the crosses between S and Royal there is a grossly asymmetric response to selection. Whether one selects for high or low plant mass there is an inevitable decline. This may be because the S genotroph

introduces factors that induce changes in gene regulation in the same direction as that in S, or because the level of gene regulation in S interacts unfavourably with the regulation in Royal. The environments in which they were grown over the years were either unable to switch the level of regulation, or were necessary for the decline to occur. This trend has the appearance and characteristics of inbreeding depression, arising from selfing the reciprocal crosses between S and Royal, but it is due to a level of regulation in S different from that in L.

Although these experiments give some insight into the behaviour of products of unstable gentoypes, they are too meagre to justify general conclusions as to their potential contribution to plant breeding, but some general remarks can be made, which are probably not in disagreement with the results of analyses on the induced changes in *Nicotiana rustica* (Perkins *et al.* 1971; Towey & Jinks 1976). First, the *Linum* crosses probably reveal the hidden behaviour of unrecorded unstable genotypes having similar or more moderate effects on plant size and yield. Secondly, phenotypic differences between relatively stable products of unstable genotypes are not essentially different from any other differences between plants, genetic or environmental, and they could be dispersed unnoticed among the rest of the genetic and environmental variation to which they could make major contributions. Unstable genotypes supplying additional variation can be generated by crosses between the more stable products of unstable genotypes. Thirdly, in so far as unstable genotypes, unrecognized, have contributed to plant improvement in the past, their conscious induction and manipulation could be advantageous in the future. Fourthly, newly introduced, unselected material from natural habitats may respond to the environments into which they are brought in a more direct way than that normally attributed to selection.

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*Discussion*

H. Rees, F.R.S. (*Department of Agricultural Botany, University College of Wales, Aberystwyth, U.K.*). It is interesting to compare the induced changes in chromosomal DNA in flax with those induced by hybridization in *Nicotiana*. In the hybrid *N. tabacum* × *N. otophora*, certain chromosome segments become amplified to a prodigious degree. In this case the 'conditioning' is a response to a genetic as distinct from an external stimulus. It would be of interest to find out if the molecular changes in the chromosomes of *Nicotiana* are of the same kind as in flax.

A. Durrant. It is also interesting to note that the amplification occurs in a genus in which heritable changes have been induced by external stimuli. Genetic requirements for environmentally induced heritable changes in amount of DNA have been studied in flax by crossing and backcrossing between a plastic flax variety and a stable linseed variety. The plastic variety apparently possesses a nuclear factor and a cytoplasmic factor that are both lacking in the stable variety, and the nuclear factor to be operative must be transmitted in a nucleus from a plant that possessed the plastic cytoplasm.

C. A. Cullis (*John Innes Institute, Norwich, U.K.*). The DNA from a number of the genotrophs described by Dr Durrant has been characterized. The ribosomal RNA gene number and a fraction of the intermediately repetitive sequences have been shown to vary between genotrophs. Three intermediately repetitive sequences, which vary in copy number between genotrophs, have been cloned from total flax DNA. These sequences were hybridized to total DNA from a number of genotrophs, which had been digested by the restriction endonuclease *Eco*RI. For one of the cloned sequences, a band of hybridization appeared in the DNA from genotroph $L_3$ that was not present in the DNA from either $L_1$ or $L_6$. The origin of this band, whether or not it is due to transposition, deletion or some other event, is being investigated.

A. Durrant. The $L_3$ genotroph has the same phenotype as the L genotroph, but its total amount of DNA has been reduced to the same intermediate amount as in the original variety, by growing the L plants out of doors for three generations. DNA hybridization studies will become increasingly important in establishing the nature of the DNA changes, if not the mechanism of the changes, and one looks forward to seeing these results.




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